Summary

Bovine leptospirosis is the infection of cattle by any serotype belonging to the genus Leptospira. It has now been reported from almost every part of the world and infections by strains belonging to the pomona, grippotyphosa, icterohaemorrhagiae and hebdomadis serogroups are of world wide significance. A variety of clinical signs such as haemoglobinuria, jaundice, mastitis and abortion have been associated with infection.

Very little is known about the serotypes of Leptospira which may infect cattle in the United Kingdom. The purpose of the study described here was to carry out investigations into bovine leptospirosis in Scotland. The work consisted of two parts, firstly, a field study and secondly, an experimental study.

The field study consisted of:

(a) a serological survey of 3,600 cattle. This was carried out using the microscopic agglutination test incorporating antigens representing sixteen Leptospira serogroups on sera from "cast" cows, cows which had aborted and cows in 29 herds. In this way the serogroups which infect cattle were identified and their relative importance was assessed;

(b) an attempt to correlate the serological findings with the clinical findings in the 29 herds by means of an epizootiological model and on a farm where an outbreak of icterohaemorrhagiae infection occurred in calves;

(c) attempts to isolate leptospires from the kidneys of cows with serological evidence of infection;

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Antibodies to serotypes representing one or more of ten Leptospira serogroups were detected in the sera of 1,766 (49.1 per cent) cattle. Antibodies to sejroe (hebdomadis serogroup) were found in 1,503 (41.8 per cent) sera; to icterohaemorrhagiae in 278 (7.7 per cent) sera; to ballum in 264 (7.3 per cent) sera. Antibodies to bratislava (australis serogroup), javanica,

canicola, panama, cynopteri, pyrogenes and autumnalis were detected in a small number of animals. There was no evidence for pomona and grippotyphosa infections.

It was concluded that infection with strains (probably serotype hardjo) belonging to the hebdomadis serogroup in susceptible pregnant cattle could result in abortion. This conclusion was based on:

1) Reports by farmers and practising veterinary surgeons of abortions of unknown aetiology in herds showing serological evidence of infection.
2) The occurrence of a much higher incidence of antibody titres of 1:300 or greater to sejroe (33.7 per cent) in cows from herds with a history of undiagnosed abortion, than in cows from other groups of animals tested.
3) The close relationship found between the incidence of cows with antibody titres of 1:10 or more and 1:300 or more to sejroe and the incidence of abortion of unknown aetiology in 29 herds over the 18 month model period.

This conclusion was supported by the demonstration, for the first time, of leptospires belonging to the hebdomadis serogroup in the internal organs of two aborted foetuses and a premature live calf.

The incidence of abortion in infected herds varied between 0 and 14 per cent per 9 month period and showed a marked seasonal variation. Abortions were more common in the months from October to March than in the remaining months.

The results of the field survey suggested that Scottish cattle act as maintaining hosts for strains belonging to the hebdomadis serogroup whereas infections by other serogroups were incidental events resulting from the contamination of their environment by other maintaining hosts.

Of the other serogroups infecting cattle only icterohaemorrhagiae was obviously associated with clinical disease. Icterohaemorrhagiae infection was diagnosed in calves on one farm and was associated with marked jaundice in one

calf and stunted growth in two treated, in-contact calves.

In the experimental study twenty pregnant heifers at various stages of gestation and seven young calves were infected with J10 strain. J10 strain had been isolated from the kidneys of a cow which had aborted. It belonged to the hebdomadis serogroup and was closely related antigenically to serotype hardjo. The major findings of the experimental study are summarised below.

Following infection there was an incubation period of 3 to 5 days followed by a marked pyrexia (up to 106.2°F) which lasted for up to 5 days. Heifers showed minimal clinical signs but more severe clinical signs were observed in the calves, one of which died 9 days post-infection. Fever (up to 106.9°F) recurred in 19 animals.

One infected heifer aborted a near-term foetus 58 days after infection and another produced a live, weak, premature (240-245 days gestation) calf 60 days after infection.

A leptospiraemia of 1 to 5 days duration was demonstrated in 10 of the 20 experimentally infected heifers and in the 7 experimental calves. It was usually associated with the period of initial pyrexia, and disappeared with appearance of circulating antibodies.

Leptospires localised in the kidney tubules where they were demonstrated as early as 9 days after infection and as late as 174 days after infection. Urinary excretion of leptospires was light and intermittent.

Localisation of leptospires was demonstrated on and between the cells of the trophoblast layer of the cotyledons of five live, full-term calves born 14, 15, 16, 32 and 55 days after infection, and in the placenta of the premature calf born 60 days after infection.

Leptospires were demonstrated in the liver, kidneys and lung of the aborted foetus and in its placenta, and were isolated from a mixed inoculum of blood and liver from calf 21 which died. They were also demonstrated in histological sections of its kidneys.

Antibodies to hardjo were detected in the sera of susceptible experimental cattle from the sixth day post-infection and maximum titres of up to 1:100,000

BOVINE LEPTOSPIROSIS: FIELD AND EXPERIMENTAL STUDIES

A Thesis for the Degree of

DOCTOR OF PHILOSOPHY

in the

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Submitted by

WILLIAM ARMSTRONG ELLIS, B.V.M.S., M.R.C.V.S.

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Antibodies to hardjo were detected in the sera of susceptible experimental cattle from the sixth day post-infection and maximum titres of up to 1:100,000

were reached between 11 and 21 days post-infection. These maximum titres persisted for 3 to 21 days after which time they began to decline. Secondary rises in antibody were detected in four heifers between 10 and 21 weeks after infection.

Antibodies were detected in the milk whey of freshly calved heifers but levels declined rapidly over the first three days of lactation although antibody persisted at a low level for some time.

Non-fatal in utero infection was demonstrated by the finding of an antibody titre of 1:10 to hardjo in a sample of serum collected from the premature live calf immediately after birth.

Passive antibody titres of up to 1:30,000 were found in the sera of calves born to infected dams within 3 days of birth. These titres persisted from 21 to more than 56 days.

All the experimental animals developed a mild interstitial nephritis.

These studies indicated that leptospires of the hebdomadis serogroup were capable of causing abortion in experimental cattle and confirmed the conclusions reached in the field study.

Chapter 1

INTRODUCTION

Leptospirosis as a Disease Entity and the Background to the present Investigation

The term "leptospirosis" applies to any infection attributable to a member of the genus Leptospira which belongs to the family Treponemataceae of the Order Spirochaetales.

Leptospirosis was first recognised as a disease entity in the latter part of the nineteenth century when Landouzy (1883 a and b) separated, what was to prove to be leptospiral jaundice, in sewerage workers, from other forms of infectious jaundice on the basis of clinical observations. The term Weil's disease was first used by Goldschmidt (1887) following the publication of Adolf Weil (1886). Weil described a febrile illness in four men which was characterised by severe nervous symptoms, enlargement of the liver and spleen, jaundice and signs of renal involvement.

The organisms now referred to as Leptospira were first recognised by Stimson (1907) in silver impregnated sections of kidney of a patient who was believed to have died from Yellow Fever and living leptospires were seen in pond water a few years later by Wolbach and Binger (1914).

The causal organism of Weil's disease was demonstrated in November 1914 in Japan (Inada et al., 1916) and independently at a later date by the German workers Hubener and Reiter (1915, 1916) and Uhlenhuth and Fromme (1915, 1916a and b).

The recognition of leptospirosis as a disease in animals quickly followed its diagnosis in man. Krumbein and Frieling (1916) considered it to be the cause of an attack of jaundice in a dog because two men in close contact with it developed Weil's disease, and Courmont and Durand (1917) showed that fatal jaundice could be regularly produced in young dogs by the injection of guinea-pig liver containing leptospires, while Uhlenhuth and

Fromme (1919) identified leptospiral infection in a dog by finding leptospires in smears of kidney and liver of a guinea-pig inoculated with dog tissue.

Nearly twenty years elapsed before leptospiral infection was first recognised in cattle, when "infectious haemoglobinuria" in calves caused by serotype grippotyphosa was reported by Michin and Azhinov (1935) in Russia. Semskow (1941) isolated this organism from a natural case and proved experimentally that it was pathogenic for calves. Later in Israel a bovine disease with a similar clinical picture was shown to be caused by leptospires (Bernkopf, 1946 a and b). Evidence that leptospirosis was the cause of a variety of clinical syndromes in cattle soon followed from different parts of the world. In the U.S.A., Baker and Little (1948) showed that leptospires could cause atypical mastitis in cows and haemoglobinuria in calves, while in Australia, Sutherland et al. (1949) demonstrated that "red-water" in calves could be caused by leptospires and in England, Field and Sellers (1950) showed that serotype icterohaemorrhagiae could cause jaundice in calves. Mathews (1946) noted the high incidence of abortion in leptospire infected herds in the U.S.A. and Te Punga and Bishop (1953) demonstrated leptospires in kidney sections from an aborted bovine foetus in New Zealand.

Bovine leptospirosis has now been reported from almost every part of the world, and in many countries it constitutes a major problem in the cattle industry rivalling brucellosis (W.H.O., 1959). Morse (1955) estimated that infection by serotype pomona alone caused an annual loss of £100 million to the American farming community. Animals of all age groups and both sexes may be affected by a variety of serotypes; antibodies to at least fifteen serogroups have now been demonstrated in cattle sera. The major problems have been infections with pomona in the U.S.A. and grippotyphosa in the U.S.S.R. and Eastern Europe, but with the extensive use of bacterins to these serotypes, infection appears to be under control and more attention has recently been paid to the less virulent serogroups, especially the hebdomadis

serogroup. Other serogroups which have been shown to contain serotypes pathogenic for cattle are bataviae, australis, canicola and icterohaemorrhagiae.

Bovine leptospirosis has been classified into three main clinical forms (Freund, 1947; Michna, 1970).

1) A PERACUTE form which is characterised by sudden onset, pyrexia, anorexia, haemoglobinuria and jaundice. The mortality rate may be high. This form is seen mainly in calves and yearlings but older animals may also be affected. Pregnant cows may abort.

In the U.S.A. and Australia, pomona is the pathogen most frequently associated with this form, and acute pomona infection has also been recorded in Europe (Witting et al., 1967). Epizootics of peracute infection due to grippotyphosa have been described in Russia by Nikolajev (1946), in Israel by Bernkopf (1946, a and b) and in Hungary by Bodi et al., (1964). Van der Hoeden (1955) reported epizootics due to canicola infection in Israel and Kiryanov (1968) described two outbreaks of acute bataviae infection in young cattle in the far-East of the U.S.S.R., in one of which there was a mortality rate of 12 per cent.

2) The SUB-ACUTE form is seen in adult cattle and is characterised by reduced milk yield; the milk resembles colostrum, although the udder feels and appears normal. There may be a marked pyrexia, slight jaundice and impaired ruminal movements. The affected cows gradually return to normal. They may abort.

3) The CHRONIC form in which abortion and retention of foetal membranes may be the only clinical manifestations. Pomona was recognised as a cause of abortion early in the history of bovine leptospirosis (Mathews, 1946; Reinhard, 1953; Te Punga and Bishop, 1953). More recently, there have been reports associating various members of the hebdomadis serogroup with abortion (Lyubashenko et al., 1966) and Bellani and Ruggeri (1968) reported endemic abortions and infertility associated with australis infection.

Atypical forms of clinical disease occur. Gerlach (1956) described a disease of Turkish cattle, due to pomona, characterised by sudden onset and inco-ordination of movement followed by death. Jaundice and fever were absent and meningitis was the principal pathological change noted. Hoag and Bell (1954b) isolated pomona from the aqueous humour of a calf which had a transient bilateral uveitis.

Despite the considerable amount of work which has been done in many parts of the world and the wide range of clinical signs attributed to leptospiral infection, very little attention has been paid to the infection of cattle by serotypes which occur in the United Kingdom. In the seventeen years following the work of Field and Sellers (1950), only two reports of bovine leptospirosis in the U.K. were published (Ingram et al., 1952; Baxter and Pearson, 1956) and both of these referred to icterohaemorrhagiae infection in calves. The possibility of the infection of cattle by other serotypes went unnoticed until Michna (1967) reported the presence of antibodies to sejroe (hebdomadis group) and ballum as well as to icterohaemorrhagiae. Coghlan and Norval (1967) confirmed Michna's findings with regard to sejroe and ballum antibodies and in addition reported the findings of antibodies to bratislava (australis group) and canicola in cattle sera.

Following the isolation of serotype sejroe (hebdomadis serogroup) from the kidneys of a cow which had aborted (Michna and Campbell, 1969) and the further recovery of serotype hardjo (hebdomadis serogroup) from similar material in 1969 (Michna et al., 1974), a grant (ARC AG 17/71) was awarded by the Agricultural Research Council for further investigations into bovine leptospirosis. This investigation was approached in two ways:

I A field study involving:

1) a serological survey of bovine sera and an attempt to correlate these findings with the clinical picture;
2) attempts to isolate leptospires from material from cases of leptospirosis.

II Experimental infections with a member of the hebdomadis serogroup, isolated from the kidneys of an aborting cow, in

1) pregnant heifers at various stages of gestation; and

2) young calves.

It is the purpose of this thesis to describe the results of this investigation.

Classification of the Genus Leptospira

The term "Leptospira" was first used by Noguchi (1918) following a morphological and serological study of:

a) the Spirochaeta icterohaemorrhagiae of Inada et al. (1916);

b) organisms isolated from cases of Weil's disease in British soldiers in Flanders;

c) strains from wild rats in the U.S.A., and

d) the Spirochaeta biflexa which Wolbach and Binger (1914) had isolated from water.

The genus Leptospira now includes all the organisms morphologically identical with the type organism of the genus Leptospira icterohaemorrhagiae (Inada et al., 1916).

A leptospire resembles a rigid partially extended helical spring. Electron microscopy shows it to consist of a spiral protoplast (approximately 0.1 μ diameter) helically coiled around a thin rigid axial filament. The pitch of the spiral protoplast is extremely fine with an amplitude of 0.4 to 0.5 μ. Both protoplast and axial filament are within a well defined cell envelope. The ends of the cell are thinner than the body and are usually curved although straight forms exist. Live leptospires spin rapidly on their long axis in such a manner that the curved ends give the organism a "button-hole" appearance. They also display forward and backward movements. The average length is 4 - 20 μ but occasionally much longer forms are seen.

The genus Leptospira is currently sub-divided into two complexes* designated "interrogans" and "biflexa". The interrogans complex includes most of the pathogenic and parasitic strains. The biflexa complex consists mainly of the so-called saprophytic strains with no known hosts. Within each complex, strains are classified into serogroups and serotypes by means of cross-agglutination reactions and cross-agglutinin absorption studies, using antisera prepared in rabbits (Turner, 1967).

The "serotype" is the basic taxon and is represented by a designated reference strain. Two strains are considered to belong to different serotypes if, after cross-absorption with adequate amounts of heterologous antigen, 10% or more of the homologous titre regularly remains in at least one of the two antisera in repeated tests.

A "serogroup" is a group of two or more serotypes that show marked antigenic relationships in the cross-agglutination test. It is not a taxonomic sub-division nor can leptospiral serogroups be defined precisely at present. The current arrangement of serogroups has the serious limitation that certain strains can be allocated to more than one serogroup. The interrogans complex now consists of over 130 serotypes grouped in 16 serogroups (W.H.O., 1967) and many more await classification. As well as these 16 serogroups there are two pathogenic serotypes, semaranga and andamana, whose cultural and biochemical characteristics resemble more closely the non-pathogenic biflexa strains (Turner, 1967).

Assuming that the antigenic structure of leptospires remains constant, their serological classification is a valuable aid in epidemiology as it can be used to distinguish between infection due to different serotypes. The concept of leptospiral serogroups has practical value in the selection of antigens and antisera for diagnostic and serological investigations, as it

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*The term complex has no official standing as a taxon but has been proposed for provisional use in dividing leptospires into two groups for which the species names, interrogans and biflexa were previously used but which cannot at present be defined with certainty (W.H.O.,1967).

allows the number of antigens used in diagnostic work to be reduced to a representative from each of the serogroups known to exist in the area.

Chapter 2

GENERAL MATERIALS AND METHODS

It is the purpose of this section to describe only the materials and technical procedures used throughout this study.

Materials and methods specific to a particular section are described in the section concerned.

Antigens used in the serological tests

Antigens representing the sixteen serogroups belonging to the "interrogans" complex were maintained in the laboratory for use in the microscopic agglutination test. These had previously been obtained from Dr. L.H. Turner of the Leptospirosis Reference Laboratory (Public Health Laboratory Service, WHO/FAO), London School of Hygiene and Tropical Medicine, by Dr. S.W. Michna and were maintained in his laboratory. They were as follows:-

	SEROGROUP	SEROTYPE
1.	Canicola	canicola (Hond Utrecht IV)
2.	Icterohaemorrhagiae	icterohaemorrhagiae (Field strain)
3.	Pomona	pomona (Johnson)
4.	Ballum	ballum (Mus 127)
5.	Grippotyphosa	grippotyphosa
6.	Hebdomadis	hebdomadis (strain hebdomadis) and sejroe (strain M84)
7.	Australis	bratislava (strain Jez. Bratislava)
8.	Autumnalis	autumnalis (strain Akiyami)
9.	Panama	panama (strain cristobali)
10.	Cynopteri	cynopteri
11.	Javanica	javanica
12.	Tarassovi	tarassovi (strain Mitis Johnson)
13.	Pyrogenes	pyrogenes
14.	Shermani	shermani

15. Bataviae — bataviae (strain Van Tienen)

16. Celledoni — whitcombi (Whitcomb)

Hardjo (204 strain), a member of the hebdomadis serogroup, isolated from the kidneys of a cow which had aborted (Michna et al., 1974), was also maintained. This strain grew quite readily and was used to follow the serological response in the experimental animals as the newly recovered J10 strain, to which it was very closely related antigenically, was extremely difficult to cultivate.

Culture Media and Antigen Production

The strains were cultivated in Stuart's (1946) modification of Schuffner's medium. Not all strains grew satisfactorily in medium containing commercial rabbit serum*, therefore sejroe, hebdomadis, hardjo, panama, cynopteri, javanica and whitcombi were grown in media containing serum collected from selected departmental stock rabbits.

The freshly prepared medium, containing 10% of suitable rabbit serum, was distributed in 4 ml. amounts into bijou bottles and incubated aerobically at 37°C for 48 hours, at which time any contaminated media and those of unsuitable pH were discarded.

Each bijou, after inoculation with ¼ to ½ ml. of the corresponding culture, was incubated at 30°C for 5-7 days. Sub-cultures were made at weekly intervals. Only densely growing, actively motile cultures, 4-9 days old, were used as antigens throughout the investigation.

The cultures were subjected to regular control tests against known standard rabbit leptospiral antisera to confirm that they remained unchanged antigenically.

Standard Leptospiral Antisera

These were prepared in rabbits by Kitaoka's method (Broom, 1957) which involved the intraperitoneal inoculation of living whole cultures into 4-6 months old rabbits. Three graded doses were given at weekly intervals.

*Wellcome Reagents Ltd., Beckenham, Kent.

These were 5 ml., 10 ml. and 20 ml. respectively. Two weeks after the last inoculation a test bleeding was carried out. When antibody was found to have attained a satisfactory titre the rabbits were bled out. When antibody titres were not satisfactory the rabbit was given a further 20 ml. of culture intraperitoneally.

Table 1 shows the final titres of the hyperimmune sera when titrated against homologous live antigen.

J10 antiserum was not titrated against its own antigen as the culture was never grown to a density suitable for use in titrations, but in common with the other antisera to members of the hebdomadis serogroup, it was checked against the other members of the group maintained in the laboratory (Table 2).

The production of high titre antisera proved much more simple for some antigens than for others and, indeed, with cynopteri, panama and shermani, high titres were never obtained although the antisera were satisfactory for the purposes of the present study.

Preparation of Bovine Sera

Blood samples were collected using individual "Vacutainers"* and allowed to clot. The clotted sample was centrifuged at 500g for 10 minutes. The serum was removed and stored at -20°C without inactivation in sealed bijou bottles until required.

Microscopic Agglutination Test

This was carried out using live leptospiral antigens prepared by the method described above. Sera were first screened at dilutions of 1:30 and 1:300 against all sixteen serogroups and any positive reactors were then titrated against the antigen to which they reacted, serial serum dilutions of 1/10, 1/30, 1/100, 1/300 etc., being used to at least three dilutions higher than the maximum screening test titre.

*Beckton, Dickinson U.K. Ltd., Wembley, Middlesex.

Table 1. Titres of the hyperimmune rabbit sera when titrated against the homologous live antigen

Antigen	Titre of homologous antiserum
canicola	++1/300,000
icterohaemorrhagiae	++1/300,000
pomona	++1/10,000
ballum	++1/10,000
grippotyphosa	++1/3,000
hebdomadis	++1/300,000
sejroe	++1/10,000
J10	- not carried out
hardjo (strain 204)	++1/30,000
bratislava	++1/100,000
autumnalis	++1/30,000
panama	++1/3,000
cynopteri	++1/1,000
javanica	++1/30,000
tarassovi	++1/10,000
pyrogenes	++1/10,000
shermani	++1/3,000
bataviae	++1/30,000
whitcombi	++1/30,000

Table 2. Titres of the hyperimmune rabbit sera to members of the hebdomadis serogroup when titrated against other members of that group

	Antigen		
Antiserum to:	hebdomadis	sejroe	hardjo (strain 204)
hebdomadis	++1/300,000	++1/1,000	++1/1,000
sejroe	++1/1,000	++1/10,000	++1/10,000
hardjo	++1/300	++1/10,000	++1/30,000
J10	++1/100	++1/10,000	++1/100,000

The tests were carried out in perspex trays containing 10 rows of 8 wells each. For the screening test two plates were placed end to end as shown in Fig. 1 and dilutions prepared in a manner similar to that developed by Schuffner and Mochtar (1927) and Schuffner and Bohlander (1939) (Fig. 2(1)). Dilutions of 1:5 and 1:50 of the test serum were prepared in the bottom wells of the tray using pasteur pipettes with tips of standard diameter. One drop of the diluted test serum was then placed in each well in the vertical column. Two drops of diluent and three drops of the required antigen were added to each well to give final serum dilutions of 1:30 and 1:300. Titrations were carried out according to the scheme depicted in Fig. 2(2).

The trays were then shaken thoroughly, stacked, covered to prevent evaporation and left in the incubator at 30°C for four hours. One drop from each well was then examined for agglutination by means of dark field microscopy at a final magnification of X200.

A serum sample was considered to be positive in the screening test if any agglutination occurred at 1:30 or 1:300 dilutions. All such positive sera were then titrated against the antigen to which they reacted.

