Clinical & Serological Studies

Of

Canine Atopic Dermatitis

by

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Abstract

Canine atopic dermatitis is a skin condition of an allergic origin which usually becomes apparent in dogs between the ages of one and three years. This is a particular problem for Guide Dogs for the Blind Association (GDBA) dogs as this is the age that dogs are finishing training and beginning their working life. Hence much time and money is spent on dogs which may have to be retired early due to this skin condition. Therefore, if dogs likely to go on and develop atopic dermatitis could be identified before beginning their training more efficient use of funds and facilities could be made by trainers and the society.

A group of dogs passing through the GDBA kennels were studied for a period of three years. This involved collecting clinical and serological data, with the aim of identifying factors which could be used to isolate dogs likely to develop clinical signs of atopic dermatitis. In addition to GDBA dogs, groups of racing greyhounds, laboratory beagles and pet dogs were also examined in order to identify factors peculiar to the GDBA population.

GDBA dogs provided an ideal opportunity to study a large number of dogs in a particular environment with excellent husbandry and recording of clinical histories.

Both clinical and serological parameters were studied. Examination of clinical histories revealed that GDBA dogs demonstrating four or more episodes of atopic type skin disease before 15 months of age were at an increased risk of developing atopic dermatitis.

Serological studies revealed that serum total IgE concentrations are unrelated to the age or parasite status of a dog. Rather there appears to be a range of serum total IgE concentrations in the canine population with some dogs showing high levels and other low. Although it has been suggested (de Weck *et al,* 1998) that only dogs with high serum total IgE concentrations will go on to develop atopic dermatitis this was not always found to be the case as a number of atopic dogs was found to have low serum total IgE concentrations.

Unlike serum total IgE, serum total IgG_1 concentrations were found to be significantly higher in dogs affected by atopic dermatitis and/or parasitism. In addition serum total IgG_1 concentrations were found to increase in dogs following hyposensitisation therapy and this appeared to be associated with the success of hyposensitisation. It is possible that measurement of serum total IgG_1 concentrations could be used as an indicator of the success of hyposensitisation before a clinical improvement becomes apparent.

When comparing intradermal skin test results and serological results in the same dogs, results do not often agree, possibly due to the different methodologies involved.

Allergen exposure appears to influence antibody levels with dogs in different environments and at different times of year showing different serological results. Although allergen exposure can be assumed to be different in different environments an interesting method of identifying exactly which pollens a dog has been exposed to was developed. This involved examination of faecal samples for their pollen contents and revealed a large variety of pollens. This method could be used in the design of intradermal skin tests or serological tests which to date are primarily based on human pollen exposures.

Examination of individual allergen responses revealed that atopic dogs appear more likely to demonstrate higher serological results against mould allergens than non-atopic dogs. This was the case even though very few dogs actually demonstrated positive intradermal skin test results against mould allergens.

In summary, this study has disproved a number of hypotheses, including the belief that serum total IgE concentrations depend on the parasite status of an animal, and that it is not possible to compare different serological tests and expect a good correlation.

The author has demonstrated that by examining the number of episodes of skin disease in dogs by particular ages, the serological response of individuals to particular allergens, especially moulds, and by assessing serum total IgG_1 concentrations it is possible to identify dogs at risk of developing atopic dermatitis.

Contents

Volume 1

Chapter 9 Final discussion & conclusions

Volume 2

Appendices

Index of Tables

Index of Figures

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Mary A. Fraser

Abbreviations

°C	degrees Celsius
%	per cent
Ab	antibody
Ag	antigen
AMD	acute moist dermatitis
ANOVA	analysis of variance
A. siro	*Acarus siro*
cAMP	cyclic adenosine monophosphate
CD	cluster of differentiation
cGMP	cyclic guanine monophosphate
CCR	curly coated retriever
Conc.	concentration
CSF	colony stimulating factor
D	Daltons
Df	Degrees of freedom
D. farinae	*Dermatophagoides farinae*
DL-A	dog leukocyte antigen
D. pteronyssinus	*Dermatophagoides pteronyssinus*
ELISA	Enzyme linked immunosorbent assay
et al.	and others
Fab	Variable end of antibody molecule
Fc	Constant end of antibody molecule
GDBA	Guide Dogs for the Blind Association
g/h	Greyhound
GR	Golden retriever
GSD	German shepherd dog
GSP	German shorthaired pointer
GUVS	Glasgow University Veterinary School
H1	type 1 histamine receptor
H2	type 2 histamine receptor
I.D.	Identification
IDST	Intradermal skin test
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgG_1	Immunoglobulin G subgroups.
IgG_2	
IgG_3	
IgG_4	
IgG_d	

IgM	Immunoglobulin M
IFNß	Interferon beta
IFNγ	Interferon gamma
IL	Interleukin
Immbrown	Immunodot (Indoor allergens)
Immgreen	Immunodot (Outdoor allergens)
Immred	Immunodot (Topscreen)
L	Labrador Retriever
mg/dl	milligrams per decilitre
MHC I	Major Histocompatability complex type 1
MHC II	Major Histocompatability complex type 2
ml	millilitres
mm	millimetres
ng	nanograms
nm	nanometres
NU	Noon units
O.D.	optical density
PCA	passive cutaneous anaphylaxis
PK	Prausnitz Kustner
PNU	protein nitrogen units
RAST	Radio-allergosorbent assay
R.D.	Reflective density
RSV	Respiratory syncitial virus
SBT	Staffordshire bull terrier
TBS	Tris buffered saline
TCR	T cell receptor
Terr	terrier
Th	T helper lymphocyte
TMB	tetramethylbenzidine hydrochloride
TNF-α	tumour necrosis factor alpha
TNF-γ	tumour necrosis factor gamma
T. putrescentiae	*Tyrophagus putrescentiae*
Ts	T suppressor lymphocyte
ug	micrograms
ul	microlitres
um	microns
WHWT	West Highland White Terrier
x	Crossed with when breeding

Preface

This study is designed to follow the progress of dogs passing through the Guide Dogs for the Blind Association (GDBA) kennels at Forfar. The main area of interest in the study is atopic dermatitis, which usually becomes evident in dogs between the ages of one and three years. As guide dogs often begin their working life at two years of age it is not uncommon for a dog to go through a year's training after which clinical signs of atopic dermatitis become. Atopic dermatitis interferes with a dog's ability to work and is distressing for an owner. Indeed, in cases where medical management is not successful it can lead to the early retirement of a dog, which is obviously costly for the GDBA.

Thus, the main aim of the study is to create a predictive model, which will be able to identify those dogs likely to develop atopic dermatitis before they begin their training at a year old.

A number of areas are incorporated in the study including clinical examinations, clinical histories and serological studies. The GDBA population provides a situation where a detailed examination of a large number of dogs from a closed population can take place. This is advantageous, as these dogs undergo regular clinical examinations and highly detailed clinical histories are kept. In addition, all dogs undergo a rigorous parasite control programme and both endo- and ectoparasites are rare in the GDBA population at this particular kennel. Thus factors such as parasitism or even owner observation, which may influence results, can be minimised.

For comparative purposes, groups of kennelled beagles, racing greyhounds and pet dogs will also be studied, to identify whether or not findings are peculiar to the GDBA population. These measures will allow findings to be applied to the canine population as a whole.

Chapter 1 Literature Review

1.1 History of atopic dermatitis

Atopic dermatitis is a skin condition found in both humans and animals which is assumed to have an allergic pathogenesis. A common feature is evidence of pruritus usually presenting at a young age.

The term atopy was first introduced in 1923 by Coca & Cook. At that time it was restricted to a 'hypersensitivity reaction of the asthma and hayfever group.' The term atopy was derived from the Greek ατοπια (atopia) meaning strange disease, an apt description to this day. Among the criteria devised by Coca and Cook were features such as, the condition was inherited by way of a dominant gene; a hypersensitivity reaction against substances such as tuberculin was present and that by injecting the active substance the severity of clinical signs could be lessened. As will be discussed later these clinical findings have stood the test of time. However, Coca and Cook also suggested that the disease could not be transferred passively to normal individuals *via* the blood of hypersensitive individuals – a fact later proved false.

The term 'atopic dermatitis' was first introduced in human medicine in 1935 by Hill & Sulzberger to describe a pruritic skin condition often associated with hay fever and asthma. In veterinary medicine atopy was first described in the dog in 1941 by Wittich, and later by Patterson (1960), and Schwartzman & Rockey (1967). The first description by Wittich (1941) noted that the main clinical features were respiratory signs of a hayfever like condition, associated with pruritus and urticaria. In addition this dog demonstrated positive direct skin tests.

In later reports of canine atopy, the condition was described as, either a pruritic skin condition, or a respiratory problem (Schwartzman & Rockey, 1967). Criteria for a diagnosis of atopy by Schwartzman & Rockey (1967) included the presence of reaginic antibodies in the serum of a hypersensitive patient; that these antibodies could be demonstrated by means of a Prausnitz Kustner test and that positive skin tests could be obtained for allergens such as

pollens, house dust or mould. These authors also noted that the clinical signs of atopy were seasonal and that the condition appeared to be familial.

The majority of recent work on canine atopic dermatitis has been carried out by Willemse. His review of atopic skin disease (1986) defined atopy as a hereditary, IgE mediated hypersensitivity to environmental allergens. Currently diagnosis of canine atopic dermatitis is based on Willemse's criteria. This will be discussed in some detail later.

1.2 Pathogenesis of atopic dermatitis

Atopic dermatitis is a condition where the patient (human or animal) becomes sensitised to environmental antigens that in non-atopic animals create no disease (Scott *et al.*, 1995). Type 1 hypersensitivity reactions involving the production of Immunoglobulin E (IgE) and mast cell degranulation are believed to be the major pathogenic process (Scott *et al.*, 1995), although late phase reactions and Type IV hypersensitivity reactions may also be involved.

Substances such as pollens and house dust mites are believed to come in contact with the immune system in the respiratory tract, with initial presentation resulting in the formation of antibodies, generally of the IgE class, which bind to mast cells. On subsequent exposure to the same substance (or allergen), cross linkage of IgE molecules *via* allergen results in the degranulation of mast cells and release of vaso-active mediators such as histamine. These mediators are responsible for the clinical signs such as pruritus associated with atopic dermatitis.

Although this is the accepted basis to the pathogenesis of atopic dermatitis much controversy exists about this theory and other ideas have been put forward by various authors. Szentivanyi (1968) put forward the beta adrenergic theory which suggests an abnormal response in the sympathetic branch of the autonomic nervous system. Both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are involved in the mediation of cell reactions. The beta adrenergic theory suggests that reduced cAMP or elevated cGMP levels tend to labilize cells and

promote the release of inflammatory mediators. This is stimulated by substances such as cholinergic drugs, oestrogen or levamisole.

A further theory of atopy is that of the Basenji greyhound model suggested by Chan *et al.* (1985). Again, this is based on studies of cAMP, where increased phosphodiesterase activity results in a lowering of cAMP responses and resultant airway hypersensitivity.

The route by which allergens come into contact with the immune system is currently under examination. The traditional theory of allergen presentation, as mentioned above, is *via* the respiratory tract. However, there is some proof that percutaneous absorption of allergens may also be involved.

The clinical presentation of atopic dermatitis in both humans and dogs would suggest that percutaneous absorption of allergens may take place. In humans, the commonly affected sites are the flexural areas of the arms and legs, or periocularly where the skin is relatively thin. In the dog, dermatitis is often present around the eyes, on the abdomen, flexural areas of the lower legs, and between the pads of the feet. These areas are subject to friction and/or have poor hair covering allowing easy contact between allergens and the skin.

In addition, increased numbers of Langerhans cells have been demonstrated in the skin of atopic humans and dogs compared to non-atopics (de Vries, 1994) and from this Olivry *et al.* (1996) suggested that transepidermal capture of allergens is possible.

1.2.1 Cellular components of immune response

1.2.1.1 Langerhans cells

Langerhans cells were first described in 1868 by Paul Langerhans. These cells are derived from the bone marrow, from which they migrate to the suprabasal epidermis (Stingl *et al.*, 1993). The identifying feature is the Birbeck granule (Yager, 1998), which is thought to represent internalised ligand receptor complexes and is present in the Langerhans cells of humans, cats, mice and horses, but is absent in avians and amphibians. In the dog, the presence of Birbeck granules is controversial (Yager, 1998).

Langerhans cells are the chief antigen presenting cell in the skin immune system (Stingl *et al.*, 1989). Surface molecules such as Major Histocompatability Complex II (MHC II) and receptors for the Fc portion of IgE and IgG are present on Langerhans cells and are important in hypersensitivity reactions in humans (Bieber *et al.*, 1992).

As mentioned above, increased numbers of Langerhans cells have been identified in the skin of human atopics compared with non-atopics (de Vries, 1994) suggesting that the Langerhans cell may be important in the pathogenesis of atopic dermatitis.

1.2.1.2 Keratinocytes

The traditional functions of keratinocytes are well known, including the production of keratin, surface lipids and intercellular substances (Scott *et al.*, 1995). Keratinocytes are also capable of producing cytokines involved in the inflammatory response; releasing inflammatory mediators such as histamine and carrying out phagocytosis under certain conditions (Scott *et al.*, 1995).

Release of preformed interleukin-1 (IL-1) takes place after injury to the keratinocyte (Yager, 1998). This cytokine is pro-inflammatory and is involved in the T cell response. Keratinocytes can also release histamine following exposure to ultraviolet light. (Yager, 1998).

Activated keratinocytes can express the Major Histocompatability Complex molecule II (MCH II) on their surface (Reviewed by Chu & Morris, 1997). The MHC II molecule is usually expressed on the surface of B lymphocytes and macrophages and is required for the presentation of antigenic epitopes to T helper cells in order to stimulate an immune response. Theoretically, keratinocytes could process and present antigens to T lymphocytes (Stingl *et al.*, 1989). If this is the case then percutaneous absorption of allergen could take place with the keratinocyte playing a major role. However, although activated keratinocytes can express MHC II molecules on their surface they have been shown to lack B7-1 (otherwise known as CD80) receptor molecules which are required for presentation of antigen to T lymphocytes (Yager, 1998). It is more probable that keratinocytes have a role to play in antigen

processing but do not contribute to the pathogenesis of atopic dermatitis by presenting antigens to T lymphocytes (Yager, 1998). Indeed it has been suggested that presentation of antigens by keratinocytes may well result in a down regulation of the immune response or the induction of tolerance (Yager, 1998). From this it can be seen that much work is still required on the role of the keratinocyte in the pathogenesis of atopic dermatitis.

1.2.1.3 Monocytes / macrophages

Monocytes are derived from the bone marrow. These cells enter the blood stream and then enter the tissues where they will reside. In these tissues monocytes differentiate into macrophages, where their main function is phagocytosis of foreign material and presentation of this to B and T lymphocytes. In addition to phagocytosis, macrophages can act as secretory cells and produce enzymes, proteins in the complement cascade, interferon gamma (IFN γ), IFN β and IL-1; IL-1 being required to activate T cells (Chang *et al.*, 1990).

1.2.1.4 Lymphocytes

Lymphocytes are immune system cells which can be divided into three groups. These are bone marrow derived cells (B cells), thymus dependent cells (T cells) and null cells. Null cells do not demonstrate any of the characteristic markers of B and T lymphocytes and will not receive any further mention. T cells have received by far the most attention in both canine and human atopic dermatitis.

T Lymphocytes

Although produced in the bone marrow T lymphocytes are activated and released from the thymus. From here they disperse to the secondary lymphoid organs such as the spleen and lymph nodes. T lymphocytes can express a number of surface molecules. Groups of surface molecules which can be identified by a single monoclonal antibody have been described as Clusters of Differentiation (CD) (Cobbold & Metcalfe, 1994) and each monoclonal antibody has been given an identifying number.

All mature T lymphocytes express surface molecules which can be identified with the use of CD3 antibodies and surface molecules called T Cell Receptors (TCR). Two forms of T cell receptors (TCR) have been described. Firstly the α/β TCR which is found on 90% of human cutaneous lymphocytes, and secondly the γ/δ TCR which is the predominant cutaneous T cell in ruminants. T cells expressing α/β TCR can be further sub-divided into two distinct groups depending on whether they express CD4+ or CD8+ markers. Those cells which express CD4+ are designated T helper cells (Th) and those that express CD8+, suppressor cells (Ts).

T helper cells are involved in the stimulation of B lymphocytes and these T helper cells can be further divided according to the cytokines which they produce. Th1 cells produce interleukin–2 (IL-2), interferon-γ (IFN-γ), and tumour necrosis factor α (TNF-α) whereas Th2 cells produce IL-4, IL-5 and IL-10.

Th1 cells preferentially develop against bacterial infection and are important in delayed hypersensitivity reactions (Chang *et al.*, 1990). Th2 cells are involved in activation and proliferation of mast cells, eosinophils and lymphocytes (Hnilca & Angarano, 1996) *via* their cytokine production. They also promote antibody dependent immunity especially IgE production (Yager, 1993), with reactions predominantly against helminths and environmental allergens. As such, Th2 cells are important in the pathogenesis of atopic dermatitis (de Vries, 1994).

Defective cell mediated immunity has been demonstrated in both canine and human atopic patients (Leung *et al.*, 1993; Nimmo Wilkie, 1990), with a lower proportion of CD8+ T suppressor cells present in the circulation of atopics. This leads to an increase in the ratio of CD4+:CD8+ cells (Leung *et al.*, 1993; Kapsenberg *et al.*, 1991; van der Heijden *et al.*, 1991; Stingl *et al.*, 1981). In atopic dermatitis this decrease in T suppressor cell numbers may be important (Chiorazzi *et al.*, 1977) as it may allow excessive IgE production by B cells.

In addition to decreased T suppressor cell numbers, atopic patients have been shown to have increased concentrations of T helper cells, in particular those

patients suffering from disease (Wiernga *et al.*, 1990) and much research has concentrated on this subset of cells.

van der Hiejden (1991) demonstrated that Th2 cells specific for the house dust mite *Dermatophagoides pteronyssinus* from the skin of atopic people produced IL-4. As IL-4 stimulates the production of IgE this would suggest that Th2 cells are important in the pathogenesis of raised serum IgE levels in atopic patients.

External factors such as drug therapy can augment Th2 cell reactions. Glucocorticoids, calcitrol and progesterone (Romagnani, 1994) may enhance the differentiation of Th2 cells and thus tend towards the production of IgE and the proliferation of mast cells. This would appear to be an anomaly in the treatment of atopic dermatitis. However, as glucocorticoids have an obvious beneficial effect in the treatment of atopic dermatitis there must be other factors involved. Hyposensitisation also appears to influence the activity of Th2 cells. Secrist *et al.* (1993) demonstrated that allergic patients receiving hyposensitisation produced lower levels of IL-4 but these authors did not provide an explanation for this.

B lymphocytes

B lymphocytes make up between 5 and 15% of the peripheral blood lymphocytes and unlike T lymphocytes, B lymphocytes are capable of interacting directly with antigens. Antigen presenting cells present antigens to B cells in association with T helper cells in the lymph nodes. The resultant activated B cells divide and thus generate large numbers of antigen specific effector cells. These will develop into plasma cells capable of producing antigen specific antibodies and memory cells capable of responding on subsequent exposure to the same allergen (reviewed by Day, 1993). Most research on atopic dermatitis in both humans and animals has concentrated on T lymphocytes rather than B lymphocytes even though B cells are the source of IgE, central to the pathogenesis of atopic dermatitis.

1.2.1.5 Mast cells

Mast cells are one of the most important cells in the pathogenesis of atopic dermatitis due to the pathological effects caused by their degranulation. Mast cells are derived from the bone marrow and are located mainly in connective tissues or near mucosal surfaces. Mast cells contain numerous granules which give the cells a distinctive appearance. These granules contain preformed mediators such as histamine, heparin and serotonin. Mast cells can also release cytokines, IL-1 and TNFα. On the surface of the mast cell are surface receptors for the Fc region of IgE and IgG antibodies and complement (Aalberse *et al.*, 1998). Cross linkage of IgE molecules bound to these Fc receptors results in the degranulation of mast cells following re-exposure to antigen or directly by substance P from irritated cutaneous nerve endings (Cooper, 1994).

A heterogeneity of mast cells has been suggested by Becker *et al.*, (1985, 1986). These authors observed two populations of mast cells which could be differentiated histologically and physiologically. One population of cells was observed to take part in the early response to antigens and a different population was associated with the late phase reaction. The exact role of this finding in the pathogenesis of atopic dermatitis was not explained.

1.2.1.6 Granulocytes

Other cells such as eosinophils, basophils and neutrophils can be involved in atopic dermatitis. Eosinophils are generally associated with parasitism and hypersensitivity and are often found in association with mast cells. On the surface of eosinophils are low affinity receptors for IgE which may be involved in atopy. Their characteristic granules contain histaminase and peroxidases. Eosinophils are influenced by IL-5 from T cells.

Unlike eosinophils, basophils possess a high affinity receptor for IgE and similar to mast cells, activation of basophils takes place *via* cross linkage of IgE molecules on their surface. This results in degranulation and release of histamine and other physiologically active substances contained in basophil granules – hence their role in the pathogenesis of atopic dermatitis.

Neutrophils are involved in inflammation and are capable of phagocytosis and enzyme release. They also possess surface receptors for the Fc portion of immunoglobulins and complement but little is known about the function of these receptors.

1.2.2 Humoral components of immune response

1.2.2.1 Immunoglobulins

Immunoglobulins or antibodies are a group of serum proteins produced by plasma cells as part of the immune response. The function of these antibodies is to bind to the antigen which induced their formation, and then to interact with neutrophils, macrophages or complement in order to facilitate destruction of that antigen. Immunoglobulins are composed of two identical heavy chains (50,000-55,000 Daltons) and two light chains (20,000-25,000D), each of which possess a constant region and a variable region of amino acids. Either end of the immunoglobulin molecule has a particular function. The variable end (N-terminal) deals with binding to antigen and is called the Fab region. The constant (carboxyl terminal) end interacts with host cells and complement and is called the Fc region.

There are five major classes of antibody, IgM, IgE, IgG, IgA and IgD. The class of antibody depends on the amino acid sequence in the constant region of the heavy chain. When animals and humans are first exposed to antigens the first antibody formed is IgM (Schultz & Halliwell, 1985). Due to the influence of cytokines and T cells these B cells switch, to produce a different heavy chain constant region, which results in the production of a different antibody class (Stavnezer, 1996). Interleukin-4 is specifically responsible for the induction of switching to IgE production (del Prete *et al.*, 1988).

IgE

The presence of reaginic antibodies has been suggested as a major feature of atopy / atopic dermatitis in both humans and animals (Willemse, 1986). IgE is the predominant antibody associated with atopy although IgG has also been shown to be involved (Willemse *et al.*, 1985b).

In humans IgE is present in very low concentrations in the serum (300ng/ml, Johansson *et al.*, 1970) due to its short half life of two days (Tizard, 1987b). Increased levels of serum IgE are associated with both parasitism (Bloch *et al.*, 1972) and hypersensitivities (Tizard, 1987b).

IgE production is induced by IL-4, and IL-13 but is reduced by IFNγ. Analogous properties, such as heat instability and the ability to induce passive cutaneous anaphylactic reactions have been illustrated for both human and canine IgE (Patterson *et al.*, 1963, Rockey & Schwartzman, 1967, Schwartzman *et al.*, 1971).

Increases in serum IgE levels have been demonstrated in human atopic subjects, which are significantly higher than in non-atopics, or people suffering from contact dermatitis or psoriasis (Gurevitch *et al.*, 1973; Juhlin *et al.*, 1969; Jones *et al.*, 1975; Ogawa *et al.*, 1971; Wittig *et al.*, 1980). The degree of increase in serum IgE concentrations is highest in patients suffering from disease at the time of sampling (Jones *et al.*, 1975; Johansson & Juhlin, 1970). Hence IgE concentrations can be used as a diagnostic parameter for atopic dermatitis in human medicine.

The kinetics of serum IgE in the dog reveal a picture different to that painted for human serology. Generally canine serum IgE concentrations are much higher than that of humans with levels of 350ug/ml standardly recorded (Schwartzman & Rockey, 1967). The high levels of parasitism present in the canine population has been put forward as the reason for these increased serum IgE concentrations (Halliwell, 1990).

In contrast to the findings in humans, non-atopic dogs can demonstrate a wide variety of serum IgE concentrations. Initial work on IgE by Katz, (1978) demonstrated that there were two populations of mice that could be divided into 'high' responders with high serum IgE concentrations and 'low' responders with low concentrations. These responses were genetically inherited. Work by de Weck *et al.* (1998) has found similar results in the canine population. These authors demonstrated that the phenotype to produce high levels of serum IgE was inherited in a dominant fashion and that only

dogs which demonstrated this trait had the ability to go on to develop atopic dermatitis. This is perhaps the most exciting area of serological research in canine atopy at the present time.

It has also been shown that both parasitism and viral infection have the ability to potentiate the IgE response to other allergens and may have a role in the pathogenesis of atopic dermatitis. Frick *et al.* (1979) demonstrated that young children with viral infections had increased levels of serum total IgE; Frick & Brooks (1983) demonstrated that following vaccination against distemper virus, dogs demonstrated increased levels of pollen allergen specific IgE antibodies. Similarly increased levels of serum total IgE were found in people and rats with concomitant parasite infestations (Bloch *et al.*, 1972).

Although the half life of serum IgE in non-atopic humans is only two days, in atopic dogs the half life was found to be two months (Frick & Brooks, 1983). No reason was given for this difference but prolonged, high serum concentrations of IgE may have a role in the pathogenesis of atopic dermatitis. The exact relationship between IgE bound to mast cells and that free in the serum is not known but it is possible that where there are high serum concentrations of total IgE, this leads to increased binding of IgE to mast cells with an increased potential for degranulation.

Hence it is apparent that the role of IgE in both human and canine atopic dermatitis is not as simple as once believed – there are many arguments for and against the part which IgE plays in the pathogenesis of atopic dermatitis.

IgG

Immunoglobulin G (IgG) antibodies are the most abundant antibody class in the body and are the major antibodies produced during the secondary phase of the immune response. Total IgG is composed of at least four different sub-groups in the dog, namely IgG_1, IgG_2, IgG_3, and IgG_4. This nomenclature is based on their physicochemical properties and follows a similar naming system used in human medicine. In addition to these four groups, a further subgroup IgG_d has been described in the dog by Willemse *et al.* (1985a, 1985b). It has been suggested that IgG_d is equivalent to IgG_1 (Halliwell,

1990). However, this is disputed by Day *et al.* (1996) who found that IgG_d antibody did not react with antiserum to any of the four recognised IgG antibody subgroups. Personal communication with Willemse confirms that IgG_d is not equivalent to any of the known IgG subgroups.

Normal dogs have been shown to develop IgG antibodies against environmental allergens (Wheeler, 1993; Shaw, 1996) but disease, parasites and hypersensitivity have all been shown to influence serum IgG levels. Hill *et al.* (1995) demonstrated that serum total IgG levels are higher in atopic and parasitised dogs than in healthy dogs. Willemse *et al.* (1985a) demonstrated that dogs suffering from *Toxocara canis* had elevated levels of serum IgG_d. In addition Halliwell & Longino (1985) demonstrated that dogs with flea hypersensitivity had raised levels of allergen specific IgG. However, these authors also found that the levels of allergen specific IgG were higher in dogs with flea allergy than in those dogs chronically exposed to fleas which did not demonstrate any allergic reaction towards the fleas. This would suggest that an increase in allergen specific IgG levels was directed more towards a hypersensitivity reaction than parasitism. In contrast however, Hill *et al.* (1995) observed that the profiles of serum total IgG in both atopic and parasitised dogs followed similar patterns and therefore there may be a similar immunoregulatory mechanism occurring in both of these conditions.

There still appears to be some disagreement as to the circumstances necessary for the production of specific IgG subgroups. It has been suggested that in humans and animals the genetic background, type of antigen, the route and duration of exposure may all affect the subclass of IgG produced in an immune response (Day *et al.*, 1996; Mazza *et al.*, 1994). However, there has not been a study of the functional properties of these IgG subclasses (Day *et al.*, 1996) which would be very helpful in determining their importance in disease.

Both humans and dogs with atopic dermatitis have shown variations in specific IgG subgroups compared to non-atopics. Djurup & Malling (1985)

and McHugh *et al.* (1990) demonstrated a dominant allergen specific IgG_1 and IgG_4 response in human atopics.

In the dog, Day *et al.* (1996) found that IgG_1 and IgG_4 were the dominant IgG subclasses present in atopic individuals. However, the levels of these antibodies varied between different causative allergens. IgG_4 was the dominant subgroup against *Dermatophagoides farinae* and *D. pteronyssinus* whereas IgG_1 was the dominant subgroup against Timothy grass. This is probably due to the various factors such as route and duration of exposure mentioned earlier.

A further factor contributing to the production of IgG is the suggestion that some of the IgG antibodies present in the serum are actually directed against IgE, termed IgG anti-IgE antibodies. These have been shown to be present in both dogs and humans (Scheur *et al.*, 1991; Carini & Fratazzi 1992; Hammerberg *et al.*, 1997). These antibodies are found in normal, parasitised and atopic humans and dogs but the highest levels are found in atopics. In atopics IgG_1 and IgG_3 subgroups contribute to the bulk of these antibodies. In humans the highest levels of IgG anti-IgE was found in those patients with the highest levels of IgE and there was a direct correlation between these two parameters. From a diagnostic viewpoint IgG anti-IgE is important because in serological tests these antibodies could mask IgE and result in low levels of IgE being recorded.

Perhaps the most important function of IgG antibodies in allergic disease is in hyposensitisation therapy of both human and canine subjects. Hyposensitisation has been shown to cause an increase in serum total IgG levels in the dog (Hites *et al.*, 1989) and it has been suggested that it is these antibodies which mediate the clinical response to hyposenstisation therapy (McHugh *et al.*, 1990). Allergen specific subgroups IgG_1 and IgG_4 have been shown to dominate different stages of immunotherapy in people (Djurup & Malling, 1985; McHugh *et al.*, 1990). Allergen specific IgG_1 has been shown by McHugh *et al.* (1990) to increase after three months of hyposensitisation therapy and then to plateau, whereas although allergen specific IgG_4 also

increases over this time it continues to rise until the end of therapy after twelve months and dominates the response (Aalberse *et al.*, 1983). In general increased levels of allergen specific IgG_4 have been shown to be associated with clinical improvement of human patients receiving hyposensitisation (McHugh *et al.*, 1990; Devey *et al.*, 1976; Rowntree *et al.*, 1985). This is contradicted by one author (Djurup & Malling, 1987) who observed that high allergen specific IgG_4 levels were associated with a failure of immunotherapy. In an attempt to explain this anomaly, Aalberse *et al.* (1998) suggested that the failure of hyposensitisation in association with increased allergen specific IgG_4 levels, may have been due to the production of IgG_4 antibodies against allergens not involved in the disease process.

The way in which these IgG antibodies contribute to the success of hyposensitisation is still much debated. The classical theory is that they act as 'blocking' antibodies, binding to the allergens before IgE, and thus preventing degranulation of mast cells (Aalberse *et al.*, 1998). It has been shown that serum with high levels of IgG_4 can block IgE mediated histamine release from peripheral blood basophils (Clinton *et al.*, 1989). Aalberse *et al.* (1998) suggested that IgG binds to a particular allergen and this antigen antibody (AgAb) complex then binds to the mast cell surface *via* allergen specific IgG. The allergen bound IgG then interacts with the Fc gamma receptor which causes down-regulation of mast cell activation (Daëron, 1997).

It can be seen from this that IgG has an important role to play in the pathogenesis and therapy of atopic dermatitis in both human and veterinary medicine with much remaining to be discovered.

IgA

Immunoglobuin A (IgA) is the major antibody associated with mucous membranes and is the major mediator of surface immunity. IgA is generally involved in binding to bacteria and inhibiting infection. In atopic humans reduced secretions of IgA have been observed although actual serum levels were no different between atopics and non-atopics (Imayama *et al.*, 1994).

In the dog Hill *et al.* (1995) observed that serum total IgA concentrations were significantly lower in atopic and parasitised dogs compared with healthy dogs ($p<0.05$) and suggested that this was caused by similar down-regulatory mechanisms in atopic and parasitised dogs due to altered, rather than primary, immune functions.

1.2.2.2 Inflammatory mediators involved in the pathogenesis of atopic dermatitis

As mentioned above inflammatory mediators such as histamine, cytokines and complement are all involved in the pathogenesis of atopic dermatitis. It is beyond the scope of this review to include all of the current knowledge of these mediators but a short discussion of the major players will be given.

Histamine

Histamine is probably the most important factor in the pathogenesis of atopic dermatitis. Its effects are easily observed and quantified and as such histamine is used as a control factor in intradermal skin testing. As mentioned earlier histamine is released from degranulating mast cells. Histamine generally acts as a pro-inflammatory mediator but can have anti-inflammatory effects (Scott, *et al.*, 1995). Pro-inflammatory effects are mediated *via* H1 receptors and induce pruritus. Anti-inflammatory effects are mediated through H2 receptors and an increase in cAMP.

Cytokines

Cytokines are glycoproteins, produced by leukocytes and other cells, which act as intercellular messengers. They are mainly involved in inflammatory processes and are important in the activation of T and B cells and the clinical presentation of atopic dermatitis. Interleukins, tumour necrosis factors (TNF) and colony stimulating factors (CSF) are all classified as cytokines.

Many cytokines contribute to the pathogenesis of atopic dermatitis. However, the main cytokines involved include, interleukin 1 (IL-1) which is necessary for the Th2 response and interleukin 4 which is the most important interleukin in the promotion of Th2 differentiation. Th2 cells produce IL-4 which allows a

positive feedback loop. The original source of IL4 is not known although it has been suggested that it may come from T cell subsets, mast cells or basophils (Zlotnil, 1993).

Continued elevation of IL-4 has been demonstrated in allergic human infants after six months of age whereas in non-atopics IL-4 levels decreased after five months of age (Bjorkstén, 1999). It is possible that this is the original source of IL-4. It has been suggested that the exaggerated production of IL-4, IL-13 and IL-5, which promote Th2 production, may be the underlying pathogenesis of allergy (Hnilca & Angarano 1996).

Arachidonic acid pathway

The arachidonic acid pathway is important in the pathogenesis of inflammation and contributes to the clinical signs observed in atopic dermatitis. Arachidonic acid is the major fatty acid in the cell membrane. Following damage to tissues, phospholipase A2 is activated and arachidonic acid is released from the cell membrane. Enzymes can then act on this and depending on the dominant enzymes, either follow the lipoxygenase pathway or the cyclooxygenase pathway both of which result in the formation of pro-inflammatory mediators.

Lipoxygenase products have been shown to be formed in significant amounts in canine atopic skin (Thomsen *et al.*, 1991). Interfering with the formation of these inflammatory mediators from arachidonic acid is the basis for using essential fatty acids in the treatment of atopic dermatitis.

1.2.3 Pathophysiology of Pruritus

The main clinical feature of atopic dermatitis is pruritus. As such the pathophysiology of pruritus requires some mention. The main problem in veterinary medicine is that it is impossible to say for definite that a dog is pruritic. We can only observe the clinical consequences likely to be caused by pruritus such as scratching and self trauma.

Pruritus is a sensation mediated by way of nerve fibres and humoral mediators mentioned earlier. Physiologists have divided the sensation of itch into two types – firstly a rapid localised itch and secondly a slow burning

diffuse itch. Both of these different sensations are transmitted by different nerve fibres. Myelinated nerves which transmit rapid impulses (called A fibres) are associated with the rapid itch and non-myelinated fibres which transmit slow impulses (C fibres) are associated with the diffuse itch (Sture, 1994). The humoral mediators of pruritus are wide and varied, some of which have been mentioned earlier.

1.2.4 Genetic background to the pathogenesis of atopic dermatitis.

The actual mode of inheritance of atopic dermatitis is not clear, although it is widely accepted that there is a familial predisposition for the disease (Reedy *et al.*, 1997a). Studies of human atopy as early as 1916, by Cooke and Vander Veer, concluded that atopy was inherited as a dominant trait with variable penetrance. Current genetic theories on human atopy consider recessive or multiple genes (Hopkin 1995). Humans have an IgE associated marker on chromosome 5q31.1 specifically localised to the IL-4 gene region (Romagnani, 1994). Near here are segments coding for IL-13, IL-5 and GM-CSF which are all involved in the Th2 response (Marsh *et al.*, 1995; Holgate *et al.*, 1995). It has been suggested by Romagnani (1994) that if promoter regions here are over-expressed under normal stimulation, there is increased IL-4 production and thus Th2 stimulation. Early reports of canine atopic dermatitis by Schwartzman & Rockey (1967) observed that often, involvement of dam, sire or siblings was found in relation to a number of atopic cases.

Schwartzman *et al.*, (1971) attempted to breed a colony of atopic dogs. They found that there was a familial tendency to develop atopy but results were not consistent with a simple recessive or simple incomplete dominant hypothesis. However, they were consistent with the inheritance of an autosomal dominant gene mutation controlling histamine release if one assumed that the gene only determined whether release occurred, the amount of histamine released depending on other factors.

As mentioned earlier, de Weck *et al.* (1998) demonstrated that the predisposition to become a high IgE responder was inherited in a dominant

manner. The high IgE responder trait was found to be present in all offspring of a couple in which one of the parents was also a high responder. It was also observed that only dogs with the high IgE responder trait could go on to develop atopic dermatitis (Mayer, *et al.*, 1998). However, this is not the only controlling factor for the development of atopy as not all high responders will go on to demonstrate clinical signs of atopy and therefore other factors must also be involved.

1.2.5 Influence of external factors on the pathogenesis of atopic dermatitis

It has been suggested that infection with both bacteria and/or viruses can influence the development of atopic dermatitis. Various authors in human medicine have suggested that exposure to viruses such as parainfluenza and respiratory syncitial virus (RSV) can be related to the development of atopy (reviewed by Frick & Brooks, 1983). A similar finding was shown in the dog by Frick & Brooks (1983) who demonstrated that pups vaccinated against canine distemper before receiving pollen extracts had significantly more reaginic antibodies to subsequent pollen exposure than in control dogs. These authors associated this response to the breakthrough theory of Katz (1978) and suggested that virus infections or virus exposure may selectively delete suppressor T lymphocytes and result in a naturally occurring allergic breakthrough.

Parasitism has also been suggested as a contributing factor to the development of atopic dermatitis. Parasites naturally cause an increase in the production of IgE antibodies in both animals and humans. A potentiation effect of parasite antigens has been suggested by Bloch *et al.* (1972) with both people and rats suffering from parasite infestations demonstrating high serum IgE levels against pollen allergens.

1.3 Clinical aspects of atopic dermatitis

1.3.1 Clinical presentation of canine atopic dermatitis

It is widely accepted that the principal clinical finding in atopic dermatitis is that of pruritus (reviewed by Scott, 1981; Willemse, 1986; Carlotti & Costargent, 1994). This pruritus can present itself in a variety of ways over a number of different sites. The only evidence that a dog is pruritic may be increased licking or chewing without any resultant skin lesion. This can however escalate into frequent scratching and self trauma.

The main areas affected in atopic dermatitis are the ears, periocular area, muzzle, feet, ventrum, extensor surface of the carpal joints and flexor aspect of tarsal joints (Scott, 1981; Willemse & Van den Brom, 1983; Willemse, 1986; Nesbitt, 1978). Any of these areas can be affected alone but it is more usual for multiple areas to be affected, especially as the disease progresses, often resulting in generalized pruritus (reviewed by Scott, 1981). Otitis externa is by far the commonest presenting sign followed by conjunctivitis (Scott, 1981; Willemse & Van den Brom, 1983). Scott (1981) observed that 55% of a group of 100 atopic dogs suffered from otitis externa in addition to other skin problems. However, only 3% of the atopic dogs in that study had otitis externa alone. The clinical findings in atopic dogs with otitis externa can be confused with non-atopic dogs with ear disease. Scott (1981) described atopic dogs as having pruritus, erythema and oedema of the ear canal with a minimum amount of exudation in addition to ear disease flaring in conjunction with skin disease and an excellent response to glucocorticoids and hyposensitisation.

Primary lesions such as erythema, papules and wheals have been reported but are rarely seen (reviewed by Scott, 1981; Willemse & Van den Brom, 1983). It is more common to find secondary lesions such as excoriations, alopecia, pyoderma or even lichenification in the worst cases. Lichenification of the extensor and flexor surfaces was found to be significantly less common ($p<0.001$) in atopic dogs that showed no skin test reactivity when compared with those who did react (Willemse, 1986).

Respiratory problems such as those observed in human atopics are rare in the dog (Schwartzman, 1984). However, Willemse & Van den Brom (1983) observed that 22.4 % of a group of atopic dogs suffered from increased sneezing and reverse sneezing has been included as one of the diagnostic criteria by Willemse (1986). Other clinical findings which are inconsistently seen include seborrhoeic skin disease, hyperhidrosis (Scott, 1981) and discoloration of the coat (Willemse & Van den Brom, 1983; Willemse, 1986). Schwartzman *et al.* (1971) reported that once affected the dog maintains its atopic state throughout life.

1.3.2 Epidemiology

1.3.2.1 Prevalence

Atopic dermatitis is a relatively common disease of people with one study demonstrating that 2-3% of children younger than five years of age were affected by atopic dermatitis (Walker & Warin, 1956).

Atopy is the second most common allergic skin disease of dogs next to flea bite hypersensitivity (Reedy *et al.*, 1997a). The reported incidence varies between studies and depends on the groups of dogs in those studies. Of the general population of dogs, between 10% and 15% were reported as being atopic (Chamberlain, 1974). At dermatological referral centres the lowest prevalence of atopy was 3-4 % of the cases seen by Halliwell & Schwartzman (1971). Scott (1981) diagnosed 8% of referral cases as atopic and 30% of cases seen by Nesbitt (1978) were diagnosed as atopic.

1.3.2.2 Age of onset

The age at which clinical signs consistent with atopy are first recognized is of major importance in the diagnosis of atopic dermatitis - indeed this parameter is included in Willemse's diagnostic criteria (1986). The usual age of onset is between one and three years (reviewed by Scott, 1981; Halliwell & Schwartzman, 1971) with a first occurrence of atopy being rare in dogs younger or older than this. Scott (1981) noted that 58% of dogs presenting

with atopic dermatitis were one year of age with only 12% being two or four years of age. Willemse (1986) reported similar findings with 75.5% of atopic dogs presenting at less than 3 years of age.

It is rare for dogs to be diagnosed as atopic between six months and one year of age (Scott, 1981). Scott only observed 2% of atopic dogs in the six to eight months age group. However it should be noted that these studies examined dogs in referral clinics and often dogs may have been affected by atopic dermatitis for a period of months before being seen at a referral centre.

In a study of progeny of atopic dogs by Schwartzman (1984) no clinical evidence of atopic dermatitis was found in dogs less than 12 months of age, and no positive intradermal skin tests were demonstrated by dogs less than six months old. However, one exception to this was a group of highly inbred atopic dogs observed by Reedy *et al.* (1997a) which demonstrated significant clinical signs consistent with atopic dermatitis by the time they were six months old.

The incidence of atopy also decreases in dogs older than three years. Again, Scott (1981) only found 2% of dogs presenting with atopy in the six to seven years age group. A possible explanation for older dogs developing atopy is if they have been moved frequently as young dogs and have not remained in the same environment long enough to demonstrate clinical allergy. (Reedy *et al.*, 1997a).

1.3.2.3 Breed predisposition

Breed predispositions for the development of atopic dermatitis have been shown by a number of studies. However, the predominant breeds often differ between different areas of the world, probably due to distinct gene pools in these areas. Even, within the United Kingdom breed differences were found by Sture *et al.* (1995) with a higher percentage of atopic West Highland white terriers and boxers in London than in Edinburgh. (14.3% and 9.5% compared to 8% and 4.6% respectively). However, no information as to the difference in

the numbers of these breeds in the general population in Edinburgh and London was given, which may bias the findings.

In America the breeds mainly affected have been reported as the wire-haired terrier (Halliwell & Schwartzman, 1971), Cairn terrier, West Highland white terrier, lhasa apso, wire-haired fox terrier, dalmatian, pug, Irish setter, Boston terrier and miniature schnauzer (Scott 1981).

In Europe, Carlotti & Costargent (1994) found that setters (Irish, English, and Gordon) were the breeds most often diagnosed as atopic with the Pyrenean shepherd, fox terriers and Labradors also over-represented. In the Netherlands, Willemse & van den Brom (1983) found boxers (13.5%) terriers (13.5%), German shepherd dogs (13%) and poodles (5.8%) to be the breeds most often diagnosed as atopic.

A decreased incidence of atopic dermatitis was found by Scott (1981) in the cocker spaniel, dachshund, standard poodle, toy poodle and German shorthaired pointer. Carlotti & Costargent (1994) also found that the cocker spaniel and dachshund were under-represented. Although most studies have concentrated on pedigree breeds, mongrels can also be affected by atopic dermatitis (Reedy *et al.*, 1997a). The breed predilections seen in dogs are somewhat different to the findings in human medicine. Caucasians have been reported as being more prone to developing atopic dermatitis but no significant difference has been shown between races (Leung *et al.*, 1993).

1.3.2.4 Sex

Sex predilections towards the development of atopic dermatitis for both male and female dogs have been suggested by different authors. Halliwell & Schwartzman (1971) and Scott (1981) found that females were more likely to be atopic. Schick & Fadok, (1986) observed that bitches had a significant predilection ($p<0.05$) for developing clinical signs of atopy. Scott (1981) also observed this. An explanation for this predisposition for female atopics was given by Reedy *et al.* (1997a) who suggested that intracellular cGMP levels were raised due to oestrogens. As cGMP is involved in the degranulation of

mast cells, higher levels of cGMP could lead to increased release of histamine and other mediators of pruritus, and thus more female animals could present with clinical signs of atopic dermatitis. However, Nesbitt (1978) found that male dogs were more likely to be atopic with 56.1% of the atopic population being male. No sex predilection was observed by Willemse & van den Brom (1983).

From this it appears likely that due to the number of factors involved in the pathogenesis of atopic dermatitis it would be very difficult to find a sex predilection for atopic dermatitis.

1.3.2.5 Seasonality

Clinical signs of atopic dermatitis are often seasonal coinciding with pollination of grasses, weeds or trees, although this depends on an individual's hypersensitivity (Halliwell & Schwartzman, 1971). Early studies on atopic dermatitis in the dog concentrated on hypersensitivity to ragweed pollen where dogs had a definite seasonal pattern, (Schwartzman & Rockey, 1967), with clinical signs paralleling the ragweed pollen season. Halliwell & Schwartzman (1971) also found the majority of ragweed allergy cases (53%) were seasonal. Seasonality of clinical signs can help with a diagnosis of atopic dermatitis. Seasonal dermatitis is more likely to be caused by pollen from grasses, trees, flowering plants; a perennial problem is more likely to be caused by house dust or household antigens (Willemse, 1986). Also, cases which initially present as seasonal can become perennial problems (Halliwell & Schwartzman, 1971). That study found 22% of cases were originally seasonal progressing to non-seasonal and 25% were completely non-seasonal. Scott *et al.* (1995) found that a progression from seasonal to non-seasonal disease occurred in 15% of atopic dogs.

1.4 Environment

As mentioned earlier atopy in the dog was associated with positive intradermal skin tests against allergens such as pollens, house dust mites or mould. The

environment that an animal is kept in is therefore important as this will control the level of these allergens to which an animal is exposed.

1.4.1 Pollens

For a pollen to be a major allergen there are a number of prerequisites that must be met. Namely, there must be a large amount of that pollen in the environment, the pollen must be small enough to become windborne (pollens spread by animal means are rarely a cause of human allergy) - this usually means less than 50um in size; and the pollen allergens must be water soluble to be absorbed. (Matthiesen *et al.*, 1991; Reedy *et al.*, 1997b). Ragweed is the best known example of a pollen that meets these criteria and most studies have concentrated on this. However, the United Kingdom is essentially free of ragweed.

In the United Kingdom, Hyde (1960) observed that the pollen season lasted from mid-January to late September. This comprised three phases - firstly mainly tree pollens from January to May; secondly grass pollens in June/July; and finally dicotyledenous herbs in August and September. Moulds, depending on their methods of spore release, tend to peak during wet conditions in spring and autumn (Reedy *et al.*, 1997b).

Pollen counts are affected by a variety of environmental factors - firstly the weather. As a large number of plants release their pollen after rainfall, pollen counts can increase after wet weather. However, during periods of rainfall, pollen in the atmosphere can be washed out of the sky, hence lowering the pollen counts (Hyde, 1960). Generally, rainfall of more than 2mm can cause a reduction in pollen levels.

The time of day can also influence pollen counts as pollen shedding is not uniform throughout the day (Hyde, 1960; Reedy *et al.*, 1997b). Usually the best conditions for pollination are mid-day/afternoon when the air is likely to be at its warmest.

The location of the pollen station is important with most stations being located in towns, on the roofs of tall buildings. Obviously pollen levels here are going to be different to those encountered by a dog at ground level.

Therefore pollen calendars can only be used as a rough guide to the likely types and levels of pollen that an animal will be exposed to. Indeed some of the pollens likely to be found at ground level, such as the heavier or sticky pollens, are not important in human medicine but may well be very important in canine medicine (Nesbitt, 1978).

Another difference between human and canine medicine is that most of the problems associated with pollens in people are due to their being inhaled and resulting in asthma/hay fever. However, in the dog cutaneous disease is the predominant clinical finding. Although it was initially believed that allergens were inhaled by the dog, this theory is being questioned and the possibility of percutaneous absorption of allergens receiving more attention (Reedy *et al.* 1997a). This means that the pollens thought to be important in human and canine medicine may not be the same and species not thought important in human medicine may indeed be pathogenic in canine medicine.

In addition to a pollen allergy, there is some proof that dogs could be allergic to the actual constituents of a plant, for example when a dog walks over freshly cut grass. This may explain the predominance of pedal dermatitis in atopic dogs. People have been shown to develop signs of hayfever when in contact with cut grass (Varney *et al.*, 1991). It has also been suggested that cut grass can produce a 'grass juice' which is released as an aerosol (Brown 1989).

The major pollens in the United Kingdom are the grasses (*Poacaea*) between the months of June and August and the *Urticacaea* (Bagni, *et al.*, 1976). Grasses are responsible for between 10 and 30% of all pollen mediated allergies (Reedy *et al.* 1997b) and are the most common pollen allergy in Europe (Weeke & Spieksma, 1991). The most widely distributed grasses in Europe associated with grass pollen allergens are *Poa pratensis* (Kentucky), *Festuca eliator* (meadow fescue), *Dactylis glomerata* (orchard), *Lolium perenne* (perennial ryegrass) and *Phleum pratense* (Timothy). In the United Kingdom, *Anthozantum odoratum* (sweet vernal grass), *Holcus lanatus* (velvet) and *Agrostis alba* (redtop) may also be the cause of pollinosis.

Cross reactivity has been demonstrated between most temperate zone grasses with one study (Dirksen & Osterballe, 1980) noting that Timothy grass could be used to identify grass allergy in 75% of allergic individuals. Studies of the particular grass allergens have found that single grass species can have 20-30 allergenic components (Mathiesen & Lowenstein, 1991), with the naming of these allergens being the subject of international agreement (Marsh, *et al.*, 1986). In general, pollen allergens have to be water soluble and have a molecular size of 5000 -70000 daltons. Again these characteristics are based on human medicine but there are no such figures for canine models. Suggestions have been made that these allergens have biochemical properties such as enzymic activity but no such findings have been made with the only physiological finding being that they can produce an IgE response. (Leiferman & Gleich, 1976; Esch & Klapper, 1989.)

Other plants have been shown to be involved in allergies (See Table 1.1). However, a lot of plants such as mugwort and plantain do not tend to produce very high levels of pollen.

Cross reactivity between groups of trees or plants is possible (Halmepuro *et al.*, 1984). Alder (*Alnus*), Birch (*Betula*), Ash (*Fraxinus)* and Hazel (*Corylus*) are all related and there may be some cross reactivity between these species. These trees shed large amounts of windspread pollens which are very good at releasing their allergenic potential when they come into contact with mucous membranes. Due to the cross reaction between these species hyposensitisation of people against birch often protects against other tree pollens.

Oak (*Fagacaea*), Beech (*Fagus*) and Chestnut (*Castanea*) only play a small part in human allergies. Olive pollen, although important in the Mediterranean has not been isolated in Britain.

Table 1.1 Pollens of major importance in United Kingdom and their pollination period (Modified from Reedy *et al.*, 1997b).

Group	**Genus**	**Species**	**Common name**	**Pollinating period**
Trees	*Quercus*	*alba*	White Oak	May
	Betula		Birch	March-April
Weeds	*Parietaria*	*judaica*	Pellitory of the wall	June-Sept
	Rumex	*crispus*	Yellow dock	June-Aug
	Chenopodium	*album*	Lamb's quarter	Aug
	Artemisia	*vulgaris*	Common mugwort	July-Aug
	Urtica	*dioica*	Nettle	June-Sept
	Plantago	*lanceolata*	English plantain	June-July
Grasses	*Poa*	*pratensis*	Kentucky	May-Aug
	Festuca	*eliator*	Meadow fescue	May-Aug
	Dactylis	*glomerata*	Orchard	May-July
	Lolium	*perenne*	Perennial rye	June-Aug
	Anthoxantum	*odoratum*	Sweet vernal	April-June
	Phleum	*pratense*	Timothy	May-Aug
	Holcus	*lanatus*	Velvet	June-Aug
	Agrostis	*alba*	Redtop	June-Aug

1.4.2 Fungal Spores

Fungi are universal in distribution and fungal spores can comprise the bulk of the suspended allergenic peptides in an area depending on the weather conditions and land usage. Most fungi of allergenic significance are nonpathogenic saprophytes and disperse their spores in dry weather conditions (Reedy *et al.*, 1997b) eg. *Cladosporium* spp., *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp. *and Rhizopus* spp.

Cladosporium is the most abundant single type of fungus in Great Britain and is the commonest fungal cause of asthma. *Botrytis* and *Alternaria* are less common but accepted causes of summer asthma. Larger numbers of *Cladosporium, Alternaria* and *Botrytis* are found in the summer whereas *Penicillium* is found in the same quantities all year round (Hyde, 1960). Tee *et al.* (1987) demonstrated that most humans allergic to a fungus tended to be sensitive to all antigenically related fungi.

1.4.3 House Dust Mites

Dust mites have been described as the major allergens in house dust (Halliwell & Kunkle, 1978). There are two species of house dust mites in the domestic environment - *Dermatophagoides pteronyssinus* and *D. farinae.* From each of these species, three groups of allergens have been isolated (Noli *et al.*, 1996). These are named *Der p* I-III and *Der f* I-III. Within groups I - III of each species these allergens seem to be homologues but between *D. farinae* and *D. pteronyssisinus* they seem to be antigenically unrelated. Groups I and II of both species have shown immediate type hypersensitivity reactions in mite allergic humans but are probably not important in the dog (Noli *et al.*, 1996).

Reactions to both *D. farinae* and *D. pteronyssinus* have been demonstrated in dogs. However reactions seem to be more common to *D. farinae* (Sture *et al.*, 1995) than *D. pteronyssinus.* Noli *et al.* (1996) observed that *D. pteronyssinus* was a relatively unimportant allergen in the dog. This agrees with work by Carlotti & Costargent (1994).

1.4.4 Ectoparasites

Flea allergy is the most common allergic skin disorder in dogs and cats. (Reedy *et al.*, 1997c). It is a problem in its own right and can complicate other allergic conditions. Many dogs have been shown to have high concentrations of IgE to biting insects and other insects. Pucheu-Haston *et al.* (1996) demonstrated that there was some cross reactivity as well as some distinct allergens between flea (*Ctenocephalides felis*), black ant (*Camponotus spp*), cockroach, and black fly (*Simulium spp.*). Allergy to ectoparasites can complicate the diagnosis of atopic dermatitis and may also interfere with serological tests as dogs can suffer from both conditions.

1.5 Diagnosis of atopic dermatitis

In addition to examining the clinical history and presenting signs many diagnostic tests have been incorporated into the diagnosis of atopic dermatitis in both canine and human medicine. One of the earliest tests was the Prausnitz Kustner test.

1.5.1 Prausnitz Kustner test

This test was recorded in 1921 (Prausnitz & Kustner) and demonstrated the existence of a skin sensitising antibody in the serum of allergic individuals which could transfer the clinical signs of allergy *via* injection of this serum to non-allergic individuals.

1.5.2 Passive Cutaneous Anaphylaxis (PCA) Test

This test is derived from the PK test and is a classic method used to measure reaginic IgE antibodies in various species of mammals (Peng *et al.*, 1993). Disadvantages are that it takes a long time to run – three days, and the sacrifice of a dog is required. In addition results are not consistent from dog to dog. Hence this method could only be used for research purposes and has been replaced with other more effective tests as discussed below.

1.5.3 Intradermal skin testing

Intradermal skin testing is a method of assessing an individual's response to a variety of allergens in an attempt to identify those which are pathogenic, and is used in both human and veterinary medicine. Intradermal skin testing was first recorded in the medical literature by Blackley in 1873, but is now utilised in the diagnosis of atopic skin disease in humans, dogs, cats and even birds.

Allergens are made up in solutions and injected intradermally. The way in which allergens are prepared differs between companies and there are no standards for this. Different preparations may contain different antigenic epitopes and this can account for differing IDST results between animals tested with different allergen preparations. In addition most of the allergens are designed for use in human dermatology rather than specifically for use in cats and dogs.

There are different ways of expressing the concentration of an allergen solution as described below (modified from Halliwell & Gorman, 1989):

1. **Protein Nitrogen Units (PNU)** –One PNU is equivalent to 10^{-5}mg of nitrogen precipitated with phosphotungstic acid.
2. **Noon units** – One noon unit is 1ml of an extract made from 1mg of pollen in 1 litre of extracting fluid.
3. **Micrograms/ml** – Extracts are standardised to the content of a particular protein allergen.
4. **Weight/volume** – The weight of allergen extracted in a given volume of extraction fluid.

The concentrations of these allergens can also differ between manufacturers. There is much controversy as to the ideal allergen concentrations to use in veterinary dermatology. The average concentration used for IDST was 100-300PNU/ml (Bunde *et al.*, 1997). However, Codner & Lessard (1993) applied different allergen concentrations to different allergens ranging from 100 PNU/ml for house dust, 250 PNU/ml for *Rhizopus* up to 500 PNU/ml for wool.

Allergens can sometimes be used as mixtures, however, this can lead to problems. Firstly there is a dilution factor when allergens are present as mixtures (Schick & Fadok, 1986) and mould extracts have been shown to contain proteolytic enzymes which may reduce the allergenic activity of a protein (Rosenbaum *et al.*, 1996).

Storage of allergens can also influence their stability. The material which allergens are kept in (whether it is a glass or plastic bottle), the presence or not of preservative and the temperature at which they are stored have all been shown to adversely affect the antigenicity of allergen extracts (Halliwell, 1987). Glycerine is often used as a preservative for allergen extracts and Kleinbeck *et al.* (1989) demonstrated that 6.25% glycerine could give a positive IDST result making a diagnosis of atopy difficult.

Allergens are generally kept at 4°C but Halliwell (1987) demonstrated that allergens kept at this temperature could lose up to 52% of their biological activity. These suggestions have been opposed by Rees *et al.* (1997) who did not find any change in biological activity between allergens stored at 4°C or 22°C. Similarly, Halliwell (1987) and Campbell & Hall (1993) demonstrated that allergens stored in plastic syringes lost a higher percentage of their biological activity than those stored in glass syringes. It seems that the veterinary dermatologist cannot win!

When it comes to the interpretation of IDST there exists as much confusion. There is no standard method for the assessment of a positive IDST result in veterinary medicine. Most practitioners use a scale of 0 or 1-4 with 0 or 1 representing a negative result equal to the negative control injection (saline) and a score of 4 equal to the positive histamine control. Results between this are applied arbitrarily.

However, the confusion does not end here. Cross reactions between allergens have been observed. Kleinbeck *et al.* (1989) observed that there was cross reaction between phylogenetically related species of pollen. In addition, although a positive intradermal skin test proves that a dog is sensitised to an allergen (if for the time being we ignore the possibility of cross reaction) it does not mean that

the dog is necessarily allergic. These positive results in non-atopic animals are often referred to as false positive results, although whether this is a true description or not is debatable. Such clinically irrelevant positive IDST results are known to be relatively common in the dog especially to allergens such as house dust (DeBoer, 1989).

From these findings it is clear that the practice of intradermal skin testing in veterinary medicine requires standardisation in both the allergens used and in the interpretation of results in order to allow sensible analysis of results between different authors. Only after this will advances be made in the study of veterinary allergic skin disease.

1.5.4 Serology

In humans serological tests to measure IgE antibodies utilise an IgE myeloma, to obtain pure fractions of IgE. However, no such myeloma has been found in the dog and this makes accurate measurement of canine IgE difficult. (Halliwell & Kunkle, 1978).

Polyclonal or monoclonal antibodies can be used in serological tests each of which has their own advantages and disadvantages. For example, monoclonal antibodies recognise a limited number of epitopes and therefore should be more specific than polyclonal antibodies. However, due to the small number of epitopes to which the monoclonal antibodies are attracted the affinity these antibodies have for the IgE molecule may be reduced (Peng *et al.*, 1993).

1.5.4.1 Radioallergosorbent assay

The first serological test developed to measure allergen specific IgE in serum was the radioallergosorbent assay or RAST (Wide *et al.* 1967). This involved binding of allergens to a solid phase such as a paper disc and then incubating them with the serum sample being tested. Following washing the discs were then incubated with a radio-labelled anti-IgE antibody which would bind to any IgE that had bound to the allergen. After washing the radioactivity present was measured thus facilitating assessment of the amount of allergen specific IgE present.

Intradermal skin testing is regarded as the 'gold standard' (Esch & Grier, 1997) in both human and veterinary medicine. Due to this authors often attempt to correlate serological results with IDST. Halliwell & Kunkle (1978) found good agreement between RAST and IDST results for ragweed allergen in dogs with 89% agreement between positive IDST and positive RAST. Where negative IDST results are obtained alongside positive RAST results it has been suggested that this may be due to high levels of serum IgE and that these RAST results are false positives (Griffin *et al.* 1990). This does however assume that IDST is demonstrating a true result and any test which does not agree with this is giving a false result. This hypothesis remains to be proved and it is possible that the positive results in the RAST test are indeed positive and that allergen specific serum IgE is present in dogs which do not demonstrate a clinical allergy. Halliwell & Kunkle (1978) suggested that the reason for the poor correlation between RAST and IDST results was due to the dynamics of IgE being different in both tests and that there were different levels of allergen purity in both tests.

1.5.4.2 Enzyme linked immunosorbent assay

The enzyme linked immunosorbent assay or ELISA was first reported in 1971 in human medicine (Engvall & Perlmann, 1971) and is now widely used in both human and veterinary medicine. The ELISA test is based on a similar method to that used in the RAST test. However, instead of using radio-labelled anti-IgE antibody the ELISA incorporates enzyme linked anti-IgE antibody which following incubation with an enzyme activator, causes a colour change which indicates the amount of IgE present.

A number of problems with the ELISA test have been observed. Halliwell (1994) reported that high levels of IgE can cause non-specific binding; incorrect cut off points between positive and negative results can lead to errors; detection of IgG instead of IgE can occur; or poor techniques such as inadequate washing of wells may be present. He also suggested that false negatives could be obtained due to poor allergen preparation; poor adsorption onto the solid phase; poor anti-IgE preparation; outdated labelled antibody; a high level of IgG antibody which may

compete with IgE for allergen; poor technique such as inadequate incubation; testing for allergens in groups; or testing at the incorrect season when the level of allergen specific IgE may be lower.

As in the case of RASTs the results of ELISA tests are often compared with IDST. Good correlation between allergen specific circulating IgE and IDST / challenge test results in atopic humans has been found (reviewed by Lockey *et al.*, 1992). Bunde *et al.*, (1997) observed excellent correlation between ELISA and IDST where identical allergens were used in both tests.

In comparing these tests, sensitivity and specificity are usually examined. Sensitivity is defined as the proportion of true positive results detected and specificity as the proportion of true negative results (Thrusfield, 1995). In the dog, the sensitivity of ELISA tests is often found to be good (Bond *et al.*, 1994; Codner & Lessard, 1993) reaching 100% for some individual allergens (Miller *et al.*, 1993). However, very poor specificity has been recorded for different allergens with a mean of 43.8% (Bond *et al.*, 1994). The reason for this poor specificity is the high number of positive ELISA results in clinically normal dogs. The predictive value of a positive test has been found to be poor but that of a negative result good (Bond *et al.*, 1994). As such, negative ELISA results are believed to be reliable (Kleinbeck *et al.* 1989; Codner & Lessard, 1993); indeed Codner & Lessard (1993) went as far as to state that a negative ELISA result could reliably rule out a diagnosis of atopy.

Correlation is another method by which IDST and ELISAs are compared. In veterinary medicine correlation coeficients between IDST and ELISA tests were found to be different for different allergens, but in general correlation was poor (Anderson & Sousa, 1993; Codner & Lessard, 1993; Bond *et al.*, 1994).

As mentioned above the main area of discrepancy between ELISA and IDST results is that of positive ELISA results but negative IDST (Halliwell & Kunkle, 1978; Kleinbeck *et al.*, 1989; Griffin *et al.*, 1990; Anderson & Sousa, 1993; Codner & Lessard, 1993; Bond *et al.* 1994; Griffin, 1994). These disparities may be due to differing levels of bound and free circulating IgE. If mast cells are saturated with allergen specific IgE which is directed against a different allergen to

that against which there is circulating IgE then differing results will be obtained on each test (Kleinbeck *et al.*, 1989). It is also possible that dogs with high serum total IgE but a low IDST score may possess fewer cutaneous mast cells or have mast cells in a state of continuous degranulation, with only a few able to respond at the time if IDST. However, if this were the case then surely these dogs would demonstrate mild clinical signs of atopic dermatitis as the clinical signs of atopic skin disease are primarily controlled by the release of such mediators of pruritus.

Other explanations have been put forward by different authors to explain the disagreement between IDST and serological tests. For example it is possible that serological tests contain allergenic epitopes which are not exposed in natural allergens (Alaba, 1997, Lowenstein & Marsh, 1983). This would lead to the development of false negative results.

Different allergens will have different abilities to bind to microwell plates and so serological results will differ for different allergens (Canterero *et al.*, 1980, Pesce *et al.*, 1977).

In the majority of reports comparing IDST and serological tests the allergens incorporated into each test are produced by different companies and so the allergenic epitopes that are present may be different. This was illustrated by Bunde *et al.*, (1997) who observed that when identical allergens were used for both tests agreement between clinical presentation, IDST and ELISA was very good at 82.3%.

The interpretation of IDST is subjective (as discussed earlier). Where dogs have a high degree of positive IDST results correlation with ELISA has been shown to be good at 90%. However, where IDST results were not clear cut, the correlation with ELISA fell to 50% (Esch & Grier, 1997).

The use of mixed allergens in ELISA tests can lead to discrepancies due to a lower concentration of allergen resulting in false negatives (Bond *et al.*, 1994).

The specificity of the serological test for IgE is very important. Where the ELISA is not totally specific for IgE, other antibodies such as IgG can be measured as well. This is a common problem where polyclonal antibodies are incorporated into the assay.

It has also been suggested that there may be different types of IgE present – one free in the circulation, and one bound to mast cells (Griffin *et al.*, 1990).

The presence of IgG anti-IgE antibodies may interfere with the measurement of IgE concentrations with lower levels of serum IgE being recorded than were actually present. This has been shown to be a problem by Hämmerberg *et al.*, (1997).

Non-specific binding of IgE may take place in the wells of the ELISA where there is a high serum IgE concentration. This will result in the development of so called false positive or clinically irrelevant ELISA results. Indeed Griffin *et al.* (1990) and Codner & Lessard (1993) observed that there was a statistically significant correlation between the level of serum IgE and the number of positive ELISA results in non-atopic dogs.

The relationship between bound and circulating IgE has not been fully explained. Schwartzman (In Anderson & Sousa, 1993) commented that serum levels of total IgE are probably in dynamic equilibrium with IgE bound to mast cells. If IgE levels were low then most would be bound and not free in the circulation and so the IDST would be more sensitive. He also suggested that there may be a difference in mast cell affinity for IgE and this could affect the levels of bound or free IgE.

Of the positive ELISA results in non-atopic dogs found by a number of authors, the highest incidence were found against mould allergens (Griffin *et al.*, 1990; Anderson & Sousa, 1993; Day *et al.*, 1996). Bunde *et al.* (1997) suggested that there may be a difference in the sensitivity of ELISA in detecting fungal specific IgE but were unsure as to the significance of this. In comparing atopic and non-atopic dogs Codner & Lessard observed that ELISA results for fungal allergens were significantly higher in atopics than non-atopics. This was the only group of allergens in which such a difference was observed. In addition sensitivity to fungal allergens was more common with serological testing than IDST. However, the reason for these differences was not known.

A seasonal variation in ELISA results has been observed by Halliwell & Kunkle (1978). These authors found a marked variation in IgE levels against ragweed in

September and June and from this suggested that it is important to take into account the time of year that a dog is being tested.

From these findings it can be seen that correlation between ELISA and IDST testing is often poor but the reasons for this are wide and varied and much work is still required in this area.

1.5.4.3 Immunodot

The immunodot test was first described in 1994 by Aubert & Frei for use in human medicine as a means of determining serum allergen specific IgE levels. This was later adapted for use in the dog (de Weck, personal communication).

The immunodot test consists of a nitrocellulose strip to which various groups of allergens are bound. Following incubation of test serum and washing, monoclonal anti-IgE antibodies are added. The test is then similar to an ELISA with washing steps and the addition of developing antibodies which in the presence of canine IgE develop a blue colour which can be evaluated by the naked eye or by measuring reflective density.

The immunodot test is easy to run and is reported to have a sensitivity of 84.5% and specificity of 94% in human medicine (Aubert & Frei, 1994). The immunodot test has been predominantly used by Hämmerling & de Weck (1998) in the dog, the main advantage of this method being the use of monoclonal dog anti-IgE antibodies in the detection of canine IgE. This assay has been reported to have a sensitivity ranging from 54-100% for different allergen groups when compared with IDST (Hämmerling & de Weck, 1998).

1.5.4.4 Other serological methods

Heska® ALLERCEPT™ is a modified ELISA test, which has recently been introduced. This test is based around the high affinity Fc IgE receptor (FcεRI) found on mast cells and basophils. The α subunit of this receptor has been reproduced by Heska and is incorporated into an ELISA test in the place of anti-IgE monoclonal or polyclonal antibodies. Comparing IDST and the ALLERCEPT

methods have shown an overall sensitivity of 86%, specificity of 92% and an overall accuracy of 90% (Bevier *et al,* 1997).

Other serological methods such as liquid gold methodology (Alaba, 1997) and the polymerase chain reaction (Foster, 1997) have been used in the study of atopic dermatitis but are generally research tools rather than diagnostic tests. No further explanation of the methodology behind them will be given here.

1.6 Therapeutics of atopic dermatitis

1.6.1 Hyposensitisation

Hyposensitisation has been used in human medicine since 1911 (Noon & Cantab) and in veterinary medicine since 1941 (Wittich). Hyposensitisation or immunotherapy involves sequential injections of a solution containing small amounts of allergens to which a person or animal is allergic. The quantities of allergens given are gradually increased and in so doing, the immune system is gradually exposed to these allergens. The exact regime used varies between vaccine manufacturers. There is however, always a risk of anaphylaxis when using hyposensitisation vaccines.

The immunological responses to hyposensitisation have not been fully explained but the widest believed theory is that hyposensitisation causes the formation of IgG antibodies which act as 'blocking' antibodies. These IgG molecules bind to allergen before IgE can do so. In addition IgG may be able to bind to the mast cell and prevent degranulation by interfering with IgE cross linkage.

Hyposensitisation vaccines can be based on IDST, ELISA or a standard mixture of allergens. The main aim of hyposensitisation is to eliminate the allergic reaction which occurs when the dog is exposed to particular allergens. The degree of improvement demonstrated by animals receiving hyposensitisation therapy is however variable with a slight reduction in pruritus through to a complete cure being classed as a success. A lot depends on the expectations of the owners and generally success is defined as any improvement in the degree of pruritus demonstrated by the dog.

The success rate of hyposensitisation in the dog is reported to be somewhere around 63% (Chamberlain, 1969). Nesbitt (1978) reported a success rate of between 82.1-85.7% after six months therapy with up to 51.4% of dogs showing some improvement after three months therapy.

Where hyposensitisation was based on IDST results Willemse *et al.* (1984) achieved a success rate of 70% and DeBoer reported a success rate of 68% (1989). Kleinbeck *et al.* (1989) observed that hyposensitisation protocols based on ELISA results were equal to or greater than those immunotherapies based on IDST. Scott (1981) suggested that a hyposensitisation vaccine does not need to contain all of the allergens to which a dog is allergic.

Reasons for the variation in the success of hyposensitisation have been suggested by Miller *et al.* (1993). These include an animals inherent ability to respond, the accuracy of the diagnosis of atopy, the accuracy of the allergy test results, the type and numbers of allergens in the immunotherapy protocol, the immunotherapy protocol used, the exogenous allergy load during immunotherapy and the development of other non-allergic pruritic skin disease during the course of the treatment. From this it may appear surprising that any animals respond favourably to hyposensitisation.

Miller *et al.* (1993) observed that there was no correlation between the age of onset, duration of disease, number of serological allergens included in the vaccine or the number of positive IDST results not included in the vaccine and the success of hyposensitisation. However, Nesbitt *et al.* (1978) found that the younger dogs showed the best response to hyposensitisation.

Possible complications with hyposensitisation include the risk of anaphylaxis, nodule development at the site of injection; increased pruritus following vaccination and pain and swelling at the injection site following injection with aqueous allergens in propylene glycol (Nesbitt, 1978).

There is as much variation in the concentration of hyposensitisation vaccines as there is in the test allergens. Nesbitt (1978) suggested that the maximum dose of alum precipitated extracts is 10,000PNU/ml and exceeding this can result in

pruritus and restlessness. Chamberlain (1969) used the same concentration initially but gradually increased this by 2500PNU/ml per allergen.

1.6.2 Other therapeutic measures

Other methods of treatment for atopic dermatitis include avoidance of the inciting agent, and symptomatic therapy including the use of glucocorticoids, antihistamines and essential fatty acids which will all relieve pruritus. However, a discussion of the pharmacological background to these treatments is beyond the scope of this review.

1.7 Summary

It can be seen from this review that a lot of work has been carried out on the pathogenesis, diagnosis and treatment of atopic dermatitis in both people and dogs. Many factors appear to be involved ranging from genetics through to the environment that animals are kept in. Common factors have been shown between humans and animals but there are many discrepancies as well.

The aims of this study are to examine both clinical and serological parameters in atopic and non-atopic dogs. Much work has been carried out on serum total IgE but it is intended to expand on this, especially as the GDBA dogs are known to have very low levels of parasite exposure. In addition work on serum IgG has concentrated more on human atopics than canine, something which it is intended to rectify with this study.

Much work in the veterinary field has concentrated on the clinical aspects of atopic dermatitis. Due to the excellent clinical histories of the GDBA dogs the aim is to identify important clinical parameters and ages of onset which could be applied in the prediction and diagnosis of atopic dermatitis.

The environment that animals are kept in is known to be important in the pathogenesis of atopic dermatitis, with dogs exposed to pollens, house dust mites and moulds being at a greater risk of developing atopy. Hence it is hoped to

identify significant environmental factors and possibly ways of preventing dogs from coming into contact with them.

Chapter 2 Materials & Methods

2.1 Animals

The clinical, historical and serological parameters of 240 dogs were examined (see Appendix A for details). Dogs came from four distinct environmental groups, namely Guide Dogs for the Blind Association (GDBA) working dogs, Glasgow University Veterinary School referral cases (GUVS), racing greyhounds and laboratory beagles.

GDBA dogs are kept both as household pets and in kennels during their training and working lives. Pet dogs and laboratory beagles were therefore included for comparative purposes. Both GDBA dogs and laboratory beagles received stringent parasite control measures throughout their lives. Greyhounds were examined as a population known to be exposed to high levels of parasites, although they also received some form of intermittent anthelmintic therapy.

2.1.1 GDBA Dogs

Clinical and serological parameters of 143 GDBA dogs were studied over a period of three years. In addition the clinical records of a further 21 GDBA dogs were examined. GDBA dogs are predominantly Golden Retriever and Labrador Retriever crosses. In addition there are a small number of German Shepherd Dogs and other breeds such as curly coated Retrievers. Breeding and whelping of GDBA dogs mainly takes place at the breeding centre in Tollgate, England. However, a small number of GDBA dogs are born in breeders' houses. Bitches and puppies are kept in the one area until the pups are fully weaned by six weeks of age. At this age they are taken to puppy walkers' homes where they will remain until entering training kennels at nine to twelve months of age. Training of dogs at the kennels takes twelve to eighteen months after which dogs will begin their working lives and stay in the homes of their owners.

Dogs were first examined by the Centre Veterinary Surgeon on entering kennels at nine to twelve months of age. When they entered kennels dogs were randomly

assigned to the study by kennel staff and their clinical progress was followed throughout their time in kennels. After leaving kennels, dogs are sent anywhere in the United Kingdom so a detailed clinical examination was not always possible but correspondence with the home vet was undertaken in order to allow dogs in the study to be monitored. Any dogs at Forfar kennels found to develop skin disease, which had not been initially assigned to the study, were thereafter included.

As the majority of GDBA dogs are bred at the GDBA centre in England very few dogs under eight weeks of age were seen at Forfar and so only low numbers are included in the study.

Whilst with the puppy walker the dogs are kept as pets in a household and are exposed to a wide variety of allergens including house dust mites. They are also exposed to other household pets such as cats, other dogs or even pet birds.

Once in kennels the dogs are kept in groups of two or three. These groups do not remain constant and the animals are continually exposed to a large number of other GDBA dogs. Whilst training individuals are given access to a town environment and thus meet dogs outwith the GDBA population. In the kennels dogs are kept on a tiled floor with shredded paper bedding. Outside is a concrete run. Both kennels and runs are cleaned twice daily with dilute bleach. Dogs are allowed access on a daily basis to a fenced grass area for exercise. This area is surrounded by trees and farmland.

All dogs are kept under a strict regime of veterinary care and parasite control. Vaccinations against Distemper, Parvovirus (Nobi-vac Puppy DP®, Intervet) and Leptospirosis (Nobi-vac L®, Intervet) are given at six weeks old, followed by Distemper, Parvovirus, Hepatitis, Parainfluenza (Nobi-vac DHPPi®, Intervet) and Leptospirosis (Nobi-vac L®, Intervet) at twelve weeks of age and a final Leptospirosis (Nobi-vac L®, Intervet) vaccine at sixteen weeks old. Yearly full booster vaccinations are given thereafter. Intranasal kennel cough vaccinations are not routinely given.

Parasite control begins with the treatment of nursing bitches and their pups with fenbendazole (Panacur®, Hoechst UK Ltd.), at a dose of 1g/2.2kg, at two, five, and eight weeks post whelping. Subsequently, the pups received febentel/praziquantel/pyrantel combination (Drontal Plus®, Bayer), at a dose of 5mg praziquantel/kg (i.e. one tablet/10kg) at twelve weeks, six months and twelve months of age. The dogs were then routinely wormed with Drontal Plus® at six monthly intervals. Faecal samples taken from all the dogs at eight-ten months of age were uniformly negative. All the dogs were treated monthly with fipronil (Frontline®, Rhône Mérieux) or permethrin (Exspot®, Mallinkrodt Veterinary) spot-on once a month to control ectoparasites and no parasites were found on clinical examination. These dogs are considered to be essentially free of parasites.

GDBA dogs recruited into the study were ultimately assigned to one of three groups: atopic dogs, dogs with clinical signs consistent with atopic dermatitis but skin test negative and non-atopic dogs. Dogs which showed signs of skin disease which did not meet the criteria of atopic dermatitis were classified as skin problem dogs and were removed from this study. This was essential to ensure that potential atopics which had not been given a definitive diagnosis prior to the termination of the study were excluded from the non-atopic group.

A. A diagnosis of atopic dermatitis was based on the following three criteria:

1. Meet Willemse's criteria of at least three major and three minor categories of skin disease (Willemse, 1986) listed below.

Major features:	Pruritus
	Morphology & distribution – involvement of the face, digits, lichenification of the flexor surface of the tarsal joint and/or extensor surface of the carpal joint.
	Chronic/ relapsing dermatitis
	Family history and/or breed predisposition.
Minor features:	Onset before three years of age
	Facial erythema and cheilitis
	Bilateral conjunctivitis
	Superficial staphylococcal pyoderma
	Hyperhydrosis
	Immediate skin test reactivity to inhalents
	Elevated allergen specific IgE

Willemse also included elevated allergen specific IgG_d as a minor criterion but this could not be included as it was not possible to carry out this serological assay.