A serum sample was considered to be positive when almost all of the organisms in the well had agglutinated at the 1:10 dilution or if approximately 50% (++) or more of the organisms were agglutinated at a higher dilution (1:30 or over). The titre of a serum sample was taken as the highest dilution in which there was agglutination of 50% or more of the organisms. The degree of agglutination was assessed in terms of the proportion of free leptospires present, e.g., at 100% (++++) agglutination there were no free leptospires visible, while at 50% (++) agglutination about half the leptospires remained free when compared with a negative control.

The series of photographs in Fig. 3 illustrate the agglutination pattern in different dilutions of rabbit serum containing antibodies to sejroe. Thus Fig. 3(a) illustrates the density of the sejroe culture diluted

Fig. 1 Scheme showing the application of the microscopic agglutination test to the screening of sera with sixteen Leptospira serotypes

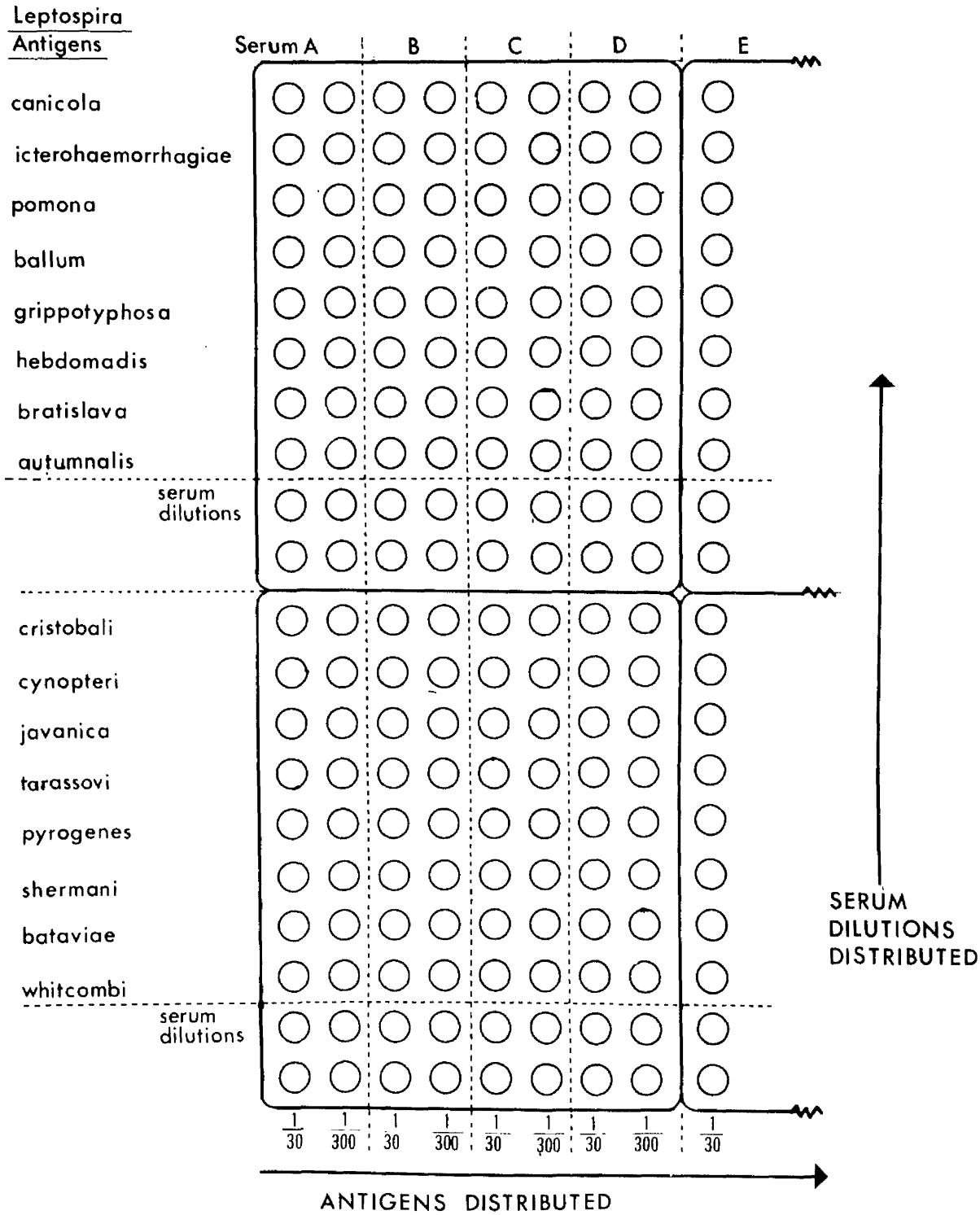
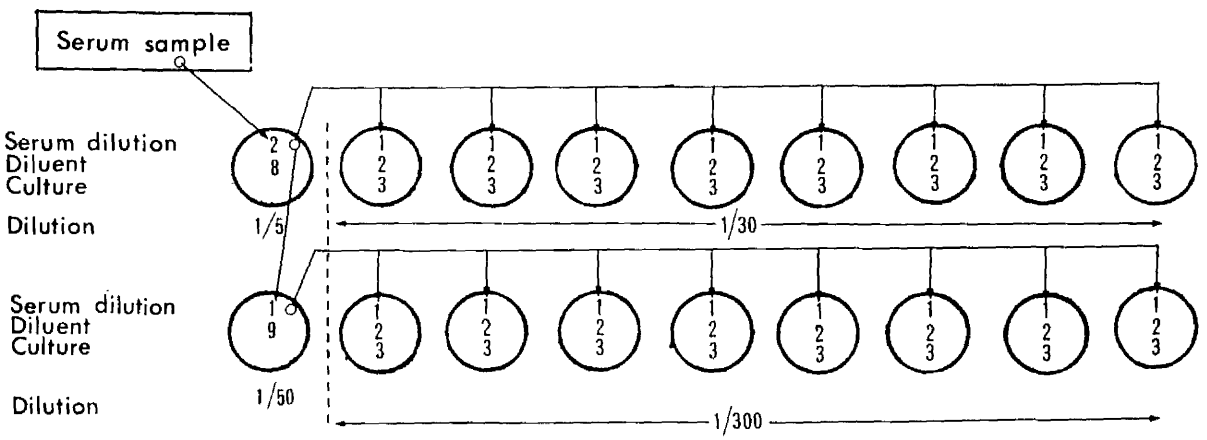


Fig. 2 The microscopic agglutination test

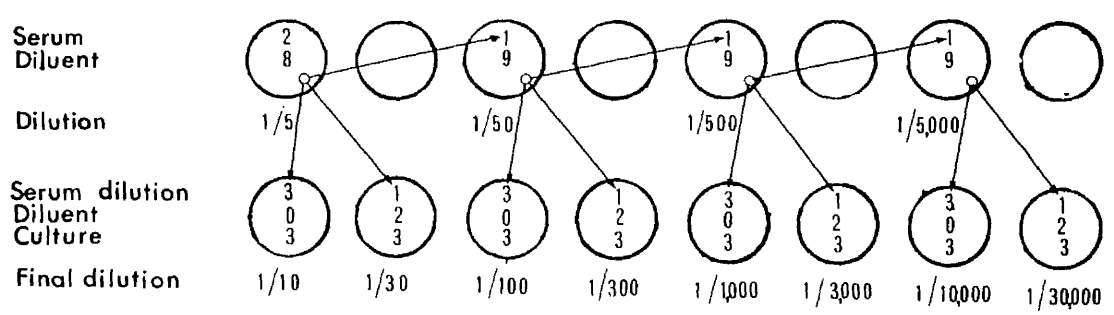
----- after Schüffner & Mochtar (1927)
and " " Bohlander (1939)

Scheme for preparing dilutions of serum as used in

(1) the sceening of sera

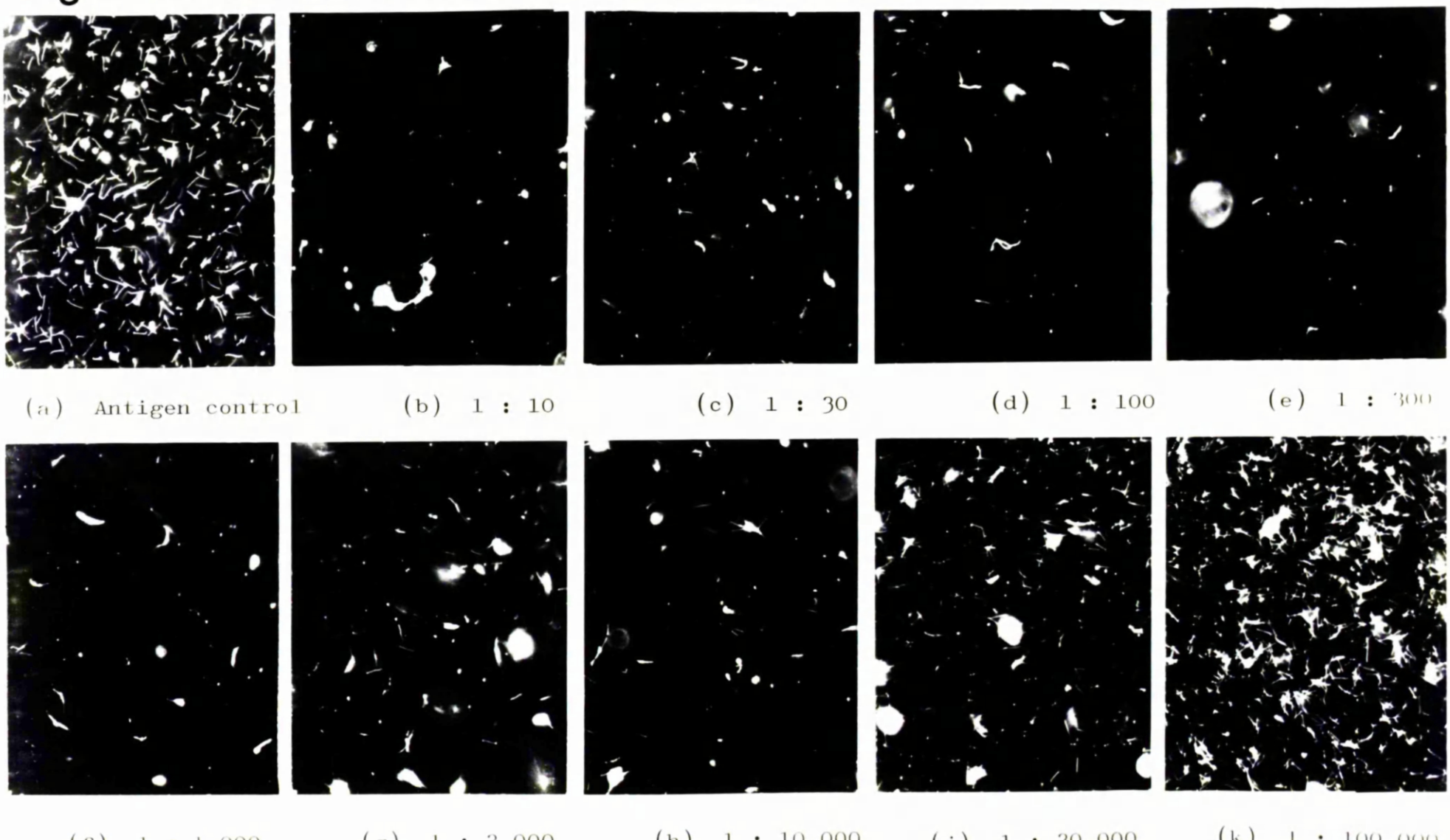


(2) the titration of positive sera



The numbers represent the number of drops added to each well.

Fig.3 Microscopic Agglutination Test on rabbit serum containing antibodies to sejroe.



(a) Antigen control (b) 1 : 10 (c) 1 : 30 (d) 1 : 100 (e) 1 : 300

(f) 1 : 1,000 (g) 1 : 3,000 (h) 1 : 10,000 (j) 1 : 30,000 (k) 1 : 100,000

1:2 to act as a negative control during the test. In Fig. 3(b) to (f) there is complete absence of motile leptospires while in Fig. 3(g) and (h) there are a few organisms, and many more are present in Fig. 3(j). There is little if any difference between Fig. 3(k) and (a). Since the density of the antigen in Fig. 3(j) appears to be about half that of the control, the titre of the serum is considered to be ++1:30,000.

Standard antigens were not used in the microscopic agglutination tests although visual inspection of the culture was used to give an approximate measure of uniformity. For this reason antibody titres obtained by this method were only approximate.

Isolation Procedures

(a) Direct culture

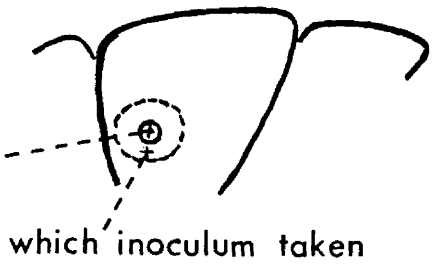
(i) From bovine kidney: This was carried out under a bright bench light, as in some cases powerful illumination of the kidney surface was necessary in order to see the gross lesions. The renal capsule was stripped off and the surface of the kidney examined immediately for the presence of pale foci or areas of hyperaemia. An area adjacent to a suitable focus was seared using a heated spatula as shown in Fig. 4 and a sterile pipette (attached via rubber tubing to a glass mouth piece) was inserted through the seared area. The pipette was rotated and probed into the lesion to obtain as many plugs of kidney tissue as possible. The renal tissue was then mixed with Stuart's (1946) medium. Graded amounts of this inoculum were then distributed to bottles containing 2 ml. amounts of culture medium which were incubated aerobically at 30°C and examined at weekly intervals by dark ground microscopy. All cultures, unless contaminated, were kept for eight weeks, after which negative ones were discarded.

Fifteen bottles of medium were used for each adult bovine kidney and 8 bottles of medium per kidney were used in the case of the calves. Only media containing sera from stock rabbits were used for isolation attempts.

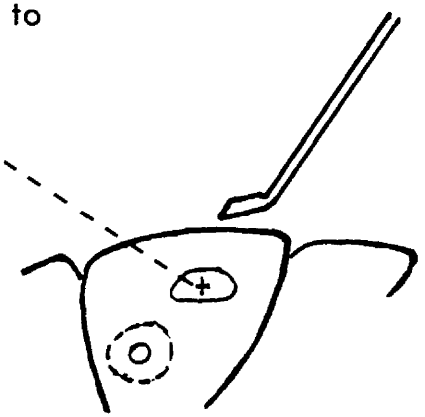
(ii) From other organs: This was carried out in a manner similar to that described above, after the organ had been removed from the carcase using aseptic techniques. No attempt was made to identify any gross lesions.

Fig.4 TECHNIQUE FOR ISOLATING LEPTOSPIRA FROM BOVINE KIDNEY

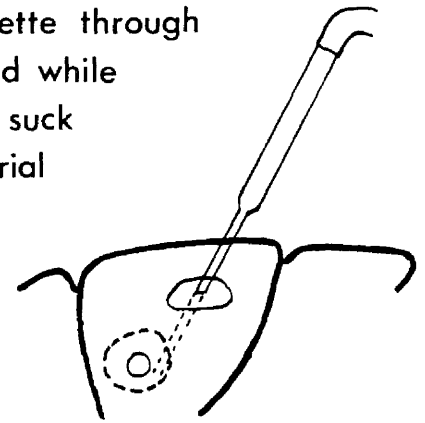
1/ Strip off capsule Examine for small pale foci under a bench lamp.



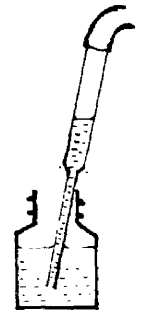
2/ Sear area adjacent to lesion with a hot spatula.



3/ Insert a sterile pipette through the seared area and while pushing in and out suck in the kidney material macerated up by this action.



4/ Mix with culture medium



5/ Distribute graded amounts of inoculum to each of 15 bottles containing Stuart's medium.

6/ Incubate at 31°C. for up to 8wks. Examine at weekly intervals.

In the case of hamster or guinea-pig material only 6 bottles of medium were used per organ.

(b) The inoculation of laboratory animals

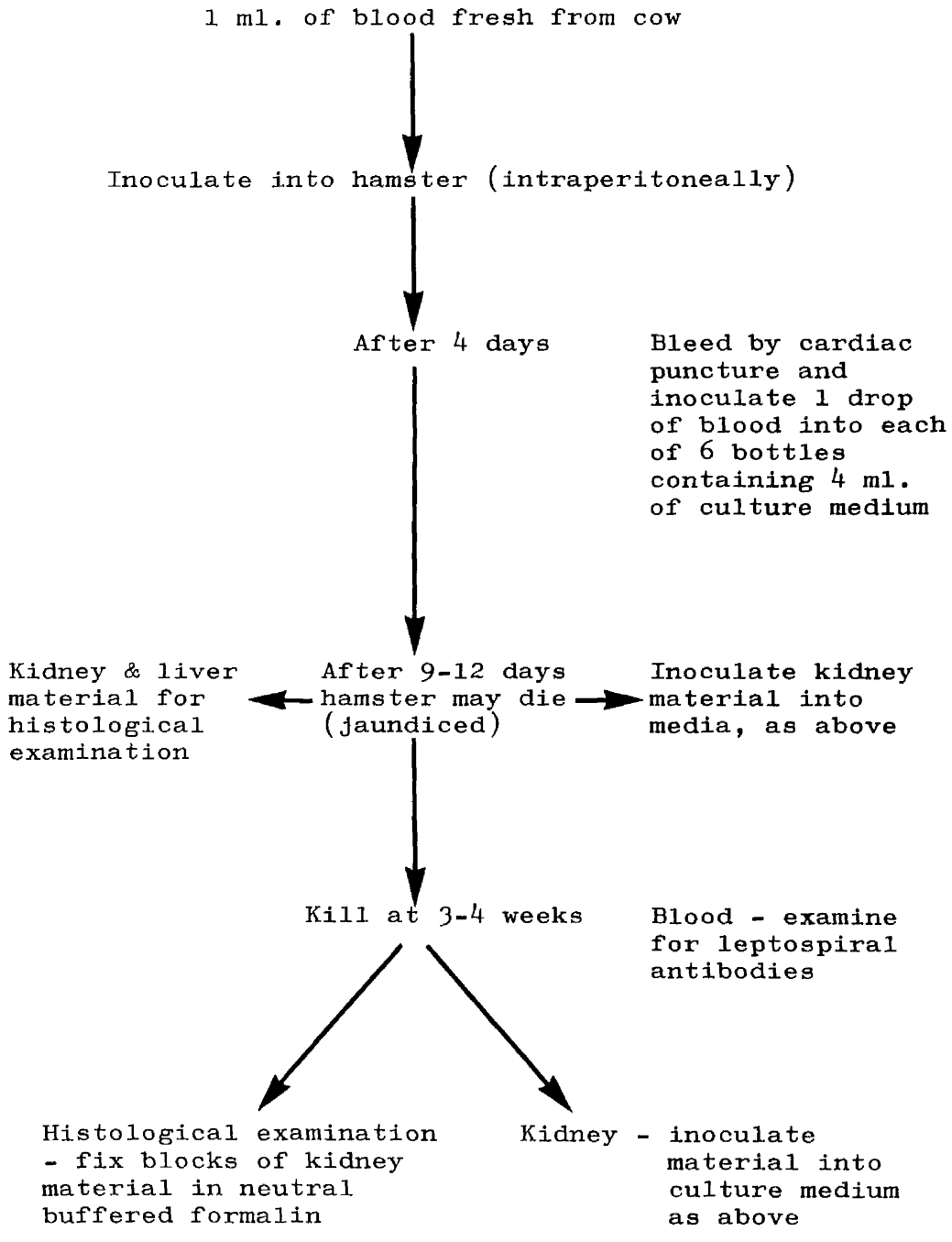
Laboratory animal inoculation was the routine procedure used for the isolation of leptospires in the following circumstances:

(i) From contaminated material: Approximately 2 gms. of the material was ground in a mortar with sterile sand, suspended in sterile physiological saline and placed in a test tube. The suspension was then centrifuged for 2-3 mins. at 500g so that the gross tissue fragments might be deposited. The centrifuged suspension was then allowed to stand for 5 mins. and 1 ml. of the supernatant fluid was removed and was inoculated intraperitoneally into either a young guinea-pig or, preferably, into a Syrian hamster.

(ii) Demonstration of Leptospiraemia: Approximately 1 ml. of blood was withdrawn aseptically from the jugular vein of animals suspected of having leptospiraemia and inoculated intraperitoneally into a young guinea-pig or a hamster. The small laboratory animals used were brought to the byre to reduce the delay between withdrawal of blood from the affected animal and inoculation of the laboratory animal.

The procedure for (i) and (ii) was the same after the inoculation of the guinea-pig or hamster and is outlined diagrammatically in Fig. 5. After 4 days the laboratory animals were bled by cardiac puncture and 1 or 2 drops of the blood were inoculated into each of 6 bijou bottles containing 4 ml. of Stuart's medium. If the small laboratory animals survived, they were killed 3-4 weeks after inoculation, their sera were examined for leptospiral antibodies, kidney material was cultured in isolation medium and blocks of kidney were fixed for histological examination. In those cases where the animals died as a result of acute leptospirosis, both kidneys and liver were taken for isolation of the pathogen and for histological examination.

Fig. 5 Summary of technique for demonstrating leptospiraemia



Demonstration of Leptospires in Bovine Urine

Mid-stream urine samples were collected into 1 litre wide-mouth flasks and taken to the laboratory for examination as soon as possible after collection. Two aliquots (A and B) of about 8 ml. of urine were taken from each urine sample. Sample A was centrifuged at 10,000g for 20 minutes and a drop of the sediment examined by dark ground microscopy at a magnification of X900. 40% formaldehyde solution was added to sample B to give a final concentration of 2% formalin. This was centrifuged at 3,000g for 40 mins. and the sediment examined in the same way as sample A.

Histological Techniques

Blocks of tissue were routinely fixed in 10% formalin or formal saline, but blocks in which it was wished to demonstrate plasma cells were fixed in Carnoy's fixative.

Three silver impregnation techniques were used for the demonstration of leptospires in tissues.

(i) Tissue blocks were stained by Levaditi's (1905) method.

(ii) 5 μ paraffin sections were stained by Young's (1969) method.

(iii) 5 μ paraffin sections were stained by Faine's (1965) method and counterstained with haematoxylin and eosin.

Haematoxylin and Eosin (H & E) were used for the routine staining of histological sections.

Other stains which were used on various tissues were:

(1) Grocott's modification of Gomori's Methamine silver method for the demonstration of fungi (Drury and Wallington, 1967).

(2) Gram-Twort (Drury and Wallington, 1967) for the presence of other bacteria.

(3) Unna-Papenheim stain for the demonstration of plasma cells (Mallory, 1961).