2. At least one positive result on IDST
3. There was no suggestion of food hypersensitivity based on an eight week restriction diet and subsequent challenge.

B. Dogs were assigned to the skin test negative group if:

1. They met Willemse's criteria of at least three major and three minor categories of skin disease (Willemse, 1986).
2. Negative results were obtained on intradermal skin testing.
3. There was no suggestion of food hypersensitivity based on an eight week restriction diet and subsequent challenge.

C. Dogs were assigned to the non-atopic group if they were:

1. Aged over three years by 31/8/98
2. Had no history of multiple episodes of atopic type skin disease consistent with Willemse's criteria (1986).
3. Had no evidence of recurring episodes of any skin disease.

Application of these criteria to the GDBA population (143 dogs) allowed the allocation of dogs to the different groups as follows:

A. Atopic dogs found which demonstrated positive results on IDST (n=18).

B. Atopic dogs which demonstrated negative IDST results but met all of the other criteria for a diagnosis of atopic dermatitis (n=2).

C. Non-atopic dogs without any recurring skin disease (n=96).

D. Problem dogs which demonstrated recurrent episodes of skin disease but did not meet the criteria for atopic dermatitis (n=27). These dogs were excluded from further studies.

2.1.2 Greyhounds

23 racing greyhounds undergoing routine biochemical and haematological examinations were also studied, details of which are shown in Appendix A. These dogs were all National Racing Greyhound Club (NRGC) registered and were being blood sampled as part of a clinical work up due to a poor performance whilst racing. Clinical examination of these dogs was carried out by the referring veterinary surgeon and all dogs studied were free of clinical skin disease at the

time of blood sampling and had no history of recurrent skin disease. All dogs were housed in outdoor kennels either built of concrete or wood and are kept on newspaper or sawdust bedding. In general these dogs were not allowed into a household environment. However, it was noted that such dogs are exposed to large numbers of fleas both in kennels and at the race track. All twenty three dogs in this study were known to be treated regularly (minimum of three to four times a year) with proprietary anthelmintics (personal communication with referring veterinary surgeon). All dogs had received up to date vaccinations against distemper, parvovirus, leptospirosis and hepatitis.

2.1.3 Laboratory beagles

33 laboratory beagles were also studied (described in Appendix A). In each case excess serum from blood samples taken for assessment of antiviral antibodies was available for analysis. These dogs were from a closed colony of dogs in England. It was not possible to examine them personally but communication with the referring veterinary surgeon confirmed that these dogs were free of skin disease at the time of sampling and had no history of recurrent skin disease. These dogs were kept indoors at all times and did not have any access to outdoor allergens. Clinical examinations have not revealed the presence of ectoparasites and these dogs did not receive any ectoparasite therapy. They were housed in concrete and steel buildings with sawdust and paper bedding and were allowed plastic toys. All dogs received vaccinations against distemper, hepatitis, parvovirus, parainfluenza (Nobivac DHPPi®, Intervet) and leptospirosis (Nobivac L®, Intervet) at six, nine and twelve weeks of age. This vaccination was then repeated annually. Breeding bitches were also vaccinated with the same regime at every oestrus. Endoparasite control was given with fenbendazole (Panacur, ® Hoechst Roussel Vet Ltd.) at two, five, eight and twelve weeks of age at a dosage of 50mg fenbendazole/kg body weight for three consecutive days. At twenty eight weeks of age and every four months thereafter fenbendazole was given on one occasion at 100mg/kg body weight. Pregnant bitches were treated with fenbendazole at a dosage of

25mg/kg body weight daily from day forty two of pregnancy until two days post whelping. These dogs are believed to be free of parasites.

2.1.4 Glasgow University Veterinary School (GUVS) Referral Cases

Cases referred to the Dermatology Clinic at Glasgow University Veterinary School (GUVS) with clinical signs consistent with atopic dermatitis were included in this study. Theseforty seven dogs were kept as household pets and were exposed to both indoor and outdoor allergens. All dogs had access to grass areas and other animals. A variety of breeds and ages of dogs were examined as shown in Appendix A. The parasite control measures of these dogs before attending GUVS varied widely often being irregular or unknown although all dogs had received up to date vaccinations against distemper, leptospirosis, parvovirus and hepatitis. The criteria required for a diagnosis of atopic dermatitis were based on those described above for GDBA dogs. Of these forty seven dogs, forty were found to give at least one positive reaction on IDST and were classified as atopic and seven gave negative IDST and were classified as skin test negative.

2.2 Clinical examination

GDBA and GUVS referral cases were given a full clinical examination by the author. A full skin examination involving collection and examination of coat brushings, skin scrapings and hair pluckings was carried out on all dogs with evidence of skin disease. Fungal culture and microscopic examination were performed on these samples. Impression smears of skin lesions were also taken where deemed necessary. Skin biopsies were taken from any swellings or non-responsive skin lesions. Where internal medical problems were detected haematological and biochemical profiles were examined and appropriate therapy instigated.

A standard treatment protocol was followed for both GDBA and GUVS dogs with skin disease. All GUVS referred cases were treated with fipronil (Frontline® spray, Rhône Mérieux) at a dosage of 3ml per kg bodyweight. This treatment was not required for GDBA dogs as these were all routinely given treatment for

ectoparasites. All GDBA dogs with recurrent skin disease were treated with phosmet (VetKem Sponge On®, Sanofi Animal Health) as part of the GDBA protocol on skin disease.

Where a pyoderma was evident on clinical examination antibacterial therapy with cephalexin (Ceporex®, Mallinkrodt Veterinary Ltd.) at 30mg/kg body weight twice daily was given for a minimum period of two weeks. Most cases required a minimum of four weeks treatment. Yeast infections were treated with chlorhexidine/enilconazole shampoo (Malaseb® Leo Laboratories Ltd.), according to manufacturer's advice twice weekly for a minimum of two weeks.

Any dogs which demonstrated recurrent skin disease were placed on restriction diets of either home prepared chicken and rice or commercially available catfish and rice (Pedigree Select Protein). Dogs were not allowed any food other than this diet and water for a minimum of six weeks up to a maximum time of twelve weeks. All dogs which underwent intradermal skin testing were also placed on this restriction diet and were only diagnosed as atopic if there was no improvement whilst on this diet.

2.3 Intradermal skin test

Prior to performing an intradermal skin test all therapy with corticosteroids, antihistamines and evening primrose oil was stopped. Corticosteroid therapy was stopped for a minimum of four weeks up to a maximum of three months where depot injections had been given. Antihistamine therapy and evening primrose oil were stopped for at least two weeks prior to intradermal skin testing.

At the time of intradermal skin testing dogs were sedated with domosedan (Domitor®, Pfizer Ltd.) at a dosage of 0.4mg per 10kg bodyweight given intramuscularly along with butorphanol (Torbugesic®, Willows Francis Veterinary) at 4mg per 10kg body weight also given intramuscularly. Once sedation had taken effect, usually after 10-15 minutes, the dog was placed in lateral recumbency and the lateral thorax clipped. A total of 48 marker pen spots were applied to the flank in order to highlight the site of the intradermal

injections. Allergens produced and supplied by ARTU Biologicals (Netherlands) were used for intradermal skin testing (See Table 2.1). Injections of 0.05ml of each allergen, a positive control (histamine, concentration unknown) and a negative control (saline) were each administered intradermally with a 27 gauge needle. After thirty minutes the diameter, colour and degree of oedema of any wheals which developed at the site of injections were recorded. A result was considered positive where the diameter of the wheal was equal to or greater than the average of the positive and negative controls.

Table 2.1 Intradermal skin test allergens used in study.

Group	Allergen	Concentration
Controls	Histamine	0.1mg/ml
	Physiological phosphate buffer	Unknown
Mites	*Dermatophagoides farinae*	100NU/ml
	Dermatophagoides pteronyssinus	100NU/ml
	Acarus siro	100NU/ml
	Tyrophagus putrescentiae	100NU/ml
Indoor	Flea	1,000NU/ml
	Mosquito	Unknown
	Cat epithelia	100ug/ml
	Human epithelia	10ug/ml
	Sheep's wool	10ug/ml
	Feathers	Unknown
	Tobacco	Unknown
Trees	Alder	1,000NU/ml
	Ash	1,000NU/ml
	Beech	1,000NU/ml
	Birch	1,000NU/ml
	Elder	1,000NU/ml
	Elm	1,000NU/ml
	Hazel	1,000NU/ml
	Horse chestnut	1,000NU/ml
	Oak	1,000NU/ml
	Poplar	1,000NU/ml
	Willow	1,000NU/ml
Grass	Bent grass	1,000NU/ml
	Cocksfoot	1,000NU/ml
	Couch grass	1000NU/ml
	Meadow fescue	1,000NU/ml
	Orchard grass	1,000NU/ml
	Perennial ryegrass	1,000NU/ml
	Sweet vernal grass	1,000NU/ml
	Timothy	1,000NU/ml
Plants	Daisy	1,000NU/ml
	Dandelion	1,000NU/ml
	Dwarf ragweed	1,000NU/ml
	Golden Rod	1,000NU/ml
-	Lamb's quarter	1,000NU/ml
	Mugwort	1,000NU/ml
	Nettle	1,000NU/ml
	Plantain	1,000NU/ml
	Rape	1,000NU/ml
	Sorrel	1,000NU/ml
	Wall pellitory	1,000NU/ml
Moulds	*Alternaria alternata*	100ug/ml
	Aspergillus	100ug/ml
	Cladosporium	100ug/ml
	Penicillium	100ug/ml
	Phomae betae	100ug/ml

2.4 Blood sampling

Dogs from the GDBA and GUVS populations which were undergoing intradermal skin testing were blood sampled at the time of testing whilst under sedation. Blood samples of at least 5ml were taken by jugular venipuncture, placed in a glass tube without any additives and allowed to clot at room temperature. Samples were then centrifuged at 3,000 rpm and 5° Celsius for ten minutes. The resultant serum was then removed and divided into 0.5ml aliquots. These were stored at -20°C until required for testing.

Serum from other GDBA dogs and greyhounds was obtained from blood samples taken for health profiles prior to elective surgery or at the time of routine health checks. Serum obtained from laboratory beagles was surplus to samples taken for viral antibody assessment as explained earlier.

At the time of sampling the ninety six non-atopic GDBA dogs ranged in age from 56 to 3161 days with an average of 619 days; eighteen atopic GDBA dogs ranged in age from 125-2892 (average of 1265 days); nine IDST negative dogs with recurrent skin disease ranged in age from 246-2885 days (average 1388). Ages of beagles and greyhounds were only available in years rather than days – twenty three greyhounds ranged in age from 3-12 years (average of 1667days) and thirty three beagles ranged in age from 2-6 years (average of 1360 days); forty atopic GUVS dogs ranged in age from 288-2941 days (average 1265 days). Ages of dogs at sampling are shown in Appendix A.

2.5 Serological Tests

Due to the limitations of the amount of serum obtained from each dog it was not possible to carry out all of the serological tests on every dog. In addition the various serological kits and reagents and intradermal skin test used in this study by necessity came from different manufacturers. This is an important point when considering the correlation of serological and IDST results but could not be avoided.

2.5.1 Enzyme Linked Immunosorbent Assay (ELISA)

A commercially available ELISA test for the detection of canine allergen specific serum IgE produced by AlerCHEK Inc. Portland, USA was used for this analysis. Allergens incorporated into this test are designed specifically for the European market and the test itself has been validated by the company (personal communication Richard Robinson, Operations Manager, AlerCHEK Inc.).

Serum samples were defrosted at room temperature for ten minutes and 0.6ml of each serum sample mixed with 2.4 ml of unspecified specimen diluent provided by AlerCHEK. This mixture was incubated at 4°C for twenty four hours. This incubation was required in order to bind serum proteins which can bind non-specifically to the plates (Kemeny & Challacombe, 1988).

Following this incubation, 100ul of each diluted sample was added to each allergen coated well. Each serum was tested against eleven outdoor antigen panels and a negative control on one plate and eleven indoor antigen panels and a positive control on another plate. All wells contained one allergen as described in Table 2.2 apart from house dust mites which contained both *Dermatophagoides farinae* and *D. pteronyssinus;* feathers which contained a mixture of feathers and *Mucor* which contained different species of mucor. Both plates were incubated at 22°C for two hours. The wells were then decanted and washed five times with the wash buffer provided which had been diluted with deionised water at a concentration of one to fifteen.

To each well, 100ul of rabbit anti-canine IgE was added, followed immediately with 100ul of conjugated peroxidase goat anti-rabbit IgG. This was incubated at 22°C for three hours. The well contents were then decanted and washed five times with buffer as described above.

To each well, 100ul of 3,3',5-5' tetramethylbenzidine hydrochloride (TMB)/peroxide was added and the plate incubated at 22°C for thirty minutes. This resulted in the formation of a blue colour in the positive control well, a clear colourless negative control and varying shades of blue in the test samples (See Fig. 2.1). The intensity of blue colour formation was assessed subjectively,

compared to a reference panel and graded from zero (colourless) to four (intense blue).

In order to stop the colour development 100ul of 0.18N sulphuric acid was added to each well resulting in a change of the blue colour to yellow where present (See Fig 2.2). The colour intensity was then read on a microplate reader (Bio-tek Instruments, Inc. Winooski, VT), at 450nm with the plate zeroed on the negative control well. The resulting optical density correlates with the degree of positivity where 0-0.149 is equal to a negative result; 0.150-0.249 is equal to a Grade 1 positive; 0.250-0.349 is equal to a Grade 2 positive; 0.350-0.449 is equal to a Grade 3 positive and greater than 0.45 is equal to a Grade 4 positive which was the highest result.

Results were then tabulated and examined with statistical packages where required.

Table 2.2 Allergens included in ELISA

Group	Allergens
OUTDOOR ALLERGENS	Orchard grass
	Timothy grass
	Kentucky blue
	Fescue.
	Poplar
	Birch
	Sheep's sorrel
	English plantain
	Mugwort
	Dandelion
	Nettle
INDOOR ALLERGENS	Flea
	Mixed dust mites – *D. farinae* *D. pteronyssinus*
	Mixed feathers
	Alternaria
	Aspergillus
	Rhizopus
	Kapok
	House dust
	Cat epithelium
	Human epithelium
	Mixed *Mucor*

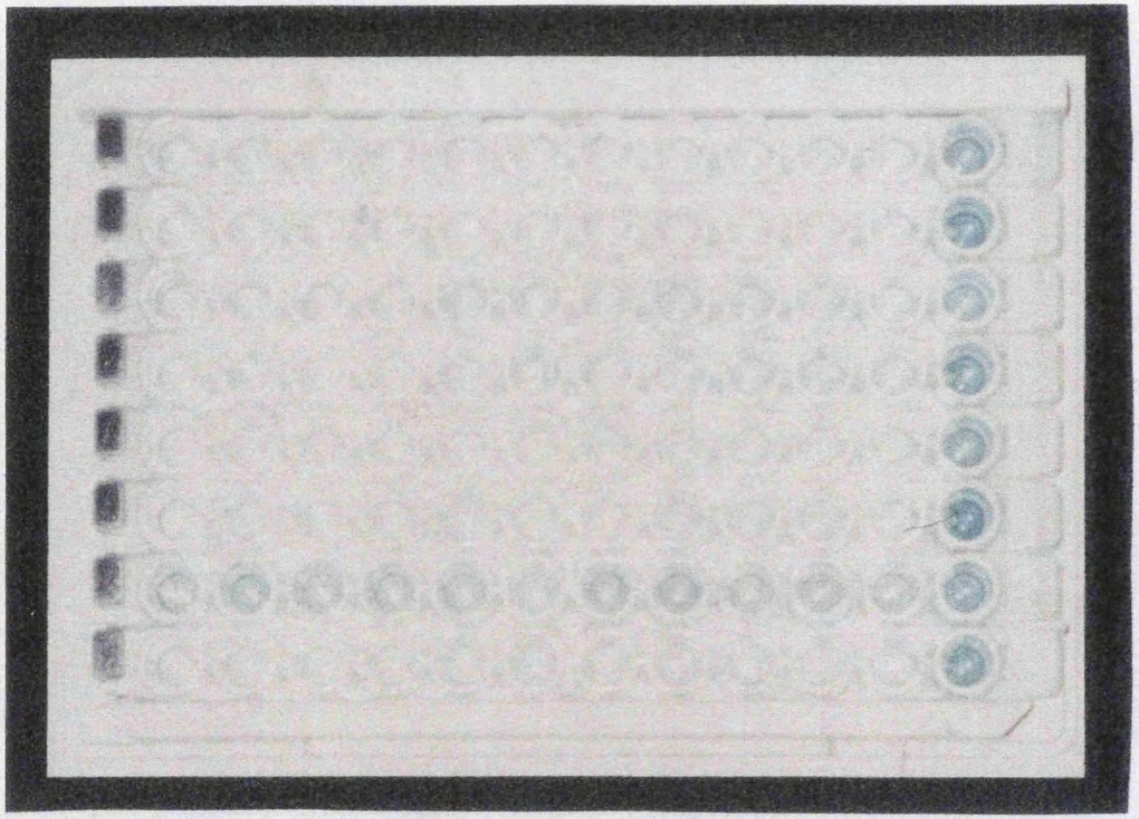


Figure 2.1 ELISA test wells demonstrating eight rows of allergen coated wells described in Table 2.2 and a positive control well (blue).

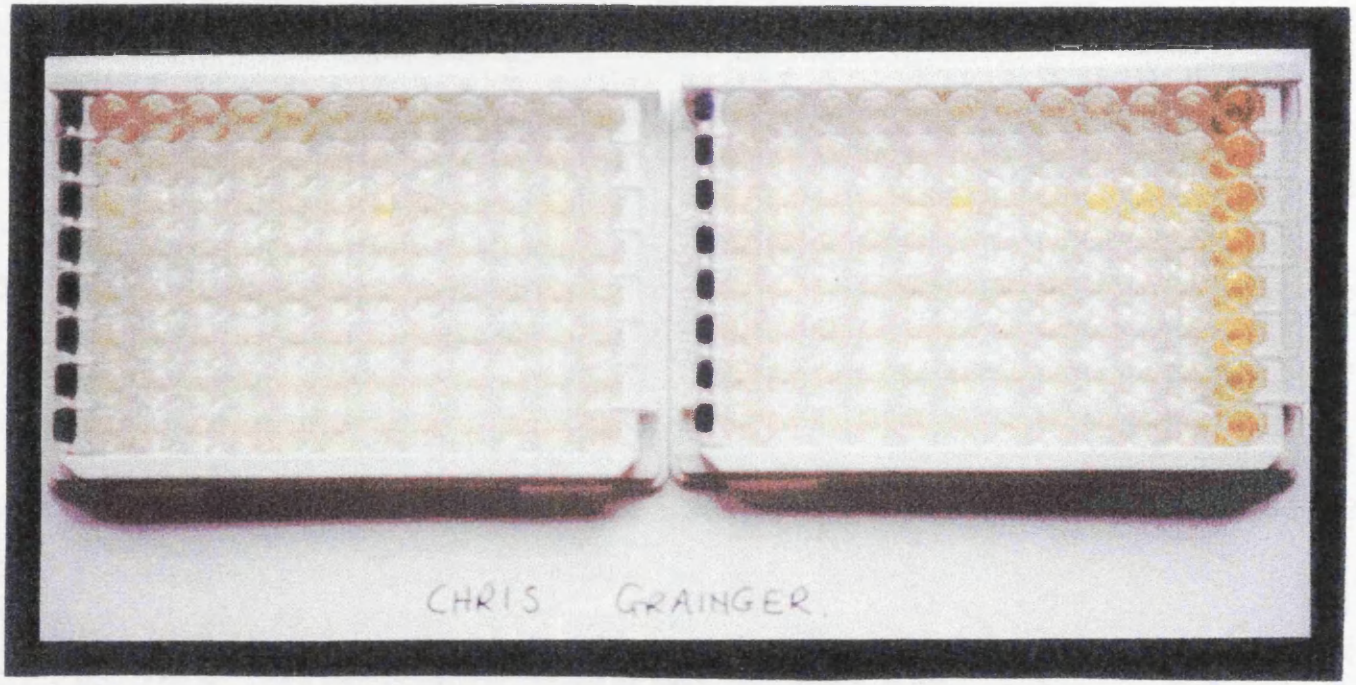


Fig 2.2 Indoor and Outdoor allergen ELISA plates following addition of 100ul 0.18N sulphuric acid with the development of a resultant yellow colour

2.5.2 Immunodot

The Immunodot test (Centre Medical des Grand Places/HESKA, Switzerland) consists of multiple nitrocellulose strips with spots containing various allergens which when incubated with serum bind to IgE and IgG. Following the addition of an anti-IgE antibody, which binds specifically to IgE, and the subsequent addition of an enzyme colour development takes place where IgE is detected (See Fig. 2.3). Different groups of allergens are present on different strips with the three strips used in this study being the Outdoor panel, Indoor panel and a Topscreen (See Table 2.3 for further details).

Serum samples were defrosted at room temperature for ten minutes. 0.5ml of the serum was then added to 0.5ml Tris Buffered Saline (TBS) which had previously been diluted one in ten with distilled water and contained 4% Microcide III. The resulting 1ml solution was then added to a well containing the appropriate strip and incubated at 22°C on a horizontal shaker for two hours for both Indoor and Outdoor panels and overnight for the Topscreen panel.

After this time serum was removed from the well and the strips washed three times for five minutes each time with 1.5 ml of the diluted TBS described above on a horizontal shaker at 22°C. To each strip 1ml of the revealing horseradish peroxidase monoclonal anti-IgE antibody mixture was added and incubated at 22°C on the horizontal shaker for two hours. Revealing antibody was then removed and the strips washed for fifteen minutes as described above.

Developer was prepared by adding fifteen graduated drops of chromogen (4-chloro-1-naphthol) and two graduated drops of enzyme substrate containing 3% hydrogen peroxide to 10ml distilled water. To each strip, 1ml of the resultant developer was then added and incubated for fifteen minutes.

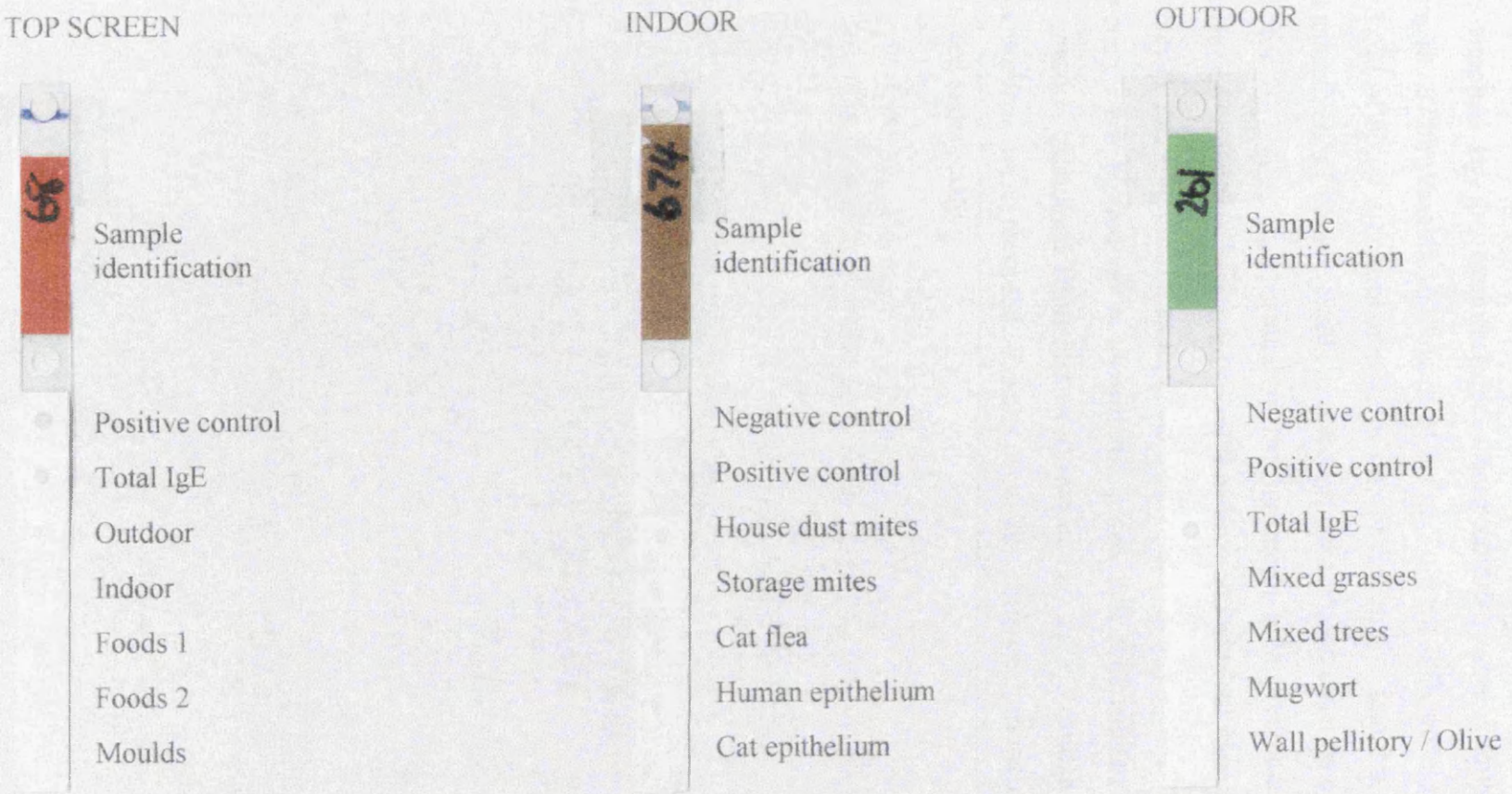
Developer was then removed and the strips washed under running tap water for one minute. The strips were then blotted dry and allowed to completely dry at room temperature for twenty four hours. Resultant colour development was assessed subjectively on a scale of 1-4 (where 1= negative, no colour; 2= positive control, some colour; 3= strong positive, 4= highly positive). Strips were also

scanned with an optical densitometer (AGFA Scanner, model 420oe at a reflective density of 1kV) and the resultant optical density recorded for statistical analysis.

Table 2.3 Allergens included in Topscreen, Outdoor and Indoor Immunodot allergen panels.

TEST STRIP	GROUP	CONSTITUENTS OF GROUP
TOP SCREEN		Total IgE
	Outdoor	Six grasses, Rye, Birch, Oak, Hazel, Ribwort, Olive, Wall pellitory, Jerusalem Cedar
	Indoor	*Dermatophagoides. farinae, D. pteronyssinus, Acarus siro, Tyrophagus putrescentiae*, Cat flea, Human dander, Cat epithelia.
	Foods A	Cow's milk, Hen's egg, Soybean, Maize flour, Wheat.
	Foods B	Lamb, Beef, Pork, Cod, Sole, Peanut.
	Moulds	*Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum, Penicillium notatum, Candida albicans.*
OUTDOOR		Total IgE
	Grasses	Cocksfoot, meadow fescue, perennial ryegrass, timothy, kentucky blue grass, velvet grass /yorkshire.
	Trees	Birch, Oak, Hazel.
		Mugwort/Ribwort
		Olive/Wall pellitory.
INDOOR	House dust mites	*D. farinae, D. pteronyssinus*
	Storage mites	*A. siro, T. putrescentiae*
		Cat flea
		Human dander
		Cat epithelia

Fig. 2.3 Immunodot test strips



2.5.3 Radial Immunodiffusion

Radial immunodiffusion is a quantitative method used in the analysis of immunoglobulins. Antibody against IgG_1 is incorporated into agar. The test serum (containing IgG_1) is placed in an antigen well in the agar and allowed to diffuse through the agar. This results in the development of an antigen-antibody precipitin ring around the well. The diameter of the precipitate ring reflects the concentration of IgG_1 present. By applying known concentrations of anti IgG_1 antibody, it is possible to create a logarithmic graph from which unknown IgG_1 concentrations can be calculated.

Analysis of serum total IgG_1 levels was carried out with a commercially available radial immunodiffusion method (Bethyl Laboratories, Inc., Montgomery, USA).

In addition to the test samples, three control samples of known IgG_1 concentration were supplied by the manufacturer. These controls were known to contain 205mg/dl, 825mg/dl and 1650mg/dl of IgG_1. To each diffusion plate well was added 5ul of either a control sample or one of eleven test samples, so that each plate contained all three controls and eleven tests. Plates were then incubated at 22°C for twenty four hours. This resulted in the formation of a precipitation ring, the diameter of which was measured in millimeters and recorded (See Fig 2.4). Ring diameters were plotted on a logarithmic graph and the resultant serum IgG_1 concentration calculated from this as shown in Fig. 2.5. Resultant serum IgG_1 concentrations were recorded. Results from the different groups of dogs were examined statistically.

Figure 2.4 Radial Immunodiffusion plate showing control serum (wells 1-3) and test serum (wells 4-16) with the development of a white precipitin ring.

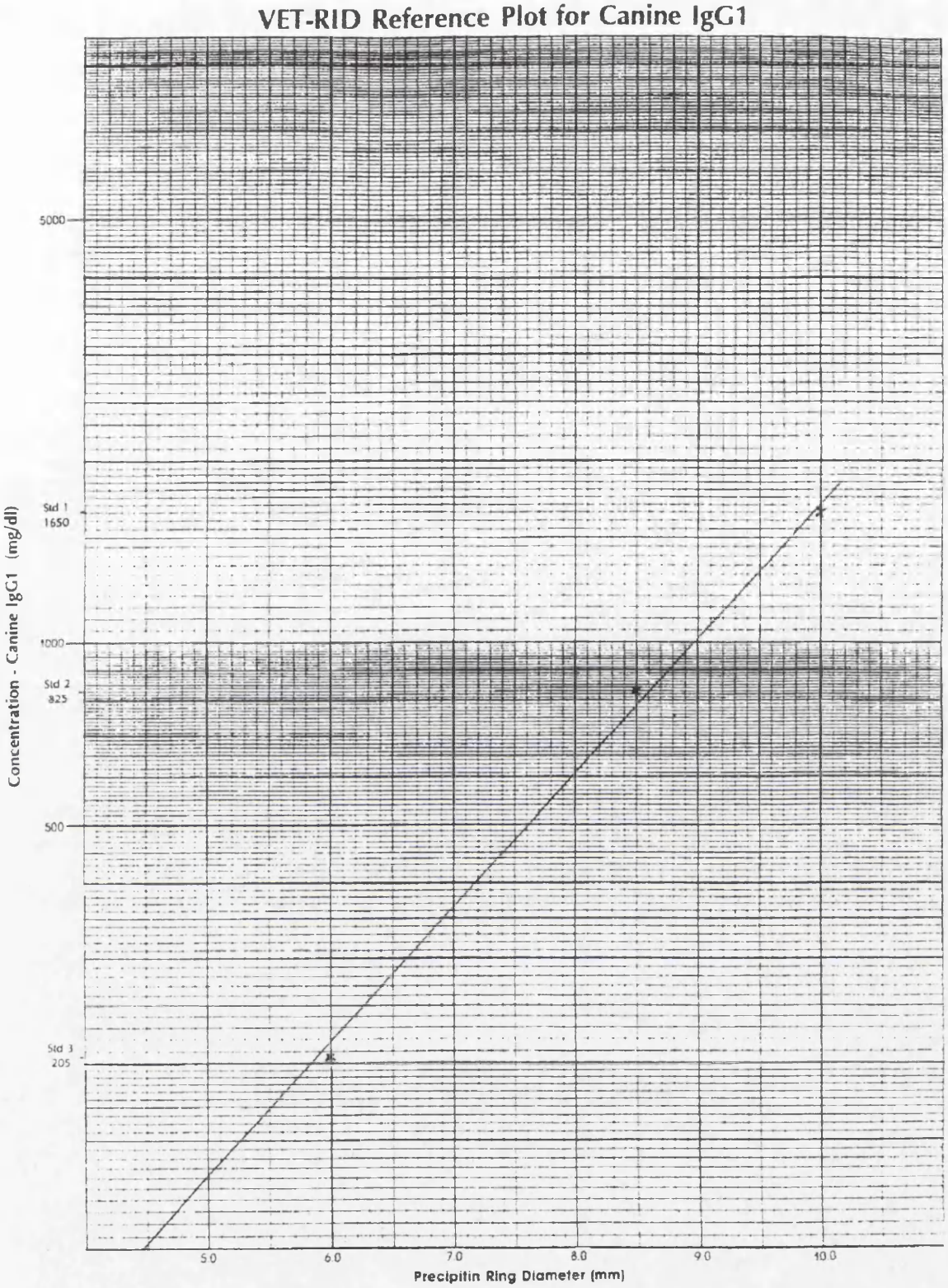


Fig. 2.5 Logarithmic graph of reference serum IgG_1 concentration in relation to precipitin ring diameter.

2.6 Statistical methods

Various statistical tests were used in the analysis of data collected in this study. A brief summary of the background to these tests follows.

The paired t test is a way of comparing two groups of data from a population with normal distribution and the same variances. The test gives a probability value (p) to the two groups being the same and that the differences between them are due to chance. A low p value, usually less than 0.05 or 0.01 is considered indicative of a significant difference between the two groups and thus not due to chance.

The Kolmogorov-Smirnov two sample test is a method of detecting whether or not the results are from one population or two distinct populations. Where the only variations between the two groups are considered to be within random variation no significant difference will be found between the groups. The Kolmogorov-Smirnov test is a method of detecting when the results are sufficiently far apart to be considered significant and indicative of two populations.

One way analysis of variance (ANOVA) is a method of comparing data when different levels of only one factor are being compared. The number of observations in each group do not need to be the same size but it is assumed that the data comes from a normally distributed population – otherwise transformation is required prior to analysis. The test indicates evidence of a significant difference between the groups.

Two way analysis of variance is a method of comparing data classified according to two variables, however the method is most effective when there are the same number of observations in each group. Where different numbers of observations are present in each group a General Linear model may be used in order to deal with the imbalance in the design. Following the identification of significant differences amongst groups using ANOVA, the Newman Keuls multiple range test was used to test for differences between particular groups.

The association between different serological tests and intradermal skin testing was examined using correlations. Pearson's correlation coefficient (r) can take a value between –1 to +1. The correlation between two variables is positive if high values of one variable correspond with high values of the other variable whereas a negative correlation is obtained if one variable increases when the other decreases. A correlation coefficient close to zero indicates that there is no linear correlation between the variables and they are unrelated.

The Mann Whitney test is a non-parametric method for comparing data from two independent groups. This test requires that the observations in each group are ranked in order and the sum of ranks used to obtain a p value which is indicative of the significance between the two groups.

The confidence interval is a range of values which we can be confident contains the true population parameter. In assessing the confidence interval of an estimated mean that interval extends either side of the mean by a multiple of the standard error. Confidence intervals are usually based on a 90%, 95% or 99% chance of including the true population value. Where confidence intervals are calculated whether 90%, 95% or 99% was used will be stated.

Sensitivity is defined as the proportion of true positives which are correctly detected by the test. Specificity is defined as the proportion of true negatives that are correctly detected by the test (Thrusfield, 1995). The predictive value of a test depends on sensitivity, specificity and prevalence of a disease in a particular population. The efficacy is a means of determining how well the diagnostic method works in both affected and non-affected animals.

Data was stored on Microsoft Excel version 5.0, 1995 and statistical analyses carried out using Minitab for Windows Release 11.21, 1996.

Chapter 3 Examination of the clinical histories of atopic and non-atopic GDBA dogs

3.1 Introduction

The aim of this part of the study was to determine if any differences could be identified between atopic and non-atopic dogs in the age of onset of skin problems and the type of skin conditions presented.

The usual age of onset of clinical signs of atopic dermatitis in the dog are between one and three years (Scott, 1981). It is rare for dogs younger than this to present with atopic dermatitis (Scott, 1981) with only 2% of atopics presenting between six-eight months of age. In support of this is work by Schwartzman (1984) who could not find any evidence of atopic dermatitis in the progeny of atopic dogs by the age of twelve months. The only exception to this is work by Reedy *et al.* (1997b) who observed clinical evidence of atopic dermatitis by the age of six months.

It was anticipated that differences in the type of skin disease presented and the age of onset of these conditions could contribute to a diagnostic plan so that dogs which would go on to develop atopic dermatitis could be identified before a year of age. This prediction would be of benefit because GDBA dogs begin their training at a year old but do not develop clinical signs, diagnostic for atopic dermatitis, until two to three years old. At this point dogs are often unable to work after much time and money has been spent on their training (as explained in 2.1.1).

3.2 Materials & Methods

Clinical histories of thirty dogs from the GDBA population described in section 2.1.1 were examined. Fifteen of these dogs had previously been diagnosed as atopic, based on clinical findings and intradermal skin testing. At the time of the study these dogs ranged in age from two to eleven years. Fifteen non-atopic dogs

were chosen at random by the Kennel Assistants based on the criteria that, all dogs were older than four years of age by 31/8/98 and that there were no skin problems suggestive of atopy in the clinical history *i.e.* although otitis externa and pyoderma are included in Willemse's criteria as clinical signs of atopic dermatitis, dogs were only excluded from being atopic if they had a history of repeated episodes of these conditions as non-atopic dogs can have isolated cases of otitis externa. A lower age limit of four years rather than three years was chosen to ensure that the chance of these dogs still developing atopic dermatitis was minimised as much as possible, although it is recognised that it is possible for a dog to develop atopic dermatitis after 4 years of age, this is uncommon. Willemse & Van den Brom (1983) reported that 75.5% of cases of atopic dermatitis developed before three years of age. The dogs chosen were aged between four and eleven years. All thirty dogs were either Golden Retrievers, Labrador Retrievers or crosses of these two breeds. Detailed clinical histories were available for all of the dogs from birth to twenty one months of age. Further details of these thirty dogs are given in Appendix A.

Clinical histories were examined for mention of any skin problems, excluding viral papillomas and pressure sores. These conditions were excluded as both occurred at specific times in the history of the kennels. For example bedding was only introduced to the kennels at the start of this study and prior to this pressure sores were very common. However, due to the introduction of bedding materials younger dogs had fewer cases of pressure sores. Also, outbreaks of viral papillomas were seen during the history of the kennels but there were times when no dogs were affected. As it was not consistently present throughout the time being studied it was not felt appropriate to include this criterion as this could adversely influence the results. During this discussion atopic type skin conditions were based on the criteria of Willemse (1986) and included pruritus, otitis externa, conjunctivitis, pedal dermatitis, pyoderma, erythema, dandruff and anal gland disease. 'Any skin condition' describes atopic type conditions in addition

to skin problems such as alopecia and wounds but does not include viral papillomas or pressure sores.

The age at which dogs first presented any skin condition or an atopic type skin problem was recorded and a survival type curve of time to event produced for both of these findings. Atopic and non-atopic dogs were compared for any significant differences in the age at which they first presented with atopic or any skin conditions using a Kolmogorov-Smirnov two sample statistical test.

Also, the frequency of presentation of particular skin conditions in atopic and non-atopic dogs was examined. In order to compare the two groups of dogs, the expected length of time a particular skin condition took to resolve, had to be determined. This was defined in non-atopic dogs as the length of time between first being examined by the centre vet until complete resolution of the problem. Only conjunctivitis, otitis externa, pedal dermatitis and acute moist dermatitis or pyoderma were observed often enough to allow statistical evaluations to be carried out. Acute moist dermatitis and pyoderma were both used by the centre vet to describe infectious skin disease and could not be differentiated on the clinical records – therefore both conditions had to be included as one. Statistical analysis of these results was carried out to obtain a 90% non-parametric confidence interval for the median times to resolution in order to define the expected duration of each skin condition.

These results for non-atopic dogs were then applied to the clinical histories of atopics. This meant that where an episode of skin disease in an atopic dog lasted twice as long as expected in a non-atopic dog, the atopic dog was described as being affected by two episodes of skin problems. This is necessary because atopic dogs can suffer from prolonged episodes of skin disease which do not respond quickly to treatment and this fact has to be taken into account when comparing both groups of dogs. However, as not all atopic dogs suffer from longer episodes of skin disease, the duration of skin disease in atopic and non-atopic dogs was not compared.

The cumulative number of episodes of particular skin conditions in atopic and non-atopic dogs, at various ages, were analysed using a Mann Whitney statistical test. This allowed the identification of the most significant diagnostic parameters. Analyses were undertaken using the Minitab version 11.21 (1996) statistical software package.

Sensitivity, specificity and predictive value of the above classifications were examined in order to identify the most suitable model to use as a diagnostic indicator for atopic dermatitis based on the number of episodes of skin problems at difference ages.

3.3 Results

Studies of the skin conditions in atopic and non-atopic dogs revealed that otitis externa was the commonest condition in both atopic and non-atopic dogs followed by conjunctivitis (See Appendix B). Conditions other than atopic type skin conditions observed in both atopic and non-atopic dogs included juvenile cellulitis, wounds, alopecia, lick granulomas and infected nailbeds.

Examination of the age at which dogs first demonstrated any kind of skin condition, (Figure 3.1) excluding viral papillomas and pressure sores, revealed that 50% of atopic dogs had demonstrated skin problems by the age of seven months compared with twelve months for the non-atopic dogs. Examination of the demonstration of atopic type skin conditions (Figure 3.2) revealed that 50% of atopic dogs were affected by the age of eight months compared with twelve months for non-atopics. All fifteen atopic dogs had suffered from some form of skin condition by the age of eleven months and atopic type skin conditions by fifteen months compared with twenty two months for the development of both atopic and any kind of skin condition in all fifteen non-atopic dogs (Figs 3.1 & 3.2).

Survival curve analyses of the number of atopic and non-atopic dogs affected by skin problems by particular ages revealed that atopic dogs were consistently affected by skin disease at a younger age than the non-atopic dogs.

Comparison of the survival type curves of atopic and non-atopic dogs with a Kolmogorov-Smirnov test (Table 3.1) revealed a significant difference between both groups of dogs at ten months of age for any skin conditions ($p<0.01$) and atopic type skin conditions ($p<0.02$).

Examination of the length of an episode of atopic type skin conditions in non-atopic dogs (Table 3.2 and Appendix C) revealed the estimated expected duration of each particular skin condition as: seven days for conjunctivitis; seven days for otitis externa; seven days for pedal dermatitis and fourteen days for pyoderma.

Examination of the number of episodes of skin disease in atopic and non-atopic dogs at differing ages, shown in Appendix B, was carried out with a Mann Whitney test (results shown in Table 3.3 and Appendix D). This revealed a significant difference between atopics and non-atopics ($p<0.025$, $p<0.042$) at nine months for the total number of episodes of both atopic type skin conditions and any skin conditions respectively. No significant difference was evident until twelve months between atopics and non-atopic for otitis externa ($p<0.002$) and eighteen months for conjunctivitis ($p<0.02$). No difference was observed between the two groups in the number of episodes of pedal dermatitis or pyoderma.

Sensitivity, the number of atopic dogs from the fifteen atopics in the study which agreed with the criteria of x number of episodes by y months of age, and specificity, the number of non-atopic dogs in the study which were not affected by x number of episodes by y months of age were found to be best for four episodes or more of atopic type skin conditions by the age of fifteen months (Table 3.4). Sensitivity for these criteria was 60% and specificity 93.3%.

For any skin condition, excluding viral papillomas and pressure sores, sensitivity of 66.7% and specificity of 86.7% were found for four episodes or more by the age of 15 months (Table 3.5). The prevalence of atopic dermatitis in the general population has been reported as between 10% and 15% (Chamberlain, 1974). Therefore a prevalence of 10% was used for statistical evaluation. Using this, the

best predictive value for a diagnosis of atopy in these thirty dogs was four or more episodes of atopic type skin disease by the age of fifteen months (Table 3.4).

Fig. 3.1 Survival type curve of time to the development of any skin disease for atopic and non-atopic dogs.

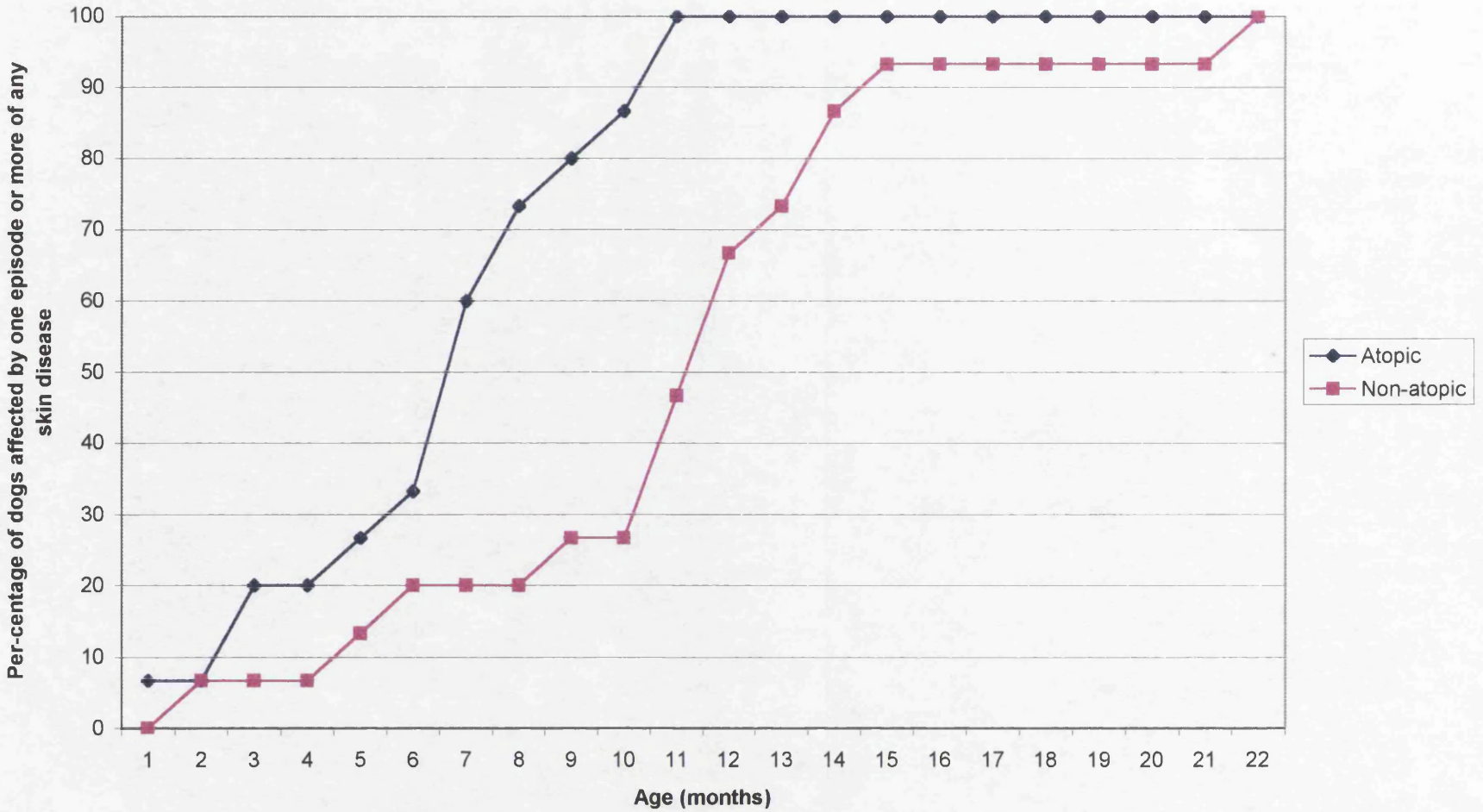


Fig. 3.2 Survival type curve of time to the development of atopic type skin disease for atopic and non-atopic dogs

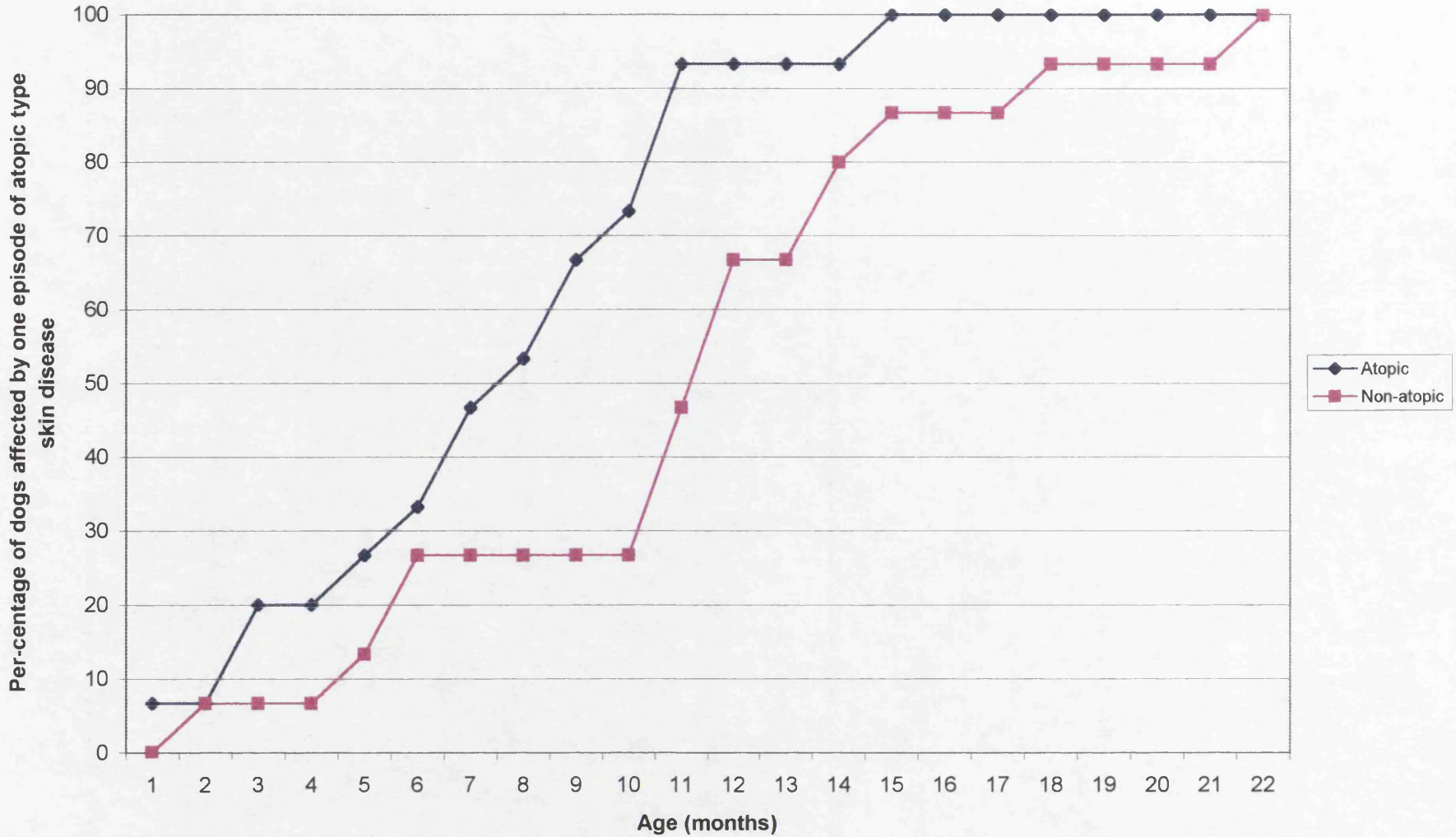


Table 3.1 Results of Kolmogorov-Smirnov two sample test examining the age which any skin disease or atopic type skin disease developed in groups of atopic and non-atopic dogs

Formula required for Kolmogorov Smirnov test:

$$X^2 = 4D^2_{m,n} \frac{mn}{m + n}$$

Where X^2 = Chi Squared distribution
D = The maximum difference between survival curves of atopic and non-atopic dogs.
m= Number of atopic dogs.
n= Number of non-atopic dogs.

1. For any skin condition with a maximum difference between the two groups at ten months old:

$$X^2 = 4 \times (60/100)^2 \times \frac{15 \times 15}{15+15}.$$

$$X^2 = 10.8$$

From this a critical value of $p<0.01$ was obtained for df=2.*

2. For atopic type skin conditions with a maximum difference between the two groups at ten months old:

$$X^2 = 4 \times (53.3/100)^2 \times \frac{15 \times 15}{15 + 15}.$$

$$X^2 = 8.5$$

From this a critical value of $p<0.02$ was obtained for df=2*

* From Siegel & Castellan, 1988.

Table 3.2. The duration of episodes of atopic type skin conditions in 15 non-atopic dogs between birth and 21 months of age and results of 90% non-parametric confidence interval.

	Duration of problem (days)			
Episode number	Conjunctivitis	Otitis externa	pedal dermatitis	Pyoderma
1	5	5	7	17
2	26	7	7	12
3	7	7	7	10
4	17	7	5	10
5	7	7	5	7
6	7	7	*	5
7	5	5	*	20
8	6	22	*	7
9	5	7	*	7
10	7	10	*	5
11	7	7	*	14
12	7	7	*	14
13	5	5	*	*
14	7	7	*	*

* No more episodes of this particular skin condition were observed.

Results of 90% non-parametric confidence interval analysis of above data (See Appendix C) revealed the expected duration of each skin condition as:

Conjunctivitis	7 days
Otitis externa	7 days
Pedal dermatitis	7 days
Pyoderma	14 days

Table 3.3 Examination of the number of episodes of individual skin diseases in atopic and non-atopic GDBA dogs by different ages with a Mann Whitney test. (Original data in Appendix B; calculations in Appendix D).

Parameter	**Age (months)**	**Level at which test is significant (p value).**
Otitis externa	3mths	1.000
	6mths	0.756
	9mths	0.507
	12mths	**0.010**
	15mths	0.071
	18mths	**0.013**
	21mths	**0.005**
Conjunctivitis	3mths	*
	6mths	1.000
	9mths	0.756
	12mths	0.678
	15mths	0.101
	18mths	**0.029**
	21mths	**0.040**
Pedal dermatitis	3mths	*
	6mths	*
	9mths	*
	12mths	1.000
	15mths	0.709
	18mths	0.152
	21mths	0.078
Pyoderma	3mths	*
	6mths	*
	9mths	*
	12mths	0.272
	15mths	0.229
	18mths	0.199
	21mths	0.300

Table 3.3 continued

Parameter	Age	Level at which test is significant. (p value)
Atopic skin conditions	3mths	0.804
	6mths	0.237
	9mths	**0.025**
	12mths	**0.004**
	15mths	**0.01**
	18mths	**0.001**
	21mths	**0.0003**
Any skin conditions	3mths	0.804
	6mths	0.351
	9mths	**0.042**
	12mths	**0.002**
	15mths	**0.003**
	18mths	**0.002**
	21mths	**0.001**

*Test could not be carried out as results identical / virtually identical.
Those figures in bold are significant. ($p < 0.05$).

Table 3.4 Sensitivity, specificity and predictive value results for the number of episodes of atopic type skin conditions demonstrated by atopic dogs by different ages.

Sensitivity of number of episodes of atopic type skin conditions

	Age (months)		
No. episodes	12mths	15mths	18mths
3	46.6%	73.3%	100%
4	33.3%	**60%**	86.7%
5	26.7%	53.3%	80%

Specificity of number of episodes of atopic type skin conditions

	Age (months)		
No. episodes	12mths	15mths	18mths
3	100%	73.3%	40%
4	100%	**93.3%**	53.3%
5	100%	100%	66.7%

Predictive value of a positive test for 10% prevalence

$$\text{Predictive value} = \frac{\text{Prevalence x Sensitivity}}{(\text{Prevalence x Sensitivity}) + [(1-\text{Prevalence})\text{x}(1-\text{Specificity})]}$$

	Age (months)		
No. episodes	12mths	15mths	18mths
3	1	0.234	0.156
4	1	**0.49**	0.171
5	1	1	0.211

Note: Figures in bold indicate best result

Table 3.5 Sensitivity, specificity and predictive value results for the number of episodes of any skin conditions demonstrated by atopic dogs by different ages.

Sensitivity of number of episodes of any skin conditions

	Age (months)		
No. episodes	9mths	12mths	15mths
3	5.3%	86.7%	100%
4	40%	**66.7%**	86.7%
5	26.7%	60%	86.7%

Specificity of number of episodes of any skin conditions

	Age (months)		
No. episodes	9mths	12mths	15mths
3	86.7%	40%	20%
4	100%	**86.7%**	26.7%
5	100%	100%	46.7%

Predictive value of a positive test for 10% prevalence.

	Age (months)		
No. episodes	9mths	12mths	15mths
3	0.308	0.138	0.122
4	1	**0.359**	0.116
5	1	1	0.153

Note: Figures in bold indicate best result

3.4 Discussion

Atopic dermatitis is characterised as a pruritic skin condition, which is often familial and generally affects particular breeds (Scott, 1981, Willemse, 1986). All thirty dogs in this particular study were Retrievers, Labrador Retrievers and their crosses which are breeds known to be predisposed to developing atopy (Willemse, 1986).

Most cases of atopic dermatitis become clinically evident between the ages of one and three years (Scott, 1981). However, clinical signs of skin problems can present themselves before this age but as they are often minor they may not be regarded as significant by an owner. The clinical histories of the working dogs in this study are highly detailed and in this respect minor skin conditions which may not be observed in pet dogs have been recorded.

Diagnosis of atopic dermatitis before one year of age is difficult as diagnosis often relies on a history of chronic relapsing skin problems. Willemse & van den Brom (1983) observed that only 27% of atopic dogs had developed clinical signs of atopic dermatitis by one year of age, whereas Scott (1981) found that 64% of atopic dogs had developed signs by one year old.

Examination of the clinical histories of the working dogs described here revealed that there was indeed a significant difference between atopic and non-atopic dogs in the age at which dogs first demonstrated skin problems. This difference was evident by ten months of age. Examination of both atopic type skin conditions and any skin conditions revealed that the atopic dogs were significantly younger than the non-atopic dogs in the age at which they first demonstrated such skin problems.

Atopic dogs were found to first present with atopic type skin conditions or any skin problems at a younger age than non-atopics. From this it can be concluded that the age at which dogs first demonstrate skin problems can be included in the diagnostic criteria of atopic dermatitis and examination of a dog's history is important in the diagnosis of atopy. The only problem is that these conditions may often be so minor that when applied to pet dogs, episodes may not be recognised by owners. However, this should not

be a problem in the GDBA population where puppy walkers could be informed of the clinical signs to look for.

Evidence of pruritus is the most common presenting clinical sign in canine atopic dermatitis (Scott, 1981). However, it can be difficult to assess as it is subjective in nature and relies on the opinion of the owner. As it is normal for all dogs to demonstrate a degree of pruritus, diagnosis can prove difficult. In the kennels, working dogs are not observed at all times and pruritus may not be diagnosed unless it is very obvious. Therefore pruritus is not mentioned regularly in the clinical histories and it could not be included as a major sign in this study.

Most commonly mentioned skin problems were conjunctivitis, otitis externa, pyoderma and pedal dermatitis and statistical analysis of these parameters was possible. Other conditions such as anal gland disease were too infrequent to include in statistical analysis and each mention of such condition had to be included as an episode of disease.

Otitis externa and conjunctivitis were the most common conditions recorded. It is possible that this is because both conditions are easy to identify when a dog is being groomed, whereas pedal dermatitis and pyoderma can go unnoticed.

In the diagnosis of atopic dermatitis the main problem is that the initial presentation of skin disease alone is not sufficient to make a diagnosis.Instead diagnosis is commonly based on repeated episodes which obviously take time to become apparent. In addition, these repeated episodes of skin disease in both humans and dogs can become chronic, taking a long time to resolve. It was therefore necessary to determine the expected duration of atopic type skin conditions in non-atopic dogs. This gave some surprising results. The expected duration of otitis externa in non-atopic dogs was found to be only seven days which is rather low as usually cases of otitis externa in GDBA dogs would be treated for a minimum of fourteen days. It is possible that this is due to iatrogenic cases of ear disease as a result of over zealous cleaning, and the misdiagnosis of cases of otitis externa by kennel staff. It has been noted that individual dogs with large amounts of wax have been referred to the centre veterinary surgeon as otitis externa and such cases are often recorded as otitis

externa. These had to be included in this study as it was not possible to differentiate them from true cases of otitis externa. Similarly cases of pedal dermatitis were expected to last for seven days which again appears a rather short time. It is possible that these cases are due to trauma as many dogs tend to jump up onto the wire mesh kennel doors. However, such cases are equally presented in atopic and non-atopic populations and it is fair to include them in this study.

Comparison of atopic and non-atopic dogs revealed that there was indeed a significant difference in the number of episodes of both atopic type skin lesions and any skin lesions as early as nine months of age. This is younger than would be expected as most diagnoses of atopy are not made before one year of age. However, it is likely that most atopic dogs do indeed begin to show clinical signs of disease at this young age, but that the process of diagnosis, often involving relapsing skin conditions, with dogs often being referred to dermatologists means that the recorded age of presentation is older. Indeed, Reedy *at al.* (1997a) reported that a group of inbred atopic dogs demonstrated clinical signs of atopic dermatitis at six months of age.

There was very little difference in the numbers of dogs presenting with atopic type skin lesions or all skin lesions. This is due to the fact that the majority of skin conditions presenting in all these dogs are of an atopic type. This makes diagnosis of atopy difficult.

Of the individual skin conditions described, a significant difference was noted between the number of episodes of otitis externa experienced by atopics and non-atopics by the age of twelve months ($p<0.01$). A significant difference was found for the number of episodes of conjunctivitis by eighteen months of age ($p<0.029$). There was no significant difference between atopic and non-atopic dogs in the number of episodes of pedal dermatitis or pyoderma. Examination of the cumulative total of all episodes of atopic type skin problems revealed the most significant difference between atopics and non-atopics was at nine months of age ($p<0.025$). However, there was very little difference between the total number of episodes of atopic type skin problem and the total number of episodes of any skin problem, with a significant difference also present

for any skin condition ($p<0.042$) at nine months of age. From this it appears that for diagnostic purposes it is better to examine the total number of episodes of atopic type skin conditions or indeed any skin conditions rather than relying on one particular skin condition.

The finding that atopic dogs as young as nine months of age have significantly more episodes of skin disease than non-atopics contradicts work by Schwartzman (1984) who could not find any clinical evidence of atopic dermatitis in dogs less than twelve months of age. However, the work described in the present study could not detect atopy in dogs as young as those studied by Reedy *et al.* (1997b), who observed that dogs could demonstrate clinical signs consistent with a diagnosis of atopic dermatitis by the time they were six months of age. In order for such an observation to withstand statistical scrutiny increased numbers of dogs would have to be studied.

Retrospective examination of clinical findings revealed a significant difference between atopic and non-atopic dogs by nine months of age. However, in order to apply this data in the prediction of atopic dermatitis, dogs who show four or more episodes of atopic type skin disease by fifteen months should be considered at risk of developing atopic dermatitis. Sensitivity of this finding was 60% and specificity 93%. Although the sensitivity is lower than desired the high specificity means that there would be a low chance of non-atopic dogs being identified as possible atopics. This would allow dogs to be assessed at the beginning of their training and a decision could be made about their future before they go through two years of training.

3.5 Conclusions

It is apparent from this study that atopic dogs demonstrate clinical evidence of skin problems both of an atopic and non-atopic nature at a younger age than non-atopic dogs. It is therefore important that the clinical history of a suspected atopic dog is examined in some detail and that the age at which dermatological disease of any sort was first recognised is included in the diagnostic parameters of canine atopic dermatitis.

In addition, examination of the cumulative number of episodes of atopic type skin conditions or any skin problems are a better diagnostic indicator than episodes of any one particular form of skin disease. Analysis of the number of episodes of skin problems should therefore be carried out in suspected atopic dogs. Those animals affected by four or more episodes of skin problems by the age of fifteen months should be investigated further as candidates for the development of atopic dermatitis.

Chapter 4 Studies of serum total IgE in non-atopic and atopic dogs.

4.1 Introduction

In human medicine serum total IgE concentrations are used as a diagnostic indicator of atopic dermatitis (Gurevitch *et al.* 1973; Jones *et al.*, 1975; Juhlin *et al.*, 1969). In general atopic people have significantly higher concentrations of serum total IgE than non-atopics. However, in the dog no such differences have been found; indeed dogs as a population have been shown to have much higher serum total IgE levels than humans (Schwartzman & Rockey, 1967). It has been suggested (Halliwell & Kunkle, 1978) that this is due to the high levels of parasites to which dogs are exposed, as the major antibody response to parasites is IgE .

The first aim of this study was to assess the serum total IgE concentrations of atopic and non-atopic GDBA dogs which are considered free of parasites in order to determine whether or not atopics and non-atopics could be differentiated on the basis of serum total IgE concentrations. Serum total IgE concentrations of atopic GUVS dogs, non-atopic greyhounds and non-atopic laboratory beagles were also examined for comparative purposes.

It has been suggested (Griffin *et al.*, 1990) that where non-atopic dogs have high serum total IgE concentrations non-specific binding of IgE in the ELISA can occur and lead to positive results. These positive ELISA results in non-atopic animals are often called false positives. The second aim was to estimate the number of positive ELISA results in non-atopic GDBA dogs, in relation to serum total IgE concentrations, to establish whether or not our findings agreed with current theories.

Thirdly, serum total IgE concentrations of non-atopic GDBA dogs were examined in relation to age. In human medicine serum total IgE concentrations have been shown to increase with age up to twenty-thirty years old (Johansson *et al.*, 1970). The aim here was to assess if any age related differences in serum total IgE levels were identifiable in the dog.

4.2 Materials & Methods

Initial assessment of the immunodot test was carried out. This involved applying serial dilutions of an IgE containing serum to the Topscreen test (as described below) and assessing the results. The initial serum was diluted 50:50 with Tris Buffered Saline (TBS).The resultant diluted serum was then diluted again 50:50 with TBS. This was repeated a further four times.

The serum total IgE concentrations of five groups of dogs were examined. Blood samples were obtained from non-atopic GDBA dogs, atopic GDBA dogs, non-atopic greyhounds, non-atopic beagles and atopic GUVS dogs as described section 2.1. Details of these dogs are given in Appendix A.

Serum total IgE concentrations were assessed in fifty five non-atopic GDBA dogs. These were predominantly Labrador Retrievers, Golden Retrievers (and their crosses) and German Shepherd Dogs. In addition Curly Coat Retrievers, Flat Coated Retrievers and Collie crosses were represented. At the time of sampling these dogs were aged from 56 – 2365 days, with a mean age of 575 days.

Twelve atopic GDBA dogs were included in this study. These dogs were of similar breeds to the non-atopic dogs and were aged from 429-2687 days, with a mean age of 1182 days, at the time of sampling.

Serum total IgE concentrations were examined from fifteen non-atopic greyhounds and twenty five non-atopic laboratory beagles. At the time of sampling greyhounds ranged in age from 3-6 years (accurate ages in days were not available for greyhounds or beagles) with a mean of 1606 days, and the age of beagles ranged from 3-5 years, with a mean of 1299 days.

A total of twenty eight atopic GUVS dogs were also examined. A wide variety of breeds were represented including Staffordshire Bull Terriers and English Setters in addition to Labradors and German Shepherd Dogs. The age of atopic GUVS dogs ranged from 305 to 2941 days (mean 1272 days).

All non-atopic dogs were older than three years by 31/8/98 and did not have any history of multiple episodes of atopic type skin disease based on Willemse's criteria (1986). Atopic dogs were diagnosed as such based on

clinical history, intradermal skin testing and serological testing as described in section 2.3-2.5.

GDBA dogs and beagles were assumed to be essentially free of parasites, although no post mortems were carried out to confirm this assumption. However, the treatment regimes for both groups of animals are such that the influence of parasites is minimal.

Blood samples were obtained and stored as described in section 2.4. Serum total IgE concentrations were evaluated using an Immunodot serological test kit (CMG / Heska, Switzerland) described in 2.5.2. Initially this involved an Outdoor panel of test strips but was later replaced by the manufacturer with a Topscreen panel. Resultant colour formation on this test strip indicated a positive result. The degree of colour formation is proportional to the concentration of serum total IgE and was assessed using an Optical Densitometer (AGFA, Scanner, model 420oe) to give a reflective density value, for comparative purposes.

Allergen specific ELISA analysis of the serum samples was carried out on forty three of the dogs undergoing total serum IgE analysis. It was not possible to assess allergen specific IgE in all fifty five non-atopic dogs due to the limitations of the amount of serum available. ELISA assays were carried out with a commercially available ELISA produced by AlerCHEK Inc., Portland, USA as described in section 2.5.1. Positive results obtained in the ELISA for these non-atopic dogs were considered clinically irrelevant results.

Statistical analysis of these results was carried out using one way Analysis of Variance (ANOVA) and the Newman Keuls multiple range test. Trends of association between variables were examined using Pearson's correlation coefficient, with r values close to 0 indicating no evidence of association. Analyses were undertaken using the Minitab version 11.21 (1996) statistical software package.

4.3 Results

Examination of serial dilutions of IgE did reveal a gradual decrease in colour formation of the total IgE spot on the Immunodot strip (Fig. 4.1).

Analysis of serum total IgE levels in atopic and non-atopic dogs revealed a high degree of overlap between groups (See Fig. 4.2). All five groups contained dogs with a wide range of serum total IgE concentrations.

Comparing serum total IgE concentrations of atopic and non-atopic GDBA dogs did not reveal any significant difference between these two groups (See Table 4.1, 4.2 & Appendix E6). Both groups of dogs contained individuals with high and low serum IgE concentrations. In addition, there was no significant difference between any combination of non-atopic beagles, atopic GUVS dogs, non-atopic GDBA dogs and atopic GDBA dogs. Indeed, non-atopic GDBA dogs with excellent parasite control could not be differentiated from atopic GUVS dogs receiving variable parasite control measures.

However, the one group where a significant difference in serum IgE concentrations was noted, was the group of non-atopic greyhounds, which demonstrated significantly higher serum IgE concentrations than all other groups of dogs ($p<0.05$) (See Table 4.1 & 4.2).

A large number of positive results were observed in non-atopic GDBA dogs, against a wide variety of allergens. No particular allergen was found to predominate (See Fig.4.3). Examination of the number of these positive ELISA results for each dog and that dog's serum total IgE concentration revealed a Pearson's correlation coefficient of –0.102 (see Appendix E7), indicating that there was no correlation between these two parameters (See Fig. 4.4). Indeed closer examination of these results revealed that some dogs with high serum total IgE reflective density levels up to 0.567 did not produce any positive ELISA results.

Further examination of the clinical histories of atopic dogs with low serum total IgE concentrations revealed that the dog with the lowest serum total IgE concentration had received ear treatment containing prednisolone two weeks prior to IDST/blood sampling and intermittent prednisolone tablets for three months up until eight weeks before IDST. The dog with the second lowest serum total IgE concentration had received an injection of dexamethasone four months prior to IDST/blood sampling.

Examination of the age of non-atopic GDBA dogs at the time of sampling and serum total IgE concentration did not reveal any correlation,

(Pearson's correlation 0.000, Appendix E8), with young and old dogs exhibiting a wide variety of serum total IgE levels as shown in Fig. 4.5.

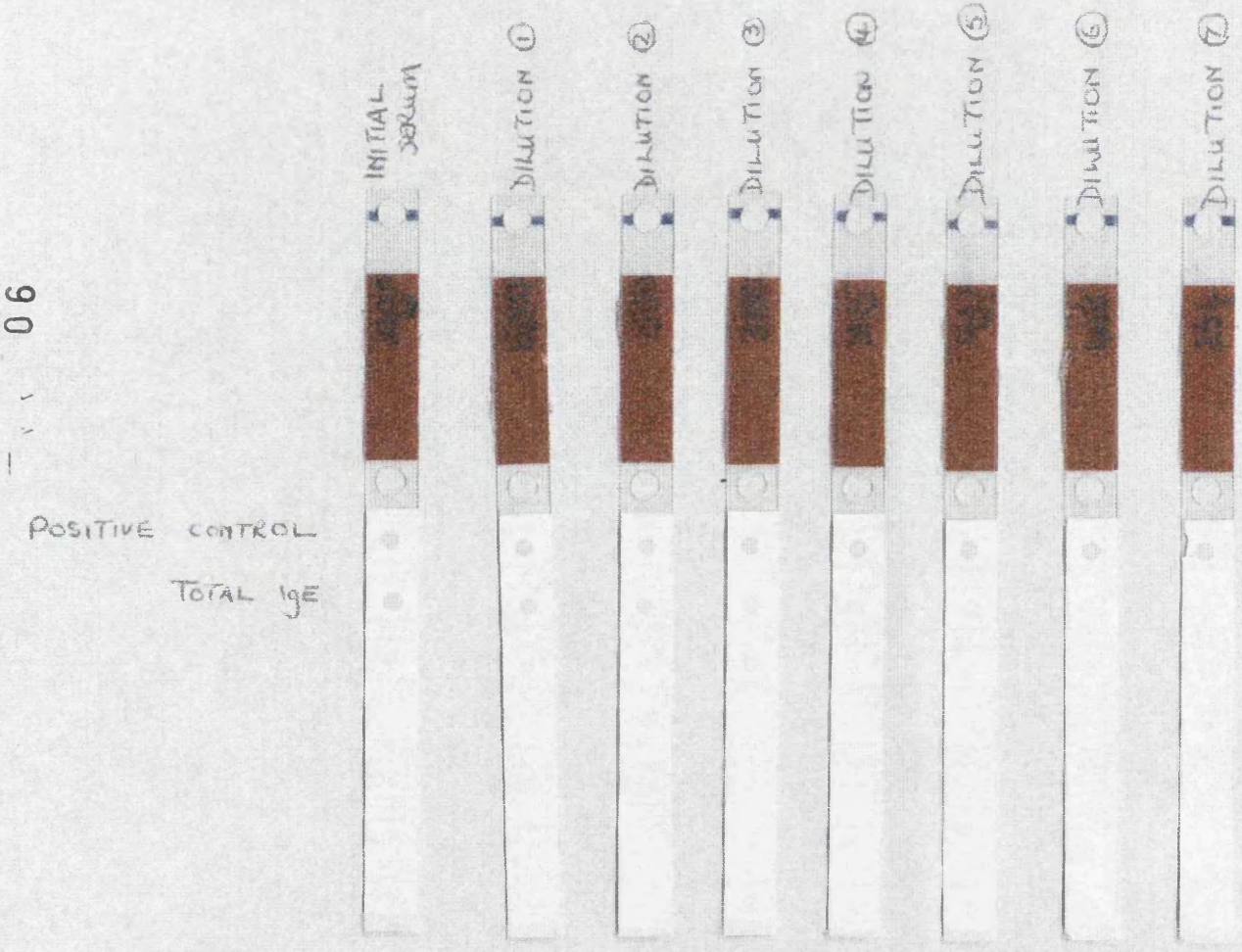


Fig. 4.1 Serial dilutions of serum IgE demonstrating a gradual decrease in colour formation on the Total IgE spot.

Fig. 4.2 Serum total IgE reflective density results in atopic and non-atopic dogs (See Appendix E)

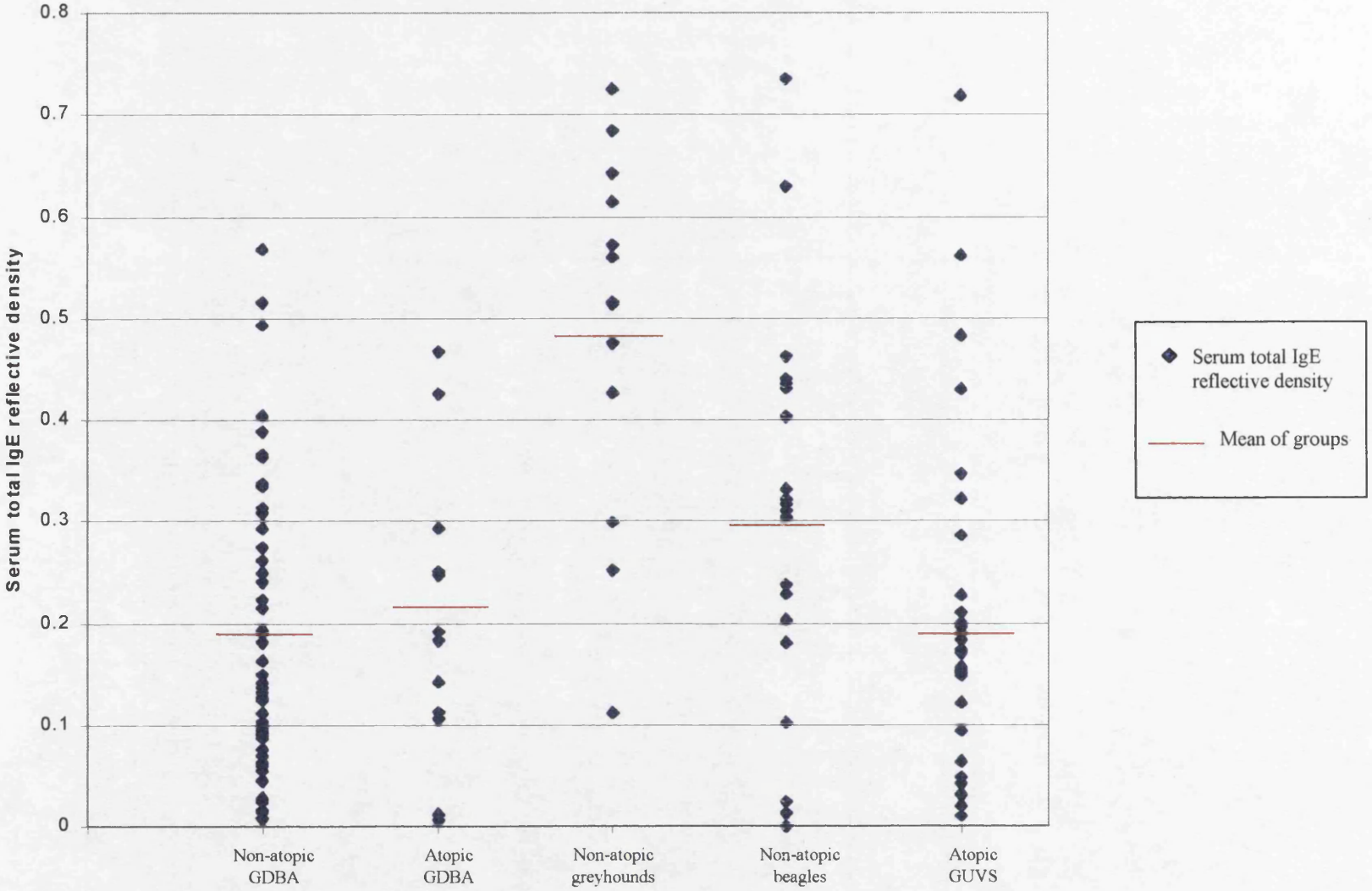


Table 4.1 – Comparison of serum total IgE reflective densities in different groups of dogs (Appendix E.6)

Group	Mean	Range	Standard deviation	Sample size
Non-atopic GDBA	0.182	0.008-0.567	0.143	55
Atopic GDBA	0.203	0.006-0.467	0.144	12
Greyhounds	0.498	0.011-0.684	0.167	15
Beagles	0.275	0.000-0.735	0.202	25
Atopic GUVS	0.206	0.010-0.718	0.172	28

Table 4.2 One way analysis of variance/ Newman Keuls Multiple Range Test. (See Appendix E.6).