Chapter 3

FIELD STUDIES OF LEPTOSPIROSIS IN CATTLE

PART A. SEROLOGICAL AND CLINICAL SURVEY

Introduction and Review of the Literature

Serological Surveys

Surveys of cattle sera for the presence of leptospiral antibodies have been conducted in almost every part of the world in an attempt to determine the serotypes which cause infection in cattle and their relative importance. Greater awareness of bovine leptospirosis, combined with an increasing knowledge of the Leptospira serotypes which occur, has resulted in the instigation of repeated and progressively larger surveys using a greater number of Leptospira serotypes as antigens. Unfortunately there has been no recent or comprehensive review of these surveys and it was found necessary to review the literature on this subject.

Terskikh (1940) was apparently the first investigator to report the results of a serological survey when he recorded varying antibody titres in all but 7 of 147 cattle sera to a leptospire isolated from a human case of leptospirosis. The following year Semskow (1941) detected antibodies to the causal leptospire in the sera of 29 out of 34 cattle which had recovered from the natural disease. Since then, Russian workers have reported antibodies to each of the following serogroups in cattle sera: canicola, icterohaemorrhagiae, pomona, grippotyphosa, hebdomadis, (Rostomyan, 1964) bataviae (Kiryanov, 1968) and tarassovi (Lavrova, 1969). The relative importance of the different serogroups in cattle varies in different parts of the country. Bezdenezhnukh and Kashanova (1956) demonstrated antibodies to canicola in apparently healthy cattle on Sakhalin Island, in the far east of the U.S.S.R. Also in Eastern Russia, Kiryanov (1968) reported outbreaks of batavia infection. In the Caucasus region the dominant leptospiral infections in cattle are pomona and

grippotyphosa (Sosov et al., 1965) and the same authors reported that examination of 2,415 sera from the Volga region showed grippotyphosa and hebdomadis infection to be the most common; field evidence suggested an association between hebdomadis infection and abortion. Genis et al. (1969) reported that in the Kirgizia region of southern Russia the relative importance of hebdomadis and tarassovi infections was increasing with a relative decline in the importance of pomona, grippotyphosa and icterohaemorrhagiae infection. In the Kaliningrad region bovine leptospirosis, due mainly to the members of the hebdomadis serogroup, is widespread (Negoda et al., 1971).

In North America pomona has been incriminated as the cause of major outbreaks of clinical bovine leptospirosis and numerous reports of the incidence of antibody titres in cattle have been published, indeed separate reports have now been published for almost every state in the U.S.A. The incidence of positive sera has varied from 4.2 per cent of 10,000 samples in Ohio (Bohl, 1955) to 33 per cent of some 7,000 cattle sampled in Alabama (Roberts et al., 1961). Boulanger and Smith (1957) reported an 8.1 per cent incidence in Canada. Surveys have, however, shown the widespread incidence of infection by other serogroups, notably the hebdomadis serogroup. Reactor rates as high as 43 per cent to sejroe, a member of the hebdomadis group, have been recorded (Hale, 1959). There was considerable confusion as to how to interpret these reactions. This arose largely from the lack of apparent clinical illness in reactors, failure to isolate the organism, or the presence of concurrent pomona infection. Stoenner et al. (1956) considered sejroe antibodies to be either non-specific or due to cross reactions to pomona. The problem was resolved by the isolation of hardjo by Roth and Galton (1960) and the agglutinin-absorption study of sera from eight states by Alexander and Evans (1962); they concluded that the titres reputedly due to sejroe were probably largely due to hardjo infection. The Committee on Leptospiroses under the auspices of the U.S. Livestock and Sanitary Association (Twiehaus et al., 1966) reported the presence of antibodies to canicola, icterohaemorrhagiae, grippothphosa, autumnalis and ballum as well as pomona

and hardjo in American cattle and Carroll (1966) reported titres to australis, cynopteri, hyos, pyrogenes and bataviae as well in a serological survey of cattle in California.

Leptospiral infection of cattle is widespread in South America. Savino and Rennella (1945/8) detected antibodies to hyos, pomona, icterohaemorrhagiae and canicola in Argentinian cattle and, in a review of the position in Argentina, Cacchione (1967) states that hebdomadis infection is the most common, accounting for 42 per cent of the bovine reactors, followed by pomona (31 per cent) and hyos (18 per cent). Cacchione et al. (1970) reported finding antibodies to ballum, bataviae, pyrogenes, grippotyphosa, wolfii (hebdomadis serogroup) and icterohaemorrhagiae in cattle from Terra del Fuega. In Brazil, positive serological reactions to icterohaemorrhagiae, pomona, grippotyphosa and canicola (Guida et al., 1959, Santa Rosa et al., 1961) and bataviae (Guida and Barros, 1958) have been recorded.

Murnane et al. (1963) reported antibodies to sejroe (hebdomadis group) grippotyphosa, autumnalis, canicola, pyrogenes, pomona, icterohaemorrhagiae, australis and bataviae in Panamanian cattle. Sejroe was by far the most prevalent serotype, 19.5 per cent of 333 apparently normal cattle having antibodies to it.

In the Carribean, Alexander et al. (1963) showed that infection by the hebdomadis serogroup was the most widespread; 20 per cent of Puerto Rican cattle had antibodies to borincana. They also recorded the presence of antibodies to djatzi (bataviae group), pomona, icterohaemorrhagiae, ballum and grippotyphosa.

In Japan, antibodies have been recorded to australis, autumnalis and hebdomadis (Yamamato, 1951) icterohaemorrhagiae and grippotyphosa (Yanagawa et al., 1955), and pomona and canicola (Yanagawa, 1970). Yanagawa et al. (1958) claimed that autumnalis infection was responsible for much of the clinical bovine leptospirosis in Japan, and Iwata (1961) reported that of 67 cattle with haemoglobinuria, 45 developed antibodies to autumnalis, 11 to hebdomadis and 11 to australis.

In Taiwan, icterohaemorrhagiae infection appears to predominate (Young et al., 1963) but antibodies have also been recorded to pomona, autumnalis, australis and canicola.

Leptospiral infection of Australian cattle was first reported by Johnson (1942) who detected antibodies to mitis. Wannan (1955) reviewed the position up to that year and stated that antibodies to icterohaemorrhagiae, pomona, grippotyphosa, bataviae, tarassovi, and to members of the pyrogenes, australis and hebdomadis serogroups had been detected. Subsequently, Spradbrow (1964) described the presence of canicola and celledoni antibodies. In a review in 1970 (Anon.) it was stated that pomona was the only serotype known to cause clinical leptospirosis in Australia, but that tarassovi was also suspected to cause leptospirosis. Economic loss was said to result mainly from death in calves and abortion in cows. In recent years there have been a number of reports of abortion and mastitis due to infection with hardjo, a member of the hebdomadis serogroup, in Queensland (Sullivan and Callan, 1970), in Tasmania (Corbould, 1971) and in New South Wales (Hoare and Claxton, 1972).

Pomona infection was recognised in New Zealand (Te Punga and Bishop, 1953) associated with the full spectrum of clinical and sub-clinical disease. Kirschner (1954) reported the presence of antibodies to pomona in 20 per cent, to icterohaemorrhagiae in 8 per cent and to mitis in 3 per cent of cattle sera in a small abattoir survey. Dodd and Brakenridge (1960) reported an outbreak of icterohaemorrhagiae infection in calves. Recently, Lake (1973) reported finding antibodies to hebdomadis in 18 per cent of 890 cattle sera, to pomona in 5 per cent, to andamana in 1.7 per cent, to autumnalis in 0.9 per cent and to icterohaemorrhagiae in 0.5 per cent. Ris et al. (1973) reported ballum antibodies in the sera of healthy calves.

Leptospiral infections have been demonstrated serologically in cattle from northern, central and southern Africa. Mailloux (1970) reported antibodies in 27 per cent of 554 sera from Moroccan cattle; icterohaemorrhagiae infection was most common followed by infection with ballum, hyos, australis and hebdomadis. Brownlow and Dedeaux (1964) reported finding canicola,

icterohaemorrhagiae and bataviae antibodies in Egyptian cattle. In Central Africa, van Riel and van Riel (1955) found serological evidence of leptospiral infection in 42 of 124 cattle; the leptospires concerned were australis, bataviae, grippotyphosa, hebdomadis, pomona, icterohaemorrhagiae and butembo (cynopteri group). Van der Merwe (1967) recorded antibodies to pomona, australis, autumnalis, canicola, grippotyphosa, icterohaemorrhagiae, pyrogenes and saxkoebing (hebdomadis group) in South African cattle. So far only grippotyphosa has been reported as causing clinical disease in African cattle by Cordier (1953) in Tunisia, and Burdin et al. (1958) in Kenya.

Sawhney and Saxena (1969) reported the presence of antibodies to icterohaemorrhagiae, sejroe, canicola and grippotyphosa in Indian cattle and in the same country Rao (1970) reported antibodies to pomona, australis, autumnalis in the sera of aborting cattle.

Widespread clinical leptospirosis associated with grippotyphosa infection has been reported from Israel (Freund, 1947; van der Hoeden et al., 1953). Van der Hoeden (1953) described clinical leptospirosis due to canicola infection and to a member of the hebdomadis serogroup (van der Hoeden, 1964). The latter outbreak was characterised mainly by udder involvement.

Brewer et al. (1960) reported antibody titres to grippotyphosa, sejroe, autumnalis, semaranga, andamana and bataviae of 1:100 or over in Turkish cattle. Antibodies to sejroe (36 per cent) and grippotyphosa (15 per cent) were by far the most common.

In Europe serological surveys have been conducted in almost every country.

In Switzerland, Wikerhauser (1951) reported antibody titres of 1:400 or more to sejroe in 25 per cent of Swiss cattle and much smaller percentages of reactors to icterohaemorrhagiae, mitis, pomona, grippotyphosa and australis, and Weisman (1951) detected antibodies to ballum. Pomona antibodies were found in 4.3 per cent of 1,287 sera from aborting cattle in Switzerland during the period 1960-1963 (Burki, 1964).

Investigations in different parts of Germany have shown significant titres to icterohaemorrhagiae, grippotyphosa, sejroe, mitis and australis in bovine sera (Kathe, 1943, 1950, Rimpau, 1950, Schlossberger, 1951).

Borg-Petersen and Fennestad (1956) reported antibodies to icterohaemorrhagiae, canicola, ballum, pyrogenes, cynopteri, autumnalis, pomona, grippotyphosa, sejroe, bataviae, tarassovi and the javanica serogroup (poi and javanica) in Danish cattle.

Buchmeiser (1962) reported antibodies to grippotyphosa, sejroe, pomona and icterohaemorrhagiae in Austrian cattle.

Low levels of antibody (1:30 to 1:600) to icterohaemorrhagiae, pomona and grippotyphosa were detected in a survey of sera from Swedish cattle submitted for brucellosis examination (von Wendt, 1956) but no definite clinical cases had been demonstrated in Sweden up to that time.

In France, antibody titres of 1:100 or more to grippotyphosa, australis, icterohaemorrhagiae, pomona and hyos have been detected in cattle sera (Lataste-Dorelle, 1965, Gaumont, 1966). Lataste-Dorelle reported that australis antibodies were the most common (8.3 per cent of sera) followed closely by grippotyphosa (6.8 per cent), icterohaemorrhagiae (1.4 per cent) and pomona (0.7 per cent) but Gaumont recorded a higher frequency of reactors to grippotyphosa than to australis. Corrias and Valpreda (1966) noted the presence of sejroe antibodies in cattle imported into Italy from France.

Antibody titres to sejroe, bratislava (australis group), canicola, pomona, icterohaemorrhagiae, tarassovi, bataviae and pyrogenes have been recorded in Italian cattle (Farina, 1955, Farina et al., 1966, Ioli et al., 1967). Farina et al. (1966) pointed out that infection by the hebdomadis serogroup (sejroe and saxkoebing) is by far the most common infection in cattle in south east Italy, accounting for 70 per cent of the positive reactions.

Wolff and Bohlander (1952) found antibodies to icterohaemorrhagiae, grippotyphosa, canicola, sejroe, ballum and pomona in the sera of Dutch cattle; icterohaemorrhagiae and grippotyphosa were the most common.

Antibodies to pomona, grippotyphosa, tarassovi, saxkoebing and bratislava have been recorded in Greek cattle (Tomopoulos, 1967).

Sebek and Janicek (1964) detected antibodies to grippotyphosa, icterohaemorrhagiae, sejroe, bratislava, ballum, pyrogenes, pomona, bataviae and tarassovi in Czechoslovakia; 16.2 per cent of bovine sera examined had antibody titres of 1:400 or more to grippotyphosa, 14.5 per cent had antibody to sejroe and 5.6 per cent had antibody to icterohaemorrhagiae. Similar results were later obtained by Konrad and Vosta (1967).

Antibodies to poi, pomona, sejroe (Fuzi, 1962) and to grippotyphosa (Bodi et al., 1964) have been detected in the sera of Hungarian cattle.

Hebdomadis, grippotyphosa and cynopteri antibodies have been reported in Polish cattle (Zwierz et al., 1966; Ugorski, 1967).

In Bulgaria, antibodies to pomona, hebdomadis, grippotyphosa, mitis, poi, bataviae, icterohaemorrhagiae and australis have been reported (Popov and Rashev, 1964; Popov and Pavlov, 1967). Pomona infection was by far the most common.

Zaharija and Peric (1966) found leptospiral antibodies in 31.6 per cent of 3,372 cattle sera in Yugoslavia. Infection by hyos accounted for 40 per cent of these reactions, followed by sejroe (23 per cent), pomona (20 per cent), icterohaemorrhagiae (8.5 per cent), australis (3.7 per cent), grippotyphosa (3.3 per cent), ballum (0.8 per cent) and one case of canicola.

Sturdza et al. (1966) recorded titres of 1:400 or more in 30.4 per cent of 638 cattle in Romania. Infection by the hebdomadis serogroup was the most widespread and was responsible for 34 per cent of the reactions, followed by tarassovi (23 per cent), pomona (20 per cent) and bratislava, autumnalis, icterohaemorrhagiae, canicola, ballum and bataviae which accounted for the remainder.

In Britain, Michna (1967) reported the presence of antibodies to leptospires in 108 of 270 sera from Scottish cattle; 46 had antibodies to icterohaemorrhagiae, 37 to sejroe and 25 to ballum. Coghlan and Norval (1967)

also reported finding antibodies in 260 of 406 cattle sera collected at Edinburgh abattoir from animals from herds in various parts of Scotland, the north of England and Northern Ireland. They found antibodies to sejroe, ballum, bratislava and canicola but failed to find any evidence of infection by icterohaemorrhagiae apart from one serum that agglutinated both icterohaemorrhagiae and ballum at a dilution of 1:100. Like Michna, they found that a high proportion of cattle (20 per cent) had antibody to sejroe.

Michna (1971) reported the results of a larger survey (2,036 cattle sera) which again emphasised the high incidence of sejroe (hebdomadis serogroup) antibodies. In addition, he showed a much higher incidence of leptospiral antibodies in sera from cattle which had aborted and sera from herds with breeding problems, than in other groups of cattle.

In a survey of eight herds in the south of England, Twigg et al. (1972) reported low levels of antibody in 12.3 per cent of the cattle. Antibodies to canicola, icterohaemorrhagiae, ballum, erinacei auriti (autumnalis group), bratislava, grippotyphosa, bataviae, saxkoebing and poi were detected but titres of 1:100 or more were only found to icterohaemorrhagiae, ballum, erinacei auriti and bataviae.

Only two serogroups have been associated with clinical illness in cattle in the United Kingdom. Icterohaemorrhagiae infection of calves has been reported by Field and Sellers (1950), Ingram et al. (1952) and Baxter and Pearson (1956). Michna and Campbell (1969) and Michna et al. (1974) reported the isolation of members of the hebdomadis serogroup (sejroe and hardjo) from the kidneys of four cows with a history of abortion. An outbreak of mastitis accompanied by transient fever in a herd in the south of England infected by a member of the hebdomadis serogroup was described by Howell et al. (1969).

A survey of the literature has shown that antibodies to members of at least fifteen of the sixteen recognised Leptospira serogroups have been recorded in cattle sera. Antibodies to the andamana and semaranga groups

have also been demonstrated. Infection by some of these groups has been shown to be related to clinical disease, but only pomona, grippotyphosa, hebdomadis and icterohaemorrhagiae serogroup infections appear to be of worldwide significance, although others are of local importance e.g., canicola infection in Israel and autumnalis infection in Japan. Considerable variation occurs in the pathogenicity of strains belonging to the same serotype in different geographical locations. The severity of leptospiral infection in cattle shows a distinct correlation with the age of the animals involved; young calves appear more likely to develop acute disease than adults.

Disease Syndromes associated with Leptospiral Infection

It is proposed to describe the disease syndromes which have been associated with infection by the more important serogroups within the context of the three main syndromes defined in Chapter 1.

Pomona Infection

Pomona is the most important leptospiral pathogen in cattle in the U.S.A., Canada, Australia, New Zealand and parts of Europe. In outbreaks of pomona infection in susceptible cattle populations the full spectrum of clinical syndromes from peracute to sub-clinical may be found. Peracute infection is most commonly seen in calves and yearlings but it can occur in adult cattle. Outbreaks have been reported in Canada (Barnum and Grinyer, 1957), in Australia (Simmons et al., 1952, Wellington et al., 1953), in New Zealand (Ensor and McClure, 1953), in Hungary (Kemenes, 1956), in Yugoslavia (Zaharija, 1959), in Germany (Witting et al., 1967), and in the U.S.A. (Marsh, 1945, Mathews, 1946). Clinical signs include pyrexia, anorexia, jaundice, haematuria and haemaglobinuria. Mortality rates of up to 50 per cent have been recorded.

In adult cattle, in the U.S.A., abortion is often the most prominent clinical feature of pomona infections and usually occurs in the last

trimester (Bohl, 1955). It is usually observed two to five weeks following suspected exposure to infection with abortion rates of up to 50 per cent in susceptible herds (Morse et al., 1955). Abortion has also been reported in Denmark (Fennestad and Borg-Petersen, 1958a) and New Zealand (Te Punga and Bishop, 1953).

Mastitis has been noted frequently. Hengl et al. (1958) reported that, in adult cattle in Hungary, the only manifestation of disease was mastitis which lasted for a few days after a short febrile illness. In the U.S.A. Baker and Little (1948) isolated pomona from the milk of three cows and Mitchell and Boulanger (1959) recovered pomona from the milk of two cows in Canada.

Meningitis caused by pomona infection has been reported in Turkey by Gerlach (1956) and in the U.S.A. by Hoag and Bell (1954a). Stoenner et al. (1963) isolated pomona from the cerebrospinal fluid of a cow showing signs of meningitis.

Grippotyphosa Infection

Epizootics of acute disease have been described in Russia (Nikolajev, 1946), in Israel (Freund, 1947; van der Hoeden et al., 1953), in Hungary (Bodi et al., 1964), and in Kenya (Burdin et al., 1958). Bernkopf et al. (1947) reported the full range of acute, subacute, chronic and subclinical infection in cattle in Israel and pointed out that abortion was a common feature of infection. In the U.S.A. where clinical disease associated with grippotyphosa infection has not been recorded, Hanson et al. (1965) isolated the organism from the urine of two cows within 10 days of their having aborted. Gayot (1955) studied the condition in Tunisia and noted the occurrence of cutaneous ulcers in acute cases; ulcers have not been reported by other authors.

Hebdomadis Infection

In Australia and North America hardjo is the important member of the hebdomadis serogroup found in cattle and Stoenner (1968) reported that

hardjo is probably the second most common serotype in cattle in the U.S.A. It causes a mild disease characterised by a drop in milk production and by mastitis (Robertson et al., 1964; Sullivan and Callan, 1970) and abortion (Hanson and Brodie, 1967, Corbould, 1971, and Hoare and Claxton, 1972).

In the United Kingdom, Michna (1967, 1971) reported finding sejroe antibodies in the sera of cows which had aborted and Michna and Campbell (1969) reported the isolation of sejroe from the kidneys of a cow which had aborted. Howell et al. (1969) described an outbreak of mastitis in a herd associated with infection by a member of this group.

Acute clinical disease characterised by haemoglobinuria and associated with hebdomadis infection has been recorded in Japan (Inui et al., 1959; Iwata, 1961).

Icterohaemorrhagiae Infection

Outbreaks of infection by icterohaemorrhagiae have been recorded by Maria and Quevedo (1947) in Argentina, by Field and Sellers (1950) and Ingram et al. (1952) in England, by Baxter and Pearson (1956) in Northern Ireland, by Mantovani (1953) in Italy and by Markov and Ruibkina (1957) in Russia. Jaundice and haematuria were the outstanding clinical features and mortality was often high.

Dodd and Brakenridge (1960) described the clinical signs in some fifty cases of copenhageni infection in New Zealand. All the cases were in animals of less than twelve weeks of age. The highest incidence and mortality encountered in any one outbreak was 20 per cent and the morbidity and mortality rates were generally similar. The syndrome described in New Zealand differed from that recorded elsewhere in that jaundice was not a prominent clinical feature. Symptomless copenhageni infection of calves was observed in Italy by Babudieri and Gaspardis (1965) and in New Zealand by Ris et al. (1973).

Icterohaemorrhagiae infection has been associated with abortion in

South America. Santa Rosa et al. (1961) isolated the organism from an aborted foetus in Brazil as did Fernandez and Acosta (1966) in Peru.

Canicola Infection

Outbreaks of canicola infection have been reported in Israel (van der Hoeden, 1955; Bar Moshe, 1962). Canicola infection caused transient fever, inappetence, jaundice, haemoglobinuria and decreased milk yield in cattle, however in the majority of cases the infection remained clinically inapparent. Abortion seemed to be associated with canicola infection in heifers. Turner et al. (1958) reported the isolation of canicola from the urine of a two-day old calf which had developed jaundice, haemoglobinuria and listlessness within the preceding 24 hours. Infection had presumably taken place in utero. In the same country, Roberts et al. (1961) isolated canicola from an aborted foetus and Carroll (1966) reported the isolation of canicola from a pooled sample of blood and urine from a cow which aborted at mid-gestation.

Australis Infection

There are very few reports of australis infection associated with clinical disease. Kita et al. (1960) described the isolation of australis from the urine of a cow with haemoglobinuria in Japan. In Italy, Bellani and Ruggeri (1968) reported endemic abortions and infertility in cattle due to australis infection. Fennestad et al. (1967) produced a severe haemorrhagic nephritis syndrome with haematuria but without haemoglobinuria in four newborn and four three-week old calves experimentally infected with strains of bratislava isolated from Danish hedgehogs.

Autumnalis Infection

Clinical disease associated with autumnalis infection has only been reported in Japan where the full range of symptoms has been observed (Kawashima et al., 1954; Yanagawa et al., 1955; Kita et al., 1960; Iwata, 1961).

Bataviae Infection

Kiryanov (1968) recorded two outbreaks of acute bataviae infection in cattle, in the far east of the U.S.S.R., in one of which there was a mortality rate of 12 per cent.

Ballum Infection

Although antibodies to ballum have been recorded in cattle sera from many parts of the world, infection by this serotype has never been associated with disease and it has only recently been isolated from cattle. It was isolated by Ris et al. (1973) from the urine of two healthy calves in New Zealand.

Javanica Infection

Javanica infection has not been reported to cause natural outbreaks of clinical leptospirosis in cattle although Fennestad and Borg-Petersen (1956) produced abortion in one out of three heifers inoculated subcutaneously with a culture of poi which had been isolated from a mouse.

Semaranga Infection

Carroll and Le Clair (1969) reported the isolation of serotype patoc from the blood of a cow with signs of acute leptospiral infection and from the urine of another cow which had been mildly pyrexic and had shown a drop in milk production.

Andamana Infection

O'Brien (1974) reported the isolation of serotype andamana from the urine of a bull which showed stiffness of gait, a staring coat, inappetence jaundiced mucous membranes and a temperature of 104.5°F.

Tarassovi Infection (syn. mitis and hyos)

Infection by this serogroup(hyos)rs in many parts of the world, and in some countries large numbers of cattle may have antibodies to members of this group. Winks (1962) reported that 47.8 per cent of beef cattle in Central Queensland had antibody titres of 1:30 or more to tarassovi and in Yugoslavia, Zaharija and Peric (1966) reported that 13 per cent of cattle had

antibodies to hyos. Malakhov et al. (1973) reported the isolation of 27 strains of tarassovi from the kidneys of cattle in Russia. Despite their widespread occurrence and their pathogenicity for other animals and man, members of this serogroup have never been reported to cause clinical illness in cattle.

Other serogroups

While antibodies to them have been recorded in cattle sera they have never been incriminated as causal agents of cattle disease.

The Concept of Maintaining and Incidental Hosts in the Study of the Parasitic Leptospires

The concept of principal and incidental hosts has been inherent in the definition of zoonoses but Audy (1958) gave it precision by introducing the term "maintaining hosts" and drawing a clear distinction between maintaining and incidental hosts in the animal population. A maintaining host ensures the perpetuation of a particular local population of parasites without the intervention of other incidental hosts. An incidental host can acquire the parasite accidentally from other host-species which maintain the parasite.

Localisation of leptospires in the kidney, especially in the convoluted tubules, occurs as a sequel to infection of an animal by a Leptospira strain. The organisms form colonies in the lumen, are shed into the urine and are voided into the hosts' environment. This shedding into the environment is the most important factor in the spread of infection. The carrier and shedder state may last for a short time (1 - 4 weeks) - a convalescent (temporary) carrier state, or may persist for many months or years, even for the duration of the host's life (Turner, 1967). These long-term carriers and shedders are the reservoir or maintaining hosts. The state of biological equilibrium necessary for this long-term carrier state occurs much more readily between some strains of Leptospira and some animal species than between others. A particular

species of host may thus act as a reservoir of one or more serotypes of Leptospira but not of other serotypes. However, the same species of host may be infected by and become a temporary shedder of many serotypes, thereby acting as an incidental host for these serotypes.

Emmanuel et al. (1964) developed the concept of incidental and maintaining hosts in the study of the epidemiology of the parasitic leptospires. They suggested that leptospiral maintenance hosts could be recognised in one of two ways. The first approach is based on a knowledge of:

a) the total number of animals showing serological evidence of infection;

b) the excretion rate, which is the number of urinary excretors per 100 of the population examined;

c) the excretion index, which is the ratio of the number of animals excreting leptospires to the total number known to have been infected.

The alternative approach is based on the recognition of the fact that leptospires usually cause one of three grades of illness in a host.

I Severe with obvious illness and high mortality, the survivors becoming the chronic urinary carriers.

II Moderate to mild, with few clinical signs, usually ending in recovery and continued urinary excretion of leptospires.

III Inapparent with no clinical signs, sometimes becoming urinary carriers.

An animal is a good host for those serotypes which cause a grade II response. Those serotypes which cause a grade I response might survive as local populations (but probably with marked epizootic fluctuations if they did) while those in grade III, although not harming the host, are unlikely to reach the exterior in sufficient numbers to ensure maintenance of the population.

The review of the literature indicates that cattle can act as

maintenance and incidental hosts for a variety of Leptospira serotypes.

Excretion indices or excretion rates are impractical techniques in the study of bovine leptospirosis because of the shortcomings and difficulties associated with the isolation of leptospires from, and their demonstration in bovine kidneys and urine coupled with the organisational and financial difficulties of obtaining either sufficient bovine kidneys or urine samples.

The Present Investigation

This study records the results of a survey carried out to investigate the Leptospira serotypes which may infect Scottish cattle and, on the basis of (1) the number of animals showing serological evidence of infection and (2) clinical symptoms (if any) attributable to infection by the serotypes found, determine those serotypes for which Scottish cattle might act as maintenance and incidental hosts. An attempt was also made to correlate the clinical findings with the immunological status of the animals, environmental factors and management practices.

Materials and Methods

Blood Samples

Blood samples were collected from four groups of cattle:

a) cows at Glasgow and Ayr abattoirs;

b) individual cows which had aborted;

c) calves on a farm on which a case of clinical *icterohaemorrhagiae* infection had occurred; and

d) 29 herds.

a) *Abattoir samples.* A total of 402 blood samples were collected at Glasgow and Ayr abattoirs during the summer and autumn of 1972. The animals from which the samples were taken were all mature females which had calved at least once, and were being slaughtered for a variety of reasons, (principally old age, brucellosis and mastitis).

b) *Individual cows which had aborted.* Blood samples from cows which had aborted were submitted by the Veterinary Investigation Laboratories and by private veterinarians. These samples were from brucellosis-negative cases where no other cause of abortion had been found. A total of 511 samples were examined.

c) *Samples from calves.* Blood samples were collected from 18 calves on a farm where a case of clinical *icterohaemorrhagiae* infection had occurred in a calf.

d) *Herd samples.* Twenty-one herds in the study were located in south west Scotland, one on the island of Islay, six in north-east Scotland and one in Westmoreland (Fig. 6). Twenty of the herds were selected at random. The other nine herds were chosen because samples from individual animals had been found to contain antibodies to *sejroe*, or there had been an abortion problem of unknown aetiology on the farm.

Blood samples were collected from the adult female stock, replacement heifers and, occasionally, from calves, yearlings and bulls on these farms. The total number of cows and heifers involved was 2,687.

Fig. 6 Map showing the location of the 29 herds tested

The samples were either collected directly by the author or were obtained through the Veterinary Investigation Laboratories of the West of Scotland College of Agriculture at Ayr and Oban, and the North of Scotland College of Agriculture, Aberdeen.

Clinical History

Wherever possible the farm was visited and a detailed history obtained from the farmer, the local veterinary surgeon and the staff of the Veterinary Investigation Laboratory.

Serological Tests

Serum samples were tested against all 16 antigens, using the microscopic agglutination test.

Interpretation of Results

The results of the herd serological survey were analysed using the following criteria; an antibody titre of ++++ 1:10 or greater to sejroe was taken to indicate past exposure to infection by a member of the hebdomadis serogroup, and a titre of ++ 1:300 or more to indicate recent exposure to infection. The data was examined for possible relationships between the immunological status of the herd, various age groups within the herd and the pattern of disease, management and environmental factors on the farm.

An 18 month period was taken as a model in order to determine whether there was any connection between the prevalence of abortion of unknown aetiology and the immune status of the herd with respect to the hebdomadis serogroup of Leptospira, as indicated by the presence of antibody titres of ++++ 1:10 or more to sejroe. The period of study was from nine months before the adult female stock and heifers were tested until nine months after testing.

The findings in the abattoir sera and the sera from aborting cattle were considered with respect to findings in the herd survey, and the known host ranges of Leptospira serotypes in the United Kingdom at that time.

Meteorological Data

The data on rainfall and temperature was supplied by the Meteorological Office.

Results

Serological Survey

Leptospiral antibodies were detected in 1,766 (49.1 per cent) of sera in a survey of 3,600 sera from adult cows and heifers (Table 3).

Table 3: The total number of sera, from various groups of cattle, which had antibody titres to any serotype

Sera from:	No. positive (antibody to any serotype)	Total No. of sera in sample	Percentage Positive
Cows at the abattoirs	291	402	72.4
Individual aborting cows	255	511	50.0
Herd survey	1,220	2,687	45.4
Total number of cows and heifers tested	1,766	3,600	49.1

Antibodies to serotypes belonging to 10 Leptospira serogroups were found. Antibodies to sejroe (hebdomadis group) were the most prevalent and were detected in 1,503 (41.8 per cent) sera (Table 4). Antibodies to icterohaemorrhagiae (7.7 per cent) and to ballum (7.3 per cent) were the next most common, while antibodies to bratislava (2.3 per cent) (australis serogroup), javanica (1.9 per cent), canicola (1.8 per cent), panama (1.7 per cent), cynopteri (1 per cent), pyrogenes (0.4 per cent) and autumnalis (0.3 per cent) were detected in small numbers of animals. Antibodies to pomona, grippotyphosa or bataviae were not detected. The numbers of cattle with antibodies to a particular serotype and the distribution of the antibody titres found are summarised in Table 4.

Figures 7 and 8 illustrate the relative frequency of antibody titres of 1:10 or greater and 1:300 or greater to various Leptospira serotypes. They show clearly that infection by the hebdomadis serogroup was the predominant

Table 4. The distribution of antibody titres to Leptospira serotypes in 3,600 cow and heifer sera tested

Serotype	Antibody titres								No. positive	% positive
	1/10	1/30	1/100	1/300	1/1,000	1/3,000	1/10,000	1/30,000		
sejroe	94	321	493	356	180	47	11	1	1,503	41.8
icterohaemorrhagiae	90	111	62	13	2				278	7.7
canicola	25	23	13	2					63	1.8
ballum	50	164	41	6	2	1			264	7.3
bratislava	23	43	15	1					82	2.3
javanica	12	24	23	6		1	1		67	1.9
panama	20	30	6	3	1				60	1.7
cynopteri	13	20	2						35	1
autumnalis		8	1						9	0.3
pyrogenes	1	8	3	1					13	0.4

Fig. 7 The distribution of antibody titres (1 : 10 or greater) to Leptospira serotypes in all positive sera tested (Total no. 1,503)

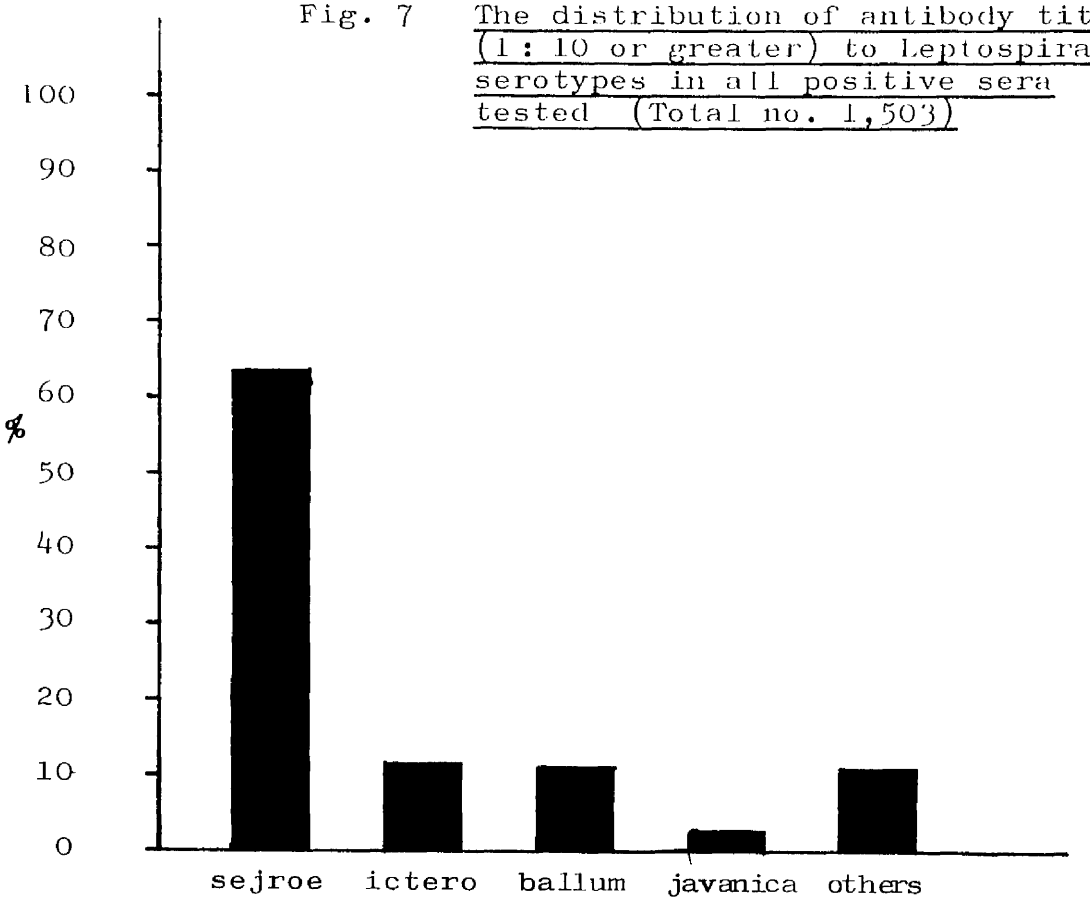
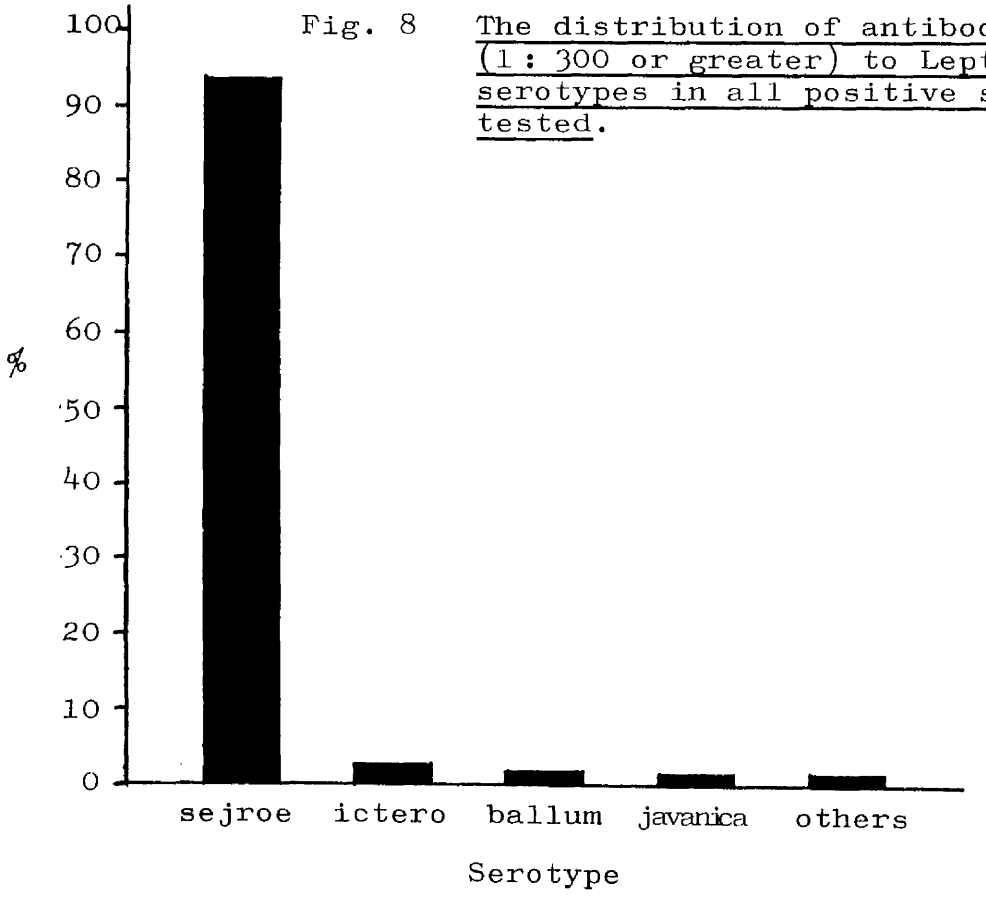


Fig. 8 The distribution of antibody titres (1 : 300 or greater) to Leptospira serotypes in all positive sera tested.



leptospiral infection in the cattle sampled. Antibody titres of 1:10 or greater to sejroe were present in 63.3 per cent of the positive sera, to icterohaemorrhagiae in 11.7 per cent, to ballum in 11.1 per cent and the remaining seven serogroups accounted for 13.9 per cent of the positive sera (Fig. 7). If antibody titres of 1:300 or more were considered alone then the importance of the hebdomadis serogroup relative to the other groups is much more noticeable (Fig. 8); it accounts for 93.7 per cent of reactors while icterohaemorrhagiae (2.4 per cent), ballum (1.4 per cent) and others (2.5 per cent) account for the remainder.

Four hundred and seventeen sera had antibody titres of 1:10 or greater to more than one serotype; however, only 5 sera had antibody titres of 1:300 or greater to two serotypes (Table 5).

Antibody titres of 1:300 or more were detected to eight serotypes, namely, sejroe, icterohaemorrhagiae, canicola, ballum, bratislava, javanica, panama and pyrogenes.

Table 5. The number of sera with antibodies to more than one serotype

Sera from:	Antibody titres to more than one serotype		
	1:10 or more	1:100 or more	1:300 or more
Cows at the abattoir	145 (49.8%*)	14	3
Individual aborting cows	47 (9.2%)	4	1
Herd survey	225 (8.4%)	52	1
Total	417 (23.6%)	70 (4%)	5 (0.3%)

* (%) refers to per cent of positive sera.

The proportion of cattle found to have leptospiral antibodies varied considerably in the three groups of adult cattle examined. In the herd survey sample 45.4 per cent (Table 3) of the cows had antibody to at least one Leptospira serotype, while in the sample of sera from individual aborting

cows 50.0 per cent had leptospiral antibodies and in the abattoir sample 72.4 per cent of sera had leptospiral antibodies. The proportion of sera with antibodies to more than one serotype varied considerably between the three groups (Table 5). 49.8 per cent of positive abattoir sera had antibodies to more than one serotype but only 9.2 per cent of positive sera from cows which had aborted and 8.4 per cent of positive herd sera had antibodies to more than one serotype.

a) Survey of abattoir sera

The number of sera with antibodies to the various serotypes and the distribution of antibody titres are enumerated in Table 6. Antibodies to eight serotypes were detected. Sejroe antibodies were the most common, being detected in 47.5 per cent sera, followed by ballum (30.1 per cent) and icterohaemorrhagiae (22.4 per cent) antibodies. Antibodies to javanica (6.5 per cent), panama (2.7 per cent), cynopteri (2.5 per cent), bratislava (1.5 per cent) and canicola (1 per cent) were detected less frequently.

The higher incidence of leptospiral antibodies in abattoir cattle than in the other two groups was largely due to the high incidence of ballum and icterohaemorrhagiae antibodies in that group (Fig. 9).

b) Sera from aborting cows

The number of sera with antibodies to the various serotypes and the distribution of antibody titres are listed in Table 7. Antibodies to nine Leptospira serotypes were detected. Antibodies to sejroe (41.3 per cent) were the most common followed by icterohaemorrhagiae (12.3 per cent). Antibodies to ballum (4.3 per cent), canicola (2.5 per cent), cynopteri (1.6 per cent), bratislava (1.2 per cent), javanica (1.2 per cent), panama (0.6 per cent), autumnalis (0.2 per cent) were detected in a small number of sera.

A javanica antibody titre of 1:3,000 was recorded in one case of abortion. Attempts to purchase this animal failed.

Table 6. The distribution of antibody titres to Leptospira serotypes in 402 sera collected from "cast" cows at the abattoir

Serotype	Antibody Titres							Total Positive	% Positive
	1/10	1/30	1/100	1/300	1/1,000	1/3,000	1/10,000		
sejroe	19	43	68	48	8	4	1	191	47.5
icterohaemorrhagiae	47	24	17	1	1			90	22.4
canicola	1	2	1					4	1
ballum	27	76	15	2	1			121	30.1
bratislava	2	3	1					6	1.5
javanica	5	10	9	1			1	26	6.5
panama	6	4	1					11	2.7
cynopteri	6	4						10	2.5

Fig. 9 The incidence of antibody to Leptospira serotypes in the sera of 3,600 cattle

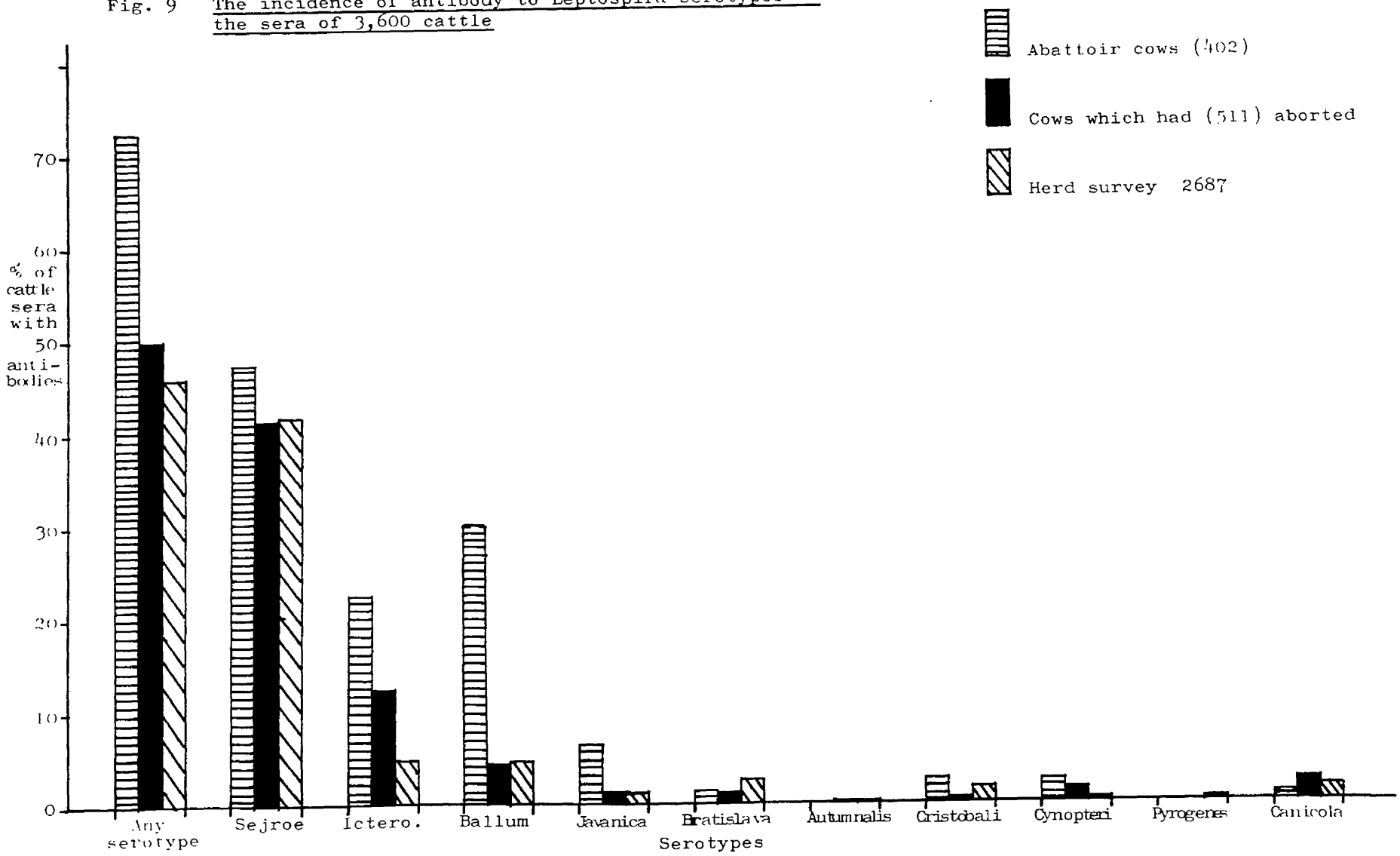


Table 7. The distribution of antibody titres to Leptospira serotypes in 511 sera collected from aborting cows

Serotype	Antibody titres								Total positive	% positive
	1/10	1/30	1/100	1/300	1/1,000	1/3,000	1/10,000	1/30,000		
sejroe	12	72	43	51	22	9	1	1	211	41.3
icterohaemorrhagiae	12	33	15	2	1				63	12.3
canicola	6	6	1						13	2.5
ballum	5	11	4		1	1			22	4.3
bratislava	2	2	1	1					6	1.2
javanica	2	1	1	1		1			6	1.2
cynopteri	5	2	1						8	1.6
autumnalis		1							1	0.2
panama	1	2							3	0.6

c) Sera from calves on a farm on which a case of clinical icterohaemorrhagiae infection had occurred

The case of clinical icterohaemorrhagiae infection was in a three month old calf which subsequently developed an antibody titre of 1:100,000. Six of eighteen other calves (3-5 months old) which had been housed with the clinical case had antibody titres to icterohaemorrhagiae ranging from 1:10 to 1:30,000.

d) Herd survey

Antibodies to ten serotypes were detected in sera examined in the course of the herd survey (Table 8). Antibodies to sejroe (41.0 per cent) were by far the most common. A small number of sera had antibodies to icterohaemorrhagiae (4.7 per cent), ballum (4.5 per cent), bratislava (2.6 per cent), canicola (1.7 per cent), panama (1.7 per cent), javanica (1.3 per cent), cynopteri (0.6 per cent), pyrogenes (0.5 per cent) and autumnalis (0.3 per cent).