A significant difference ($p<0.05$) was observed amongst the five groups of dogs described above. Further examination with a Newman Keuls multiple range test revealed that :

1. Greyhounds were significantly different to all other groups

2. No significant difference was present between non-atopic GDBA dogs, atopic GDBA dogs, beagles and atopic GUVS dogs

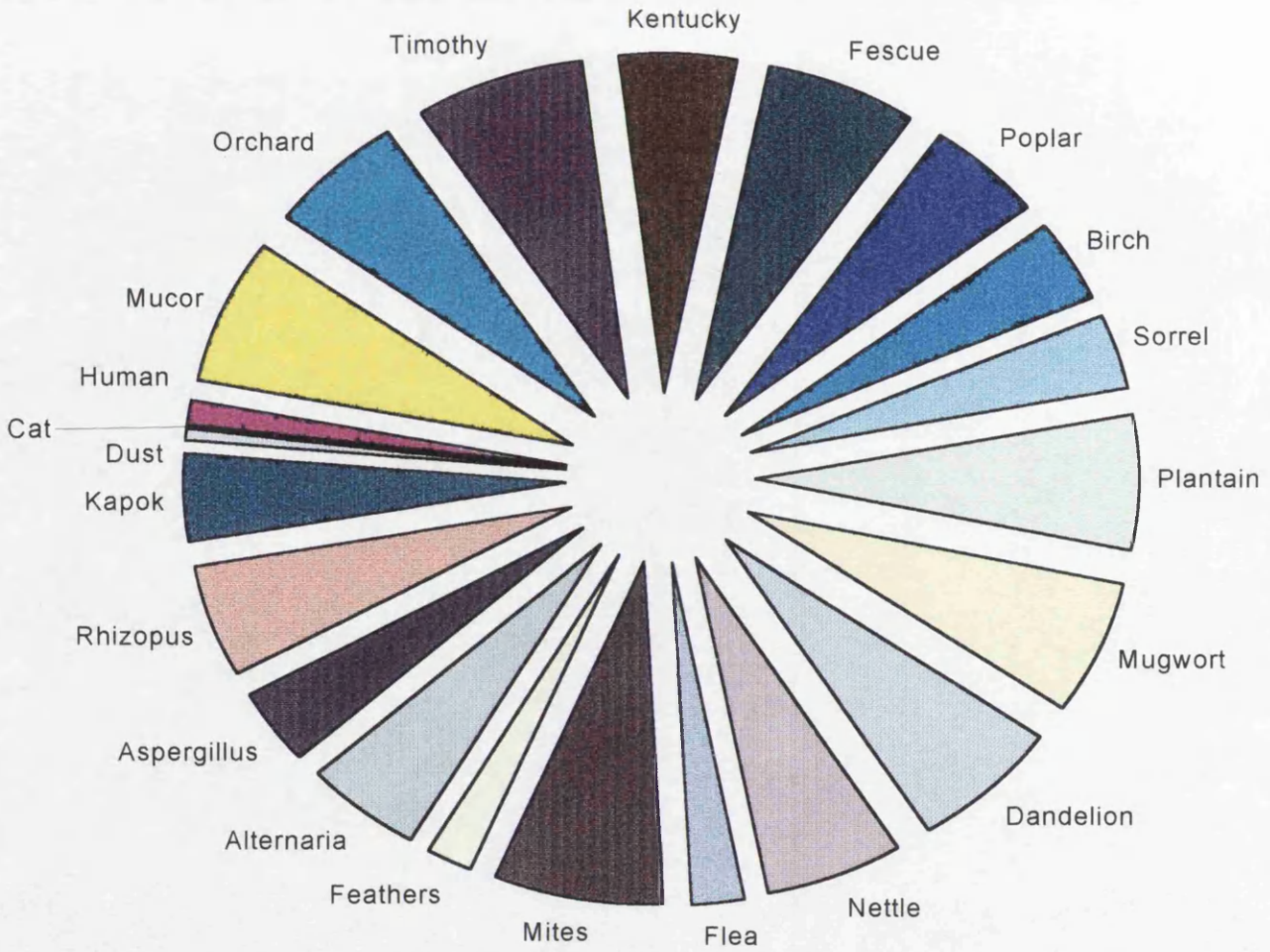


Figure 4.3. Breakdown of allergen specific positive ELISA results in non-atopic GDBA dogs demonstrating no overall prevalence of any one allergen

Fig 4.4 Serum total IgE reflective density as determined by Immunodot in relation to the number of false positive ELISA results in individual GDBA dogs

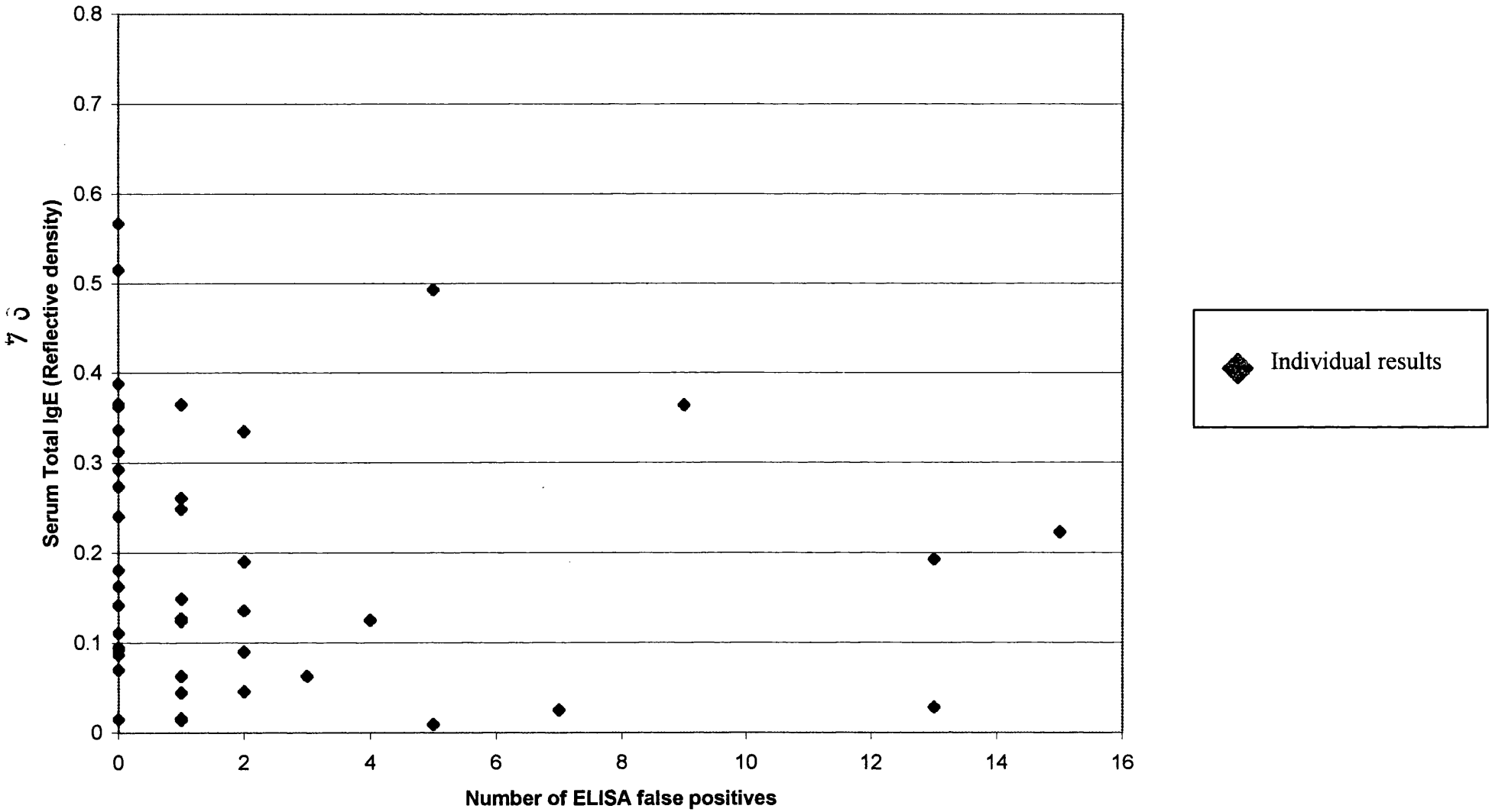
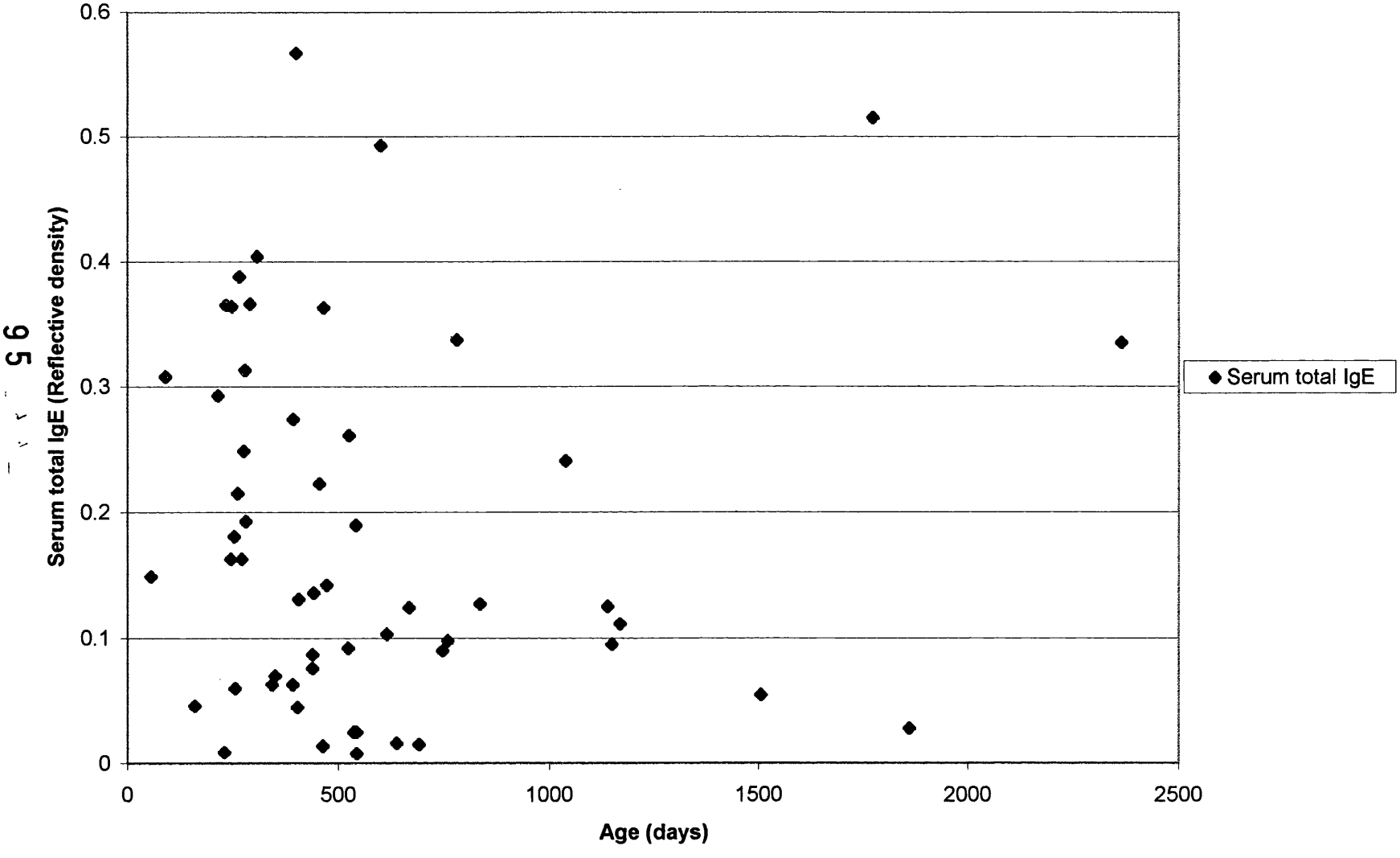


Fig. 4.5 Serum total IgE reflective density of non-atopic GDBA dogs in relation to age



4.4 Discussion

The production of IgE antibodies is mainly directed against parasites and is involved in hypersensitivity reactions (Bloch *et al.*, 1972, Tizard, 1987b). Serum IgE levels in non-atopic humans are relatively low at 300ng/ml (Johansson *et al.*, 1970), compared with that of dogs where serum total IgE levels can be as high as 350ug/ml (Rockey & Schwartzman, 1967).

In human medicine serum IgE levels have been shown to be significantly higher in atopic patients compared with non-atopics (Gurevitch *et al.*, 1973, Jones *et al.*, 1975, Juhlin *et al.*, 1969). However, this is not the case in the dog with no such significant difference being observed in veterinary medicine (Halliwell & Kunkle, 1978). The reason for the high level of total serum IgE in the dog is assumed to be the high level of parasitism to which dogs are exposed and subsequently infested (Halliwell & Kunkle, 1978). This results in parasitised atopic and non-atopic dogs having similar serum total IgE concentrations.

It is known that due to the excellent parasite control measures applied by the GDBA these dogs have extremely low levels of parasite exposure and are known to be free of endoparasites. Therefore it would be expected that atopic GDBA dogs would have significantly higher serum total IgE concentrations than non-atopic GDBA dogs. However, this is not the case. Non-atopic GDBA dogs in this study demonstrated a wide variety of serum total IgE concentrations similar to those observed in atopic GDBA dogs. No significant difference was found in serum total IgE levels between atopic and non-atopic GDBA dogs. Similar findings were reported by DeBoer & Hill (1999) who did not find and correlation between serum total IgE concentrations at six-twelve weeks of age and the development of atopic dermatitis in West Highland Terriers. These dogs also showed a wide range of serum total IgE concentrations.

Examining only the non-atopic GDBA dogs and non-atopic beagles it appears rather surprising that a number of these dogs had high levels of serum total IgE. Both of these groups of dogs underwent stringent parasite control

measures and it is unlikely that parasite burdens are responsible for inducing serum IgE production. A more likely reason for these serum total IgE concentrations is the 'high' and 'low' responder theory put forward by de Weck *et al.*, (1998) following original work by Katz (1978). Katz demonstrated that a group of inbred mice exhibited a wide range of serum total IgE concentrations and he divided these animals into groups of 'high' or 'low' responders. This phenotype was found to be restricted to the IgE antibody class and was not found with IgG. Katz (1978) also observed that the low responder phenotype corresponded to the non-atopic population. Similar work by de Weck (1998) demonstrated similar findings in the canine population – namely that particular dogs demonstrated high levels of serum total IgE and that other dogs demonstrated low levels. Results in this study from the non-atopic GDBA dogs and beagles would appear to agree with this. Although parasitism and hypersensitivity reactions may influence serum IgE levels, there appears to be a dominant phenotypical variation also controlling serum total IgE concentrations. The division of animals into groups of high or low responders would suggest that there is a bimodal distribution of serum total IgE response, however this is not the case. There is some area of overlap between both high and low responders as can be seen from Fig 4.1.

The non-atopic GDBA dogs studied here have shown no evidence of being atopic. However, some of these dogs were high responders. As de Weck (1998) suggested that only the dogs with increased serum total IgE concentrations went on to develop atopic dermatitis it would be reasonable to expect that these dogs could develop atopic dermatitis. From the results of this study it would appear that although a dog may have the ability to become atopic (i.e. high responder) they do not necessarily become so and other controlling factors must be involved. Mayer *et al.* (1998) suggested that genes other than those controlling serum IgE concentrations may be involved in the development of atopic dermatitis.

Conversely, as de Weck, *et al.*, (1998), suggested that only high responders go on to develop atopic dermatitis it appears surprising that examination of

atopic GDBA dogs revealed dogs with low serum total IgE concentrations. It is possible that these findings are due to previous corticosteroid therapy, as the two dogs with the lowest serum total IgE concentrations had received some form of corticosteroid medication before blood sampling. Dexamethasone has been shown to have approximately twenty times the anti-inflammatory activity of prednisolone (Intervet UK Ltd., data sheet). It is possible that these steroid therapies are responsible for the low serum total IgE concentrations. Although, it has been stated that there is no requirement to stop corticosteroid therapy prior to ELISA testing (Anderson & Sousa, 1993) recent work has suggested that such treatment may indeed influence serum IgE concentrations (McCall *et al.*, 1998).

Comparison of non-parasitised GDBA atopic dogs or parasitised GUVS atopic dogs did not reveal any significant difference. It is fair to assume that if serum total IgE concentrations are influenced by parasite burdens then the serum total IgE concentrations of GUVS dogs would be higher than GDBA. However, from our results this does not appear to be the case.

Examination of non-atopic greyhounds revealed significantly higher levels of serum total IgE. Greyhounds as a breed are known to be exposed to high levels of parasites (Jacobs, 1978, Walker & Jacobs, 1982). As this examination was retrospective it was not possible to examine the faecal parasite egg count of these dogs. However, a negative result on such an examination does not conclusively prove that a dog does not have a parasite burden. It is possible that dogs possessing latent larvae of *Toxocara canis* may have elevated serum IgE levels.

Also, fleas are known to be a common problem in many greyhound kennels (personal communication with referring veterinary surgeon) and it is possible that this may have an influence on the serum total IgE concentrations. However, this is unlikely as these dogs had no history of demonstrating flea allergy. Halliwell *et al* (1987) demonstrated that where dogs underwent continual exposure to fleas low only levels of serum total IgE were present.

It is also possible that as a breed greyhounds may inherently produce higher levels of serum total IgE, although no work has been carried out in this area. It is also possible that because only fifteen greyhounds were examined (in comparison to fifty five non-atopic GDBA dogs) we may by chance have serum total IgE levels of high responding dogs. Further work is required in this area.

It has been suggested by Griffin *et al.* (1990) that there may be a correlation between serum total IgE concentration in non-atopic dogs and the number of so called false positive ELISA results *i.e.* where non-atopic dogs demonstrate positive ELISA results. Griffin suggested that where non-atopic dogs have high levels of serum total IgE non-specific binding of IgE to the test well is more likely to occur with the production of a positive result. However, this study did not reveal any such correlation. This difference may be due to the fact that Griffin's study only included fifteen dogs whereas this study examined forty three non-atopic dogs.

It has been shown that IgG anti-IgE antibodies are present in both atopic and non-atopic dogs (Hammerberg *et al.*, 1997) and it has been suggested that these antibodies may interfere with the assessment of serum total IgE levels, due to non-specific binding of IgG antibodies to the well resulting in lower levels of serum total IgE being recorded than are actually present. In this study the use of Immunodot technique should have minimised this error. However, if IgG anti-IgE antibodies did manage to interfere with the detection of serum total IgE it would mean that lower levels of total serum IgE were recorded than were present and therefore dogs which actually had high levels of serum total IgE and a high number of positive ELISA results were identified as having low levels of serum total IgE. However, this appears unlikely. Although IgG anti-IgE allergen specific antibodies can interfere with ELISA testing resulting in a low number of positive ELISA results, it is possible that the IgG anti-IgE antibodies are directed against different epitopes from those detected by the ELISA test. An assessment of IgG anti-IgE antibodies was not possible in this study and further work in this area is required.

Examination of serum total IgE levels in non-atopic people have demonstrated that there is an increase in such levels until twenty-thirty years of age (Johansson *et al.*, 1970). Examination of this parameter in the non-atopic canine population did not reveal such a correlation; indeed there was no obvious correlation between age and serum IgE levels. This may be due to the high and low responders discussed above with this trait being obvious at a very young age. No work has demonstrated the youngest age at which this trait can be identified. Secondly, the lack of correlation may be due to the small number of samples from dogs under six months of age. It is possible that any correlation in the dog is present at a much younger age and this was not obvious from the results that were available.

4.5 Conclusions

This study has demonstrated that dogs as a population can exhibit a wide range of serum total IgE concentrations. This means that even in non-parasitised animals serum total IgE concentrations cannot be used to distinguish atopic and non-atopic dogs. Therefore, although serum total IgE concentrations can be used as a diagnostic parameter in human medicine this is not that case in canine medicine.

A group of greyhounds has been shown to have significantly higher serum total IgE concentrations than GDBA and GUVS atopic dogs. Whether this is due to a breed predisposition or a high level of parasitism requires further study.

No correlation could be observed between serum total IgE concentrations and either the number of positive ELISA results in non-atopic animals or the animal's age.

In summary, serum total IgE concentrations cannot be used as a diagnostic indicator of atopic dermatitis with further work being required on the environmental and genetic factors controlling the production of serum IgE.

Chapter 5 Seasonal variation of serological results

5.1 Introduction

Clinical presentations of canine atopic dermatitis are often seasonal, being more common during the summer when pollens and house dust mites (common allergens) are present at higher levels (Halliwell & Schwartzman, 1971). In the diagnosis of atopic dermatitis serological tests in both human and canine medicine incorporate cut off points used to identify positive reactions for individual allergens. However, these values remain constant throughout the year and there is no allowance for any increase in serum IgE levels in non-atopic individuals due to increased exposure to particular allergens at different times of the year.

Assessing allergen exposure in canine medicine presents particular problems, not normally encountered in human medicine. Dogs at ground level are exposed to different allergens than humans and therefore pollens which are not regarded as pathogenic in human medicine may well be involved in the pathogenesis of canine atopy. In addition, pollen grains may gather on the ground after pollination periods have finished so that canine exposure to these pollens may be longer than that expected for people.

The aim of this study was to examine whether or not there was any seasonal variation in the serum allergen specific IgE levels of non-atopic dogs. Due to the complicating factors of canine pollen exposure a study of the pollens to which dogs are exposed was initiated. This was carried out by examining faecal pollen content. Previously faecal pollen content has been studied in herbivores to identify areas where they have been feeding (Caulton, 1988). However, to date no such study has been carried out in carnivores to identify the pollens that are ingested either by inhalation then swallowing, by grooming themselves or others or by licking the environment.

5.2 Materials & Methods

A total of seventy four non-atopic GDBA dogs (described in section 2.1.1) were included in the serological study. These dogs were blood sampled on one occasion prior to elective surgery and excess serum was analysed for an allergen specific IgE response with an AlerCHECK ELISA as described in section 2.5.1. These serological results are summarised in Appendix F.

The samples were collected over the three years of the study and in order to investigate seasonal effects the dogs were grouped according to the month of sampling. For each Indoor and Outdoor allergen the mean optical density value of allergen specific IgE detected by ELISA was plotted against each month of sampling.

Similar evaluation of results could not be carried out for Immunodot results due to the smaller numbers tested and thus the small numbers of dogs in each month. Comparison of ELISA results for individual allergens on a monthly basis was carried out using one way Analysis of Variance (ANOVA) and the Newman Keuls multiple range test.

Fresh faecal samples were collected from fourteen GDBA dogs chosen at random by the kennel staff. Two dogs were sampled each month for six months from February to July. Samples from four dogs were available in June, two in the early part of the month and two later on. These fourteen dogs had been resident in the kennels for a minimum of two months prior to sampling and were not part of the serological study.

It had been hoped to extend this study for a longer period of time but this was not possible. Faecal analysis was carried out by Dr E Caulton of the Scottish Centre for Pollen Studies in Edinburgh. The basic method involved the desiccation of each faecal sample. Of the resultant material one gram then underwent acetolysation and slide preparation (as described in Appendix H). Slides of faecal material were examined at x40 magnification and pollen types identified and counted. Dependent on the amount of faecal material available either two or three slides were prepared for each dog. For the purpose of

comparison the mean number of each species of pollen grain present on a slide was calculated for each dog.

5.3 Results

The influence of month of sampling on the mean optical density value of allergen specific IgE detected by ELISA is illustrated in Figures 5.1 and 5.2. These revealed an apparent seasonal variation in the mean allergen specific ELISA results for outdoor allergens with peak ELISA results in August for a number of outdoor allergens including Kentucky grass, poplar, Timothy grass, sorrel and nettle. A smaller peak in mean ELISA results for outdoor allergens was also noted earlier in the year around May for birch, dandelion, nettle and mugwort. The manufacturer's cut off point for a positive reaction with the ELISA test is an optical density of above 0.15. It can be seen from Fig. 5.2 that in August the mean ELISA results for all of the outdoor allergens were above 0.15 and that in January, February, May, July and September at least one outdoor allergen gave a mean value above this cut off point.

No clear cut seasonal variation was observed for indoor allergens (Fig 5.1). Fewer mean indoor allergen optical density results were above 0.15, with the highest mean ELISA results being observed for *Rhizopus* in August. *Mucor*, house dust mites and *Rhizopus* were the only allergens that gave mean results above 0.15. ELISA results for mite allergens peaked in July. This contrasts with the mean ELISA optical density results for dust allergens which were consistently low (< 0.08) throughout the year.

Statistical evaluation (One Way ANOVA & Newman Keuls MRT) of these results revealed that allergen specific IgE concentrations against Kentucky grass (*Poa pratensis)* in August were indeed significantly higher than all other months (Table 5.1 & Appendix G). Allergen specific IgE concentrations against poplar allergen were significantly higher in August than February and September. *Alternaria* specific IgE concentrations were found to be significantly higher in November than all other months. The only anomaly would appear to be the ELISA results for cat epithelium which were

significantly higher in February than in January. However, examination of these results with one way Analysis of Variance did not find reveal any significant difference between concentrations of cat epithelium specific IgE throughout the year.

Pollen grains were present in faecal samples throughout the months from January to July (See Table 5.2). The lowest mean number of pollen grains was observed in January with only 6.6 pollen grains present per slide for Andy. This contrasts with the greatest average number of pollen grains per slide of 120 in May for Eddie. Overall the greatest number of pollen grains observed per slide was in May and June.

A wide and varied selection of pollen taxa were observed throughout the year with the most predominant pollen found in faecal samples being *Graminae* which was present in all fourteen faecal samples throughout the six months (See Table 5.4, Fig 5.3 & Fig 5.4). Unfortunately it is not possible to determine particular species of *Graminae* present in faecal samples as the damage these pollen grains receive in passing through the dog's digestive system eradicates the subtle differences between different species of pollen grains. The number of *Graminae* pollen grains counted was 36 times greater than pine pollen grains, which was the next most common pollen observed. This was followed by dandelion, ragwort and ash.

The largest number of taxa observed in the one faecal sample was in March for dog Weaver (Table 5.3) when 11 different taxa were found; these included *Graminae, Pinus, Acer* and *Plantago*. This contrasts with the faecal sample from Rachel in July where only *Graminae* were observed.

Peak levels of pollen were present in the faecal samples in May. Individual types of pollen which were found to peak at this time of year included *Graminae*, pine, dandelion, and legumes.

Figure 5.1 Distribution of mean serum allergen specific IgE optical density for indoor allergens throughout the year as determined by ELISA

Figure 5.2 Distribution of mean serum allergen specific IgE optical density for outdoor allergens throughout the year as determined by ELISA

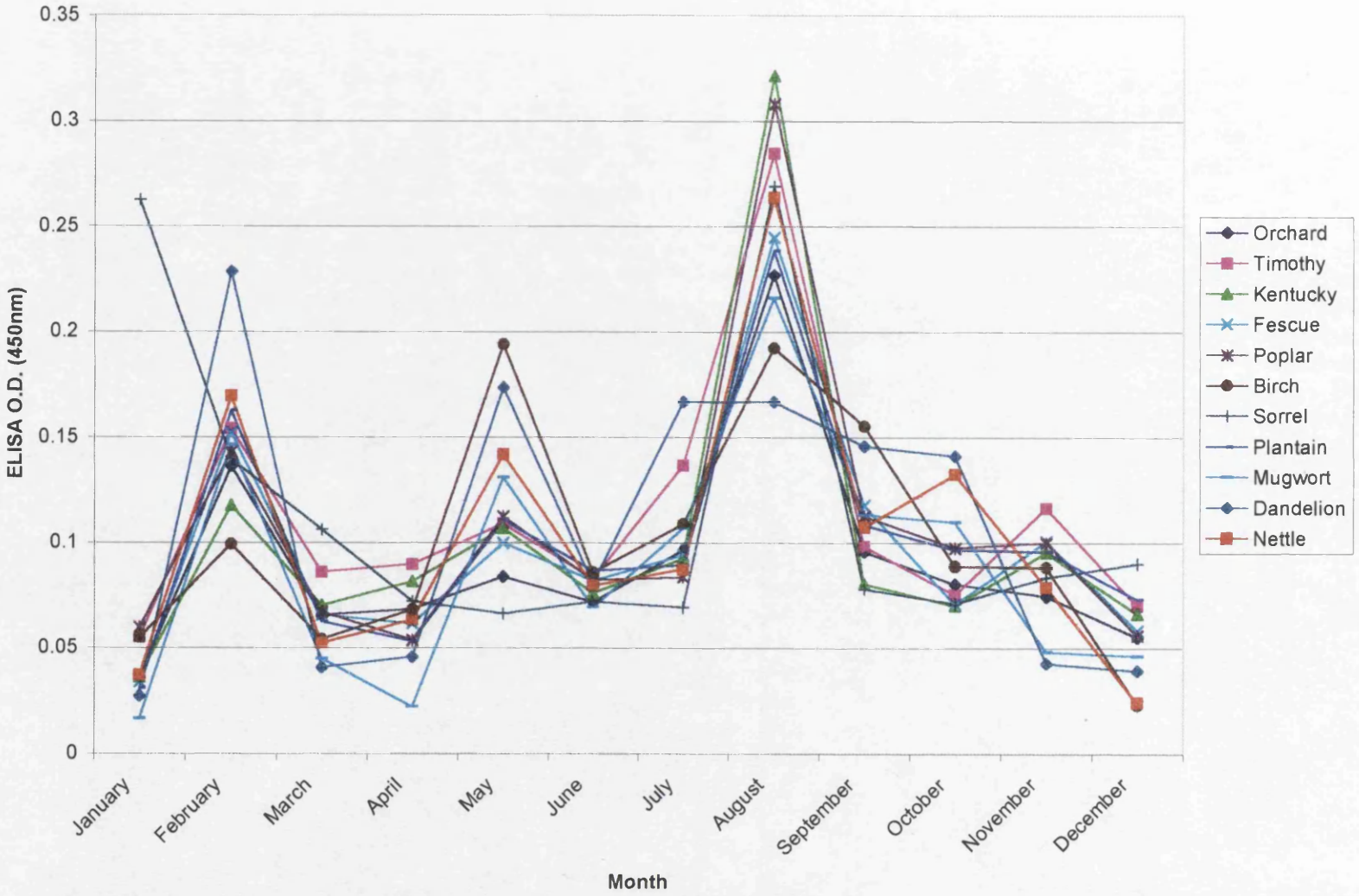


Table 5.1 Summary of One Way Analysis of Variance and Newman Keuls Multiple Range Test findings for seasonal variation of ELISA results

(See Appendix G)

Allergen	p value	Multiple Range Test results
Flea	0.510	No significant difference amongst seasonal groups
Mites	0.124	No significant difference amongst seasonal groups
Feathers	0.294	No significant difference amongst seasonal groups
Alternaria	**0.026**	**November greater than all other groups**
Aspergillus	0.625	No significant difference amongst seasonal groups
Rhizopus	0.410	No significant difference amongst seasonal groups
Kapok	0.356	No significant difference amongst seasonal groups
Dust	0.428	No significant difference amongst seasonal groups
Cat epithelium	0.122	**February greater than January**
Human epithelium	0.844	No significant difference amongst seasonal groups
Mucor	0.734	No significant difference amongst seasonal groups
Orchard grass	0.066	No significant difference amongst seasonal groups
Timothy grass	**0.024**	No significant difference amongst seasonal groups
Kentucky grass	**0.001**	**August greater than all other months**
Fescue	0.077	No significant difference amongst seasonal groups
Poplar	**0.037**	**August greater than September and February**
Birch	0.577	No significant difference amongst seasonal groups
Sorrel	**0.048**	No significant difference amongst seasonal groups
Plantain	0.056	No significant difference amongst seasonal groups
Mugwort	0.517	No significant difference amongst seasonal groups
Dandelion	0.698	No significant difference amongst seasonal groups
Nettle	0.178	No significant difference amongst seasonal groups

NOTE – Entries in bold are significant.

Figure 5.3 Faecal sample pollen analysis results showing pollen counts for *Graminae* at various times during spring/summer.

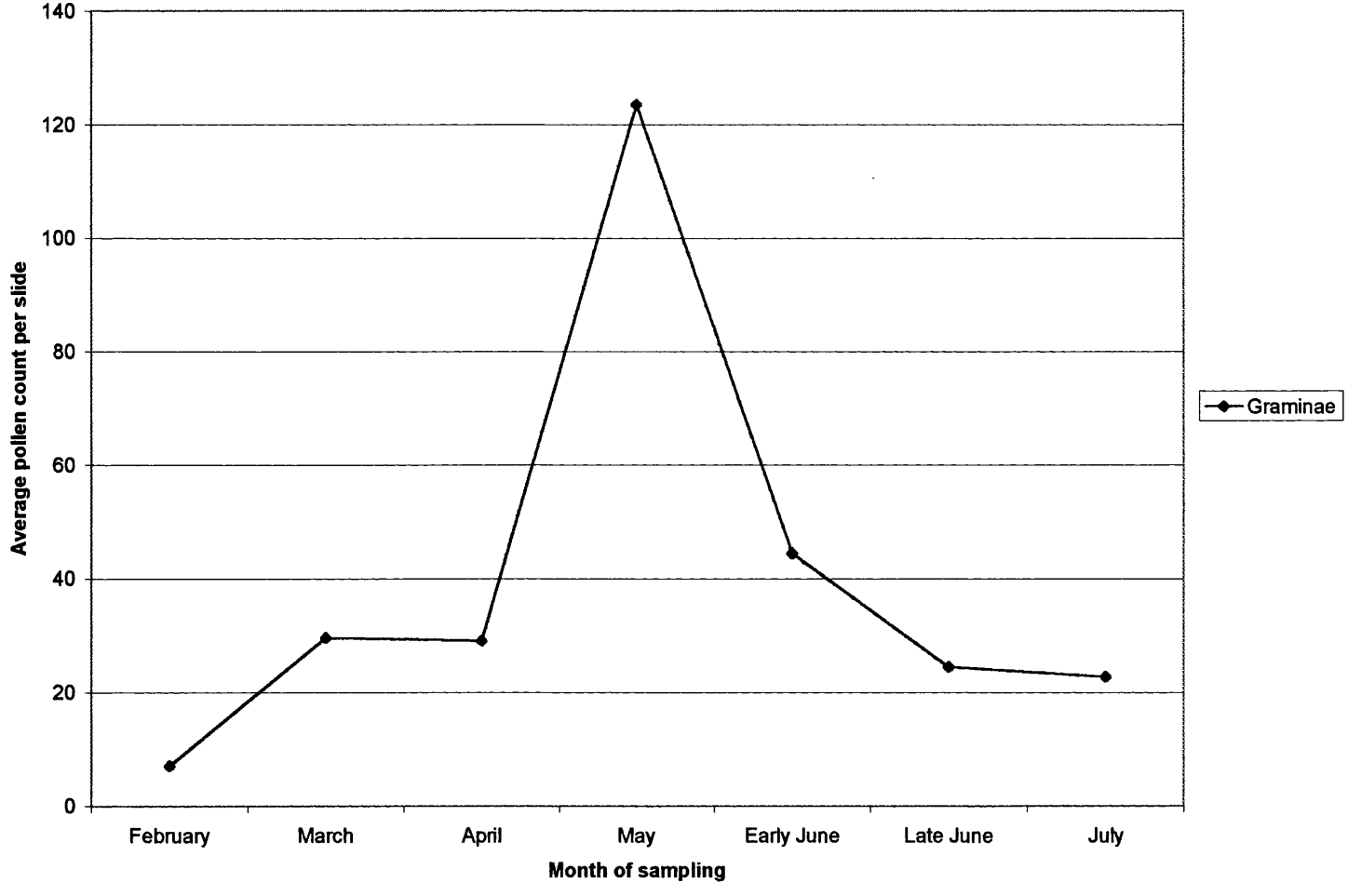


Figure 5.4 Faecal sample pollen analysis results (excluding *Graminae*) showing pollen counts for different plants at various times during spring/summer.

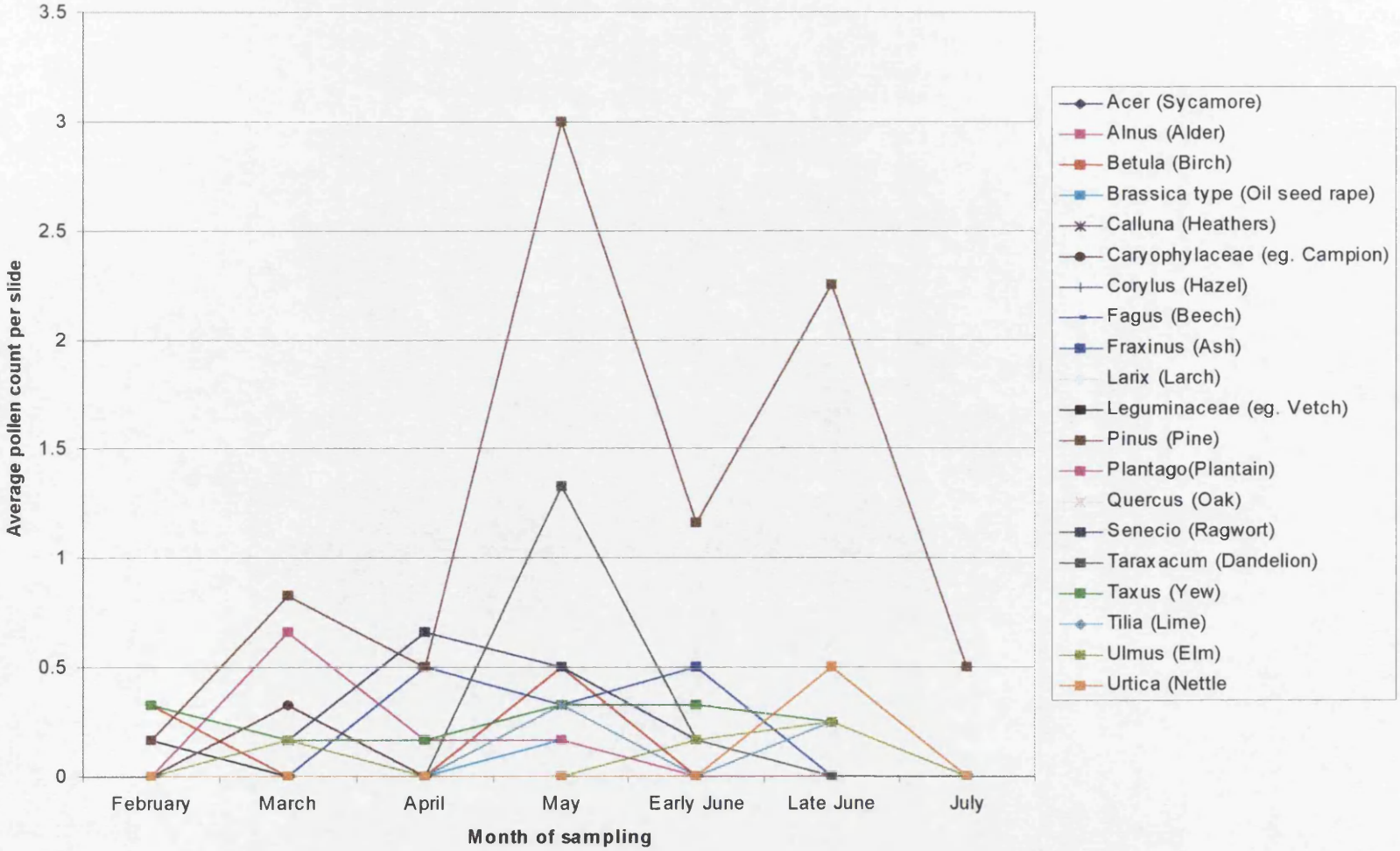


Table 5.2 Total number of taxa and means of pollen grains found in faecal samples.

No.	Name	Date sampled	Mean number of pollen grains observed per slide	Total number of taxa observed per faecal sample
1	Jenny	Feb 1998	9.6	4
2	Andy	Feb 1998	6.6	3
3	Yulie	Mar 1998	17.0	5
4	Weaver	Mar 1998	49.6	12
5	Jed	Apr 1998	12.0	3
6	Uffa	Apr 1998	50.0	5
7	Lana	May 1998	14.2	8
8	Eddie	May 1998	120.0	10
9	Fraser	June 1998	50.0	2
10	Mary	June 1998	44.6	8
11	Amos	June 1998	36.5	8
12	Mungo	June 1998	27.5	2
13	Omar	July 1998	33,5	2
14	Rachel	July 1998	15.5	1

Table 5.3 Different taxa found in dog Weaver's faecal sample in March.

Pollen	Average number of grains per slide
Graminae (Grasses)	43.66
Corylus (Hazel)	0.33
Ulmus (Elm)	0.33
Taxus (Yew)	0.33
Caryophylaceae (eg Campion)	0.33
Acer (Sycamore)	0.66
Pinus (Pine)	1.66
Plantago (Plantain)	1
Taraxacum (Dandelion)	0.33
Larix (Larch)	0.33
Senecio (Ragwort)	0.33
Alnus (Alder)	0.33

Table 5.4 Detailed examination of pollen distribution amongst faecal samples

Taxon	Total number of pollen grains counted	Number of faecal samples where Taxa present	Mean (standard deviation) number of pollen grains per faecal sample	Per-centage of total recorded for each taxon
Graminae (grass)	1586	14	113.3 (118.3)	91.04
Pinus (Pine)	44	10	4.4 (3.5)	2.52
Taraxacum (Dandelion)	10	4	2.5 (1.9)	0.57
Senecio (Ragwort)	9	4	2.3 (1.5)	0.51
Fraxinus (Ash)	9	4	2.3 (0.9)	0.51
Taxus (Yew)	8	5	1.6 (0.5)	0.45
Plantago (Plantain)	6	4	1.5 (1.0)	0.34
Betula (Birch)	5	2	2.5 (2.1)	0.28
Calluna/Erica (Heathers)	4	3	1.3 (0.6)	0.22
Ulmus (Elm)	3	3	1.0 (0.0)	0.17
Tilia (Lime)	3	3	1.0 (0.0)	0.17
Acer (Sycamore/Mapel)	2	1	-	0.11
Brassica type (Oil seed rape)	2	1	-	0.11
Alnus (Alder)	2	1	-	0.11
Caryophylaceae (eg Campion)	2	1	-	0.11
Urtica (Nettle)	2	1	-	0.11
Larix (Larch)	1	1	-	0.06
Leguminosae (eg. Vetch)	1	1	-	0.06
Corylus (Hazel)	1	1	-	0.06
Quercus (Oak)	1	1	-	0.06
Fagus (Beech)	1	1	-	0.06
Unidentified	20	5	4.0 (4.0)	1.16

- Sample size too small for meaningful examination of data.

5.4 Discussion

The seasonal variation of serum allergen specific IgE is an area which has received little attention. Recent work by McCall *et al.* (1998) has illustrated that there is indeed a seasonal variation in allergen specific antibody concentrations in suspected atopic dogs tested in the USA. This agrees with work by Halliwell & Kunkle (1978) who demonstrated there was a seasonal variation in RAST results for ragweed specific IgE in atopic dogs. In addition this author suggested that the time of year when dogs are tested should be taken into account in the interpretation of results. However, work by Miller *et al.* (1992) did not find any such seasonal variation in allergen specific IgE concentrations in atopic dogs.

Commercially available ELISA tests used for the diagnosis of canine atopic dermatitis utilise a cut off point where optical density or reflectivity results greater than a set value are deemed as indicative or suggestive of atopic dermatitis. In general these cut off points remain constant throughout the year and are assumed to incorporate any variations in serum IgE concentration which might occur in non-atopic dogs due to differences in exposure. The ELISA test used in this study uses a cut off point of 0.15, where any optical density results greater than this are deemed positive and suggestive of atopic dermatitis in that particular dog.

This study of non-atopic dogs revealed that there is indeed a seasonal variation for pollen allergens in allergen specific IgE concentrations assessed by ELISA. Although different numbers of dogs were examined each month, the lowest numbers were present between December and March, whereas the significant difference was found most often for August in comparison with the rest of the year.

In addition many of these non-atopic dogs demonstrated allergen specific IgE concentrations which were above the positive cut off point for this test. If these serological results were to be relied upon in the diagnosis of atopic dermatitis then these dogs would have been classed as atopic. Although it has been suggested by Willemse (1986) that serological results alone cannot be

relied upon in the diagnosis of atopic dermatitis and that other clinical parameters need to be included, it is known that many practitioners rely on such serological tests in the diagnosis of atopic dermatitis. The situation is further complicated by the fact that dogs with skin diseases such as pyoderma, flea allergic dermatitis or food allergy can present with clinical signs very similar to those observed in atopic dermatitis and if these dogs gave an ELISA result above 0.15 they could be falsely diagnosed as atopic. It is clear that in the interpretation of serological results, the time of year or particular pollen levels should be taken into account in order that non-atopic dogs are not falsely diagnosed as atopic. It may also be of benefit to vary the positive cut off point throughout the year in order to avoid positive ELISA results in non-atopic animals. One potential problem is that dogs in different areas of the country will be exposed to different allergen levels at different times of the year and from year to year. Results presented here therefore support the work by Willemse (1986), and suggest that serological tests alone cannot be used in the diagnosis of atopic dermatitis due to the variation in basal IgE levels following allergen exposure in non-atopic dogs.

Examination of the pollens with which dogs come into contact provided interesting findings. As expected, grasses were the predominant pollens and were present in all samples between February to July. Unfortunately it was not possible to identify particular species of grasses from faecal samples due to the damage that they receive in passing through the digestive system. This would have been helpful in the design of an intradermal skin test as the predominant grass allergens could have been included.

The second most common pollen was *Pinus*. This is not thought to be allergenic in human medicine but pine extracts are available for IDST (ARTU Biologicals, Netherlands) in the dog although at present pine is not incorporated into IDST panels for GDBA dogs. It is hoped that in future pine can be included in IDST panels.

For pollens to be regarded as allergens they have to meet a number of criteria including being present in large quantities and being water soluble

(Matthiesen *et al.*, 1991). Pine pollen is certainly present in large quantities although its water solubility remains to be observed. The high levels of pine pollen with which dogs are coming into contact are probably due to the number of pine trees situated above the kennel runs. Hence, the pollen does not need to be windborne as it merely has to fall onto the dogs. In addition to pine, ragwort and yew allergens should also be incorporated into the IDST as these were also relatively common.

In humans, pollens need to be relatively small to become windborne before they are regarded as possible allergens. However, dogs come into contact with pollens on the ground and so different pollens may be important. In general, pollen counts are carried out on top of buildings and the pollens present at that level may be different from those with which dogs are coming into contact. Both dandelion and plantain pollens were present in the faecal samples. Both of these pollens are present in the air at low concentrations and are not thought to be important in human allergies. However, due to their presence here in appreciable quantities it is important that their potential significance is investigated.

Comparing pollen levels throughout the year with allergen specific IgE levels from the ELISA test did reveal a similar pattern for grasses. However, it has to be taken into account that the serological results are the mean of samples from three subsequent years whereas the faecal pollen levels were taken in the one year. Therefore any comparisons do not take into account any annual variation in the pollen concentrations (Fig. 5.6). These demonstrate the difference in total pollen levels throughout two years of the study – to date no such pollen calendars have been made for the final year of the study from the same source. However, certain plants are known to pollinate at the same time each year with little variation from year to year.

Mean allergen specific IgE concentrations as determined by optical density for outdoor allergens in general showed a dramatic peak in August. Unfortunately it was not possible to obtain faecal pollen analysis for August which would have been most interesting. On initial examination this peak in grass specific

IgE levels appears an anomaly as grass pollen levels are known to decrease from July onwards. However, personal communication with Dr Caulton (Scottish Centre for Pollen Studies, Edinburgh) has revealed that in August during harvesting there is a temporary increase in pollen levels due to settled pollen becoming airborne again and this is demonstrated on the pollen count from Edinburgh in 1943 (See Fig 5.5, Hyde, 1960) shown below. This may well be relevant here as the kennels at Forfar are surrounded by arable land.

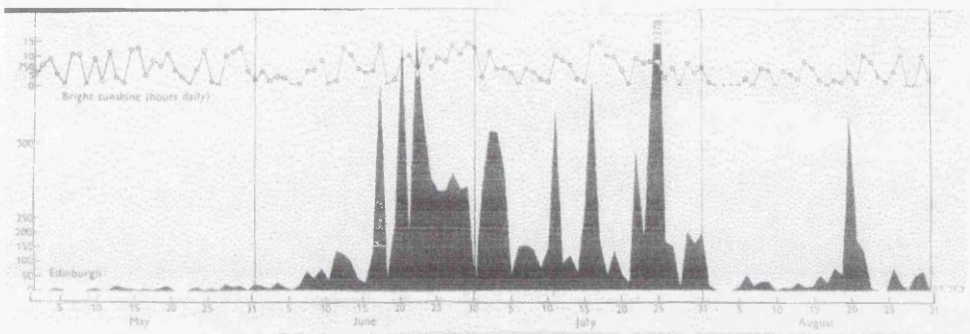


Fig. 5.5 Total pollen levels in Edinburgh, 1943 (From Hyde, 1960)

It is also possible that an increase in serum allergen specific IgE concentrations takes time to develop and therefore would not be expected to be simultaneous with the pollen levels. Primary immune responses are known to take fifteen-twenty days to reach a peak and secondary responses seven-ten days (Tizard, 1987a). Whether these responses are primary or secondary is not known but it is feasible that the peak serum IgE concentrations seen in August are due to the increased levels of pollen in July.

Serum allergen specific IgE levels are higher in August than those observed for May. This may be due to very high levels of allergen exposure or alternatively may be due to a second stimulation allergen response with even higher levels of serum IgE developing on subsequent exposure to an allergen.

5.5 Conclusion

In conclusion this study has provided evidence to support the existence of a seasonal variation in serum allergen specific IgE concentrations at different times of the year. Although it must be taken into consideration that different numbers of dogs were present in each month it appears likely that these levels correspond with the variation in allergen exposure. Care must therefore be taken in the interpretation of ELISA results in the diagnosis of atopic dermatitis as there is a real risk of non-atopic dogs exposed to high levels of allergens being falsely diagnosed as atopic.

In addition we have shown that canine pollen exposure can be assessed by faecal examination. The findings suggest that dogs are exposed to levels of particular pollens different to those thought to be important in human medicine and additional pollens may need to be incorporated into the standard canine IDST.

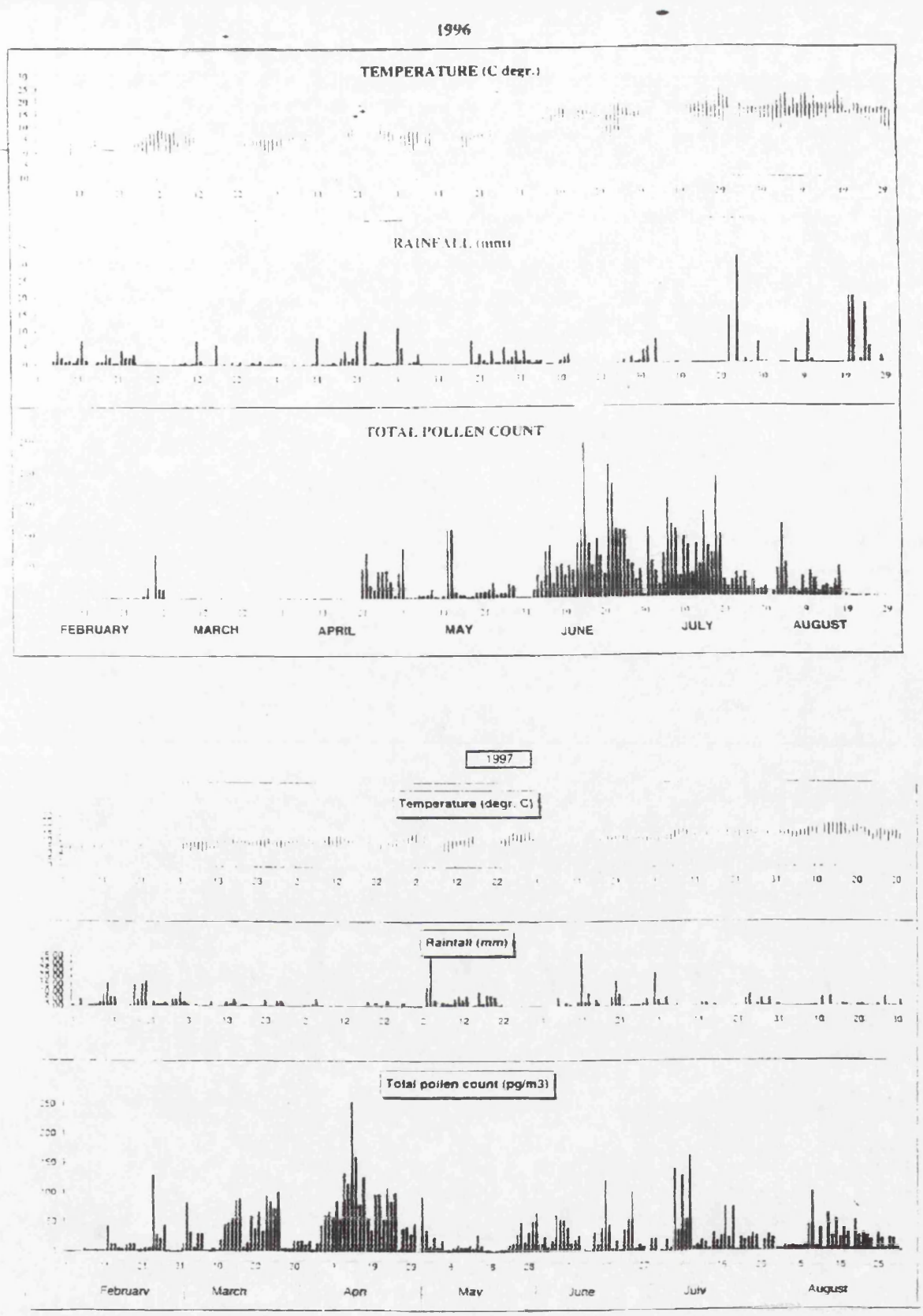


Fig 5.6 Total pollen counts in 1996 and 1997 in the Edinburgh area (data from E.Caulton).

Chapter 6. Examination of serological results in atopic and non-atopic dogs.

6.1 Introduction

The objectives of this study were to examine allergen specific IgE levels by ELISA and Immunodot in atopic and non-atopic working GDBA dogs, atopic pet GUVS dogs, and non-atopic greyhounds and beagles. The aim was to identify whether or not there were any significant differences in the mean allergen specific serum IgE optical/reflective densities between atopics and non-atopics which could be used as markers in the diagnosis of atopic dermatitis. In addition the author hoped to pinpoint any differences between working GDBA dogs, greyhounds, beagles and GUVS dogs in their response to different allergens in order to assess the influence which exposure may have on serological results.

6.2 Materials & Methods

Five groups of atopic and non-atopic dogs described in section 2.1 were included in this study. Serum samples underwent serological testing with ELISA and Immunodot tests as described in sections 2.5.1 and 2.5.2. A breakdown of the dogs included in each group is shown below.

Description	**ELISA**	**Immunodot**		
		Indoor	**Outdoor**	**Topscreen**
Non-atopic GDBA	74	42	50	11
Atopic GDBA	14	8	7	4
Non-atopic greyhounds	12	4	4	2
Non-atopic beagles	8	4	6	9
Atopic GUVS	32	24	21	15

Table 6.1 Number of serological tests carried out on individual dogs in each group.

Due to a shortage of serum it was not possible to perform every serological test on every sample. When comparing atopic and non-atopic groups of dogs, beagles and greyhounds were separated as these dogs were kept under different environmental conditions and received different parasite control measures. For other comparative purposes beagles and greyhounds were examined as one group. Serological results of both ELISA and Immunodot tests for these dogs were examined for statistical significance with a General Linear Model method in Minitab version 11.21 (1996). The general linear model allows us to compare the four groups of dogs described when there are different numbers of dogs in each group and is a similar method to the two way ANOVA

6.3 Results

Results are shown in Tables 6.2-6.37 and Appendix F. Statistical analysis of this data is shown in Appendices I and J.

Examination of the mean allergen specific serum IgE optical densities, of each group of atopic and non-atopic dogs determined by ELISA revealed many serological results, which were below that considered significant by the manufacturers (optical density of 0.15). A similar finding was made with the Immunodot test with most results below the level of a positive reflective density of 0.147.

Mean ELISA results greater than 0.15, and thus considered positive, were found in non-atopic greyhounds for fescue grass (0.162) (Table 6.16) and poplar allergens (0.154) (Table 6.17). Atopic GDBA dogs only demonstrated a positive mean ELISA result for dandelion allergen (0.153) (Table 6.22).

A similar examination of Immunodot results in non-atopic greyhounds and non-atopic beagles revealed a mean reflective density of greater than 0.147 (equal to a Grade 2, positive result) for house dust mite allergens (0.168) (Table 6.24) and the group of Indoor allergens (0.155) (Table 6.34) by both groups.

Non-atopic beagles demonstrated a particularly high mean reflective density of 0.265 against the group of moulds (Table 6.37).

The lowest mean optical densities assessed by ELISA were shown by all four groups against cat (mean < 0.051) and human epithelium (mean < 0.057) (Tables 6.10 & 6.11 respectively). Similarly the lowest reflective densities assessed by Immunodot were shown by all four groups against Flea allergens with the beagles having the lowest results of all (mean of 0.000) against flea allergens (Table 6.26).

Beagles as a group demonstrated low serum IgE optical/reflective densities for many allergens including human epithelium assessed on both ELISA and Immunodot (Tables 6.11 & 6.24 respectively) and dust mites, storemites and flea, assessed by Immunodot (Tables 6.24, 6.25 & 6.26). This contrasts with allergen specific IgE optical densities of the beagles against dust mites, assessed by ELISA, where beagles had the highest mean (Table 6.3).

Comparison of atopic and non-atopic dogs revealed that ELISA results were significantly higher in atopics (GDBA and GUVS) than non-atopics (GDBA and greyhounds), against *Alternaria* ($p<0.036$) and *Aspergillus* ($p<0.05$) (Tables 6.5 & 6.6). A difference was seen in results when beagles rather than greyhounds were included in the non-atopic group. Here atopic dogs had significantly higher optical density results against *Aspergillus* ($p<0.013$) than non-atopics but no such significant difference for *Alternaria* (Tables 6.6 & 6.5).

A similar examination of Immunodot results did not reveal any significant differences between atopic and non-atopic dogs (GDBA and greyhounds) against any allergens (Tables 6.24-6.37). Replacement of non-atopic greyhounds with non-atopic beagles, revealed atopic dogs to have significantly higher allergen specific IgE reflective densities against dustmites ($p<0.029$) (Table 6.24), the Indoor group of allergens ($p<0.034$) (Table 6.34) and tree allergens ($p<0.030$) (Table 6.30). Non-atopic dogs were found to have significantly higher serum IgE reflective densities against foods group 2 ($p<0.027$) (Table 6.36) and moulds ($p<0.000$) (Table 6.37).

Where serological results of greyhounds and beagles were grouped together for a particular allergen atopic dogs (GDBA & GUVS) had significantly higher optical/reflective density results than non-atopics (GDBA, greyhounds & beagles) against *Alternaria* ($p<0.024$) and *Aspergillus* ($p<0.021$) as assessed by ELISA (Tables 6.5 & 6.6) and the dustmites ($p<0.028$) and the Indoor group of allergens ($p<0.035$) assessed by Immunodot (Tables 6.24 & 6.34).

Non-atopic dogs were found to have a significantly higher reflective density than atopics against foods group 2 allergens ($p<0.045$) and moulds ($p<0.000$) assessed by Immunodot (Tables 6.36 & 6.37).

Comparison of GDBA (atopic and non-atopic) and non-GDBA (GUVS and greyhounds) revealed that GDBA dogs had significantly higher optical/reflective densities against *Mucor* ($p<0.043$) assessed by ELISA (Table 6.12) and cat epithelium ($p<0.004$) assessed by Immunodot (Table 6.28). Non-GDBA dogs (GUVS and greyhounds) were found to have significantly higher serum IgE optical/reflective densities than GDBA dogs against Fescue ($p<0.030$) assessed by ELISA (Table 6.16) and Grasses ($p<0.001$) and Trees ($p<0.001$) assessed by Immunodot (Tables 6.29 & 6.30).

Replacement of greyhounds with beagles in the non-atopic group revealed atopic and non-atopic GDBA dogs to have significantly higher optical/reflective densities than atopic and non-atopic non-GDBA dogs against *Mucor* ($p<0.010$) assessed by ELISA (Table 6.12), and cat epithelium ($p<0.000$) and olive/wall pellitory ($p<0.026$) assessed by Immunodot (Tables 6.28 & 6.32). Non-GDBA dogs were found to have significantly higher reflective densities of serum IgE than GDBA dogs against grasses ($p<0.000$) and moulds ($p<0.000$) as assessed by Immunodot (Tables 6.29 & 6.37).

Grouping beagles and greyhounds together in the non-GDBA group along with GUVS atopic dogs revealed GDBA dogs to have significantly higher optical/reflective densities against *Mucor* ($p<0.008$) assessed by ELISA (Table 6.12) and cat epithelium ($p<0.000$) assessed by Immunodot (Table 6.28).

Non-GDBA dogs (beagles, greyhounds and GUVS) were found to have significantly higher reflective densities than GDBA dogs against grass

($p<0.000$) and moulds ($p<0.000$) assessed by Immunodot (Tables 6.29 & 6.37).

Interaction, where there is a relationship between the atopic status of a dog and whether the dog is a GDBA dog or not was found for flea allergens ($p<0.050$), human epithelium ($p<0.034$) tree allergens ($p<0.043$) and moulds ($p<0.001$) all assessed by Immunodot (Tables 6.26, 6.27, 6.30 & 6.37) when beagles rather than greyhounds were included in the non-atopic group. In addition grouping greyhounds and beagles revealed an interaction between the groups for moulds ($p<0.019$) also assessed by Immunodot (Table 6.37).

The way in which dogs were grouped influenced the statistical results. Different results were found between the use of beagles or greyhounds alone or grouped together for *Alternaria*, Fescue grass, dust mite, flea, human epithelium, tree, olive/wall pellitory, group of Indoor allergens, Foods group 1, and moulds.

As a significant difference was found between atopic and non-atopic dogs for *Alternaria* and *Aspergillus* with the ELISA test these findings were applied to the GDBA population. The mean ELISA result for non-atopic GDBA dogs for *Alternaria* was 0.088. Assuming that an ELISA result greater than 0.088 would suggest that a dog was atopic gave a sensitivity of 64.3% and a specificity of 60.8%. The mean ELISA result for *Aspergillus* in non-atopic GDBA dogs was 0.092. Assuming that an ELISA result greater than 0.092 would suggest that a dog was atopic gave a sensitivity of 50% and a specificity of 58.1%.

Examination of the correlation between serum total IgE concentrations assessed by Immunodot and the responses for *Alternaria* and *Aspergillus* assessed by ELISA (see Appendix K) did not reveal any correlation (Pearson's correlation coefficient of –0.031 for *Alternaria* and –0.284 for *Aspergillus* respectively).

Table 6.2 Mean flea specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for Flea (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.058	0.077
NON-ATOPIC	0.073	0.069

No significant difference between atopic and non-atopic groups (F=0.03, p=0.852)

No significant difference between GDBA and non-GDBA groups (F=0.15, p=0.704)

No significant interaction between atopic and GDBA groups (F=0.38, p=0.538)

Mean ELISA Results for Flea (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.058	0.077
NON-ATOPIC	0.073	0.060

No significant difference between atopic and non-atopic groups (F=0.00, p=0.957)

No significant difference between GDBA and non-GDBA groups (F=0.02, p=0.901)

No significant interaction between atopic and GDBA groups (F=0.63, p=0.429)

Mean ELISA Results for Flea (non-atopic / non-GDBA = Greyhound & Beagle)

	GDBA	NON-GDBA
ATOPIC	0.058	0.077
NON-ATOPIC	0.073	0.065

No significant difference between atopic and non-atopic groups (F=0.01, p=0.921)

No significant difference between GDBA and non-GDBA groups (F=0.1, p=0.751)

No significant interaction between atopic and non-atopic groups (F=0.65, p=0.422)

Table 6.3 Mean house dust mite specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for house dust mites (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.123	0.128
NON-ATOPIC	0.117	0.139

No significant difference between atopic and non-atopic groups (F=0.03, p=0.859)

No significant difference between GDBA and non-GDBA groups (F=1.00, p=0.320)

No significant interaction between atopic and GDBA groups (F=0.41, p=0.521)

Mean ELISA Results for house dust mites (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.123	0.128
NON-ATOPIC	0.117	0.141

No significant difference between atopic and non-atopic groups (F=0.05, p=0.832)

No significant difference between GDBA and non-GDBA groups (F=0.91, p=0.343)

No significant interaction between atopic and GDBA groups (F=0.40, p=0.528)

Mean ELISA Results for house dust mites (non-atopic / non-GDBA = Greyhound & Beagle)

	GDBA	NON-GDBA
ATOPIC	0.123	0.128
NON-ATOPIC	0.117	0.140

No significant difference observed between atopic and non-atopic groups (F=0.05, p=0.825)

No significant difference observed between GDBA and non-GDBA groups (F=0.126, p=0.263)

No significant interaction observed between atopic and GDBA groups (F=0.54, p=0.465)

Table 6.4 Mean feather specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for feathers (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.060	0.088
NON-ATOPIC	0.071	0.077

No significant difference observed between atopic and non-atopic groups (F=0.00, p=0.981)

No significant difference observed between GDBA and non-GDBA groups (F=1.92, p=0.168)

No significant interaction observed between atopic and GDBA groups (F=0.82, p=0.366)

Mean ELISA Results for feathers (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.060	0.088
NON-ATOPIC	0.071	0.085

No significant difference observed between atopic and non-atopic groups (F=0.11, p=0.740)

No significant difference observed between GDBA and non-GDBA groups (F=2.55, p=0.113)

No significant interaction observed between atopic and GDBA groups (F=0.28, p=0.597)

Mean ELISA Results for feathers (non-atopic /non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.060	0.088
NON-ATOPIC	0.071	0.080

No significant difference observed between atopic and non-atopic groups (F=0.03, p=0.860)

No significant difference observed between GDBA and non-GDBA groups (F=0.292, p=0.090)

No significant interaction observed between atopic and GDBA groups (F=0.76, p=0.386)

Table 6.5 Mean Alternaria specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for *Alternaria* (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.109	0.107
NON-ATOPIC	0.088	0.071

Significant difference observed between atopic and non-atopic groups (F=4.50, p=0.036).

No significant difference observed between GDBA and non-GDBA groups (F=0.49, p=0.484).

No significant interaction observed between atopic and GDBA groups (F=0.29, p=0.591).

Mean ELISA Results for *Alternaria* (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.109	0.107
NON-ATOPIC	0.088	0.076

No significant difference observed between atopic and non-atopic groups (F=3.04, p=0.084).

No significant difference observed between GDBA and non-GDBA groups (F=0.22, p=0.640).

No significant interaction observed between atopic and GDBA groups (F=0.10, p=0.748).

Mean ELISA Results for *Alternaria* (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.109	0.107
NON-ATOPIC	0.088	0.062

Significant difference observed between atopic and non-atopic groups (F=5.22, p=0.024)

No significant difference observed between GDBA and non-GDBA groups (F=0.49, p=0.483)

No significant interaction observed between atopic and GDBA groups (F=0.27, p=0.603)

Table 6.6 Mean Aspergillus specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for *Aspergillus* (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.106	0.115
NON-ATOPIC	0.092	0.092

Significant difference observed between atopic and non-atopic groups (F=3.92, p=0.050).

No significant difference observed between GDBA and non-GDBA groups (F=0.00, p=0.952).

No significant interaction observed between atopic and GDBA groups (F=0.01, p=0.941).

Mean ELISA Results for *Aspergillus* (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.106	0.115
NON-ATOPIC	0.092	0.066

Significant difference observed between atopic and non-atopic groups (F=6.35, p=0.013).

No significant difference between GDBA and non-GDBA groups (F=0.47, p=0.494).

No significant interaction between atopic and GDBA groups (F=1.86, p=0.175).

Mean ELISA Results for *Aspergillus* (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.106	0.115
NON-ATOPIC	0.092	0.081

Significant difference observed between atopic and non-atopic groups (F=5.46, p=0.021)

No significant difference observed between GDBA and non-GDBA groups (F=0.01, p=0.927)

No significant interaction observed between atopic and GDBA groups (F=0.85, p=0.358)

Table 6.7 Mean Rhizopus specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for *Rhizopus* (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.086	0.063
NON-ATOPIC	0.100	0.076

No significant difference observed between atopic and non-atopic groups (F=0.380, p=0.541).

No significant difference observed between GDBA and non-GDBA groups (F=1.18, p=0.280).

No significant interaction observed between atopic and GDBA groups (F=0.00, p=0.973).

Mean ELISA Results for *Rhizopus* (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.086	0.063
NON-ATOPIC	0.100	0.037

No significant difference observed between atopic and non-atopic groups (F=0.07, p=0.794).

No significant difference observed between GDBA and non-GDBA groups (F=3.20, p=0.076).

No significant interaction observed between atopic and GDBA groups (F=0.71, p=0.401).

Mean ELISA Results for *Rhizopus* (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.086	0.063
NON-ATOPIC	0.100	0.060

No significant difference observed between atopic and non-atopic groups (F=0.08, p=0.778)

No significant difference observed between GDBA and non-GDBA groups (F=2.65, p=0.106)

No significant interaction observed between atopic and GDBA groups (F=0.p20, p=0.657)

Table 6.8 Mean kapok specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for kapok (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.084	0.065
NON-ATOPIC	0.077	0.049

No significant difference observed between atopic and non-atopic groups ($F=0.40$, $p=0.529$).

No significant difference observed between GDBA and non-GDBA groups ($F=1.75$, $p=0.188$).

No significant interaction observed between atopic and GDBA groups ($F=0.06$, $p=0.811$).

Mean ELISA Results for kapok (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.084	0.065
NON-ATOPIC	0.077	0.044

No significant difference observed between atopic and non-atopic groups ($F=0.48$, $p=0.492$).

No significant difference observed between GDBA and non-GDBA groups ($F=1.72$, $p=0.193$).

No significant interaction observed between atopic and GDBA groups ($F=0.11$, $p=0.736$).

Mean ELISA Results for kapok (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.084	0.064
NON-ATOPIC	0.077	0.047

No significant difference observed between atopic and non-atopic groups ($F=0.60$, $p=0.441$)

No significant difference observed between GDBA and non-GDBA groups ($F=2.41$, $p=0.123$)

No significant interaction observed between atopic and GDBA groups ($F=0.11$, $p=0.741$)

Table 6.9 Mean dust specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for dust (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.044	0.042
NON-ATOPIC	0.050	0.046

No significant difference between atopic and non-atopic groups (F=0.47, p=0.496).

No significant difference between GDBA and non-GDBA groups (F=0.17, p=0.683).

No significant interaction between atopic and GDBA groups (F=0.01, p=0.920).

Mean ELISA Results for dust (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.044	0.042
NON-ATOPIC	0.050	0.057

No significant difference observed between atopic and non-atopic groups (F=1.58, p=0.212).

No significant difference between GDBA and non-GDBA groups (F=0.09, p=0.768).

No significant interaction between atopic and GDBA groups (F=0.32, p=0.572).

Mean ELISA Results for dust (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.044	0.042
NON-ATOPIC	0.050	0.051

No significant difference observed between atopic and non-atopic groups (F=1.12, p=0.292)

No significant difference observed between GDBA and non-GDBA groups (F= 0.01, p=0.905)

No significant interaction observed between atopic and GDBA groups (F=0.05, p=0.832)

Table 6.10 Mean cat epithelium specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for cat epithelium (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.034	0.036
NON-ATOPIC	0.038	0.051

No significant difference between atopic and non-atopic groups (F=2.57, p=0.111).

No significant difference between GDBA and non-GDBA groups (F=1.67, p=0.198).

No significant interaction between atopic and GDBA groups (F=0.83, p=0.363).

Mean ELISA Results for cat epithelium (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.034	0.036
NON-ATOPIC	0.038	0.037

No significant difference observed between atopic and non-atopic groups (F=0.15, p=0.700).

No significant difference between GDBA and non-GDBA groups (F=0.01, p=0.923).

No significant interaction between atopic and GDBA groups (F=0.07, p=0.798).

Mean ELISA Results for cat epithelium (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.034	0.036
NON-ATOPIC	0.038	0.046

No significant difference observed between atopic and non-atopic groups (F=1.53, p=0.218)

No significant difference observed between GDBA and non-GDBA groups (F=-0.81, p=0.371)

No significant interaction observed between atopic and GDBA groups (F=0.23, p=0.632)

Table 6.11 Mean human epithelium specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for human epithelium (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.033	0.034
NON-ATOPIC	0.047	0.057

No significant difference between atopic and non-atopic groups (F=2.96, p=0.088).

No significant difference between GDBA and non-GDBA groups (F=0.26, p=0.612).

No significant interaction between atopic and GDBA groups (F=0.14, p=0.706).

Mean ELISA Results for human epithelium (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.033	0.034
NON-ATOPIC	0.047	0.025

No significant difference observed between atopic and non-atopic groups (F=0.04, p=0.835).

No significant difference between GDBA and non-GDBA groups (F=0.81, p=0.370).

No significant interaction between atopic and GDBA groups (F=1.04, p=0.310).

Mean ELISA Results for human epithelium (non-atopic non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.033	0.034
NON-ATOPIC	0.047	0.044

No significant difference observed between atopic and non-atopic groups (F=1.58, p=0.211)

No significant difference observed between GDAB and non-GDBA groups (F=0.01, p=0.922)

No significant interaction observed between atopic and GDBA groups (F=0.06, p=0.808).

Table 6.12 Mean Mucor specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for *Mucor* (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.141	0.076
NON-ATOPIC	0.109	0.083

No significant difference between atopic and non-atopic groups (F=0.33, p=0.564).

Significant difference between GDBA and non-GDBA groups (F=4.19, p=0.043).

No significant interaction between atopic and GDBA groups (F=0.75, p=0.387).

Mean ELISA Results for *Mucor* (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.141	0.076
NON-ATOPIC	0.108	0.044

No significant difference observed between atopic and non-atopic groups (F=1.71, p=0.193).

Significant difference between GDBA and non-GDBA groups (F=6.92, p=0.010).

No significant interaction between atopic and GDBA groups (F=0.00, p=0.996).

Mean ELISA Results for *Mucor* (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.141	0.076
NON-ATOPIC	0.109	0.067

No significant difference observed between atopic and non-atopic groups (F=1.09, p=0.298)

Significant difference observed between GDBA and non-GDBA groups (F=7.27, p=0.008)

No significant interaction observed between atopic and GDBA groups (F=0.34, p=0.560)

Table 6.13 Mean Orchard grass specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for Orchard grass (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.089	0.118
NON-ATOPIC	0.093	0.109

No significant difference between atopic and non-atopic groups (F=0.01, p=0.911).

No significant difference between GDBA and non-GDBA groups (F=1.35, p=0.247).

No significant interaction between atopic and GDBA groups (F=0.13, p=0.722).

Mean ELISA Results for Orchard grass (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.089	0.118
NON-ATOPIC	0.093	0.061

No significant difference observed between atopic and non-atopic groups (F=1.50, p=0.223).

No significant difference between GDBA and non-GDBA groups (F=0.00, p=0.944).

No significant interaction between atopic and GDBA groups (F=2.09, p=0.151).

Mean ELISA Results for Orchard grass (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.089	0.118
NON-ATOPIC	0.93	0.090

No significant difference observed between atopic and non-atopic groups (F=0.47, p=0.496)

No significant difference observed between GDBA and non-GDBA groups (F=0.56, p=0.456)

No significant interaction observed between atopic and GDBA groups (F=0.92, p=0.340)

Table 6.14 Mean Timothy grass specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for Timothy grass (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.113	0.144
NON-ATOPIC	0.116	0.148

No significant difference between atopic and non-atopic groups (F=0.02, p=0.880).

No significant difference between GDBA and non-GDBA groups (F=2.02, p=0.158).

No significant interaction between atopic and GDBA groups (F=0.00, p=0.983).

Mean ELISA Results for Timothy grass (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.113	0.144
NON-ATOPIC	0.116	0.092

No significant difference observed between atopic and non-atopic groups (F=1.04, p=0.309).

No significant difference between GDBA and non-GDBA groups (F=0.02, p=0.895).

No significant interaction between atopic and GDBA groups (F=1.30, p=0.257).

Mean ELISA Results for Timothy grass (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.113	0.144
NON-ATOPIC	0.116	0.125

No significant difference observed between atopic and non-atopic groups (F=0.16, p=0.690)

No significant difference observed between GDBA and non-GDBA groups (F=1.03, p=0.313)

No significant interaction observed between atopic and GDBA groups (F=0.29, p=0.588)

Table 6.15 Mean Kentucky grass specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for Kentucky grass (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.103	0.132
NON-ATOPIC	0.103	0.133

No significant difference between atopic and non-atopic groups (F=0.00, p=0.983).

No significant difference between GDBA and non-GDBA groups (F=1.63, p=0.204).

No significant interaction between atopic and GDBA groups (F=0.00, p=0.995).

Mean ELISA Results for Kentucky grass (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.102	0.132
NON-ATOPIC	0.103	0.098

No significant difference observed between atopic and non-atopic groups (F=0.45, p=0.503).

No significant difference between GDBA and non-GDBA groups (F=0.23, p=0.635).

No significant interaction between atopic and GDBA groups (F=0.49, p=0.487).

Mean ELISA Results for Kentucky grass (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.103	0.132
NON-ATOPIC	0.103	0.119

No significant difference observed between atopic and non-atopic groups (F=0.10, p=0.753)

No significant difference observed between GDBA and non-GDBA groups (F=1.20, p=0.276)

No significant interaction observed between atopic and GDBA groups (F=0.12, p=0.730)

Table 6.16 Mean Fescue grass specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for Fescue grass (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.093	0.126
NON-ATOPIC	0.102	0.162

No significant difference between atopic and non-atopic groups (F=1.15, p=0.286).

Significant difference between GDBA and non-GDBA groups (F=4.79, p=0.030).

No significant interaction between atopic and GDBA groups (F=0.44, p=0.510).

Mean ELISA Results for Fescue grass (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.093	0.126
NON-ATOPIC	0.102	0.084

No significant difference observed between atopic and non-atopic groups (F=0.52, p=0.474).

No significant difference between GDBA and non-GDBA groups (F=0.11, p=0.744).

No significant interaction between atopic and GDBA groups (F=1.22, p=0.272).

Mean ELSA Results for Fescue grass (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.093	0.126
NON-ATOPIC	0.102	0.095

No significant difference observed between atopic and non-atopic groups (F=0.14, p=0.710)

No significant difference observed between GDBA and non-GDBA groups (F=2.60, p=0.109)

No significant interaction observed between atopic and GDBA groups (F=0.01, p=0.934)

Table 6.17 Mean poplar specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for poplar (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.113	0.101
NON-ATOPIC	0.109	0.154

No significant difference between atopic and non-atopic groups (F=0.97, p=0.328).

No significant difference between GDBA and non-GDBA groups (F=0.45, p=0.505).

No significant interaction between atopic and GDBA groups (F=1.34, p=0.249).

Mean ELISA Results for poplar (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.113	0.101
NON-ATOPIC	0.109	0.080

No significant difference observed between atopic and non-atopic groups (F=0.22, p=0.642).

No significant difference between GDBA and non-GDBA groups (F=0.58, p=0.449).

No significant interaction between atopic and GDBA groups (F=0.09, p=0.761).

Mean ELISA Results for poplar (non-atopic /non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.113	0.101
NON-ATOPIC	0.109	0.124

No significant difference observed between atopic and non-atopic groups (F=0.19, p=0.664)

No significant difference observed between GDBA and non-GDBA groups (F=0.01, p=0.993)

No significant interaction observed between atopic and GDBA groups (F=0.40, p=0.530)

Table 6.18 Mean birch specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for birch (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.140	0.104
NON-ATOPIC	0.112	0.095

No significant difference between atopic and non-atopic groups (F=0.42, p=0.517).

No significant difference between GDBA and non-GDBA groups (F=0.85, p=0.359).

No significant interaction between atopic and GDBA groups (F=0.12, p=0.730).

Mean ELISA Results for birch (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.140	0.104
NON-ATOPIC	0.112	0.057

No significant difference observed between atopic and non-atopic groups (F=1.42, p=0.235).

No significant difference between GDBA and non-GDBA groups (F=2.07, p=0.153).

No significant interaction between atopic and GDBA groups (F=0.09, p=0.770).

Mean ELISA Results for birch (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.140	0.104
NON-ATOPIC	0.112	0.080

No significant difference observed between atopic and non-atopic groups (F=1.07, p=0.303)

No significant difference observed between GDBA and non-GDBA groups (F=1.79, p=0.183)

No significant interaction observed between atopic and GDBA groups (F=0.01, p=0.930)

Table 6.19 Mean sorrel specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for sorrel (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.070	0.092
NON-ATOPIC	0.101	0.104

No significant difference between atopic and non-atopic groups (F=0.85, p=0.359).

No significant difference between GDBA and non-GDBA groups (F=0.27, p=0.605).

No significant interaction between atopic and GDBA groups (F=0.16, p=0.689).

Mean ELISA Results for sorrel (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.071	0.092
NON-ATOPIC	0.101	0.079

No significant difference observed between atopic and non-atopic groups (F=0.11, p=0.740).

No significant difference between GDBA and non-GDBA groups (F=0.00, p=0.976).

No significant interaction between atopic and GDBA groups (F=0.74, p=0.391).

Mean ELISA Results for sorrel (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.071	0.092
NON-ATOPIC	0.101	0.094

No significant difference observed between atopic and non-atopic groups (F=0.61, p=0.434)

No significant difference observed between GDBA and non-GDBA groups (F=0.11, p=0.739)

No significant interaction observed between atopic and GDBA groups (F=0.49, p=0.487)

Table 6.20 Mean plantain specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for plantain (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.116	0.108
NON-ATOPIC	0.103	0.116

No significant difference between atopic and non-atopic groups (F=0.01, p=0.918).

No significant difference between GDBA and non-GDBA groups (F=0.01, p=0.919).

No significant interaction between atopic and GDBA groups (F=0.25, p=0.618).

Mean ELISA Results for plantain (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.116	0.108
NON-ATOPIC	0.103	0.082

No significant difference observed between atopic and non-atopic groups (F=0.67, p=0.413).

No significant difference between GDBA and non-GDBA groups (F=0.40, p=0.528).

No significant interaction between atopic and GDBA groups (F=0.07, p=0.791).

Mean ELISA Results for plantain (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.116	0.108
NON-ATOPIC	0.103	0.103

No significant difference observed between atopic and non-atopic groups (F=0.22, p=0.639)

No significant difference observed between GDBA and non-GDBA groups (F=0.06, p=0.811)

No significant interaction observed between atopic and GDBA groups (F=0.03, p=0.836)

Table 6.21 Mean mugwort specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for mugwort (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.119	0.093
NON-ATOPIC	0.095	0.106

No significant difference between atopic and non-atopic groups (F=0.03, p=0.861).

No significant difference between GDBA and non-GDBA groups (F=0.07, p=0.796).

No significant interaction between atopic and GDBA groups (F=0.40, p=0.528).

Mean ELISA Results for mugwort (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.119	0.093
NON-ATOPIC	0.095	0.044

No significant difference observed between atopic and non-atopic groups (F=1.28, p=0.261).

No significant difference between GDBA and non-GDBA groups (F=1.45, p=0.230).

No significant interaction between atopic and GDBA groups (F=0.15, p=0.698).

Mean ELISA Results for mugwort (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.119	0.093
NON-ATOPIC	0.095	0.104

No significant difference observed between atopic and non-atopic groups (F=0.45, p=0.502)

No significant difference observed between GDBA and non-GDBA groups (F=0.59, p=0.445)

No significant interaction observed between atopic and GDBA groups (F=0.06, p=0.814)

Table 6.22 Mean dandelion specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for dandelion (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.153	0.097
NON-ATOPIC	0.118	0.072

No significant difference between atopic and non-atopic groups (F=0.71, p=0.401).

No significant difference between GDBA and non-GDBA groups (F=1.97, p=0.163).

No significant interaction between atopic and GDBA groups (F=0.02, p=0.901).

Mean ELISA Results for dandelion (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.153	0.097
NON-ATOPIC	0.118	0.042

No significant difference observed between atopic and non-atopic groups (F=1.27, p=0.262).

No significant difference between GDBA and non-GDBA groups (F=2.66, p=0.105).

No significant interaction between atopic and GDBA groups (F=0.07, p=0.799).

Mean ELISA Results for dandelion (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.153	0.097
NON-ATOPIC	0.117	0.060

No significant difference observed between atopic and non-atopic groups (F=0.129, p=0.258)

No significant difference observed between GDBA and non-GDBA groups (F=3.13, p=0.079)

No significant interaction observed between atopic and GDBA groups (F=0.00, p=0.965)

Table 6.23 Mean nettle specific IgE optical densities, as determined by ELISA, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean ELISA Results for nettle (non-atopic / non-GDBA = Greyhound)

	GDBA	NON-GDBA
ATOPIC	0.121	0.088
NON-ATOPIC	0.109	0.087

No significant difference between atopic and non-atopic groups (F=0.08, p=0.784).

No significant difference between GDBA and non-GDBA groups (F=1.14, p=0.288).

No significant interaction between atopic and GDBA groups (F=0.04, p=0.836).

Mean ELISA Results for nettle (non-atopic / non-GDBA = Beagle)

	GDBA	NON-GDBA
ATOPIC	0.121	0.088
NON-ATOPIC	0.109	0.087

No significant difference observed between atopic and non-atopic groups (F=0.78, p=0.379).

No significant difference between GDBA and non-GDBA groups (F=2.55, p=0.113).

No significant interaction between atopic and GDBA groups (F=0.20, p=0.654).

Mean ELISA Results for nettle (non-atopic / non-GDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.121	0.088
NON-ATOPIC	0.109	0.090

No significant difference observed between atopic and non-atopic groups (F=0.39, p=0.531)

No significant difference observed between GDBA and non-GDBA groups (F=2.31, p=0.131)

No significant interaction observed between atopic and GDBA groups (F=0.01, p=0.933)

Table 6.24 Mean dustmite specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for dust mite (non-atopic / nonGDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.051	0.168
NON-ATOPIC	0.027	0.063

No significant difference observed between atopic and non-atopic groups (F=2.23, p=0.140)

No significant difference observed between GDBA and non-GDBA groups (F=3.12, p=0.081)

No significant interaction observed between atopic and GDBA groups (F=0.85, p=0.360)

Mean Immunodot Results for dust mite (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.052	0.168
NON-ATOPIC	0.027	0.000

Significant difference observed between atopic and non-atopic groups (F=4.97, p=0.029)

No significant difference observed between GDBA and non-GDBA groups (F=1.08, p=0.303)

No significant interaction observed between atopic and GDBA groups (F=0.2.75, p=0.102)

Mean Immunodot Results for dust mites (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.051	0.168
NON-ATOPIC	0.027	0.032

Significant difference observed between atopic and non-atopic groups (F=5.05, p=0.028)

No significant difference observed between GDBA and non-GDBA groups (F=2.87, p=0.094)

No significant interaction observed between atopic and GDBA groups (F=2.42, p=0.124)

Table 6.25 Mean storemite specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for storemites (non-atopic / nonGDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.038	0.081
NON-ATOPIC	0.046	0.077

No significant difference observed between atopic and non-atopic groups (F=0.01, p=0.911)

No significant difference observed between GDBA and non-GDBA groups (F=2.81, p=0.098)

No significant interaction observed between atopic and GDBA groups (F=0.08, p=0.776)

Mean Immunodot Results for storemites (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.038	0.081
NON-ATOPIC	0.046	0.000

No significant difference observed between atopic and non-atopic groups (F=2.65, p=0.0.108)

No significant difference observed between GDBA and non-GDBA groups (F=0.00, p=0.951)

No significant interaction observed between atopic and GDBA groups (F=4.10, p=0.046)

Mean Immunodot Results for storemites (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.038	0.081
NON-ATOPIC	0.046	0.039

No significant difference observed between atopic and non-atopic groups (F=0.81, p=0.370)

No significant difference observed between GDBA and non-GDBA groups (F=0.92, p=0.339)

No significant interaction observed between atopic and GDBA groups (F=1.89, p=0.173)

Table 6.26 Mean flea specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for flea (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.012	0.019
NON-ATOPIC	0.016	0.010

No significant difference observed between atopic and non-atopic groups (F=0.16, p=0.690)

No significant difference observed between GDBA and non-GDBA groups (F=0.01, p=0.921)

No significant interaction between atopic and GDBA groups (F=1.22, p=0.272)

Mean Immunodot Results for flea (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.012	0.019
NON-ATOPIC	0.016	0.000

No significant difference observed between atopic and non-atopic groups (F=1.66, p=0.202)

No significant difference observed between GDBA and non-GDBA groups (F=0.62, p=0.435)

Significant interaction observed between atopic and GDBA groups (F=3.98, p=0.050)

Mean Immunodot Results for flea (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.012	0.019
NON-ATOPIC	0.016	0.005

No significant difference observed between atopic and non-atopic groups (F=1.03, p=0.312)

No significant difference observed between GDBA and non-GDBA groups (F=0.17, p=0.681)

No significant interaction observed between atopic and GDBA groups (F=3.49, p=0.065)

Table 6.27 Mean human epithelium specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for human epithelium (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.048	0.051
NON-ATOPIC	0.062	0.059

No significant difference between atopic and non-atopic groups (F=0.94, p=0.355)

No significant difference between GDBA and non-GDBA groups (F=0.00, p=0.998)

No significant interaction between atopic and GDBA groups (F=0.07, p=0.787)

Mean Immunodot Results for human epithelium (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.048	0.051
NON-ATOPIC	0.062	0.015

No significant difference observed between atopic and non-atopic groups (F=0.087, p=0.355)

No significant difference observed between GDBA and non-GDBA groups (F=3.57, p=0.063)

Significant interaction observed between atopic and GDBA groups (F=4.64, p=0.034)

Mean Immunodot Results for human epithelium (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.048	0.051
NON-ATOPIC	0.062	0.037

No significant difference observed between atopic and non-atopic groups (F=0.00, p=0.988)

No significant difference observed between GDBA and non-GDBA groups (F=1.26, p=0.265)

No significant interaction observed between atopic and GDBA groups (F=2.07, p=0.154)

Table 6.28 Mean cat epithelium specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for cat epithelium (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.043	0.011
NON-ATOPIC	0.038	0.025

No significant difference observed between atopic and non-atopic groups (F=0.46, p=0.501)

Significant difference observed between GDBA and non-GDBA groups (F=8.96, p=0.004)

No significant interaction observed between atopic and GDBA groups (F=1.54, p=0.218)

Mean Immunodot Results for cat epithelium (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.043	0.011
NON-ATOPIC	0.038	0.000

No significant difference observed between atopic and non-atopic groups (F=1.10, p=0.299)

Significant difference observed between GDBA and non-GDBA groups (F=23.04, p=0.000)

No significant interaction observed between atopic and GDBA groups (F=0.22, p=0.642)

Mean Immunodot Results for cat epithelium (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.043	0.011
NON-ATOPIC	0.038	0.013

No significant difference observed between atopic and non-atopic groups (F=0.04, p=0.836)

Significant difference observed between GDBA and non-GDBA groups (F=21.01, p=0.000)

No significant interaction observed between atopic and GDBA groups (F=0.22, p=0.641)

Table 6.29 Mean grass specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for grass (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.021	0.056
NON-ATOPIC	0.022	0.044

No significant difference observed between atopic and non-atopic groups (F=0.50, p=0.481)

Significant difference observed between GDBA and non-GDBA groups (F=12.13, p=0.001)

No significant interaction observed between atopic and GDBA groups (F=0.62, p=0.433)

Mean Immunodot Results for grass (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.021	0.056
NON-ATOPIC	0.022	0.046

No significant difference observed between atopic and non-atopic groups (F=0.39, p=0.536)

Significant difference observed between GDBA and non-GDBA groups (F=14.45, p=0.00)

No significant interaction observed between atopic and GDBA groups (F=0.50, p=0.481)

Mean Immunodot Results for grass (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.021	0.056
NON-ATOPIC	0.022	0.045

No significant difference observed between atopic and non-atopic groups (F=0.58, p=0.450)

Significant difference observed between GDBA and non-GDBA groups (F=17.94, p=0.000)

No significant interaction observed between atopic and GDBA groups (F=0.73, p=0.395)

Table 6.30 Mean tree specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for tree (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.020	0.030
NON-ATOPIC	0.019	0.045

No significant difference observed between atopic and non-atopic groups (F=2.12, p=0.149)

Significant difference observed between GDBA and non-GDBA groups (F=12.94, p=0.001)

No significant interaction observed between atopic and GDBA groups (F=2.56, p=0.113)

Mean Immunodot Results for tree (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.020	0.030
NON-ATOPIC	0.019	0.010

Significant difference observed between atopic and non-atopic groups (F=4.91, p=0.030)

No significant difference observed between GDBA and non-GDBA groups (F=0.03, p=0.857)

Significant interaction observed between atopic and GDBA groups (F=4.22, p=0.043)

Mean Immunodot Results for tree (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.020	0.030
NON-ATOPIC	0.019	0.024

No significant difference observed between atopic and non-atopic groups (F=0.48, p=0.492)

No significant difference observed between GDBA and non-GDBA groups (F=3.08, p=0.083)

No significant interaction observed between atopic and GDBA groups (F=0.28, p=0.600)

Table 6.31 Mean mugwort specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for mugwort (non-atopic /non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.056	0.061
NON-ATOPIC	0.051	0.061

No significant difference observed between atopic and non-atopic groups (F=0.07, p=0.787)

No significant difference observed between GDBA and non-GDBA groups (F=0.88, p=0.351)

No significant interaction observed between atopic and GDBA groups (F=0.09, p=0.759)

Mean Immunodot Results for mugwort (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.056	0.061
NON-ATOPIC	0.051	0.036

No significant difference observed between atopic and non-atopic groups (F=3.80, p=0.055)

No significant difference observed between GDBA and non-GDBA groups (F=0.39, p=0.534)

No significant interaction observed between atopic and GDBA groups (F=1.73, p=0.192)

Mean Immunodot Results for mugwort (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.056	0.061
NON-ATOPIC	0.051	0.046

No significant difference observed between atopic and non-atopic groups (F=2.05, p=0.156)

No significant difference observed between GDBA and non-GDBA groups (F=0.00, p=0.969)

No significant interaction observed between atopic and GDBA groups (F=0.53, p=0.469)

Table 6.32 Mean olive/wall pellitory specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for olive/wall pellitory (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.083	0.056
NON-ATOPIC	0.083	0.071

No significant difference observed between atopic and non-atopic groups (F=0.09, p=0.765)

No significant difference observed between GDBA and non-GDBA groups (F=0.57, p=0.452)

No significant interaction observed between atopic and GDBA groups (F=0.09, p=0.768)

Mean Immunodot Results for olive/wall pellitory (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.083	0.056
NON-ATOPIC	0.083	0.007

No significant difference observed between atopic and non-atopic groups (F=1.18, p=0.281)

Significant difference observed between GDBA and non-GDBA groups (F=5.14, p=0.026)

No significant interaction observed between atopic and GDBA groups (F=1.19, p=0.279)

Mean Immunodot Results for olive/wall pellitory (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.083	0.056
NON-ATOPIC	0.083	0.032

No significant difference observed between atopic and non-atopic groups (F=0.33, p=0.566)

No significant difference observed between GDBA and non-GDBA groups (F=3.59, p=0.061)

No significant interaction observed between atopic and GDBA groups (F=0.34, p=0.562)

Table 6.33 Mean Outdoor specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for Outdoor group of allergen (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.067	0.079
NON-ATOPIC	0.058	0.063

No significant difference observed between atopic and non-atopic groups (F=0.13, p=0.726)
No significant difference observed between GDBA and non-GDBA groups (F=0.06, p=0.802)
No significant interaction observed between atopic and GDBA groups (F=0.01, p=0.920)

Mean Immunodot Results for Outdoor group (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.067	0.079
NON-ATOPIC	0.058	0.061

No significant difference observed between atopic and non-atopic groups (F=0.31, p=0.578)
No significant difference observed between GDBA and non-GDBA groups (F=0.11, p=0.739)
No significant interaction observed between atopic and GDBA groups (F=0.04, p=0.852)

Mean Immunodot Results for Outdoor group (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.067	0.079
NON-ATOPIC	0.058	0.062

No significant difference observed between atopic and non-atopic groups (F=0.33, p=0.568)
No significant difference observed between GDBA and non-GDBA groups (F=0.13, p=0.723)
No significant interaction observed between atopic and GDBA groups (F=0.04, p=0.852)

Table 6.34 Mean Indoor specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for Indoor group (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.123	0.155
NON-ATOPIC	0.100	0.080

No significant difference observed between atopic and non-atopic groups (F=1.26, p=0.271)

No significant difference observed between GDBA and non-GDBA groups (F=0.01, p=0.916)

No significant interaction observed between atopic and GDBA groups (F=0.30, p=0.589)

Mean Immunodot Results for Indoor group (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.123	0.155
NON-ATOPIC	0.100	0.044

Significant difference observed between atopic and non-atopic groups (F=4.86, p=0.034)

No significant difference observed between GDBA and non-GDBA groups (F=0.18, p=0.673)

No significant interaction observed between atopic and GDBA groups (F=1.88, p=0.179)

Mean Immunodot Results for Indoor group (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.123	0.155
NON-ATOPIC	0.100	0.051

Significant difference observed between atopic and non-atopic groups (F=4.80, p=0.035)

No significant difference observed between GDBA and non-GDBA groups (F=0.11, p=0.740)

No significant interaction observed between atopic and GDBA groups (F=1.74, p=0.195)

Table 6.35 Mean foods group 1 specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for Foods group 1 (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.018	0.028
NON-ATOPIC	0.028	0.023

No significant difference observed between atopic and non-atopic groups (F=0.09, p=0.762)
No significant difference observed between GDBA and non-GDBA groups (F=0.11, p=0.744)
No significant interaction observed between atopic and GDBA groups (F=0.93, p=0.342)

Mean Immunodot Results for Foods group 1 (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.018	0.028
NON-ATOPIC	0.028	0.026

No significant difference observed between atopic and non-atopic groups (F=0.19, p=0.662)
No significant difference observed between GDBA and non-GDBA groups (F=0.21, p=0.647)
No significant interaction observed between atopic and GDBA groups (F=0.58, p=0.453)

Mean Immunodot Results for Foods group 1 (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.018	0.028
NON-ATOPIC	0.028	0.025

No significant difference observed between atopic and non-atopic groups (F=0.19, p=0.668)
No significant difference observed between GDBA and non-GDBA groups (F=0.21, p=0.651)
No significant interaction observed between atopic and GDBA groups (F=0.68, p=0.414)

Table 6.36 Mean foods group 2 specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for Foods group 2 (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.022	0.029
NON-ATOPIC	0.039	0.025

No significant difference observed between atopic and non-atopic groups (F=0.33, p=0.572)
No significant difference observed between GDBA and non-GDBA groups (F=0.09, p=0.763)
No significant interaction observed between atopic and GDBA groups (F=0.78, p=0.384)

Mean Immunodot Results for Foods group 2 (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.022	0.029
NON-ATOPIC	0.039	0.054

Significant difference observed between atopic and non-atopic groups (F=5.36, p=0.027)
No significant difference observed between GDBA and non-GDBA groups (F=1.34, p=0.254)
No significant interaction observed between atopic and GDBA groups (F=0.16, p=0.696)

Mean Immunodot Results for Foods group 2 (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.022	0.029
NON-ATOPIC	0.039	0.049

Significant difference observed between atopic and non-atopic groups (F=4.29, p=0.045)
No significant difference observed between GDBA and non-GDBA groups (F=0.80, p=0.378)
No significant interaction observed between atopic and GDBA groups (F=0.01, p=0.911)

Table 6.37 Mean mould specific IgE reflective densities, as determined by Immunodot, in different groups of atopic and non-atopic dogs, and results of General Linear Model examination of this data.

Mean Immunodot Results for moulds (non-atopic / non-GDBA = Greyhounds)

	GDBA	NON-GDBA
ATOPIC	0.039	0.082
NON-ATOPIC	0.089	0.104

No significant difference observed between atopic and non-atopic groups (F=3.24, p=0.083)

No significant difference observed between GDBA and non-GDBA groups (F=2.18, p=0.151)

No significant interaction observed between atopic and GDBA groups (F=0.51, p=0.480)

Mean Immunodot Results for moulds (non-atopic / nonGDBA = Beagles)

	GDBA	NON-GDBA
ATOPIC	0.039	0.082
NON-ATOPIC	0.089	0.265

Significant difference observed between atopic and non-atopic groups (F=41.54, p=0.000)

Significant difference observed between GDBA and non-GDBA groups (F=37.08, p=0.000)

Significant interaction observed between atopic and GDBA groups (F=13.65, p=0.001)

Mean Immunodot Results for moulds (non-atopic / nonGDBA = Greyhounds & Beagles)

	GDBA	NON-GDBA
ATOPIC	0.039	0.082
NON-ATOPIC	0.089	0.235

Significant difference observed between atopic and non-atopic groups (F=23.07, p=0.000)

Significant difference observed between GDBA and non-GDBA groups (F=20.25, p=0.000)

Significant interaction observed between atopic and GDBA groups (F=6.04, p=0.019)

6.4 Discussion

Serological tests have been shown by a number of authors to have a tendency for a high sensitivity and poor specificity due to the large number of so called false positive results which these tests produce (Bond *et al.*, 1994; Codner & Lessard, 1993). This is said to be the case when non-atopic dogs are shown to have levels of allergen specific serum IgE which would be positive by these tests.

Based on these reports it would be expected that no differences would be observed between atopic and non-atopic dogs in their serological results. Indeed Bond *et al.*, (1994), in a study of thirty dogs, did not find any significant difference in allergen specific serum IgE concentrations between atopic and non-atopic dogs. However, this was not the case in this present study. Here atopic dogs were shown to have significantly higher optical densities of allergen specific serum IgE against *Alternaria, Aspergillus,* indicated by ELISA; and significantly higher serum IgE reflective densities for Indoor allergens, dustmites and trees as indicated by Immunodot.

Other authors have found atopic dogs to give higher ELISA results for mould allergens than non-atopics. Codner & Lessard (1993) noted that dogs suspected of being atopic demonstrated significantly higher ELISA results for fungal allergens than clinically normal dogs but these authors did not offer any explanations for this. A high number of clinically irrelevant positive ELISA results for mould allergens were observed by Griffin *et al.* (1990), Anderson & Sousa (1993) and Day *et al.* (1996). These results are classed as false positive because they did not agree with any IDST positive results towards mould allergens. This is similar to the findings in this study where very few positive IDST results were observed against mould allergens (See Appendix L).

It is possible that the higher optical density results observed against mould allergens are not true reflections of the immune response. Instead they may reflect the attraction which serum IgE has for mould allergens in particular and that non-specific binding of IgE is taking place in the ELISA test. From this it would be expected that animals with higher serum total IgE concentrations would have higher optical density results against moulds.

However examination of the correlation between serum total IgE reflective densities (assessed by Immunodot) and the optical density of ELISA results for *Alternaria* and *Aspergillus* did not reveal any significant correlation with a correlation coefficient of –0.031 and –0.284 respectively (See Appendix K). It is possible that this discrepancy could be due to the different methods of measuring serum total IgE and allergen specific IgE.

It is possible that there is a cross reaction occurring between *Malassezia* and *Alternaria / Aspergillus.* Cross reactions between serum against *Malassezia furfur* and moulds have been reported in humans (Nordvall & Johansson, 1990). Although clinical disease due to *Malassezia* was rare in the atopic dogs, *Malassezia* was recovered from ears (both diseased and normal). Further work is required in this area.

Examination of the sensitivity and specificity of *Alternaria* and *Aspergillus* ELISA results in identifying atopic dogs demonstrated better results for *Alternaria.* These results were fair with a sensitivity of 64.3% and specificity of 60.8%. It is proposed that these findings for *Alternaria* be included in the predictive model of atopic dermatitis, but that these findings cannot be examined in isolation due to the lower sensitivity and specificity than desired.

House dust mites have been shown to be important allergens in the pathogenesis of canine atopic dermatitis (Sture *et al.,* 1995, DeBoer, 1989). It is not surprising therefore that atopic dogs had significantly higher ($p<0.029$) reflective density values for house dust mite specific serum IgE than non-atopics assessed by Immunodot. However it is surprising that no such difference was noted with the ELISA test.

The likeliest reason for these findings is that the ELISA is showing falsely increased levels of house dust mite specific serum IgE. However why this should be the case for house dust mites and not for all of the other allergens is not known. It is possible that there may be some cross reaction between house dust mites and storage mites, but it is unlikely that the greyhounds and beagles are exposed to storage mites either. Storage mites have been shown to be present in cereal products (Reedy *et al.,* 1997b) and it is possible that storage mites could be present in dry dog food. If the dogs are fed such a diet they

may be exposed to these allergens. However no information was available on the food supply of the beagles and greyhounds.

It is also possible that the increased levels of house dust mite specific serum IgE may indeed be a true reflection of the antibody status of these dogs. Non-atopic dogs have been shown to demonstrate positive IDST results to house dust mite allergens (DeBoer, 1989). It possible that these dogs do indeed have circulating levels of house dust mite specific IgE and yet do not demonstrate clinical evidence of atopic dermatitis. However, if this were to be the case these dogs would have to either have met house dust mites at some point in their lifetime or to have been exposed to another allergen which can cross react with house dust mite specific IgE. The possibility of a cross reacting epitope seems the likeliest explanation due to the difference in results between ELISA and Immunodot tests. As different serological tests incorporate different allergen epitopes (Esch, 1997) this seems the most likely explanation for the differing results.

As the groups of dogs were kept in different environments it was interesting to note differences in the serum IgE responses of the dogs. Indeed differences were observed against a number of allergens including grass, trees, cat epithelium, wall pellitory and moulds.

GDBA dogs had significantly higher levels of serum IgE against cat epithelium than non-GDBA dogs ($p<0.004$, where the non-GDBA group included greyhounds, and $p<0.000$ where the non-GDBA group included beagles), even though none of the results for any dogs were considered positive. As the GDBA dogs are exposed to cats on a daily basis as part of their training this would suggest that exposure has a stimulatory effect on serum IgE concentrations against cat allergens. It is however, interesting to note that this difference was only detected with the Immunodot test and not the ELISA.

GDBA dogs were found to have significantly lower allergen specific serum IgE optical/reflective densities against Fescue, grasses (as a group) and trees than non-GDBA dogs. This shows agreement between the ELISA test in assessing fescue specific IgE and the Immunodot in assessing grass specific IgE. The main reason for these increased levels of allergen specific IgE in the

non-GDBA dogs was the higher levels of IgE directed against fescue and trees by the greyhounds. Although all dogs other than the beagles are exposed to grass allergens it is possible that the greyhounds are exposed to higher levels of grass than the other groups due to training methods. This supports the theory that increased exposure can lead to increased serum IgE concentrations. Examination of the actual optical density results for fescue in these greyhounds revealed that many of these non-atopic dogs had levels consistent with a diagnosis of atopic dermatitis. This would suggest that the level of a positive reaction based on the ELISA test may need to be altered and may have to be different for different allergens.

It was expected that the beagles would have significantly lower grass specific serum IgE optical/reflective densities than the other groups, as these dogs are kept indoors. It is not known whether beagles could be exposed to pollen allergens *via* the air conditioning but this may explain these results.

Mould specific IgE reflective densities assessed by Immunodot were highest in the non-atopic beagles. Indeed many of these results were considered positive. Although no study has been carried out to assess mould exposure in these animals it is possible that these dogs are exposed to high levels of mould although whether this would be any higher than that to which the GDBA kennels dogs are exposed is not known.

The way in which dogs are grouped had an obvious influence on statistical results. This was demonstrated by comparing atopic dogs, non-atopic GDBA dogs and either non-atopic greyhounds or non-atopic beagles. When greyhounds and beagles were examined individually different results were obtained than when these two groups were considered together. This is due to the fact that greyhounds and beagles demonstrated different serological results with one group often having significantly higher optical/reflective densities for individual allergens than the other, and on considering these dogs together the predominant group influenced the final result. Although both of these groups consisted of non-atopic dogs it illustrated the fact that different backgrounds can influence the results and suggests that allergen exposure has an effect on serum IgE concentrations.

Interaction was found between the groups for a number of allergens. Interaction demonstrates that two factors, whether dogs belong to the GDBA population or not and whether dogs are atopic or not, both have an effect on the results. The combined effect of both factors gives a result in excess of that which would be expected from the combined effects of each parameter. Interaction was highest for mould specific IgE assessed by Immunodot where the highest mean was found for non-atopic, non-GDBA dogs. The reason for this is not known but suggests that there may be a background level of serum IgE produced in non-atopic dogs against mould allergens. Whether this result is due to an increased level of exposure in these dogs is not known, but is a possibility worth further study.

6.5 Conclusion

In summary, significantly higher mould specific serum IgE optical densities were found in atopic dogs than non-atopics with the ELISA test. Similar results have been observed by a number of other authors. Sensitivity and specificity of *Alternaria* results were 64.3% and 60.8% respectively. Although these results were below the level considered positive by the manufacturers and therefore does not suggest that the atopic dogs are allergic to mould allergens, this is a useful finding and should be incorporated as a parameter in the diagnosis of canine atopic dermatitis, combined with other factors.

It also appears that a dog's surroundings may have an influence on allergen specific serological results. It would therefore be useful to be aware of the environment in which animals are kept when assessing serological parameters.

Chapter 7 Comparison of intradermal skin testing, ELISA and Immunodot assays in atopic and non-atopic dogs.

7.1 Introduction

Many authors have attempted to correlate the results from intradermal skin testing with those of serological tests in both human and veterinary medicine with varying success (Lockey, *et al,* 1992; Bunde *et al,* 1997; Bond *et al,* 1994). The general opinion appears to be that the degree of correlation between IDST and serological tests for negative results is good but correlation is poor when results are positive in either test. The aim of this study was to examine the correlation between intradermal skin testing, ELISA and Immunodot test results to determine if any one serological test gave a better correlation with intradermal skin testing.

7.2 Materials & Methods

A total of forty six atopic dogs, fourteen from the GDBA population and thirty two from the GUVS pet dog population were included in this study. All of these dogs underwent IDST and ELISA testing and had at least one positive result on IDST. Different numbers of these dogs also underwent Immunodot testing dependent on the availability of serum for each dog – thirty five dogs underwent Indoor Immunodot testing, thirty one dogs underwent Outdoor Immunodot testing and twenty seven underwent Topscreen testing. Further details of these dogs are given in Appendix L.

In addition to these atopic dogs, IDST and serological results of a total of nine dogs with clinical signs consistent with a diagnosis of atopic dermatitis (as described in section 2.1.1) but with negative IDST results were included (called skin problem dogs). As it was not possible to carry out IDST on healthy non-atopic dogs for ethical reasons, serological results for the seventy four non-atopic GDBA dogs also described in section 2.1.1 were included for comparative purposes. Serological results of all dogs are given in Appendix F.

A positive result on ELISA was defined as an optical density (450nm) of equal to or greater than 0.15. A positive result to any house dust mite was recorded as a positive for house dust mites as a group. A positive Immunodot test was defined as any blue colour formation on the Immunodot strip. The number of dogs with a positive result was calculated for each individual IDST allergen and each serological test allergen panel.

Sensitivity, specificity and efficacy of serological test results in relation to IDST results were examined. Sensitivity was defined as the number of dogs with positive results on both IDST and serological testing as a proportion of the total number of dogs with positive results on IDST alone. Specificity was defined as the number of dogs with negative results on both IDST and serological testing as a proportion of the total number of dogs with negative results on IDST alone. Efficacy was defined as the percentage of concordant positive or negative reactions among all cases. Sensitivity, specificity and efficacy of ELISA testing in relation to Immunodot was also examined.

Examination of the correlation coefficient of IDST and serological results for atopic and non-atopic groups of dogs was carried out. In addition correlation of Topscreen and individual Indoor or Outdoor Immunodot panels was carried out only in IDST positive dogs as this was the only group of dogs where both types of Immunodot tests had been carried out. For the purposes of correlation only the presence or not of a positive result was recorded and not the degree of positivity.

7.3 Results

Tabulated results are shown in Appendices L and M, and Tables 7.1-7.6.

The number of positive results for each allergen produced by IDST and serological testing in atopic dogs are shown in Table 7.1. This revealed house dust mites to be the predominant allergens with the highest number of positive results by both IDST and Immunodot screening methods i.e. 89.1% positive by IDST and 45.7% positive by Immunodot. The ELISA method also revealed 37% of atopic dogs with positive reactions against HDM, but this was lower

than the number of positive reactions obtained against grasses with 45.7% of atopic dogs demonstrating positive reactions. In addition, with ELISA the number of positive reactions against HDM was the same as the number of positives against moulds (37%).

When examining the serological results of IDST negative dogs (Table 7.2), results were similar to those described above with the highest number of positive reactions present against house dust mites and different grasses. One difference was the greater proportion of positive reactions against different tree allergens on ELISA. Also, no positive reactions against HDM were found on Immunodot, although fewer IDST negative dogs were tested.

Serological results of non-atopic dogs were similar to those of atopic and skin problem dogs (See Table 7.3) in that non-atopic dogs demonstrated a number of positive reactions to various allergens, the predominant allergens being moulds, house dust mites and grasses. In this instance the proportion of positive reactions was less with the Immunodot test methods than the ELISA.

Overall when comparing different combinations of IDST and serological tests the sensitivity was found to be poor but the specificity was in general quite good (See Table 7.4). The best sensitivity was 53.3% when comparing IDST and Immunodot results for house dust mites. Specificities of 100% were found for a number of allergens but were more common when the Immunodot test was compared with ELISA or IDST than when comparing IDST with ELISA. Efficacy was variable but was greater than 75% for a number of allergens for all comparisons of IDST and serological tests.

Comparison of Topscreen Immunodot and either indoor or outdoor Immunodot screens (see Appendix M) revealed a correlation of 0.73 for Indoor results. Outdoor results could not be correlated as all results were negative. Examination of the sensitivity, specificity and efficacy of these two tests revealed the best results for house dust mites. An efficacy of 100% was achieved for grasses and mugwort but as all results were negative this may be an overestimation.

Correlation values between serological tests and IDST were not particularly good (Table7.6). The best results were obtained for house dust mites followed by storage mites, comparing Immunodot with IDST. House dust mite analysis

with Immunodot gave a better correlation with IDST than ELISA. Other comparisons gave a low and often negative correlation.

Table 7.1. Comparison between IDST and serological testing for allergen specific IgE in atopic dogs. (* indicates that a particular allergen was not present on that serological test).

Allergen	IDST			ELISA			Topscreen			Immunodot		
	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%
House dust mites	46	41	**89.1**	46	17	**37.0**				35	16	**45.7**
Storage mites	46	30	**65.2**	*	*	*				35	5	**14.3**
Cat flea	46	3	**6.5**	46	5	**10.9**				35	0	**0**
Cat epithelia	46	4	**8.7**	46	0	**0**	27	13	**48.1**	35	0	**0**
Human epithelia	46	2	**4.3**	46	0	**0**				35	0	**0**
Trees	46	4	**8.7**	46	12	**26.1**				31	0	**0**
Grasses	46	8	**17.4**	46	21	**45.7**	**27**	1	**3.7**	31	0	**0**
Mugwort	46	3	**6.5**	46	9	**19.6**				31	0	**0**
Sorrel	46	1	**2.2**	46	6	**13.0**	*	*	*	*	*	*
Plantain	46	3	**6.5**	46	9	**19.6**	*	*	*	*	*	*
Dandelion	46	3	**6.5**	46	9	**19.6**	*	*	*	*	*	*
Nettle	46	2	**4.3**	46	6	**13.0**	*	*	*	*	*	*
Moulds	46	1	**2.2**	46	17	**37.0**	27	0	**0**	*	*	*

Table 7.2. Comparison between IDST and serological testing for allergen specific IgE in IDST negative dogs with atopic type skin disease. (* indicates that a particular allergen was not present on that serological test).

Allergen	ELISA			Topscreen			Immunodot		
	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%
House dust mites	6	3	**50**	2	1	**50**	6	0	**0**
Storage mites	*	*	*				6	0	**0**
Cat flea	6	1	**16.7**				6	0	**0**
Cat epithelia	6	0	**0**				6	0	**0**
Human epithelia	6	0	**0**				6	0	**0**
Trees	6	3	**50**	2	0	**0**	6	0	**0**
Grasses	6	3	**50**				6	0	**0**
Mugwort	6	0	**0**				6	0	**0**
Sorrel	6	0	**0**	*	*	*	*	*	*
Plantain	6	1	**16.7**	*	*	*	*	*	*
Dandelion	6	1	**16.7**	*	*	*	*	*	*
Nettle	6	0	**0**	*	*	*	*	*	*
Moulds	6	1	**16.7**	2	1	**50**	*	*	*

Table 7.3 Comparison of different serological results in non-atopic GDBA dogs. (* indicates that a particular allergen was not present on that serological test).

Allergen	ELISA			Topscreen			Immunodot		
	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%
House dust mites	74	17	**23.0**	10	2	**20**	33	2	**6.1**
Storage mites	*	*	*				33	0	**0**
Cat flea	74	4	**5.4**				33	0	**0**
Cat epithelia	74	0	**0**				33	0	**0**
Human epithelia	74	0	**0**				33	1	**3.0**
Trees	74	11	**14.9**	10	0	**0**	39	0	**0**
Grasses	74	18	**24.3**				39	0	**0**
Mugwort	74	13	**17.6**				39	0	**0**
Sorrel	74	6	**8.1**	*	*	*	*	*	*
Plantain	74	12	**16.2**	*	*	*	*	*	*
Dandelion	74	11	**14.9**	*	*	*	*	*	*
Nettle	74	10	**13.5**	*	*	*	*	*	*
Moulds	74	24	**32.4**	10	0	**0**	*	*	*

Table 7.4 Sensitivity, specificity and efficacy of IDST and serological tests in skin tested dogs with atopic type skin disease (See Appendix M). (* indicates that a particular allergen was not present on that serological test).

Allergen	IDST/ELISA			IDST/Immdot			ELISA/Immdot		
	Sens	Spec	**Effic**	Sens	Spec	**Effic**	Sens	Spec	**Effic**
House dust mites	32.5%	41.7%	**34.6%**	53.3%	100%	**65.9%**	46.7%	63.6%	**56.8%**
Storage mites	*	*	*	20.8%	100%	**53.7%**	27.3%	100%	**63.6%**
Cat flea	0%	87.8%	**82.7%**	0%	100%	**97.6%**	0%	100%	**84.2%**
Cat epithelia	0%	100%	**92.3%**	0%	100%	**90.2%**	0%	100%	**100%**
Human epithelia	0%	100%	**96.2%**	0%	100%	**97.6%**	0%	100%	**100%**
Trees	40%	74.5%	**71.2%**	0%	100%	**91.9%**	0%	100%	**70.6%**
Grass	37.5%	52.3%	**59.6%**	0%	100%	**75.7%**	0%	100%	**61.8%**
Mugwort	33.3%	83.7%	**80.8%**	0%	100%	**97.3%**	0%	100%	**76.5%**
Sorrel	0%	88.2%	**86.5%**	*	*	*	*	*	*
Plantain	0%	79.6%	**75%**	*	*	*	*	*	*
Dandelion	0%	79.6%	**75%**	*	*	*	*	*	*
Nettle	0%	88%	**84.6%**	*	*	*	*	*	*
Moulds	0%	65.4%	**65.4%**	0%	96.6%	**96.6%**	0%	93.8%	**51.7%**

Table 7.5 Sensitivity, specificity and efficacy of Immunodot Topscreen compared with individual Immunodot allergen panels (See Appendix M).

Allergen	Sensitivity	Specificity	Efficacy
House dust mites	81.8	88.9	77.3
Storage mites	27.3	100	63.6
Cat Flea	0	100	50
Cat epithelia	0	100	50
Human epithelia	0	100	50
Trees	0	100	94.7
Grasses	0	100	100
Mugwort	0	100	100
Sorrel	0	88	84.6

Table 7.6 Correlation between IDST and serological tests in skin tested dogs with skin disease (See Appendix M).

Allergen	Correlation coefficient		
	IDST/ELISA	IDST/Immunodot	ELISA/Immunodot
House dust mites	-0.171	0.454	0.075
Storage mites	*	0.330	*
Cat flea	-0.089	-	-
Trees	0.135	-	-
Grasses	0.033	-	-
Mugwort	0.105	-	-
Sorrel	-0.051	*	*
Plantain	-0.121	*	*
Dandelion	-0.121	*	*
Nettle	-0.072	*	*
Moulds	-0.102	-0.036	-0.170

- All values were identical therefore correlation coefficient could not be calculated .

* no figures available as allergens not present on ELISA or Immunodot test.

7.4 Discussion

Many authors have attempted to compare IDST and serological tests with most authors not finding any evidence of correlation between the two methods. (Miller *et al*, 1993; Bond *et al*, 1994; Codner & Lessard, 1993). Most work has concentrated on the RAST and ELISA methods of serological testing with little published work being available for the Immunodot test as this is a relatively recent development in veterinary medicine (Hämmerling & de Weck, 1998, Zunic, 1998).

In this present study the majority of positive results on both IDST and serology were obtained against house dust mites and grasses. Positive serological results were not restricted to atopic dogs and were seen in IDST negative, skin problem dogs and in non-atopic dogs. Positive results against these allergens in atopic dogs are commonly found (DeBoer, 1989). These substances are the main allergens to which dogs are exposed and subsequently develop allergies. However, the reason for the high number of positive reactions against these allergens in non-atopic dogs is not known.

However it is possible that the IDST negative dogs with atopic type skin disease may indeed be allergic to these allergens. Hyposensitisation of atopic dogs based entirely on ELISA results has been shown to be as successful as hyposensitisation based on IDST (Willemse *et al*, 1984). The underlying mechanisms in atopic dermatitis may involve more than percutaneous exposure of allergens to sensitised mast cells. If this was the case then not all atopic dogs would react on IDST.

This does not explain the positive serological results in non-atopic dogs. Positive IDST results have been recorded in non-atopic dogs against house dust mites and it is known that IDST results cannot be relied upon alone in the diagnosis of atopic dermatitis (Willemse, 1986). It therefore appears that this finding also applies to serological results. Positive results for allergens to which an animal is not clinically allergic are considered to be the major problem with ELISA tests evaluated by Bond *et al*, (1994), and Codner &

Lessard (1993). Many reasons for the development of these positive serological results have been put forward including non-specific binding of IgE; differences in the concentrations of bound or free serum IgE; and the detection of different antigenic epitopes by different test methods (Esch, 1997) . In this study very few positive results were observed with the Immunodot test for IDST negative dogs and non-atopic dogs. From this it would appear that the Immunodot test gives results which agree better with IDST than ELISA and on this basis the Immunodot results appear more reliable than ELISA.

The ELISA test demonstrated a high number of positive results against mould allergens for all three groups of atopic and non-atopic dogs. This was not demonstrated on IDST or Immunodot tests. The high number of positive ELISA results for mould allergens agrees with work by a number of authors (Griffin *et al*, 1990, Anderson & Sousa, 1993, Day *et al*, 1996) who also found a high number of positive reactions against mould allergens. The reason for this is unknown, but it has been suggested that there may be a high degree of non-specific binding between IgE and moulds. The sensitivity of the ELISA test for mould allergens was thought to be an important factor by Bunde *et al* (1997) but the reason behind this was not given. When atopic and non-atopic dogs were compared, atopics were found to have significantly higher ELISA results for fungal allergens than non-atopics (Codner & Lessard, 1993), as discussed in the previous chapter. However, the reason for this was not known. In this study a greater percentage of atopic dogs demonstrated positive results for moulds than IDST negative or non-atopic dogs and it would appear that this is an important finding.

Examination of the sensitivity and specificity of serological tests compared with IDST revealed a poor sensitivity but a good specificity with many results being 100% specific. This is unexpected as most work to date has found that serological tests have a good sensitivity but poor specificity often around 10% (Miller *et al,* 1993; Bond *et al,* 1994; Codner & Lessard, 1993). The main reason for our results was the high number of negative results for a number of

allergens, in particular with the Immunodot test. Most other authors have found that serological tests are prone to giving positive results in atopic and non-atopic dogs with a sensitivity of between 43-100% (Miller et al, 1993, Bond et al, 1994, Codner & Lessard, 1993). Sensitivity in this study was found to be between 0-40% when IDST and ELISA tests were compared. Sensitivity of Immunodot compared to IDST was also poor ranging from 0-53.3%. Previous studies have concentrated on between twenty-forty dogs. This study examined a total of 129 atopic and non-atopic dogs and it is possible that the differences observed between this study and other authors work is due to the differing numbers examined.

Although sensitivity was poor, specificity for most allergens was good and as such the efficacy of serological tests in comparison to IDST and each other was good. The one allergen for which results were not particularly good was house dust mites where a higher number of positive results were obtained on IDST than serology. It is possible that this finding is due to percutaneous exposure of allergens with differing levels of skin associated house dust mite specific IgE and free circulating IgE although further work is required in this area. Both ELISA and Immunodot serological tests are designed so that a positive result is indicated by a colour change which is the same for all allergens. It is possible that this indicator requires to be different for different allergens and this so called 'cut off point' requires to be increased for house dust mite allergens.

Correlation between serology and IDST was poor as has been described by other authors (Anderson & Sousa, 1993; Bond *et al,* 1994; Codner & Lessard, 1993). The best correlation was obtained between house dust mites on Immunodot and IDST (correlation coefficient of 0.454)

Comparison of IDST and Immunodot tests by Hämmerling & de Weck (1998) for house dust mites demonstrated a sensitivity of 100% and specificity of 60%. This compares with results in this study of 53.3% sensitivity and 100% specificity. However, differences between these two comparisons are due to the fact that Hämmerling & de Weck combined results for Topscreen and

Indoor Immunodot *i.e*, where a dog demonstrated a positive result on either Topscreen or Indoor Immunodot panels, then this was considered as a positive result for house dust mites. This assumes that a positive Topscreen Indoor result is due to house dust mites. However, on comparing results from Topscreen and Indoor in our study although the best correlation of 0.730 was obtained for HDM (i.e. the majority of the positive results on Topscreen were due to house dust mite reactions) it was not 100%. The efficacy of results in the present study for house dust mites and those for Hämmerling & de Weck (1998) were similar at 66% and 78% respectively. Comparison of IDST with Immunodot, examining storage mites, revealed a poor sensitivity, better specificity and similar efficacy for our results with those of Hämmerling & de Weck.

When comparing ELISA results with Immunodot from house dust mites, again Hämmerling & de Weck grouped Topscreen and Indoor results. However, even with this discrepancy results of this study compare favourably (with those of Hämmerling & de Weck) with sensitivity of 46.7% (48%), specificity 63.6% (75%) and efficacy 56.8% (67%) respectively. Overall, however both groups of results were poor.

We have to ask however, if direct comparison of these different test results is justified. IDST has been regarded as the 'gold standard' for many years (Esch, 1997). Measurement of serum IgE is a comparatively recent development in allergy diagnosis yet therapies based entirely on serological testing rather than IDST have been shown to be successful (Willemse *et al*, 1984). In addition clinically irrelevant positive results are known to occur in IDST as indicated earlier. Exactly how bound and free IgE are related in humans and in dogs is not known and because of this it might not be possible to directly correlate IDST and serological tests.

In addition, when comparing different tests a reasonable comparison can only be made when the same epitopes are being detected. This is supported by the work by Bunde *et al.* (1997) where identical allergens were used in both IDST and ELISA and the correlation between both tests was found to be very good

with 80% correlation between IDST and serology overall. Ragweed and grasses had an even better agreement of 96.8% between IDST and serology. This suggests that animals can produce IgE against different epitopes on the one compound as positive results can be obtained on one test and not another and thus using different allergens sources (as is the case in most studies) will result in lower correlation. As correlation results in this study were best for house dust mites with the Immunodot and IDST this suggested that the epitopes in these two tests may be very similar.

On measuring serology, complicating factors such as IgG anti-IgE need to be taken into account (Hämmerling *et al,* 1997). Manufacturers of the ELISA test acknowledge that IgG can interfere with results either by binding to IgE in the sample or binding to IgE sites in the test kit. However, the Immunodot test claims to be more specific for IgE. This might explain the difference in the sensitivity and specificity of both tests and hence the better correlation of the Immunodot test with IDST.

7.5 Conclusion

In conclusion this study has demonstrated that although good sensitivity and specificity results can be obtained for particular allergens with serological tests in comparison to IDST no one test gives results which are identical to those obtained with IDST. Positive results were obtained against a number of allergens in non-atopic dogs with the predominant allergens being house dust mites. Whether these results are true 'false positives' or do indeed illustrate the presence of house dust mite specific serum IgE remains to be proved. However, a high proportion of atopic dogs have positive results for mould specific serum IgE suggesting that this may be important in the diagnosis of canine atopic dermatitis.

Sensitivity and specificity of serological tests in comparison to IDST was better for Immunodot than ELISA suggesting that the Immunodot method may be more accurate than the ELISA methods. This does however assume

that the IDST method is the most reliable, a fact which still remains to be proved.

Chapter 8 Studies of serum total IgG1

8.1 Introduction

The majority of work on atopic dermatitis in both human and veterinary medicine has concentrated on the role of IgE antibodies. However, it has been suggested that IgG antibodies may also be important (Djurup & Malling, 1987; McHugh *et al.* 1990; Hill *et al.* 1995). Serum IgG levels are influenced by a number of factors including hypersensitivity reactions, parasitic infestations and infections. Hill *et al.* (1995) demonstrated that serum total IgG levels of atopic dogs were significantly higher than those of healthy non-atopic dogs. Similarly, Hites *et al.* (1989) found that allergen specific IgG was higher in atopic than non-atopic dogs. In addition the presence of endoparasites has been associated with a significant increase in serum total IgG levels as compared to healthy controls (Hill *et al.*, 1995). Flea allergic dermatitis (Halliwell & Longino, 1985) and staphylococcal antigens (Morales *et al.*, 1994) can also induce raised serum IgG levels.

Four different IgG subgroups have been identified in both people and dogs termed IgG_1, IgG_2, IgG_3, and IgG_4. Subgroups IgG_1 and IgG_4 have been shown to be the dominant subgroups in both human and canine subjects with atopic disease (Djurup & Malling, 1987; McHugh *et al.*, 1990; and Day & Corato 1996).

IgG antibodies have been shown to be produced against IgE antibodies in both humans and dogs (Scheur *et al.*, 1991; Carini *et al.* 1992, Hammerberg *et al.*, 1997). These have been shown to be mainly antibodies of the IgG_1 and IgG_3 subgroups.

Increased levels of antigen specific IgG have been found in response to hyposensitisation in both human and veterinary medicine (Hites *et al.*, 1989) and these antibodies are thought to act as 'blocking' antibodies. Correlation with the success of hyposensitisation has been attempted by various authors

but there is some disagreement in this area. Djurup & Malling (1987) concluded that an increase in antigen specific IgG_4 was associated with failure of hyposensitisation. However, this contradicts McHugh *et al.* (1990) and other authors (Devey *et al.*, 1976; van der Giessen *et al.*, 1976) who found that serum antigen specific levels of IgG_4 rose significantly in successfully hyposensitised patients. The initial response to hyposensitisation in humans has been shown to be dominated by IgG_1 followed later by an increase in serum IgG_4. (Aalberse, 1983).

In the dog, antigen specific IgG has been shown to rise following hyposensitisation (Hites *et al.*, 1989) but no mention was made of the clinical success of this treatment. Carlotti (1996) has suggested that no correlation between serum IgG levels following hyposensitisation and clinical success in the dog may exist.

The aims of this study were firstly to assess the total serum IgG_1 concentration in atopic and non-atopic dogs kept under different parasite control regimes. Secondly, the response to hyposensitisation with respect to serum IgG_1 levels before and after hyposensitisation therapy, was examined.

8.2 Materials & Methods

A total of seventy eight atopic and non-atopic dogs were examined. These were thirteen non-atopic GDBA dogs without skin disease; ten non-atopic GDBA dogs with skin disease at the time of sampling (pyoderma diagnosed on clinical examination); seventeen atopic GDBA dogs; fourteen non-atopic greyhounds without skin disease at the time of sampling; and twenty four atopic GUVS pet dogs, as described in Appendix N. All dogs underwent a dermatological examination before sampling and atopic dermatitis was diagnosed as described in section 2.1 and 2.2.

Parasite control measures varied between different groups. Both atopic and non-atopic GDBA dogs received excellent parasite control measures as

described in section 2.1.1. GUVS dogs received a variety of parasite control measures and their parasite status prior to examination at GUVS was not known. Greyhounds were known to receive regular parasite control therapy but were also known to be exposed to large numbers of both endo- and ectoparasites.

Atopic dogs undergoing hyposensitisation treatment were given an alum precipitated vaccine containing up to eight different allergens (ARTU Biologicals, Netherlands. See Table 8.2) based firstly on the results of IDST then serological tests. This hyposensitisation vaccine was given by subcutaneous injection in the scruff. All dogs received the same injection regime of increasing doses of vaccine based on manufacturer's instructions (See Table 8.3). After six months' therapy dogs were re-assessed and examined for the presence of skin disease. During this period dogs had been treated by the home veterinary surgeon and had not been examined by the author. Owners were questioned as to how they felt about the dog's overall condition – whether there had been any improvement or not; if the dog was still pruritic and if so was this any better then previously; had any anti-pruritic treatment been prescribed for the dog at any time in the past six months.

The dog's response was then scaled, based on these findings, and the dog allocated to one of the following groups:

1. Dogs required no or only occasional steroid or antihistamine therapy to relieve pruritus – the clinical response was defined as GOOD.
2. Dogs were still pruritic and required intermittent therapy – the clinical response was defined as FAIR.
3. There was no improvement in condition and the dogs required continuous therapy. The clinical response was defined as POOR.

Blood samples from twelve dogs receiving hyposensitisation were obtained at the time of IDST and after six months hyposensitisation therapy. In addition serum was available from three dogs after twelve months therapy, and in one dog six months after the end of twelve months hyposensitisation. Serum IgG_1 concentrations were analysed using a radial immunodiffusion assay (VetRid, Bethyl :Laboratories Ltd., Texas) as described in section 2.5.3. Analyses were undertaken using the Minitab version 11.21 (1996) statistical software package.

A One Way Analysis of Variance and Newman Keuls multiple range test of the $\log_e$ of serum IgG_1 concentrations was carried out.

Serum values of IgG_1 were compared before and after hyposensitisation using a paired t test.

8.3 Results

Full results are given in Appendix N.

Comparison of serum IgG_1 levels in atopic and non-atopic dogs revealed the highest mean value of 418.5mg/dl in atopic pet dogs and the widest range of values, from 10mg/dl to 1550mg/dl in GDBA atopic dogs (Fig. 8.1). The lowest mean values of 12.0mg/dl were observed in the non-atopic GDBA dogs with skin disease followed by a mean of 24.6mg/dl in the non-atopic GDBA dogs without skin disease. However, there was an area of overlap in all five groups.

Statistical evaluation of data, required the examination of the $\log_e$ of serum IgG_1 concentrations in order to obtain results which were not distorted due to the skewed nature of this data (Table 8.1). This is due to the majority of results tending towards the lower range of concentrations. One Way Analysis of Variance of this data revealed that non-atopic working dogs with or without skin disease had significantly lower serum IgG_1

concentrations than atopic working dogs, non-atopic greyhounds and atopic pet dogs ($p<0.000$). There were no significant differences between any of the other groups. No significant difference was noted between those non-atopic dogs with pyoderma and those without, indeed non-atopic working dogs with pyoderma had a lower mean serum IgG_1 concentration than dogs without skin disease.

Serum IgG_1 concentrations of twelve atopic dogs after six months of hyposensitisation therapy were significantly higher than before the initiation of hyposensitisation ($p<0.01$) (Table 8.5).

In addition seven of the twelve atopic dogs which demonstrated a good clinical response to hyposensitisation were found to exhibit an increase in serum total IgG_1 concentrations varying from 51% to 583% of the original serum IgG_1 levels (Table 8.4, Fig. 8.2). This compares with three dogs which demonstrated a poor clinical response to hyposensitisation whose serum IgG_1 concentrations after six months were similar to or lower than the initial levels. No correlation was evident between the degree of increase in serum IgG_1 concentration and the degree of clinical improvement shown by an individual dog as the increase in IgG_1 ranged from 70mg/dl to 455mg/dl where a good response was found. However, comparison of the increase in serum total IgG_1 concentrations pre- and post-hyposensitisation in dogs with a good response and those with a fair or poor response revealed a significant difference between the two groups ($p<0.016$).

Twelve months after initiation of hyposensitisation therapy, serum IgG_1 concentrations available from two dogs were found to be higher than the initial serum IgG_1 concentrations but lower than the levels after six months therapy (Table 8.6). The serum IgG_1 concentration was available for one dog eighteen months after initiation of therapy and six months after the cessation of therapy. In this dog serum IgG_1 concentration was higher

eighteen months after therapy than at the initiation of therapy. In addition this dog still demonstrated a good clinical response to hyposensitisation.

Fig. 8.1 Serum total IgG1 concentration in atopic and non-atopic dogs

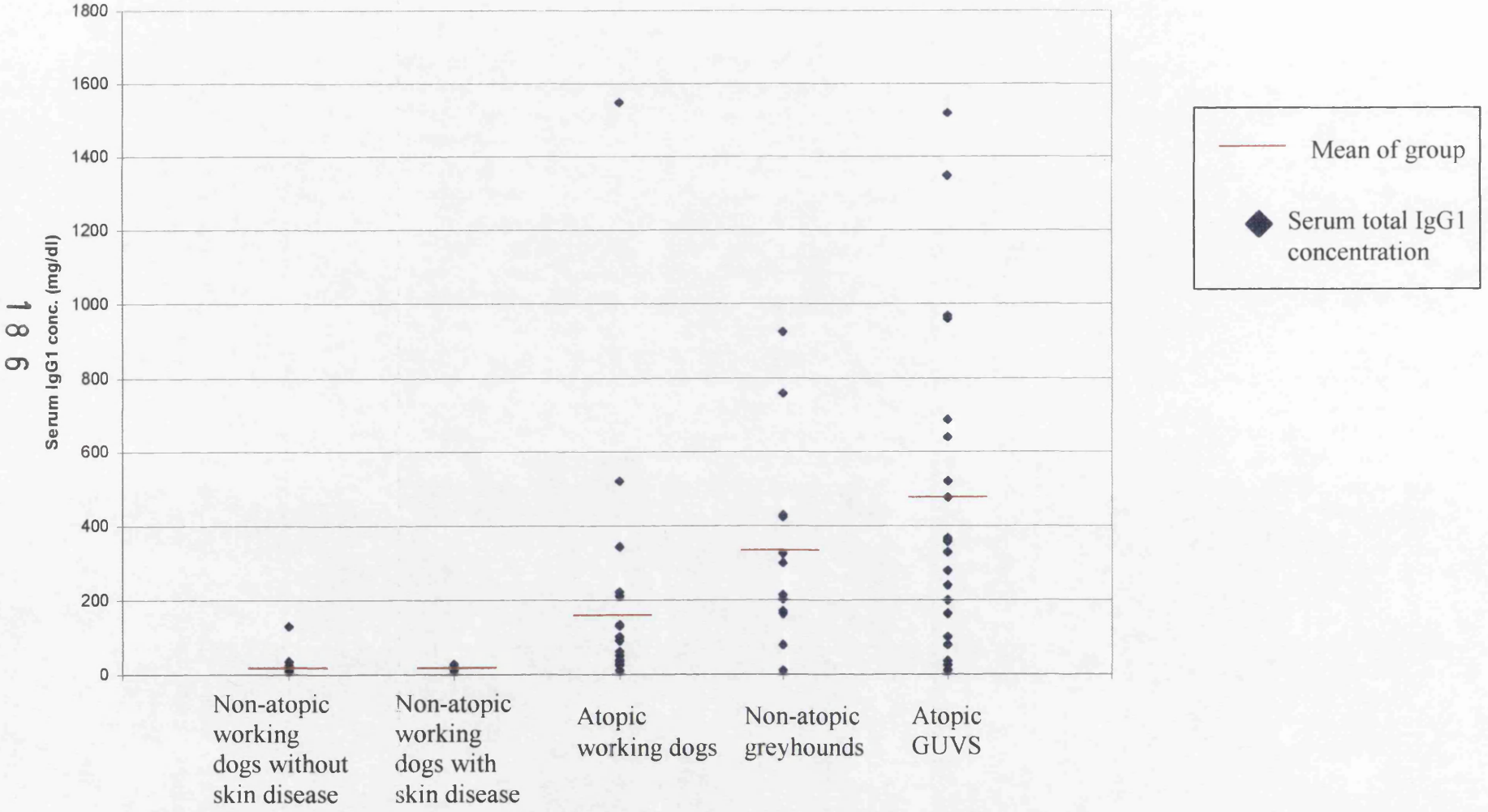


Table 8.1 Statistical analysis of serum total IgG_1 concentrations in different groups of atopic and non-atopic dogs.

a. Examination of serum IgG_1 concentrations.

Group	Number of dogs	Mean IgG_1 concentration (mg/dl)	Standard deviation
Non-atopic GDBA dogs without skin disease	13	24.6	32.6
Non-atopic GDBA dogs with skin disease	10	12.0	6.3
Atopic GDBA dogs	17	188.8	377.2
Non-atopic greyhounds	14	276.4	283.8
Atopic GUVS dogs	24	418.5	419.3

b. Results of Newman Keuls multiple range test on $\log_e$ of serum IgG_1 concentrations (See Appendix N):

Non-atopic working dogs with or without skin disease significantly lower than other three groups.

c. Correlation of serum total IgE and serum IgG_1 concentrations (See Appendix N):

Pearson correlation of 0.099. No correlation between serum total IgE and IgG_1.

Table 8.2. Allergens included in individual hyposensitisation vaccines

Dog Identification	Allergens in hyposensitisation vaccine
Palmer	*Dermatophagoides farinae, D. pteronyssinus, Acarus siro, Tyrophagus putrescentiae*
Paul	*D. farinae, D. pteronyssinus*, Fescue, Sorrel, Nettle
Kai	*D. farinae, D. pteronyssinus,* Timothy, Italian ryegrass, Cat dander, Human epithelium
Chris	*D. farinae, D. pteronyssinus, A. siro, T. putrescentiae,* Poplar, Horse Chestnut, Hazel, Elm.
Andrea	*D. farinae, D. pteronyssinus.*
Macauly	*D. farinae, D. pteronyssinus, A. siro, T. putrescentiae.*
Kerry	*D. farinae, A. siro*, T. *putrescentiae*, Mosquito.
Rhuri	Meadow fescue, Sweet vernal grass, Perennial ryegrass, Timothy
Brandy	*D. farinae, D. pteronyssinus, A. siro, T. putrescentiae*, Timothy, Plantain, Dandelion, Birch,
Kerry II	*D. farinae, D. pteronyssinus, A. siro, T. putrescentiae*, Orchard, Timothy, Fescue.
Budd	*D. farine, D. pteronyssinus, A. siro, T. putrescentiae*, Orchard, Fescue.
Sally	*D. farine, D. pteronyssinus, A. siro, T. putrescentiae*, Orchard

Table 8.3 Injection regime when administering hyposensitisation therapy.

Day of injection	Amount of vaccine injected subcutaneously
Day 1.	0.2ml
Day 15	0.4ml
Day 29	0.6ml
Day 43	0.8ml
Day 64	1.0ml
Day 85	1.0ml
Day 113	1.0ml
Day 141	1.0ml
Day 169	1.0ml

Injections of 1ml of vaccine are continued at four week intervals thereafter for the lifetime of the dog.

Table 8.4. Serum total IgG_1 concentrations in 12 atopic dogs before and after 6 months of hyposensitisation therapy

DOG	Environment	Breed	Initial IgG_1 conc. (mg/dl)	IgG_1 conc. after 6mths (mg/dl)	Difference between pre- & post-hyposensitisation.	Percentage increase	Clinical Response
1 Palmer.	Kennel	Golden RetrieverxLabrador	30	205	175	583%	good
2.Paul	Kennel	Golden RetrieverxLabrador	35	215	180	514%	good
3.Kai	Kennel	Golden RetrieverxLabrador	40	230	190	575%	good
4.chris	Kennel	Labrador	50	120	70	140%	good
5.Andrea	Kennel	German Shepherd Dog	135	65	-70	-52%	poor
6.Macauly	Home	German Shepherd Dog	165	250	85	51%	good
7.Kerry	Home	Terrier	200	445	245	122%	good
8.Rhuri	Home	German Shepherd Dog	365	820	455	125%	good
9.Brandy	Home	Dalmatian	25	120	95	380%	fair
10.Kerry II	Home	German Shepherd DogxLabrador	35	150	115	328%	fair
11.Budd	Home	Labrador	240	215	-25	-10%	poor
12.Sally	Home	Labrador	960	975	15	1%	poor

Fig 8.2 Serum total IgG1 concentration (mg/dl) before and after hyposensitisation therapy

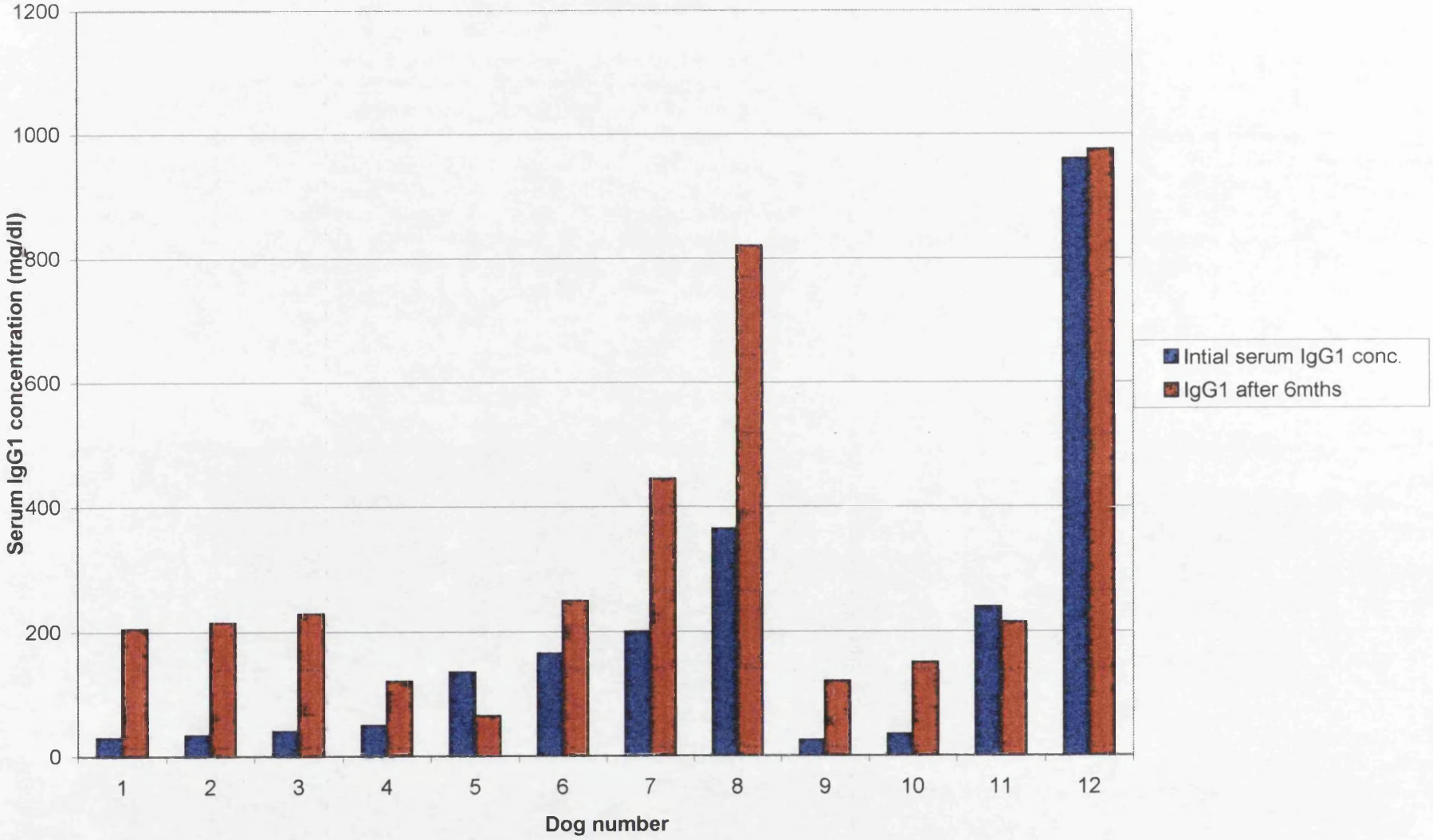


Table 8.5 Results of paired t test on the difference between serum total IgG_1 concentrations pre- and post-hyposensitisation

Mean = 127.5

Standard deviation = 138.6

$p = 0.0087$.

Therefore a significant difference was observed between the serum IgG_1 concentrations before and following 6 months hyposensitisation ($p<0.01$).

Table 8.6 Examination of change in serum total IgG_1 concentration post hyposensitisation in relation to clinical response.

	Good response	Poor/fair response
Number of dogs	7	5
Mean serum IgG_1 concentration (mg/dl)	200	26

$p = 0.016$

Therefore a significant difference was observed between the serum IgG_1 concentrations before and following 6 months hyposensitisation in those dogs with a good response compared to those dogs with a poor or fair response.

Table 8.7 Serum total IgG_1 concentrations in three dogs at 12 and 18 months after the initiation of hyposensitisation therapy.

Dog number	Initial IgG_1 (mg/dl)	6mths IgG_1 (mg/dl)	12 mths IgG_1 (mg/dl)	18mths IgG_1 (mg/dl)
2.	35	215	250	-
3.	40	230	130	150
8.	365	820	430	-

8.4 Discussion

Total serum IgG levels have been shown to be raised in both atopic and parasitised dogs (Hill *et al.*, 1995). This report demonstrates a similar pattern with the subgroup IgG_1. Serum levels of IgG_1 were significantly raised in both groups of atopic dogs as compared to non-atopic working dogs with or without skin disease. The reason for this increase in serum total IgG_1 levels in atopic dogs is unknown. Allergen specific IgG antibodies have been demonstrated in atopic dogs (Day *et al.*, 1996), but the exact conditions required for IgG_1 production have not yet been identified although genetics, route of administration and dosage of antigen have all been suggested as important (Day & Mazza, 1995).

No significant difference was found between non-atopic GDBA dogs with or without pyoderma. This suggests that although IgG_1 may be influenced by the presence of infection this effect is not as potent as hypersensitivity or parasitism.

From this finding it appears that as serum total IgG_1 concentrations are higher in atopic dogs than non-atopics and that the presence of pyoderma has no effect on total IgG_1 concentrations measurement of serum total IgG_1 could be incorporated into the predictive model for the diagnosis of atopic dermatitis in the GDBA population.

Serum IgG_1 levels were also raised in greyhounds known to be exposed to higher numbers of parasites than non-atopic GDBA dogs. It has previously been suggested that encysted larvae of *Toxocara canis* may influence serum levels of IgE (Hill *et al.*, 1995). There are no studies to demonstrate the effect such larvae might have on IgG_1 but the presence of such encysted larvae might perhaps explain the raised levels of serum IgG_1 found in dogs of unknown parasite status.

In general the serum IgG_1 levels found in the present study were lower than those described by Mazza *et al.* (1994), although the GUVS dogs and greyhounds were within those higher ranges. However, in that study no mention was made of the parasite control and parasitism might account for the higher levels of IgG_1.

The increase in serum IgG_1 levels following hyposensitisation therapy observed in this study follows a similar pattern to that found in human medicine. Serum antigen specific IgG_1 levels have been shown to increase following initiation of hyposensitisation and then decrease three months after beginning treatment (Aalberse, 1983). In the dogs studied here a similar picture was found where serum total IgG_1 concentrations increased initially and then decreased twelve months after beginning therapy. Although serum total IgG_1 concentrations twelve months after initiation of therapy were lower than those recorded after six months therapy they still remained higher than before beginning hyposensitisation. In the one dog where serum total IgG_1 concentrations were available eighteen months after beginning hyposensitisation serum total IgG_1 levels were still higher than at the beginning of therapy.

The fact that serum IgG_1 levels were found to remain above initial levels eighteen months after initiation of hyposensitisation would suggest that IgG_1 may be part of the long term response to hyposensitisation. Also this dog remained clinically well after six months without hyposensitisation, suggesting that IgG_1 may be linked to a clinical improvement.

Evidence to support the suggestion that an increase in serum IgG_1 concentrations is associated with clinical improvement is shown by the seven dogs which demonstrated an increase in serum IgG_1 concentrations and an obvious clinical improvement and the three dogs with a poor clinical response whose serum total IgG_1 concentrations following hyposensitisation were similar or lower than those at the beginning of therapy. Although only small numbers of dogs were examined here, this finding does suggest that an increase in serum total IgG_1 concentrations is associated with a good clinical response to hyposensitisation and further work is required in this area. As in most cases it can take 6-9 months for a clinical response to hyposensitisation to become evident (Reedy *et al.*, 1997c), it is possible that measurement of serum total IgG_1 concentrations could be used as an indicator of the clinical response a dog will make to hyposensitisation before that clinical response is evident.

It is not surprising that there is no correlation between the degree of increase in serum IgG_1 levels and the degree of clinical improvement as in a vaccine

response there is often no correlation between the size of increase in antibody levels – only that there is an increase.

Although two dogs with a large increase in serum IgG_1 levels were thought to only have made a minor clinical improvement, there are two possible explanations for this. Firstly, the clinical response was partly based on the owner's opinion which is subjective and influenced by each owner's expectations; secondly some dogs do not manifest any clinical response to hyposensitisation until they have received nine months' therapy. It is possible that these dogs are showing an increase in serum total IgG_1 levels prior to demonstrating a clinical response and that measurement of total IgG_1 levels will therefore prove useful in predicting a dog's response to hyposensitisation.

Further work on allergen specific IgG_1 levels following hyposensitisation would be useful in order to compare canine results with those of human medicine more fully. In addition as the IgG_1 test is quick and easy to run this may prove a valuable test in the examination of a dog's response to hyposensitisation.

8.5 Conclusion

In conclusion this study has demonstrated that concentrations of serum IgG_1 are greater in atopic dogs than those of non-atopic dogs. In addition dogs with stringent parasite control measures had lower serum total IgG_1 concentrations than dogs where measures were less stringent. This implies that in the assessment of canine serum total IgG_1 concentrations it is necessary to investigate the parasite status of a particular animal in order to determine whether or not any increase is due to an allergic or a parasitic pathogenesis.

In addition, although it has been demonstrated that in cases of pyoderma there is an increase in serum IgG concentrations this is not shown with the IgG_1 subgroup and therefore infection does not interfere with the assessment of serum IgG_1 concentrations.

Perhaps the most important finding was that the majority of serum concentrations of IgG_1 were raised following hyposensitisation suggesting that IgG_1 is involved in the response to hyposensitisation. In addition, although only small numbers were examined there is an indication that an increase in

serum IgG_1 concentrations is associated with a clinical response to hyposensitisation.

Thus it is evident that IgG_1 is important in canine atopic dermatitis and further work is required in this area. In addition it is also apparent that measurement of serum total IgG_1 concentrations can be incorporated into the GDBA predictive model for the diagnosis of atopic dermatitis.

Chapter 9. Final Discussion & Conclusions

9.1 Discussion

Canine atopic dermatitis is a disease which is commonly found in particular breeds and ages of dogs. Diagnosis can often be difficult with a number of different parameters being examined in any one dog.

The most obvious sign that a dog has atopic dermatitis is the clinical evidence of pruritus and skin lesions. Examination of the clinical presentation of dogs with atopic dermatitis and the types of skin conditions presented in this study agree with that described in the literature (Scott, 1981, Willemse & van den Brom, 1983, Willemse, 1986) – namely repeated episodes of otitis externa, conjunctivitis, and pedal dermatitis.

Atopic dermatitis in dogs is usually diagnosed when animals are over one year of age (Scott, 1981, Willemse, 1986). However, this study has demonstrated that it is possible to differentiate atopic and non-atopic dogs younger than one year of age. Indeed groups of atopic and non-atopic dogs had significant differences in the number of episodes of skin disease by the age of nine months. It is possible that most atopic dogs in the general population demonstrate atopic dermatitis at this young age. Most published papers on the age of presentation of atopic dermatitis are from referral clinics (Halliwell & Schwartzman, 1971; Scott, 1981; Willemse 1986) and dogs will obviously be older before they are referred to such hospitals.

It was possible to demonstrate that the best predictive model for the diagnosis of atopic dermatitis in an individual dog was the presence of four or more episodes of atopic type skin disease (as described by Willemse, 1986) by the age of fifteen months. It was also shown that this finding could be used as a diagnostic indicator for individual GDBA dogs. Although this is within the age limit recorded in the literature for the diagnosis of atopic dermatitis (Scott, 1981) it is towards the younger end of the range. In addition it would coincide with the beginning of GDBA dog training and if a diagnosis of atopic dermatitis could be made at this age then the dog could be withdrawn from the working dog population.

The role of the antibody, IgE, in the pathogenesis of atopic dermatitis is undisputed. Some further information as to the role of serum total IgE in the diagnosis of atopic dermatitis in the dog has been gleaned in this study. In human medicine, serum concentrations of total IgE have been found to be greater in atopics than in non-atopics (Gurevitch *et al.*, 1973, Juhlin *et al.*, 1969, Jones *et al.*, 1975, Ogawa *et al.*, 1971 and Wittig *et al.*, 1980). In addition serum IgE concentrations have been shown to be associated with the degree of disease at the time of sampling, with higher levels recorded when atopic people are suffering from active skin disease (Jones *et al.*, 1975, Johansson & Juhlin, 1970). It had already been suggested that the general dog population differed, (Halliwell & Kunkle, 1978, Schwartzman & Rockey, 1967) with serum IgE concentrations in atopic and non-atopic dogs being indistinguishable. Indeed dogs have been shown to have much greater concentrations of serum total IgE than humans (Schwartzman & Rockey, 1967). This has always been assumed to be due to the high levels of parasitism believed to exist in dogs (Halliwell & Kunkle 1978).

Results in this study have shown that in GDBA dogs with no history of parasite burdens, there is still no correlation between serum total IgE concentrations and the presence of atopy. Indeed non-atopic GDBA dogs studied here were found to have a wide range of serum total IgE concentrations which could not be differentiated from the atopic GDBA or GUVS dogs. Therefore serum total IgE cannot be used as a diagnostic indicator in the diagnosis of atopy in the dog even when the influence of parasitism is excluded.

Non-atopic GDBA dogs showed a wide distribution of serum total IgE concentrations. These findings are similar to work by Katz (1978) and de Weck *et al.*, (1997). In work by de Weck *et al.*, (1998) only high responders (i.e. dogs with high levels of serum total IgE) went on to develop atopic dermatitis. Examination of the non-atopic dogs in the GDBA population revealed dogs with serum total IgE concentrations towards the high end of the range. From de Weck's results these dogs would be expected to develop atopic dermatitis. However, this is not the case as many of these dogs were over five years of age and are thus unlikely to develop atopic dermatitis. This suggests

that the presence of high serum total IgE concentrations is associated with the development of atopic dermatitis but that this alone is not responsible for the development of atopic dermatitis and that other factors are involved.

Conversely de Weck *et al.*, (1998) observed that atopic dogs were within the group of high responders. However, in this present study atopic dogs were found with lower than expected serum total IgE concentrations. One possible explanation for this is that dogs had received some form of steroid therapy within three months of the tests. As corticosteroids have an immunosuppressive effect it is possible that they can depress serum total IgE concentrations. This agrees with recent work by McCall *et al.*, (1998) where it has been suggested that corticosteroid therapy does indeed reduce concentrations of serum total IgE.

Theoretically then, based on the work of de Weck, it is possible to use measurements of serum total IgE concentrations, in order to find out if a dog is a 'high' or 'low' responder and to use this as an indicator of the likelihood of an animal developing atopic dermatitis,. If a dog is a 'low' responder (possibly less than the mean reflective density result of 0.182) then it would be less likely that that animal would be atopic. However, if the dog is a 'high' responder (*i.e.* reflective density above 0.182) then it does not confirm that the dog is atopic, but indicates the possibility of the dog becoming atopic. However, applying this data to the atopic GDBA population in this study this was not found to be the case as atopic dogs can have low serum total IgE concentrations and further work is required to identify all the factors controlling IgE production.

In human medicine serum total IgE concentrations have been shown to increase with age until the mid-twenties. Examination of the age of an animal in relation to serum total IgE concentrations did not reveal any correlation in non-atopic dogs. This suggests that it is not necessary to take into account the age of an animal when assessing serum total IgE concentrations and that age had no bearing on the results found in the present study. This contrasts with recent work by Racine *et al.*, (1999) who found that serum total IgE concentrations increased in a population of beagles until the age of 4 years. It is possible that the differences between this present study and that of Racine *et*

al., are due to the different methods of serum total IgE analysis used in each study - Racine's study used an ELISA whereas this present study used the Immunodot method to detect serum total IgE concentrations.

Examination of serum IgG_1 concentrations in atopic and non-atopic dogs revealed some important findings. IgG antibodies develop in normal dogs in response to allergens but the concentrations of serum total IgG has been shown to be significantly increased in dogs suffering from atopy, infections and parasitism (Hill, *et al.*, 1995). In atopic humans the response to allergens has been shown to be dominated by subgroups IgG_1 and IgG_4.

This study has shown that in the dog serum total IgG_1 concentrations are increased in dogs affected by atopic dermatitis and exposed to parasites. Non-atopic dogs with less stringent parasite control measures had significantly higher concentrations of serum IgG_1 than all GDBA dogs. Thus it seems likely that IgG_1 is influenced by both the presence of parasites and allergies. Although infection has been shown to have an influence on serum total IgG concentrations, examination of serum total IgG_1 concentrations in non-atopic dogs with or without skin disease did not reveal any significant difference. This is important because it means that examination of serum total IgG_1 concentration can be used as a diagnostic indicator in the diagnosis of atopic dermatitis in GDBA dogs even where there is concurrent skin disease.