Panama antibodies at titres ranging from 1:30 to 1:1,000 were found in six animals from the same herd. The only other reactors in this herd were three heifers with antibodies to sejroe. The absence of evidence of infection by other serogroups precludes the possibility of the reactions to panama being due to cross reactions to members of other serogroups.

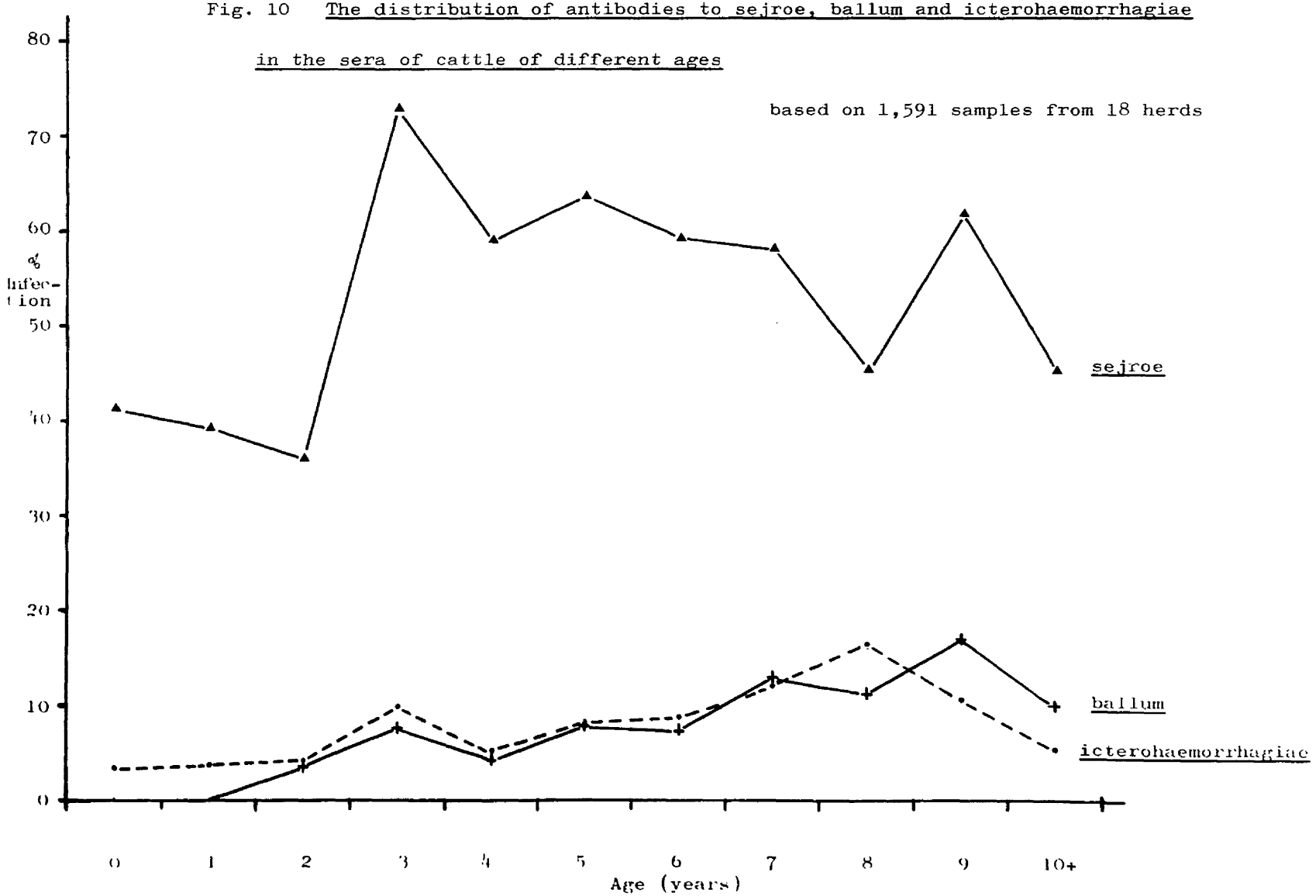
The incidence of antibodies to the hebdomadis serogroup (sejroe) in the herds tested ranged from 0 to 96.2 per cent (Table 25, Appendix 1) although the incidence in particular groups of animals on a farm could reach 100 per cent e.g., the heifers on farm LH4. Herds with a high incidence of antibodies to sejroe were widely distributed geographically. Infected herds were located on the islands of Bute and Islay, in N.E. and S.W. Scotland and in Westmoreland.

Incidence of antibody titres in cattle of various ages. Figure 10 shows the results of an analysis of the incidence of ballum, icterohaemorrhagiae and sejroe antibodies in cattle of different ages from 18 farms where the age of every animal tested was known.

Table 8. The distribution of antibody titres to Leptospira serotypes in 2,687 sera from cows and heifers in 29 herds

Serotype	Antibody titres							Total positive	% positive
	1/10	1/30	1/100	1/300	1/1,000	1/3,000	1/10,000		
sejroe	63	306	382	257	150	34	9	1,101	41.0
icterohaemorrhagiae	31	54	30	10				125	4.7
canicola	18	15	11	2				46	1.7
ballum	18	77	22	4				121	4.5
bratislava	19	38	13					70	2.6
javanica	5	13	13	4				35	1.3
panama	13	24	5	3	1			46	1.7
cynopteri	2	14	1					17	0.6
autumnalis		7	1					8	0.3
pyrogenes	1	8	3	1				13	0.5

Fig. 10 The distribution of antibodies to sejroe, ballum and icterohaemorrhagiae in the sera of cattle of different ages



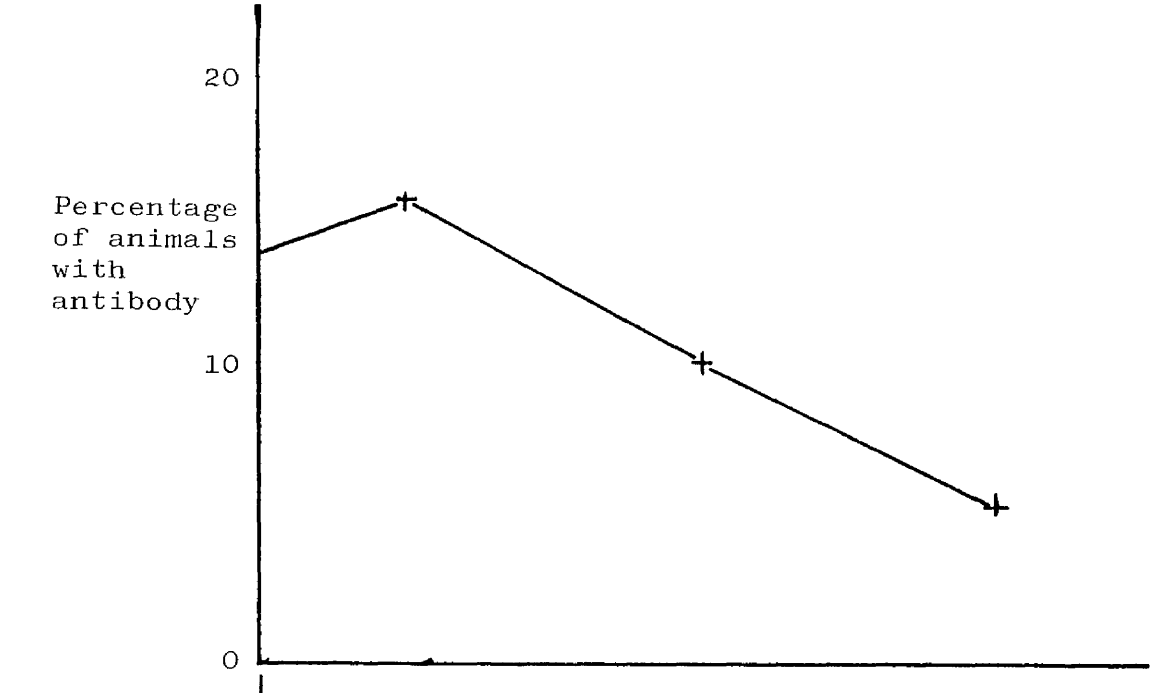
Antibodies to sejroe were found in a high proportion (37-72 per cent) of the animals. The major feature in the distribution of sejroe antibodies was the increase in incidence from 37 per cent in two year old heifers to 72 per cent in three year old cows. This increase in the rate of infection at this time reflects the acquisition of antibodies to sejroe by heifers as a result of infection following their introduction into the main herd before or after calving.

The incidence of antibodies to ballum and icterohaemorrhagiae was much lower than the incidence of antibodies to sejroe. Antibodies to both ballum and icterohaemorrhagiae showed a steady increase in incidence up to 8 years of age for icterohaemorrhagiae and up to 9 years for ballum and thereafter they declined. This slow increase in incidence with age represented the difference in incidence between those that had acquired antibody titres and those in which titres had disappeared.

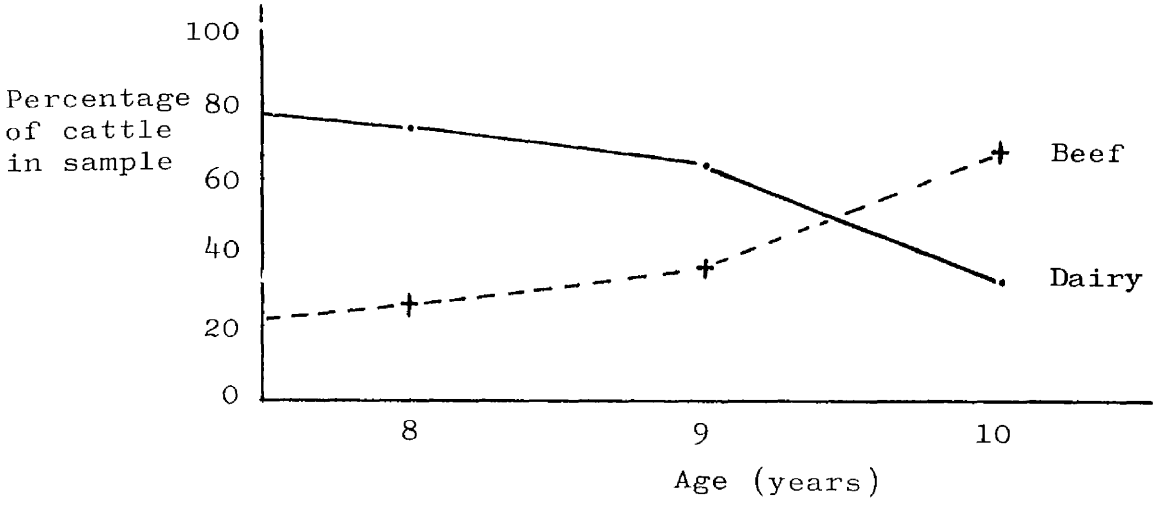
The incidence of icterohaemorrhagiae antibodies was higher in dairy cattle (11 per cent) than in beef cattle (2.7 per cent) in the 10 years and older age group (Fig. 11). The ratio of beef to dairy cows altered drastically (Fig. 11) in the older age groups (8 years and over). The decrease in the proportion of dairy cattle relative to beef cattle in the older age groups coupled with a higher incidence of antibodies to icterohaemorrhagiae in dairy cattle would account for the decline in the overall incidence of icterohaemorrhagiae antibodies (Fig. 10) in the older age groups.

The increase in incidence of ballum and icterohaemorrhagiae antibodies with age may explain (1) the greater incidence of ballum and icterohaemorrhagiae antibodies in abattoir sera as most of the abattoir samples were obtained from old cows, and (2) the much higher incidence of multiple antibody titres in the abattoir cows when compared with the other groups.

Fig. 11 (a) The percentage of cattle in different age groups which had antibody to icterohaemorrhagiae.



(b) The ratio of beef to dairy cattle.



A Clinical Investigation into Infection by the Hebdomadis Serogroup and Attempts to Correlate the Clinical and Serological Findings

The details of the individual herds are summarised in Table 26 (Appendix 1).

Clinical history obtained from the owners of infected herds and from private veterinary surgeons

The most consistent feature reported was the presence of abortion of unknown cause in the latter half of gestation and of premature birth. The incidence was generally low and the pattern suggested that the condition was self limiting. In some herds the picture was often of a spate of abortions in which up to 10 per cent of the cows aborted over a period of several months. This was followed by a period of several years during which abortions did not occur or occurred only sporadically and which was in turn followed by another small spate of abortions. In other herds, abortion recurred every year in certain age groups of cows.

A number of owners of infected herds complained of a high rate of retained foetal membranes but in the course of the investigation it was impossible to form an objective assessment of whether this occurred more frequently in infected than in uninfected herds.

On one farm (LH26) a "mastitis-like" syndrome was noticed in several groups of cows by the farmer. It was characterised by a drop in milk yield and the presence of blood-tinged milk in all four quarters of the udder. The udder did not become hard and taut but remained soft. Intra-mammary penicillin therapy produced a rapid response.

The incidence of antibodies to sejroe in herds with a history of abortion of unknown aetiology

The incidence of antibodies to sejroe in herds with a history of undiagnosed abortion was 64.9 per cent (Table 9). This was considerably higher than in the other groups of cattle tested, especially those herds with no history of abortion (16.0 per cent). Of more significance, probably,

Table 9. The incidence of antibody titres to sejroe in various groups of cattle

	No. Positive		Total No. of Animals	Percentage Positive	
	1:10 or more	1:300 or more		1:10 or more	1:300 or more
1. Abattoir cows	191	61	402	47.5	15.2
2. Individual aborting cows	211	84	511	41.3	16.4
3. Herds with history of abortion	843	425	1,299	64.9	33.7
4. Herds with no history	80	18	279	28.6	6.5
5. Herds with no history of abortion	178	7	1,109	16.0	0.6
6. All herds	1,101	450	2,687	41.0	16.7
7. All cows and heifers tested	1,503	595	3,600	41.8	16.5

Line 6 was obtained by adding lines 3, 4 and 5.

Line 7 was obtained by adding lines 1, 2 and 6.

was the much higher incidence (33.7 per cent) of sera with a titre of 1:300 or more in herds with a history of abortion than in other groups (Table 9). Only 7 cows (0.6 per cent) of 1,109 cows from herds with no history of abortion during the 18 month model period had a titre of 1:300 or more to sejroe.

The relationship between the incidence of abortion and the incidence of sejroe antibodies

Abortions, on the infected farms, were reported under the Brucellosis Eradication Scheme, and, during the 18 month model period, varied from 0 to 14 per cent per 9 month period (Fig. 12).

The herds examined could be divided into three groups on the basis of the incidence of antibodies to sejroe at titres of 1:10 or greater and 1:300 or greater, and on the incidence of abortion in the 9 months prior to sampling and the 9 months after sampling.

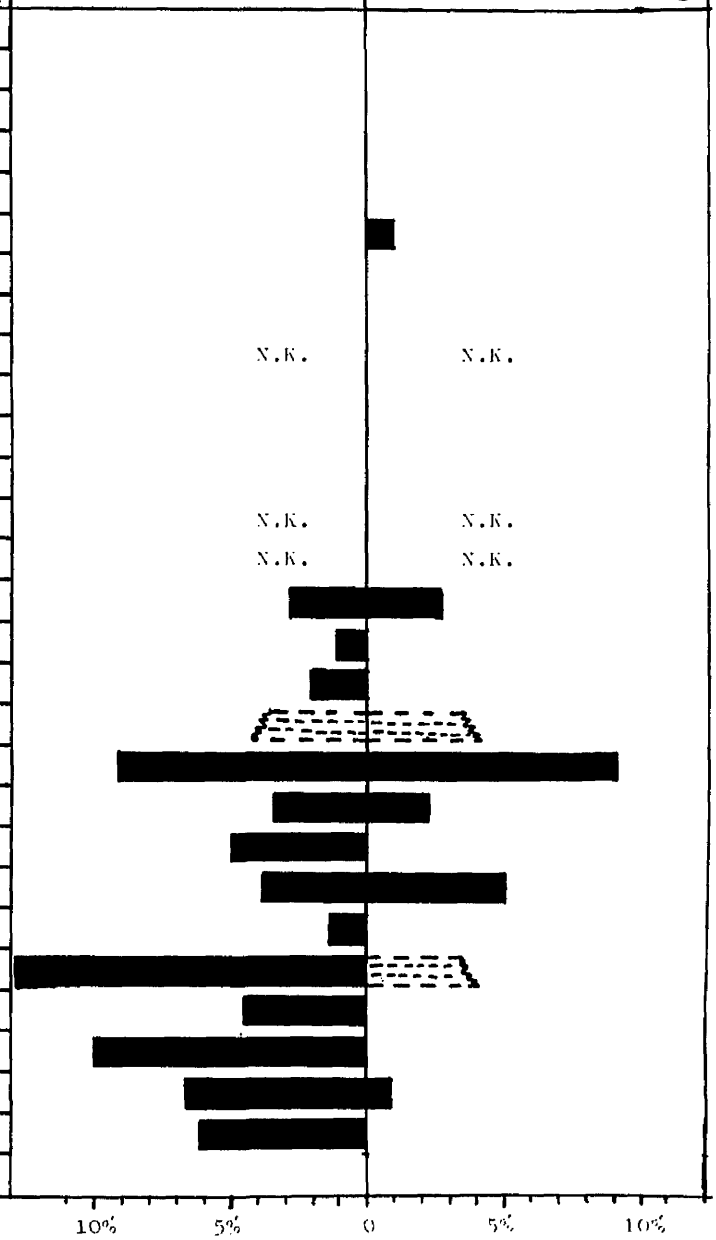
The first group consisted of herds in which there was either no serological evidence of infection or in which up to 40 per cent of the animals possessed antibody to sejroe but in which less than approximately 8 per cent of the total showed evidence of recent exposure i.e. had antibody titres of 1:300 or more. In this group abortion of unknown cause was virtually absent.

The second group (Fig. 12) consisted of herds in which the incidence of sejroe antibodies of 1:10 or more ranged from about 40 per cent to about 70 per cent and the incidence of antibody titres of 1:300 or greater ranged from 10 per cent to 30 per cent. In these herds abortion took place both in the periods before and after testing.

The distinction between the groups was not absolutely clear cut, for example, herds LH27 and LH11 fell between categories 1 and 2 in that, while the incidence of antibody titres of 1:10 or more to sejroe (44.7 per cent and 51.3 per cent respectively) suggested a group 2 clinical picture, the low incidence (5.9 per cent and 5.1 per cent) of titres of 1:300 or more

Fig. 12 The relationship between the incidence of abortions and the incidence of sejroe antibodies on the 29 farms during the 18 month model period.

Group	Herd Survey No.	Percentage of cattle with antibodies to sejroe		Percentage of cows in a herd aborting during the 18 month model period	
		1 : 10 or more	1 : 300 or more	9 months before testing	9 months after testing
Group 1	LH 2	0	0		
	LH 3	0	0		
	LH 15	0	0		
	LH 5	1.8	0		
	LH 8	2.0	0		
	LH 16	2.4	0		
	LH 6	4.7	0		
	LH 22	8.2	0		
	LH 10	14.2	0.9	N.K.	N.K.
	LH 18	14.8	0		
	LH 19	21.7	0		
	LH 21	22.1	7.4		
Group 2	LH 7	37.5	10.4	N.K.	N.K.
	LH 17	37.7	9.1	N.K.	N.K.
	LH 25	43.3	10.0		
	LH 27	44.7	5.9		
	LH 11	51.3	5.1		
	LH 9	51.5	37.6		
	LH 29	54.5	27.3		
	LH 12	58.2	18.0		
	LH 14	61.5	15.4		
	LH 23	69.1	30.9		
Group 3	LH 20	69.1	29.1		
	LH 1	70.9	29.1		
	LH 13	72.2	21.1		
	LH 26	84.3	27.0		
	LH 28	84.3	37.2		
	LH 24	89.6	33.3		
	LH 4	96.2	40.4		



The herds are listed in order of ascending values for the incidence of sejroe antibodies (1 : 10 or more) in the serum samples.

N.K. = History unknown

Abortions occurred but the exact number was not known

indicated a group one picture. The clinical picture was in fact intermediate between that of groups one and two as only one abortion occurred in each herd in the 9 month period before testing and no abortions occurred in the period after testing.

The third group consisted of herds in which the incidence of infection was more than approximately 70 per cent. In these, abortions took place in the period prior to sampling but were uncommon or did not occur at all in the period after testing, i.e., the infection was self-limiting. Examples were herds LH13, LH26 and LH 24 (Fig. 12).

In two herds (LH1 and LH23) with high rates of infection, infection was self-limiting in the older cattle but abortions in the herds continued because management practices were such that batches of susceptible animals were introduced into the herds at regular intervals.

It would appear therefore that:-

(1) For abortion to occur there has to be a mixed population of recently infected cattle (as evidenced by antibody titres of 1:300 or more) and susceptible animals.

(2) A threshold level of active infection may be necessary before abortions occur in a herd.

(3) Once all the animals in a herd have been exposed to infection, abortions cease to occur.

The incidence of abortion in various age groups of cattle

In some herds, abortion was limited to certain age groups, while in others, abortions occurred in cattle of different ages. The restriction of abortion to a given age group of cattle could often be attributed to the system of management on the farms concerned which ensured the exposure to infection of a particular age group of susceptible animals. In many areas of Scotland it is common practice for the heifer replacement stock to be kept either on a separate farm from the main herd, or on a different part of the same farm. This was the practice on a number of farms included in

this survey in which infection by the hebdomadis serogroup was enzootic. The heifers were invariably susceptible (seronegative) to infection when they returned to the main herd. The clinical picture seen depended, to a certain extent, upon the stage of gestation reached by the heifers when introduced into the main herd. Examples were seen in:-

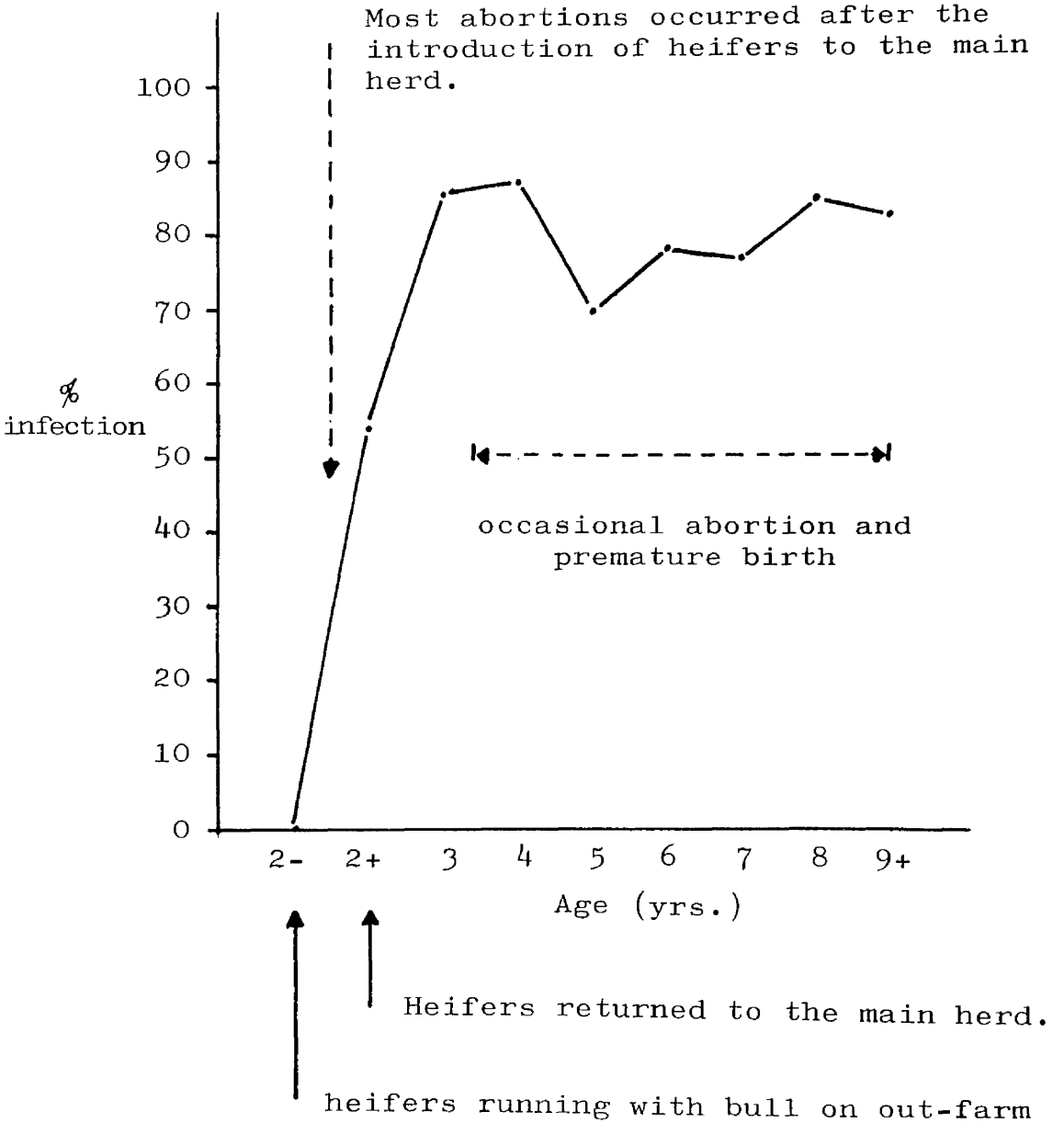
(1) Herd LH28 - Abortions occurred largely in the heifers. Seronegative heifers were returned to the main farm after running with the bull on an out-farm at the end of October, by which time they were 3-5 months pregnant. During the next 5 months, abortions occurred. In one batch of 24 heifers, 7 aborted (all had developed antibodies to sejroe). Infection was enzootic in the main herd; 84.3 per cent of animals had antibodies to sejroe. The serological status of various age groups of cattle within this herd is summarised in Figure 13.

(2) Herd LH23 - Abortions occurred mainly in the heifers. As in herd LH28 the heifers were initially kept separate from the main milking herd in which infection was enzootic (Table 26, Appendix 1). The heifers were introduced into the main herd while in the latter half of gestation and abortions occurred.

(3) Herd LH1 - Abortions occurred mainly during the second gestation period. The heifers were kept on a separate farm which was free from infection with the hebdomadis serogroup and were seronegative on their return to the main herd at calving in late winter and early spring, and most abortions occurred during the following gestation period (the second).

The pattern of infection in herd LH4 contrasted markedly with the above examples. Yearling and replacement heifers were kept with the milking cows under very damp conditions and infection was enzootic in all age groups; 96.2 per cent of the cows and 100 per cent of the heifers had antibodies to sejroe. Abortions did not occur in any age group, although one live premature birth was reported. The immunological status of the various age

Fig. 13 The percentage of animals with antibodies to sejroe in different age groups in herd LH 28



groups with respect to sejroe is summarised in Figure 14.

In herd LH26 infection was not related to age. Seven of 52 cows of various ages aborted during the four months prior to testing and a "mastitis", similar to that ascribed to leptospiral infection, was seen in groups of cows during this period. 82.7 per cent of the adult cows and 88.9 per cent of the heifers had antibodies to sejroe when tested and 27 per cent of animals had antibody titres of 1:300 or more. Cows and heifer stock were kept together on this farm. No abortions occurred subsequent to testing. This picture of abortion in all age groups, the occurrence of the mastitis-like syndrome and the relatively high incidence of antibody titres of 1:300 or more indicated that an epizootic of infection had occurred in the herd in the period prior to testing and that by the time of testing the herd had become immune and abortions stopped.

The stage of gestation at which abortion occurred

While it was the impression of farmers and private veterinary surgeons that abortion occurred in the second half of gestation it was impossible to obtain detailed information on the stage of gestation for many of the abortions, as Veterinary Investigation Laboratory records at the time of the investigation merely indicated whether it occurred before or after 256 days.

In herd LH23, 45 abortions were reported between the 1st January, 1971, and 30th April, 1973. Twenty five of these occurred in the last month of gestation, one occurred in the sixth month, one occurred in the seventh month and the remaining eighteen occurred between the sixth and eighth months of gestation. No abortions occurred before the sixth month.

Other factors which appeared to modify the clinical picture

Factors, other than those mentioned above, appeared to influence the incidence of abortion, given that infection was active within a herd and that there was a susceptible population of pregnant animals. They were:-

a) Temperature, Rainfall and Season. The lowest seasonal incidence of abortion coincided with the period of maximum temperature and minimum rainfall for the area (Fig. 15) while the period of maximum

Fig. 14 The percentage of animals in different age groups with antibodies to sejroe in herd LH 4

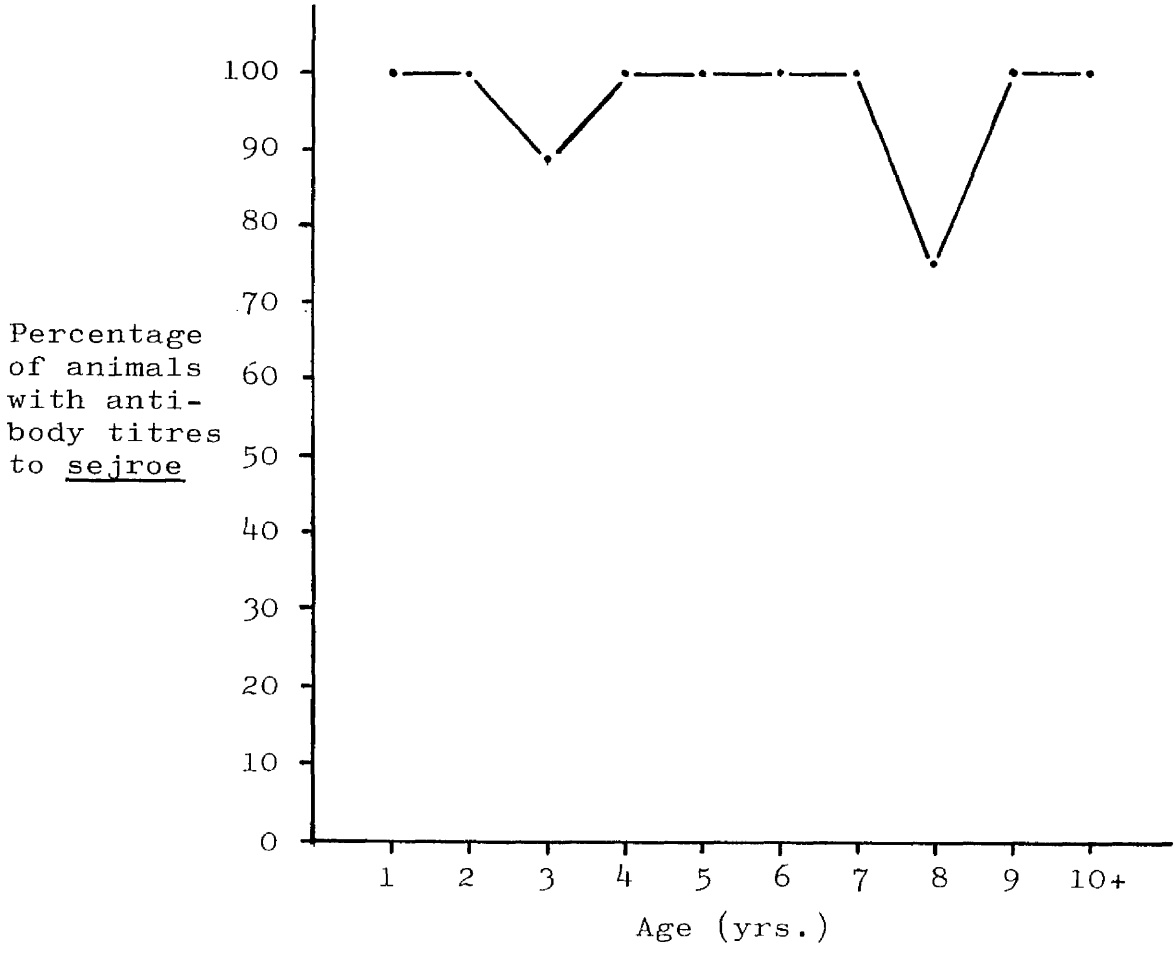
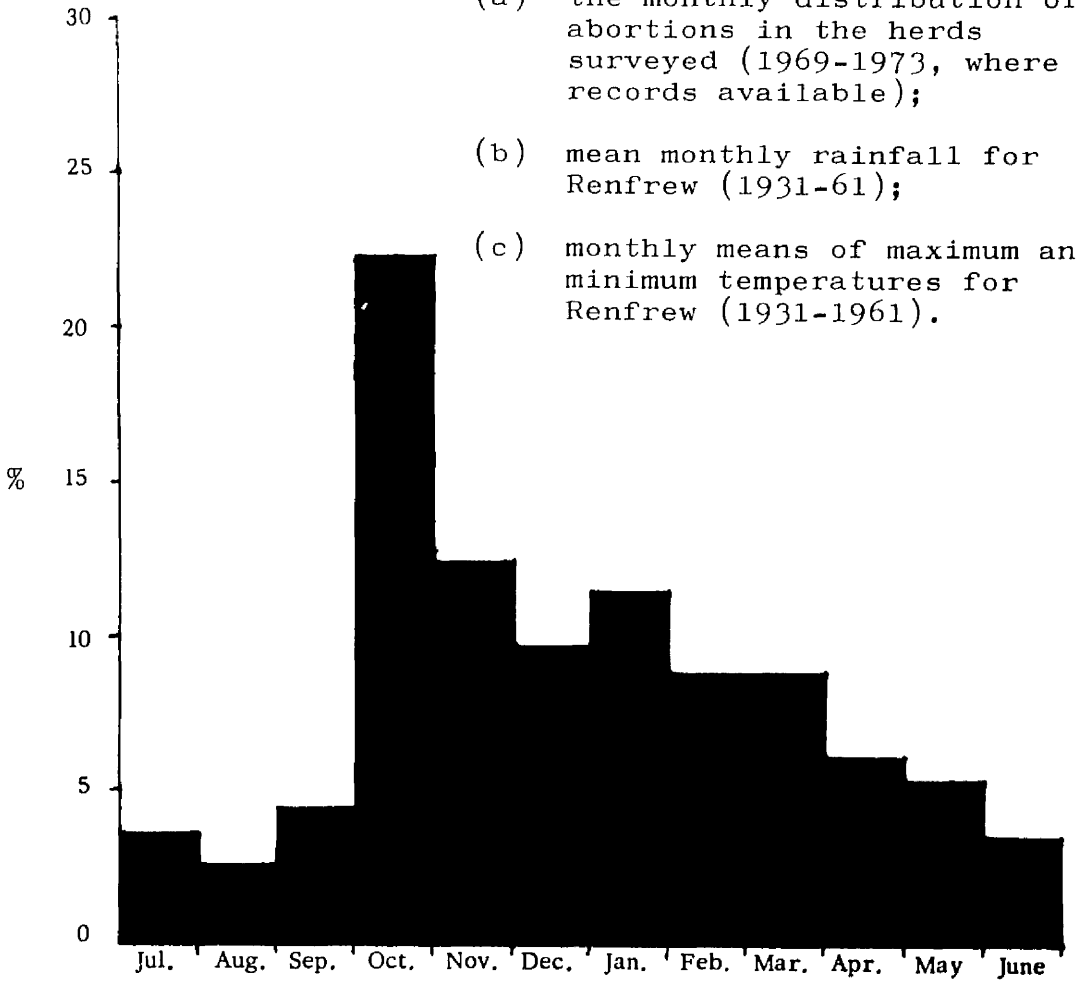


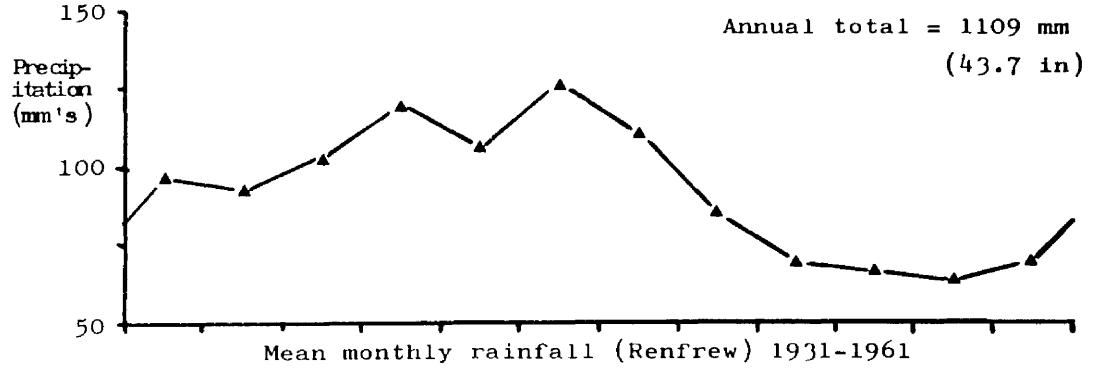
Fig. 15 A composite diagram illustrating:

(a) the monthly distribution of abortions in the herds surveyed (1969-1973, where records available);

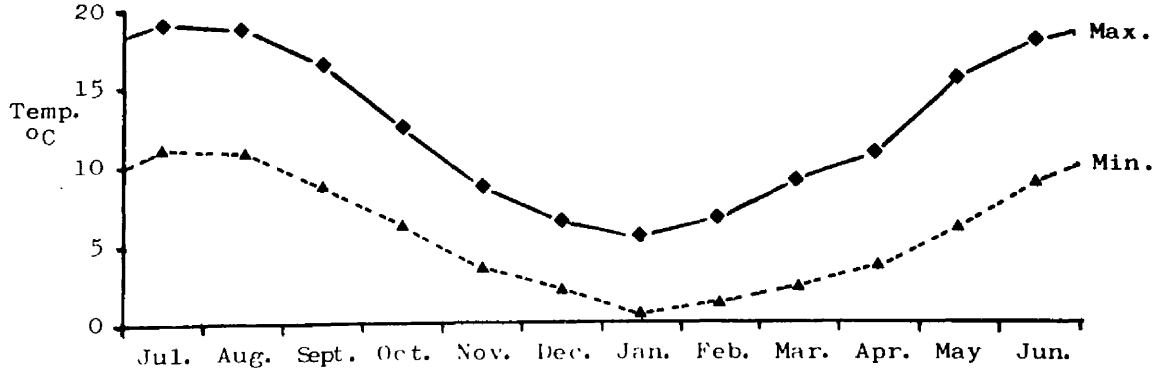
(b) mean monthly rainfall for Renfrew (1931-61);

(c) monthly means of maximum and minimum temperatures for Renfrew (1931-1961).





Mean monthly rainfall (Renfrew) 1931-1961



Monthly means of maximum and minimum temperatures (Renfrew) 1931-1961

incidence occurred when temperatures were falling but had not reached the winter low and when rainfall was at its highest.

b) Concentration of animals. The lowest seasonal incidence of abortion occurred when cattle were at grass and were therefore more widely dispersed, urine dilution was maximal, and the chance of cow to cow transmission was lowest.

The peak seasonal incidence occurred when cattle had either been brought indoors for the winter (Fig. 15) or, when, if they were still at grass, they spent most of their time around the small area where they were given supplementary feeding. This area often became heavily poached and there was an accumulation of surface water. In any case, the cattle spent all, or part of the day, in a confined area with a high cattle density and the maximum chance of cow to cow transmission of infection. This suggests that cow to cow transmission was the most important method of spread within the herd.

Other points of epizootiological interest

Contact between infected cattle and susceptible cattle was not always direct, e.g., in herd LH28 the heifers were not introduced into the herd as such but were kept in a paddock with a self-feed silo where their only source of drinking water was a small stream into which drained the effluent from the covered yard housing the main herd (84.3 per cent had antibodies to sejroe).

Of the nine herds where infection was either absent or there were no animals with titres of 1:300 or more to sejroe, seven were either closed or virtually closed herds.

In two herds (LH16 and LH18) the only animals with titres to sejroe were bought-in animals and in no case was the antibody titre greater than 1:100.

Clinical Disease Associated with other Serogroups

Javanica

In the survey of sera from cows which had aborted antibodies were detected at a dilution of 1:3,000 to javanica. Attempts to purchase this cow were unsuccessful.

Icterohaemorrhagiae

During the course of the study icterohaemorrhagiae infection was detected in calves on one farm. A 3 month old calf was admitted to the Veterinary School with marked jaundice of the sclera, gingivae and skin. It recovered following treatment with penicillin, streptomycin and electrolytes. It developed an antibody titre of 1:100,000 to icterohaemorrhagiae. None of the calves on the farm of origin showed evidence of jaundice however, two of the calves (titres 1:30,000 and 1:10,000) were very stunted. The farmer had noticed that these calves were off colour and had treated them with penicillin.

The calves were kept in three pens in an old grey stone building and were bedded on straw on top of approximately 12-15 inches of manure. The areas around the drinking bowls were very wet and there were a number of rat holes in the crumbling mortar between the stones in the wall of the building.

Other serogroups

No clinical symptoms could be attributed to infection by strains belonging to any other serogroup.

Discussion

The Significance of the Serological Findings

The microscopic agglutination test using live antigens is the most sensitive test for leptospiral antibodies available. Since serotypes which belong to the same serogroup cross react at higher titres, it is not necessary to identify the individual serotypes present in a country before embarking on a serological survey, provided that antigens belonging to all the known serogroups are used. The information obtained during such a survey will indicate the serogroups to which strains likely to cause infection of the host species being surveyed belonged and their relative importance. Where a serogroup is not known to occur, the selection of a serotype to represent that serogroup as a test antigen may not be entirely appropriate. For example, cattle sera were found to react with sejroe in the United States; eventually strains of hardjo not sejroe were isolated, and the same happened in Canada (Turner, 1968). As strains isolated in the west of Scotland were shown to be closely related to sejroe (Michna and Campbell, 1969), sejroe was used to represent the hebdomadis serogroup in this survey, however, the subsequent isolation of serotype hardjo (204 strain) from a cow in the same area (Michna et al., 1974) may mean that hardjo is the important serotype infecting cattle in this area. The use of sejroe rather than hardjo may mean that the maximum titres observed were slightly lower and the overall incidence of antibodies to the hebdomadis serogroup slightly lower than they would have been had hardjo been used. This lower incidence of cattle with antibodies would result from the failure of sejroe to detect low levels of antibody to hardjo. As serotypes hardjo and sejroe are very closely related serologically this decreased ability to detect low levels of hardjo antibody may be more imaginary than real, and subsequent experimental results (Chapter 4) bear this out.

The argument that the serotypes employed to represent the various serogroups may not be those most appropriate applies to all the other

serogroups, as well as the hebdomadis serogroup. Consequently, the overall incidence of cattle having antibodies to members of the various serogroups may well have been lower than in fact they were.