Perhaps the most exciting finding was the observation that there is an increase in serum total IgG_1 concentration in dogs after receiving hyposensitisation vaccinations. Although previous work (Hites *et al.*, 1989) has demonstrated that there is an increase in serum total IgG levels after hyposensitisation, no work has been carried out on the subgroup IgG_1. In addition, in the published papers no mention has been made of the clinical response of the dogs that were receiving hyposensitisation vaccinations. In this study an increase was found in serum total IgG_1 concentrations post hyposensitisation. However, the degree of increase in different dogs was variable. Comparison of pre- and post-hyposensitisation serum total IgG_1 concentrations revealed a significant difference in the change in IgG_1 concentrations between dogs with a good response and those with a fair/poor response ($p<0.016$).

Although the numbers of dogs in this study were small, there is enough evidence to warrant further investigation of these findings. It is possible that

the measurement of serum total IgG_1 concentrations could be used as an indicator of the clinical response of an animal receiving hyposensitisation therapy before a clinical response is evident.

The clinical presentation of dogs with atopic dermatitis has been shown to be seasonal (Halliwell & Schwartzman, 1971). In another study, the production of serum allergen specific IgE was shown not to be seasonal (Miller *et al.*, 1992a). However, this present study has demonstrated that there is indeed a seasonal variation in allergen specific IgE concentrations in a group of non-atopic dogs when a sufficiently large number of dogs are examined over a three year period. This was found to be most evident for outdoor allergens but was not present for indoor allergens such as house dust mites possibly due to the greater seasonal variation in outdoor allergen concentrations than indoor allergens. In addition when examining the serological allergen specific results of non-atopic GDBA dogs, many dogs gave results which would have been considered indicative of atopic dermatitis based on the manufacturer's design of the test. This suggests that exposure levels need to be considered when using IgE assays as indicators of atopic dermatitis.

The author has also utilised a novel method of detecting which allergens dogs are exposed to. By examining faecal samples it was possible to detect the pollens to which dogs were exposed. The technique demonstrated that dogs were exposed to a wide variety of pollens including pine which is not at present included in the IDST panel. Fecal pollen counts could be used to tailor IDST or serology panels for individual dogs based on their exposure. Another possible use is for the identification of allergens thought not to be important in human allergies which may be important in canine atopy.

In examining the results of serological tests large numbers of positive results which did not correspond with IDST results were found. ELISA results alone demonstrated a significantly higher level of serum IgE specific for moulds in atopic dogs than non-atopic dogs, although very few of these results would have been considered positive by the ELISA test manufacturers. A number of other authors (Griffin *et al.*, 1990, Codner & Lessard, 1993, Anderson & Sousa, 1993, Day *et al.*, 1996) have demonstrated finding increased allergen specific IgE results for mould allergens in atopic dogs compared to non-

atopics, when using the ELISA method, but did not offer an explanation. However, it seems likely that it is due to the design of the ELISA test as this phenomenon does not occur with the Immunodot serological test and may be due to non-specific binding of IgE. Whatever the reason, as there was a significant difference in this response between atopic and non-atopic dogs it is possible that this test could be used as a diagnostic parameter in the diagnosis of atopy in the GDBA dogs.

The level of exposure to allergens was also found to be important when serological results of different groups of dogs were examined. This was most evident between non-atopic greyhounds and beagles where serum IgE specific for grass allergens was found to be significantly higher in greyhounds than beagles. This is probably due to the fact that greyhounds are exposed to high levels of grass, whereas laboratory beagles are not directly exposed to grass at all, although whether beagles come into contact with grass pollens or not is not known. It was also shown that the non-atopic greyhounds had significantly higher concentrations of grass specific serum IgE than atopic GDBA and GUVS dogs and that the mean of fescue specific IgE concentrations of non-atopic greyhounds was above that considered as positive by the manufacturer. From this it can be seen that exposure levels need to be taken in consideration when assessing if an animal may be allergic to a specific allergen, as high exposure levels may give a false positive result as far as a diagnosis of atopy is considered.

No correlation was found to be present between high serum total IgE concentrations and the number of positive ELISA results. Other authors (Griffin *et al.*, 1990; Codner & Lessard, 1993) have demonstrated that positive ELISA results could be obtained in non-atopic dogs if there was a high concentration of serum total IgE due to non-specific binding of IgE in the ELISA. Although that was not the case in this study, it is possible that it was due to the fact that most positive results were obtained with the ELISA test whereas serum total IgE concentrations were assessed with the Immunodot test.

The correlation between IDST and serological tests was, in general, poor. This agrees with previous work by other authors (Anderson & Sousa, 1993, Codner & Lessard, 1993, Bond *et al.*, 1994) where no agreement could be found

between IDST and serological test methods. It was suggested that this was due to the IDST and serological tests being produced by different manufacturers and that these tests incorporated different antigenic epitopes. This was further illustrated by the good correlation found by Esch, (1997) when the same epitopes were incorporated into both IDST and serological tests. The best correlation in this present study was found between the Immunodot IDST for house dust mites, illustrating that there is probably some overlap in the epitopes detected by both tests.

9.2 Conclusions

In conclusion this study has led to the identification of a number of novel parameters which can be used in the diagnosis of canine atopic dermatitis. These findings can be usefully applied both to the group of GDBA dogs studied and to the wider canine population.

In summary:

- Dogs from the GDBA population which demonstrate four or more episodes of atopic type skin disease before fifteen months of age are at an increased risk of developing atopic dermatitis
- Serum total IgE concentrations are unrelated to the assumed parasite status of a dog population
- Both high and low responders are present even in a dog population with long-term rigorous parasite control and high responders do not necessarily go on to develop atopic dermatitis
- There is no correlation between serum total IgE concentration and age
- Total serum IgE concentration cannot be used in the diagnosis of canine atopic dermatitis
- There is a significant difference in serum total IgG_1 concentration between atopic and non-atopic groups of dogs in a population with long term rigorous parasite control.
- Serum total IgG_1 concentrations in atopic dogs rise after hyposensitisation and there is a statistically significant difference in IgG_1 levels between those dogs which respond to hyposensitisation and those which do not
- Agreement between IDST results and serological results was poor

- In the population examined atopic dogs have a variety of recurrent skin disease and as a group have an earlier onset of any sort of skin problems and a greater number of episodes of skin disease than non-atopics
- Atopic dogs have higher levels of allergen specific serum IgE directed against moulds than non-atopics and are more likely to have a positive ELISA results for mould specific (*Alternaria)* IgE as determined by ELISA
- It is possible to demonstrate canine pollen exposure by faecal analysis
- There is a seasonal variation in allergen specific serum IgE concentrations in the dog
- Exposure to allergens influences serum allergen specific IgE concentrations such that non-atopic dogs can demonstrate positive serological results

Thus by applying these findings to the environmental, clinical and serological examination of GDBA dogs a more reliable diagnosis of atopic dermatitis can be made at a younger age than is currently possible.

9.3 Further work

Areas in this study where further work could be carried out are:

- Studies of serum total IgG_1 in a larger group of dogs receiving hyposensitisation vaccinations
- Faecal pollen analysis over a twelve month period and relate this to the pollen calendar
- Incorporate pollens identified on faecal analysis in IDST.
- Examine serum total IgE concentrations in greyhounds as a breed and in relation to parasite status
- Analyse serum IgE specific for *Malassezia* and relate this to *Alternaria/Aspergillus* specific serum IgE
- Examine seasonal variations in serological results in a larger group of dogs, with the same number of dogs examined every month
- Implement predictive model, including both clinical and serological findings, to the GDBA population to determine practical applications of these results

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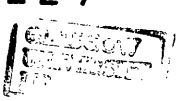

Clinical & Serological Studies

Of

Canine Atopic Dermatitis

by

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Dissertation submitted for the Degree of Doctor of Philosophy

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Volume 2 - Appendix

Appendix

Appendix A

Demographic Data of Dogs Included in Clinical and Serological Studies.

Appendix A1 Atopic and non-atopic GDBA dogs in clinical study

Atopic dogs

Name	Breed	Sex
Clova	Labrador	Female
Cassie	Golden Retriever	Female
Carlo	Golden Retriever x Labrador	Male
Cedar	Golden Retriever x Labrador	Female
Estelle	Golden Retriever x Labrador	Male
Enton	Labrador x Golden Retriever/Labrador	Male
Freya	Labrador	Female
Griff	Golden Retriever	Male
Kai	Golden Retriever x Labrador	Male
Illya	Labrador	Female
Jos	Golden Retriever x Labrador	Female
Opal	Labrador	Female
Pedro	Golden Retriever x Labrador	Male
Prince	Labrador	Male
Venice	Labrador	Female

Non-atopic dogs

Name	Breed	Sex
Alice	Labrador x Golden Retirever/Labrador	Female
Barney	Labrador	Male
Bertie	Golden Retriever x Labrador	Male
Brodie	Labrador x Golden Retriever	Male
Bunty	Retriever	Female
Clancy	Labrador x Labrador/Curly Coated Retriever	Female
Duke	Labrador	Male
Godfrey	Labrador/Golden Retriever x Golden Retriever	Male
Innis	Golden Retriever x Labrador	Male
June	Labrador x Golden Retriever	Female
Nyle	Labrador	Male
Quintus	Labrador	Male
Wayne	Golden Retriever x Labrador	Male
Winnie	Labrador	Female
Yvette	Labrador	Female

Appendix A2 Non-atopic GDBA dogs included in serological studies

	Name	Breed	Description	Age at sampling (days)
1.	Aaron	GSD	Tan	281
2.	Abbie	GSD	Tan	524
3.	Andy	LxGR	Yellow	1861
4.	Angus	LxGR	Black	261
5.	Babs	GSD	Tan	519
6.	Barley	L	Yellow	452
7.	Barney	L	Yellow	2365
8.	Becky	L	Yellow	472
9.	Betsy	GRxL	Yellow	464
10.	Betsy II	GRxL	Yellow	429
11.	Blair	GRxL	Yellow	291
12.	Bobby	L	Yellow	355
13.	Bradley	L	Yellow	1139
14.	Briar	FCR	Black	1168
15.	Brodie	GSD	Tan	836
16.	Broom	CCR	Black	90
17.	Callum	L	Black	265
18.	Carmel	L	Other	56
19.	Casper	L	Black	2375
20.	Cedar	L	Black	393
21.	Cherie	L	Yellow	615
22.	Chips	L	Yellow	255
23.	Clive	GRxL	Yellow	668
24.	Clover	L	Black	473
25.	Craig	L	Yellow	3161
26.	Curtis	L	Black	644
27.	Daisy	GRxL	Yellow	69
28.	Dalby	GRxL	Yellow	374
29.	Daniel	L	Black	684
30.	Darcy	L	Black	234
31.	Delaney	GRxL	Yellow	279
32.	Duke	GRxL	Yellow	1506
33.	Duncan	GR	Golden	507
34.	Ellie	L	Black	351
35.	Emily	L	Yellow	1005
36.	Eva	GRxL	Black	1833
37.	Farley	L	Yellow	759
38.	Freya	L	Black	392
39.	Gabby	GR	Golden	461
40.	Gabby II	GR	Golden	429

	Name	Breed	Description	Age at sampling (days)
41.	Gaynor	L	Yellow	601
42.	Glen	L	Yellow	634
43.	Glennie	GSD	Tan	344
44.	Grace	GR	Golden	457
45.	Harry	GR	White	245
46.	Henry	GR	Golden	284
47.	Henry II	GR	Golgen	465
48.	Hunter	GRxL	Yellow	262
49.	Ike	GSD	Tan	318
50.	Illis	GRxL	Yellow	276
51.	Isla	L	Yellow	747
52.	Judy	GR	Golden	428
53.	Katy	L	Yellow	439
54.	Kay	GR	Golden	404
55.	Lionel	L	Yellow	608
56.	Lucy	L	Yellow	554
57.	Magnus	CCRxL	Black	258
58.	Martha	GRxL	Yellow	407
59.	Mary	GR	Golden	215
60.	Max	L	Yellow	638
61.	Mel L	GRxL	Yellow	1039
62.	Melody	GRxL	Yellow	1500
63.	Mick	L	Yellow	160
64.	Morven	GR	Golden	1149
65.	Nash	L	Yellow	542
66.	Nelson	L	Yellow	542
67.	Norma	GRxL	Yellow	423
68.	Onyx	LxGR	Yellow	1708
69.	Paddy	LxGR	Yellow	691
70.	Pascoe	GRxL	Black	280
71.	Perry	GRxL	Yellow	793
72.	Phoebe	GRxL	Yellow	564
73.	Pippa	L	Yellow	781
74.	Quizz	GR	Golden	439
75.	Ria	L	Yellow	401
76.	Rigsby	L	Yellow	251
77.	Ryan	GRxL	Yellow	270
78.	Sally	GR	Golden	455
79.	Sally II	GSD	Tan	520
80.	Sherry	GRxL	Yellow	472
81.	Sid	L	Black	544
82.	Star	GR	Golden	442
83.	Tara	L	Black	882
84.	Teal	GRxL	Black	247
85.	Thomas	L	Yellow	544

	Name	Breed	Description	Age at sampling (days)
86.	Velma	GRxL	Yellow	396
87.	Victor	L	Black	253
88.	Wade	CCRxL	Black	230
89	Wellington	L	Yellow	537
90.	William	GR	Golden	526
91.	Willow	L	Black	491
92.	Wilson	CCRxL	Black	858
93.	Yogi	GRxC	Yellow	308
94.	Yuma	GRxL	Black	73
95.	Yusef	LxGR	Yellow	1773
96.	Zeus	LxGR	Yellow	466

Appendix A3 Atopic GDBA dogs included in serological studies

	Name	Breed	Description	Age at sampling (days)
1.	Abel	GSD	Black & Tan	2689
2.	Alana	GSD	Black & Tan	1709
3.	Andrea	GSD	Black & Tan	1325
4.	Carlo	GRxL	Yellow	429
5.	Cedar II	GRxL	Yellow	565
6.	Chris	L	Yellow	2687
7.	Claire	L	Yellow	2892
8.	Dusty	GRxL	Yellow	1830
9.	Griff	GR	Golden	1820
10.	Harris	LxGR	Yellow	630
11.	Herbie	GRxL	Yellow	125
12.	Keaton	L	Yellow	413
13.	Kai	GRxL	Yellow	784
14.	Opal	L	Yellow	1473
15.	Palmer	GRxL	Black	916
16.	Paul	GRxL	Black	908
17.	Pedro	GRxL	Black	796
18.	Reo	L	Yellow	776

Appendix A4 Non-atopic greyhounds included in serological studies

Dog identification	Age at sampling (years)*
1. 334	3
2. 335	4
3. 337	4
4. 338	3
5. 340	4
6. 341	4
7. 342	3
8. 343	3
9. 344	6
10. 345	6
11. 346	6
12. 347	6
13. 348	6
14. 349	6
15. 350	3
16. 371	3
17. 372	5
18. 373	12
19. 374	5
20. 381	4
21. 408	3
22. 460	3
23. 496	3

* Accurate ages in days were not available for these dogs

Appendix A5 Non-atopic beagles included in serological studies

	Dog identification	Age at sampling (years)*
1.	14985	3
2.	14989	3
3.	15363	3
4.	15364	3
5.	15923	4
6.	15926	4
7.	15945	3
8.	15946	3
9.	15947	3
10.	15948	5
11.	16161	5
12.	16166	5
13.	16167	3
14.	16168	5
15.	16169	5
16.	16615	3
17.	16619	4
18.	16636	3
19.	16660	3
20.	16663	3
21.	16849	3
22.	16850	3
23.	16860	6
24.	16863	3
25.	16867	4
26.	16868	3
27	16869	5
28.	17527	4
29.	17531	4
30.	17536	3
31.	17541	4
32.	17545	3
33.	17550	4

* Accurate ages in days were not available for these dogs

Appendix A6 GUVS atopic dogs included in serological studies

	Name	Breed	Description	Age at sampling (days)
1.	Alfred	GSP	Brown	1266
2.	Ben	Bull Terrier	Brown	1100
3.	Brandy	Dalmatian	Black & White	750
4.	Budd	Labrador	Yellow	915
5.	Fudge	Labrador	Yellow	815
6.	Holly	Yorkshire Terr	Black & Tan	820
7.	Islay	SBT	Black	2770
8.	Jess	Cross breed	Black	1440
9.	Jock	Labrador	Yellow	640
10.	Kara	Cross Breed	Bloack	770
11.	Kassie	GSD	Black & Tan	468
12.	Kayla	Boxer	Brown	550
13.	Kerry	Lakeland Terr	Brown	643
14.	Kerry II.	GSDxL	Tan	2187
15.	Kim	Labrador	Yellow	1865
16.	Kyle	Irish Setter	Red	2941
17.	Libby	Bulldog	Black	607
18.	Lucy	English Setter	Tricolour	730
19.	Lucy II	Retriever	Golden	607
20.	Luke	Great Dane	Blue	388
21.	Macauly	GSD	Black &Tan	305
22.	Maisie	WHWT	White	2653
23.	Megan	Labrador	Yellow	2521
24.	Molly	JRT	Brown & White	2203
25.	Olwen	I Wolfhound	Grey	498
26.	Rhuri	GSD	Black & Tan	719
27.	Raver	SBT	Black	1454
28.	Sally	Labrador	Black	1092
29.	Sammie	G Retriever	Golden	2166
30.	Shane	GSD	Black & Tan	1007
31.	Shannon	Cairn Terrier	Black	730
32.	Sheena	Cross Breed	Black	2520
33.	Shogun	GSD	Black & Tan	1640
34.	Shona	Cairn Terrier	Black	740
35.	Skerry	SBT	Black	1939
36.	Tanya	GSD	Black & Tan	1470
37.	Teena	Cross breed	Black	1081
38.	Tia	Cross breed	Black	1490
39.	Toby	Labrador	Yellow	1825
40.	Tyson	Boxer	Brown	288

Appendix A7 GDBA dogs with negative IDST and recurrent skin disease included in serological studies

	Name	Breed	Description	Age at sampling (days)
1.	Barry	GSD	Black & Tan	1989
2.	Riley	CCR	Chocolate	246

Appendix A8 GUVS dogs with recurrent skin disease but negative IDST results included in serological studies

	Name	Breed	Description	Age at sampling (days)
1.	Buster	GSD	Black & Tan	1492
2.	Kyle	Keeshond	Black & Tan	1103
3.	Oscar	GR	Golden	1929
4.	Oscar II	Cross breed	Black	2885
5.	Skye	GSP	Tan	513
6.	Winston	Rhodesian Ridgeback	Tan	710
7.	Zac	GSD	Black & Tan	1629

Appendix B

Numbers of episodes of different skin conditions in atopic and non-atopic GDBA dogs

&

statistical analysis of this data.

Appendix B1 Cumulative number of episodes of conjunctivitis at particular ages in atopic and non-atopic GDBA dogs.

Dog No.	Atopic Guide dogs - age							Non-atopic guide dogs - age						
	3 mths	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth
1	0	0	0	1	1	3	6	1	1	1	1	1	5	6
2	0	0	0	0	0	0	0	0	0	0	1	3	3	3
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	2	2	0	0	0	0	0	0	0
5	0	0	0	0	0	1	1	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	2	4	9	10	0	0	0	0	0	0	0
9	0	0	1	1	2	2	2	0	0	0	0	0	0	0
10	0	0	0	0	1	3	3	0	0	0	0	0	0	1
11	0	1	2	2	3	5	5	0	0	0	0	0	0	0
12	0	0	0	0	2	2	2	0	0	0	0	0	0	0
13	0	0	0	0	1	1	1	0	0	0	1	1	1	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	2	6	8	0	0	0	0	0	0	0

Appendix B2 Cumulative number of episodes of otitis externa at particular ages in atopic and non-atopic GDBA dogs.

Dog No.	Atopic Guide dogs - age							Non-atopic guide dogs - age						
	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth
1	0	0	0	1	1	2	4	1	1	1	1	1	2	3
2	0	0	0	1	1	2	5	0	0	0	0	0	1	2
3	0	0	0	0	1	2	5	0	0	0	0	0	1	1
4	0	0	0	0	0	4	7	0	0	0	0	1	2	3
5	0	0	0	2	3	5	6	0	0	0	0	0	0	1
6	1	2	3	4	8	8	8	0	0	0	0	1	5	7
7	0	0	0	0	0	3	3	0	0	0	0	1	2	2
8	0	0	0	0	2	10	15	0	0	0	0	0	1	1
9	0	0	0	0	0	0	1	0	0	0	0	1	5	6
10	0	0	0	1	1	2	2	0	0	0	0	2	3	4
11	0	0	0	0	0	1	1	0	0	0	0	1	2	3
12	0	0	3	4	5	5	5	0	0	0	0	1	2	2
13	0	0	0	3	6	10	11	0	0	0	0	1	1	1
14	0	1	3	3	8	9	9	0	0	0	0	0	0	0
15	0	0	0	1	5	6	8	0	0	0	0	0	0	0

Appendix B3 Cumulative number of episodes of pedal dermatitis at particular ages in atopic and non-atopic dogs.

Dog No.	Atopic Guide dogs - age							Non-atopic guide dogs - age						
	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth
1	1	1	1	1	2	2	2	0	0	0	0	1	1	1
2	0	0	0	0	0	1	5	0	0	0	0	0	0	4
3	0	0	0	0	0	1	1	0	0	0	1	1	1	1
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	3	0	0	0	0	1	1	1
6	0	0	0	0	1	1	1	0	0	0	0	0	0	0
7	0	0	0	0	0	1	1	0	0	0	0	0	0	0
8	0	0	0	0	0	2	7	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	1	1
10	0	0	0	0	1	1	2	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	1	1
12	0	0	0	0	1	2	2	0	0	0	1	1	3	4
13	0	0	0	0	0	0	1	0	0	0	0	0	0	0
14	0	1	1	1	1	2	2	0	0	0	0	0	0	1
15	0	0	0	0	0	1	1	0	0	0	0	0	0	0

Appendix B4 Cumulative number of episodes of pyoderma at particular ages in atopic and non-atopic GDBA dogs

Dog No.	Atopic Guide dogs - age							Non-atopic guide dogs - age						
	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth
1	0	0	0	0	1	1	1	0	0	0	0	0	0	1
2	0	0	0	0	0	0	0	0	0	0	0	1	1	1
3	0	0	0	0	0	0	0	0	0	0	1	2	2	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	1	4	0	0	0	0	0	0	1
6	0	1	4	4	5	10	11	0	0	0	0	1	1	1
7	0	0	0	1	1	1	1	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	1	1	1	0	0	0	0	0	0	0
10	0	0	1	3	3	3	3	0	0	0	1	1	1	1
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	1	1	1	1	0	0	0	0	0	0	0
13	0	0	2	2	2	3	3	0	0	0	0	0	1	1
14	0	0	1	1	2	2	3	0	0	0	1	1	1	1
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix B5 Cumulative number of episodes of atopic type skin disease at particular ages in atopic and non-atopic GDBA dogs

Dog No.	Atopic Guide dogs - age							Non-atopic guide dogs - age						
	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth
1	1	1	1	3	5	8	14	2	2	2	2	3	8	11
2	0	0	0	1	1	4	11	0	0	0	1	4	5	10
3	0	0	0	0	1	3	6	0	0	0	2	3	4	4
4	0	0	0	0	0	6	9	0	0	0	0	1	2	3
5	0	1	1	3	4	8	15	0	0	0	0	1	1	3
6	1	3	7	8	14	19	20	0	0	0	0	2	6	8
7	0	0	0	1	1	5	5	0	0	0	0	1	2	2
8	0	0	0	2	6	21	32	0	0	0	0	0	1	1
9	0	0	1	1	3	3	6	0	0	0	0	1	6	7
10	0	0	1	4	6	10	11	0	0	1	1	3	4	6
11	0	1	2	2	3	6	6	0	0	0	0	1	3	4
12	0	0	3	5	9	10	11	0	0	0	1	2	5	6
13	0	0	2	5	9	15	17	0	0	0	1	2	3	3
14	0	2	5	5	11	13	14	0	0	0	1	1	1	2
15	0	0	0	1	7	13	17	0	0	0	0	0	0	0

Appendix B6 Cumulative number of episodes of all skin conditions (except warts and bedsores) at particular ages in atopic and non-atopic GDBA dogs

Dog No.	Atopic Guide dogs - age							Non-atopic guide dogs - age						
	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth	3 mth	6 mth	9 mth	12 mth	15 mth	18 mth	21 mth
1	1	1	1	3	5	8	13	2	2	2	2	3	8	11
2	0	0	0	1	3	5	12	0	0	0	1	4	5	10
3	0	0	0	0	1	3	6	0	0	0	3	4	5	5
4	0	0	0	4	6	13	18	0	0	0	0	3	4	5
5	0	1	1	3	4	8	15	0	0	0	0	1	2	6
6	1	3	7	8	15	21	22	0	0	0	0	3	7	9
7	0	0	0	2	2	6	6	0	0	0	0	2	3	3
8	0	0	0	2	6	21	32	0	0	0	0	0	1	2
9	0	0	1	1	3	3	6	0	0	0	0	1	6	9
10	0	0	1	4	6	10	11	0	0	1	1	3	4	6
11	0	1	2	2	3	6	6	0	0	0	0	2	4	5
12	0	0	3	5	9	10	11	0	0	0	1	3	6	7
13	0	0	2	6	10	16	19	0	01	0	2	3	5	5
14	0	3	6	6	12	14	15	0	0	1	3	3	6	8
15	0	0	0	1	9	15	19	0	0	0	0	0	0	0

Appendix C

90% non-parametric confidence interval analysis of the duration of episodes of atopic type skin conditions in 15 atopic GDBA dogs between birth and 21 months of age.

```
MTB > print c1-c5
```

Data Display

Row	Conj.	Otitis	Pedal	AMD	other
1	*	*	*	17	*
2	*	*	7	*	*
3	5	*	*	*	*
4	26	*	*	*	*
5	*	*	*	12	*
6	*	5	*	*	*
7	*	*	7	*	*
8	*	7	*	*	*
9	*	7	*	*	*
10	*	*	7	*	*
11	7	*	*	*	*
12	17	*	*	*	*
13	7	*	*	*	*
14	*	7	*	*	*
15	*	*	*	10	*
16	*	*	5	*	*
17	*	7	*	*	*
18	*	7	*	*	*
19	7	*	*	*	*
20	5	*	*	*	*
21	*	*	*	10	*
22	*	*	*	7	*
23	*	*	*	5	*
24	*	*	5	*	*
25	*	*	*	*	3
26	*	5	*	*	*
27	*	*	*	20	*
28	*	*	*	7	*
29	*	22	*	*	*
30	*	7	*	*	*
31	*	10	*	*	*
32	*	*	*	7	*
33	*	*	*	5	*
34	*	7	*	*	*
35	*	7	*	*	*
36	*	*	*	14	*
37	7	*	*	*	*
38	*	5	*	*	*
39	5	*	*	*	*
40	*	7	*	*	*
41	7	*	*	*	*
42	7	*	*	*	*
43	7	*	*	*	*
44	5	*	*	*	*
45	*	*	*	14	*
46	7	*	*	*	*
47	*	*	*	*	*

```
MTB > Describe mean median c1
```

Descriptive Statistics

Variable	N	N*	Mean	Median	Tr Mean	StDev	SE Mean
Conj.	14	33	8.50	7.00	7.33	5.85	1.56

Variable	Min	Max	Q1	Q3
Conj.	5.00	26.00	5.00	7.00

```
MTB > SInterval 95.0  'Conj.'.
```

Sign Confidence Interval

```
Sign confidence interval for median

                                 Achieved
             N    N*   Median   Confidence     Confidence Interval   Position
Conj.       14    33    7.000       0.9426   (    5.000,    7.000)          4
                               *     0.9500   (    5.000,    7.000)        NLI
                               *     0.9871   (    5.000,    7.000)          3
```

```
MTB > Describe mean median c2
```

Descriptive Statistics

```
Variable       N       N*      Mean    Median  Tr Mean     StDev   SE Mean
Otitis        14       33      7.86      7.00      6.92      4.26      1.14

Variable     Min      Max        Q1        Q3
Otitis      5.00    22.00      6.50      7.00
```

```
MTB > SInterval 95.0  'Otitis'.
```

Sign Confidence Interval

```
Sign confidence interval for median

                                 Achieved
             N    N*   Median   Confidence     Confidence Interval   Position
Otitis      14    33    7.000       0.9426   (    7.000,    7.000)          4
                               *     0.9500   (    6.897,    7.000)        NLI
                               *     0.9871   (    5.000,    7.000)          3
```

```
MTB > Describe mean median c3
```

Descriptive Statistics

```
Variable       N       N*      Mean    Median  Tr Mean     StDev   SE Mean
Pedal          5       42     6.200     7.000     6.250     1.095     0.490

Variable     Min      Max        Q1        Q3
Pedal      5.000    7.000     5.000     7.000
```

```
MTB > SInterval 95.0  'Pedal'.
```

Sign Confidence Interval

```
Sign confidence interval for median

                                 Achieved
             N    N*   Median   Confidence     Confidence Interval   Position
Pedal        5    42    7.000       0.9375   (    5.000,    7.000)          1
          The highest attainable confidence has been achieved.
```

```
MTB > Describe mean median c4
```

Descriptive Statistics

```
Variable       N       N*      Mean    Median  Tr Mean     StDev   SE Mean
AMD           12       35     10.67     10.00     10.30      4.83      1.39

Variable     Min      Max        Q1        Q3
AMD         5.00    20.00      7.00     14.00
```

```
MTB > SInterval 95.0  'AMD'.
```

Sign Confidence Interval

```
Sign confidence interval for median

                                 Achieved
                N     N*  Median  Confidence     Confidence Interval   Position
AMD            12     35   10.00      0.8540  (     7.00,    14.00)          4
                                      0.9500  (     7.00,    14.00)        NLI
                                      0.9614  (     7.00,    14.00)          3
```

```
MTB > Describe mean median c5
```

Descriptive Statistics

```
Variable        N       N*      Mean    Median  Tr Mean     StDev  SE Mean
other           1       46    3.0000    3.0000   3.0000         *        *

Variable      Min      Max        Q1        Q3
other      3.0000   3.0000         *         *
```

```
MTB > SInterval 95.0  'other'.
```

Sign Confidence Interval

```
Sign confidence interval for median

                                 Achieved
                N     N*  Median  Confidence     Confidence Interval   Position
other      Not enough data.

MTB >
```

Appendix D

Mann Whitney confidence interval and test of the cumulative number of episodes of particular skin conditions in 15 atopic and 15 non-atopic GDBA dogs.

Appendix D1

Mann Whitney confidence interval and test of the cumulative number of episodes of otitis externa in 15 atopic and 15 non-atopic GDBA dogs.

MTB > Mann-Whitney 95.0 '3mths' '3';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

3mths N = 15 Median = 0.0000
3 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is -0.0000
95.4 Percent CI for ETA1-ETA2 is (-0.0000,0.0000)
W = 232.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000
The test is significant at 1.0000 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '6mths' '6';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

6mths N = 15 Median = 0.0000
6 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is 0.0000
95.4 Percent CI for ETA1-ETA2 is (0.0001,-0.0002)
W = 240.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7557
The test is significant at 0.5501 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '9mths' '9';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

9mths N = 15 Median = 0.000
9 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 0.000
95.4 Percent CI for ETA1-ETA2 is (-0.000,0.000)
W = 249.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5069
The test is significant at 0.2609 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '12mths' '12';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

12mths N = 15 Median = 1.000
12 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 1.000
95.4 Percent CI for ETA1-ETA2 is (0.000,2.000)
W = 295.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0101
The test is significant at 0.0021 (adjusted for ties)

MTB > Mann-Whitney 95.0 '15mths' '15';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

15mths N = 15 Median = 1.000
15 N = 15 Median = 1.000
Point estimate for ETA1-ETA2 is 1.000
95.4 Percent CI for ETA1-ETA2 is (-0.001,4.000)
W = 276.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0712
The test is significant at 0.0570 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '18mths' '18';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

18mths N = 15 Median = 4.000
18 N = 15 Median = 2.000
Point estimate for ETA1-ETA2 is 2.000
95.4 Percent CI for ETA1-ETA2 is (-0.001,5.000)
W = 293.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0128
The test is significant at 0.0113 (adjusted for ties)

MTB > Mann-Whitney 95.0 '21mths' '21';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

21mths N = 15 Median = 5.000
21 N = 15 Median = 2.000
Point estimate for ETA1-ETA2 is 3.000
95.4 Percent CI for ETA1-ETA2 is (0.999,6.000)
W = 300.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0051
The test is significant at 0.0048 (adjusted for ties)

MTB >

Appendix D2

Mann Whitney confidence interval and test of the cumulative number of episodes of conjunctivitis in 15 atopic and 15 non-atopic GDBA dogs.

MTB > Mann-Whitney 95.0 '3mths' '3';
SUBC> Alternative 0.

* ERROR * Completion of computation impossible.

* ERROR * All values in column are identical.

MTB > Mann-Whitney 95.0 '6mths' '6';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

6mths N = 15 Median = 0.0000
6 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is -0.0000
95.4 Percent CI for ETA1-ETA2 is (-0.0000,0.0000)
W = 232.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000
The test is significant at 1.0000 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '9mths' '9';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

9mths N = 15 Median = 0.0000
9 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is 0.0000
95.4 Percent CI for ETA1-ETA2 is (0.0001,-0.0002)
W = 240.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7557
The test is significant at 0.5501 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '12mths' '12';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

12mths N = 15 Median = 0.0000
12 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is 0.0000
95.4 Percent CI for ETA1-ETA2 is (0.0002,-0.0001)
W = 243.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6783
The test is significant at 0.5742 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '15mths' '15';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

15mths N = 15 Median = 1.000
15 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 0.000
95.4 Percent CI for ETA1-ETA2 is (-0.001,1.000)

W = 272.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1013
The test is significant at 0.0570 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '18mths' '18';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

18mths N = 15 Median = 2.000
18 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 1.000
95.4 Percent CI for ETA1-ETA2 is (0.000,3.001)
W = 285.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0294
The test is significant at 0.0159 (adjusted for ties)

MTB > Mann-Whitney 95.0 '21mths' '21';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

21mths N = 15 Median = 2.000
21 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 1.000
95.4 Percent CI for ETA1-ETA2 is (-0.001,3.000)
W = 282.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0401
The test is significant at 0.0255 (adjusted for ties)

MTB >

Appendix D3

Mann Whitney confidence interval and test of the cumulative number of episodes of pedal dermatitis in 15 atopic and 15 non-atopic GDBA dogs.

MTB > Mann-Whitney 95.0 '3mths' '3';
SUBC> Alternative 0.

* ERROR * Completion of computation impossible.

* ERROR * All values in column are identical.

MTB > Mann-Whitney 95.0 '6mths' '6';
SUBC> Alternative 0.

* ERROR * Completion of computation impossible.

* ERROR * All values in column are identical.

MTB > Mann-Whitney 95.0 '9mths' '9';
SUBC> Alternative 0.

* ERROR * Completion of computation impossible.

* ERROR * All values in column are identical.

MTB > Mann-Whitney 95.0 '12mths' '12';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

12mths N = 15 Median = 0.0000
12 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is -0.0000
95.4 Percent CI for ETA1-ETA2 is (0.0001,-0.0001)
W = 232.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000
The test is significant at 1.0000 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '15mths' '15';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

15mths N = 15 Median = 0.0000
15 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is -0.0000
95.4 Percent CI for ETA1-ETA2 is (0.0000,-0.0000)
W = 242.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7089
The test is significant at 0.6404 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '18mths' '18';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

18mths N = 15 Median = 1.000
18 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 0.000
95.4 Percent CI for ETA1-ETA2 is (-0.000,1.000)
W = 267.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1524
The test is significant at 0.1201 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '21mths' '21';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

21mths N = 15 Median = 1.000
21 N = 15 Median = 1.000
Point estimate for ETA1-ETA2 is 1.000
95.4 Percent CI for ETA1-ETA2 is (-0.001,2.001)
W = 275.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0779
The test is significant at 0.0649 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB >

Appendix D4

Mann Whitney confidence interval and test of the cumulative number of episodes of pyoderma in 15 atopic and 15 non-atopic GDBA dogs.

MTB > Mann-Whitney 95.0 '3mths' '3';
SUBC> Alternative 0.

* ERROR * Completion of computation impossible.

* ERROR * All values in column are identical.

MTB > Mann-Whitney 95.0 '6mths' '6';
SUBC> Alternative 0.

* ERROR * Completion of computation impossible.

* ERROR * All values in column are identical.

MTB > Mann-Whitney 95.0 '9mths' '9';
SUBC> Alternative 0.

* ERROR * Completion of computation impossible.

* ERROR * All values in column are identical.

MTB > Mann-Whitney 95.0 '12mths' '12';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

12mths N = 15 Median = 0.000
12 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 0.000
95.4 Percent CI for ETA1-ETA2 is (-0.000,1.000)
W = 259.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2717
The test is significant at 0.1726 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '15mths' '15';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

15mths N = 15 Median = 1.000
15 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 0.000
95.4 Percent CI for ETA1-ETA2 is (0.001,1.000)
W = 262.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2290
The test is significant at 0.1784 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '18mths' '18';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

18mths N = 15 Median = 1.000
18 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is -0.000
95.4 Percent CI for ETA1-ETA2 is (-0.000,0.999)
W = 264.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1985
The test is significant at 0.1602 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '21mths' '21';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

21mths N = 15 Median = 1.000
21 N = 15 Median = 1.000
Point estimate for ETA1-ETA2 is -0.000
95.4 Percent CI for ETA1-ETA2 is (0.001,1.999)
W = 258.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2998
The test is significant at 0.2660 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB >

Appendix D5

Mann Whitney confidence interval and test of the cumulative number of episodes of atopic type skin conditions in 15 atopic and 15 non-atopic GDBA dogs.

MTB > Mann-Whitney 95.0 '3mths' '3';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

3mths N = 15 Median = 0.0000
3 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is -0.0000
95.4 Percent CI for ETA1-ETA2 is (-0.0001,0.0001)
W = 239.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.8035
The test is significant at 0.6326 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '6mths' '6';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

6mths N = 15 Median = 0.000
6 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is -0.000
95.4 Percent CI for ETA1-ETA2 is (-0.000,1.000)
W = 261.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2372
The test is significant at 0.0903 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '9mths' '9';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

9mths N = 15 Median = 1.000
9 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 1.000
95.4 Percent CI for ETA1-ETA2 is (0.000,2.001)
W = 287.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0251
The test is significant at 0.0093 (adjusted for ties)

MTB > Mann-Whitney 95.0 '12mths' '12';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

12mths N = 15 Median = 2.000
12 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 2.000
95.4 Percent CI for ETA1-ETA2 is (1.001,3.000)
W = 303.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0037
The test is significant at 0.0027 (adjusted for ties)

MTB > Mann-Whitney 95.0 '15mths' '15';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

15mths N = 15 Median = 5.000
15 N = 15 Median = 1.000
Point estimate for ETA1-ETA2 is 3.000
95.4 Percent CI for ETA1-ETA2 is (1.000,6.000)
W = 295.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0095
The test is significant at 0.0084 (adjusted for ties)

MTB > Mann-Whitney 95.0 '18mths' '18';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

18mths N = 15 Median = 8.000
18 N = 15 Median = 3.000
Point estimate for ETA1-ETA2 is 5.000
95.4 Percent CI for ETA1-ETA2 is (2.002,9.000)
W = 314.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0008
The test is significant at 0.0007 (adjusted for ties)

MTB > Mann-Whitney 95.0 '21mths' '21';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

21mths N = 15 Median = 11.000
21 N = 15 Median = 4.000
Point estimate for ETA1-ETA2 is 8.000
95.4 Percent CI for ETA1-ETA2 is (4.000,10.998)
W = 320.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0003
The test is significant at 0.0003 (adjusted for ties)

MTB >

Appendix D6

Mann Whitney confidence interval and test of the cumulative number of episodes of any skin conditions in 15 atopic and 15 non-atopic GDBA dogs.

MTB > Mann-Whitney 95.0 '3mths' '3';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

3mths N = 15 Median = 0.0000
3 N = 15 Median = 0.0000
Point estimate for ETA1-ETA2 is -0.0000
95.4 Percent CI for ETA1-ETA2 is (-0.0001,0.0001)
W = 239.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.8035
The test is significant at 0.6326 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '6mths' '6';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

6mths N = 15 Median = 0.000
6 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 0.000
95.4 Percent CI for ETA1-ETA2 is (0.000,1.000)
W = 255.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3507
The test is significant at 0.2071 (adjusted for ties)

Cannot reject at alpha = 0.05

MTB > Mann-Whitney 95.0 '9mths' '9';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

9mths N = 15 Median = 1.000
9 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 1.000
95.4 Percent CI for ETA1-ETA2 is (-0.000,2.000)
W = 282.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0421
The test is significant at 0.0210 (adjusted for ties)

MTB > Mann-Whitney 95.0 '12mths' '12';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

12mths N = 15 Median = 3.000
12 N = 15 Median = 0.000
Point estimate for ETA1-ETA2 is 2.000
95.4 Percent CI for ETA1-ETA2 is (1.000,4.000)
W = 306.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0023
The test is significant at 0.0018 (adjusted for ties)

MTB > Mann-Whitney 95.0 '15mths' '15';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

15mths N = 15 Median = 6.000
15 N = 15 Median = 3.000
Point estimate for ETA1-ETA2 is 3.000
95.4 Percent CI for ETA1-ETA2 is (1.000,6.001)
W = 305.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0026
The test is significant at 0.0021 (adjusted for ties)

MTB > Mann-Whitney 95.0 '18mths' '18';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

18mths N = 15 Median = 10.000
18 N = 15 Median = 5.000
Point estimate for ETA1-ETA2 is 5.000
95.4 Percent CI for ETA1-ETA2 is (1.999,10.000)
W = 307.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0020
The test is significant at 0.0019 (adjusted for ties)

MTB > Mann-Whitney 95.0 '21mths' '21';
SUBC> Alternative 0.

Mann-Whitney Confidence Interval and Test

21mths N = 15 Median = 13.000
21 N = 15 Median = 6.000
Point estimate for ETA1-ETA2 is 7.000
95.4 Percent CI for ETA1-ETA2 is (3.002,12.000)
W = 316.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0006
The test is significant at 0.0005 (adjusted for ties)

MTB >

Appendix E

Serum total IgE reflective density results in atopic and non-atopic dogs

&

statistical analysis of this data.

Appendix E1 Serum total IgE reflective densities in non-atopic GDBA dogs

Name	Age in days	Total IgE (R. D.)	No. false positive ELISA results
Thomas	544	0.008	*
Wade	230	0.009	5
Betsy	464	0.014	1
Paddy	691	0.015	0
Max	638	0.016	1
Wellington	537	0.025	7
Sid	544	0.025	*
Andy	1861	0.028	13
Kay	404	0.045	1
Mick	160	0.046	2
Duke	1506	0.055	*
Chips	255	0.060	*
Freya	392	0.063	1
Glennie	344	0.063	3
Ellie	351	0.070	0
Quizz	439	0.076	*
Katy	439	0.087	0
Isla	747	0.090	2
Abbie	524	0.092	0
Morven	1149	0.095	0
Farley	759	0.098	*
Cherie	615	0.103	*
Briar	1168	0.111	0
Clive	668	0.124	1
Bradley	1139	0.125	4
Brodie	836	0.127	1
Martha	407	0.131	*
Star	442	0.136	2
Clover	473	0.142	0
Carmel	56	0.149	1
Harry	245	0.163	0
Ryan	270	0.163	*
Victor	253	0.181	0
Nelson	542	0.190	2
Aaron	281	0.193	13
Angus	261	0.215	*
Sally	455	0.223	15
Mel	1039	0.241	0
Illis	276	0.249	1

Name	Age in days	Total IgE (R. D.)	No. false positive ELISA results
William	526	0.261	1
Cedar	393	0.274	0
Mary	215	0.293	0
Broom	90	0.308	*
Delaney	279	0.313	0
Barney	2365	0.335	2
Pippa	781	0.337	0
Zeus	466	0.363	0
Teal	247	0.364	9
Darcy	234	0.365	1
Blair	291	0.366	0
Callum	265	0.388	0
Yogi	308	0.404	*
Gaynor	601	0.493	5
Yusef	1773	0.515	0
Ria	401	0.567	0

NOTE: * No ELISA results were available for these samples due to a shortage of serum.

Appendix E2 Total serum IgE reflective densities in atopic GDBA dogs at the time of IDST.

Name	Age (days)	IgE Reflective density
Carlo	429	0.006
Cedar	565	0.012
Paul	908	0.106
Opal	1473	0.112
Kai	784	0.142
Chris	2687	0.183
Palmer	916	0.191
Griff	1820	0.247
Andrea	1325	0.25
Alana	1709	0.293
Reo	776	0.425
Pedro	796	0.467

Appendix E3 Total serum IgE reflective densities in non-atopic greyhounds

Dog identification	Age (years)*	Total serum IgE (reflective density)
345	6	0.111
344	6	0.252
346	6	0.299
496	3	0.426
335	4	0.475
381	4	0.513
348	6	0.514
408	3	0.515
342	3	0.559
334	3	0.571
349	6	0.571
460	3	0.614
341	4	0.642
340	3	0.684
347	6	0.725

* Accurate ages in days were not available for these dogs.

Appendix E4 Serum total IgE reflective densities in non-atopic beagles

Dog identification	Age (years)*	Total serum IgE (reflective density)
14989	3	0.000
15948	5	0.000
15946	3	0.000
15945	3	0.013
16169	5	0.013
16167	3	0.024
17531	4	0.102
16168	5	0.180
16849	3	0.203
16636	3	0.229
16663	3	0.237
16660	3	0.304
17541	4	0.310
14985	3	0.317
15926	4	0.321
15947	3	0.322
16161	5	0.331
17527	4	0.403
17550	4	0.431
17536	3	0.436
16868	3	0.440
17545	3	0.440
16615	3	0.462
16619	4	0.629
16850	3	0.735

* Accurate ages in days were not available for these dogs.

Appendix E5 Serum total IgE reflective densities in atopic GUVS dogs at the time of IDST

Name	Age (days)	IgE (Reflective density)
Sally	1092	0.010
Kim	1865	0.020
Tanya	1470	0.030
Toby	1825	0.031
Brandy	750	0.041
Lucy	607	0.048
Rhuri	719	0.063
Olwen	498	0.094
Maisie	2653	0.121
Kyle	2491	0.148
Ben	1100	0.151
Luke	388	0.153
Shane	1007	0.156
Sam	2166	0.170
Macauly	305	0.173
Islay	2770	0.183
Shona	740	0.190
Molly	2203	0.196
Budd	915	0.200
Fudge	815	0.210
Kerry	643	0.227
Jock	640	0.286
Skerry	1939	0.322
Lucy	730	0.347
Tia	1490	0.429
Sheena	2520	0.482
Kayla	550	0.561
Tyson	288	0.718

Appendix E6

One way ANOVA of serum total IgE reflective density results in atopic and non-atopic dogs.

MTB > print c1 c2.

Data Display

Row	Group	IgE Reflective density
1	1	0.008
2	1	0.009
3	1	0.014
4	1	0.015
5	1	0.016
6	1	0.025
7	1	0.025
8	1	0.028
9	1	0.045
10	1	0.046
11	1	0.055
12	1	0.060
13	1	0.063
14	1	0.063
15	1	0.070
16	1	0.076
17	1	0.087
18	1	0.090
19	1	0.092
20	1	0.095
21	1	0.098
22	1	0.103
23	1	0.111
24	1	0.124
25	1	0.125
26	1	0.127
27	1	0.131
28	1	0.136
29	1	0.142
30	1	0.149
31	1	0.163
32	1	0.163
33	1	0.181
34	1	0.190
35	1	0.193
36	1	0.215
37	1	0.223
38	1	0.241
39	1	0.249
40	1	0.261
41	1	0.274
42	1	0.293
43	1	0.308
44	1	0.313
45	1	0.335
46	1	0.337
47	1	0.363
48	1	0.364
49	1	0.365
50	1	0.366

51	1	0.388
52	1	0.404
53	1	0.493
54	1	0.515
55	1	0.567
56	2	0.006
57	2	0.012
58	2	0.106
59	2	0.112
60	2	0.142
61	2	0.183
62	2	0.191
63	2	0.247
64	2	0.250
65	2	0.293
66	2	0.425
67	2	0.467
68	3	0.111
69	3	0.252
70	3	0.299
71	3	0.426
72	3	0.475
73	3	0.513
74	3	0.514
75	3	0.515
76	3	0.559
77	3	0.571
78	3	0.571
79	3	0.614
80	3	0.642
81	3	0.684
82	3	0.725
83	4	0.000
84	4	0.000
85	4	0.000
86	4	0.013
87	4	0.013
88	4	0.024
89	4	0.102
90	4	0.180
91	4	0.203
92	4	0.229
93	4	0.237
94	4	0.304
95	4	0.310
96	4	0.317
97	4	0.321
98	4	0.322
99	4	0.331
100	4	0.403
101	4	0.431
102	4	0.436
103	4	0.440
104	4	0.440
105	4	0.462
106	4	0.629
107	4	0.735
108	5	0.010

109	5	0.020
110	5	0.030
111	5	0.031
112	5	0.041
113	5	0.048
114	5	0.063
115	5	0.094
116	5	0.121
117	5	0.148
118	5	0.151
119	5	0.153
120	5	0.156
121	5	0.170
122	5	0.173
123	5	0.183
124	5	0.190
125	5	0.196
126	5	0.200
127	5	0.210
128	5	0.227
129	5	0.286
130	5	0.322
131	5	0.347
132	5	0.429
133	5	0.482
134	5	0.561
135	5	0.718

MTB >

One-Way Analysis of Variance

Analysis of Variance for IgE Refl

Source	DF	SS	MS	F	P
Group	4	1.2666	0.3166	11.76	0.000
Error	130	3.5007	0.0269		
Total	134	4.7672			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	----+---------+---------+---------+---
1	55	0.1817	0.1431	(--*--)
2	12	0.2028	0.1441	(------*------)
3	15	0.4981	0.1671	(----*-----)
4	25	0.2753	0.2020	(---*----)
5	28	0.2057	0.1717	(---*---)
				----+---------+---------+---------+---
Pooled StDev =		0.1641		0.15 0.30 0.45 0.60

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

Newman-Keuls Multiple Range Test

Please enter the following info at the DATA> prompt :

Number of groups
DATA> 5

MS(error)
DATA> 0.0269

Df(error)
DATA> 130

means of each group
DATA> 0.1817
DATA> 0.2028
DATA> 0.4981
DATA> 0.2753
DATA> 0.2057

number of observations in each group
DATA> 55
DATA> 12
DATA> 15
DATA> 25
DATA> 28

Results of Newman-Keuls multiple range test

Data Display

MS error: 0.0269 df error: 130

Group Mean Count

Data Display

1	0.1817	55
2	0.2028	12
3	0.4981	15
4	0.2753	25
5	0.2057	28

Data Display

Group 3 significantly different to group 1

Data Display-

Group 3 significantly different to group 2

Data Display

Group 3 significantly different to group 5

Data Display

Group 3 significantly different to group 4

MTB >

Appendix E7

Correlation of serum total IgE reflective density results and the number of false positive ELISA results in non-atopic GDBA dogs.

Worksheet size: 100000 cells

MTB > print c1 c2

Data Display

Row	Total IgE	No. false positive ELISAs
1	0.008	*
2	0.009	5
3	0.014	1
4	0.015	0
5	0.016	1
6	0.025	7
7	0.025	*
8	0.028	13
9	0.045	1
10	0.046	2
11	0.055	*
12	0.060	*
13	0.063	1
14	0.063	3
15	0.070	0
16	0.076	*
17	0.087	0
18	0.090	2
19	0.092	0
20	0.095	0
21	0.098	*
22	0.103	*
23	0.111	0
24	0.124	1
25	0.125	4
26	0.127	1
27	0.131	*
28	0.136	2
29	0.142	0
30	0.149	1
31	0.163	0
32	0.163	*
33	0.181	0
34	0.190	2
35	0.193	13
36	0.215	*
37	0.223	15
38	0.241	0
39	0.249	1
40	0.261	1

41	0.274	0
42	0.293	0
43	0.308	*
44	0.313	0
45	0.335	2
46	0.337	0
47	0.363	0
48	0.364	9
49	0.365	1
50	0.366	0
51	0.388	0
52	0.404	*
53	0.493	5
54	0.515	0
55	0.567	0

MTB >

Correlations (Pearson)

Correlation of Total IgE and No. false positive ELISAs = -0.102

Appendix E8

Correlation between age and serum total IgE reflective density results in non-atopic GDBA dogs.

Worksheet size: 100000 cells

MTB > print c1 c2

Data Display

Row	Age in days	Total IgE
1	544	0.008
2	230	0.009
3	464	0.014
4	691	0.015
5	638	0.016
6	537	0.025
7	544	0.025
8	1861	0.028
9	404	0.045
10	160	0.046
11	1506	0.055
12	255	0.060
13	392	0.063
14	344	0.063
15	351	0.070
16	439	0.076
17	439	0.087
18	747	0.090
19	524	0.092
20	1149	0.095
21	759	0.098
22	615	0.103
23	1168	0.111
24	668	0.124
25	1139	0.125
26	836	0.127
27	407	0.131
28	442	0.136
29	473	0.142
30	56	0.149
31	245	0.163
32	270	0.163
33	253	0.181
34	542	0.190
35	281	0.193
36	261	0.215
37	455	0.223
38	1039	0.241
39	276	0.249
40	526	0.261
41	393	0.274
42	215	0.293
43	90	0.308
44	279	0.313
45	2365	0.335
46	781	0.337
47	466	0.363

48	247	0.364
49	234	0.365
50	291	0.366
51	265	0.388
52	308	0.404
53	601	0.493
54	1773	0.515
55	401	0.567

MTB >

Correlations (Pearson)

Correlation of Age in days and Total IgE = 0.000

Appendix F

Allergen specific serological results

Appendix F1 ELISA optical density results for indoor allergen specific IgE at various times of the year in non-atopic GDBA dogs.

Name	Month sampled	Age at sampling (days)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
Aaron	February	281	0.028	0.119	0.072	0.164	0.084	0.146	0.177	0.123	0.115	0.048	0.223
Abbie	August	524	0.040	0.072	0.062	0.132	0.094	0.051	0.050	0.065	0.033	0.018	0.062
Andy	August	1861	0.071	0.152	0.180	0.044	0.054	0.406	0.319	0.033	0.091	0.082	0.245
Babs	June	519	0.014	0.086	0.037	0.118	0.102	0.051	0.036	0.043	0.079	0.028	0.087
Barley	August	452	0.033	0.054	0.042	0.082	0.087	0.290	0.073	0.070	0.022	0.042	0.093
Barney	July	2365	0.045	0.070	0.044	0.155	0.161	0.044	0.070	0.114	0.040	0.055	0.062
Becky	June	472	0.022	0.083	0.034	0.225	0.124	0.022	0.025	0	0.019	0.030	0.175
Betsy	August	464	0.055	0.115	0.106	0.052	0.078	0.124	0.093	0.035	0.051	0.197	0.081
Betsy II	November	429	0.033	0.146	0.103	0.198	0.095	0.130	0.044	0.070	0.045	0.011	0.152
Blair	June	429	0.03	0.026	0.055	0.109	0.088	0.021	0.060	0.084	0	0	0.017
Bobby	October	355	0.05	0.073	0.060	0.033	0.092	0.081	0.054	0.056	0.041	0.050	0.062
Bradley	February	1139	0.11	0.181	0.213	0.131	0.168	0.074	0.121	0.136	0.083	0.064	0.096
Briar	April	1168	0	0.120	0	0	0.036	0	0.015	0	0	0	0.019
Brodie	July	836	0.045	0.116	0.063	0.089	0.108	0.066	0.061	0.075	0.065	0.052	0.083
Callum	September	265	0.034	0.030	0.011	0.034	0.123	0.122	0.087	0.024	0.008	0.018	0.107
Carmel	July	56	0.046	0.287	0.065	0.037	0.043	0.039	0.043	0.047	0.014	0.032	0.059
Caspar	November	2375	0.073	0.164	0.128	0.268	0.151	0.137	0.138	0.115	0.103	0.085	0.206
Cedar	November	393	0.057	0.090	0.030	0.079	0.064	0.040	0.009	0.012	0.013	0.013	0.096

Name	Month sampled	Age at sampling (days)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
Clive	March	668	0.317	0.110	0.080	0.184	0.053	0.073	0.029	0.046	0.034	0.042	0.109
Clover	April	473	0.059	0.064	0.117	0.068	0.133	0.093	0.079	0.059	0.072	0.074	0.127
Craig	October	3161	0.053	0.135	0.043	0.024	0.020	0.077	0.030	0.014	0.006	0.023	0.052
Curtis	October	644	0.018	0.083	0.016	0.073	0.065	0.064	0.021	0.033	0.031	0.043	0.064
Daisy	October	69	0.149	0.068	0.029	0.037	0.058	0.218	0.130	0.055	0.042	0.047	0.184
Dalby	June	374	0.058	0.083	0.064	0.209	0.082	0.074	0.060	0.051	0.050	0.044	0.121
Darcy	July	234	0.047	0.117	0.043	0.063	0.069	0.006	0.029	0	0	0.025	0.026
Delaney	October	279	0.019	0.024	0.019	0.039	0.116	0.058	0.047	0.037	0.010	0.027	0.092
Duncan	July	507	0.216	0.276	0.121	0.107	0.118	0.588	0.383	0.071	0.044	0.104	0.449
Ellie	June	351	0.060	0.100	0.071	0.080	0.111	0.080	0.072	0.067	0.058	0.063	0.054
Freya	September	392	0.049	0.099	0.028	0.192	0.148	0.111	0.056	0.049	0.028	0.043	0.115
Gabby	June	461	0.090	0.131	0.140	0.153	0.127	0.116	0.092	0.075	0.076	0.057	0.228
Gabby II	May	429	0.079	0.109	0.091	0.093	0.107	0.133	0.082	0.067	0.079	0.087	0.163
Gaynor	June	601	0.058	0.089	0.067	0.072	0.102	0.035	0.078	0.028	0.009	0.007	0.098
Glennie	April	344	0.059	0.159	0.103	0.186	0.141	0.111	0.045	0.102	0.056	0.062	0.093
Harry	September	245	0.062	0.062	0.066	0.142	0.078	0.080	0.072	0.077	0.057	0.063	0.088
Hunter	March	262	0.026	0.072	0.038	0.047	0.067	0.040	0.040	0.041	0.022	0.041	0.019
Ike	August	318	0.087	0.186	0.089	0.071	0.063	0.239	0.188	0.060	0.044	0.053	0.235
Illis	April	276	0.031	0.130	0.080	0.113	0.137	0.076	0.076	0.023	0.017	0.024	0.018
Isla	April	747	0.105	0.041	0.046	0.011	0.057	0	0	0	0	0	0
Judy	May	428	0.474	0.303	0.147	0.086	0.077	0.396	0.320	0.077	0.076	0.226	0.751

Name	Month sampled	Age at sampling (days)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
Katy	June	439	0.019	0.106	0.032	0.049	0.071	0.059	0.035	0.027	0.019	0.029	0.091
Kay	June	404	0.055	0.184	0.064	0.081	0.116	0.046	0.064	0.057	0.041	0.062	0.058
Lionel	November	608	0.077	0.153	0.079	0.051	0.065	0.047	0.048	0.025	0.067	0.048	0.044
Lucy	July	554	0.153	0.147	0.110	0.059	0.153	0.185	0.060	0.064	0.058	0.058	0.107
Magnus	February	258	0.068	0.149	0.083	0.061	0.083	0.072	0.086	0.063	0.046	0.037	0.068
Mary	March	215	0.079	0.131	0.092	0.126	0.063	0.037	0.032	0.067	0.039	0.042	0.076
Max	July	638	0	0.276	0	0	0.015	0.127	0	0	0	0	0
Mel	May	1039	0.105	0.095	0.075	0.096	0.059	0.036	0.071	0.067	0.053	0.035	0.112
Melody	July	1500	0.086	0.275	0.104	0.062	0.065	0.072	0.049	0.044	0.057	0.434	0.067
Mick	May	160	0.025	0.084	0.032	0.043	0.038	0.043	0.037	0.009	0.032	0.007	0.061
Morven	June	1149	0.066	0.086	0.075	0.054	0.050	0.048	0.017	0.025	0.023	0.024	0.089
Nash	September	542	0.061	0.159	0.291	0.123	0.104	0.052	0.109	0.056	0.047	0.032	0.372
Nelson	September	542	0.069	0.146	0.075	0.059	0.089	0.123	0.035	0.038	0.022	0.033	0.046
Norma	July	423	0.002	0.090	0.026	0.026	0.013	0	0	0.001	0	0	0.025
Onyx	September	1708	0.128	0.137	0.070	0.067	0.091	0.615	0.410	0.034	0.038	0.117	0.286
Paddy	June	691	0.053	0.087	0.090	0.078	0.074	0.074	0.062	0.069	0.041	0.038	0.070
Phoebe	June	564	0.090	0.092	0.107	0.095	0.075	0.060	0.020	0.014	0.014	0.018	0.126
Pippa	November	781	0.045	0.140	0.043	0.138	0.077	0.084	0.071	0.102	0.033	0.048	0.079
Ria	May	401	0.003	0.060	0.013	0	0.029	0	0.006	0	0	0.002	0.012
Sally	June	455	0.050	0.231	0.138	0.233	0.188	0.198	0.169	0.093	0.057	0.035	0.329
Sally II	September	520	0.008	0.060	0.019	0.049	0.090	0.004	0.051	0.038	0.101	0.033	0.047

Name	Month sampled	Age at sampling (days)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
Sherry	November	472	0.011	0.077	0.036	0.109	0.075	0.039	0.026	0.002	0.009	0	0.020
Star	May	443	0.103	0.137	0.136	0.083	0.147	0.165	0.107	0.079	0.091	0.085	0.122
Tara	December	882	0.039	0.076	0.045	0.091	0.084	0.028	0.021	0.028	0.025	0.020	0.092
Teal	August	247	0.656	0.061	0.152	0.104	0.226	0.055	0.168	0.057	0.032	0.013	0.032
Velma	January	396	0	0.013	0	0	0	0	0	0	0	0	0
Victor	June	253	0.047	0.089	0.061	0.086	0.116	0.030	0.051	0.036	0.018	0.022	0.046
Wade	October	230	0.027	0.069	0.019	0.053	0.057	0.210	0.225	0.036	0	0.015	0.093
Wellington	April	537	0.049	0.203	0.097	0.080	0.151	0.050	0.039	0.051	0.030	0.032	0.037
William	January	526	0.012	0.137	0.043	0.036	0.119	0.017	0.024	0.030	0.024	0.044	0.070
Willow	February	491	0.098	0.160	0.077	0.078	0.069	0.048	0.063	0.043	0.057	0.035	0.054
Wilson	July	858	0.033	0.091	0.040	0.037	0.110	0.042	0.026	0.046	0.012	0.024	0.034
Yuma	September	73	0.052	0.090	0.062	0.117	0.159	0.161	0.077	0.066	0.066	0.066	0.200
Yusef	May	1773	0.060	0.063	0.057	0.031	0.063	0.034	0.030	0.160	0.015	0.017	0.041
Zeus	January	466	0.068	0.072	0.027	0.042	0.106	0.022	0.024	0.041	0.006	0.024	0.035

Appendix F.2 ELISA optical density results for outdoor allergen specific IgE at various times of the year in non-atopic GDBA dogs.

Name	Month sampled	Age at sampling (days)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
Aaron	February	281	0.235	0.234	0.168	0.168	0.289	0.150	0.230	0.313	0.356	0.062	0.406
Abbie	August	524	0.053	0.078	0.115	0.079	0.083	0.055	0.066	0.095	0.067	0.039	0.050
Andy	August	1861	0.623	0.618	0.669	0.682	0.877	0.341	0.790	0.619	0.507	0.430	0.56
Babs	June	519	0.074	0.055	0.063	0.065	0.053	0.064	0.072	0.050	0.036	0.011	0.041
Barley	August	452	0.113	0.089	0.054	0.081	0.068	0.110	0.044	0.067	0.032	0.034	0.080
Barney	July	2365	0.072	0.085	0.068	0.081	0.074	0.133	0.074	0.063	0.028	0.068	0.090
Becky	June	472	0.074	0.070	0.063	0.081	0.046	0.056	0.008	0.043	0.038	0.065	0.030
Betsy	August	464	0.158	0.576	0.613	0.245	0.231	0.324	0.400	0.258	0.306	0.199	0.161
Betsy II	November	429	0.079	0.136	0.108	0.131	0.176	0.144	0.092	0.141	0.059	0.052	0.088
Blair	June	429	0.055	0.076	0.061	0.078	0.048	0.043	0.098	0.086	0.040	0.022	0.007
Bobby	October	355	0.049	0.047	0.056	0.038	0.044	0.060	0.059	0.074	0.006	0.028	0.048
Bradley	February	1139	0.108	0.102	0.071	0.212	0.084	0.083	0.125	0.142	0.078	0.055	0.078
Briar	April	1168	0.019	0	0	0.020	0.005	0	0.018	0.002	0	0	0
Brodie	July	836	0.089	0.143	0.098	0.141	0.104	0.116	0.101	0.164	0.073	0.127	0.128
Callum	September	265	0.050	0.049	0.076	0.046	0.083	0.075	0.037	0.067	0.082	0.103	0.110
Carmel	July	56	0.088	0.109	0.098	0.099	0.063	0.067	0.090	0.074	0.054	0.034	0.053
Caspar	November	2375	0.159	0.176	0.148	0.135	0.177	0.141	0.117	0.178	0.064	0.092	0.176
Cedar	November	393	0.070	0.119	0.081	0.090	0.043	0.063	0.053	0.056	0.034	0.022	0.058

Name	Month sampled	Age at sampling (days)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
Clive	March	668	0.068	0.106	0.077	0.075	0.058	0.058	0.085	0.059	0.042	0.046	0.049
Clover	April	473	0.090	0.054	0.078	0.041	0.054	0.099	0.070	0.091	0.010	0.069	0.070
Craig	October	3161	0.206	0.212	0.188	0.216	0.148	0.103	0.234	0.187	0.153	0.168	0.265
Curtis	October	644	0.058	0.038	0.050	0.034	0.056	0.061	0.042	0.043	0	0.063	0.048
Daisy	October	69	0.133	0.123	0.092	0.093	0.173	0.191	0.067	0.168	0.180	0.190	0.205
Dalby	June	374	0.041	0.058	0.058	0.055	0.051	0.063	0.049	0.057	0.020	0.082	0.060
Darcy	July	234	0.084	0.245	0.043	0.027	0.006	0.081	0.074	0.023	0.004	0	0.023
Delaney	October	279	0.018	0.026	0.032	0.034	0.037	0.047	0.021	0.050	0	0.033	0.045
Duncan	July	507	0.219	0.290	0.221	0.237	0.283	0.460	0.118	0.277	0.433	0.520	0.325
Ellie	June	351	0.078	0.081	0.078	0.081	0.083	0.092	0.062	0.092	0.048	0.058	0.072
Freya	September	392	0.042	0.075	0.075	0.064	0.051	0.091	0.132	0.084	0.064	0.045	0.104
Gabby	June	461	0.130	0.100	0.083	0.077	0.090	0.089	0.093	0.089	0.053	0.123	0.076
Gabby II	May	429	0.066	0.075	0.097	0.170	0.097	0.112	0.086	0.102	0.033	0.098	0.079
Gaynor	June	601	0.106	0.096	0.068	0.069	0.142	0.134	0.063	0.139	0.339	0.162	0.136
Glennie	April	344	0.109	0.124	0.222	0.086	0.068	0.114	0.076	0.080	0.043	0.061	0.085
Harry	September	245	0.072	0.068	0.055	0.061	0.084	0.097	0.066	0.083	0.023	0.058	0.055
Hunter	March	262	0.053	0.071	0.045	0.047	0.072	0.038	0.127	0.057	0.036	0.041	0.056
Ike	August	318	0.191	0.168	0.279	0.234	0.461	0.236	0.127	0.203	0.269	0.179	0.630
Illis	April	276	0.031	0.056	0.039	0.056	0.049	0.044	0.110	0.030	0.010	0.021	0.021
Isla	April	747	0	0.093	0.009	0	0.001	0.032	0	0.009	0	0	0.001
Judy	May	428	0.212	0.261	0.305	0.223	0.324	0.792	0.131	0.306	0.601	0.681	0.570

Name	Month sampled	Age at sampling (days)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
Katy	June	439	0.047	0.053	0.056	0.057	0.051	0.068	0.040	0.060	0	0.037	0.050
Kay	June	404	0.067	0.085	0.109	0.099	0.134	0.129	0.081	0.111	0.057	0.052	0.097
Lionel	November	608	0.073	0.098	0.076	0.074	0.084	0.102	0.073	0.083	0.054	0.044	0.069
Lucy	July	554	0.105	0.116	0.071	0.131	0.084	0.057	0.080	0.112	0.072	0.059	0.117
Magnus	February	258	0.170	0.196	0.138	0.154	0.139	0.098	0.143	0.123	0.110	0.760	0.120
Mary	March	215	0.075	0.081	0.088	0.074	0.071	0.066	0.106	0.071	0.056	0.035	0.052
Max	July	638	0	0	0	0	0	0	0	0	0	0	0
Mel	May	1039	0.040	0.120	0.057	0.072	0.083	0.086	0.007	0.034	0.029	0.043	0.055
Melody	July	1500	0.223	0.320	0.246	0.162	0.147	0.108	0.103	0.144	0.381	0.092	0.126
Mick	May	160	0.051	0.069	0.037	0.034	0.096	0.131	0.040	0.084	0.168	0.186	0.098
Morven	June	1149	0.068	0.078	0.087	0.086	0.068	0.086	0.088	0.092	0.038	0.090	0.090
Nash	September	542	0.089	0.187	0.104	0.115	0.098	0.067	0.121	0.093	0.078	0.046	0.103
Nelson	September	542	0.069	0.086	0.086	0.065	0.121	0.121	0.052	0.073	0.041	0.036	0.052
Norma	July	423	0.062	0.004	0.012	0	0.039	0.035	0	0	0.001	0.053	0
Onyx	September	1708	0.324	0.196	0.161	0.460	0.343	0.656	0.107	0.332	0.582	0.756	0.308
Paddy	June	691	0.047	0.093	0.078	0.077	0.052	0.044	0.060	0.056	0.026	0.042	0.042
Phoebe	June	564	0.077	0.097	0.088	0.095	0.104	0.096	0.091	0.093	0.074	0.147	0.092
Pippa	November	781	0.040	0.141	0.136	0.124	0.079	0.060	0.130	0.093	0.060	0.042	0.064
Ria	May	401	0.036	0.060	0.060	0.020	0.020	0.026	0.038	0.006	0	0.017	0.015
Sally	June	455	0.128	0.188	0.133	0.193	0.205	0.212	0.166	0.216	0.184	0.206	0.286
Sally II	September	520	0.029	0.049	0.019	0.054	0.021	0.052	0.044	0.045	0.001	0.005	0.041

Name	Month sampled	Age at sampling (days)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
Sherry	November	472	0.025	0.028	0.022	0.049	0.040	0.019	0.035	0.023	0.017	0.003	0.018
Star	May	443	0.106	0.092	0.124	0.104	0.126	0.118	0.104	0.152	0.049	0.099	0.096
Tara	December	882	0.055	0.070	0.066	0.058	0.056	0.023	0.090	0.073	0.046	0.039	0.024
Teal	August	247	0.223	0.179	0.199	0.147	0.129	0.088	0.187	0.188	0.115	0.118	0.101
Velma	January	396	0	0	0	0	0	0	0	0	0	0	0
Victor	June	253	0.009	0.033	0.038	0.034	0.024	0.026	0.035	0.030	0.015	0.033	0.035
Wade	October	230	0.018	0.005	0.001	0.009	0.127	0.071	0.002	0.057	0.318	0.364	0.183
Wellington	April	537	0.161	0.210	0.141	0.165	0.144	0.120	0.158	0.106	0.069	0.122	0.201
William	January	526	0.062	0.096	0.056	0.050	0.057	0.049	0.730	0.057	0	0.042	0.071
Willow	February	491	0.032	0.084	0.093	0.074	0.060	0.066	0.060	0.071	0.041	0.036	0.073
Wilson	July	858	0.021	0.053	0.065	0.063	0.035	0.031	0.051	0.035	0.020	0.713	0.011
Yuma	September	73	0.090	0.075	0.066	0.074	0.099	0.083	0.065	0.088	0.033	0.117	0.084
Yusef	May	1773	0.075	0.086	0.068	0.075	0.039	0.094	0.056	0.089	0.036	0.090	0.080
Zeus	January	466	0.045	0.068	0.052	0.051	0.122	0.115	0.057	0.035	0.049	0.039	0.039

Appendix F3 ELISA optical density results for indoor allergen specific IgE in atopic GDBA dogs.

Name	Age at sampling (days)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
Alana	1709	0.035	0.091	0.066	0.095	0.078	0.074	0.048	0.017	0	0.022	0.150
Andrea	1325	0.039	0.044	0.041	0.090	0.062	0.039	0.019	0.022	0.019	0.021	0.063
Carlo	429	0	0.111	0	0.089	0.050	0	0	0.007	0.007	0	0.058
Cedar II	565	0.083	0.170	0.096	0.117	0.183	0.084	0.078	0.066	0.055	0.053	0.185
Chris	2687	0.182	0.171	0.067	0.091	0.143	0.149	0.479	0.155	0.048	0.041	0.423
Claire	2892	0.020	0.057	0.027	0.070	0.045	0.067	0.017	0.025	0.014	0.018	0.052
Dusty	1830	0.096	0.151	0.069	0.044	0.077	0.091	0.055	0.021	0.040	0.037	0.094
Griff	1820	0.035	0.074	0.019	0.060	0.079	0.063	0.035	0.037	0.027	0.022	0.053
Kai	784	0.057	0.230	0.078	0.353	0.186	0.313	0.203	0.087	0.052	0.057	0.433
Opal	1473	0.077	0.184	0.047	0.104	0.146	0.070	0.036	0.049	0.058	0.054	0.108
Palmer	916	0.060	0.121	0.120	0.131	0.132	0.099	0.081	0.074	0.058	0.074	0.094
Paul	908	0.017	0.118	0.071	0.041	0.106	0.030	0.043	0.001	0.025	0.016	0.005
Pedro	796	0.070	0.113	0.055	0.164	0.166	0.096	0.050	0.022	0.036	0.021	0.181
Reo	776	0.042	0.091	0.083	0.077	0.033	0.028	0.036	0.038	0.033	0.027	0.074

Appendix F4 ELISA optical density results for outdoor allergen specific IgE in atopic GDBA dogs.

Name	Age at sampling (days)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
Alana	1709	0.058	0.068	0.067	0.057	0.131	0.059	0.109	0.143	0.016	0.014	0.035
Andrea	1325	0.098	0.068	0.071	0.042	0.036	0.030	0.033	0.026	0.059	0.042	0.051
Carlo	429	0.113	0.059	0	0.029	0.010	0.022	0	0.025	0	0	0
Cedar II	565	0.078	0.115	0.162	0.125	0.109	0.137	0.084	0.068	0.082	0.074	0.094
Chris	2687	0.162	0.231	0.164	0.223	0.332	0.770	0.119	0.313	0.706	0.737	0.423
Claire	2892	0.030	0.032	0.039	0.046	0.039	0.038	0.028	0.045	0.035	0.064	0.062
Dusty	1830	0.017	0.235	0.093	0.184	0.150	0.085	0.112	0.160	0.096	0.091	0.102
Griff	1820	0.146	0.144	0.140	0.124	0.213	0.167	0.100	0.219	0.212	0.167	0.146
Kai	784	0.107	0.119	0.133	0.084	0.223	0.272	0.058	0.125	0.242	0.686	0.436
Opal	1473	0.070	0.071	0.081	0.077	0.064	0.096	0.043	0.064	0.023	0.032	0.071
Palmer	916	0.131	0.111	0.125	0.102	0.078	0.080	0.075	0.108	0.013	0.036	0.094
Paul	908	0.025	0.096	0.167	0.064	0.024	0.082	0.076	0.105	0.057	0.033	0.042
Pedro	796	0.163	0.167	0.132	0.079	0.131	0.073	0.109	0.184	0.094	0.128	0.103
Reo	776	0.045	0.067	0.062	0.070	0.041	0.050	0.044	0.043	0.033	0.031	0.033

Appendix F5 ELISA optical density results for indoor allergen specific IgE in non-atopic greyhounds.

Dog I.D.	Age at sampling (years)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
340	4	0.003	0.092	0.024	0.033	0.062	0.024	0.018	0.016	0.017	0.040	0.043
341	4	0.015	0.080	0.027	0.055	0.145	0.046	0.058	0.041	0.027	0.027	0.044
342	3	0.037	0.072	0.029	0.038	0.031	0.055	0.032	0.035	0.021	0.035	0.066
343	3	0.015	0.086	0.016	0.039	0.078	0.051	0.025	0.031	0.018	0.037	0.035
334	3	0.012	0.089	0.024	0.039	0.059	0.048	0.026	0.017	0.021	0.038	0.162
345	6	0.049	0.133	0.075	0.065	0.105	0.069	0.035	0.097	0.065	0.031	0.056
346	6	0.185	0.153	0.112	0.110	0.098	0.093	0.075	0.077	0.086	0.088	0.125
347	6	0.063	0.187	0.155	0.050	0.124	0.084	0.037	0.032	0.038	0.030	0.066
348	6	0.075	0.195	0.137	0.059	0.050	0.119	0.078	0.042	0.081	0.051	0.075
349	6	0.059	0.163	0.096	0.119	0.062	0.101	0.031	0.038	0.048	0.094	0.071
350	3	0.095	0.186	0.104	0.113	0.149	0.124	0.111	0.081	0.094	0.072	0.138
371	3	0.215	0.237	0.126	0.134	0.133	0.094	0.065	0.049	0.100	0.140	0.111

Appendix F6 ELISA optical density results for outdoor allergen specific IgE in non-atopic greyhounds.

Dog I.D.	Age at sampling (years)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
340	4	0.033	0.042	0.032	0.039	0.035	0.032	0.111	0.022	0.031	0.024	0.054
341	4	0.045	0.042	0.049	0.056	0.054	0.063	0.039	0.033	0.037	0.028	0.035
342	3	0.086	0.071	0.085	0.165	0.085	0.046	0.096	0.073	0.049	0.042	0.053
343	3	0.091	0.117	0.069	0.075	0.094	0.035	0.069	0.055	0.065	0.067	0.060
334	3	0.159	0.266	0.292	0.418	0.338	0.230	0	0	0	0	0
345	6	0.146	0.232	0.076	0.105	0.144	0.119	0.106	0.097	0.372	0.054	0.096
346	6	0.180	0.179	0.156	0.185	0.143	0.183	0.183	0.221	0.105	0.153	0.133
347	6	0.155	0.141	0.101	0.122	0.404	0.111	0.088	0.174	0.095	0.082	0.095
348	6	0.120	0.167	0.244	0.201	0.154	0.122	0.202	0.155	0.107	0.083	0.126
349	6	0.101	0.116	0.163	0.156	0.109	0.077	0.053	0.116	0.075	0.049	0.137
350	3	0.085	0.237	0.160	0.205	0.141	0.079	0.190	0.197	0.194	0.083	0.136
371	3	0.106	0.163	0.164	0.227	0.152	0.046	0.113	0.252	0.145	0.193	0.114

Appendix F7 ELISA optical density results for indoor and outdoor allergen specific IgE in non-atopic beagles.

Dog I.D.	Age at sampling (years)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
15363	3	0.064	0.163	0.110	0.118	0.148	0.041	0.076	0.123	0.095	0.036	0.038
15364	3	0.065	0.250	0.091	0.134	0.078	0.065	0.081	0.039	0.031	0.024	0.077
15923	4	0.052	0.147	0.066	0.040	0.051	0.048	0.049	0.123	0.043	0.034	0.043
16166	5	0.041	0.098	0.079	0.090	0.044	0.044	0.034	0.026	0.042	0.023	0.070
16867	4	0.037	0.199	0.123	0.104	0.043	0.045	0.032	0.032	0.021	0.03	0.045
16860	6	0.035	0.063	0.060	0.036	0.043	0.012	0.028	0.022	0.019	0.019	0.023
16869	5	0.125	0.121	0.081	0.036	0.047	0.017	0.037	0.046	0.017	0.02	0.034
16863	3	0.057	0.087	0.072	0.050	0.070	0.020	0.018	0.046	0.027	0.014	0.021

Dog I.D.	Age at sampling (years)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
15363	3	0.062	0.161	0.097	0.111	0.094	0.053	0.076	0.081	0.064	0.077	0.081
15364	3	0.068	0.173	0.123	0.169	0.097	0.093	0.167	0.132	0.067	0.082	0.045
15923	4	0.092	0.106	0.070	0.083	0.088	0.089	0.156	0.113	0.075	0.031	0.064
16166	5	0.030	0.032	0.038	0.055	0.101	0.040	0.065	0.122	0	0.028	0.041
16867	4	0.051	0.056	0.115	0.087	0.109	0.040	0.030	0.048	0.041	0.047	0.049
16860	6	0.058	0.026	0.175	0.042	0.038	0.047	0.052	0.052	0.028	0.014	0.037
16869	5	0.048	0.091	0.103	0.068	0.071	0.045	0.039	0.033	0.032	0.036	0.032
16863	3	0.082	0.087	0.060	0.060	0.041	0.049	0.043	0.078	0.041	0.021	0.055

Appendix F.8 ELISA optical density results for indoor allergen specific IgE in atopic GUVS dogs.