The total number of animals having antibody to a Leptospira serogroup was also lowered by the screening technique employed in the investigation. The lower screening dilution used was 1:30 consequently only sera which caused slight agglutination at 1:30 in the screening test were repeated at a dilution of 1:10 and may subsequently have been found to have an antibody titre of ++++1:10. If there was less than 50 per cent agglutination in the 1:30 dilution these sera were recorded as having titres of 1:10.

The importance of this lack of sensitivity was negligible as the main points to be ascertained from the serological survey were: firstly, which serogroups were present in Scottish cattle; secondly, the relative importance and approximate incidence of the various serogroups so that an assessment could be made as to whether cattle could act as maintenance or incidental hosts for strains belonging to them; thirdly, the immune status of the herds with respect to the hebdomadis serogroup.

Many authors have used an arbitrary "significant" titre when reporting the results of serological surveys. Only titres equal to or greater than this significant titre were included in their results. The titres used have varied from 1:30 to 1:400, with 1:100 being that most commonly used. The level chosen was quite arbitrary and was often a matter of expedience, the minimum dilution used in the screening of the sera being used (Turner, 1968). The use of an arbitrary significant titre can be of practical value in (1) eliminating many low antibody titres which may result from cross reactions and (2) recording those antibody titres most likely to have resulted from recent exposure to infection.

For the purposes of this survey a titre of ++++1:10 or greater was taken as signifying the presence of residual antibodies or early infection by a leptospire. Such a figure was regarded as significant by Turner (1968) and

no one has yet demonstrated an alternative "non-specific" cause of such reactions. The use of this base titre proved very useful in determining the immune status of a herd when attempting to correlate the clinical and serological findings within a given herd. The results of Stalheim (1971) suggest that cattle may still be resistant to reinfection when their serum antibody titres have fallen below 1:10.

An arbitrary titre of 1:300 proved valuable in this investigation, firstly, as a means of establishing those serological reactions which were unlikely to be due to cross-reactions by strains belonging to a different serogroup (only 5 sera out of 1,766 positive sera had titres of 1:300 or more to two serotypes) and, secondly, as a guide to whether infection was active within a herd. The presence of such an antibody titre does not mean that the animal is actively infected by and excreting leptospires of that serogroup, just as the converse is also true, i.e., an animal can be excreting leptospires but have no detectable serum antibodies to that leptospire (Seibold et al., 1961; Gorshanova, 1966, 1969). The presence of antibody titres of 1:300 or more is a useful guide to the number of animals which have recently been exposed to infection.

Cattle may be infected by any of the parasitic leptospires which exist in their environment. These will come directly or indirectly from two sources; either other carrier cattle or other hosts which inhabit the same environment. Since strains belonging to the hebdomadis, icterohaemorrhagiae, canicola, ballum, australis, javanica and autumnalis serogroups of Leptospira have been isolated in the United Kingdom (Table 10) it was to some extent predictable that some cattle would have antibodies to some or all of the above serogroups, especially as the known hosts for these are found in the rural environment.

Strains belonging to the panama, cynopteri and pyrogenes serogroups have, so far, not been isolated from animals infected in this country, although a strain belonging to the pyrogenes serogroup has been recovered

Table 10. Strains of Leptospira isolated in the United Kingdom and their sources*

SEROGROUP	SEROTYPE	HOSTS
Icterohaemorrhagiae	copenhageni	Man, rats, dogs, cattle.
	icterohaemorrhagiae	Rats.
Javanica	Not determined	Hedgehogs.
Canicola	canicola	Man, dogs, pigs.
Ballum	Not determined	White mice, field mice, voles.
Autumnalis	erinacei-auriti	Water vole.
Australis	bratislava	Hedgehog.
	Not determined	Hedgehogs, voles, field mice.
Hebdomadis	Not determined	Field mice, voles, cattle.

* from Turner (1969).

Also members of

(1) the Ballum serogroup from a rat (Michna and Ellis, 1974);

(2) the Icterohaemorrhagiae serogroup from piglets (Field and Sellers, 1951);

and

serotype hardjo from a cow (Michna et al., 1974).

in the United Kingdom from a human infected while in Jamaica (Turner, 1974). The finding that antibodies to these groups were present in cattle sera examined in this survey cannot be dismissed on the grounds of their being cross-reactions as the titres to them were often present in sera in which antibodies to other serotypes of Leptospira were not found, for example, antibodies to panama, with titres ranging from 1:30 to 1:1,000, were found in six cows in a herd (LH6) in which the only other reactors were three heifers with antibodies to sejroe. It would seem probable therefore, that members of the panama, pyrogenes and possibly cynopteri serogroups exist in Scotland.

The Epizootiology of Infection with the Hebdomadis Serogroup

The high incidence of antibodies to sejroe observed in this survey (41.8 per cent) confirms the findings of Michna (1967, 1971) and Coghlan and Norval (1967), in smaller surveys, that infection by the hebdomadis serogroup is the most significant leptospiral infection of Scottish cattle. The isolation of serotype hardjo from a cow by Michna et al. (1974) mentioned above suggests that hardjo may be the important serotype of the hebdomadis serogroup infecting cattle. It is probable that Scottish cattle act as maintaining hosts for hardjo (or closely related strains) as they fulfil two of the criteria suggested by Emmanuel et al. (1964) for the identification of maintaining hosts; firstly, a high incidence of antibodies to the organism and a mild clinical syndrome, and secondly, that cow to cow transmission appears to be the most important method of transmission within an infected herd.

Michna (1971) and Hoare and Claxton (1972) noted a much higher incidence of antibodies to sejroe and hardjo respectively in Brucella negative herds with a history of abortion than in healthy herds and Robertson et al. (1964), Sulzer et al. (1964), Michna and Campbell (1969) and Hoare and Claxton (1972) have reported the occurrence of abortion in herds infected by strains belonging to the hebdomadis serogroup. The simple

demonstrating the close association between the incidence of cows with antibody titres of 1:300 or higher to sejroe and the incidence of abortion in a herd of unknown cause, provided that there was also a population of susceptible animals (Fig. 12). This finding suggests that, in Scotland, leptospirosis due to strains belonging to the hebdomadis serogroup (probably hardjo) may be an important cause of bovine abortion of unknown aetiology. There are several factors which may limit such a conclusion. The first factor is the sample size; only 29 herds were studied and of these 9 were selected on the basis of either the presence of known serologically-positive animals in them or the presence of an abortion problem of unknown cause on the farm. The second factor is that 5 of the herds in which abortions were not reported were hill beef herds in which abortions may have remained undetected. The third factor is that the method of determining whether abortion had occurred depended on the willingness of the farmer to report any abortions which occurred in compliance with the terms of the Brucellosis Eradication Scheme. The fourth factor is that no detailed information was available for three herds, while only partial information was available for a further two herds (Fig. 12).

There is insufficient information in the literature to allow a proper comparison to be made of the abortion rates observed in infected herds in Britain with those observed abroad. Sulzer et al. (1964) and Robertson et al. (1964) reported abortions in infected herds but the number of animals were small. Hoare and Claxton (1972) reported abortion rates of up to 20 per cent in individual herds; a higher rate than that observed in this study although high incidences were recorded in specific groups of animals. The incidence of abortion associated with hardjo infection is lower than in some outbreaks of pomona infection in which abortion rates up to 40 per cent have been recorded (Stoenner, 1968).

herd in this investigation, while abortion was a constant finding. In contrast to the clinical picture observed in Scotland, mastitis and an associated drop in milk yield are commonly observed in outbreaks of hardjo infection in Australia (Hoare and Claxton, 1972). This apparent difference in clinical picture may be due either to differences in the pathogenicity of strains which exist in Scotland and Australia or to differences in the epizootiology of infection. Infection by hardjo or related serotypes appear to be enzootic in the cattle population in Scotland, as shown by the high incidence of antibodies to sejroe in sera from herds in different parts of the country, and this has been the case for some years (Michna (1967); Coghlan and Norval, 1967). Epizootics most frequently occur in heifers in which infection may manifest itself as abortion but will not cause mastitis as these animals are not lactating. When infection occurs in adult cattle only sporadic cases occur and any associated mastitis may pass unnoticed. In the one herd in which a mastitis-like syndrome was recorded, retrospective examination of the evidence suggested that there had been an epizootic of infection in the herd, as abortion occurred in all age groups of pregnant cattle and a high incidence of sejroe antibodies was found in all age groups of cattle tested. A high incidence of antibody titres of 1:300 or more to sejroe was also detected which suggested recent exposure to infection. In contrast to the situation in Scotland, reports from Australia describe epizootics of infection by leptospires of the hebdomadis serogroup in areas where infection has not been present previously. In New South Wales, Keast et al. (1964) reported that only a small percentage of cattle gave serological reactions to leptospires of the hebdomadis serogroup. Subsequently, Hoare and Claxton (1972) reported widespread infection of cattle by hardjo: 16.8 per cent of 2,817 sera from 406 properties in New South Wales had antibody titres to hardjo of 1:300 or higher. Both abortion and mastitis were a feature of those outbreaks

investigated by Hoare and Claxton.

Most abortions due to leptospirosis occur in the last trimester of gestation (Fennestad and Borg-Petersen, 1960; Stoenner, 1968), and infection must take place in the latter half of gestation for abortion to be a sequel of infection, as the interval between clinical infection and abortion is usually 2-3 weeks (Stoenner, 1968). The time interval between infection and abortion due to leptospires of the hebdomadis serogroup appears to be much longer (6-12 weeks) (Hoare and Claxton, 1972; Ellis and Michna, 1974) than the 2-3 weeks reported for other serogroups by Stoenner (1968). This longer interval, and the fact that infection must occur in the latter half of gestation, may account for the large number of cases which occurred in the last month of gestation in herd LH23.

The fact that spring calving cows are passing through the most susceptible stage of gestation, i.e., the latter half, during the period of winter confinement, may contribute to the peak of abortions noticed in late autumn and winter. Stoenner (1968) noted high abortion rates in beef herds which practised spring calving, whereas abortion rates were much lower in herds which practised continuous breeding.

The marked seasonal incidence of abortion in the infected herds has been noted by other investigators. In the northern hemisphere, abortions have occurred primarily during the late autumn and early winter (Mathews, 1946; Stoenner et al., 1956; Hanson et al., 1965).

The period when abortions reached a peak (October to March) (Fig. 15) is the time of the year when, rainfall is at its highest resulting in the maximum accumulation of surface water, temperatures are low, cattle are kept in confined areas and spring calving cows are in the latter half of gestation.

The importance of rainfall in bovine leptospirosis due to leptospires of other serogroups has been noted by a number of authors. According to Seddon (1953), most cases of bovine leptospirosis occur in Queensland in the first half of the year and in the southern states of Australia chiefly in the spring, both times corresponding to the period of highest rainfall.

Doherty (1967a) failed to establish infection by introducing 4 pomona carriers to a group of in-contact heifers over the dry months of August to October, but succeeded by introducing 3 infected steers during the wet month of November. In contrast, in New Zealand which has a steady rainfall all the year round, there is no strict seasonal incidence to leptospirosis in cattle (Salisbury, 1954).

Hanson et al. (1964) considered a wet environment to be one of the major factors in the spread of leptospirosis in a beef herd which they had kept under surveillance for an 11 year period.

That rainfall and a wet environment should be of importance in the epizootiology of bovine leptospirosis is consistent with the known experimental evidence on factors which favour the survival of viable leptospires without a host. Okazaki and Ringen (1957) found that leptospires could not be detected in dry soil in vitro after 2½ hours, while the organisms could be cultured from damp soil in vitro for 5 days and from super-saturated soil in vitro, leptospires were cultured for 183 days. Obviously, the longer leptospires remain viable outside the host, the greater will be the contamination of the environment and, consequently, the greater the chance of an animal becoming infected.

Water may also be important in the transmission and dissemination of leptospirosis. A contaminated stream appeared to be the source of infection for the heifer stock in LH28. Ponds have been implicated as sources of spread of leptospirosis in enzootics in cattle studied in Russia (Byalik, 1961), Hungary (Fuzi and Kiszel, 1962) and in the United States (Stoenner et al., 1956). In the latter country, pomona has been isolated from water associated with outbreaks in cattle (Gillespie et al., 1957; Gillespie and Ryno, 1963).

During the period when most abortions occurred in infected herds, the cows were permanently housed or were kept in confined areas close to the farmyard for supplementary winter feeding. Hanson et al. (1965) noted that hardjo and pomona infections occurred primarily during winter feed-lot

confinement and appeared to result from cow to cow transmission. Clearly, if susceptible and excretor animals are packed close together, direct transmission of organisms from carrier host to susceptible animal by urine splashing will become increasingly important. Also, the greater the number of shedder animals in a given area, the greater will be the contamination of the environment.

The possible relationship between abortion in Leptospira infected herds and the mean ambient temperatures is shown in Figure 15. Okazaki and Ringen (1957) have reported that leptospires are killed rapidly by extremes of heat (40°C) or cold (2°C). Temperatures below 2°C occur regularly during the period when most abortions occur. It is unlikely that this affects the epizootiology, as under conditions of close confinement where direct transmission is probably the method of spread, survival outside the host is not important. Also, as the cattle are usually indoors, the temperature of their environment may not fall to the levels recorded outside.

Two factors which may be of importance in the epizootiology of infection with hardjo or related strains in Scottish cattle and which were not considered in this investigation were (1) the role of wild animals and (2) the effect of soil and surface water pH.

Untyped strains belonging to the hebdomadis serogroup have been isolated from field mice and voles (Turner, 1969) in the United Kingdom. While cow to cow transmission would seem to be the important mode of transmission within infected herds wild rodents could act as a reservoir of infection for cattle as they may contaminate pasture.

The survival of leptospires in the environment is favoured by pH values around neutrality (Turner, 1967). The pH of the soil has been reported to influence the epizootiology of bovine leptospirosis. Musaev (1960) found that the number of infected farms with an acid soil was one sixth of the number of infected premises with neutral or alkaline soil.

The low incidence of antibodies to serogroups other than the hebdomadis serogroup suggests that, in Scotland, cattle act solely as incidental hosts

for strains belonging to these groups.

The Epizootiology of Infection with other Serogroups

The maintenance host for icterohaemorrhagiae is the brown rat, which is commonly found around farm buildings and yards. Twigg et al. (1972) noted that a large proportion of permanently housed cattle had antibodies to icterohaemorrhagiae, the inference being that this was because they shared the same environment as the brown rat. In this study, the higher incidence of icterohaemorrhagiae antibodies found in older dairy cattle (11 per cent) than in beef cattle (2.7 per cent) may reflect the greater time spent by dairy cattle around the farm buildings and yards than that spent by beef cattle.

Clinical icterohaemorrhagiae infection has previously been reported in calves in the United Kingdom (Field and Sellers, 1950; Ingram et al., 1952; Baxter and Pearson, 1956) but it has not been reported in adult cattle. This is consistent with the observation by Fennestad (1963) that the severity of leptospirosis increases with the decreasing age of the animal.

Serotype icterohaemorrhagiae has occasionally been incriminated as a cause of abortion. Fennestad and Borg-Petersen (1956) demonstrated by experimental infection of pregnant heifers that serotype icterohaemorrhagiae could cause abortion. It has since been isolated from a naturally aborted calf in Brazil (Santa Rosa et al., 1961) and also in Peru (Fernandez and Acosta, 1966). The higher incidence of icterohaemorrhagiae antibodies in cows which had aborted (12.3 per cent) (Table 7) than in cows in the herd survey (4.7 per cent) (Table 8) may indicate that icterohaemorrhagiae occasionally causes abortion or it may just reflect a lower standard of rat control on those farms from which the abortion samples originated.

Dogs and pigs are the main hosts for canicola in the west of Scotland. Under modern farm management regimes, opportunities for contact between pigs and cattle are minimal. The dog population on a farm is usually low, therefore opportunities for dog to cow contact are very limited. The low

incidence of antibodies (1.8 per cent) to canicola in the cattle sampled (Table 4) probably reflects this minimal contact between cattle and pigs or dogs.

The known hosts of strains belonging to the ballum, australis, javanica and autumnalis serogroups, in the United Kingdom, are the small wild mammals which may be found on pasture. Antibodies to these serogroups have previously been reported (Michna, 1967, 1971; Coghlan and Norval, 1967; Twigg et al., 1972). Fennstad and Borg-Petersen (1956) showed that poi (javanica group) could cause abortion in experimentally infected heifers. It is possible that strains belonging to the javanica serogroup may occasionally cause abortion in Britain.

In view of the recent isolation of patoc (semaranga serogroup) from a cow in the United States (Carroll and Le Clair, 1969) and andamana from a bull in Eire (O'Brien, 1974), it may have been an error to exclude these two antigens from the battery of antigens used to screen the sera.

The review of the literature indicated that infection of cattle with strains belonging to four serogroups of Leptospira is of world-wide significance. This survey has demonstrated that antibodies to two of these serogroups, namely the hebdomadis and icterohaemorrhagiae serogroups, are present in Scotland (Table 4) and confirms the findings of Michna (1967; 1971) and Coghlan and Norval (1967). Antibodies to pomona and grippotyphosa have not been detected in Scottish cattle, even in low serum dilutions.

In the past there has been some evidence of the presence of serotype pomona in Scotland. Michna (1958; 1967) found pomona antibodies in dilutions of from 1:10 to 1:1,000 in pig sera collected early in his investigations, i.e. during the later part of the 1950's and early 1960's. From the mid-1960's until the present time pomona antibodies have not been detected in pig sera (Michna, personal communication). These results suggest that there may have been a focus of pomona infection in the pig population but that this has died out.

Twigg et al. (1973) reported finding low titres (up to 1:100) to grippotyphosa in 11 small rodents and 3 hares, while in 7 out of 21 wild goats in Galloway they detected antibody titres ranging from 1:30 to 1:1,000 to grippotyphosa. It is possible that infection with this particular serotype may be enzootic in places such as the moors of Galloway, but again, antibodies to this serotype were not found in this survey.

The absence of evidence of infection by pomona and grippotyphosa serotypes in the cattle population is a highly desirable state and every effort should be made to maintain this freedom from infection as both these serotypes can be serious animal and human pathogens.

Why these two infections are either absent or only present as a very localised focus in some remote part, as may be the case with grippotyphosa, is obscure, but there are a number of factors which may be of importance.

Firstly, the sera of imported livestock are checked for antibodies to pomona and grippotyphosa and this prevents the importation of infected livestock, except for the seronegative carrier.

Secondly, a review of the world literature shows that while in a given geographical area pomona, grippotyphosa, icterohaemorrhagiae or strains belonging to the hebdomadis serogroup may be the main group of leptospires infecting cattle, high rates of infection by pomona, grippotyphosa and icterohaemorrhagiae do not co-exist, although any one of these may co-exist with a high rate of infection by strains of the hebdomadis serogroup. It appears that for a given geographical area, the prevalent leptospire may limit or even exclude the presence of serologically distinct serotypes, e.g., in Northern Europe grippothphosa infection prevails and pomona infection is inhibited while in Central Europe the reverse applies (Kemenes, 1964). Kemenes suggested that the overall prevalence of icterohaemorrhagiae and canicola inhibits the existence and spread of pomona in the United Kingdom. He demonstrated varying degrees of cross-immunity in guinea pigs, inoculated with pomona and icterohaemorrhagiae and to a lesser extent grippotyphosa due

to previous exposure to one or other serotype but not with or to sejroe. Alexander et al. (1971) made a similar observation in sheep.

Thirdly, there may not be a suitable ecological niche, involving a suitable host, for either grippotyphosa or pomona in the United Kingdom. In Denmark, pomona infection is restricted exclusively to the islands of Lolland-Falster, where its maintenance host is the striped field mouse which, in turn, is only found on those islands (Fennestad and Borg-Petersen, 1972).

Whatever the reason for the failure of pomona and grippotyphosa infections to establish themselves in cattle in the United Kingdom it would seem sensible to continue with the present import restrictions which apply to leptospirosis.

Summary of Findings

Antibodies to serotypes representing one or more of ten Leptospira serogroups were detected in the sera of 1,766 (49.1 per cent) cows and heifers out of a total of 3,600 sera tested. Antibodies to sejroe (hebdomadis serogroup) were the most common, being found in 1,503 (41.8 per cent) sera. Antibodies to icterohaemorrhagiae (7.7 per cent) and to ballum (7.3%) were the next most common, while antibodies to bratislava (australis group), javanica, canicola, panama, cynopteri, pyrogenes and autumnalis were detected in a small number of animals.

The incidence of antibodies to Leptospira varied considerably between the various groups of cattle tested: 72.4 per cent of sera from the older cows collected at the abattoir had antibodies to Leptospira, whereas antibodies to Leptospira were only detected in 50.0 per cent of the sera from cows which had aborted and 45.4 per cent of the sera from cows in the herd survey.

Antibodies to more than one serogroup were found in 417 (23.6 per cent) of the 1,766 positive sera, however, only 5 of these sera had antibody titres of 1:300 or more to two serogroups.

The results suggest that Scottish cattle act as maintaining hosts for strains belonging to the hebdomadis serogroup and that infection by these strains is associated with abortion.

The association with abortion is based on:

1) Reports by farmers and veterinary practitioners of abortion of unknown aetiology in infected herds.
2) The occurrence of a much higher incidence of antibodies to sejroe in herds with a history of undiagnosed abortion than in the other groups of cattle tested, especially those herds with no history of abortion. In herds with a history of abortion 33.7 per cent of cows had antibody titres to sejroe whereas in herds with no history of abortion during an 18 month

model period only 0.6 per cent had antibody titres to serotype sejroe of 1:300 or greater.

3) The close relationship between the incidence of cows with antibody titres to sejroe of 1:10 or more and 1:300 or more and the incidence of abortion of unknown aetiology in 29 herds during the nine months prior to testing and the nine month period after testing.

Infection of heifers often occurred when they were introduced into the adult herd. Thirty seven per cent of two-year-old heifers in 18 of the herds tested had antibodies to sejroe whereas 72 per cent of three-year-old cows had antibodies to sejroe. In several herds abortion was restricted to the heifer stock.

The incidence of abortion varied from 0 to 14 per cent per 9 month period in infected herds, however, higher incidences were recorded in particular groups of cattle, e.g., 29 per cent of a batch of heifers aborted in herd LH28.

The incidence of abortion showed a marked seasonal variation, abortions being more common in the months from October to March than in the period from April to September. The period of greatest incidence of abortion coincided with the period when climatic conditions were most conducive to the survival of leptospires in the environment and the close confinement of cattle at this time allowed the best chance of direct cow to cow transmission.

Infection within the herds studied was self-limiting. As the number of cattle with antibodies to sejroe tended towards 100 per cent abortions ceased to occur.

The results suggested that infection of Scottish cattle by leptospires belonging to serogroups, other than the hebdomadis serogroup, was incidental, and due to the contamination of their environment by other maintaining hosts, chiefly rodents.

Icterohaemorrhagiae infection was diagnosed in calves on one farm. It was associated clinically with marked jaundice in one calf and stunted growth in two treated, in-contact calves.