Name	Age at sampling (days)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
Ben	1100	0.090	0.119	0.115	0.071	0.059	0.052	0.035	0.039	0.057	0.045	0.078
Brandy	750	0.090	0.110	0.121	0.190	0.232	0.077	0.150	0.062	0.08	0.053	0.124
Budd	915	0.184	0.275	0.208	0.224	0.205	0.188	0.210	0.088	0.083	0.085	0.178
Fudge	815	0.069	0.145	0.060	0.221	0.147	0.064	0.059	0.090	0.050	0.040	0.099
Islay	2770	0.082	0.062	0.053	0.114	0.059	0.027	0.009	0.011	0.016	0.012	0.135
Jock	640	0	0	0	0.016	0.030	0	0	0	0	0	0
Kara	770	0	0.034	0	0	0.001	0.041	0	0	0	0	0.028
Kayla	550	0.073	0.116	0.072	0.185	0.122	0.118	0.063	0.048	0.058	0.076	0.148
Kerry	643	0.047	0.073	0.053	0.041	0.083	0.042	0.024	0.020	0.023	0.017	0.028
Kerry II	2187	0.179	0.223	0.156	0.133	0.135	0.318	0.308	0.100	0.082	0.060	0.094
Kim	1865	0.052	0.061	0.048	0.037	0.047	0.044	0.015	0.020	0.026	0.052	0.033
Kyle	2941	0.051	0.154	0.132	0.130	0.067	0.076	0.062	0.070	0.066	0.040	0.105
Lucy	730	0	0.169	0.075	0.046	0.142	0.088	0.099	0.036	0.043	0.048	0.060
Lucy II	607	0.068	0.150	0.072	0.086	0.149	0.048	0.119	0.063	0.045	0.042	0.088
Luke	388	0.069	0.098	0.073	0.161	0.175	0.027	0.047	0.048	0.061	0.029	0.104
Macauly	305	0.088	0.160	0.072	0.226	0.195	0.068	0.063	0.070	0.038	0.041	0.121
Maisie	2653	0.073	0.184	0.097	0.144	0.059	0.045	0.060	0.062	0.054	0.071	0.122
Molly	2203	0.060	0.087	0.069	0.047	0.057	0.018	0.050	0.030	0.021	0.029	0.044

Name	Age at sampling (days)	Flea	Mites	Feathers	Alternaria	Aspergillus	Rhizopus	Kapok	Dust	Cat	Human	Mucor
Olwen	498	0.071	0.113	0.173	0.192	0.132	0.048	0.044	0.050	0.041	0.073	0.068
Rhuri	719	0.187	0.186	0.388	0.072	0.082	0.227	0.126	0.075	0.043	0.059	0.141
Sally	1092	0.075	0.130	0.051	0.086	0.072	0.029	0.094	0.022	0.039	0.041	0.060
Sammie	2166	0.109	0.154	0.090	0.097	0.119	0.053	0.084	0.064	0.045	0.030	0.100
Shane	1007	0.071	0.067	0.051	0.142	0.226	0.002	0.022	0.027	0	0.010	0.092
Sheena	2520	0	0.013	0.034	0.083	0.051	0	0	0	0	0	0
Shogun	1640	0.047	0.143	0.058	0.078	0.092	0.069	0.041	0.039	0.049	0.035	0.009
Shona	740	0.059	0.163	0.078	0.068	0.039	0.030	0.022	0.029	0.023	0.014	0.026
Skerry	1939	0.043	0.105	0.057	0.137	0.056	0	0	0.033	0	0	0
Tanya	1470	0.298	0.139	0.125	0.068	0.126	0.078	0.056	0.030	0.040	0.037	0.085
Teena	1081	0.079	0.263	0.065	0.042	0.116	0.028	0.067	0.042	0.016	0.027	0.062
Tia	1490	0.047	0.202	0.067	0.075	0.333	0.052	0.073	0.043	0.024	0.010	0.071
Toby	1825	0.068	0.128	0.071	0.072	0.109	0.042	0.037	0.028	0.030	0.011	0.085
Tyson	288	0.021	0.075	0.027	0.134	0.155	0.021	0.037	0.009	0	0.016	0.051

Appendix F9 ELISA optical density results for outdoor allergen specific IgE in atopic GUVS dogs.

Name	Age at sampling (days)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
Ben	1100	0.096	0.122	0.123	0.226	0.076	0.140	0.079	0.080	0.285	0.075	0.068
Brandy	750	0.126	0.177	0.211	0.147	0.118	0.114	0.122	0.092	0.071	0.084	0.070
Budd	915	0.205	0.229	0.175	0.249	0.219	0.242	0.194	0.294	0.238	0.159	0.177
Fudge	815	0.068	0.097	0.091	0.099	0.079	0.097	0.083	0.110	0.035	0.047	0.057
Isay	2770	0.057	0.129	0.118	0.107	0.059	0.046	0.064	0.026	0.005	0.042	0.025
Jock	640	0	0.092	0.003	0	0	0	0.020	0.048	0.004	0	0.054
Kara	770	0	0.165	0.195	0	0	0	0	0	0	0.500	0
Kayla	550	0.207	0.135	0.130	0.154	0.162	0.194	0.082	0.158	0.197	0.165	0.138
Kerry	643	0.133	0.008	0.042	0.052	0.031	0.055	0.046	0.031	0.041	0.111	0.051
Kerry II	2187	0.257	0.366	0.289	0.325	0.385	0.381	0.184	0.487	0.464	0.421	0.482
Kim	1865	0.040	0.068	0.081	0.039	0.068	0.033	0.021	0.009	0.023	0.025	0.040
Kyle	2941	0.107	0.168	0.177	0.083	0.035	0.112	0.087	0.039	0.020	0.031	0.066
Lucy	730	0.210	0.417	0.389	0.253	0.296	0.141	0.162	0.328	0.169	0.153	0.240
Lucy II	607	0.433	0.378	0.545	0.435	0.352	0.181	0.375	0.248	0.222	0.179	0.171
Luke	388	0.050	0.097	0.088	0.071	0.060	0.072	0.064	0.059	0.045	0.050	0.077
Macauly	305	0.061	0.149	0.075	0.112	0.102	0.071	0.108	0.134	0.064	0.025	0.113
Maisie	2653	0.070	0.070	0.073	0.116	0.088	0.058	0.143	0.083	0.054	0.067	0.095

Name	Age at sampling (days)	Orchard	Timothy	Kentucky	Fescue	Poplar	Birch	Sorrel	Plantain	Mugwort	Dandelion	Nettle
Molly	2203	0.101	0.142	0.132	0.109	0.094	0.074	0.067	0.101	0.072	0.094	0.141
Olwen	498	0.099	0.095	0.088	0.092	0.045	0.026	0.100	0.001	0.029	0.038	0.091
Rhuri	719	0.165	0.192	0.153	0.240	0.139	0.167	0.116	0.145	0.189	0.167	0.113
Sally	1092	0.244	0.112	0.078	0.140	0.127	0.058	0.094	0.136	0.118	0.111	0.074
Sam	2166	0.123	0.152	0.260	0.086	0.138	0.178	0.088	0.080	0.076	0.107	0.095
Shane	1007	0.056	0	0.027	0.068	0.036	0.017	0.096	0.060	0.062	0.053	0.041
Sheena	2520	0	0.030	0	0.080	0	0	0	0.026	0	0	0
Shogun	1640	0.070	0.150	0.125	0.197	0.063	0.047	0.041	0.057	0	0.033	0.079
Shona	740	0.077	0.108	0.122	0.080	0.087	0.077	0.084	0.102	0.135	0.139	0.044
Skerry	1939	0.032	0.024	0.031	0.050	0.006	0.021	0.055	0.032	0.019	0.005	0
Tanya	1470	0.075	0.098	0.079	0.078	0.071	0.046	0.056	0.074	0.049	0.036	0.043
Teena	1081	0.087	0.332	0.097	0.095	0.122	0.525	0.084	0.100	0.092	0.110	0.084
Tia	1490	0.150	0.086	0.069	0.062	0.040	0.048	0.052	0.048	0.028	0.006	0.042
Toby	1825	0.348	0.172	0.148	0.160	0.105	0.056	0.172	0.240	0.160	0.060	0.040
Tyson	288	0.026	0.046	0.018	0.023	0.018	0.048	0.008	0.019	0	0.023	0.014

Appendix F10 Immunodot reflective density results for indoor allergen specific IgE in non-atopic GDBA dogs.

Name	Age at sampling (days)	Dustmites	Storemites	Flea	Human	Cat
Aaron	281	0.047	0.030	0.017	0.020	0.056
Abbie	524	0.011	0.027	0.013	0.043	0.025
Andy	1861	0.027	0.068	0.035	0.063	0.009
Angus	261	0.063	0.028	0.010	0.062	0.018
Barney	2365	0.025	0.080	0.018	0.068	0.036
Betsy	464	0.035	0.038	0.007	0.038	0.023
Blair	291	0.014	0.053	0.002	0.071	0.034
Bradley	1139	0.134	0.023	0.010	0.182	0.059
Brodie	836	0.009	0.046	0.002	0.067	0.038
Broom	90	0.058	0.083	0.020	0.088	0.040
Cherie	615	0.016	0.028	0.007	0.062	0.025
Clive	668	0.010	0.029	0.015	0.081	0.034
Clover	473	0.006	0.029	0.003	0.020	0.059
Darcy	234	0.150	0.079	0.004	0.057	0.039
Delaney	279	0.025	0.061	0.021	0.054	0.015
Duke	1506	0.012	0.029	0.026	0.061	0.029
Ellie	351	0.009	0.031	0.095	0.180	0.077
Emily	1005	0.006	0.027	0.001	0.054	0.025
Farley	759	0.006	0.031	0.004	0.019	0.049
Freya	392	0.014	0.033	0.011	0.022	0.014
Gaynor	601	0.052	0.077	0.031	0.106	0.011
Glennie	344	0.006	0.038	0.005	0.053	0.024
Harry	245	0.045	0.077	0.034	0.055	0.017
Illis	276	0.008	0.035	0.002	0.046	0.049
Isla	747	0.016	0.027	0.027	0.028	0.019
Katy	439	0.029	0.063	0.027	0.070	0.019
Kay	404	0.014	0.061	0.027	0.077	0.020
Martha	407	0.008	0.030	0.008	0.028	0.068
Mary	215	0.032	0.092	0.008	0.095	0.017
Max	638	0.008	0.030	0.019	0.057	0.061
Mick	160	0.007	0.035	0.004	0.075	0.088
Morven	1149	0.012	0.031	0.035	0.080	0.027
Nelson	542	0.019	0.062	0.007	0.059	0.038
Paddy	691	0.007	0.032	0.003	0.043	0.019
Thomas	544	0.008	0.031	0.024	0.054	0.066
Victor	253	0.059	0.072	0.011	0.069	0.052

Name	Age at sampling (days)	Dustmites	Storemites	Flea	Human	Cat
Wade	230	0.008	0.035	0.015	0.034	0.085
Wellington	537	0.016	0.043	0.005	0.028	0.141
William	526	0.030	0.030	0.009	0.053	0.037
Yogi	308	0.014	0.126	0.020	0.074	0.012
Yusef	1773	0.043	0.035	0.031	0.082	0.026
Zeus	466	0.005	0.032	0.003	0.044	0.008

Appendix F11 Immunodot reflective density results for indoor allergen specific IgE in atopic GDBA dogs.

Name	Age at sampling (days)	Dustmites	Storemites	Flea	Human	Cat epithelium
Chris	2687	0.007	0.035	0.007	0.055	0.014
Andrea	1325	0.009	0.032	0.009	0.040	0.050
Carlo	429	0.007	0.026	0.003	0.047	0.067
Cedar II	565	0.058	0.049	0.014	0.049	0.038
Griff	1820	0.014	0.030	0.002	0.066	0.024
Kai	784	0.039	0.029	0.004	0.021	0.056
Opal	1473	0.019	0.053	0.024	0.047	0.038
Reo	776	0.258	0.046	0.033	0.059	0.053

Appendix F12 Immunodot reflective density results for indoor allergen specific IgE in non-atopic greyhounds.

Dog I.D.	Age at sampling (years)	Dustmites	Storemites	Flea	Human	Cat epithelium
340	4	0.043	0.067	0.005	0.062	0.008
342	3	0.054	0.086	0.016	0.055	0.060
334	3	0.024	0.059	0.005	0.057	0.015
335	4	0.132	0.097	0.015	0.063	0.018

Appendix F13 Immunodot reflective density results for indoor allergen specific IgE in non-atopic beagles.

Dog I.D.	Age at sampling (years)	Dustmites	Storemites	Flea	Human	Cat epithelium
15945	3	0	0	0	0	0
14989	3	0	0	0	0	0
15948	5	0	0	0	0.059	0
15946	3	0	0	0	0	0

Appendix F14 Immunodot reflective density results for indoor allergen specific IgE in atopic GUVS dogs.

Name	Age at sampling (days)	Dustmites	Storemites	Flea	Human	Mould
Brandy	750	0.014	0.023	0.039	0.061	0.002
Budd	915	0.609	0.282	0	0	0
Fudge	815	0.256	0.101	0.029	0.107	0.011
Islay	2770	0.050	0.005	0	0	0
Jock	640	0.130	0.088	0.043	0.070	0.019
Kayla	550	0.008	0.024	0.012	0.061	0.020
Kerry II	2187	0.022	0	0	0	0
Kim	1865	0.039	0.056	0.013	0.071	0.024
Kyle	2941	0	0.002	0	0.021	0
Lucy	730	0.258	0.068	0.009	0.059	0.034
Lucy	607	0	0.028	0	0	0
Luke	388	0.044	0.100	0.012	0.102	0.007
Macauly	305	0.202	0.042	0.050	0.118	0.007
Maisie	2653	0.004	0.037	0.074	0.103	0.047
Olwen	498	0.159	0.118	0.068	0.143	0.012
Rhuri	719	0	0.019	0	0	0
Sally	1092	0	0	0	0	0
Shane	1007	0.005	0.040	0.024	0.070	0.003
Sheena	2520	0.562	0.261	0.021	0.040	0.015
Shona	740	0.740	0.505	0.011	0.055	0.004
Skerry	1939	0.175	0.067	0.024	0.052	0.018
Tanya	1470	0.063	0.038	0.010	0.049	0
Toby	1825	0.575	0.015	0	0	0
Tyson	288	0.107	0.028	0.017	0.045	0.041

Appendix F15 Immunodot reflective density results for outdoor allergen specific IgE in non-atopic GDBA dogs.

Name	Age at sampling (days)	Grass	Tree	Mugwort	Olive
Aaron	281	0.015	0.008	0.024	0.054
Abbie	524	0.042	0.026	0.039	0.052
Andy	1861	0.037	0.030	0.090	0.103
Angus	261	0.013	0.006	0.032	0.034
Barney	2365	0.022	0.032	0.100	0.138
Betsy	464	0.016	0.027	0.069	0.057
Blair	291	0.007	0.007	0.052	0.070
Bradley	1139	0.008	0.036	0.117	0.086
Brodie	836	0.014	0.025	0.040	0.053
Broom	90	0.009	0.022	0.063	0.075
Callum	265	0.008	0.015	0.038	0.066
Cherie	615	0.009	0.018	0.052	0.109
Clive	668	0.011	0.016	0.049	0.142
Clover	473	0.011	0.011	0.025	0.120
Darcy	234	0.031	0.045	0.076	0.108
Delaney	279	0.141	0.044	0.050	0.022
Duke	1506	0.012	0.008	0.047	0.073
Ellie	351	0.007	0.018	0.024	0.103
Farley	759	0.006	0.005	0.019	0.025
Freya	392	0.030	0.012	0.030	0.010
Gaynor	601	0.034	0.040	0.070	0.040
Glennie	344	0.021	0.011	0.005	0.127
Harry	245	0.006	0.009	0.019	0.051
Illis	276	0.010	0.009	0.075	0.107
Isla	747	0.020	0.042	0.078	0.070
Katy	439	0.004	0.005	0.033	0.074
Kay	404	0.007	0.007	0.020	0.018
Martha	407	0.011	0.017	0.027	0.034
Mary	215	0.003	0.019	0.066	0.068
Max	638	0.039	0.010	0.093	0.066
Mick	160	0.022	0.013	0.050	0.078
Morven	1149	0.008	0.010	0.042	0.061
Nelson	542	0.021	0.020	0.062	0.050
Paddy	691	0.009	0.009	0.014	0.669
Pippa	781	0.021	0.008	0.046	0.056
Quizz	439	0.009	0.013	0.042	0.015
Ria	401	0.010	0.010	0.043	0.074
Ryan	270	0.008	0.021	0.126	0.008
Sally	455	0.021	0.004	0.029	0.099

Name	Age at sampling (days)	Grass	Tree	Mugwort	Olive
Sid	544	0.039	0.019	0.068	0.061
Star	442	0.008	0.017	0.021	0.131
Teal	247	0.014	0.013	0.041	0.044
Thomas	544	0.012	0.018	0.072	0.079
Victor	253	0.021	0.045	0.070	0.052
Wade	230	0.008	0.007	0.021	0.122
Wellington	537	0.035	0.009	0.023	0.130
William	526	0.056	0.046	0.083	0.069
Yogi	308	0.037	0.023	0.071	0.066
Yusef	1773	0.125	0.051	0.052	0.055
Zeus	466	0.009	0.006	0.047	0.060

Appendix F16 Immunodot reflective density results for outdoor allergen specific IgE in atopic GDBA dogs.

Name	Age at sampling (days)	Grass	Tree	Mugwort	Olive
Chris	2687	0.019	0.013	0.064	0.083
Kai	784	0.009	0.003	0.020	0.053
Opal	1473	0.047	0.040	0.066	0.077
Carlo	429	0.017	0.008	0.022	0.063
Reo	776	0.023	0.035	0.084	0.065
Andrea	1325	0.008	0.025	0.069	0.130
Griff	1820	0.026	0.013	0.065	0.107

Appendix F17 Immunodot reflective density results for outdoor allergen specific IgE in non-atopic greyhounds.

Dog I.D.	Age at sampling (years)	Grass	Tree	Mugwort	Olive
340	4	0.046	0.060	0.067	0.05
342	3	0.046	0.059	0.067	0.071
334	3	0.041	0.021	0.057	0.093
335	4	0.042	0.041	0.054	0.07

Appendix F18 Immunodot reflective density results for outdoor allergen specific IgE in non-atopic beagles.

Dog I.D.	Age at sampling (years)	Grass	Tree	Mugwort	Olive
15945	3	0.057	0.035	0.053	0.020
16167	3	0.079	0.018	0.056	0.007
16169	5	0.078	0	0.038	0.013
14989	3	0.048	0.009	0.050	0
15948	5	0.011	0	0.010	0
15946	3	0.001	0	0.010	0

Appendix F19 Immunodot reflective density results for outdoor allergen specific IgE in atopic GUVS dogs.

Name	Age at sampling (days)	Grass	Tree	Mugwort	Olive
Lucy II	607	0.050	0.031	0.081	0.061
Brandy	750	0.063	0.018	0.038	0.068
Budd	915	0.017	0.003	0.058	0.041
Fudge	815	0.056	0.032	0.067	0.046
Islay	2770	0.054	0.027	0.108	0.071
Jock	640	0.083	0.021	0.032	0.038
Kayla	550	0.063	0.016	0.069	0.091
Kerry II	2187	0.030	0.043	0.059	0.083
Kim	1865	0.066	0.009	0.061	0
Kyle	2941	0.070	0.068	0.062	0.061
Lucy	730	0.053	0.026	0.051	0.097
Luke	388	0.043	0.007	0.053	0.074
Macauly	305	0.041	0.031	0.042	0.111
Maisie	2653	0.032	0.040	0.051	0.013
Olwen	498	0.029	0.005	0.017	0.018
Rhuri	719	0.114	0.023	0.072	0
Sally	1092	0.057	0.044	0.061	0.014
Shane	1007	0.068	0.039	0.078	0.059
Tanya	1470	0.084	0.055	0.069	0.097
Toby	1825	0.073	0.069	0.074	0.054
Tyson	288	0.027	0.017	0.077	0.077

Appendix F20 Immunodot reflective density results for Topscreen allergen specific IgE in non-atopic GDBA dogs.

Name	Age at sampling (days)	Outdoor	Indoor	Foods 1	Foods 2	Moulds
Abbie	524	0.022	0.049	0.041	0.029	0.081
Andy	1861	0.107	0.057	0.021	0.019	0.020
Betsy	464	0.020	0.017	0.026	0.022	0.098
Bradley	1139	0.042	0.092	0.008	0.013	0.070
Briar	1168	0.058	0.105	0.027	0.017	0.099
Callum	265	0.066	0.173	0.011	0.145	0.115
Carmel	56	0.054	0.059	0.021	0.040	0.127
Cedar	393	0.082	0.217	0.007	0.016	0.038
Chips	255	0.123	0.125	0.086	0.059	0.094
Delaney	279	0.027	0.072	0.021	0.027	0.129
Mel	1039	0.034	0.132	0.042	0.047	0.103

Appendix F21 Immunodot reflective density results for Topscreen allergen specific IgE in atopic GDBA dogs.

Name	Age at sampling (days)	Outdoor	Indoor	Foods 1	Foods 2	Moulds
Andrea	1325	0.041	0.043	0.008	0.007	0.010
Paul	908	0.067	0.179	0.030	0.055	0.081
Pedro	796	0.120	0.132	0.023	0.018	0.057
Reo	776	0.038	0.149	0.010	0.007	0.008

Appendix F22 Immunodot reflective density results for Topscreen allergen specific IgE in non-atopic greyhounds.

Name	Age at sampling (years)	Outdoor	Indoor	Foods 1	Foods 2	Moulds
345	6	0.029	0.068	0.021	0.024	0.070
344	6	0.097	0.092	0.025	0.026	0.137

Appendix F23 Immunodot reflective density results for Topscreen allergen specific IgE in non-atopic beagles.

Dog I.D.	Age at sampling (years)	Outdoor	Indoor	Foods 1	Foods 2	Moulds
16849	3	0.057	0.034	0.007	0.060	0.285
16850	3	0.057	0.022	0.119	0.091	0.250
16868	3	0.050	0.023	0.002	0.036	0.272
17527	4	0.056	0.031	0.010	0.065	0.280
17531	4	0.090	0.079	0.019	0.068	0.249
17536	3	0.087	0.134	0.059	0.019	0.096
17541	4	0.051	0.024	0.002	0.021	0.278
17545	3	0.043	0.026	0.005	0.073	0.306
17550	3	0.060	0.023	0.008	0.051	0.367

Appendix F24 Immunodot reflective density results for Topscreen allergen specific IgE at in atopic GUVS dogs.

Name	Age at sampling (days)	Outdoor	Indoor	Foods 1	Foods 2	Moulds
Brandy	750	0.026	0.097	0.012	0.022	0.093
Budd	915	0.012	0.205	0.023	0.040	0.133
Fudge	815	0.196	0.340	0.013	0.015	0.142
Islay	2770	0.064	0.076	0.016	0.014	0.061
Jock	640	0.139	0.172	0.049	0.048	0.142
Kerry	643	0.017	0.037	0.053	0.033	0.117
Kyle	2941	0.056	0.093	0.020	0.023	0.060
Luke	388	0.096	0.102	0.018	0.024	0.043
Macauly	305	0.015	0.158	0.053	0.043	0.054
Maisie	2653	0.019	0.054	0.020	0.023	0.033
Olwen	498	0.039	0.098	0.037	0.022	0.058
Sam	2166	0.065	0.163	0.016	0.005	0.022
Shane	1007	0.023	0.060	0.020	0.033	0.056
Shona	740	0.041	0.493	0.036	0.042	0.051
Skerry	1939	0.374	0.179	0.041	0.045	0.167

Appendix G

Statistical analysis of ELISA results in non-atopic GDBA dogs for each month over a three year period

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Worksheet size: 100000 cells


Retrieving worksheet from file: D:\OLD3SEAS.MTW
Worksheet was saved on  9/ 7/1998
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One-Way Analysis of Variance

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Analysis of Variance for Flea
Source     DF        SS        MS        F        P
Month      11   0.09792   0.00890     0.94    0.510
Error      62   0.58777   0.00948
Total      73   0.68569

                                                    Individual 95% CIs For Mean
                                                    Based on Pooled StDev
Level       N      Mean     StDev  ---+---------+---------+---------+---
 1          3   0.02667   0.03630        (--------*---------)
 2          4   0.07600   0.03655             (-------*-------)
 3          3   0.14067   0.15499                 (---------*--------)
 4          6   0.05050   0.03478             (-----*------)
 5          7   0.12129   0.16011                   (-----*-----)
 6         14   0.05086   0.02346               (---*----)
 7         10   0.06730   0.06802               (-----*----)
 8          6   0.15700   0.24526                       (------*------)
 9          8   0.05788   0.03431               (-----*-----)
10          6   0.05267   0.04956             (-----*------)
11          6   0.04933   0.02506           (------*------)
12          1   0.03900   0.00000  (---------------*---------------)
                                    ---+---------+---------+---------+---
Pooled StDev =  0.09737             -0.12       0.00      0.12      0.24
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One-Way Analysis of Variance

```
Analysis of Variance for Mites
Source     DF        SS        MS        F        P
Month      11   0.06262   0.00569     1.59    0.124
Error      62   0.22190   0.00358
Total      73   0.28452

                                                    Individual 95% CIs For Mean
                                                    Based on Pooled StDev
Level       N      Mean     StDev  -------+---------+---------+---------
 1          3   0.07400   0.06202         (---------*--------)
 2          4   0.15225   0.02584                   (--------*-------
 3          3   0.10433   0.02991              (---------*---------)
 4          6   0.11950   0.05981                   (------*------)
 5          7   0.12157   0.08431                    (-----*------)
 6         14   0.10521   0.04942                   (----*----)
 7         10   0.17450   0.09182                             (----*----
 8          6   0.10667   0.05381                (------*------)
 9          8   0.09787   0.04625                (-----*-----)
10          6   0.07533   0.03565              (------*------)
11          6   0.12833   0.03587                     (------*------)
12          1   0.07600   0.00000  (-----------------*-----------------)
                                    -------+---------+---------+---------
Pooled StDev =  0.05982              0.000     0.070     0.140
```

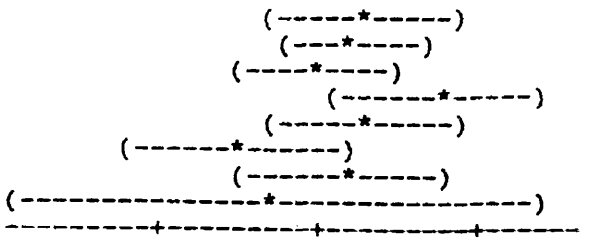
One-Way Analysis of Variance

```
Analysis of Variance for Feathers
Source     DF        SS        MS        F        P
Month      11   0.03242   0.00295     1.22    0.294
Error      62   0.14994   0.00242
Total      73   0.18236

                                                    Individual 95% CIs For Mean
                                                    Based on Pooled StDev
Level       N      Mean     StDev  ---------+---------+---------+-------
 1          3   0.02333   0.02173     (---------*--------)
 2          4   0.11125   0.06798                    (--------*-------)
 3          3   0.07000   0.02835              (---------*---------)
 4          6   0.07383   0.04362                  (-----*------)
```

5	7	0.07871	0.05014
6	14	0.07393	0.03433
7	10	0.06160	0.03925
8	6	0.10517	0.05274
9	8	0.07775	0.08969
10	6	0.03100	0.01733
11	6	0.06983	0.04005
12	1	0.04500	0.00000

Pooled StDev = 0.04918



One-Way Analysis of Variance

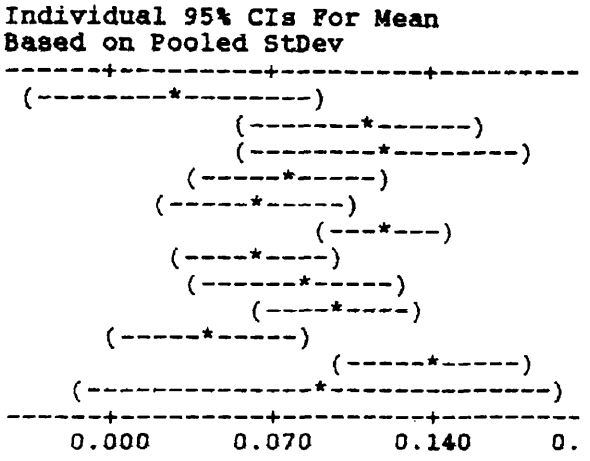
Analysis of Variance for Alternar

Source	DF	SS	MS	F	P
Month	11	0.06945	0.00631	2.20	0.026
Error	62	0.17813	0.00287		
Total	73	0.24758			

Level	N	Mean	StDev
1	3	0.02600	0.02272
2	4	0.10850	0.04751
3	3	0.11900	0.06877
4	6	0.07633	0.06863
5	7	0.06171	0.03719
6	14	0.11729	0.06275
7	10	0.06350	0.04445
8	6	0.08083	0.03299
9	8	0.09787	0.05443
10	6	0.04317	0.01739
11	6	0.14050	0.08044
12	1	0.09100	0.00000

Pooled StDev = 0.05360

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

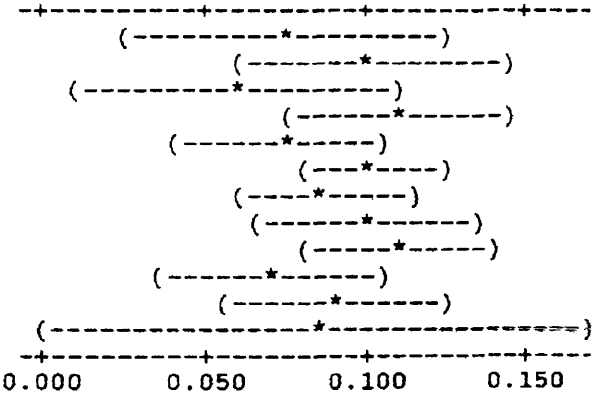
Analysis of Variance for Aspergil

Source	DF	SS	MS	F	P
Month	11	0.01651	0.00150	0.82	0.625
Error	62	0.11407	0.00184		
Total	73	0.13058			

Level	N	Mean	StDev
1	3	0.07500	0.06528
2	4	0.10100	0.04519
3	3	0.06100	0.00721
4	6	0.10917	0.04936
5	7	0.07429	0.04102
6	14	0.10186	0.03369
7	10	0.08550	0.05286
8	6	0.10033	0.06332
9	8	0.11025	0.02994
10	6	0.06800	0.03291
11	6	0.08783	0.03290
12	1	0.08400	0.00000

Pooled StDev = 0.04289

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

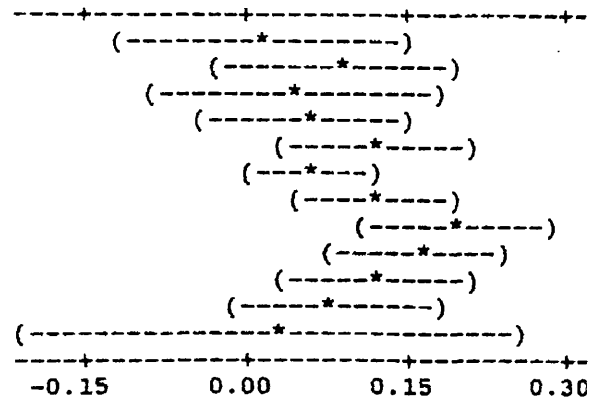
Analysis of Variance for Rhizopus

Source	DF	SS	MS	F	P
Month	11	0.1549	0.0141	1.06	0.410
Error	62	0.8259	0.0133		
Total	73	0.9808			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev
1	3	0.0130	0.0115
2	4	0.0850	0.0423
3	3	0.0500	0.0200
4	6	0.0550	0.0471
5	7	0.1153	0.1372
6	14	0.0653	0.0460
7	10	0.1169	0.1746
8	6	0.1942	0.1420
9	8	0.1585	0.1907
10	6	0.1180	0.0749
11	6	0.0795	0.0450
12	1	0.0280	0.0000

Pooled StDev = 0.1154



One-Way Analysis of Variance

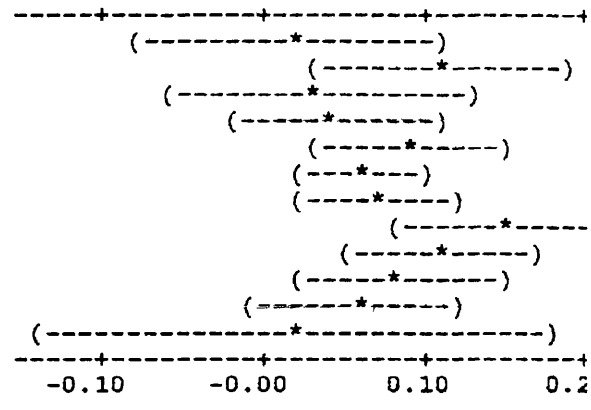
Analysis of Variance for Kapok

Source	DF	SS	MS	F	P
Month	11	0.08160	0.00742	1.13	0.356
Error	62	0.40787	0.00658		
Total	73	0.48947			

Level	N	Mean	StDev
1	3	0.01600	0.01386
2	4	0.11175	0.04961
3	3	0.03367	0.00569
4	6	0.04233	0.03173
5	7	0.09329	0.10568
6	14	0.06007	0.03858
7	10	0.07210	0.11190
8	6	0.14850	0.09949
9	8	0.11213	0.12250
10	6	0.08450	0.07893
11	6	0.05600	0.04531
12	1	0.02100	0.00000

Pooled StDev = 0.08111

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

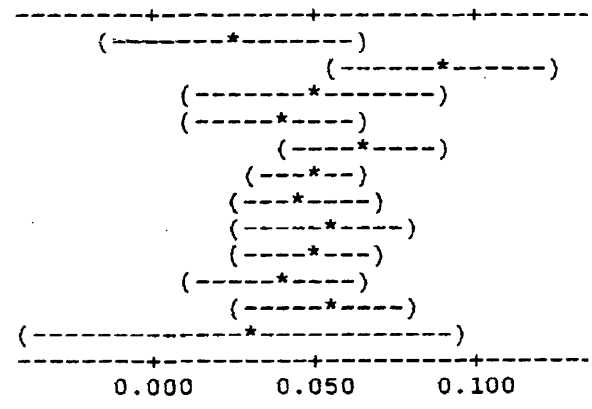
Analysis of Variance for Dust

Source	DF	SS	MS	F	P
Month	11	0.01300	0.00118	1.04	0.428
Error	62	0.07081	0.00114		
Total	73	0.08381			

Level	N	Mean	StDev
1	3	0.02367	0.02122
2	4	0.09125	0.04523
3	3	0.05133	0.01380
4	6	0.03917	0.03952
5	7	0.06557	0.05281
6	14	0.04779	0.02765
7	10	0.04620	0.03753
8	6	0.05333	0.01563
9	8	0.04775	0.01773
10	6	0.03850	0.01560
11	6	0.05433	0.04814
12	1	0.02800	0.00000

Pooled StDev = 0.03379

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

Analysis of Variance for Cat

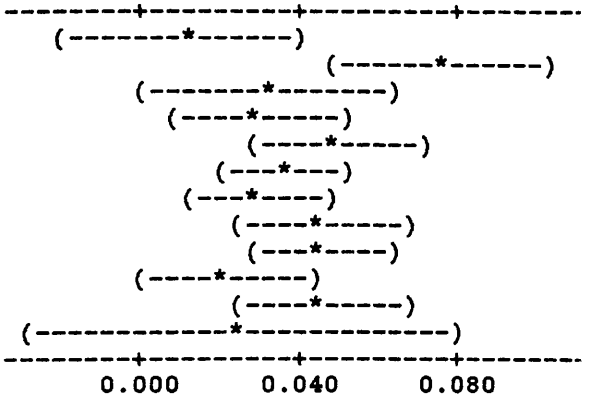
Source	DF	SS	MS	F	P

Month	11	0.013168	0.001197	1.60	0.122
Error	62	0.046442	0.000749		
Total	73	0.059610			

Level	N	Mean	StDev
1	3	0.01000	0.01249
2	4	0.07525	0.03071
3	3	0.03167	0.00874
4	6	0.02917	0.02968
5	7	0.04943	0.03477
6	14	0.03600	0.02512
7	10	0.02900	0.02647
8	6	0.04550	0.02447
9	8	0.04588	0.02917
10	6	0.02167	0.01858
11	6	0.04500	0.03553
12	1	0.02500	0.00000

Pooled StDev = 0.02737

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

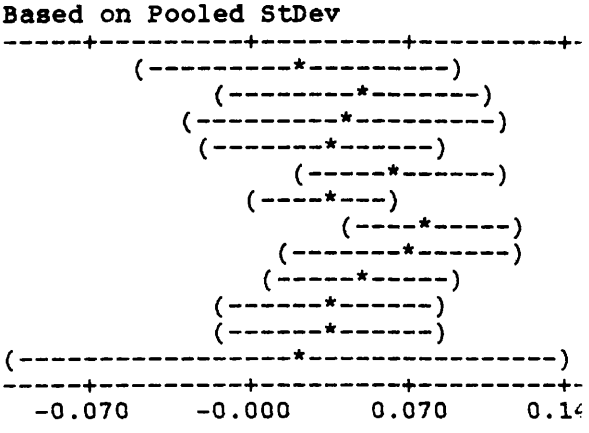
Analysis of Variance for Human

Source	DF	SS	MS	F	P
Month	11	0.02369	0.00215	0.57	0.844
Error	62	0.23365	0.00377		
Total	73	0.25735			

Level	N	Mean	StDev
1	3	0.02267	0.02203
2	4	0.04600	0.01329
3	3	0.04167	0.00058
4	6	0.03200	0.03091
5	7	0.06557	0.07889
6	14	0.03264	0.01903
7	10	0.07840	0.12868
8	6	0.06750	0.06821
9	8	0.05063	0.03136
10	6	0.03417	0.01440
11	6	0.03417	0.03200
12	1	0.02000	0.00000

Pooled StDev = 0.06139

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

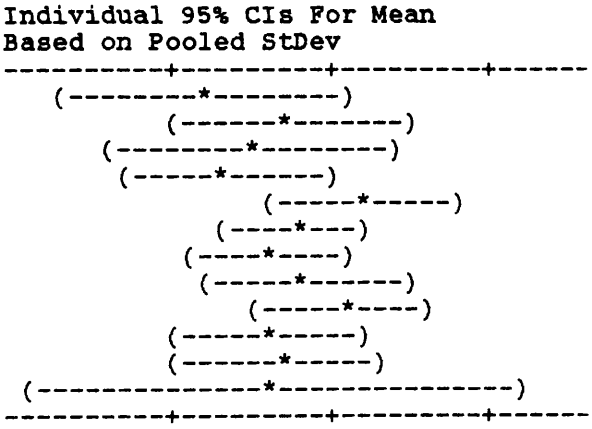
Analysis of Variance for Mucor

Source	DF	SS	MS	F	P
Month	11	0.1052	0.0096	0.70	0.734
Error	62	0.8478	0.0137		
Total	73	0.9531			

Level	N	Mean	StDev
1	3	0.0350	0.0350
2	4	0.1102	0.0772
3	3	0.0680	0.0455
4	6	0.0490	0.0499
5	7	0.1803	0.2569
6	14	0.1135	0.0823
7	10	0.0912	0.1295
8	6	0.1247	0.0917
9	8	0.1576	0.1184
10	6	0.0912	0.0485
11	6	0.0995	0.0692
12	1	0.0920	0.0000

Pooled StDev = 0.1169

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

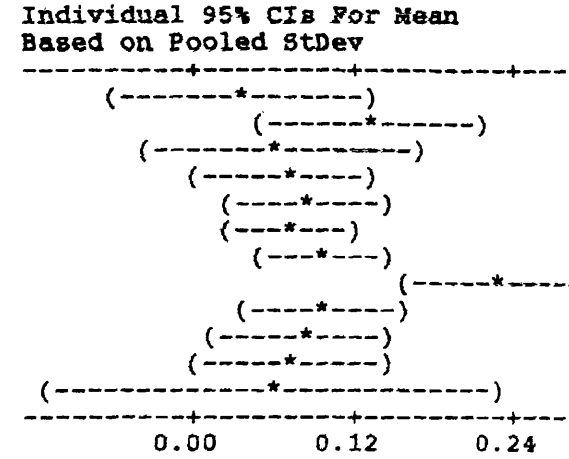
Analysis of Variance for Orchard

Source	DF	SS	MS	F	P
Month	11	0.14248	0.01295	1.84	0.066
Error	62	0.43677	0.00704		
Total	73	0.57924			

Level	N	Mean	StDev
1	3	0.03567	0.03204
2	4	0.13625	0.08671
3	3	0.06533	0.01124
4	6	0.06833	0.06198
5	7	0.08371	0.06138
6	14	0.07150	0.03309
7	10	0.09630	0.07316
8	6	0.22683	0.20306
9	8	0.09563	0.09479
10	6	0.08033	0.07459
11	6	0.07433	0.04653
12	1	0.05500	0.00000

Pooled StDev = 0.08393

Individual 95% CIs For Mean Based on Pooled StDev



One-Way Analysis of Variance

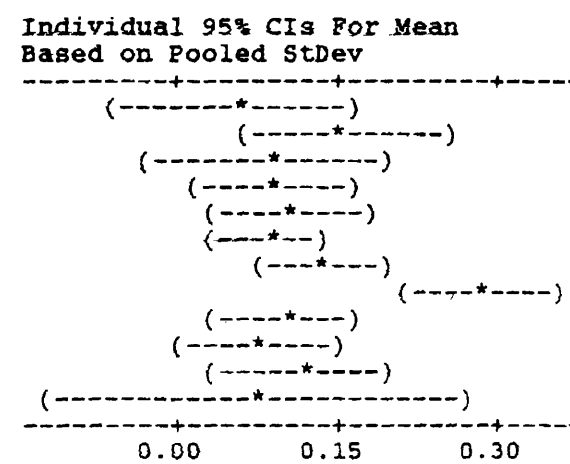
Analysis of Variance for Timothy

Source	DF	SS	MS	F	P
Month	11	0.22907	0.02082	2.23	0.024
Error	62	0.57890	0.00934		
Total	73	0.80797			

Level	N	Mean	StDev
1	3	0.05467	0.04937
2	4	0.15400	0.07250
3	3	0.08600	0.01803
4	6	0.08950	0.07227
5	7	0.10900	0.06976
6	14	0.08307	0.03599
7	10	0.13650	0.11355
8	6	0.28467	0.24567
9	8	0.09813	0.05907
10	6	0.07517	0.07814
11	6	0.11633	0.05040
12	1	0.07000	0.00000

Pooled StDev = 0.09663

Individual 95% CIs For Mean Based on Pooled StDev



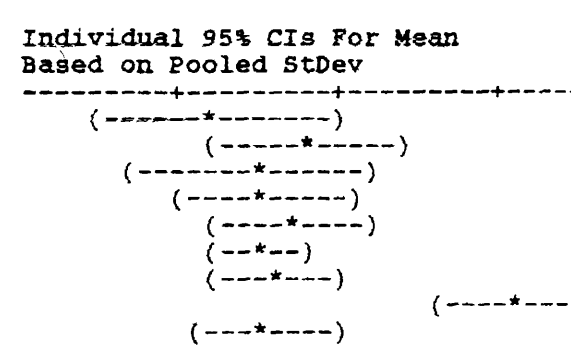
One-Way Analysis of Variance

Analysis of Variance for Kentucky

Source	DF	SS	MS	F	P
Month	11	0.33081	0.03007	3.43	0.001
Error	62	0.54355	0.00877		
Total	73	0.87435			

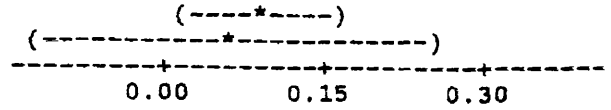
Level	N	Mean	StDev
1	3	0.03600	0.03124
2	4	0.11750	0.04371
3	3	0.07000	0.02234
4	6	0.08150	0.08602
5	7	0.10686	0.09194
6	14	0.07593	0.02395
7	10	0.09220	0.08127
8	6	0.32150	0.25951
9	8	0.08025	0.04098
10	6	0.06983	0.06513

Individual 95% CIs For Mean Based on Pooled StDev



11	6	0.09517	0.04592
12	1	0.06600	0.00000

Pooled StDev = 0.09363



One-Way Analysis of Variance

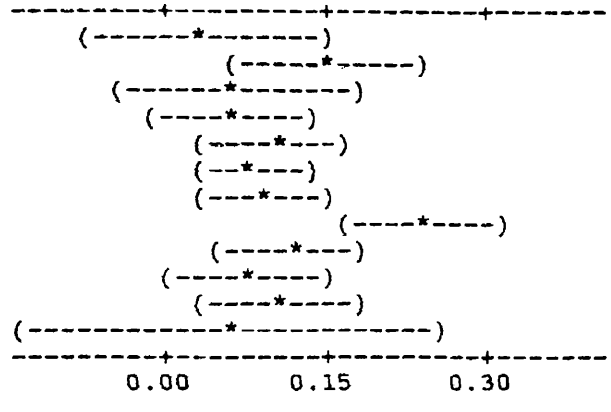
Analysis of Variance for Fescue

Source	DF	SS	MS	F	P
Month	11	0.17612	0.01601	1.78	0.077
Error	62	0.55819	0.00900		
Total	73	0.73431			

Level	N	Mean	StDev
1	3	0.03367	0.02916
2	4	0.15200	0.05757
3	3	0.06533	0.01589
4	6	0.06133	0.05877
5	7	0.09971	0.07327
6	14	0.08193	0.03614
7	10	0.09410	0.07599
8	6	0.24467	0.22590
9	8	0.11737	0.13998
10	6	0.07067	0.07639
11	6	0.10050	0.03504
12	1	0.05800	0.00000

Pooled StDev = 0.09488

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

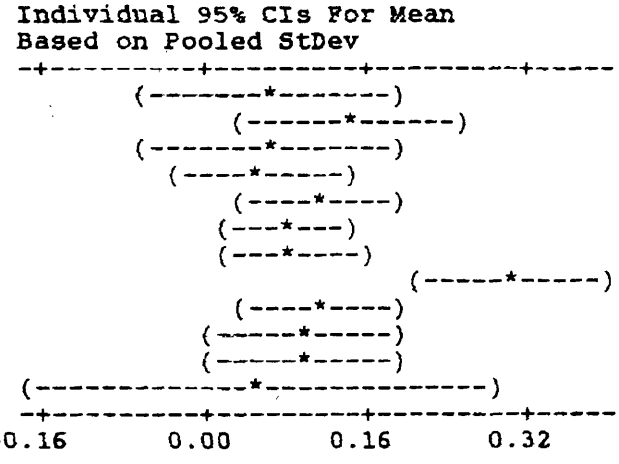
Analysis of Variance for Poplar

Source	DF	SS	MS	F	P
Month	11	0.2945	0.0268	2.06	0.037
Error	62	0.8042	0.0130		
Total	73	1.0987			

Level	N	Mean	StDev
1	3	0.0597	0.0610
2	4	0.1430	0.1028
3	3	0.0670	0.0078
4	6	0.0535	0.0520
5	7	0.1121	0.1002
6	14	0.0822	0.0493
7	10	0.0835	0.0830
8	6	0.3082	0.3141
9	8	0.1125	0.0981
10	6	0.0975	0.0589
11	6	0.0998	0.0620
12	1	0.0560	0.0000

Pooled StDev = 0.1139

Individual 95% CIs For Mean
Based on Pooled StDev



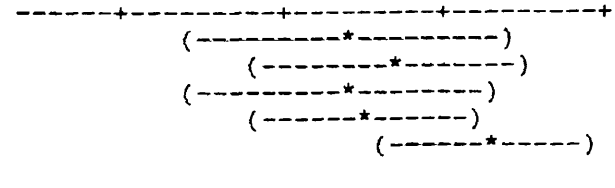
One-Way Analysis of Variance

Analysis of Variance for Birch

Source	DF	SS	MS	F	P
Month	11	0.1573	0.0143	0.87	0.577
Error	62	1.0235	0.0165		
Total	73	1.1807			

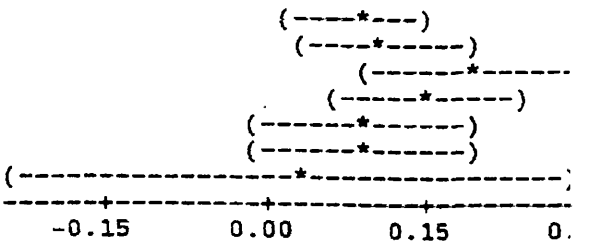
Level	N	Mean	StDev
1	3	0.0547	0.0577
2	4	0.0992	0.0363
3	3	0.0540	0.0144
4	6	0.0682	0.0496
5	7	0.1941	0.2658

Individual 95% CIs For Mean
Based on Pooled StDev



6	14	0.0859	0.0477
7	10	0.1088	0.1302
8	6	0.1923	0.1248
9	8	0.1553	0.2034
10	6	0.0888	0.0535
11	6	0.0882	0.0496
12	1	0.0230	0.0000

Pooled StDev = 0.1285



One-Way Analysis of Variance

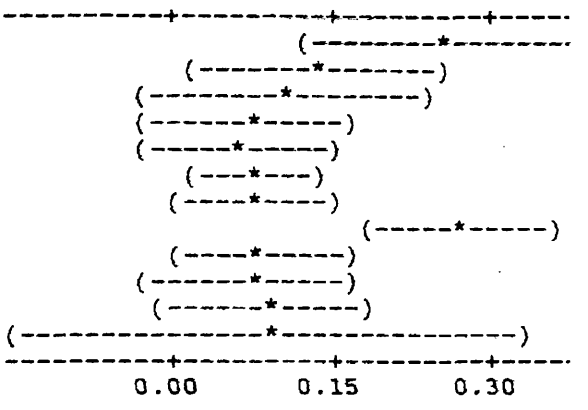
Analysis of Variance for Sorrel

Source	DF	SS	MS	F	P
Month	11	0.3008	0.0273	1.96	0.048
Error	62	0.8647	0.0139		
Total	73	1.1655			

Level	N	Mean	StDev
1	3	0.2623	0.4060
2	4	0.1395	0.0701
3	3	0.1060	0.0210
4	6	0.0720	0.0582
5	7	0.0660	0.0430
6	14	0.0719	0.0372
7	10	0.0691	0.0409
8	6	0.2690	0.2854
9	8	0.0780	0.0367
10	6	0.0708	0.0835
11	6	0.0833	0.0367
12	1	0.0900	0.0000

Pooled StDev = 0.1181

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

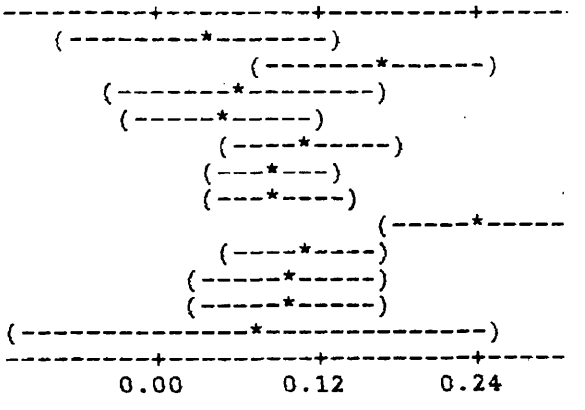
Analysis of Variance for Plantain

Source	DF	SS	MS	F	P
Month	11	0.16727	0.01521	1.91	0.056
Error	62	0.49456	0.00798		
Total	73	0.66184			

Level	N	Mean	StDev
1	3	0.03067	0.02875
2	4	0.16225	0.10489
3	3	0.06233	0.00757
4	6	0.05300	0.04483
5	7	0.11043	0.09828
6	14	0.08671	0.04736
7	10	0.08920	0.08727
8	6	0.23833	0.19949
9	8	0.10813	0.09171
10	6	0.09650	0.06386
11	6	0.09567	0.05631
12	1	0.07300	0.00000

Pooled StDev = 0.08931

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

Analysis of Variance for Mugwort

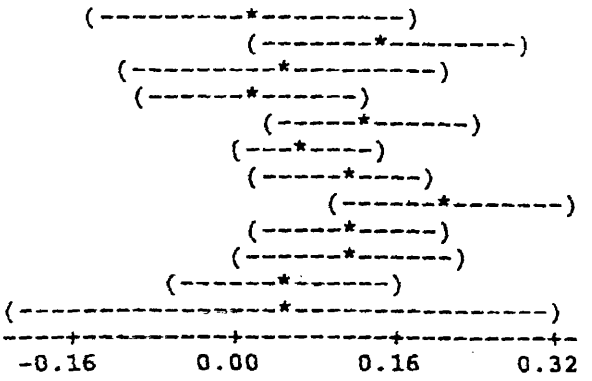
Source	DF	SS	MS	F	P
Month	11	0.1957	0.0178	0.93	0.517
Error	62	1.1837	0.0191		
Total	73	1.3794			

Level	N	Mean	StDev

Individual 95% CIs For Mean
Based on Pooled StDev

1	3	0.0163	0.0283
2	4	0.1463	0.1426
3	3	0.0447	0.0103
4	6	0.0220	0.0279
5	7	0.1309	0.2142
6	14	0.0691	0.0889
7	10	0.1066	0.1611
8	6	0.2160	0.1799
9	8	0.1130	0.1915
10	6	0.1095	0.1304
11	6	0.0480	0.0185
12	1	0.0460	0.0000

Pooled StDev = 0.1382



One-Way Analysis of Variance

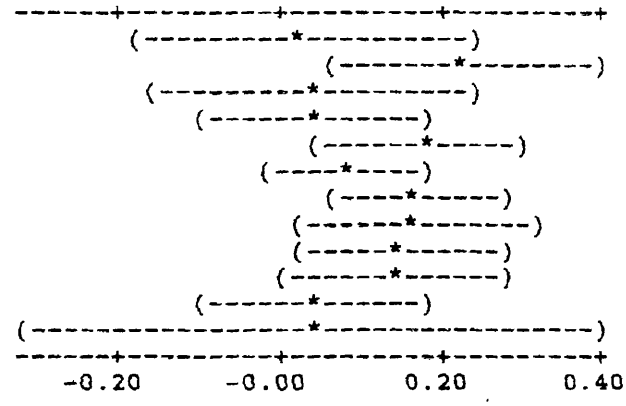
Analysis of Variance for Dandelio

Source	DF	SS	MS	F	P
Month	11	0.2514	0.0229	0.74	0.698
Error	62	1.9188	0.0309		
Total	73	2.1702			

Level	N	Mean	StDev
1	3	0.0270	0.0234
2	4	0.2283	0.3547
3	3	0.0407	0.0055
4	6	0.0455	0.0477
5	7	0.1734	0.2300
6	14	0.0807	0.0583
7	10	0.1666	0.2445
8	6	0.1665	0.1462
9	8	0.1458	0.2492
10	6	0.1410	0.1291
11	6	0.0425	0.0301
12	1	0.0390	0.0000

Pooled StDev = 0.1759

Individual 95% CIs For Mean
Based on Pooled StDev



One-Way Analysis of Variance

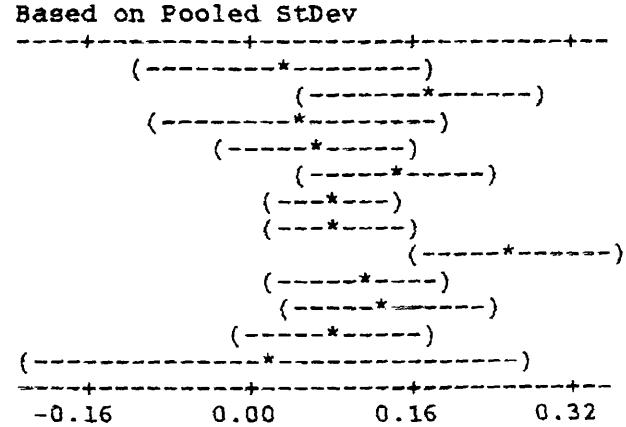
Analysis of Variance for Nettle

Source	DF	SS	MS	F	P
Month	11	0.2364	0.0215	1.44	0.178
Error	62	0.9245	0.0149		
Total	73	1.1609			

Level	N	Mean	StDev
1	3	0.0367	0.0356
2	4	0.1693	0.1592
3	3	0.0523	0.0035
4	6	0.0630	0.0764
5	7	0.1419	0.1909
6	14	0.0796	0.0681
7	10	0.0873	0.0983
8	6	0.2637	0.2602
9	8	0.1071	0.0854
10	6	0.1323	0.0973
11	6	0.0788	0.0529
12	1	0.0240	0.0000

Pooled StDev = 0.1221

Individual 95% CIs For Mean
Based on Pooled StDev



```
MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                    Newman-Keuls Multiple Range Test
                    ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.00948

            Df(error)
DATA> 62

            means of each group
DATA> 0.02667
DATA> 0.07600
DATA> 0.14067
DATA> 0.05050
DATA> 0.12129
DATA> 0.05086
DATA> 0.06730
DATA> 0.15700
DATA> 0.05788
DATA> 0.05267
DATA> 0.04933
DATA> 0.03900

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:  0.00948      df error:      62

     Group      Mean      Count
```

Data Display

1	0.02667	3
2	0.07600	4
3	0.14067	3
4	0.05050	6
5	0.12129	7
6	0.05086	14
7	0.06730	10
8	0.15700	6
9	0.05788	8
10	0.05267	6
11	0.04933	6
12	0.03900	1

No individual groups are significantly different

```
MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC
```

Newman-Keuls Multiple Range Test MITES

Please enter the following info at the DATA> prompt :

```
            Number of groups
DATA> 12

            MS(error)
DATA> 0.00358

            Df(error)
DATA> 62

            means of each group
DATA> 0.07400
DATA> 0.15225
DATA> 0.10433
DATA> 0.11950
DATA> 0.12157
DATA> 0.10521
DATA> 0.17450
DATA> 0.10667
DATA> 0.09787
DATA> 0.07533
DATA> 0.12833
DATA> 0.07600

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1
```

Results of Newman-Keuls multiple range test

Data Display

MS error: 0.00358 df error: 62

Data Display

Group	Mean	Count
1	0.07400	3
2	0.15225	4
3	0.10433	3
4	0.11950	6
5	0.12157	7
6	0.10521	14
7	0.17450	10
8	0.10667	6
9	0.09787	8

```
        10    0.07533        6
        11    0.12833        6
        12    0.07600        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                    Newman-Keuls Multiple Range Test
                    ================================

   Please enter the following info at the DATA> prompt :

              Number of groups
DATA> 12

              MS(error)
DATA> 0.00242

              Df(error)
DATA> 62

              means of each group
DATA> 0.02333
DATA> 0.11125
DATA> 0.07000
DATA> 0.07383
DATA> 0.07871
DATA> 0.07393
DATA> 0.06160
DATA> 0.10517
DATA> 0.07775
DATA> 0.03100
DATA> 0.06983
DATA> 0.04500

              number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

FEATHERS

Data Display

```
 MS error:  0.00242      df error:      62

     Group      Mean      Count
```

Data Display

```
        1    0.02333        3
        2    0.11125        4
        3    0.07000        3
        4    0.07383        6
        5    0.07871        7
        6    0.07393       14
```

```
        7    0.06160       10
        8    0.10517        6
        9    0.07775        8
       10    0.03100        6
       11    0.06983        6
       12    0.04500        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.00287

            Df(error)
DATA> 62

            means of each group
DATA> 0.02600
DATA> 0.10850
DATA> 0.11900
DATA> 0.07633
DATA> 0.06171
DATA> 0.11729
DATA> 0.06350
DATA> 0.08083
DATA> 0.09787
DATA> 0.04317
DATA> 0.41050
DATA> 0.09100

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

ALTERNARIA.

Data Display

```
 MS error:  0.00287      df error:      62

     Group      Mean      Count
```

Data Display

```
        1    0.02600        3
        2    0.10850        4
        3    0.11900        3
```

4	0.07633	6
5	0.06171	7
6	0.11729	14
7	0.06350	10
8	0.08083	6
9	0.09787	8
10	0.04317	6
11	0.41050	6
12	0.09100	1

Data Display

```
Group 11 significantly different to group  1
```

Data Display

```
Group 11 significantly different to group 10
```

Data Display

```
Group 11 significantly different to group  5
```

Data Display

```
Group 11 significantly different to group  7
```

Data Display

```
Group 11 significantly different to group  4
```

Data Display

```
Group 11 significantly different to group  8
```

Data Display

```
Group 11 significantly different to group 12
```

Data Display

```
Group 11 significantly different to group  9
```

Data Display

```
Group 11 significantly different to group  2
```

Data Display

```
Group 11 significantly different to group  6
```

Data Display

```
Group 11 significantly different to group  3

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.00184

            Df(error)
DATA> 62

            means of each group
DATA> 0.07500
DATA> 0.10100
DATA> 0.06100
DATA> 0.10917
DATA> 0.07429
DATA> 0.10186
DATA> 0.08550
DATA> 0.10033
DATA> 0.11025
DATA> 0.06800
DATA> 0.08783
DATA> 0.08400

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

ASPERGILLUS

Data Display

```
 MS error:  0.00184      df error:      62

     Group      Mean     Count
```

Data Display

```
         1   0.07500         3
         2   0.10100         4
         3   0.06100         3
```

```
         4    0.10917        6
         5    0.07429        7
         6    0.10186       14
         7    0.08550       10
         8    0.10033        6
         9    0.11025        8
        10    0.06800        6
        11    0.08783        6
        12    0.08400        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                    Newman-Keuls Multiple Range Test
                    ================================

   Please enter the following info at the DATA> prompt :

              Number of groups
DATA> 12

              MS(error)
DATA> 0.0133

              Df(error)
DATA> 62

              means of each group
DATA> 0.0130
DATA> 0.0850
DATA> 0.0500
DATA> 0.0550
DATA> 0.1153
DATA> 0.0653
DATA> 0.1169
DATA> 0.1942
DATA> 0.1585
DATA> 0.1180
DATA> 0.0795
DATA> 0.0280

              number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

RHIZOPUS

Data Display

```
 MS error:    0.0133       df error:       62

     Group      Mean      Count
```

Data Display

```
         1     0.0130        3
         2     0.0850        4
         3     0.0500        3
         4     0.0550        6
         5     0.1153        7
         6     0.0653       14
         7     0.1169       10
         8     0.1942        6
         9     0.1585        8
        10     0.1180        6
        11     0.0795        6
        12     0.0280        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.00658

            Df(error)
DATA> 62

            means of each group
DATA> 0.01600
DATA> 0.11175
DATA> 0.03367
DATA> 0.04233
DATA> 0.09329
DATA> 0.06007
DATA> 0.07210
DATA> 0.14850
DATA> 0.11213
DATA> 0.08450
DATA> 0.05600
DATA> 0.02100

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

KAPCK

Data Display

```
 MS error:  0.00658      df error:      62

     Group      Mean      Count
```

Data Display

1	0.01600	3
2	0.11175	4
3	0.03367	3
4	0.04233	6
5	0.09329	7
6	0.06007	14
7	0.07210	10
8	0.14850	6
9	0.11213	8
10	0.08450	6
11	0.05600	6
12	0.02100	1

No individual groups are significantly different

```
MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.0014

            Df(error)
DATA> 62

            means of each group
DATA> 0.02367
DATA> 0.09125
DATA> 0.05133
DATA> 0.03917
DATA> 0.06557
DATA> 0.04779
DATA> 0.04620
DATA> 0.05333
DATA> 0.04775
DATA> 0.03850
DATA> 0.05433
DATA> 0.02800

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

MS error: 0.0014 df error: 62

```
     Group      Mean      Count
```

Data Display

```
         1    0.02367        3
         2    0.09125        4
         3    0.05133        3
         4    0.03917        6
         5    0.06557        7
         6    0.04779       14
         7    0.04620       10
         8    0.05333        6
         9    0.04775        8
        10    0.03850        6
        11    0.05433        6
        12    0.02800        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.000749

            Df(error)
DATA> 62

            means of each group
DATA> 0.01000
DATA> 0.07525
DATA> 0.03167
DATA> 0.02917
DATA> 0.04943
DATA> 0.03600
DATA> 0.02900
DATA> 0.04550
DATA> 0.04588
DATA> 0.02167
DATA> 0.04500
DATA> 0.02500

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
* ERROR * Specified F format is too small for variable 2.
```

```
* Macro exiting...
MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                    Newman-Keuls Multiple Range Test
                    ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.0377

            Df(error)
DATA> 62

            means of each group
DATA> 0.02267
DATA> 0.04600
DATA> 0.04167
DATA> 0.03200
DATA> 0.06557
DATA> 0.03264
DATA> 0.07840
DATA> 0.06750
DATA> 0.05063
DATA> 0.03417
DATA> 0.03417
DATA> 0.02000

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:    0.0377      df error:      62
```

Group	Mean	Count
1	0.02267	3
2	0.04600	4
3	0.04167	3
4	0.03200	6
5	0.06557	7
6	0.03264	14
7	0.07840	10
8	0.06750	6
9	0.05063	8
10	0.03417	6
11	0.03417	6

```
       12    0.02000       1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.0137

            Df(error)
DATA> 62

            means of each group
DATA> 0.0350
DATA> 0.1102
DATA> 0.0680
DATA> 0.0490
DATA> 0.1803
DATA> 0.1135
DATA> 0.0912
DATA> 0.1247
DATA> 0.1576
DATA> 0.0912
DATA> 0.0995
DATA> 0.0920

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

MUCCR

Data Display

```
 MS error:    0.0137      df error:      62

     Group      Mean      Count
```

Data Display

```
        1     0.0350        3
        2     0.1102        4
        3     0.0680        3
        4     0.0490        6
        5     0.1803        7
        6     0.1135       14
        7     0.0912       10
        8     0.1247        6
```

```
         9     0.1576        8
        10     0.0912        6
        11     0.0995        6
        12     0.0920        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                    Newman-Keuls Multiple Range Test
                    ================================

   Please enter the following info at the DATA> prompt :

             Number of groups
DATA> 12

             MS(error)
DATA> 0.00704

             Df(error)
DATA> 62

             means of each group
DATA> 0.03567
DATA> 0.13625
DATA> 0.06533
DATA> 0.06833
DATA> 0.08371
DATA> 0.07150
DATA> 0.09630
DATA> 0.22683
DATA> 0.09563
DATA> 0.08033
DATA> 0.07433
DATA> 0.05500

             number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:  0.00704      df error:      62

     Group      Mean      Count
```

Data Display

```
         1    0.03567        3
         2    0.13625        4
         3    0.06533        3
         4    0.06833        6
         5    0.08371        7
```

```
         6    0.07150       14
         7    0.09630       10
         8    0.22683        6
         9    0.09563        8
        10    0.08033        6
        11    0.07433        6
        12    0.05500        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                    Newman-Keuls Multiple Range Test
                    ================================

   Please enter the following info at the DATA> prompt :

             Number of groups
DATA> 12

             MS(error)
DATA> 0.00934

             Df(error)
DATA> 62

             means of each group
DATA> 0.05467
DATA> 0.15400
DATA> 0.08600
DATA> 0.08950
DATA> 0.10900
DATA> 0.08307
DATA> 0.13650
DATA> 0.28467
DATA> 0.09813
DATA> 0.07517
DATA> 0.11633
DATA> 0.07000

             number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:  0.00934      df error:      62

     Group      Mean     Count
```

Data Display

```
         1    0.05467        3
         2    0.15400        4
```

TIMOTHY GRASS

```
        3    0.08600        3
        4    0.08950        6
        5    0.10900        7
        6    0.08307       14
        7    0.13650       10
        8    0.28467        6
        9    0.09813        8
       10    0.07517        6
       11    0.11633        6
       12    0.07000        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.00877

            Df(error)
DATA> 62

            means of each group
DATA> 0.03600
DATA> 0.11750
DATA> 0.07000
DATA> 0.08150
DATA> 0.10686
DATA> 0.07593
DATA> 0.09220
DATA> 0.32150
DATA> 0.08025
DATA> 0.06983
DATA> 0.09517
DATA> 0.06600

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

KENTUCKY GRAS

Data Display

```
 MS error:  0.00877      df error:      62

     Group      Mean     Count
```

Data Display

1	0.03600	3
2	0.11750	4
3	0.07000	3
4	0.08150	6
5	0.10686	7
6	0.07593	14
7	0.09220	10
8	0.32150	6
9	0.08025	8
10	0.06983	6
11	0.09517	6
12	0.06600	1

Data Display

Group 8 significantly different to group 1

Data Display

Group 8 significantly different to group 12

Data Display

Group 8 significantly different to group 10

Data Display

Group 8 significantly different to group 3

Data Display

Group 8 significantly different to group 6

Data Display

Group 8 significantly different to group 9

Data Display

Group 8 significantly different to group 4

Data Display

Group 8 significantly different to group 7

Data Display

Group 8 significantly different to group 11

Data Display

```
Group  8 significantly different to group  5
```

Data Display

```
Group  8 significantly different to group  2

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.00900

            Df(error)
DATA> 62

            means of each group
DATA> 0.03367
DATA> 0.15200
DATA> 0.06533
DATA> 0.06133
DATA> 0.09971
DATA> 0.08193
DATA> 0.09410
DATA> 0.24467
DATA> 0.11737
DATA> 0.07067
DATA> 0.10050
DATA> 0.05800

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:    0.009      df error:      62

     Group      Mean      Count
```

Data Display

```
        1    0.03367        3
        2    0.15200        4
        3    0.06533        3
        4    0.06133        6
        5    0.09971        7
        6    0.08193       14
        7    0.09410       10
        8    0.24467        6
        9    0.11737        8
       10    0.07067        6
       11    0.10050        6
       12    0.05800        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.013

            Df(error)
DATA> 62

            means of each group
DATA> 0.0597
DATA> 0.1430
DATA> 0.0670
DATA> 0.0535
DATA> 0.1121
DATA> 0.0822
DATA> 0.0835
DATA> 0.3082
DATA> 0.1125
DATA> 0.0975
DATA> 0.0998
DATA> 0.0560

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

POPLAR

Data Display

```
 MS error:     0.013       df error:       62

     Group      Mean      Count
```

Data Display

1	0.0597	3
2	0.1430	4
3	0.0670	3
4	0.0535	6
5	0.1121	7
6	0.0822	14
7	0.0835	10
8	0.3082	6
9	0.1125	8
10	0.0975	6
11	0.0998	6
12	0.0560	1

Data Display

```
Group  8 significantly different to group  9
```

Data Display

```
Group  8 significantly different to group  2

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.0165

            Df(error)
DATA> 62

            means of each group
DATA> 0.0547
DATA> 0.0992
DATA> 0.0540
DATA> 0.0682
DATA> 0.1941
DATA> 0.0859
DATA> 0.1088
DATA> 0.1923
DATA> 0.1553
DATA> 0.0888
DATA> 0.0882
DATA> 0.0230

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
```

```
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:    0.0165     df error:      62

     Group      Mean     Count
```

Data Display

```
         1    0.0547         3
         2    0.0992         4
         3    0.0540         3
         4    0.0682         6
         5    0.1941         7
         6    0.0859        14
         7    0.1088        10
         8    0.1923         6
         9    0.1553         8
        10    0.0888         6
        11    0.0882         6
        12    0.0230         1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.0139

            Df(error)
DATA> 62

            means of each group
DATA> 0.2623
DATA> 0.1395
DATA> 0.1060
DATA> 0.0720
DATA> 0.0660
DATA> 0.0719
DATA> 0.0691
DATA> 0.2690
DATA> 0.0780
DATA> 0.0708
DATA> 0.0833
DATA> 0.0900

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
```

```
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:    0.0139      df error:      62

     Group      Mean      Count
```

Data Display

```
         1    0.2623          3
         2    0.1395          4
         3    0.1060          3
         4    0.0720          6
         5    0.0660          7
         6    0.0719         14
         7    0.0691         10
         8    0.2690          6
         9    0.0780          8
        10    0.0708          6
        11    0.0833          6
        12    0.0900          1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.00798

            Df(error)
DATA> 62

            means of each group
DATA> 0.03067
DATA> 0.16225
DATA> 0.06233
DATA> 0.05300
DATA> 0.11043
DATA> 0.08671
DATA> 0.05920
DATA> 0.23833
DATA> 0.10813
DATA> 0.09650
DATA> 0.09567
DATA> 0.07300

            number of observations in each group
DATA> 3
DATA> 4
```

```
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:  0.00798      df error:      62

     Group      Mean      Count
```

Data Display

```
         1   0.03067         3
         2   0.16225         4
         3   0.06233         3
         4   0.05300         6
         5   0.11043         7
         6   0.08671        14
         7   0.05920        10
         8   0.23833         6
         9   0.10813         8
        10   0.09650         6
        11   0.09567         6
        12   0.07300         1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.0191

            Df(error)
DATA> 62

            means of each group
DATA> 0.0163
DATA> 0.1463
DATA> 0.0447
DATA> 0.0220
DATA> 0.1309
DATA> 0.0691
DATA> 0.1066
DATA> 0.2160
DATA> 0.1130
DATA> 0.1095
DATA> 0.0480
DATA> 0.0460
```

```
            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:    0.0191      df error:      62

     Group      Mean      Count
```

Data Display

```
         1    0.0163        3
         2    0.1463        4
         3    0.0447        3
         4    0.0220        6
         5    0.1309        7
         6    0.0691       14
         7    0.1066       10
         8    0.2160        6
         9    0.1130        8
        10    0.1095        6
        11    0.0480        6
        12    0.0460        1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================


   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.0309

            Df(error)
DATA> 62

            means of each group
DATA> 0.0270
DATA> 0.2283
DATA> 0.0407
DATA> 0.0455
DATA> 0.1734
DATA> 0.0807
DATA> 0.1666
DATA> 0.1665
DATA> 0.1458
DATA> 0.1410
```

```
DATA> 0.0425
DATA> 0.0390

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:   0.0309      df error:      62

     Group      Mean      Count
```

Data Display

```
         1    0.0270          3
         2    0.2283          4
         3    0.0407          3
         4    0.0455          6
         5    0.1734          7
         6    0.0807         14
         7    0.1666         10
         8    0.1665          6
         9    0.1458          8
        10    0.1410          6
        11    0.0425          6
        12    0.0390          1

 No individual groups are significantly different

MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC

                  Newman-Keuls Multiple Range Test
                  ================================

   Please enter the following info at the DATA> prompt :

            Number of groups
DATA> 12

            MS(error)
DATA> 0.0149

            Df(error)
DATA> 62

            means of each group
DATA> 0.0367
DATA> 0.1693
DATA> 0.0523
DATA> 0.0630
DATA> 0.1419
DATA> 0.0796
DATA> 0.0873
```

NEPTLK

```
DATA> 0.2637
DATA> 0.1071
DATA> 0.1323
DATA> 0.0788
DATA> 0.0240

            number of observations in each group
DATA> 3
DATA> 4
DATA> 3
DATA> 6
DATA> 7
DATA> 14
DATA> 10
DATA> 6
DATA> 8
DATA> 6
DATA> 6
DATA> 1

 Results of Newman-Keuls multiple range test
 -------------------------------------------
```

Data Display

```
 MS error:   0.0149      df error:      62

     Group      Mean      Count
```

Data Display

```
         1    0.0367          3
         2    0.1693          4
         3    0.0523          3
         4    0.0630          6
         5    0.1419          7
         6    0.0796         14
         7    0.0873         10
         8    0.2637          6
         9    0.1071          8
        10    0.1323          6
        11    0.0788          6
        12    0.0240          1

 No individual groups are significantly different
```

Appendix H

Methodology of Pollen Analysis

Faecal pollen analysis (From Caulton, 1988)

Firstly the faecal sample was desiccated at 30°C for one week. One gram of the resultant material was removed, ground to a powder and placed in a boiling tube. To this was added 20ml of 10 per cent potassium hydroxide, and placed in a boiling water bath for 20 minutes. At five minute intervals distilled water was added to maintain the 10 per cent concentration. The contents were then filtered and the filtrate centrifuged at 2000 rpm for ten minutes. Supernatant was removed and the precipitate washed and centrifuged again as above. A few ml of glacial acetic acid were then added to the precipitate and the contents centrifuged as above. To the precipitate, 2.5ml of a mixture of acetic anhydride and concentrated sulphuric acid (9/1 w/v) were added. The tube was then placed in a hot water bath for three minutes. Then a few drops of glacial acetic acid were added to stop the reaction. The resultant precipitate was then washed and centrifuged twice as above. Then the precipitate was allowed to drain over filter paper for 10 minutes.

10ml of 30 per cent dilute glycerine and five drops of basic fuschine stain were added to the precipitate and stirred. One to two drops of this now pink coloured suspension were added to warmed slides and covered. These slides were then sealed with DPX after thirty minutes. After this time analysis of these slides could take place at x 40 magnification on a standard microscope.

Appendix I

Statistical analysis of ELISA results for atopic and non-atopic GDBA and GUVS dogs, greyhounds and beagles

Appendix I1

Statisitical analysis of ELISA results for atopic and non-atopic dogs (non-GDBA/non-atopic =greyhounds).

Worksheet size: 100000 cells

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Flea

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000060	0.000239	0.000239	0.03	0.852
GDBA	1	0.000907	0.000999	0.000999	0.15	0.704
Atopic*GDBA	1	0.002621	0.002621	0.002621	0.38	0.538
Error	128	0.879609	0.879609	0.006872		
Total	131	0.883196				

Unusual Observations for Flea

Obs	Flea	Fit	StDev Fit	Residual	St Resid
19	0.317000	0.072959	0.009637	0.244041	2.96R
39	0.474000	0.072959	0.009637	0.401041	4.87R
64	0.656000	0.072959	0.009637	0.583041	7.08R
116	0.298000	0.076562	0.014654	0.221438	2.71R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.05807	0.07656	0.07093
	0.04482	0.06152	0.05711
2	74	12	86
	0.07296	0.06858	0.07235
	0.09692	0.06774	0.09308
All	88	44	132
	0.07059	0.07439	0.07186
	0.09062	0.06257	0.08211

Cell Contents --
Flea:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mites

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001316	0.000119	0.000119	0.03	0.859
GDBA	1	0.003904	0.003758	0.003758	1.00	0.320
Atopic*GDBA	1	0.001560	0.001560	0.001560	0.41	0.521
Error	128	0.481820	0.481820	0.003764		
Total	131	0.488600				

Unusual Observations for Mites

Obs	Mites	Fit	StDev Fit	Residual	St Resid
16	0.287000	0.116905	0.007132	0.170095	2.79R
27	0.276000	0.116905	0.007132	0.159095	2.61R
39	0.303000	0.116905	0.007132	0.186095	3.05R
46	0.276000	0.116905	0.007132	0.159095	2.61R
48	0.275000	0.116905	0.007132	0.158095	2.59R
94	0.275000	0.128156	0.010846	0.146844	2.43R
97	0.000000	0.128156	0.010846	-0.128156	-2.12R
117	0.263000	0.128156	0.010846	0.134844	2.23R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.12329	0.12816	0.12667
	0.05247	0.06425	0.06037
2	74	12	86
	0.11691	0.13942	0.12005
	0.06243	0.05522	0.06167
All	88	44	132
	0.11792	0.13123	0.12236
	0.06072	0.06150	0.06107

Cell Contents --
Mites:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Feathers

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001587	0.000002	0.000002	0.00	0.981
GDBA	1	0.005494	0.005706	0.005706	1.92	0.168
Atopic*GDBA	1	0.002445	0.002445	0.002445	0.82	0.366
Error	128	0.379760	0.379760	0.002967		
Total	131	0.389288				

Unusual Observations for Feathers

Obs	Feathers	Fit	StDev Fit	Residual	St Resid
3	0.180000	0.071257	0.006332	0.108743	2.01R
12	0.213000	0.071257	0.006332	0.141743	2.62R
51	0.291000	0.071257	0.006332	0.219743	4.06R
94	0.208000	0.087844	0.009629	0.120156	2.24R
110	0.388000	0.087844	0.009629	0.300156	5.60R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.05993	0.08784	0.07935
	0.03158	0.07091	0.06262
2	74	12	86
	0.07126	0.07708	0.07207
	0.04998	0.05094	0.04985
All	88	44	132
	0.06945	0.08491	0.07461
	0.04756	0.06567	0.05451

Cell Contents --
Feathers:N
Mean
StDev

MTB >

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Alternar

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.014507	0.016197	0.016197	4.50	0.036
GDBA	1	0.001857	0.001775	0.001775	0.49	0.484
Atopic*GDBA	1	0.001046	0.001046	0.001046	0.29	0.591
Error	128	0.460985	0.460985	0.003601		
Total	131	0.478394				

Unusual Observations for Alternar

Obs	Alternar	Fit	StDev Fit	Residual	St Resid
7	0.225000	0.087797	0.006976	0.137203	2.30R
17	0.268000	0.087797	0.006976	0.180203	3.02R
24	0.209000	0.087797	0.006976	0.121203	2.03R
59	0.233000	0.087797	0.006976	0.145203	2.44R
83	0.353000	0.109000	0.016039	0.244000	4.22R
106	0.226000	0.106813	0.010609	0.119188	2.02R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.10900	0.10681	0.10748
	0.07756	0.06226	0.06641
2	74	12	86
	0.08780	0.07117	0.08548
	0.05824	0.03695	0.05588
All	88	44	132
	0.09117	0.09709	0.09314
	0.06169	0.05833	0.06043

Cell Contents --
Alternar:N
Mean
StDev

MTB >

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Aspergil

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.012784	0.007260	0.007260	2.74	0.100
GDBA	1	0.000336	0.000357	0.000357	0.13	0.714
Atopic*GDBA	1	0.000386	0.000386	0.000386	0.15	0.703
Error	128	0.338983	0.338983	0.002648		
Total	131	0.352489				

Unusual Observations for Aspergil

Obs	Aspergil	Fit	StDev Fit	Residual	St Resid
64	0.226000	0.091500	0.005982	0.134500	2.63R
93	0.232000	0.114750	0.009097	0.117250	2.31R
98	0.001000	0.114750	0.009097	-0.113750	-2.25R
112	0.226000	0.114750	0.009097	0.111250	2.20R
118	0.333000	0.114750	0.009097	0.218250	4.31R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.10614	0.11475	0.11213
	0.05266	0.07067	0.06525
2	74	12	86
	0.09150	0.09133	0.09148
	0.04229	0.03992	0.04174
All	88	44	132
	0.09383	0.10836	0.09867
	0.04409	0.06418	0.05187

Cell Contents --
Aspergil:N
Mean
StDev

MTB >

MTB > glm c8=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Rhizopus

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.021017	0.003532	0.003532	0.38	0.541
GDBA	1	0.011134	0.011104	0.011104	1.18	0.280
Atopic*GDBA	1	0.000011	0.000011	0.000011	0.00	0.973
Error	128	1.205076	1.205076	0.009415		
Total	131	1.237237				

Unusual Observations for Rhizopus

Obs	Rhizopus	Fit	StDev Fit	Residual	St Resid
3	0.406000	0.099932	0.011279	0.306068	3.18R
27	0.588000	0.099932	0.011279	0.488068	5.06R
39	0.396000	0.099932	0.011279	0.296068	3.07R
54	0.615000	0.099932	0.011279	0.515068	5.34R
83	0.313000	0.085929	0.025932	0.227071	2.43R
101	0.318000	0.063125	0.017152	0.254875	2.67R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c8.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.08593	0.06313	0.07007
	0.07503	0.06724	0.06967
2	74	12	86
	0.09993	0.07567	0.09655
	0.11591	0.03156	0.10835
All	88	44	132
	0.09770	0.06655	0.08732
	0.11019	0.05955	0.09718

Cell Contents --
Rhizopus:N
Mean
StDev

MTB >

MTB > glm c9=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Kapok

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000197	0.002572	0.002572	0.40	0.529
GDBA	1	0.011398	0.011269	0.011269	1.75	0.188
Atopic*GDBA	1	0.000370	0.000370	0.000370	0.06	0.811
Error	128	0.824643	0.824643	0.006443		
Total	131	0.836609				

Unusual Observations for Kapok

Obs	Kapok	Fit	StDev Fit	Residual	St Resid
3	0.319000	0.077257	0.009331	0.241743	3.03R
27	0.383000	0.077257	0.009331	0.305743	3.84R
39	0.320000	0.077257	0.009331	0.242743	3.04R
54	0.410000	0.077257	0.009331	0.332743	4.17R
79	0.479000	0.084286	0.021452	0.394714	5.10R
101	0.308000	0.064875	0.014189	0.243125	3.08R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c9.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.08429	0.06487	0.07078
	0.12341	0.06438	0.08565
2	74	12	86
	0.07726	0.04925	0.07335
	0.08188	0.02815	0.07718
All	88	44	132
	0.07837	0.06061	0.07245
	0.08893	0.05692	0.07991

Cell Contents --
Kapok:N
Mean
StDev

MTB >

MTB > glm c10=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Dust

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001348	0.000489	0.000489	0.47	0.496
GDBA	1	0.000179	0.000176	0.000176	0.17	0.683
Atopic*GDBA	1	0.000011	0.000011	0.000011	0.01	0.920
Error	128	0.134485	0.134485	0.001051		
Total	131	0.136023				

Unusual Observations for Dust

Obs	Dust	Fit	StDev Fit	Residual	St Resid
1	0.123000	0.050027	0.003768	0.072973	2.27R
12	0.136000	0.050027	0.003768	0.085973	2.67R
17	0.115000	0.050027	0.003768	0.064973	2.02R
73	0.160000	0.050027	0.003768	0.109973	3.42R
79	0.155000	0.044357	0.008663	0.110643	3.54R

R denotes an observation with a large standardized residual

MTB > table c1 c2.
SUBC> stats c10.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.044357	0.042125	0.042804
	0.040636	0.026655	0.031106
2	74	12	86
	0.050027	0.046333	0.049512
	0.033883	0.025553	0.032744
All	88	44	132
	0.049125	0.043273	0.047174
	0.034849	0.026131	0.032223

Cell Contents --
Dust:N
Mean
StDev

MTB >

MTB > glm c11=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Cat

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0005974	0.0019071	0.0019071	2.57	0.111
GDBA	1	0.0012931	0.0012403	0.0012403	1.67	0.198
Atopic*GDBA	1	0.0006172	0.0006172	0.0006172	0.83	0.363
Error	128	0.0949040	0.0949040	0.0007414		
Total	131	0.0974117				

Unusual Observations for Cat

Obs	Cat	Fit	StDev Fit	Residual	St Resid
1	0.115000	0.037919	0.003165	0.077081	2.85R
17	0.103000	0.037919	0.003165	0.065081	2.41R
60	0.101000	0.037919	0.003165	0.063081	2.33R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c11.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.033714	0.036031	0.035326
	0.019201	0.024843	0.023083
2	74	12	86
	0.037919	0.051333	0.039791
	0.028576	0.032148	0.029273
All	88	44	132
	0.037250	0.040205	0.038235
	0.027252	0.027511	0.027269

Cell Contents --
Cat:N
Mean
StDev

MTB >

MTB > glm c12=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Human

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.006477	0.006786	0.006786	2.96	0.088
GDBA	1	0.000622	0.000595	0.000595	0.26	0.612
Atopic*GDBA	1	0.000329	0.000329	0.000329	0.14	0.706
Error	128	0.293724	0.293724	0.002295		
Total	131	0.301151				

Unusual Observations for Human

Obs	Human	Fit	StDev Fit	Residual	St Resid
8	0.197000	0.047419	0.005569	0.149581	3.14R
39	0.226000	0.047419	0.005569	0.178581	3.75R
48	0.434000	0.047419	0.005569	0.386581	8.13R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c12.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.033071	0.034469	0.034043
	0.020281	0.023903	0.022646
2	74	12	86
	0.047419	0.056917	0.048744
	0.059374	0.034797	0.056527
All	88	44	132
	0.045136	0.040591	0.043621
	0.055202	0.028704	0.047946

Cell Contents --
Human:N
Mean
StDev

MTB >

MTB > glm c13=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Mucor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.002564	0.003303	0.003303	0.33	0.564
GDBA	1	0.040385	0.041370	0.041370	4.19	0.043
Atopic*GDBA	1	0.007454	0.007454	0.007454	0.75	0.387
Error	128	1.263922	1.263922	0.009874		
Total	131	1.314326				

Unusual Observations for Mucor

Obs	Mucor	Fit	StDev Fit	Residual	St Resid
27	0.449000	0.108811	0.011552	0.340189	3.45R
39	0.751000	0.108811	0.011552	0.642189	6.51R
51	0.372000	0.108811	0.011552	0.263189	2.67R
59	0.329000	0.108811	0.011552	0.220189	2.23R
79	0.423000	0.140929	0.026558	0.282071	2.95R
83	0.433000	0.140929	0.026558	0.292071	3.05R

R denotes an observation with a large standardized residual

MTB > table c1 c2.
SUBC> stats c13.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.14093	0.07622	0.09591
	0.13171	0.04634	0.08601
2	74	12	86
	0.10881	0.08267	0.10516
	0.11426	0.04129	0.10731
All	88	44	132
	0.11392	0.07798	0.10194
	0.11699	0.04464	0.10016

Cell Contents --
Mucor:N
Mean
StDev

MTB >

MTB > glm c14=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Orchard

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.005394	0.000093	0.000093	0.01	0.911
GDBA	1	0.009784	0.009953	0.009953	1.35	0.247
Atopic*GDBA	1	0.000938	0.000938	0.000938	0.13	0.722
Error	128	0.941534	0.941534	0.007356		
Total	131	0.957650				

Unusual Observations for Orchard

Obs	Orchard	Fit	StDev Fit	Residual	St Resid
3	0.623000	0.093473	0.009970	0.529527	6.22R
54	0.324000	0.093473	0.009970	0.230527	2.71R
89	0.433000	0.117906	0.015161	0.315094	3.73R
119	0.348000	0.117906	0.015161	0.230094	2.73R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c14

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.08879	0.11791	0.10904
	0.05034	0.09949	0.08794
2	74	12	86
	0.09347	0.10892	0.09563
	0.08908	0.04525	0.08431
All	88	44	132
	0.09273	0.11545	0.10030
	0.08390	0.08761	0.08550

Cell Contents --
Orchard:N
Mean
StDev

MTB >

MTB > glm c15=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Timothy

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.006029	0.000222	0.000222	0.02	0.880
GDBA	1	0.019736	0.019701	0.019701	2.02	0.158
Atopic*GDBA	1	0.000005	0.000005	0.000005	0.00	0.983
Error	128	1.251335	1.251335	0.009776		
Total	131	1.277104				

Unusual Observations for Timothy

Obs	Timothy	Fit	StDev Fit	Residual	St Resid
3	0.618000	0.115919	0.011494	0.502081	5.11R
8	0.576000	0.115919	0.011494	0.460081	4.68R
48	0.320000	0.115919	0.011494	0.204081	2.08R
89	0.378000	0.143937	0.017479	0.234063	2.41R
101	0.366000	0.143937	0.017479	0.222063	2.28R
104	0.417000	0.143937	0.017479	0.273063	2.81R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c15.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11307	0.14394	0.13454
	0.06228	0.10350	0.09330
2	74	12	86
	0.11592	0.14775	0.12036
	0.10520	0.07440	0.10171
All	88	44	132
	0.11547	0.14498	0.12530
	0.09934	0.09561	0.09874

Cell Contents --
Timothy:N
Mean
StDev

MTB >

MTB > glm c16=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Kentucky

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00759	0.00000	0.00000	0.00	0.983
GDBA	1	0.01749	0.01748	0.01748	1.63	0.204
Atopic*GDBA	1	0.00000	0.00000	0.00000	0.00	0.995
Error	128	1.37223	1.37223	0.01072		
Total	131	1.39731				

Unusual Observations for Kentucky

Obs	Kentucky	Fit	StDev Fit	Residual	St Resid
3	0.669000	0.103203	0.012036	0.565797	5.50R
8	0.613000	0.103203	0.012036	0.509797	4.96R
89	0.545000	0.132250	0.018303	0.412750	4.05R
104	0.389000	0.132250	0.018303	0.256750	2.52R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c16.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.10257	0.13225	0.12322
	0.05128	0.11289	0.09864
2	74	12	86
	0.10320	0.13258	0.10730
	0.10944	0.07900	0.10583
All	88	44	132
	0.10310	0.13234	0.11285
	0.10219	0.10384	0.10328

Cell Contents --
Kentucky:N
Mean
StDev

MTB >

```
MTB > glm c17=c1 c2 c1*c2
```

General Linear Model

```
Factor   Levels Values
Atopic        2  1  2
GDBA          2  1  2
```

Analysis of Variance for Fescue

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000885	0.010473	0.010473	1.15	0.286
GDBA	1	0.044518	0.043705	0.043705	4.79	0.030
Atopic*GDBA	1	0.003987	0.003987	0.003987	0.44	0.510
Error	128	1.168541	1.168541	0.009129		
Total	131	1.217930				

Unusual Observations for Fescue

Obs	Fescue	Fit	StDev Fit	Residual	St Resid
3	0.682000	0.102041	0.011107	0.579959	6.11R
54	0.460000	0.102041	0.011107	0.357959	3.77R
89	0.435000	0.125875	0.016890	0.309125	3.29R
101	0.325000	0.125875	0.016890	0.199125	2.12R
125	0.418000	0.162833	0.027582	0.255167	2.79R

R denotes an observation with a large standardized residual

```
MTB > table c1 c2;
SUBC> stats c17.
```

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.09329	0.12588	0.11596
	0.05502	0.09544	0.08591
2	74	12	86
	0.10204	0.16283	0.11052
	0.10029	0.10112	0.10204
All	88	44	132
	0.10065	0.13595	0.11242
	0.09436	0.09726	0.09642

```
Cell Contents --
      Fescue:N
           Mean
           StDev

MTB >
```

MTB > glm c18=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Poplar

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00334	0.01222	0.01222	0.97	0.328
GDBA	1	0.00625	0.00565	0.00565	0.45	0.505
Atopic*GDBA	1	0.01694	0.01694	0.01694	1.34	0.249
Error	128	1.61966	1.61966	0.01265		
Total	131	1.64619				

Unusual Observations for Poplar

Obs	Poplar	Fit	StDev Fit	Residual	St Resid
3	0.877000	0.108554	0.013077	0.768446	6.88R
36	0.461000	0.108554	0.013077	0.352446	3.15R
54	0.343000	0.108554	0.013077	0.234446	2.10R
79	0.332000	0.112929	0.030064	0.219071	2.02R
89	0.352000	0.100656	0.019885	0.251344	2.27R
101	0.385000	0.100656	0.019885	0.284344	2.57R
128	0.404000	0.154417	0.032473	0.249583	2.32R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c18.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11293	0.10066	0.10439
	0.09234	0.09484	0.09323
2	74	12	86
	0.10855	0.15442	0.11495
	0.12268	0.10925	0.12135
All	88	44	132
	0.10925	0.11532	0.11127
	0.11792	0.10062	0.11210

Cell Contents --
Poplar:N
Mean
StDev

MTB >

MTB > glm c19=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Birch

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00092	0.00689	0.00689	0.42	0.517
GDBA	1	0.01356	0.01385	0.01385	0.85	0.359
Atopic*GDBA	1	0.00196	0.00196	0.00196	0.12	0.730
Error	128	2.09110	2.09110	0.01634		
Total	131	2.10754				

Unusual Observations for Birch

Obs	Birch	Fit	StDev Fit	Residual	St Resid
27	0.460000	0.111649	0.014858	0.348351	2.74R
39	0.792000	0.111649	0.014858	0.680351	5.36R
54	0.656000	0.111649	0.014858	0.544351	4.29R
79	0.770000	0.140071	0.034160	0.629929	5.11R
101	0.381000	0.103906	0.022595	0.277094	2.20R
117	0.525000	0.103906	0.022595	0.421094	3.35R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c19

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.14007	0.10391	0.11491
	0.19267	0.11163	0.13997
2	74	12	86
	0.11165	0.09525	0.10936
	0.12718	0.06143	0.12005
All	88	44	132
	0.11617	0.10155	0.11130
	0.13866	0.09982	0.12684

Cell Contents --
Birch:N
Mean
StDev

MTB >

MTB > glm c20=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Sorrel

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00790	0.00918	0.00918	0.85	0.359
GDBA	1	0.00278	0.00291	0.00291	0.27	0.605
Atopic*GDBA	1	0.00174	0.00174	0.00174	0.16	0.689
Error	128	1.38662	1.38662	0.01083		
Total	131	1.39905				

Unusual Observations for Sorrel

Obs	Sorrel	Fit	StDev Fit	Residual	St Resid
3	0.790000	0.101446	0.012099	0.688554	6.66R
8	0.400000	0.101446	0.012099	0.298554	2.89R
69	0.730000	0.101446	0.012099	0.628554	6.08R
89	0.375000	0.092094	0.018399	0.282906	2.76R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c20.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.07071	0.09209	0.08559
	0.03715	0.07197	0.06376
2	74	12	86
	0.10145	0.10417	0.10183
	0.12636	0.06220	0.11922
All	88	44	132
	0.09656	0.09539	0.09617
	0.11718	0.06895	0.10334

Cell Contents --
Sorrel:N
Mean
StDev

MTB >

MTB > glm c21=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Plantain

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000810	0.000098	0.000098	0.01	0.918
GDBA	1	0.000125	0.000095	0.000095	0.01	0.919
Atopic*GDBA	1	0.002315	0.002315	0.002315	0.25	0.618
Error	128	1.184646	1.184646	0.009255		
Total	131	1.187896				

Unusual Observations for Plantain

Obs	Plantain	Fit	StDev Fit	Residual	St Resid
1	0.313000	0.103324	0.011183	0.209676	2.19R
3	0.619000	0.103324	0.011183	0.515676	5.40R
39	0.306000	0.103324	0.011183	0.202676	2.12R
54	0.332000	0.103324	0.011183	0.228676	2.39R
79	0.313000	0.116286	0.025711	0.196714	2.12R
101	0.487000	0.107719	0.017006	0.379281	4.01R
104	0.328000	0.107719	0.017006	0.220281	2.33R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c21.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11629	0.10772	0.11033
	0.08262	0.10746	0.09972
2	74	12	86
	0.10332	0.11625	0.10513
	0.09522	0.08316	0.09328
All	88	44	132
	0.10539	0.11005	0.10694
	0.09301	0.10055	0.09523

Cell Contents --
Plantain:N
Mean
StDev

MTB >

MTB > glm c22=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mugwort

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00049	0.00055	0.00055	0.03	0.861
GDBA	1	0.00102	0.00118	0.00118	0.07	0.796
Atopic*GDBA	1	0.00707	0.00707	0.00707	0.40	0.528
Error	128	2.25906	2.25906	0.01765		
Total	131	2.26764				

Unusual Observations for Mugwort

Obs	Mugwort	Fit	StDev Fit	Residual	St Resid
3	0.507000	0.095149	0.015443	0.411851	3.12R
27	0.433000	0.095149	0.015443	0.337851	2.56R
39	0.601000	0.095149	0.015443	0.505851	3.83R
48	0.381000	0.095149	0.015443	0.285851	2.17R
54	0.582000	0.095149	0.015443	0.486851	3.69R
79	0.706000	0.119143	0.035505	0.586857	4.58R
101	0.464000	0.092688	0.023485	0.371313	2.84R
126	0.372000	0.106250	0.038350	0.265750	2.09R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c22.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11914	0.09269	0.10074
	0.18355	0.10380	0.13155
2	74	12	86
	0.09515	0.10625	0.09670
	0.13746	0.09893	0.13233
All	88	44	132
	0.09897	0.09639	0.09811
	0.14480	0.10153	0.13157

Cell Contents --
Mugwort:N
Mean
StDev

MTB >

MTB > glm c23=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Dandelio

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00026	0.01850	0.01850	0.71	0.401
GDBA	1	0.05115	0.05138	0.05138	1.97	0.163
Atopic*GDBA	1	0.00041	0.00041	0.00041	0.02	0.901
Error	128	3.33231	3.33231	0.02603		
Total	131	3.38414				

Unusual Observations for Dandelio

Obs	Dandelio	Fit	StDev Fit	Residual	St Resid
27	0.520000	0.117622	0.018757	0.402378	2.51R
39	0.681000	0.117622	0.018757	0.563378	3.52R
44	0.760000	0.117622	0.018757	0.642378	4.01R
54	0.756000	0.117622	0.018757	0.638378	3.98R
71	0.713000	0.117622	0.018757	0.595378	3.72R
79	0.737000	0.152500	0.043122	0.584500	3.76R
83	0.686000	0.152500	0.043122	0.533500	3.43R
98	0.500000	0.097375	0.028523	0.402625	2.54R
101	0.421000	0.097375	0.028523	0.323625	2.04R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c23.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.15250	0.09737	0.11415
	0.24125	0.10965	0.16048
2	74	12	86
	0.11762	0.07150	0.11119
	0.17242	0.05456	0.16179
All	88	44	132
	0.12317	0.09032	0.11222
	0.18387	0.09780	0.16073

Cell Contents --
Dandelio:N
Mean
StDev

MTB >

MTB > glm c24=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Nettle

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00159	0.00098	0.00098	0.08	0.784
GDBA	1	0.01475	0.01490	0.01490	1.14	0.288
Atopic*GDBA	1	0.00057	0.00057	0.00057	0.04	0.836
Error	128	1.67415	1.67415	0.01308		
Total	131	1.69105				

Unusual Observations for Nettle

Obs	Nettle	Fit	StDev Fit	Residual	St Resid
1	0.406000	0.108541	0.013295	0.297459	2.62R
3	0.560000	0.108541	0.013295	0.451459	3.97R
36	0.630000	0.108541	0.013295	0.521459	4.59R
39	0.570000	0.108541	0.013295	0.461459	4.06R
79	0.423000	0.120857	0.030565	0.302143	2.74R
83	0.436000	0.120857	0.030565	0.315143	2.86R
101	0.482000	0.088281	0.020217	0.393719	3.50R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c24.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.12086	0.08828	0.09820
	0.13590	0.08989	0.10551
2	74	12	86
	0.10854	0.08658	0.10548
	0.12611	0.04538	0.11825
All	88	44	132
	0.11050	0.08782	0.10294
	0.12698	0.07971	0.11362

Cell Contents --
Nettle:N
Mean
StDev

MTB >

Appendix I2

Statisitical analysis of ELISA results for atopic and non-atopic dogs (non-GDBA/non-atopic =beagles).

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Flea

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000015	0.000020	0.000020	0.00	0.957
GDBA	1	0.000405	0.000105	0.000105	0.02	0.901
Atopic*GDBA	1	0.004232	0.004232	0.004232	0.63	0.429
Error	124	0.834982	0.834982	0.006734		
Total	127	0.839634				

Unusual Observations for Flea

Obs	Flea	Fit	StDev Fit	Residual	St Resid
19	0.317000	0.072959	0.009539	0.244041	2.99R
39	0.474000	0.072959	0.009539	0.401041	4.92R
64	0.656000	0.072959	0.009539	0.583041	7.15R
116	0.298000	0.076563	0.014506	0.221438	2.74R
121	0.064000	0.059500	0.029012	0.004500	0.06 X
122	0.065000	0.059500	0.029012	0.005500	0.07 X
123	0.052000	0.059500	0.029012	-0.007500	-0.10 X
124	0.041000	0.059500	0.029012	-0.018500	-0.24 X
125	0.037000	0.059500	0.029012	-0.022500	-0.29 X
126	0.035000	0.059500	0.029012	-0.024500	-0.32 X
127	0.125000	0.059500	0.029012	0.065500	0.85 X
128	0.057000	0.059500	0.029012	-0.002500	-0.03 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.05807	0.07656	0.07093
	0.04482	0.06152	0.05711
2	74	8	82
	0.07296	0.05950	0.07165
	0.09692	0.02891	0.09249
All	88	40	128

0.07059 0.07315 0.07139
0.09062 0.05662 0.08131

Cell Contents --
Flea:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mites

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001621	0.000173	0.000173	0.05	0.832
GDBA	1	0.002890	0.003478	0.003478	0.91	0.343
Atopic*GDBA	1	0.001532	0.001532	0.001532	0.40	0.528
Error	124	0.475287	0.475287	0.003833		
Total	127	0.481331				

Unusual Observations for Mites

Obs	Mites	Fit	StDev Fit	Residual	St Resid
16	0.287000	0.116905	0.007197	0.170095	2.77R
27	0.276000	0.116905	0.007197	0.159095	2.59R
39	0.303000	0.116905	0.007197	0.186095	3.03R
46	0.276000	0.116905	0.007197	0.159095	2.59R
48	0.275000	0.116905	0.007197	0.158095	2.57R
94	0.275000	0.128156	0.010944	0.146844	2.41R
97	0.000000	0.128156	0.010944	-0.128156	-2.10R
117	0.263000	0.128156	0.010944	0.134844	2.21R
121	0.163000	0.141000	0.021889	0.022000	0.38 X
122	0.250000	0.141000	0.021889	0.109000	1.88 X
123	0.147000	0.141000	0.021889	0.006000	0.10 X
124	0.098000	0.141000	0.021889	-0.043000	-0.74 X
125	0.199000	0.141000	0.021889	0.058000	1.00 X
126	0.063000	0.141000	0.021889	-0.078000	-1.35 X
127	0.121000	0.141000	0.021889	-0.020000	-0.35 X
128	0.087000	0.141000	0.021889	-0.054000	-0.93 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

```
          1      2     All

1        14     32      46
    0.12329 0.12816 0.12667
    0.05247 0.06425 0.06037

2        74      8      82
    0.11691 0.14100 0.11926
    0.06243 0.06212 0.06243

All      88     40     128
    0.11792 0.13073 0.12192
    0.06072 0.06325 0.06156

 Cell Contents --
        Mites:N
            Mean
            StDev
```

MTB > glm c5=c1 c2 c1*c2

General Linear Model

```
Factor  Levels Values
Atopic       2  1  2
GDBA         2  1  2
```

Analysis of Variance for Feathers

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001333	0.000316	0.000316	0.11	0.740
GDBA	1	0.008199	0.007282	0.007282	2.55	0.113
Atopic*GDBA	1	0.000804	0.000804	0.000804	0.28	0.597
Error	124	0.354525	0.354525	0.002859		
Total	127	0.364861				

Unusual Observations for Feathers

Obs	Feathers	Fit	StDev Fit	Residual	St Resid
3	0.180000	0.071257	0.006216	0.108743	2.05R
12	0.213000	0.071257	0.006216	0.141743	2.67R
51	0.291000	0.071257	0.006216	0.219743	4.14R
94	0.208000	0.087844	0.009452	0.120156	2.28R
110	0.388000	0.087844	0.009452	0.300156	5.70R
121	0.110000	0.085250	0.018905	0.024750	0.49 X
122	0.091000	0.085250	0.018905	0.005750	0.11 X
123	0.066000	0.085250	0.018905	-0.019250	-0.38 X
124	0.079000	0.085250	0.018905	-0.006250	-0.12 X
125	0.123000	0.085250	0.018905	0.037750	0.75 X
126	0.060000	0.085250	0.018905	-0.025250	-0.50 X
127	0.081000	0.085250	0.018905	-0.004250	-0.08 X
128	0.072000	0.085250	0.018905	-0.013250	-0.26 X

R denotes an observation with a large standardized residual

X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.05993	0.08784	0.07935
	0.03158	0.07091	0.06262
2	74	8	82
	0.07126	0.08525	0.07262
	0.04998	0.02175	0.04806
All	88	40	128
	0.06945	0.08733	0.07504
	0.04756	0.06390	0.05360

Cell Contents --
Feathers:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Alternar

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.012789	0.011218	0.011218	3.04	0.084
GDBA	1	0.000669	0.000811	0.000811	0.22	0.640
Atopic*GDBA	1	0.000383	0.000383	0.000383	0.10	0.748
Error	124	0.457249	0.457249	0.003687		
Total	127	0.471089				

Unusual Observations for Alternar

Obs	Alternar	Fit	StDev Fit	Residual	St Resid
7	0.225000	0.087797	0.007059	0.137203	2.27R
17	0.268000	0.087797	0.007059	0.180203	2.99R
24	0.209000	0.087797	0.007059	0.121203	2.01R
59	0.233000	0.087797	0.007059	0.145203	2.41R
83	0.353000	0.109000	0.016229	0.244000	4.17R
121	0.118000	0.076000	0.021469	0.042000	0.74 X

122	0.134000	0.076000	0.021469	0.058000	1.02 X
123	0.040000	0.076000	0.021469	-0.036000	-0.63 X
124	0.090000	0.076000	0.021469	0.014000	0.25 X
125	0.104000	0.076000	0.021469	0.028000	0.49 X
126	0.036000	0.076000	0.021469	-0.040000	-0.70 X
127	0.036000	0.076000	0.021469	-0.040000	-0.70 X
128	0.050000	0.076000	0.021469	-0.026000	-0.46 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.10900	0.10681	0.10748
	0.07756	0.06226	0.06641
2	74	8	82
	0.08780	0.07600	0.08665
	0.05824	0.04014	0.05664
All	88	40	128
	0.09117	0.10065	0.09413
	0.06169	0.05938	0.06090

Cell Contents --
Alternar:N
Mean
StDev

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Aspergil

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.015816	0.016926	0.016926	6.35	0.013
GDBA	1	0.000636	0.001254	0.001254	0.47	0.494
Atopic*GDBA	1	0.004966	0.004966	0.004966	1.86	0.175

Error	124	0.330460	0.330460	0.002665
Total	127	0.351878		

Unusual Observations for Aspergil

Obs	Aspergil	Fit	StDev Fit	Residual	St Resid
64	0.226000	0.091500	0.006001	0.134500	2.62R
93	0.232000	0.114750	0.009126	0.117250	2.31R
98	0.001000	0.114750	0.009126	-0.113750	-2.24R
112	0.226000	0.114750	0.009126	0.111250	2.19R
118	0.333000	0.114750	0.009126	0.218250	4.30R
121	0.148000	0.065500	0.018252	0.082500	1.71 X
122	0.078000	0.065500	0.018252	0.012500	0.26 X
123	0.051000	0.065500	0.018252	-0.014500	-0.30 X
124	0.044000	0.065500	0.018252	-0.021500	-0.45 X
125	0.043000	0.065500	0.018252	-0.022500	-0.47 X
126	0.043000	0.065500	0.018252	-0.022500	-0.47 X
127	0.047000	0.065500	0.018252	-0.018500	-0.38 X
128	0.070000	0.065500	0.018252	0.004500	0.09 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	44	32	46
	0.10614	0.11475	0.11213
	0.05266	0.07067	0.06525
2	74	8	82
	0.09150	0.06550	0.08896
	0.04229	0.03588	0.04223
All	88	40	128
	0.09383	0.10490	0.09729
	0.04409	0.06781	0.05264

Cell Contents --
Aspergil:N
Mean
StDev

MTB > glm c8=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Rhizopus

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.016523	0.000660	0.000660	0.07	0.794
GDBA	1	0.027269	0.030833	0.030833	3.20	0.076
Atopic*GDBA	1	0.006844	0.006844	0.006844	0.71	0.401
Error	124	1.196465	1.196465	0.009649		
Total	127	1.247101				

Unusual Observations for Rhizopus

Obs	Rhizopus	Fit	StDev Fit	Residual	St Resid
3	0.406000	0.099932	0.011419	0.306068	3.14R
27	0.588000	0.099932	0.011419	0.488068	5.00R
39	0.396000	0.099932	0.011419	0.296068	3.03R
54	0.615000	0.099932	0.011419	0.515068	5.28R
83	0.313000	0.085929	0.026253	0.227071	2.40R
101	0.318000	0.063125	0.017365	0.254875	2.64R
121	0.041000	0.036500	0.034729	0.004500	0.05 X
122	0.065000	0.036500	0.034729	0.028500	0.31 X
123	0.048000	0.036500	0.034729	0.011500	0.13 X
124	0.044000	0.036500	0.034729	0.007500	0.08 X
125	0.045000	0.036500	0.034729	0.008500	0.09 X
126	0.012000	0.036500	0.034729	-0.024500	-0.27 X
127	0.017000	0.036500	0.034729	-0.019500	-0.21 X
128	0.020000	0.036500	0.034729	-0.016500	-0.18 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c8.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	44	32	46
	0.08593	0.06313	0.07007
	0.07503	0.06724	0.06967
2	4	8	82
	0.09993	0.03650	0.09374
	0.11591	0.01831	0.11178

```
All     88     40     128
  0.09770 0.05780 0.08523
  0.11019 0.06141 0.09909

 Cell Contents --
      Rhizopus:N
           Mean
           StDev
```

MTB > glm c9=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Kapok

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000314	0.003142	0.003142	0.48	0.492
GDBA	1	0.010723	0.011337	0.011337	1.72	0.193
Atopic*GDBA	1	0.000752	0.000752	0.000752	0.11	0.736
Error	124	0.819568	0.819568	0.006609		
Total	127	0.831358				

Unusual Observations for Kapok

Obs	Kapok	Fit	StDev Fit	Residual	St Resid
3	0.319000	0.077257	0.009451	0.241743	2.99R
27	0.383000	0.077257	0.009451	0.305743	3.79R
39	0.320000	0.077257	0.009451	0.242743	3.01R
54	0.410000	0.077257	0.009451	0.332743	4.12R
79	0.479000	0.084286	0.021728	0.394714	5.04R
101	0.308000	0.064875	0.014372	0.243125	3.04R
121	0.076000	0.044375	0.028743	0.031625	0.42 X
122	0.081000	0.044375	0.028743	0.036625	0.48 X
123	0.049000	0.044375	0.028743	0.004625	0.06 X
124	0.034000	0.044375	0.028743	-0.010375	-0.14 X
125	0.032000	0.044375	0.028743	-0.012375	-0.16 X
126	0.028000	0.044375	0.028743	-0.016375	-0.22 X
127	0.037000	0.044375	0.028743	-0.007375	-0.10 X
128	0.018000	0.044375	0.028743	-0.026375	-0.35 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c9.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.08429	0.06487	0.07078
	0.12341	0.06438	0.08565
2	74	8	82
	0.07726	0.04438	0.07405
	0.08188	0.02281	0.07864
All	88	40	128
	0.07837	0.06078	0.07288
	0.08893	0.05879	0.08091

Cell Contents --
Kapok:N
Mean
StDev

MTB > glm c10=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Dust

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001846	0.001771	0.001771	1.58	0.212
GDBA	1	0.000051	0.000098	0.000098	0.09	0.768
Atopic*GDBA	1	0.000361	0.000361	0.000361	0.32	0.572
Error	124	0.139392	0.139392	0.001124		
Total	127	0.141650				

Unusual Observations for Dust

Obs	Dust	Fit	StDev Fit	Residual	St Resid
1	0.123000	0.050027	0.003898	0.072973	2.19R
12	0.136000	0.050027	0.003898	0.085973	2.58R
73	0.160000	0.050027	0.003898	0.109973	3.30R
79	0.155000	0.044357	0.008961	0.110643	3.42R
121	0.123000	0.057125	0.011854	0.065875	2.10RX
122	0.039000	0.057125	0.011854	-0.018125	-0.58 X
123	0.123000	0.057125	0.011854	0.065875	2.10RX
124	0.026000	0.057125	0.011854	-0.031125	-0.99 X
125	0.032000	0.057125	0.011854	-0.025125	-0.80 X
126	0.022000	0.057125	0.011854	-0.035125	-1.12 X
127	0.046000	0.057125	0.011854	-0.011125	-0.35 X
128	0.046000	0.057125	0.011854	-0.011125	-0.35 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c10.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.044357	0.042125	0.042804
	0.040636	0.026655	0.031106
2	74	8	82
	0.050027	0.057125	0.050720
	0.033883	0.041557	0.034474
All	88	40	128
	0.049125	0.045125	0.047875
	0.034849	0.030194	0.033397

Cell Contents --
Dust:N
Mean
StDev

MTB > glm c11=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Cat

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0001829	0.0001057	0.0001057	0.15	0.700
GDBA	1	0.0000133	0.0000067	0.0000067	0.01	0.923
Atopic*GDBA	1	0.0000468	0.0000468	0.0000468	0.07	0.798
Error	124	0.0880762	0.0880762	0.0007103		
Total	127	0.0883192				

Unusual Observations for Cat

Obs	Cat	Fit	StDev Fit	Residual	St Resid

1	0.115000	0.037919	0.003098	0.077081	2.91R
3	0.091000	0.037919	0.003098	0.053081	2.01R
17	0.103000	0.037919	0.003098	0.065081	2.46R
60	0.101000	0.037919	0.003098	0.063081	2.38R
62	0.091000	0.037919	0.003098	0.053081	2.01R
121	0.095000	0.036875	0.009423	0.058125	2.33RX
122	0.031000	0.036875	0.009423	-0.005875	-0.24 X
123	0.043000	0.036875	0.009423	0.006125	0.25 X
124	0.042000	0.036875	0.009423	0.005125	0.21 X
125	0.021000	0.036875	0.009423	-0.015875	-0.64 X
126	0.019000	0.036875	0.009423	-0.017875	-0.72 X
127	0.017000	0.036875	0.009423	-0.019875	-0.80 X
128	0.027000	0.036875	0.009423	-0.009875	-0.40 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c11.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.033714	0.036031	0.035326
	0.019201	0.024843	0.023083
2	74	8	82
	0.037919	0.036875	0.037817
	0.028576	0.025470	0.028144
All	88	40	128
	0.037250	0.036200	0.036922
	0.027252	0.024640	0.026371

Cell Contents --
Cat:N
Mean
StDev

MTB > glm c12=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Human

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.003689	0.000099	0.000099	0.04	0.835
GDBA	1	0.001296	0.001832	0.001832	0.81	0.370
Atopic*GDBA	1	0.002352	0.002352	0.002352	1.04	0.310
Error	124	0.280819	0.280819	0.002265		
Total	127	0.288155				

Unusual Observations for Human

Obs	Human	Fit	StDev Fit	Residual	St Resid
8	0.197000	0.047419	0.005532	0.149581	3.16R
39	0.226000	0.047419	0.005532	0.178581	3.78R
48	0.434000	0.047419	0.005532	0.386581	8.18R
121	0.036000	0.025000	0.016825	0.011000	0.25 X
122	0.024000	0.025000	0.016825	-0.001000	-0.02 X
123	0.034000	0.025000	0.016825	0.009000	0.20 X
124	0.023000	0.025000	0.016825	-0.002000	-0.04 X
125	0.030000	0.025000	0.016825	0.005000	0.11 X
126	0.019000	0.025000	0.016825	-0.006000	-0.13 X
127	0.020000	0.025000	0.016825	-0.005000	-0.11 X
128	0.014000	0.025000	0.016825	-0.011000	-0.25 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c12.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.033071	0.034469	0.034043
	0.020281	0.023903	0.022646
2	74	8	82
	0.047419	0.025000	0.045232
	0.059374	0.007690	0.056807
All	88	40	128
	0.045136	0.032575	0.041211
	0.055202	0.021897	0.047633

Cell Contents --
Human:N
Mean
StDev

MTB > glm c13=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mucor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00127	0.01723	0.01723	1.71	0.193
GDBA	1	0.07122	0.06969	0.06969	6.92	0.010
Atopic*GDBA	1	0.00000	0.00000	0.00000	0.00	0.996
Error	124	1.24804	1.24804	0.01006		
Total	127	1.32054				

Unusual Observations for Mucor

Obs	Mucor	Fit	StDev Fit	Residual	St Resid
27	0.449000	0.108811	0.011662	0.340189	3.41R
39	0.751000	0.108811	0.011662	0.642189	6.44R
51	0.372000	0.108811	0.011662	0.263189	2.64R
59	0.329000	0.108811	0.011662	0.220189	2.21R
79	0.423000	0.140929	0.026813	0.282071	2.92R
83	0.433000	0.140929	0.026813	0.292071	3.02R
121	0.038000	0.043875	0.035470	-0.005875	-0.06 X
122	0.077000	0.043875	0.035470	0.033125	0.35 X
123	0.043000	0.043875	0.035470	-0.000875	-0.01 X
124	0.070000	0.043875	0.035470	0.026125	0.28 X
125	0.045000	0.043875	0.035470	0.001125	0.01 X
126	0.023000	0.043875	0.035470	-0.020875	-0.22 X
127	0.034000	0.043875	0.035470	-0.009875	-0.11 X
128	0.021000	0.043875	0.035470	-0.022875	-0.24 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c13.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.14093	0.07622	0.09591
	0.13171	0.04634	0.08601

2	74	8	82
	0.10881	0.04388	0.10248
	0.11426	0.02026	0.11035
All	88	40	128
	0.11392	0.06975	0.10012
	0.11699	0.04419	0.10197

Cell Contents --
Mucor:N
Mean
StDev

MTB > glm c14=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Orchard

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.010307	0.011144	0.011144	1.50	0.223
GDBA	1	0.000159	0.000037	0.000037	0.00	0.944
Atopic*GDBA	1	0.015538	0.015538	0.015538	2.09	0.151
Error	124	0.921703	0.921703	0.007433		
Total	127	0.947708				

Unusual Observations for Orchard

Obs	Orchard	Fit	StDev Fit	Residual	St Resid
3	0.623000	0.093473	0.010022	0.529527	6.18R
54	0.324000	0.093473	0.010022	0.230527	2.69R
89	0.433000	0.117906	0.015241	0.315094	3.71R
119	0.348000	0.117906	0.015241	0.230094	2.71R
121	0.062000	0.061375	0.030482	0.000625	0.01 X
122	0.068000	0.061375	0.030482	0.006625	0.08 X
123	0.092000	0.061375	0.030482	0.030625	0.38 X
124	0.030000	0.061375	0.030482	-0.031375	-0.39 X
125	0.051000	0.061375	0.030482	-0.010375	-0.13 X
126	0.058000	0.061375	0.030482	-0.003375	-0.04 X
127	0.048000	0.061375	0.030482	-0.013375	-0.17 X
128	0.082000	0.061375	0.030482	0.020625	0.26 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2,
SUBC> stats c14.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.08879	0.11791	0.10904
	0.05034	0.09949	0.08794
2	74	8	82
	0.09347	0.06137	0.09034
	0.08908	0.01960	0.08530
All	88	40	128
	0.09273	0.10660	0.09706
	0.08390	0.09198	0.08638

Cell Contents --
Orchard:N
Mean
StDev

MTB > glm c15=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Timothy

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.013004	0.010196	0.010196	1.04	0.309
GDBA	1	0.000911	0.000172	0.000172	0.02	0.895
Atopic*GDBA	1	0.012672	0.012672	0.012672	1.30	0.257
Error	124	1.211246	1.211246	0.009768		
Total	127	1.237834				

Unusual Observations for Timothy

Obs	Timothy	Fit	StDev Fit	Residual	St Resid
3	0.618000	0.115919	0.011489	0.502081	5.11R
8	0.576000	0.115919	0.011489	0.460081	4.69R
48	0.320000	0.115919	0.011489	0.204081	2.08R
89	0.378000	0.143937	0.017472	0.234063	2.41R
101	0.366000	0.143937	0.017472	0.222063	2.28R
104	0.417000	0.143937	0.017472	0.273063	2.81R
121	0.161000	0.091500	0.034943	0.069500	0.75 X
122	0.173000	0.091500	0.034943	0.081500	0.88 X
123	0.106000	0.091500	0.034943	0.014500	0.16 X

124	0.032000	0.091500	0.034943	-0.059500	-0.64 X
125	0.056000	0.091500	0.034943	-0.035500	-0.38 X
126	0.026000	0.091500	0.034943	-0.065500	-0.71 X
127	0.091000	0.091500	0.034943	-0.000500	-0.01 X
128	0.087000	0.091500	0.034943	-0.004500	-0.05 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c15.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11307	0.14394	0.13454
	0.06228	0.10350	0.09330
2	74	8	82
	0.11592	0.09150	0.11354
	0.10520	0.05450	0.10141
All	88	40	128
	0.11547	0.13345	0.12109
	0.09934	0.09746	0.09873

Cell Contents --
Timothy:N
Mean
StDev

MTB > glm c16=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Kentucky

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.01246	0.00479	0.00479	0.45	0.503
GDBA	1	0.00365	0.00241	0.00241	0.23	0.635
Atopic*GDBA	1	0.00515	0.00515	0.00515	0.49	0.487
Error	124	1.31628	1.31628	0.01062		
Total	127	1.33754				

Unusual Observations for Kentucky

Obs	Kentucky	Fit	StDev Fit	Residual	St Resid
3	0.669000	0.103203	0.011977	0.565797	5.53R
8	0.613000	0.103203	0.011977	0.509797	4.98R
89	0.545000	0.132250	0.018213	0.412750	4.07R
104	0.389000	0.132250	0.018213	0.256750	2.53R
121	0.097000	0.097625	0.036427	-0.000625	-0.01 X
122	0.123000	0.097625	0.036427	0.025375	0.26 X
123	0.070000	0.097625	0.036427	-0.027625	-0.29 X
124	0.038000	0.097625	0.036427	-0.059625	-0.62 X
125	0.115000	0.097625	0.036427	0.017375	0.18 X
126	0.175000	0.097625	0.036427	0.077375	0.80 X
127	0.103000	0.097625	0.036427	0.005375	0.06 X
128	0.060000	0.097625	0.036427	-0.037625	-0.39 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c16.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.10257	0.13225	0.12322
	0.05128	0.11289	0.09864
2	74	8	82
	0.10320	0.09762	0.10266
	0.10944	0.04259	0.10466
All	88	40	128
	0.10310	0.12532	0.11005
	0.10219	0.10321	0.10262

Cell Contents --
Kentucky:N
Mean
StDev

MTB > glm c17=c1 c2 c1*c2

General Linear Model

Factor Levels Values

Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Fescue

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.007208	0.004446	0.004446	0.52	0.474
GDBA	1	0.002125	0.000923	0.000923	0.11	0.744
Atopic*GDBA	1	0.010471	0.010471	0.010471	1.22	0.272
Error	124	1.067461	1.067461	0.008609		
Total	127	1.087266				

Unusual Observations for Fescue

Obs	Fescue	Fit	StDev Fit	Residual	St Resid
3	0.682000	0.102041	0.010786	0.579959	6.29R
54	0.460000	0.102041	0.010786	0.357959	3.88R
89	0.435000	0.125875	0.016402	0.309125	3.39R
101	0.325000	0.125875	0.016402	0.199125	2.18R
121	0.111000	0.084375	0.032804	0.026625	0.31 X
122	0.169000	0.084375	0.032804	0.084625	0.98 X
123	0.083000	0.084375	0.032804	-0.001375	-0.02 X
124	0.055000	0.084375	0.032804	-0.029375	-0.34 X
125	0.087000	0.084375	0.032804	0.002625	0.03 X
126	0.042000	0.084375	0.032804	-0.042375	-0.49 X
127	0.068000	0.084375	0.032804	-0.016375	-0.19 X
128	0.060000	0.084375	0.032804	-0.024375	-0.28 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c17.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.09329	0.12588	0.11596
	0.05502	0.09544	0.08591
2	74	8	82
	0.10204	0.08438	0.10032
	0.10029	0.04036	0.09609
All	88	40	128
	0.10065	0.11758	0.10594
	0.09436	0.08841	0.09253

Cell Contents --
Fescue:N
Mean
StDev

MTB > glm c18=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Poplar

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00005	0.00262	0.00262	0.22	0.642
GDBA	1	0.00629	0.00695	0.00695	0.58	0.449
Atopic*GDBA	1	0.00112	0.00112	0.00112	0.09	0.761
Error	124	1.49357	1.49357	0.01204		
Total	127	1.50102				

Unusual Observations for Poplar

Obs	Poplar	Fit	StDev Fit	Residual	St Resid
3	0.877000	0.108554	0.012758	0.768446	7.05R
36	0.461000	0.108554	0.012758	0.352446	3.23R
54	0.343000	0.108554	0.012758	0.234446	2.15R
79	0.332000	0.112929	0.029332	0.219071	2.07R
89	0.352000	0.100656	0.019401	0.251344	2.33R
101	0.385000	0.100656	0.019401	0.284344	2.63R
121	0.094000	0.079875	0.038802	0.014125	0.14 X
122	0.097000	0.079875	0.038802	0.017125	0.17 X
123	0.088000	0.079875	0.038802	0.008125	0.08 X
124	0.101000	0.079875	0.038802	0.021125	0.21 X
125	0.109000	0.079875	0.038802	0.029125	0.28 X
126	0.038000	0.079875	0.038802	-0.041875	-0.41 X
127	0.071000	0.079875	0.038802	-0.008875	-0.09 X
128	0.041000	0.079875	0.038802	-0.038875	-0.38 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c18.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11293	0.10066	0.10439
	0.09234	0.09484	0.09323
2	74	8	82
	0.10855	0.07988	0.10576
	0.12268	0.02725	0.11705
All	88	40	128
	0.10925	0.09650	0.10527
	0.11792	0.08575	0.10872

Cell Contents --
Poplar:N
Mean
StDev

MTB > glm c19=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Birch

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00218	0.02353	0.02353	1.42	0.235
GDBA	1	0.03288	0.03419	0.03419	2.07	0.153
Atopic*GDBA	1	0.00142	0.00142	0.00142	0.09	0.770
Error	124	2.05282	2.05282	0.01656		
Total	127	2.08930				

Unusual Observations for Birch

Obs	Birch	Fit	StDev Fit	Residual	St Resid
27	0.460000	0.111649	0.014957	0.348351	2.73R
39	0.792000	0.111649	0.014957	0.680351	5.32R
54	0.656000	0.111649	0.014957	0.544351	4.26R
79	0.770000	0.140071	0.034388	0.629929	5.08R
101	0.381000	0.103906	0.022745	0.277094	2.19R
117	0.525000	0.103906	0.022745	0.421094	3.33R
121	0.053000	0.057000	0.045490	-0.004000	-0.03 X
122	0.093000	0.057000	0.045490	0.036000	0.30 X
123	0.089000	0.057000	0.045490	0.032000	0.27 X
124	0.040000	0.057000	0.045490	-0.017000	-0.14 X
125	0.040000	0.057000	0.045490	-0.017000	-0.14 X
126	0.047000	0.057000	0.045490	-0.010000	-0.08 X
127	0.045000	0.057000	0.045490	-0.012000	-0.10 X
128	0.049000	0.057000	0.045490	-0.008000	-0.07 X

R denotes an observation with a large standardized residual

X denotes an observation whose X value gives it large influence.

```
MTB > table c1 c2;
SUBC> stats c19.
```

Tabulated Statistics

```
Rows: Atopic   Columns: GDBA

       1       2      All

1      14      32       46
    0.14007 0.10391 0.11491
    0.19267 0.11163 0.13997

2      74       8       82
    0.11165 0.05700 0.10632
    0.12718 0.02145 0.12199

All    88      40      128
    0.11617 0.09452 0.10941
    0.13866 0.10173 0.12826

 Cell Contents --
       Birch:N
          Mean
          StDev
```

```
MTB > glm c20=c1 c2 c1*c2
```

General Linear Model

```
Factor   Levels Values
Atopic        2  1  2
GDBA          2  1  2

Analysis of Variance for Sorrel

Source       DF   Seq SS   Adj SS   Adj MS      F      P
Atopic        1  0.00547  0.00122  0.00122   0.11  0.740
GDBA          1  0.00011  0.00001  0.00001   0.00  0.976
Atopic*GDBA   1  0.00815  0.00815  0.00815   0.74  0.391
Error       124  1.36396  1.36396  0.01100
Total       127  1.37768

Unusual Observations for Sorrel

Obs   Sorrel      Fit   StDev Fit  Residual  St Resid
  3  0.790000  0.101446   0.012192  0.688554     6.61R
  8  0.400000  0.101446   0.012192  0.298554     2.87R
```

69	0.730000	0.101446	0.012192	0.628554	6.03R
89	0.375000	0.092094	0.018540	0.282906	2.74R
121	0.076000	0.078500	0.037080	-0.002500	-0.03 X
122	0.167000	0.078500	0.037080	0.088500	0.90 X
123	0.156000	0.078500	0.037080	0.077500	0.79 X
124	0.065000	0.078500	0.037080	-0.013500	-0.14 X
125	0.030000	0.078500	0.037080	-0.048500	-0.49 X
126	0.052000	0.078500	0.037080	-0.026500	-0.27 X
127	0.039000	0.078500	0.037080	-0.039500	-0.40 X
128	0.043000	0.078500	0.037080	-0.035500	-0.36 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c20.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.07071	0.09209	0.08559
	0.03715	0.07197	0.06376
2	74	8	82
	0.10145	0.07850	0.09921
	0.12636	0.05332	0.12117
All	88	40	128
	0.09656	0.08938	0.09431
	0.11718	0.06825	0.10415

Cell Contents --
Sorrel:N
Mean
StDev

MTB > glm c21=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Plantain

Source	DF	Seq SS	Adj SS	Adj MS	F	P

Atopic	1	0.002411	0.006083	0.006083	0.67 0.413
GDBA	1	0.003248	0.003612	0.003612	0.40 0.528
Atopic*GDBA	1	0.000636	0.000636	0.000636	0.07 0.791
Error	124	1.118101	1.118101	0.009017	
Total	127	1.124396			

Unusual Observations for Plantain

Obs	Plantain	Fit	StDev Fit	Residual	St Resid
1	0.313000	0.103324	0.011039	0.209676	2.22R
3	0.619000	0.103324	0.011039	0.515676	5.47R
39	0.306000	0.103324	0.011039	0.202676	2.15R
54	0.332000	0.103324	0.011039	0.228676	2.42R
79	0.313000	0.116286	0.025378	0.196714	2.15R
101	0.487000	0.107719	0.016786	0.379281	4.06R
104	0.328000	0.107719	0.016786	0.220281	2.36R
121	0.081000	0.082375	0.033573	-0.001375	-0.02 X
122	0.132000	0.082375	0.033573	0.049625	0.56 X
123	0.113000	0.082375	0.033573	0.030625	0.34 X
124	0.122000	0.082375	0.033573	0.039625	0.45 X
125	0.048000	0.082375	0.033573	-0.034375	-0.39 X
126	0.052000	0.082375	0.033573	-0.030375	-0.34 X
127	0.033000	0.082375	0.033573	-0.049375	-0.56 X
128	0.078000	0.082375	0.033573	-0.004375	-0.05 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c21.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11629	0.10772	0.11033
	0.08262	0.10746	0.09972
2	74	8	82
	0.10332	0.08238	0.10128
	0.09522	0.03691	0.09126
All	88	40	128
	0.10539	0.10265	0.10453
	0.09301	0.09762	0.09409

Cell Contents --
Plantain:N
Mean
StDev

MTB > glm c22=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Mugwort

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00333	0.02220	0.02220	1.28	0.261
GDBA	1	0.02344	0.02529	0.02529	1.45	0.230
Atopic*GDBA	1	0.00263	0.00263	0.00263	0.15	0.698
Error	124	2.15565	2.15565	0.01738		
Total	127	2.18505				

Unusual Observations for Mugwort

Obs	Mugwort	Fit	StDev Fit	Residual	St Resid
3	0.507000	0.095149	0.015327	0.411851	3.14R
27	0.433000	0.095149	0.015327	0.337851	2.58R
39	0.601000	0.095149	0.015327	0.505851	3.86R
48	0.381000	0.095149	0.015327	0.285851	2.18R
54	0.582000	0.095149	0.015327	0.486851	3.72R
79	0.706000	0.119143	0.035238	0.586857	4.62R
101	0.464000	0.092688	0.023308	0.371313	2.86R
121	0.064000	0.043500	0.046616	0.020500	0.17 X
122	0.067000	0.043500	0.046616	0.023500	0.19 X
123	0.075000	0.043500	0.046616	0.031500	0.26 X
124	0.000000	0.043500	0.046616	-0.043500	-0.35 X
125	0.041000	0.043500	0.046616	-0.002500	-0.02 X
126	0.028000	0.043500	0.046616	-0.015500	-0.13 X
127	0.032000	0.043500	0.046616	-0.011500	-0.09 X
128	0.041000	0.043500	0.046616	-0.002500	-0.02 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c22.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46

0.11914 0.09269 0.10074
0.18355 0.10380 0.13155

2 74 8 82
0.09515 0.04350 0.09011
0.13746 0.02462 0.13161

All 88 40 128
0.09897 0.08285 0.09393
0.14480 0.09523 0.13117

Cell Contents --
Mugwort:N
Mean
StDev

MTB > glm c23=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Dandelio

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00045	0.03377	0.03377	1.27	0.262
GDBA	1	0.06914	0.07088	0.07088	2.66	0.105
Atopic*GDBA	1	0.00174	0.00174	0.00174	0.07	0.799
Error	124	3.30400	3.30400	0.02665		
Total	127	3.37533				

Unusual Observations for Dandelio

Obs	Dandelio	Fit	StDev Fit	Residual	St Resid
27	0.520000	0.117622	0.018975	0.402378	2.48R
39	0.681000	0.117622	0.018975	0.563378	3.47R
44	0.760000	0.117622	0.018975	0.642378	3.96R
54	0.756000	0.117622	0.018975	0.638378	3.94R
71	0.713000	0.117622	0.018975	0.595378	3.67R
79	0.737000	0.152500	0.043626	0.584500	3.72R
83	0.686000	0.152500	0.043626	0.533500	3.39R
98	0.500000	0.097375	0.028856	0.402625	2.51R
101	0.421000	0.097375	0.028856	0.323625	2.01R
121	0.077000	0.042000	0.057712	0.035000	0.23 X
122	0.082000	0.042000	0.057712	0.040000	0.26 X
123	0.031000	0.042000	0.057712	-0.011000	-0.07 X
124	0.028000	0.042000	0.057712	-0.014000	-0.09 X
125	0.047000	0.042000	0.057712	0.005000	0.03 X
126	0.014000	0.042000	0.057712	-0.028000	-0.18 X
127	0.036000	0.042000	0.057712	-0.006000	-0.04 X
128	0.021000	0.042000	0.057712	-0.021000	-0.14 X

R denotes an observation with a large standardized residual

X denotes an observation whose X value gives it large influence.

```
MTB > table c1 c2;
SUBC> stats c23.
```

Tabulated Statistics

```
Rows: Atopic   Columns: GDBA

       1       2      All

1      14      32       46
    0.15250 0.09737 0.11415
    0.24125 0.10965 0.16048

2      74       8       82
    0.11762 0.04200 0.11024
    0.17242 0.02515 0.16540

All    88      40      128
    0.12317 0.08630 0.11165
    0.18387 0.10086 0.16303

 Cell Contents --
      Dandelio:N
           Mean
           StDev
```

```
MTB > glm c24=c1 c2 c1*c2
```

General Linear Model

```
Factor   Levels Values
Atopic        2  1  2
GDBA          2  1  2


Analysis of Variance for Nettle

Source       DF   Seq SS   Adj SS   Adj MS      F      P
Atopic        1  0.00065  0.01041  0.01041   0.78  0.379
GDBA          1  0.03197  0.03404  0.03404   2.55  0.113
Atopic*GDBA   1  0.00269  0.00269  0.00269   0.20  0.654
Error       124  1.65327  1.65327  0.01333
Total       127  1.68857


Unusual Observations for Nettle

Obs   Nettle      Fit   StDev Fit  Residual  St Resid
  1  0.406000  0.108541   0.013423  0.297459     2.59R
  3  0.560000  0.108541   0.013423  0.451459     3.94R
```

36	0.630000	0.108541	0.013423	0.521459	4.55R
39	0.570000	0.108541	0.013423	0.461459	4.02R
79	0.423000	0.120857	0.030860	0.302143	2.72R
83	0.436000	0.120857	0.030860	0.315143	2.83R
101	0.482000	0.088281	0.020412	0.393719	3.46R
121	0.081000	0.050500	0.040824	0.030500	0.28 X
122	0.045000	0.050500	0.040824	-0.005500	-0.05 X
123	0.064000	0.050500	0.040824	0.013500	0.12 X
124	0.041000	0.050500	0.040824	-0.009500	-0.09 X
125	0.049000	0.050500	0.040824	-0.001500	-0.01 X
126	0.037000	0.050500	0.040824	-0.013500	-0.12 X
127	0.032000	0.050500	0.040824	-0.018500	-0.17 X
128	0.055000	0.050500	0.040824	0.004500	0.04 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

```
MTB > table c1 c2;
SUBC> stats c24.
```

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.12086	0.08828	0.09820
	0.13590	0.08989	0.10551
2	74	8	82
	0.10854	0.05050	0.10288
	0.12611	0.01595	0.12106
All	88	40	128
	0.11050	0.08073	0.10120
	0.12698	0.08187	0.11531

Cell Contents --
Nettle:N
Mean
StDev

MTB >

Appendix I3

Statisitical analysis of ELISA results for atopic and non-atopic dogs (non-GDBA/non-atopic =greyhounds & beagles).

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Flea

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000003	0.000065	0.000065	0.01	0.921
GDBA	1	0.000114	0.000661	0.000661	0.10	0.751
Atopic*GDBA	1	0.004226	0.004226	0.004226	0.65	0.422
Error	136	0.885857	0.885857	0.006514		
Total	139	0.890200				

Unusual Observations for Flea

Obs	Flea	Fit	StDev Fit	Residual	St Resid
19	0.317000	0.072959	0.009382	0.244041	3.04R
39	0.474000	0.072959	0.009382	0.401041	5.00R
64	0.656000	0.072959	0.009382	0.583041	7.27R
116	0.298000	0.076562	0.014267	0.221438	2.79R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.05807	0.07656	0.07093
	0.04482	0.06152	0.05711
2	74	20	94
	0.07296	0.06495	0.07126
	0.09692	0.05464	0.08941
All	88	52	140
	0.07059	0.07210	0.07115
	0.09062	0.05870	0.08003

Cell Contents --
Flea:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mites

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000725	0.000183	0.000183	0.05	0.825
GDBA	1	0.006656	0.004723	0.004723	1.26	0.263
Atopic*GDBA	1	0.002009	0.002009	0.002009	0.54	0.465
Error	136	0.508846	0.508846	0.003742		
Total	139	0.518236				

Unusual Observations for Mites

Obs	Mites	Fit	StDev Fit	Residual	St Resid
16	0.287000	0.116905	0.007111	0.170095	2.80R
27	0.276000	0.116905	0.007111	0.159095	2.62R
39	0.303000	0.116905	0.007111	0.186095	3.06R
46	0.276000	0.116905	0.007111	0.159095	2.62R
48	0.275000	0.116905	0.007111	0.158095	2.60R
94	0.275000	0.128156	0.010813	0.146844	2.44R
97	0.000000	0.128156	0.010813	-0.128156	-2.13R
117	0.263000	0.128156	0.010813	0.134844	2.24R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.12329	0.12816	0.12667
	0.05247	0.06425	0.06037
2	74	20	94
	0.11691	0.14005	0.12183
	0.06243	0.05646	0.06166
All	88	52	140
	0.11792	0.13273	0.12342
	0.06072	0.06108	0.06106

Cell Contents --
Mites:N
Mean

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Feathers

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001171	0.000088	0.000088	0.03	0.860
GDBA	1	0.006760	0.008241	0.008241	2.92	0.090
Atopic*GDBA	1	0.002132	0.002132	0.002132	0.76	0.386
Error	136	0.383392	0.383392	0.002819		
Total	139	0.393454				

Unusual Observations for Feathers

Obs	Feathers	Fit	StDev Fit	Residual	St Resid
3	0.180000	0.071257	0.006172	0.108743	2.06R
12	0.213000	0.071257	0.006172	0.141743	2.69R
51	0.291000	0.071257	0.006172	0.219743	4.17R
94	0.208000	0.087844	0.009386	0.120156	2.30R
110	0.388000	0.087844	0.009386	0.300156	5.74R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.05993	0.08784	0.07935
	0.03158	0.07091	0.06262
2	74	20	94
	0.07126	0.08035	0.07319
	0.04998	0.04115	0.04817
All	88	52	140
	0.06945	0.08496	0.07521
	0.04756	0.06084	0.05320

Cell Contents --
Feathers:N
Mean

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Alternar

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.016067	0.018146	0.018146	5.22	0.024
GDBA	1	0.002506	0.001715	0.001715	0.49	0.483
Atopic*GDBA	1	0.000942	0.000942	0.000942	0.27	0.603
Error	136	0.472377	0.472377	0.003473		
Total	139	0.491891				

Unusual Observations for Alternar

Obs	Alternar	Fit	StDev Fit	Residual	St Resid
7	0.225000	0.087797	0.006851	0.137203	2.34R
17	0.268000	0.087797	0.006851	0.180203	3.08R
24	0.209000	0.087797	0.006851	0.121203	2.07R
59	0.233000	0.087797	0.006851	0.145203	2.48R
83	0.353000	0.109000	0.015751	0.244000	4.30R
94	0.224000	0.106813	0.010418	0.117188	2.02R
106	0.226000	0.106813	0.010418	0.119188	2.05R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.10900	0.10681	0.10748
	0.07756	0.06226	0.06641
2	74	20	94
	0.08780	0.07310	0.08467
	0.05824	0.03728	0.05461
All	88	52	140
	0.09117	0.09385	0.09216
	0.06169	0.05611	0.05949

Cell Contents --
Alternar:N
Mean
StDev

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Aspergil

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.016147	0.014091	0.014091	5.46	0.021
GDBA	1	0.000261	0.000022	0.000022	0.01	0.927
Atopic*GDBA	1	0.002197	0.002197	0.002197	0.85	0.358
Error	136	0.351196	0.351196	0.002582		
Total	139	0.369800				

Unusual Observations for Aspergil

Obs	Aspergil	Fit	StDev Fit	Residual	St Resid
64	0.226000	0.091500	0.005907	0.134500	2.66R
93	0.232000	0.114750	0.008983	0.117250	2.34R
98	0.001000	0.114750	0.008983	-0.113750	-2.27R
112	0.226000	0.114750	0.008983	0.111250	2.22R
118	0.333000	0.114750	0.008983	0.218250	4.36R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.10614	0.11475	0.11213
	0.05266	0.07067	0.06525
2	74	20	94
	0.09150	0.08100	0.08927
	0.04229	0.03957	0.04174
All	88	52	140
	0.09383	0.10177	0.09678
	0.04409	0.06240	0.05158

Cell Contents --
Aspergil:N
Mean
StDev

MTB > glm c8=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Rhizopus

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.014106	0.000712	0.000712	0.08	0.778
GDBA	1	0.028405	0.023682	0.023682	2.65	0.106
Atopic*GDBA	1	0.001765	0.001765	0.001765	0.20	0.657
Error	136	1.214785	1.214785	0.008932		
Total	139	1.259062				

Unusual Observations for Rhizopus

Obs	Rhizopus	Fit	StDev Fit	Residual	St Resid
3	0.406000	0.099932	0.010987	0.306068	3.26R
5	0.290000	0.099932	0.010987	0.190068	2.02R
27	0.588000	0.099932	0.010987	0.488068	5.20R
39	0.396000	0.099932	0.010987	0.296068	3.15R
54	0.615000	0.099932	0.010987	0.515068	5.49R
83	0.313000	0.085929	0.025259	0.227071	2.49R
101	0.318000	0.063125	0.016707	0.254875	2.74R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c8.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.08593	0.06313	0.07007
	0.07503	0.06724	0.06967
2	74	20	94
	0.09993	0.06000	0.09144
	0.11591	0.03298	0.10506
All	88	52	140
	0.09770	0.06192	0.08441
	0.11019	0.05618	0.09517

Cell Contents --
Rhizopus:N
Mean
StDev

MTB > glm c9=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Kapok

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000000	0.003643	0.003643	0.60	0.441
GDBA	1	0.017130	0.014665	0.014665	2.41	0.123
Atopic*GDBA	1	0.000669	0.000669	0.000669	0.11	0.741
Error	136	0.828399	0.828399	0.006091		
Total	139	0.846198				

Unusual Observations for Kapok

Obs	Kapok	Fit	StDev Fit	Residual	St Resid
3	0.319000	0.077257	0.009073	0.241743	3.12R
27	0.383000	0.077257	0.009073	0.305743	3.94R
39	0.320000	0.077257	0.009073	0.242743	3.13R
54	0.410000	0.077257	0.009073	0.332743	4.29R
79	0.479000	0.084286	0.020859	0.394714	5.25R
101	0.308000	0.064875	0.013797	0.243125	3.16R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c9.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.08429	0.06487	0.07078
	0.12341	0.06438	0.08565
2	74	20	94
	0.07726	0.04730	0.07088
	0.08188	0.02562	0.07449
All	88	52	140
	0.07837	0.05812	0.07085
	0.08893	0.05327	0.07802

Cell Contents --
Kapok:N
Mean
StDev

MTB > glm c10=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Dust

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001671	0.001212	0.001212	1.12	0.292
GDBA	1	0.000006	0.000016	0.000016	0.01	0.905
Atopic*GDBA	1	0.000049	0.000049	0.000049	0.05	0.832
Error	136	0.147133	0.147133	0.001082		
Total	139	0.148859				

Unusual Observations for Dust

Obs	Dust	Fit	StDev Fit	Residual	St Resid
1	0.123000	0.050027	0.003824	0.072973	2.23R
12	0.136000	0.050027	0.003824	0.085973	2.63R
73	0.160000	0.050027	0.003824	0.109973	3.37R
79	0.155000	0.044357	0.008791	0.110643	3.49R
121	0.123000	0.050650	0.007355	0.072350	2.26R
123	0.123000	0.050650	0.007355	0.072350	2.26R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c10.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.044357	0.042125	0.042804
	0.040636	0.026655	0.031106
2	74	20	94
	0.050027	0.050650	0.050160
	0.033883	0.032307	0.033384
All	88	52	140
	0.049125	0.045404	0.047743
	0.034849	0.028952	0.032725

Cell Contents --
Dust:N
Mean
StDev

MTB > glm c11=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Cat

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0005491	0.0011332	0.0011332	1.53	0.218
GDBA	1	0.0007992	0.0005955	0.0005955	0.81	0.371
Atopic*GDBA	1	0.0001699	0.0001699	0.0001699	0.23	0.632
Error	136	0.1004483	0.1004483	0.0007386		
Total	139	0.1019665				

Unusual Observations for Cat

Obs	Cat	Fit	StDev Fit	Residual	St Resid
1	0.115000	0.037919	0.003159	0.077081	2.86R
17	0.103000	0.037919	0.003159	0.065081	2.41R
60	0.101000	0.037919	0.003159	0.063081	2.34R
140	0.100000	0.045550	0.006077	0.054450	2.06R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c11.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.033714	0.036031	0.035326
	0.019201	0.024843	0.023083
2	74	20	94
	0.037919	0.045550	0.039543
	0.028576	0.029835	0.028856
All	88	52	140
	0.037250	0.039692	0.038157
	0.027252	0.026993	0.027085

Cell Contents --
Cat:N
Mean
StDev

MTB > glm c12=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Human

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.004966	0.003474	0.003474	1.58	0.211
GDBA	1	0.000056	0.000021	0.000021	0.01	0.922
Atopic*GDBA	1	0.000131	0.000131	0.000131	0.06	0.808
Error	136	0.299027	0.299027	0.002199		
Total	139	0.304181				

Unusual Observations for Human

Obs	Human	Fit	StDev Fit	Residual	St Resid
8	0.197000	0.047419	0.005451	0.149581	3.21R
39	0.226000	0.047419	0.005451	0.178581	3.83R
48	0.434000	0.047419	0.005451	0.386581	8.30R
140	0.140000	0.044150	0.010485	0.095850	2.10R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c12.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.033071	0.034469	0.034043
	0.020281	0.023903	0.022646
2	74	20	94
	0.047419	0.044150	0.046723
	0.059374	0.031307	0.054490
All	88	52	140
	0.045136	0.038192	0.042557
	0.055202	0.027112	0.046780

Cell Contents --
Human:N
Mean
StDev

MTB > glm c13=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mucor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.000503	0.010207	0.010207	1.09	0.298
GDBA	1	0.064911	0.068082	0.068082	7.27	0.008
Atopic*GDBA	1	0.003197	0.003197	0.003197	0.34	0.560
Error	136	1.274018	1.274018	0.009368		
Total	139	1.342629				

Unusual Observations for Mucor

Obs	Mucor	Fit	StDev Fit	Residual	St Resid
27	0.449000	0.108811	0.011251	0.340189	3.54R
39	0.751000	0.108811	0.011251	0.642189	6.68R
51	0.372000	0.108811	0.011251	0.263189	2.74R
59	0.329000	0.108811	0.011251	0.220189	2.29R
79	0.423000	0.140929	0.025867	0.282071	3.02R
83	0.433000	0.140929	0.025867	0.292071	3.13R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c13.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.14093	0.07622	0.09591
	0.13171	0.04634	0.08601
2	74	20	94
	0.10881	0.06715	0.09995
	0.11426	0.03897	0.10417
All	88	52	140
	0.11392	0.07273	0.09862
	0.11699	0.04349	0.09828

Cell Contents --
Mucor:N
Mean
StDev

MTB > glm c14=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Orchard

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.008237	0.003272	0.003272	0.47	0.496
GDBA	1	0.002028	0.003927	0.003927	0.56	0.456
Atopic*GDBA	1	0.006432	0.006432	0.006432	0.92	0.340
Error	136	0.955073	0.955073	0.007023		
Total	139	0.971770				

Unusual Observations for Orchard

Obs	Orchard	Fit	StDev Fit	Residual	St Resid
3	0.623000	0.093473	0.009742	0.529527	6.36R
54	0.324000	0.093473	0.009742	0.230527	2.77R
89	0.433000	0.117906	0.014814	0.315094	3.82R
119	0.348000	0.117906	0.014814	0.230094	2.79R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c14.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.08879	0.11791	0.10904
	0.05034	0.09949	0.08794
2	74	20	94
	0.09347	0.08990	0.09271
	0.08908	0.04356	0.08135
All	88	52	140
	0.09273	0.10713	0.09808
	0.08390	0.08314	0.08361

Cell Contents --
Orchard:N
Mean
StDev

MTB > glm c15=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Timothy

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.008551	0.001510	0.001510	0.16	0.690
GDBA	1	0.007859	0.009723	0.009723	1.03	0.313
Atopic*GDBA	1	0.002790	0.002790	0.002790	0.29	0.588
Error	136	1.287316	1.287316	0.009466		
Total	139	1.306517				

Unusual Observations for Timothy

Obs	Timothy	Fit	StDev Fit	Residual	St Resid
3	0.618000	0.115919	0.011310	0.502081	5.20R
8	0.576000	0.115919	0.011310	0.460081	4.76R
48	0.320000	0.115919	0.011310	0.204081	2.11R
89	0.378000	0.143937	0.017199	0.234063	2.44R
101	0.366000	0.143937	0.017199	0.222063	2.32R
104	0.417000	0.143937	0.017199	0.273063	2.85R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c15.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11307	0.14394	0.13454
	0.06228	0.10350	0.09330
2	74	20	94
	0.11592	0.12525	0.11790
	0.10520	0.07140	0.09871
All	88	52	140
	0.11547	0.13675	0.12337
	0.09934	0.09217	0.09695

Cell Contents --
Timothy:N
Mean
StDev

MTB > glm c16=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Kentucky

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00865	0.00102	0.00102	0.10	0.753
GDBA	1	0.01108	0.01223	0.01223	1.20	0.276
Atopic*GDBA	1	0.00123	0.00123	0.00123	0.12	0.730
Error	136	1.39079	1.39079	0.01023		
Total	139	1.41176				

Unusual Observations for Kentucky

Obs	Kentucky	Fit	StDev Fit	Residual	St Resid
3	0.669000	0.103203	0.011756	0.565797	5.63R
8	0.613000	0.103203	0.011756	0.509797	5.08R
39	0.305000	0.103203	0.011756	0.201797	2.01R
89	0.545000	0.132250	0.017877	0.412750	4.15R
104	0.389000	0.132250	0.017877	0.256750	2.58R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c16.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.10257	0.13225	0.12322
	0.05128	0.11289	0.09864
2	74	20	94
	0.10320	0.11860	0.10648
	0.10944	0.06775	0.10188
All	88	52	140
	0.10310	0.12700	0.11198
	0.10219	0.09747	0.10078

Cell Contents --
Kentucky:N
Mean
StDev

MTB > glm c17=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Fescue

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001812	0.001236	0.001236	0.14	0.710
GDBA	1	0.023901	0.023129	0.023129	2.60	0.109
Atopic*GDBA	1	0.000061	0.000061	0.000061	0.01	0.934
Error	136	1.209488	1.209488	0.008893		
Total	139	1.235261				

Unusual Observations for Fescue

Obs	Fescue	Fit	StDev Fit	Residual	St Resid
3	0.682000	0.102041	0.010963	0.579959	6.19R
54	0.460000	0.102041	0.010963	0.357959	3.82R
89	0.435000	0.125875	0.016671	0.309125	3.33R
101	0.325000	0.125875	0.016671	0.199125	2.15R
133	0.418000	0.131450	0.021087	0.286550	3.12R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c17.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.09329	0.12588	0.11596
	0.05502	0.09544	0.08591
2	74	20	94
	0.10204	0.13145	0.10830
	0.10029	0.08986	0.09845
All	88	52	140
	0.10065	0.12802	0.11081
	0.09436	0.09248	0.09427

Cell Contents --
Fescue:N
Mean
StDev

MTB > glm c18=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Poplar

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00177	0.00230	0.00230	0.19	0.664
GDBA	1	0.00070	0.00009	0.00009	0.01	0.933
Atopic*GDBA	1	0.00483	0.00483	0.00483	0.40	0.530
Error	136	1.65153	1.65153	0.01214		
Total	139	1.65882				

Unusual Observations for Poplar

Obs	Poplar	Fit	StDev Fit	Residual	St Resid
3	0.877000	0.108554	0.012810	0.768446	7.02R
36	0.461000	0.108554	0.012810	0.352446	3.22R
54	0.343000	0.108554	0.012810	0.234446	2.14R
79	0.332000	0.112929	0.029452	0.219071	2.06R
89	0.352000	0.100656	0.019480	0.251344	2.32R
101	0.385000	0.100656	0.019480	0.284344	2.62R
136	0.404000	0.124600	0.024641	0.279400	2.60R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c18.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11293	0.10066	0.10439
	0.09234	0.09484	0.09323
2	74	20	94
	0.10855	0.12460	0.11197
	0.12268	0.09267	0.11667
All	88	52	140
	0.10925	0.10987	0.10948
	0.11792	0.09383	0.10924

Cell Contents --
Poplar:N
Mean
StDev

MTB > glm c19=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Birch

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00309	0.01651	0.01651	1.07	0.303
GDBA	1	0.02844	0.02771	0.02771	1.79	0.183
Atopic*GDBA	1	0.00012	0.00012	0.00012	0.01	0.930
Error	136	2.10135	2.10135	0.01545		
Total	139	2.13300				

Unusual Observations for Birch

Obs	Birch	Fit	StDev Fit	Residual	St Resid
27	0.460000	0.111649	0.014450	0.348351	2.82R
39	0.792000	0.111649	0.014450	0.680351	5.51R
54	0.656000	0.111649	0.014450	0.544351	4.41R
79	0.770000	0.140071	0.033221	0.629929	5.26R
101	0.381000	0.103906	0.021974	0.277094	2.26R
117	0.525000	0.103906	0.021974	0.421094	3.44R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c19.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.14007	0.10391	0.11491
	0.19267	0.11163	0.13997
2	74	20	94
	0.11165	0.07995	0.10490
	0.12718	0.05219	0.11585
All	88	52	140
	0.11617	0.09469	0.10819
	0.13866	0.09342	0.12388

Cell Contents --
Birch:N
Mean
StDev

MTB > glm c20=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Sorrel

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00627	0.00637	0.00637	0.61	0.434
GDBA	1	0.00031	0.00115	0.00115	0.11	0.739
Atopic*GDBA	1	0.00503	0.00503	0.00503	0.49	0.487
Error	136	1.40968	1.40968	0.01037		
Total	139	1.42130				

Unusual Observations for Sorrel

Obs	Sorrel	Fit	StDev Fit	Residual	St Resid
3	0.790000	0.101446	0.011835	0.688554	6.81R
8	0.400000	0.101446	0.011835	0.298554	2.95R
69	0.730000	0.101446	0.011835	0.628554	6.22R
89	0.375000	0.092094	0.017998	0.282906	2.82R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c20.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.07071	0.09209	0.08559
	0.03715	0.07197	0.06376
2	74	20	94
	0.10145	0.09390	0.09984
	0.12636	0.05877	0.11510
All	88	52	140
	0.09656	0.09279	0.09516
	0.11718	0.06660	0.10112

Cell Contents --
Sorrel:N
Mean
StDev

MTB > glm c21=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Plantain

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001572	0.001945	0.001945	0.22	0.639
GDBA	1	0.000341	0.000508	0.000508	0.06	0.811
Atopic*GDBA	1	0.000380	0.000380	0.000380	0.04	0.836
Error	136	1.199688	1.199688	0.008821		
Total	139	1.201981				

Unusual Observations for Plantain

Obs	Plantain	Fit	StDev Fit	Residual	St Resid
1	0.313000	0.103324	0.010918	0.209676	2.25R
3	0.619000	0.103324	0.010918	0.515676	5.53R
39	0.306000	0.103324	0.010918	0.202676	2.17R
54	0.332000	0.103324	0.010918	0.228676	2.45R
79	0.313000	0.116286	0.025102	0.196714	2.17R
94	0.294000	0.107719	0.016603	0.186281	2.02R
101	0.487000	0.107719	0.016603	0.379281	4.10R
104	0.328000	0.107719	0.016603	0.220281	2.38R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c21.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11629	0.10772	0.11033
	0.08262	0.10746	0.09972
2	74	20	94
	0.10332	0.10270	0.10319
	0.09522	0.06925	0.08998
All	88	52	140
	0.10539	0.10579	0.10554
	0.09301	0.09387	0.09299

Cell Contents --
Plantain:N
Mean
StDev

MTB > glm c22=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mugwort

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00227	0.00760	0.00760	0.45	0.502
GDBA	1	0.00897	0.00985	0.00985	0.59	0.445
Atopic*GDBA	1	0.00093	0.00093	0.00093	0.06	0.814
Error	136	2.28221	2.28221	0.01678		
Total	139	2.29438				

Unusual Observations for Mugwort

Obs	Mugwort	Fit	StDev Fit	Residual	St Resid
1	0.356000	0.095149	0.015059	0.260851	2.03R
3	0.507000	0.095149	0.015059	0.411851	3.20R
27	0.433000	0.095149	0.015059	0.337851	2.63R
39	0.601000	0.095149	0.015059	0.505851	3.93R
48	0.381000	0.095149	0.015059	0.285851	2.22R
54	0.582000	0.095149	0.015059	0.486851	3.78R
79	0.706000	0.119143	0.034621	0.586857	4.70R
101	0.464000	0.092688	0.022900	0.371313	2.91R
134	0.372000	0.081150	0.028966	0.290850	2.30R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c22.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.11914	0.09269	0.10074
	0.18355	0.10380	0.13155
2	74	20	94
	0.09515	0.08115	0.09217
	0.13746	0.08297	0.12756
All	88	52	140
	0.09897	0.08825	0.09499
	0.14480	0.09563	0.12848

Cell Contents --
Mugwort:N
Mean
StDev

MTB > glm c23=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Dandelio

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00242	0.03167	0.03167	1.29	0.258
GDBA	1	0.08237	0.07690	0.07690	3.13	0.079
Atopic*GDBA	1	0.00005	0.00005	0.00005	0.00	0.965
Error	136	3.34092	3.34092	0.02457		
Total	139	3.42576				

Unusual Observations for Dandelio

Obs	Dandelio	Fit	StDev Fit	Residual	St Resid
3	0.430000	0.117622	0.018220	0.312378	2.01R
27	0.520000	0.117622	0.018220	0.402378	2.58R
39	0.681000	0.117622	0.018220	0.563378	3.62R
44	0.760000	0.117622	0.018220	0.642378	4.13R
54	0.756000	0.117622	0.018220	0.638378	4.10R
71	0.713000	0.117622	0.018220	0.595378	3.82R
79	0.737000	0.152500	0.041889	0.584500	3.87R
83	0.686000	0.152500	0.041889	0.533500	3.53R
98	0.500000	0.097375	0.027707	0.402625	2.61R
101	0.421000	0.097375	0.027707	0.323625	2.10R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c23.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.15250	0.09737	0.11415
	0.24125	0.10965	0.16048
2	74	20	94
	0.11762	0.05970	0.10530
	0.17242	0.04665	0.15604
All	88	52	140
	0.12317	0.08288	0.10821
	0.18387	0.09198	0.15699

Cell Contents --
Dandelio:N
Mean
StDev

MTB > glm c24=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Nettle

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.00021	0.00487	0.00487	0.39	0.531
GDBA	1	0.03110	0.02862	0.02862	2.31	0.131
Atopic*GDBA	1	0.00009	0.00009	0.00009	0.01	0.933
Error	136	1.68218	1.68218	0.01237		
Total	139	1.71357				

Unusual Observations for Nettle

Obs	Nettle	Fit	StDev Fit	Residual	St Resid
1	0.406000	0.108541	0.012929	0.297459	2.69R
3	0.560000	0.108541	0.012929	0.451459	4.09R
36	0.630000	0.108541	0.012929	0.521459	4.72R
39	0.570000	0.108541	0.012929	0.461459	4.18R
79	0.423000	0.120857	0.029724	0.302143	2.82R
83	0.436000	0.120857	0.029724	0.315143	2.94R
101	0.482000	0.088281	0.019660	0.393719	3.60R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c24.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	14	32	46
	0.12086	0.08828	0.09820
	0.13590	0.08989	0.10551
2	74	20	94
	0.10854	0.07215	0.10080
	0.12611	0.04019	0.11418
All	88	52	140
	0.11050	0.08208	0.09994
	0.12698	0.07467	0.11103

Cell Contents --
Nettle:N
Mean
StDev

Appendix J

Statistical analysis of Immunodot results for atopic and non-atopic GDBA and GUVS dogs, greyhounds and beagles

Appendix J1

Statisitical analysis of Immunodot results for atopic and non-atopic dogs (non-GDBA/non-atopic =greyhounds).

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Dustmite

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.22265	0.03776	0.03776	2.23	0.140
GDBA	1	0.07148	0.05295	0.05295	3.12	0.081
Atopic*GDBA	1	0.01442	0.01442	0.01442	0.85	0.360
Error	74	1.25580	1.25580	0.01697		
Total	77	1.56435				

Unusual Observations for Dustmite

Obs	Dustmite	Fit	StDev Fit	Residual	St Resid
52	0.562000	0.167583	0.026591	0.394417	3.09R
54	0.609000	0.167583	0.026591	0.441417	3.46R
70	0.740000	0.167583	0.026591	0.572417	4.49R
73	0.575000	0.167583	0.026591	0.407417	3.19R
75	0.043000	0.063250	0.065135	-0.020250	-0.18 X
76	0.054000	0.063250	0.065135	-0.009250	-0.08 X
77	0.024000	0.063250	0.065135	-0.039250	-0.35 X
78	0.132000	0.063250	0.065135	0.068750	0.61 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.05137	0.16758	0.13853
	0.08541	0.22446	0.20406
2	42	4	46
	0.02674	0.06325	0.02991
	0.03091	0.04748	0.03360
All	50	28	78
	0.03068	0.15268	0.07447
	0.04387	0.21107	0.14253

Cell Contents --
Dustmite:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Storemit

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.008462	0.000056	0.000056	0.01	0.911
GDBA	1	0.014536	0.012607	0.012607	2.81	0.098
Atopic*GDBA	1	0.000368	0.000368	0.000368	0.08	0.776
Error	74	0.332433	0.332433	0.004492		
Total	77	0.355799				

Unusual Observations for Storemit

Obs	Storemit	Fit	StDev Fit	Residual	St Resid
52	0.261000	0.081125	0.013681	0.179875	2.74R
54	0.282000	0.081125	0.013681	0.200875	3.06R
70	0.505000	0.081125	0.013681	0.423875	6.46R
75	0.067000	0.077250	0.033512	-0.010250	-0.18 X
76	0.086000	0.077250	0.033512	0.008750	0.15 X
77	0.059000	0.077250	0.033512	-0.018250	-0.31 X
78	0.097000	0.077250	0.033512	0.019750	0.34 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.03750	0.08113	0.07022
	0.01030	0.11578	0.10167
2	42	4	46
	0.04636	0.07725	0.04904
	0.02342	0.01737	0.02444
All	50	28	78
	0.04494	0.08057	0.05773
	0.02202	0.10702	0.06798

Cell Contents --
Storemit:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Flea

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0000522	0.0000492	0.0000492	0.16	0.690
GDBA	1	0.0000442	0.0000030	0.0000030	0.01	0.921
Atopic*GDBA	1	0.0003746	0.0003746	0.0003746	1.22	0.272
Error	74	0.0226484	0.0226484	0.0003061		
Total	77	0.0231193				

Unusual Observations for Flea

Obs	Flea	Fit	StDev Fit	Residual	St Resid
17	0.095000	0.016095	0.002699	0.078905	4.56R
65	0.074000	0.019000	0.003571	0.055000	3.21R
66	0.068000	0.019000	0.003571	0.049000	2.86R
75	0.005000	0.010250	0.008747	-0.005250	-0.35 X
76	0.016000	0.010250	0.008747	0.005750	0.38 X
77	0.005000	0.010250	0.008747	-0.005250	-0.35 X
78	0.015000	0.010250	0.008747	0.004750	0.31 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.012000	0.019000	0.017250
	0.011136	0.021657	0.019634
2	42	4	46
	0.016095	0.010250	0.015587
	0.016291	0.006076	0.015718
All	50	28	78
	0.015440	0.017750	0.016269
	0.015559	0.020332	0.017328

Cell Contents --
Flea:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Human

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.002631	0.001155	0.001155	0.94	0.335
GDBA	1	0.000005	0.000000	0.000000	0.00	0.998
Atopic*GDBA	1	0.000090	0.000090	0.000090	0.07	0.787
Error	74	0.090866	0.090866	0.001228		
Total	77	0.093593				

Unusual Observations for Human

Obs	Human	Fit	StDev Fit	Residual	St Resid
8	0.182000	0.062429	0.005407	0.119571	3.45R
17	0.180000	0.062429	0.005407	0.117571	3.40R
66	0.143000	0.051125	0.007153	0.091875	2.68R
75	0.062000	0.059250	0.017521	0.002750	0.09 X
76	0.055000	0.059250	0.017521	-0.004250	-0.14 X
77	0.057000	0.059250	0.017521	-0.002250	-0.07 X
78	0.063000	0.059250	0.017521	0.003750	0.12 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.048000	0.051125	0.050344
	0.013575	0.042766	0.037422
2	42	4	46
	0.062429	0.059250	0.062152
	0.034025	0.003862	0.032506
All	50	28	78
	0.060120	0.052286	0.057308
	0.031993	0.039598	0.034864

Cell Contents --
Human:N
Mean
StDev

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Cat

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0063042	0.0002287	0.0002287	0.46	0.502
GDBA	1	0.0058001	0.0045029	0.0045029	8.96	0.004
Atopic*GDBA	1	0.0007740	0.0007740	0.0007740	1.54	0.218
Error	74	0.0371753	0.0371753	0.0005024		
Total	77	0.0500537				

Unusual Observations for Cat

Obs	Cat	Fit	StDev Fit	Residual	St Resid
31	0.088000	0.038286	0.003458	0.049714	2.24R
37	0.085000	0.038286	0.003458	0.046714	2.11R
38	0.141000	0.038286	0.003458	0.102714	4.64R
75	0.008000	0.025250	0.011207	-0.017250	-0.89 X
76	0.060000	0.025250	0.011207	0.034750	1.79 X
77	0.015000	0.025250	0.011207	-0.010250	-0.53 X
78	0.018000	0.025250	0.011207	-0.007250	-0.37 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.042500	0.011000	0.018875
	0.017501	0.013863	0.020095
2	42	4	46
	0.038286	0.025250	0.037152
	0.026572	0.023543	0.026345
All	50	28	78
	0.038960	0.013036	0.029654
	0.025238	0.015845	0.025496

Cell Contents --
Cat:N
Mean
StDev

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Grass

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0103210	0.0002849	0.0002849	0.50	0.481
GDBA	1	0.0076828	0.0069034	0.0069034	12.13	0.001
Atopic*GDBA	1	0.0003537	0.0003537	0.0003537	0.62	0.433
Error	78	0.0444076	0.0444076	0.0005693		
Total	81	0.0627650				

Unusual Observations for Grass

Obs	Grass	Fit	StDev Fit	Residual	St Resid
16	0.141000	0.021940	0.003374	0.119060	5.04R
49	0.125000	0.021940	0.003374	0.103060	4.36R
73	0.114000	0.055857	0.005207	0.058143	2.50R
79	0.046000	0.043750	0.011930	0.002250	0.11 X
80	0.046000	0.043750	0.011930	0.002250	0.11 X
81	0.041000	0.043750	0.011930	-0.002750	-0.13 X
82	0.042000	0.043750	0.011930	-0.001750	-0.08 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.021286	0.055857	0.047214
	0.013149	0.022903	0.025678
2	50	4	54
	0.021940	0.043750	0.023556
	0.025896	0.002630	0.025566
All	57	25	82
	0.021860	0.053920	0.031634
	0.024604	0.021412	0.027837

Cell Contents --
Grass:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Tree

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0007511	0.0004760	0.0004760	2.12	0.149
GDBA	1	0.0025487	0.0029016	0.0029016	12.94	0.001
Atopic*GDBA	1	0.0005747	0.0005747	0.0005747	2.56	0.113
Error	78	0.0174915	0.0174915	0.0002242		
Total	81	0.0213660				

Unusual Observations for Tree

Obs	Tree	Fit	StDev Fit	Residual	St Resid
49	0.051000	0.018840	0.002118	0.032160	2.17R
67	0.068000	0.029714	0.003268	0.038286	2.62R
77	0.069000	0.029714	0.003268	0.039286	2.69R
79	0.060000	0.045250	0.007487	0.014750	1.14 X
80	0.059000	0.045250	0.007487	0.013750	1.06 X
81	0.021000	0.045250	0.007487	-0.024250	-1.87 X
82	0.041000	0.045250	0.007487	-0.004250	-0.33 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.019571	0.029714	0.027179
	0.014022	0.018879	0.018103
2	50	4	54
	0.018840	0.045250	0.020796
	0.012913	0.018373	0.014900
All	57	25	82
	0.018930	0.032200	0.022976
	0.012924	0.019313	0.016241

Cell Contents --
Tree:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mugwort

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0011731	0.0000443	0.0000443	0.07	0.787
GDBA	1	0.0004840	0.0005277	0.0005277	0.88	0.351
Atopic*GDBA	1	0.0000567	0.0000567	0.0000567	0.09	0.759
Error	78	0.0468496	0.0468496	0.0006006		
Total	81	0.0485635				

Unusual Observations for Mugwort

Obs	Mugwort	Fit	StDev Fit	Residual	St Resid
5	0.100000	0.050900	0.003466	0.049100	2.02R
8	0.117000	0.050900	0.003466	0.066100	2.72R
38	0.126000	0.050900	0.003466	0.075100	3.10R
79	0.067000	0.061250	0.012254	0.005750	0.27 X
80	0.067000	0.061250	0.012254	0.005750	0.27 X
81	0.057000	0.061250	0.012254	-0.004250	-0.20 X
82	0.054000	0.061250	0.012254	-0.007250	-0.34 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.055714	0.060952	0.059643
	0.024662	0.019433	0.020500
2	50	4	54
	0.050900	0.061250	0.051667
	0.026920	0.006752	0.026078
All	57	25	82
	0.051491	0.061000	0.054390
	0.026492	0.017900	0.024486

Cell Contents --
Mugwort:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Olive

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.006828	0.000502	0.000502	0.09	0.765
GDBA	1	0.003751	0.003193	0.003193	0.57	0.452
Atopic*GDBA	1	0.000488	0.000488	0.000488	0.09	0.768
Error	78	0.435578	0.435578	0.005584		
Total	81	0.446645				

Unusual Observations for Olive

Obs	Olive	Fit	StDev Fit	Residual	St Resid
34	0.669000	0.082680	0.010568	0.586320	7.93R
79	0.050000	0.071000	0.037364	-0.021000	-0.32 X
80	0.071000	0.071000	0.037364	0.000000	0.00 X
81	0.093000	0.071000	0.037364	0.022000	0.34 X
82	0.070000	0.071000	0.037364	-0.001000	-0.02 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.08257	0.05590	0.06257
	0.02721	0.03280	0.03316
2	50	4	54
	0.08268	0.07100	0.08181
	0.09133	0.01757	0.08797
All	57	25	82
	0.08267	0.05832	0.07524
	0.08589	0.03110	0.07426

Cell Contents --
Olive:N
Mean
StDev

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Outdoor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.002411	0.000665	0.000665	0.13	0.726
GDBA	1	0.000470	0.000340	0.000340	0.06	0.802
Atopic*GDBA	1	0.000054	0.000054	0.000054	0.01	0.920
Error	28	0.149022	0.149022	0.005322		
Total	31	0.151957				

Unusual Observations for Outdoor

Obs	Outdoor	Fit	StDev Fit	Residual	St Resid
30	0.374000	0.078800	0.018836	0.295200	4.19R
31	0.029000	0.063000	0.051586	-0.034000	-0.66 X
32	0.097000	0.063000	0.051586	0.034000	0.66 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.06650	0.07880	0.07621
	0.03797	0.09657	0.08672
2	11	2	13
	0.05773	0.06300	0.05854
	0.03437	0.04808	0.03437
All	15	17	32
	0.06007	0.07694	0.06903
	0.03419	0.09128	0.07001

Cell Contents --
Outdoor:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Indoor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.021015	0.011254	0.011254	1.26	0.271
GDBA	1	0.000724	0.000101	0.000101	0.01	0.916
Atopic*GDBA	1	0.002667	0.002667	0.002667	0.30	0.589
Error	28	0.249674	0.249674	0.008917		
Total	31	0.274080				

Unusual Observations for Indoor

Obs	Indoor	Fit	StDev Fit	Residual	St Resid
19	0.340000	0.155133	0.024382	0.184867	2.03R
29	0.493000	0.155133	0.024382	0.337867	3.70R
31	0.068000	0.080000	0.066772	-0.012000	-0.18 X
32	0.092000	0.080000	0.066772	0.012000	0.18 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.12575	0.15513	0.14895
	0.05849	0.12088	0.10994
2	11	2	13
	0.09982	0.08000	0.09677
	0.05879	0.01697	0.05440
All	15	17	32
	0.10673	0.14629	0.12775
	0.05781	0.11587	0.09403

Cell Contents --
Indoor:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Foods 1

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0000121	0.0000282	0.0000282	0.09	0.762
GDBA	1	0.0001280	0.0000327	0.0000327	0.11	0.744
Atopic*GDBA	1	0.0002817	0.0002817	0.0002817	0.93	0.342
Error	28	0.0084387	0.0084387	0.0003014		
Total	31	0.0088605				

Unusual Observations for Foods 1

Obs	Foods 1	Fit	StDev Fit	Residual	St Resid
9	0.086000	0.028273	0.005234	0.057727	3.49R
31	0.021000	0.023000	0.012276	-0.002000	-0.16 X
32	0.025000	0.023000	0.012276	0.002000	0.16 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.017750	0.028467	0.026211
	0.010532	0.014851	0.014497
2	11	2	13
	0.028273	0.023000	0.027462
	0.022383	0.002828	0.020545
All	15	17	32
	0.025467	0.027824	0.026719
	0.020121	0.014028	0.016906

Cell Contents --
Foods 1:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Foods 2

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0007588	0.0002130	0.0002130	0.33	0.572
GDBA	1	0.0000010	0.0000604	0.0000604	0.09	0.763
Atopic*GDBA	1	0.0005095	0.0005095	0.0005095	0.78	0.384
Error	28	0.0182039	0.0182039	0.0006501		
Total	31	0.0194732				

Unusual Observations for Foods 2

Obs	Foods 2	Fit	StDev Fit	Residual	St Resid
6	0.145000	0.039455	0.007688	0.105545	4.34R
31	0.024000	0.025000	0.018030	-0.001000	-0.06 X
32	0.026000	0.025000	0.018030	0.001000	0.06 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.021750	0.028800	0.027316
	0.022765	0.012891	0.014978
2	11	2	13
	0.039455	0.025000	0.037231
	0.037843	0.001414	0.034972
All	15	17	32
	0.034733	0.028353	0.031344
	0.034636	0.012129	0.025063

Cell Contents --
Foods 2:N
Mean
StDev

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Moulds

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.002444	0.005541	0.005541	3.24	0.083
GDBA	1	0.005379	0.003718	0.003718	2.18	0.151
Atopic*GDBA	1	0.000875	0.000875	0.000875	0.51	0.480
Error	28	0.047857	0.047857	0.001709		
Total	31	0.056554				

Unusual Observations for Moulds

Obs	Moulds	Fit	StDev Fit	Residual	St Resid
30	0.167000	0.082133	0.010675	0.084867	2.12R
31	0.070000	0.103500	0.029233	-0.033500	-1.15 X
32	0.137000	0.103500	0.029233	0.033500	1.15 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.03900	0.08213	0.07305
	0.03601	0.04615	0.04689
2	11	2	13
	0.08855	0.10350	0.09085
	0.03451	0.04738	0.03480
All	15	17	32
	0.07533	0.08465	0.08028
	0.04053	0.04532	0.04271

Cell Contents --
Moulds:N
Mean
StDev

Appendix J2

Statisitical analysis of Immundot results for atopic and non-atopic dogs (non-GDBA/non-atopic =beagles).

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Dustmite

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.24577	0.08388	0.08388	4.97	0.029
GDBA	1	0.03725	0.01817	0.01817	1.08	0.303
Atopic*GDBA	1	0.04639	0.04639	0.04639	2.75	0.102
Error	74	1.24904	1.24904	0.01688		
Total	77	1.57844				

Unusual Observations for Dustmite

Obs	Dustmite	Fit	StDev Fit	Residual	St Resid
52	0.562000	0.167583	0.026520	0.394417	3.10R
54	0.609000	0.167583	0.026520	0.441417	3.47R
70	0.740000	0.167583	0.026520	0.572417	4.50R
73	0.575000	0.167583	0.026520	0.407417	3.20R
75	0.000000	0.000000	0.064959	0.000000	0.00 X
76	0.000000	0.000000	0.064959	0.000000	0.00 X
77	0.000000	0.000000	0.064959	0.000000	0.00 X
78	0.000000	0.000000	0.064959	0.000000	0.00 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.05137	0.16758	0.13853
	0.08541	0.22446	0.20406
2	42	4	46
	0.02674	0.00000	0.02441
	0.03091	0.00000	0.03047
All	50	28	78
	0.03068	0.14364	0.07123
	0.04387	0.21560	0.14318

Cell Contents –
Dustmite:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Storemit

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.014682	0.011857	0.011857	2.65	0.108
GDBA	1	0.000885	0.000017	0.000017	0.00	0.951
Atopic*GDBA	1	0.018382	0.018382	0.018382	4.10	0.046
Error	74	0.331528	0.331528	0.004480		
Total	77	0.365478				

Unusual Observations for Storemit

Obs	Storemit	Fit	StDev Fit	Residual	St Resid
52	0.261000	0.081125	0.013663	0.179875	2.75R
54	0.282000	0.081125	0.013663	0.200875	3.07R
70	0.505000	0.081125	0.013663	0.423875	6.47R
75	0.000000	0.000000	0.033467	-0.000000	-0.00 X
76	0.000000	0.000000	0.033467	-0.000000	-0.00 X
77	0.000000	0.000000	0.033467	-0.000000	-0.00 X
78	0.000000	0.000000	0.033467	-0.000000	-0.00 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.03750	0.08113	0.07022
	0.01030	0.11578	0.10167
2	42	4	46
	0.04636	0.00000	0.04233
	0.02342	0.00000	0.02596
All	50	28	78
	0.04494	0.06954	0.05377
	0.02202	0.11070	0.06889

Cell Contents —
Storemit:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Flea

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0001231	0.0005043	0.0005043	1.66	0.202
GDBA	1	0.0000292	0.0001878	0.0001878	0.62	0.435
Atopic*GDBA	1	0.0012109	0.0012109	0.0012109	3.98	0.050
Error	74	0.0225376	0.0225376	0.0003046		
Total	77	0.0239009				

Unusual Observations for Flea

Obs	Flea	Fit	StDev Fit	Residual	St Resid
17	0.095000	0.016095	0.002693	0.078905	4.58R
65	0.074000	0.019000	0.003562	0.055000	3.22R
66	0.068000	0.019000	0.003562	0.049000	2.87R
75	0.000000	0.000000	0.008726	-0.000000	-0.00 X
76	0.000000	0.000000	0.008726	-0.000000	-0.00 X
77	0.000000	0.000000	0.008726	-0.000000	-0.00 X
78	0.000000	0.000000	0.008726	-0.000000	-0.00 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.012000	0.019000	0.017250
	0.011136	0.021657	0.019634
2	42	4	46
	0.016095	0.000000	0.014696
	0.016291	0.000000	0.016212
All	50	28	78
	0.015440	0.016286	0.015744
	0.015559	0.021104	0.017618

Cell Contents --
Flea:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Human

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001189	0.001093	0.001093	0.87	0.355
GDBA	1	0.002501	0.004507	0.004507	3.57	0.063
Atopic*GDBA	1	0.005860	0.005860	0.005860	4.64	0.034
Error	74	0.093432	0.093432	0.001263		
Total	77	0.102982				

Unusual Observations for Human

Obs	Human	Fit	StDev Fit	Residual	St Resid
8	0.182000	0.062429	0.005483	0.119571	3.41R
17	0.180000	0.062429	0.005483	0.117571	3.35R
66	0.143000	0.051125	0.007253	0.091875	2.64R
75	0.000000	0.014750	0.017766	-0.014750	-0.48 X
76	0.000000	0.014750	0.017766	-0.014750	-0.48 X
77	0.059000	0.014750	0.017766	0.044250	1.44 X
78	0.000000	0.014750	0.017766	-0.014750	-0.48 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.048000	0.051125	0.050344
	0.013575	0.042766	0.037422
2	42	4	46
	0.062429	0.014750	0.058283
	0.034025	0.029500	0.036018
All	50	28	78
	0.060120	0.045929	0.055026
	0.031993	0.042693	0.036571

Cell Contents --
Human:N
Mean
StDev

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Cat

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0048805	0.0005255	0.0005255	1.10	0.299
GDBA	1	0.0112023	0.0110563	0.0110563	23.04	0.000
Atopic*GDBA	1	0.0001045	0.0001045	0.0001045	0.22	0.642
Error	74	0.0355126	0.0355126	0.0004799		
Total	77	0.0516999				

Unusual Observations for Cat

Obs	Cat	Fit	StDev Fit	Residual	St Resid
31	0.088000	0.038286	0.003380	0.049714	2.30R
37	0.085000	0.038286	0.003380	0.046714	2.16R
38	0.141000	0.038286	0.003380	0.102714	4.75R
75	0.000000	-0.000000	0.010953	0.000000	0.00 X
76	0.000000	-0.000000	0.010953	0.000000	0.00 X
77	0.000000	-0.000000	0.010953	0.000000	0.00 X
78	0.000000	-0.000000	0.010953	0.000000	0.00 X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.042500	0.011000	0.018875
	0.017501	0.013863	0.020095
2	42	4	46
	0.038286	0.000000	0.034957
	0.026572	0.000000	0.027609
All	50	28	78
	0.038960	0.009429	0.028359
	0.025238	0.013382	0.025912

Cell Contents --
Cat:N
Mean
StDev

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Grass

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0096460	0.0002411	0.0002411	0.39	0.536
GDBA	1	0.0089787	0.0090116	0.0090116	14.45	0.000
Atopic*GDBA	1	0.0003118	0.0003118	0.0003118	0.50	0.481
Error	80	0.0498742	0.0498742	0.0006234		
Total	83	0.0688107				

Unusual Observations for Grass

Obs	Grass	Fit	StDev Fit	Residual	St Resid
16	0.141000	0.021940	0.003531	0.119060	4.82R
49	0.125000	0.021940	0.003531	0.103060	4.17R
51	0.019000	0.021286	0.009437	-0.002286	-0.10 X
52	0.009000	0.021286	0.009437	-0.012286	-0.53 X
53	0.047000	0.021286	0.009437	0.025714	1.11 X
54	0.017000	0.021286	0.009437	-0.004286	-0.19 X
55	0.023000	0.021286	0.009437	0.001714	0.07 X
56	0.008000	0.021286	0.009437	-0.013286	-0.57 X
57	0.026000	0.021286	0.009437	0.004714	0.20 X
73	0.114000	0.055857	0.005449	0.058143	2.39R
79	0.057000	0.045667	0.010193	0.011333	0.50 X
80	0.079000	0.045667	0.010193	0.033333	1.46 X
81	0.078000	0.045667	0.010193	0.032333	1.42 X
82	0.048000	0.045667	0.010193	0.002333	0.10 X
83	0.011000	0.045667	0.010193	-0.034667	-1.52 X
84	0.001000	0.045667	0.010193	-0.044667	-1.96 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.021286	0.055857	0.047214
	0.013149	0.022903	0.025678
2	50	6	56
	0.021940	0.045667	0.024482
	0.025896	0.033128	0.027423
All	57	27	84
	0.021860	0.053593	0.032060

0.024604 0.025163 0.028793

Cell Contents --
Grass:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Tree

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0015972	0.0010726	0.0010726	4.91	0.030
GDBA	1	0.0000056	0.0000071	0.0000071	0.03	0.857
Atopic*GDBA	1	0.0009222	0.0009222	0.0009222	4.22	0.043
Error	80	0.0174681	0.0174681	0.0002184		
Total	83	0.0199930				

Unusual Observations for Tree

Obs	Tree	Fit	StDev Fit	Residual	St Resid
49	0.051000	0.018840	0.002090	0.032160	2.20R
51	0.013000	0.019571	0.005585	-0.006571	-0.48 X
52	0.003000	0.019571	0.005585	-0.016571	-1.21 X
53	0.040000	0.019571	0.005585	0.020429	1.49 X
54	0.008000	0.019571	0.005585	-0.011571	-0.85 X
55	0.035000	0.019571	0.005585	0.015429	1.13 X
56	0.025000	0.019571	0.005585	0.005429	0.40 X
57	0.013000	0.019571	0.005585	-0.006571	-0.48 X
67	0.068000	0.029714	0.003225	0.038286	2.65R
77	0.069000	0.029714	0.003225	0.039286	2.72R
79	0.035000	0.010333	0.006033	0.024667	1.83 X
80	0.018000	0.010333	0.006033	0.007667	0.57 X
81	0.000000	0.010333	0.006033	-0.010333	-0.77 X
82	0.009000	0.010333	0.006033	-0.001333	-0.10 X
83	0.000000	0.010333	0.006033	-0.010333	-0.77 X
84	0.000000	0.010333	0.006033	-0.010333	-0.77 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.019571	0.029714	0.027179
	0.014022	0.018879	0.018103

2	50	6	56
	0.018840	0.010333	0.017929
	0.012913	0.014067	0.013176
All	57	27	84
	0.018930	0.025407	0.021012
	0.012924	0.019484	0.015520

Cell Contents --
Tree:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mugwort

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0019886	0.0023232	0.0023232	3.80	0.055
GDBA	1	0.0002494	0.0002391	0.0002391	0.39	0.534
Atopic*GDBA	1	0.0010576	0.0010576	0.0010576	1.73	0.192
Error	80	0.0489537	0.0489537	0.0006119		
Total	83	0.0522492				

Unusual Observations for Mugwort

Obs	Mugwort	Fit	StDev Fit	Residual	St Resid
5	0.100000	0.050900	0.003498	0.049100	2.01R
8	0.117000	0.050900	0.003498	0.066100	2.70R
38	0.126000	0.050900	0.003498	0.075100	3.07R
51	0.064000	0.055714	0.009350	0.008286	0.36 X
52	0.020000	0.055714	0.009350	-0.035714	-1.56 X
53	0.066000	0.055714	0.009350	0.010286	0.45 X
54	0.022000	0.055714	0.009350	-0.033714	-1.47 X
55	0.084000	0.055714	0.009350	0.028286	1.24 X
56	0.069000	0.055714	0.009350	0.013286	0.58 X
57	0.065000	0.055714	0.009350	0.009286	0.41 X
79	0.053000	0.036167	0.010099	0.016833	0.75 X
80	0.056000	0.036167	0.010099	0.019833	0.88 X
81	0.038000	0.036167	0.010099	0.001833	0.08 X
82	0.050000	0.036167	0.010099	0.013833	0.61 X
83	0.010000	0.036167	0.010099	-0.026167	-1.16 X
84	0.010000	0.036167	0.010099	-0.026167	-1.16 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.055714	0.060952	0.059643
	0.024662	0.019433	0.020500
2	50	6	56
	0.050900	0.036167	0.049321
	0.026920	0.021170	0.026599
All	57	27	84
	0.051491	0.055444	0.052762
	0.026492	0.022067	0.025090

Cell Contents --
Mugwort:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor	Levels	Values
Atopic	2	1 2
GDBA	2	1 2

Analysis of Variance for Olive

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.002672	0.006400	0.006400	1.18	0.281
GDBA	1	0.028230	0.027955	0.027955	5.14	0.026
Atopic*GDBA	1	0.006457	0.006457	0.006457	1.19	0.279
Error	80	0.435004	0.435004	0.005438		
Total	83	0.472363				

Unusual Observations for Olive

Obs	Olive	Fit	StDev Fit	Residual	St Resid
34	0.669000	0.082680	0.010428	0.586320	8.03R
51	0.083000	0.082571	0.027871	0.000429	0.01 X
52	0.053000	0.082571	0.027871	-0.029571	-0.43 X
53	0.077000	0.082571	0.027871	-0.005571	-0.08 X
54	0.063000	0.082571	0.027871	-0.019571	-0.29 X
55	0.065000	0.082571	0.027871	-0.017571	-0.26 X
56	0.130000	0.082571	0.027871	0.047429	0.69 X
57	0.107000	0.082571	0.027871	0.024429	0.36 X
79	0.020000	0.006667	0.030104	0.013333	0.20 X
80	0.007000	0.006667	0.030104	0.000333	0.00 X
81	0.013000	0.006667	0.030104	0.006333	0.09 X
82	0.000000	0.006667	0.030104	-0.006667	-0.10 X
83	0.000000	0.006667	0.030104	-0.006667	-0.10 X
84	0.000000	0.006667	0.030104	-0.006667	-0.10 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

```
MTB > table c1 c2;
SUBC> stats c6.
```

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.08257	0.05590	0.06257
	0.02721	0.03280	0.03316
2	50	6	56
	0.08268	0.00667	0.07454
	0.09133	0.00838	0.08944
All	57	27	84
	0.08267	0.04496	0.07055
	0.08589	0.03572	0.07544

Cell Contents --
Olive:N
Mean
StDev

```
MTB >
```

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Outdoor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.002786	0.001339	0.001339	0.31	0.578
GDBA	1	0.000389	0.000481	0.000481	0.11	0.739
Atopic*GDBA	1	0.000149	0.000149	0.000149	0.04	0.852
Error	35	0.148829	0.148829	0.004252		
Total	38	0.152154				

Unusual Observations for Outdoor

Obs	Outdoor	Fit	StDev Fit	Residual	St Resid
30	0.374000	0.078800	0.016837	0.295200	4.69R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.06650	0.07880	0.07621
	0.03797	0.09657	0.08672
2	11	9	20
	0.05773	0.06122	0.05930
	0.03437	0.01628	0.02714
All	15	24	39
	0.06007	0.07221	0.06754
	0.03419	0.07645	0.06328

Cell Contents --
Outdoor:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Indoor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.053713	0.036220	0.036220	4.86	0.034
GDBA	1	0.004153	0.001347	0.001347	0.18	0.673
Atopic*GDBA	1	0.013996	0.013996	0.013996	1.88	0.179
Error	35	0.261070	0.261070	0.007459		
Total	38	0.332932				

Unusual Observations for Indoor

Obs	Indoor	Fit	StDev Fit	Residual	St Resid
19	0.340000	0.155133	0.022300	0.184867	2.22R
29	0.493000	0.155133	0.022300	0.337867	4.05R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.12575	0.15513	0.14895
	0.05849	0.12088	0.10994
2	11	9	20
	0.09982	0.04400	0.07470
	0.05879	0.03822	0.05697
All	15	24	39
	0.10673	0.11346	0.11087
	0.05781	0.11146	0.09360

Cell Contents --
Indoor:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Foods 1

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0000077	0.0001150	0.0001150	0.19	0.662
GDBA	1	0.0000541	0.0001268	0.0001268	0.21	0.647
Atopic*GDBA	1	0.0003422	0.0003422	0.0003422	0.58	0.453
Error	35	0.0207507	0.0207507	0.0005929		
Total	38	0.0211547				

Unusual Observations for Foods 1

Obs	Foods 1	Fit	StDev Fit	Residual	St Resid
9	0.086000	0.028273	0.007342	0.057727	2.49R
32	0.119000	0.025667	0.008116	0.093333	4.07R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.017750	0.028467	0.026211
	0.010532	0.014851	0.014497
2	11	9	20
	0.028273	0.025667	0.027100
	0.022383	0.039243	0.030231
All	15	24	39
	0.025467	0.027417	0.026667
	0.020121	0.025919	0.023595

Cell Contents --
Foods 1:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Foods 2

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0033652	0.0035123	0.0035123	5.36	0.027
GDBA	1	0.0010705	0.0008807	0.0008807	1.34	0.254
Atopic*GDBA	1	0.0001020	0.0001020	0.0001020	0.16	0.696
Error	35	0.0229314	0.0229314	0.0006552		
Total	38	0.0274691				

Unusual Observations for Foods 2

Obs	Foods 2	Fit	StDev Fit	Residual	St Resid
6	0.145000	0.039455	0.007718	0.105545	4.32R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.021750	0.028800	0.027316
	0.022765	0.012891	0.014978
2	11	9	20
	0.039455	0.053778	0.045900
	0.037843	0.024314	0.032498
All	15	24	39
	0.034733	0.038167	0.036846
	0.034636	0.021433	0.026886

Cell Contents --
Foods 2:N
Mean
StDev

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Moulds

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.087561	0.103940	0.103940	41.54	0.000
GDBA	1	0.125457	0.092775	0.092775	37.08	0.000
Atopic*GDBA	1	0.034154	0.034154	0.034154	13.65	0.001
Error	35	0.087582	0.087582	0.002502		
Total	38	0.334755				

Unusual Observations for Moulds

Obs	Moulds	Fit	StDev Fit	Residual	St Resid
36	0.096000	0.264778	0.016674	-0.168778	-3.58R
39	0.367000	0.264778	0.016674	0.102222	2.17R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.03900	0.08213	0.07305
	0.03601	0.04615	0.04689
2	11	9	20
	0.08855	0.26478	0.16785
	0.03451	0.07243	0.10453
All	15	24	39
	0.07533	0.15063	0.12167
	0.04053	0.10621	0.09386

Cell Contents --
Moulds:N
Mean
StDev

Appendix J3

Statisitical analysis of Immunodot results for atopic and non-atopic dogs (non-GDBA/non-atopic =greyhounds & beagles).

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Dustmite

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.24046	0.08175	0.08175	5.05	0.028
GDBA	1	0.04190	0.04648	0.04648	2.87	0.094
Atopic*GDBA	1	0.03928	0.03928	0.03928	2.42	0.124
Error	78	1.26380	1.26380	0.01620		
Total	81	1.58545				

Unusual Observations for Dustmite

Obs	Dustmite	Fit	StDev Fit	Residual	St Resid
52	0.562000	0.167583	0.025983	0.394417	3.17R
54	0.609000	0.167583	0.025983	0.441417	3.54R
70	0.740000	0.167583	0.025983	0.572417	4.59R
73	0.575000	0.167583	0.025983	0.407417	3.27R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.05137	0.16758	0.13853
	0.08541	0.22446	0.20406
2	42	8	50
	0.02674	0.03162	0.02752
	0.03091	0.04593	0.03323
All	50	32	82
	0.03068	0.13359	0.07084
	0.04387	0.20355	0.13991

Cell Contents --
Dustmite:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Storemit

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.012292	0.003588	0.003588	0.81	0.370
GDBA	1	0.003460	0.004084	0.004084	0.92	0.339
Atopic*GDBA	1	0.008361	0.008361	0.008361	1.89	0.173
Error	78	0.344368	0.344368	0.004415		
Total	81	0.368480				

Unusual Observations for Storemit

Obs	Storemit	Fit	StDev Fit	Residual	St Resid
52	0.261000	0.081125	0.013563	0.179875	2.77R
54	0.282000	0.081125	0.013563	0.200875	3.09R
70	0.505000	0.081125	0.013563	0.423875	6.52R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.03750	0.08113	0.07022
	0.01030	0.11578	0.10167
2	42	8	50
	0.04636	0.03863	0.04512
	0.02342	0.04283	0.02700
All	50	32	82
	0.04494	0.07050	0.05491
	0.02202	0.10348	0.06745

Cell Contents --
Storemit:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Flea

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0001652	0.0003032	0.0003032	1.03	0.312
GDBA	1	0.0000791	0.0000500	0.0000500	0.17	0.681
Atopic*GDBA	1	0.0010236	0.0010236	0.0010236	3.49	0.065
Error	78	0.0228585	0.0228585	0.0002931		
Total	81	0.0241265				

Unusual Observations for Flea

Obs	Flea	Fit	StDev Fit	Residual	St Resid
17	0.095000	0.016095	0.002642	0.078905	4.67R
65	0.074000	0.019000	0.003494	0.055000	3.28R
66	0.068000	0.019000	0.003494	0.049000	2.92R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.012000	0.019000	0.017250
	0.011136	0.021657	0.019634
2	42	8	50
	0.016095	0.005125	0.014340
	0.016291	0.006770	0.015657
All	50	32	82
	0.015440	0.015531	0.015476
	0.015559	0.019890	0.017259

Cell Contents --
Flea:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Human

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.001254	0.000000	0.000000	0.00	0.988
GDBA	1	0.001819	0.001577	0.001577	1.26	0.265
Atopic*GDBA	1	0.002584	0.002584	0.002584	2.07	0.154
Error	78	0.097437	0.097437	0.001249		
Total	81	0.103095				

Unusual Observations for Human

Obs	Human	Fit	StDev Fit	Residual	St Resid
8	0.182000	0.062429	0.005454	0.119571	3.42R
17	0.180000	0.062429	0.005454	0.117571	3.37R
66	0.143000	0.051125	0.007215	0.091875	2.66R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.048000	0.051125	0.050344
	0.013575	0.042766	0.037422
2	42	8	50
	0.062429	0.037000	0.058360
	0.034025	0.030743	0.034531
All	50	32	82
	0.060120	0.047594	0.055232
	0.031993	0.040112	0.035676

Cell Contents --
Human:N
Mean
StDev

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Cat

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0045706	0.0000213	0.0000213	0.04	0.836
GDBA	1	0.0102704	0.0103569	0.0103569	21.01	0.000
Atopic*GDBA	1	0.0001081	0.0001081	0.0001081	0.22	0.641
Error	78	0.0384504	0.0384504	0.0004930		
Total	81	0.0533995				

Unusual Observations for Cat

Obs	Cat	Fit	StDev Fit	Residual	St Resid
31	0.088000	0.038286	0.003426	0.049714	2.27R
37	0.085000	0.038286	0.003426	0.046714	2.13R
38	0.141000	0.038286	0.003426	0.102714	4.68R
76	0.060000	0.012625	0.007850	0.047375	2.28R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	8	24	32
	0.042500	0.011000	0.018875
	0.017501	0.013863	0.020095
2	42	8	50
	0.038286	0.012625	0.034180
	0.026572	0.020486	0.027222
All	50	32	82
	0.038960	0.011406	0.028207
	0.025238	0.015423	0.025676

Cell Contents --
Cat:N
Mean
StDev

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic .2 1 2
GDBA 2 1 2

Analysis of Variance for Grass

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0087818	0.0003419	0.0003419	0.58	0.450
GDBA	1	0.0102335	0.0106606	0.0106606	17.94	0.000
Atopic*GDBA	1	0.0004343	0.0004343	0.0004343	0.73	0.395
Error	84	0.0499037	0.0499037	0.0005941		
Total	87	0.0693533				

Unusual Observations for Grass

Obs	Grass	Fit	StDev Fit	Residual	St Resid
16	0.141000	0.021940	0.003447	0.119060	4.93R
49	0.125000	0.021940	0.003447	0.103060	4.27R
51	0.019000	0.021286	0.009213	-0.002286	-0.10 X
52	0.009000	0.021286	0.009213	-0.012286	-0.54 X
53	0.047000	0.021286	0.009213	0.025714	1.14 X
54	0.017000	0.021286	0.009213	-0.004286	-0.19 X
55	0.023000	0.021286	0.009213	0.001714	0.08 X
56	0.008000	0.021286	0.009213	-0.013286	-0.59 X
57	0.026000	0.021286	0.009213	0.004714	0.21 X
73	0.114000	0.055857	0.005319	0.058143	2.44R

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.021286	0.055857	0.047214
	0.013149	0.022903	0.025678
2	50	10	60
	0.021940	0.044900	0.025767
	0.025896	0.024759	0.026924
All	57	31	88
	0.021860	0.052323	0.032591
	0.024604	0.023679	0.028234

Cell Contents --
Grass:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Tree

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0010535	0.0001217	0.0001217	0.48	0.492
GDBA	1	0.0007179	0.0007841	0.0007841	3.08	0.083
Atopic*GDBA	1	0.0000706	0.0000706	0.0000706	0.28	0.600
Error	84	0.0214068	0.0214068	0.0002548		
Total	87	0.0232489				

Unusual Observations for Tree

Obs	Tree	Fit	StDev Fit	Residual	St Resid
49	0.051000	0.018840	0.002258	0.032160	2.04R
51	0.013000	0.019571	0.006034	-0.006571	-0.44 X
52	0.003000	0.019571	0.006034	-0.016571	-1.12 X
53	0.040000	0.019571	0.006034	0.020429	1.38 X
54	0.008000	0.019571	0.006034	-0.011571	-0.78 X
55	0.035000	0.019571	0.006034	0.015429	1.04 X
56	0.025000	0.019571	0.006034	0.005429	0.37 X
57	0.013000	0.019571	0.006034	-0.006571	-0.44 X
67	0.068000	0.029714	0.003484	0.038286	2.46R
77	0.069000	0.029714	0.003484	0.039286	2.52R
79	0.060000	0.024300	0.005048	0.035700	2.36R
80	0.059000	0.024300	0.005048	0.034700	2.29R

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.019571	0.029714	0.027179
	0.014022	0.018879	0.018103
2	50	10	60
	0.018840	0.024300	0.019750
	0.012913	0.023400	0.015041
All	57	31	88
	0.018930	0.027968	0.022114
	0.012924	0.020211	0.016347

Cell Contents --
Tree:N

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Mugwort

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0017325	0.0012331	0.0012331	2.05	0.156
GDBA	1	0.0000100	0.0000009	0.0000009	0.00	0.969
Atopic*GDBA	1	0.0003181	0.0003181	0.0003181	0.53	0.469
Error	84	0.0506005	0.0506005	0.0006024		
Total	87	0.0526611				

Unusual Observations for Mugwort

Obs	Mugwort	Fit	StDev Fit	Residual	St Resid
5	0.100000	0.050900	0.003471	0.049100	2.02R
8	0.117000	0.050900	0.003471	0.066100	2.72R
38	0.126000	0.050900	0.003471	0.075100	3.09R
51	0.064000	0.055714	0.009277	0.008286	0.36 X
52	0.020000	0.055714	0.009277	-0.035714	-1.57 X
53	0.066000	0.055714	0.009277	0.010286	0.45 X
54	0.022000	0.055714	0.009277	-0.033714	-1.48 X
55	0.084000	0.055714	0.009277	0.028286	1.24 X
56	0.069000	0.055714	0.009277	0.013286	0.58 X
57	0.065000	0.055714	0.009277	0.009286	0.41 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.055714	0.060952	0.059643
	0.024662	0.019433	0.020500
2	50	10	60
	0.050900	0.046200	0.050117
	0.026920	0.020784	0.025901
All	57	31	88
	0.051491	0.056194	0.053148
	0.026492	0.020748	0.024603

Cell Contents --
Mugwort:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Olive

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.002626	0.001763	0.001763	0.33	0.566
GDBA	1	0.023005	0.019070	0.019070	3.59	0.061
Atopic*GDBA	1	0.001796	0.001796	0.001796	0.34	0.562
Error	84	0.445863	0.445863	0.005308		
Total	87	0.473290				

Unusual Observations for Olive

Obs	Olive	Fit	StDev Fit	Residual	St Resid
34	0.669000	0.082680	0.010303	0.586320	8.13R
51	0.083000	0.082571	0.027537	0.000429	0.01 X
52	0.053000	0.082571	0.027537	-0.029571	-0.44 X
53	0.077000	0.082571	0.027537	-0.005571	-0.08 X
54	0.063000	0.082571	0.027537	-0.019571	-0.29 X
55	0.065000	0.082571	0.027537	-0.017571	-0.26 X
56	0.130000	0.082571	0.027537	0.047429	0.70 X
57	0.107000	0.082571	0.027537	0.024429	0.36 X

R denotes an observation with a large standardized residual
X denotes an observation whose X value gives it large influence.

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	7	21	28
	0.08257	0.05590	0.06257
	0.02721	0.03280	0.03316
2	50	10	60
	0.08268	0.03240	0.07430
	0.09133	0.03529	0.08645
All	57	31	88
	0.08267	0.04832	0.07057
	0.08589	0.03487	0.07376

Cell Contents --
Olive:N
Mean
StDev

MTB > glm c3=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Outdoor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.002801	0.001359	0.001359	0.33	0.568
GDBA	1	0.000414	0.000521	0.000521	0.13	0.723
Atopic*GDBA	1	0.000144	0.000144	0.000144	0.04	0.852
Error	37	0.151146	0.151146	0.004085		
Total	40	0.154505				

Unusual Observations for Outdoor

Obs	Outdoor	Fit	StDev Fit	Residual	St Resid
30	0.374000	0.078800	0.016503	0.295200	4.78R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c3.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.06650	0.07880	0.07621
	0.03797	0.09657	0.08672
2	11	11	22
	0.05773	0.06155	0.05964
	0.03437	0.02106	0.02789
All	15	26	41
	0.06007	0.07150	0.06732
	0.03419	0.07400	0.06215

Cell Contents --
Outdoor:N
Mean
StDev

MTB > glm c4=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Indoor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.055475	0.034174	0.034174	4.80	0.035
GDBA	1	0.003668	0.000794	0.000794	0.11	0.740
Atopic*GDBA	1	0.012411	0.012411	0.012411	1.74	0.195
Error	37	0.263479	0.263479	0.007121		
Total	40	0.335034				

Unusual Observations for Indoor

Obs	Indoor	Fit	StDev Fit	Residual	St Resid
19	0.340000	0.155133	0.021788	0.184867	2.27R
29	0.493000	0.155133	0.021788	0.337867	4.14R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c4.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.12575	0.15513	0.14895
	0.05849	0.12088	0.10994
2	11	11	22
	0.09982	0.05055	0.07518
	0.05879	0.03754	0.05434
All	15	26	41
	0.10673	0.11088	0.10937
	0.05781	0.10735	0.09152

Cell Contents --
Indoor:N
Mean
StDev

MTB > glm c5=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Foods 1

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0000027	0.0001051	0.0001051	0.19	0.668
GDBA	1	0.0000328	0.0001167	0.0001167	0.21	0.651
Atopic*GDBA	1	0.0003825	0.0003825	0.0003825	0.68	0.414
Error	37	0.0207703	0.0207703	0.0005614		
Total	40	0.0211882				

Unusual Observations for Foods 1

Obs	Foods 1	Fit	StDev Fit	Residual	St Resid
9	0.086000	0.028273	0.007144	0.057727	2.56R
34	0.119000	0.025182	0.007144	0.093818	4.15R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c5.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.017750	0.028467	0.026211
	0.010532	0.014851	0.014497
2	11	11	22
	0.028273	0.025182	0.026727
	0.022383	0.035128	0.028787
All	15	26	41
	0.025467	0.027077	0.026488
	0.020121	0.024896	0.023015

Cell Contents --
Foods 1:N
Mean
StDev

MTB > glm c6=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Foods 2

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.0028379	0.0028135	0.0028135	4.29	0.045
GDBA	1	0.0006031	0.0005226	0.0005226	0.80	0.378
Atopic*GDBA	1	0.0000084	0.0000084	0.0000084	0.01	0.911
Error	37	0.0242886	0.0242886	0.0006564		
Total	40	0.0277380				

Unusual Observations for Foods 2

Obs	Foods 2	Fit	StDev Fit	Residual	St Resid
6	0.145000	0.039455	0.007725	0.105545	4.32R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c6.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.021750	0.028800	0.027316
	0.022765	0.012891	0.014978
2	11	11	22
	0.039455	0.048545	0.044000
	0.037843	0.024671	0.031519
All	15	26	41
	0.034733	0.037154	0.036268
	0.034636	0.020869	0.026333

Cell Contents --
Foods 2:N
Mean
StDev

MTB > glm c7=c1 c2 c1*c2

General Linear Model

Factor Levels Values
Atopic 2 1 2
GDBA 2 1 2

Analysis of Variance for Moulds

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Atopic	1	0.080660	0.082560	0.082560	23.07	0.000
GDBA	1	0.102973	0.072452	0.072452	20.25	0.000
Atopic*GDBA	1	0.021604	0.021604	0.021604	6.04	0.019
Error	37	0.132389	0.132389	0.003578		
Total	40	0.337627				

Unusual Observations for Moulds

Obs	Moulds	Fit	StDev Fit	Residual	St Resid
31	0.070000	0.235455	0.018036	-0.165455	-2.90R
38	0.096000	0.235455	0.018036	-0.139455	-2.45R
41	0.367000	0.235455	0.018036	0.131545	2.31R

R denotes an observation with a large standardized residual

MTB > table c1 c2;
SUBC> stats c7.

Tabulated Statistics

Rows: Atopic Columns: GDBA

	1	2	All
1	4	15	19
	0.03900	0.08213	0.07305
	0.03601	0.04615	0.04689
2	11	11	22
	0.08855	0.23545	0.16200
	0.03451	0.09315	0.10174
All	15	26	41
	0.07533	0.14700	0.12078
	0.04053	0.10311	0.09187

Cell Contents --
Moulds:N
Mean
StDev

Appendix K

Correlation of positive ELISA results for mould allergens with serum total IgE concentrations as assessed by Immunodot.

Correlations (Pearson)

Correlation of Alternaria and IgE = -0.031

Correlations (Pearson)

Correlation of Aspergillus and IgE = -0.284

MTB > print c1-c3

Data Display

Row	Alternaria	Aspergillus	IgE
1	0.095	0.078	0.293
2	0.090	0.062	0.250
3	0.089	0.050	0.060
4	0.117	0.183	0.012
5	0.091	0.143	0.183
6	0.070	0.045	*
7	0.044	0.077	*
8	0.060	0.079	0.247
9	0.228	0.135	*
10	0.353	0.186	0.142
11	0.104	0.146	0.112
12	0.131	0.132	0.191
13	0.041	0.106	0.106
14	0.164	0.166	0.467
15	0.077	0.033	0.425
16	0.144	0.095	*

MTB >

Appendix L

Intradermal skin test and serological results for atopic and skin test negative dogs.

Appendix L1 Intradermal & serological results of confirmed GUVS & GDBA atopic dogs

(No results were avaliable for dogs indicated by '*')

i. Atopic GUVS dogs

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Ben	*D. farinae* *D. pteronyssinus* *A. siro* Plantain Mugwort Dandelion Nettle	Fescue Mugwort	*	*	Negative
Brandy	*D. farinae* *D. pteronyssinus* *A. siro* Birch Plantain Mugwort Dandelion	*Alternaria* *Aspergillus* Kapok Timothy Kentucky	Negative	Negative	Indoor
Budd	*D. farinae* *D. pteronyssinus* *A. siro*	Flea House dust mites Feathers *Alternaria* *Aspergillus* *Rhizopus* Kapok *Mucor*	House dust mites Storage mites	Negative	Indoor

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Budd (cont.)		Orchard Timothy Kentucky Fescue Poplar Birch Sorrel Plantain Mugwort Dandelion Nettle			
Fudge	*D. farinae* *D. pteronyssinus* *A. siro*	*Alternaria*	House dust mites	Negative	Indoor
Islay	*D. farinae* *D. pteronyssinus* *A. siro* Cat epithelium	Negative	Negative	Negative	Negative
Jock	*D. farinae* *D. pteronyssinus* *A. siro* Timothy Fescue Plantain	Negative	Negative	Negative	Indoor

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Kara	*D. farinae* *D. pteronyssinus* *A. siro*	Timothy Kentucky Dandelion	*	*	*
Kayla	*D. farinae* *D. pteronyssinus* *A. siro*	*Alternaria* Orchard Fescue Poplar Birch Plantain	Negative	Negative	*
Kerry	*D. farinae* *D. pteronyssinus* *A. siro*	Negative	*	*	*
Kerry II	*D. farinae* *D. pteronyssinus* *A. siro*	Flea House dust mites Feathers *Rhizopus* Kapok Orchard Timothy Kentucky Fescue Poplar Birch Sorrel	Negative	Negative	Negative

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Kerry II (cont.)		Plantain Mugwort Dandelion Nettle			
Kim	*D. farinae* *D. pteronyssinus*	Negative	Negative	Negative	*
Kyle	*D. farinae* *D. pteronyssinus*	House dust mites Timothy Kentucky	Negative	Negative	Negative
Lucy	*D. farinae* *D. pteronyssinus*	House dust mites Orchard Timothy Kentucky Fescue Poplar Sorrel Plantain Mugwort Dandelion Nettle	House dust mites	Negative	*

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Lucy II	*D. farinae* *D. pteronyssinus* *A. Siro* *E. maynei* *T.putrescentiaea*	House dust mites Orchard Timothy Kentucky Fescue Poplar Birch Sorrel Plantain Mugwort Dandelion Nettle	Negative	Negative	Negative
Luke	*D. farinae* *D. pteronyssinus* *A. siro*	*Alternaria* *Aspergillus*	Negative	Negative	Negative
Macauly	*D. farinae* *D. pteronyssinus* *A. siro*	House dust mites *Alternaria* *Aspergillus*	House dust mites	Negative	Indoor
Maisie	Ash	House dust mites	Negative	Negative	Negative
Molly	*D. farinae* *D. pteronyssinus* *A. siro*	Negative	House dust mites Storage mites	*	*

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Olwen	*D. farinae* *D. pteronyssinus* *A. siro*	Feathers *Alternaria*	House dust mites	Negative	Negative
Rhuri	Timothy Fescue	Flea House dust mites Feathers *Rhizopus* Orchard Timothy Kentucky Fescue Birch Mugwort Dandelion	Negative	Negative	*
Sally	*D. farinae* *D. pteronyssinus* *A. siro*	Orchard	Negative	Negative	*

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Sammie	Flea *D. farinae* *D. pteronyssinus* *A. siro* Fescue Mugwort	House dust mites Timothy Kentucky Birch	*	*	Indoor
Shane	*A. siro* Fescue	Aspergillus	Negative	Negative	Negative
Sheena	*D. farinae* *D. pteronyssinus* *A. siro* Cat epithelium Fescue Poplar	Negative	House dust mites Storage mites	Wall pellitory	Indoor
Shogun	*D. farinae* *D. pteronyssinus*	Timothy Fescue	*	*	*
Shona	*D. farinae* *D. pteronyssinus* *A. siro*	House dust mites	House dust mites Storage mites	*	Indoor

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Skerry	*D. farinae* *D. pteronyssinus* *A. siro*	Negative	House dust mites	*	Indoor Outdoor
Tanya	*D. farinae* *D. pteronyssinus* *A. siro*	Flea	Negative	Negative	*
Teena	Poplar	House dust mites Timothy Birch	*	*	*
Tia	*D. farinae* *D. pteronyssinus* *A. siro*	House dust mites Aspergillus Orchard	House dust mites	*	Indoor
Toby	*D. farinae* *D. pteronyssinus* *A. siro*	Orchard Timothy Fescue Sorrel	House dust mites	Negative	*
Tyson	*D. farinae* *D. pteronyssinus* *A. siro* Timothy Dandelion	*Aspergillus*	House dust mites	Negative	*

ii. Atopic GDBA dogs

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Alana	*D. farinae* *D. pteronyssinus*	*Mucor*	Negative	Negative	Negative
Andrea	Flea *D. farinae* *D. pteronyssinus* Feathers	Sorrel	Negative	Negative	Negative
Carlo	*Phoma betae*	*Alternaria*	Negative	Negative	*
Cedar	*D. farinae* *D. pteronyssinus* *T. putrescentiaea*	House dust mites *Aspergillus* *Mucor* Kentucky	House dust mites	Negative	Indoor
Chris	*Alternaria* Cat epithelium Orchard Poplar	Flea House dust mites Kapok Dust Mucor Orchard Timothy Kentucky Fescue	Negative	Negative	Negative

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Chris (cont.)		Poplar Birch Plantain Mugwort Dandelion Nettle			
Claire	*D. farinae*	Negative	*	*	*
Dusty	*D. farinae* *D. pteronyssinus* *A. siro*	House dustmites Timothy Fescue Poplar Plantain	*	*	*
Griff	*D. farinae* *D. pteronyssinus* *A. siro*	Poplar Birch Plantain Mugwort Dandelion	House dust mites Storage mites	Negative	*

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Kai	*D. farinae* *D. pteronyssinus* Human epithelium Cat epithelium Timothy Ryegrass	House dust mites *Alternaria* *Aspergillus* *Rhizopus* Kapok *Mucor* Poplar Birch Mugwort Dandelion Nettle	Negative	Negative	*
Opal	*D. farinae* *D. pteronyssinus*	House dust mites	House dust mites	Negative	*
Palmer	*D. farinae* *D. pteronyssinus* *A. siro*	Negative	*	*	Indoor
Paul	Flea *D. farinae* *D. pteronyssinus* Human epithelium Fescue Sorrel Nettle	Kentucky	*	*	Negative

Name	IDST	ELISA	Immbrown	Immgreen	Immred
Pedro	*D. farinae* *A. siro*	*Alternaria* *Aspergillus* Mucor Orchard Timothy Plantain	*	*	Negative
Reo	*D. farinae* *D. pteronyssinus* *A. siro*	Negative	House dust mites	Negative	Indoor

Appendix L2 Serological results of suspected atopic dogs with negative IDST results.
(No results were avaliable for dogs indicated by '*')

Name	ELISA	Immbrown	Immgreen	Immred
Barry	*	-ve	-ve	*
Buster	Orchard Kentucky Fescue Poplar Plantain	-ve	-ve	*
Kyle	Timothy Birch	*	*	*
Oscar	*	-ve	-ve	*
Oscar II	Feathers Dandelion	-ve	-ve	*
Riley	Flea Mites	-ve	-ve	*
Skye	*	-ve	-ve	*
Winston	Mites *Alternaria* *Aspegillus* Mucor	*	*	-ve
Zac	Mites Orchard Timothy Kentucky Poplar	*	*	Indoor Moulds

Appendix M

Sensitivity, specificity, efficacy and correlation of serological results compared with IDST results

Appendix M1

Sensitivity, specificity and efficacy of serological tests in relation to each other and IDST

HOUSE DUST MITES

	IDST +ve	IDST -ve
ELISA +ve	13	7
ELISA -ve	27	5

Sensitivity = 32.5%
Specificity = 41.7%
Efficacy = 34.6%

	IDST +ve	IDST -ve
Immdot +ve	16	0
Immdot -ve	14	11

Sensitivity = 53.3%
Specificity = 100%
Efficacy = 65.9%

	ELISA +ve	ELISA -ve
Immdot +ve	7	8
Immdot -ve	8	14

Sensitivity = 46.7%
Specificity = 63.6%
Efficacy = 56.8%

	Topscreen +ve	Topscreen -ve
Immdot +ve	9	1
Immdot -ve	2	8

Sensitivity = 81.8%
Specificity = 88.9%
Efficacy = 77.3%

STORAGE MITE

	IDST +ve	IDST -ve
Immdot +ve	5	0
Immdot -ve	19	17

Sensitivity = 20.8%
Specificity = 100%
Efficacy = 53.7%

	Topscreen +ve	Topscreen -ve
Immdot +ve	3	0
Immdot -ve	8	11

Sensitivity = 27.3%
Specificity = 100%
Efficacy = 63.6%

CAT FLEA

	IDST +ve	IDST -ve
ELISA +ve	0	6
ELISA -ve	3	43

Sensitivity = 0%
Specificity = 87.8%
Efficacy = 82.7%

	IDST +ve	IDST -ve
Immdot +ve	0	0
Immdot -ve	1	40

Sensitivity = 0%
Specificity = 100%
Efficacy = 97.6%

	ELISA +ve	ELISA -ve
Immdot +ve	0	0
Immdot -ve	6	32

Sensitivity = 0%
Specificity = 100%
Efficacy = 84.2%

	Topscreen +ve	Topscreen -ve
Immdot +ve	0	0
Immdot -ve	11	11

Sensitivity = 0%
Specificity = 100%
Efficacy = 50%

CAT EPITHELIA

	IDST +ve	IDST -ve
ELISA +ve	0	0
ELISA -ve	4	48

Sensitivity = 0%
Specificity = 100%
Efficacy = 92.3%

	IDST +ve	IDST -ve
Immdot +ve	0	0
Immdot -ve	4	37

Sensitivity = 0%
Specificity = 100%
Efficacy = 90.2%

	ELISA +ve	ELISA -ve
Immdot +ve	0	0
Immdot -ve	0	38

Sensitivity = 0%
Specificity = 100%
Efficacy = 100%

	Topscreen +ve	Topscreen -ve
Immdot +ve	0	0
Immdot -ve	11	11

Sensitivity = 0%
Specificity = 100%
Efficacy = 50%

HUMAN EPITHELIA

	IDST +ve	IDST -ve
ELISA +ve	0	0
ELISA -ve	2	50

Sensitivity = 0%
Specificity = 100%
Efficacy = 96.2%

	IDST +ve	IDST -ve
Immdot +ve	0	0
Immdot -ve	1	40

Sensitivity = 0%
Specificity = 100%
Efficacy = 97.6%

	ELISA +ve	ELISA -ve
Immdot +ve	0	0
Immdot -ve	0	38

Sensitivity = 0%
Specificity = 100%
Efficacy = 100%

	Topscreen +ve	Topscreen -ve
Immdot +ve	0	0
Immdot -ve	11	11

Sensitivity = 0%
Specificity = 100%
Efficacy =50%

TREES

	IDST +ve	IDST –ve
ELISA +ve	2	12
ELISA -ve	3	35

Sensitivity = 40%
Specificity = 74.5%
Efficacy = 71.2%

	IDST +ve	IDST -ve
Immdot +ve	0	0
Immdot -ve	3	34

Sensitivity = 0%
Specificity = 100%
Efficacy = 91.9%

	ELISA +ve	ELISA -ve
Immdot +ve	0	0
Immdot -ve	10	24

Sensitivity = 0%
Specificity = 100%
Efficacy = 70.6%

	Topscreen +ve	Topscreen -ve
Immdot +ve	0	0
Immdot -ve	1	18

Sensitivity = 0%
Specificity = 100%
Efficacy = 94.7%

GRASSES

	IDST +ve	IDST -ve
ELISA +ve	3	21
ELISA -ve	5	23

Sensitivity = 37.5%
Specificity = 52.3%
Efficacy = 59.6%

	IDST +ve	IDST -ve
Immdot +ve	0	0
Immdot -ve	9	28

Sensitivity = 0%
Specificity = 100%
Efficacy = 75.7%

	ELISA +ve	ELISA -ve
Immdot +ve	0	0
Immdot -ve	13	21

Sensitivity = 0%
Specificity = 100%
Efficacy = 61.8%

	Topscreen +ve	Topscreen -ve
Immdot +ve	0	0
Immdot -ve	0	19

Sensitivity = 0%
Specificity = 100%
Efficacy = 100%

MUGWORT

	IDST +ve	IDST -ve
ELISA +ve	1	8
ELISA -ve	2	41

Sensitivity = 33.3%
Specificity = 83.7%
Efficacy = 80.8%

	IDST +ve	IDST -ve
Immdot +ve	0	0
Immdot -ve	1	36

Sensitivity = 0%
Specificity = 100%
Efficacy = 97.3%

	ELISA +ve	ELISA -ve
Immdot +ve	0	0
Immdot -ve	8	26

Sensitivity = 0%
Specificity = 100%
Efficacy = 76.5%

	Topscreen +ve	Topscreen -ve
Immdot +ve	0	0
Immdot -ve	0	19

Sensitivity = 0%
Specificity = 100%
Efficacy = 100%

SORREL

	IDST +ve	IDST -ve
ELISA +ve	0	6
ELISA -ve	1	45

Sensitivity = 0%
Specificity = 88.2%
Efficacy = 86.5%

PLANTAIN

	IDST +ve	IDST -ve
ELISA +ve	0	10
ELISA -ve	3	39

Sensitivity = 0%
Specificity = 79.6%
Efficacy = 75%

DANDELION

	IDST +ve	IDST -ve
ELISA +ve	0	10
ELISA -ve	3	39

Sensitivity = 0%
Specificity = 79.6%
Efficacy = 75%

NETTLE

	IDST +ve	IDST -ve
ELISA +ve	0	6
ELISA -ve	2	44

Sensitivity = 0%
Specificity = 88%
Efficacy =84.6%

MOULDS

	IDST +ve	IDST -ve
ELISA +ve	0	18
ELISA -ve	0	34

Sensitivity = 0%
Specificity = 65.4%
Efficacy = 65.4%

	IDST +ve	IDST -ve
Immdot +ve	0	1
Immdot -ve	0	28

Sensitivity = 0%
Specificity = 96.6%
Efficacy = 96.6%

	ELISA +ve	ELISA -ve
Immdot +ve	0	1
Immdot -ve	13	15

Sensitivity = 0%
Specificity = 93.8%
Efficacy = 51.7%

Appendix M2

Correlation of serological results with IDST results

Correlations (Pearson)

Correlation of House dust mites - IDST and House dust mites - ELISA = -0.171

Correlations (Pearson)

Correlation of House dust mites - IDST and Immunodot Indoor - HDM = 0.454

Correlations (Pearson)

Correlation of House dust mites - ELISA and Immunodot Indoor - HDM = 0.075

Correlations (Pearson)

Correlation of Storage mites - IDST and Storage mites - Immunodot = 0.330

Correlations (Pearson)

Correlation of cat flea IDST and Cat flea ELISA = -0 089

Correlation 'cat flea IDST' 'Cat flea Immunodot'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

Correlation 'Cat flea ELISA' 'Cat flea Immunodot'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

Correlations (Pearson)

Correlation of Trees - IDST and Trees - ELISA = 0.135

Correlation 'Trees - IDST' 'Trees - Immunodot'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

Correlation 'Trees - ELISA' 'Trees - Immunodot'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

Correlations (Pearson)

Correlation of Grasses - IDST and grasses - ELISA = 0.033

Correlation 'Grasses - IDST' 'Grass - Immunodot'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

Correlation 'grasses - ELISA' 'Grass - Immunodot'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

Correlations (Pearson)

Correlation of Mugwort - IDST and Mugwort - ELISA = 0.105

Correlation 'Mugwort - IDST' 'Mugwort - Immdot'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

Correlation 'Mugwort - ELISA' 'Mugwort - Immdot'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

Correlations (Pearson)

Correlation of Sorrel - IDST and Sorrel - ELISA = -0.051

Correlations (Pearson)

Correlation of Plantain - IDST and Plantain - ELISA = -0.121

Correlations (Pearson)

Correlation of Dandelion - IDST and Dandelion - ELISA = -0.121

Correlations (Pearson)

Correlation of nettle - IDST and nettle - ELISA = -0.072

Correlations (Pearson)

Correlation of mould - IDST and mould - ELISA = -0.102

Correlations (Pearson)

Correlation of mould - IDST and Immunodot - moulds = -0.036

Correlations (Pearson)

Correlation of mould - ELISA and Immunodot - moulds = -0.170

Appendix M3

Correlation of Topscreen Immunodot with individual Indoor and Outdoor Immunodot panels

Worksheet size: 100000 cells

Correlations (Pearson)

Correlation of Indoor and Topscreen Indoor = 0.730

Correlation 'Outdoor' 'Topscreen Outdoor'.

* NOTE * All values in column are identical.

* ERROR * Completion of computation impossible.

* ERROR * Unknown MINITAB command:

MTB > print c1-c4

Data Display

Row	Indoor	Outdoor	Topscreen Indoor	Topscreen Outdoor
1	0	0	0	0
2	1	1	1	0
3	0	0	1	0
4	1	0	1	0
5	1	0	1	0
6	0	0	0	0
7	0	0	1	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	1	0	1	0
12	0	0	0	0
13	1	0	0	0
14	0	0	0	0
15	1	*	1	*
16	1	*	1	*
17	1	*	1	*
18	0	0	0	0
19	0	0	0	0
20	1	0	1	0
21	0	0	0	0
22	1	0	1	0

MTB >

Appendix N

Serum total IgG_1 concentrations in atopic and non-atopic dogs

&

statistical evaluation of this data.

Appendix N1 Serum total IgG_1 concentrations in non-atopic GDBA dogs with and without skin disease at the time of sampling.

Name	Breed	Age at sampling (days)	Serum IgG_1 conc. (mg/dl)
Non-atopic GDBA			
Angus	LabradorxGolden Retriever	261	25
Cherie	Labrador	615	20
Daniel	Labrador	684	10
Emily	Labrador	1005	130
Eva	Golden RetrieverxLabrador	1833	10
Glen	Labrador	634	10
Grace	Golden Retriever	457	10
Henry	Golden Retriever	465	10
Melody	Golden RetrieverxLabrador	1500	10
Paddy	LabradorxGolden Retriever	691	35
Pascoe	Golden RetrieverxLabrador	280	20
Perry	Golden RetrieverxLabrador	793	20
Rigsby	Labrador	251	10
Skin disease non-atopic GDBA			
Lucy	Labrador	554	10
Henry	Golden Retriever	284	10
Mary	Golden Retriever	215	10
Sherry	Golden RetrieverxLabrador	472	10
Curtis	Labrador	644	10
Norma	Golden RetrieverxLabrador	423	10
Duncan	Golden Retriever	507	10
Ria	Labrador	401	10
Illis	Golden RetrieverxLabrador	276	10
Onyx	LabradorxGolden Retriever	1708	30

Appendix N2 Serum total IgG_1 concentration in atopic GDBA dogs

Dog number	Breed	Age at sampling (days)	Serum IgG_1 conc. (mg/dl)
Abel	German Shepherd Dog	2689	1550
Alana	German Shepherd Dog	1709	25
Andrea	German Shepherd Dog	1325	135
Carlo	Golden RetrieverxLabrador	429	10
Cedar	Golden RetrieverxLabrador	565	10
Chris	Labrador	2687	50
Clare	Labrador	2892	520
Dusty	Golden RetrieverxLabrador	1830	220
Griff	Golden Retriever	1820	345
Harris	LabradorxGolden Retriever	630	60
Herbie	Golden RetrieverxLabrador	125	10
Kai	Golden RetrieverxLabrador	784	40
Keaton	Labrador	413	100
Opal	Labrador	1473	60
Palmer	Golden RetrieverxLabrador	916	30
Paul	Golden RetrieverxLabrador	908	35
Pedro	Golden RetrieverxLabrador	796	10

Appendix N3 Serum total IgG_1 concentrations in non-atopic greyhounds

Dog number	Age (yrs)	Serum IgG_1 conc. (mg/dl)
334	3	425
338	3	325
342	3	925
344	6	215
345	6	430
346	6	10
347	6	10
348	6	205
349	6	10
350	3	760
371	3	300
372	5	10
373	12	80
374	5	165

Appendix N4 Serum total IgG_1 concentrations in atopic GUVS dogs.

Atopic GUVS	Breed	Age at sampling (days)	IgG_1 conc. (mg/dl)
Alfred	German Shorthaired Pointer	1266	240
Brandy	Dalmatian	750	25
Budd	Labrador	915	240
Holly	Yorkshire Terrier	820	100
Islay	Staffordshire Bull Terrier	2770	1520
Jess	Cross breed	1440	520
Kassie	German Shepherd Dog	468	10
Kayla	Boxer	550	475
Kerry	Terrier	545	200
Kerry II	German Shepherd DogxLabrador	2187	35
Kim	Labrador	1865	360
Libby	Bulldog	607	475
Luke	Great Dane	388	970
Macauly	German Shepherd Dog	305	165
Megan	Labrador	2521	1350
Olwen	Irish Wolfhound	498	10
Raver	Staffordshire Bull Terrier	1454	80
Rhuri	German Shepherd Dog	719	365
Sally	Labrador	1092	960
Sammie	Golden Retriever	2166	280
Shannon	Cairn Terrier	730	10
Shogun	German Shepherd Dog	1640	640
Skerry	Staffordshire Bull Terrier	1939	330
Tyson	Boxer	288	685

Appendix N5

One Way ANOVA and Newman Keuls multiple range test of the log_e of serum total IgG_1 concentrations in atopic and non-atopic dogs

Worksheet size: 100000 cells

MTB > print c1-c3

Data Display

Row	Group	Serum IgG1	Log e serum IgG1
1	1	25	3.21888
2	1	20	2.99573
3	1	10	2.30259
4	1	130	4.86753
5	1	10	2.30259
6	1	10	2.30259
7	1	10	2.30259
8	1	10	2.30259
9	1	10	2.30259
10	1	35	3.55535
11	1	20	2.99573
12	1	20	2.99573
13	1	10	2.30259
14	2	10	2.30259
15	2	10	2.30259
16	2	10	2.30259
17	2	10	2.30259
18	2	10	2.30259
19	2	10	2.30259
20	2	10	2.30259
21	2	10	2.30259
22	2	10	2.30259
23	2	30	3.40120
24	2	1550	7.34601
25	3	25	3.21888
26	3	135	4.90527
27	3	10	2.30259
28	3	10	2.30259
29	3	50	3.91202
30	3	520	6.25383
31	3	220	5.39363
32	3	345	5.84354
33	3	60	4.09434
34	3	10	2.30259
35	3	40	3.68888
36	3	100	4.60517
37	3	60	4.09434
38	3	30	3.40120
39	3	35	3.55535
40	3	10	2.30259
41	4	425	6.05209
42	4	325	5.78383

43	4	925	6.82979
44	4	215	5.37064
45	4	430	6.06379
46	4	10	2.30259
47	4	10	2.30259
48	4	205	5.32301
49	4	10	2.30259
50	4	760	6.63332
51	4	300	5.70378
52	4	10	2.30259
53	4	80	4.38203
54	4	165	5.10595
55	5	240	5.48064
56	5	25	3.21888
57	5	240	5.48064
58	5	100	4.60517
59	5	1520	7.32647
60	5	520	6.25383
61	5	10	2.30259
62	5	475	6.16331
63	5	200	5.29832
64	5	35	3.55535
65	5	360	5.88610
66	5	475	6.16331
67	5	970	6.87730
68	5	165	5.10595
69	5	1350	7.20786
70	5	10	2.30259
71	5	80	4.38203
72	5	365	5.89990
73	5	960	6.86693
74	5	280	5.63479
75	5	10	2.30259
76	5	640	6.46147
77	5	330	5.79909
78	5	685	6.52942

MTB

One-Way Analysis of Variance

Analysis of Variance for Log e se

Source	DF	SS	MS	F	P
Group	4	89.53	22.38	11.98	0.000
Error	73	136.40	1.87		
Total	77	225.93			

			Individual 95% CIs For Mean Based on Pooled StDev
Level	N	Mean	StDev
1	13	2.827	0.756 ( )
2	10	2.412	0.347 ( )
3	17	4.090	1.489)

```
4       14    4.747    1.714                (------*------)
5       24    5.296    1.543                   (-----*-----)
                                  ------------------------------
Pooled StDev =   1.367            2.4      3.6      4.8
```

```
MTB > %nk
Executing from file: C:\MTBWIN\MACROS\nk.MAC
```

Newman-Keuls Multiple Range Test

Please enter the following info at the DATA> prompt :

```
        Number of groups
DATA> 5

        MS(error)
DATA> 1.87

        Df(error)
DATA> 73

        means of each group
DATA> 2.827
DATA> 2.412
DATA> 4.090
DATA> 4.747
DATA> 5.296

        number of observations in each group
DATA> 13
DATA> 10
DATA> 17
DATA> 4
DATA> 24
```

Results of Newman-Keuls multiple range test

Data Display

MS error: 1.87 df error: 73

Group	Mean	Count

Data Display

1	2.827	13
2	2.412	10
3	4.090	17

4	4.747	14
5	5.296	24

Data Display

Group 3 significantly different to group 2

Data Display

Group 4 significantly different to group 2

Data Display

Group 5 significantly different to group 2

Data Display

Group 3 significantly different to group 1

Data Display

Group 4 significantly different to group 1

Data Display

Group 5 significantly different to group 1

MTB