PART B. THE ISOLATION OF J10 STRAIN; A MEMBER OF THE HEBDOMADIS SEROGROUP

Introduction

The primary isolation of new serotypes belonging to the hebdomadis serogroup has been made most frequently from humans and from small rodents. Hebdomadis was the second pathogenic serotype to be discovered. It was isolated in Japan by Ido et al. (1918) from humans suffering from a mild form of leptospirosis known as "seven-day fever", which was characterised by fever (102°-105°F) meningitis, severe headache and backache. It was differentiated from icterohaemorrhagiae on the basis of the clinical disease it caused in man, by its different pathogenicity for laboratory animals, and by serological methods.

Further serotypes belonging to what is now called the hebdomadis serogroup were not isolated until Schuffner et al. (1935) recorded the recovery of medanensis from a dog in Indonesia. Subsequently wolfii was grown from a human patient in Indonesia in 1937 (Schuffner et al., 1939) and hardjo was isolated from a human patient in Sumatra in 1938 (Wolff, 1953). In Europe, sejroe was isolated from a Danish fisherman in 1937 (Borg-Petersen and Christensen, 1939) and saxkoebing was isolated in 1942 from two mice by Borg-Petersen (1944) who showed these strains to be identical antigenically with strains recovered by Mino (1941) in Italy from two rice-field workers. Today the hebdomadis group is the largest Leptospira serogroup, some twenty-eight serotypes being recognised (W.H.O., 1967).

The first recorded isolations of a member of the hebdomadis serogroup from cattle were made in Japan. Watanabe et al. (1953) recorded the isolation of two strains of hebdomadis from the kidneys of cattle which showed signs of haemoglobinuria, and, subsequently, Bezdenezhnuikh and Kashanova, (1956) isolated hebdomadis from a calf on Sakhalin Island, which, although part of the U.S.S.R., lies just to the north of Japan. Inui et al. (1959) reported the

isolation of 5 strains of hebdomadis from 9 Japanese cattle with haemoglobinuria.

In Belgium, van Riel et al. (1957) reported the isolation of sejroe from a preparation of kidneys, pooled from twenty cattle and Andreani (1968) recovered hardjo from a cow in Italy. In Britain, Michna and Campbell (1969) recorded the successful isolation of sejroe from the kidneys of aborting cows in the Glasgow area, and more recently Michna et al. (1974) recovered hardjo (204 strain), from the kidneys of a cow that had aborted.

In North America and Australia hardjo is the serotype responsible for antibodies to the hebdomadis group in cattle.

Roth and Galton (1960) reported the isolation of hardjo from the urine of an asymptomatic yearling bull in Louisiana and Clark et al. (1961) recorded its recovery from a cow in Pennsylvania. Sulzer et al. (1964) isolated hardjo from the urine of two cows following an outbreak of disease in a herd in Nebraska which was characterised by a drop in milk production and by abortion. Hanson and Brodie (1967) isolated eight strains of hardjo from cattle in Illinois; one of the eight isolations was made from the urine of a cow following an abortion, while the other isolations were made from kidney homogenates collected at the time of slaughter. Stoenner (1967) demonstrated concurrent infection by the hebdomadis and pomona serogroups when he isolated pomona and hardjo from different cows in a herd which had had serious losses from abortions during two successive breeding seasons.

In Canada, hardjo was isolated from the urine of a cow following an outbreak of mastitis in a herd (Robertson et al., 1964).

In Australia, Sullivan and Stallman (1969) recovered hardjo from the urine of a clinically normal heifer which had an intense leptospiruria and the following year Sullivan and Callan (1970) reported the isolation of hardjo from the urine of cows affected with mastitis, while in Tasmania, where infection by the hebdomadis serogroup is associated with abortion and mastitis, Corbould (1971) reported the isolation of hardjo.

Lake (1973) isolated hardjo from the urine of a clinically healthy calf in New Zealand.

Balcanica was isolated from cattle in Northern Caucasia (U.S.S.R.) by Semenova et al. (1965).

It is the purpose of this section to describe the isolation of strain J10, a member of the hebdomadis serogroup, from the kidneys of a cow which had aborted. This was an essential preliminary step before experimental studies could be initiated.

Materials and Methods

Attempts were made to isolate leptospires from the kidneys of twelve cows obtained from five herds. Four of these herds A (LH13), B (LH29), C (LH23) and D (LH1) were included in the herd serological survey. The other herd (E) was known to contain a large number of cows with sejroe antibodies and had an undiagnosed abortion and infertility problem. The individual animals from which isolation was attempted had histories of either abortion, infertility, retained placenta or endometritis (Table 11). Serum samples from each cow had previously been examined and found to contain antibodies to sejroe.

Cows (1) to (7) inclusive were purchased and slaughtered at the Veterinary School. The kidneys were removed immediately for culturing. Cows (8), (9) and (10) were slaughtered at Dyce, near Aberdeen and their kidneys dispatched by train to Glasgow. Cultures from these were not set up until the following day. Cows (11) and (12) were slaughtered at Bellshill Abattoir, and kidney cultures were set up the same day.

The kidneys were examined in the following way in the laboratory:-

(1) the capsule was removed and any gross features were noted;

(2) kidney material was inoculated into Stuart's medium;

(3) photographs of the kidney were taken;

(4) blocks approximately $\frac{1}{2}$-1 cm thick were fixed in 10 per cent formal saline.

Serial 5 μ sections of kidney were stained by the methods of Faine (1965) and Young (1969); between twenty and forty sections from each tissue block were stained. Sections were also stained with haematoxylin and eosin. Blocks of kidney were stained by Levaditi's (1905) method.

Blood samples were collected from all the cows at the time of slaughter and their sera examined for leptospiral antibodies.

The Leptospira culture, strain J10, isolated from cow (2) was sent to Dr Dikken, at the W.H.O. Leptospirosis Reference Laboratory, Institute for

Tropical Hygiene, Amsterdam, for identification. This organism was also examined in the electron microscope, and under the dark ground microscope, in order to confirm that it had the morphology of a member of the genus Leptospira.

Preparation of cultures for electron microscopy. Suspensions of leptospiral culture were spun for 25 minutes at 10,000g. After discarding the supernatant, the sediment was resuspended in sterile phosphate buffered saline (P.B.S.), mixed thoroughly and recentrifuged for 7 minutes at 15,000g. The supernatant was again discarded and the pellets resuspended in sterile P.B.S. to one-tenth of the original volume. A drop of leptospiral suspension was allowed to settle on carbon-coated, Formvar-covered grids for 2 minutes, blotted and then stained with 1 per cent phosphotungstic acid (pH 7.2) for 30 seconds, blotted again and allowed to dry. Grids were examined on an A.E.I. 6B electron microscope.

Results

Isolation

Cultures from 11 cows with antibody titres to sejroe varying from 1:30 to 1:3,000 (Table 11) proved negative and were discarded after eight weeks' incubation. Leptospires were found in only two bottles out of thirty inoculated with kidney material from cow J10. This cow had aborted five weeks prior to slaughter. A blood sample taken the day following abortion had an antibody titre of 1:10,000 to sejroe and this had fallen to 1:1,000 five weeks later.

There was a five week interval between the inoculation of cow J10 kidney material into medium and growth being observed. The leptospire isolated (strain J10) had proved extremely difficult to cultivate to the density required for use as an antigen in serological tests.

Preliminary tests with the stock rabbit sera indicated that it belonged to the hebdomadis serogroup (Table 2). Studies by Dr Dikken have confirmed that finding and have shown that the strain is closely related to serotype hardjo (strain Hardjoprajitno).

Microscopic studies of J10 strain

The dark ground and electron microscopic studies proved that J10 had the morphological characteristics of a member of the genus Leptospira.

Examination of live J10 culture by dark ground microscopy showed it was long thin fairly rigid organism, which rotated rapidly about its longitudinal axis and which could move forwards and backwards in the direction of its longitudinal axis. Dead, formalin-fixed organisms had a tight spiral structure with hooked ends.

Electron microscopy (Figs. 16 and 17) revealed that the organism possessed a spiral protoplasmic cylinder, limited by a membrane. An axial filament, composed of a single fibril, originated from a sub-terminal attachment disc at each end of the protoplasmic cylinder. The whole was surrounded by an outer envelope.

Table 11. Attempts to isolate Leptospires from Cows' Kidneys - Summary of Results

Farm	Cow No.	Antibody titre at slaughter to sejroe	Cultural examination	Histological examination for Lepto.	Source of material	History
A. LH 13	1) S10	1/30	-	-	Purchased	Aborted
"	2) J10	1/1,000	+	+	"	Aborted - had titre of 1/10,000 when first examined.
B. LH 29	3) 52	1/1,000	-	-	"	Infertility
"	4) 98	1/3,000	-	+	"	Endometritis
"	5) 100	1/1,000	-	-	"	Infertility
C. LH 23	6) 72	1/300	-	-	"	Aborted - had titre of 1/3,000 when first examined.
"	7) 163	1/30	-	-	"	Aborted - had titre of 1/300 when first examined.
D. LH 1	8) I	1/100	-	-	Kidneys collected at abattoir	Aborted
"	9) II	1/1,000	-	-	"	Infertility
"	10) III	1/300	-	-	"	Infertility
E.	11) R3	1/3,000	-	+	"	Premature calving. Retained placenta. Infertility.
	12) R9	1/1,000	-	+	"	Retained placenta.

All the kidneys showed macroscopic evidence of focal interstitial nephritis.

+ = Leptospires present

- = " absent

Pathology of the cows' kidneys

Macroscopic examination. A few pale yellowish foci, not more than 3 mm in diameter were seen on the surface of the kidneys. Sometimes these foci were surrounded by a ring of hyperaemia (Figs. 18 and 19).

Microscopic examination. Leptospires were demonstrated in sections of kidney from cows J10 (Figs. 20 and 21), 98 (Figs. 22 and 23), R3 and R9 (Figs. 24 and 25) by the methods of Faine (1965) and Young (1969) but not by Levaditi's (1905) technique. The micro-organisms were seen lying free in the lumens of the kidney tubules (Figs. 20 and 21), projecting from the tubular epithelium, between the epithelial cells and in the interstitium (Figs. 22 and 23). They were found in discrete foci; usually within groups of adjacent tubules which contained leptospires (Figs. 22 and 23). These foci of leptospires were usually found either in the cortex or at the cortico-medullary junction, however, in the kidney of cow R9 large numbers of leptospires were seen in the interstitium and in the collecting ducts, deep in the medulla, near the papilla (Figs. 24 and 25). No inflammatory changes appeared to be directly associated with the foci of leptospires. Small discrete foci of interstitial nephritis were seen in most sections (Fig. 26 and 27) but were confined to the cortex and cortico-medullary junction. Where infiltration by inflammatory cells was seen, leptospires were absent and vice versa. The foci of interstitial nephritis were composed largely of lymphocytes with occasional plasma cells. The remains of collapsed necrotic tubules could be distinguished in some of the foci (Fig. 27).

Figure 16. An electron micrograph of an organism from a culture of J10 strain, negatively stained with phosphotungstic acid. Note the tightly coiled arrangement of the organism.

x 12,600

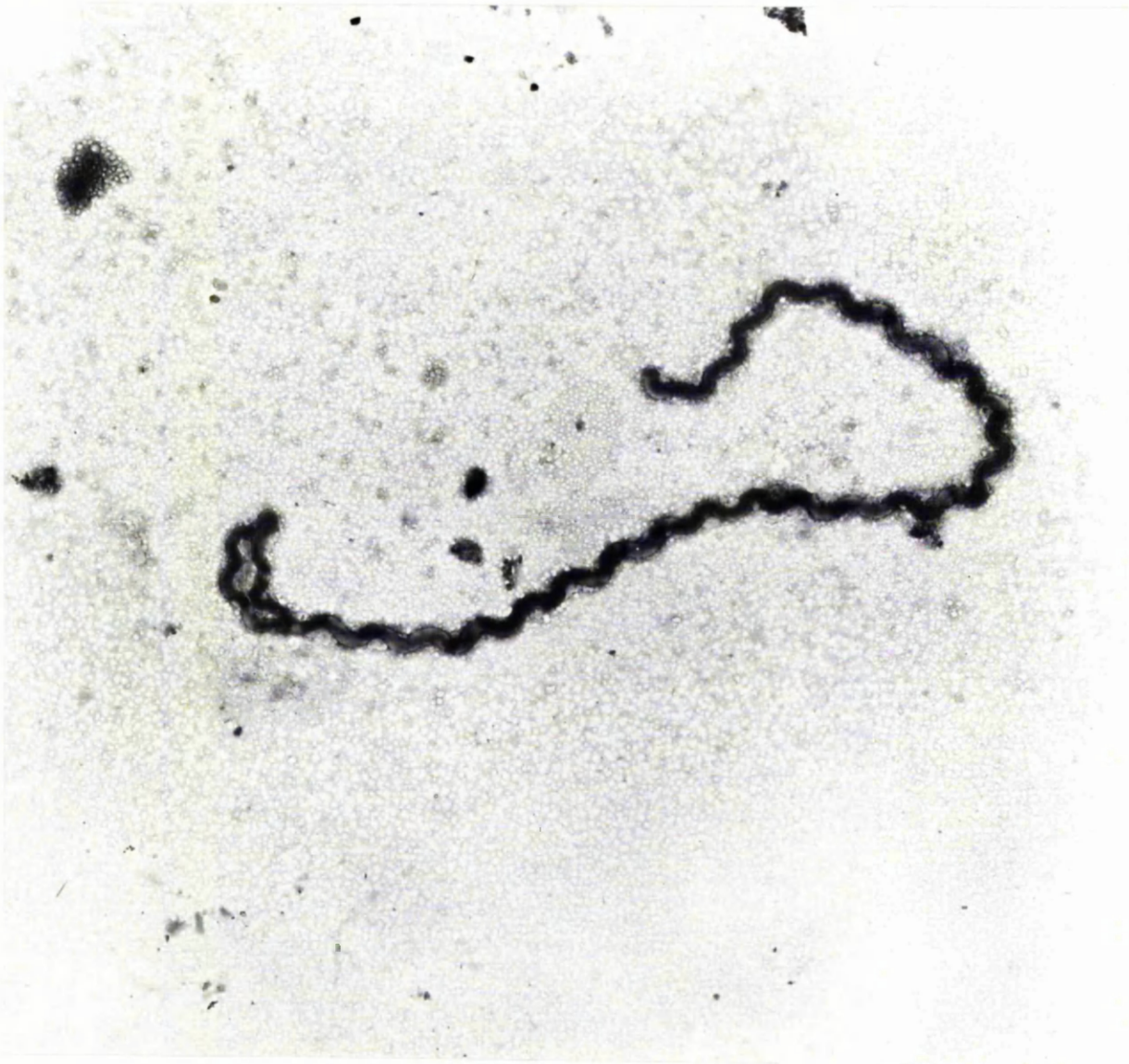


Figure 17. An electron micrograph of an organism from a culture of J10 strain. This illustrates the various ultrastructural features of the organism.

Note: the axial filament (a);
the sub-terminal attachment disc of the axial filament (d);
the limiting membrane (l.m.);
the protoplasmic cylinder (p);
the outer envelope (o.e.).

Phosphotungstic acid x 50,000

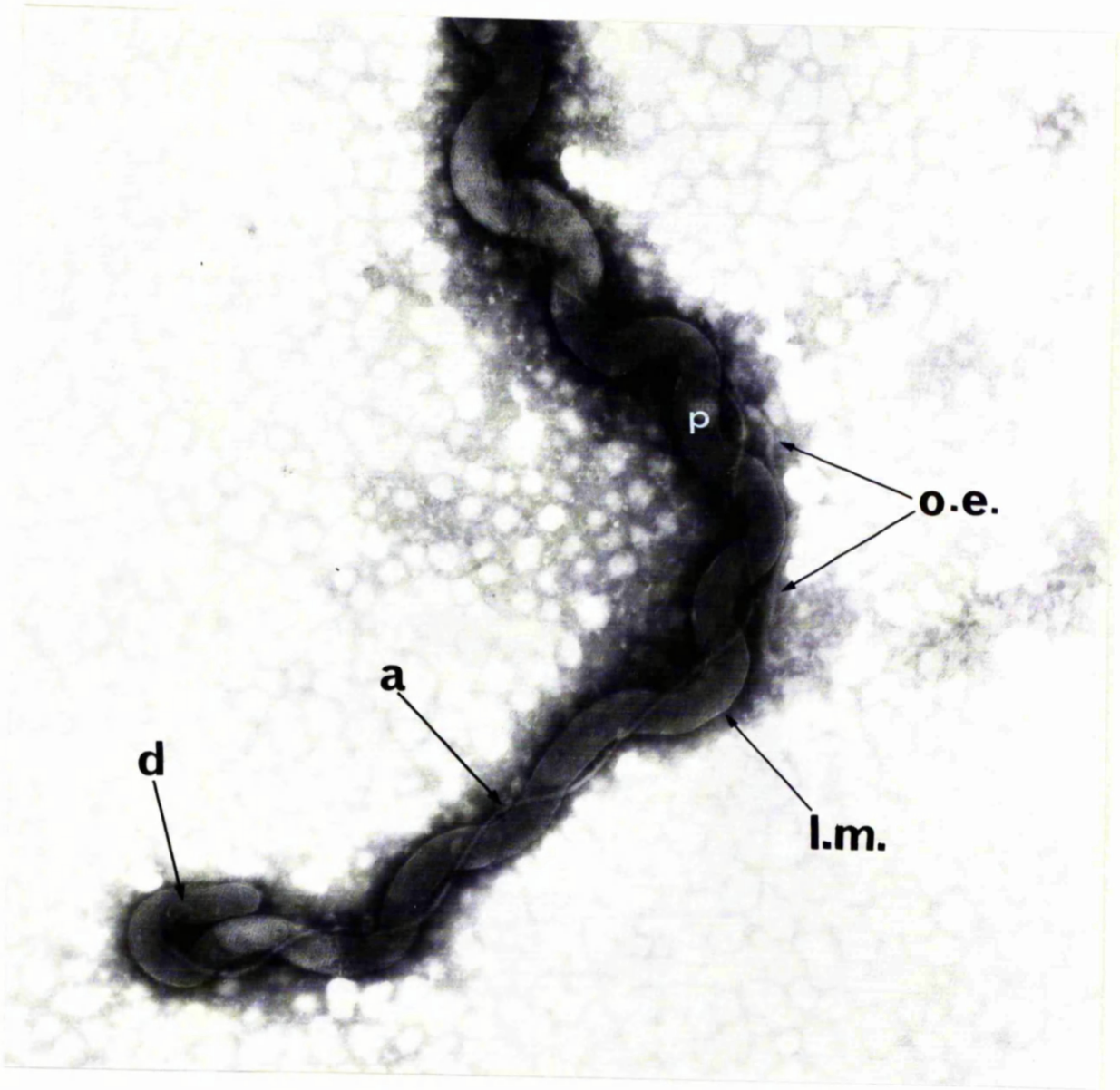
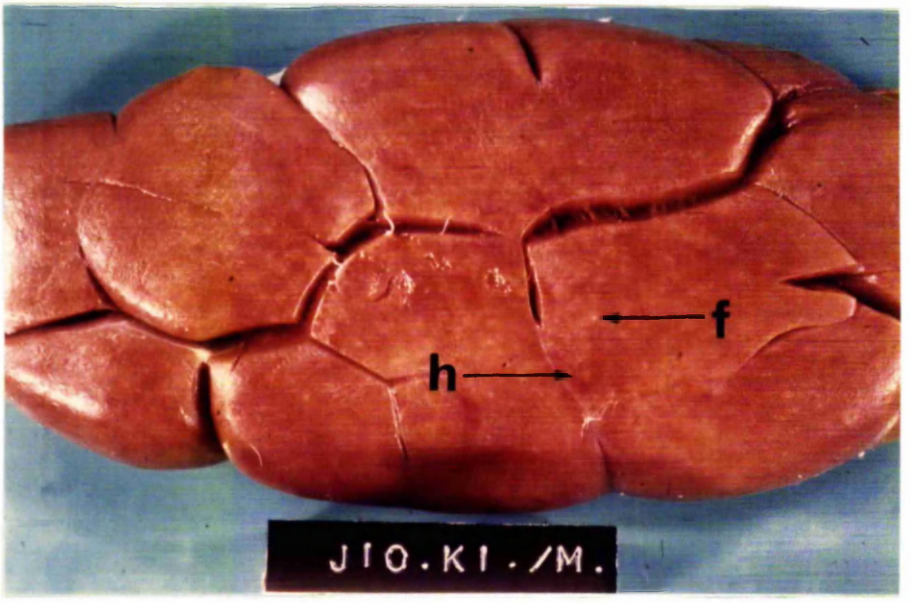

p
o.e.
a
d
l.m.

Figure 18. A photograph of a kidney, with the capsule removed, from cow J10. There are multiple small yellowish foci (f) on the surface of the kidney. Note the rings of hyperaemia (h) surrounding some of the foci.

Figure 19. A close-up of the kidney (J10) surface, showing the foci (f) and the rings of hyperaemia (h) more distinctly.


f
h
J10.K1./M.

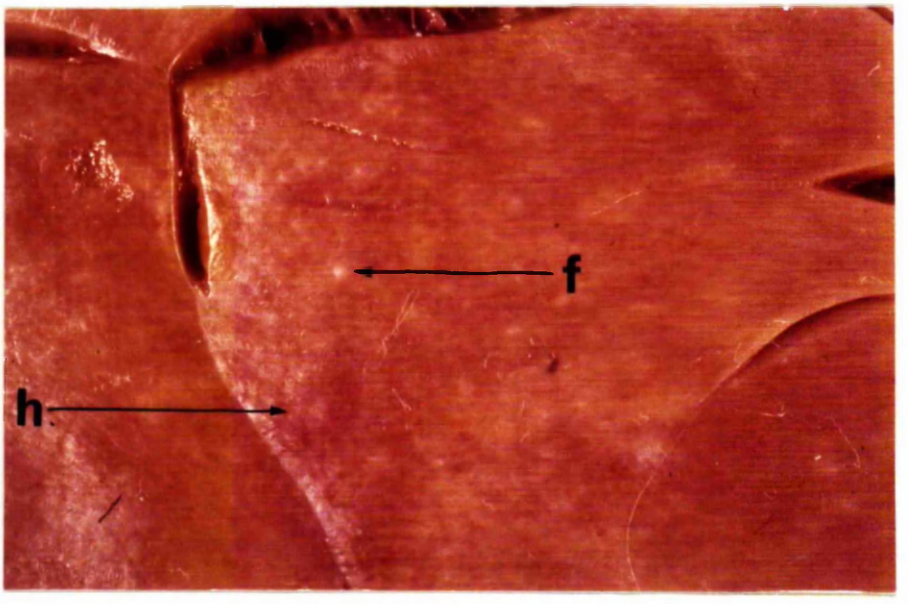
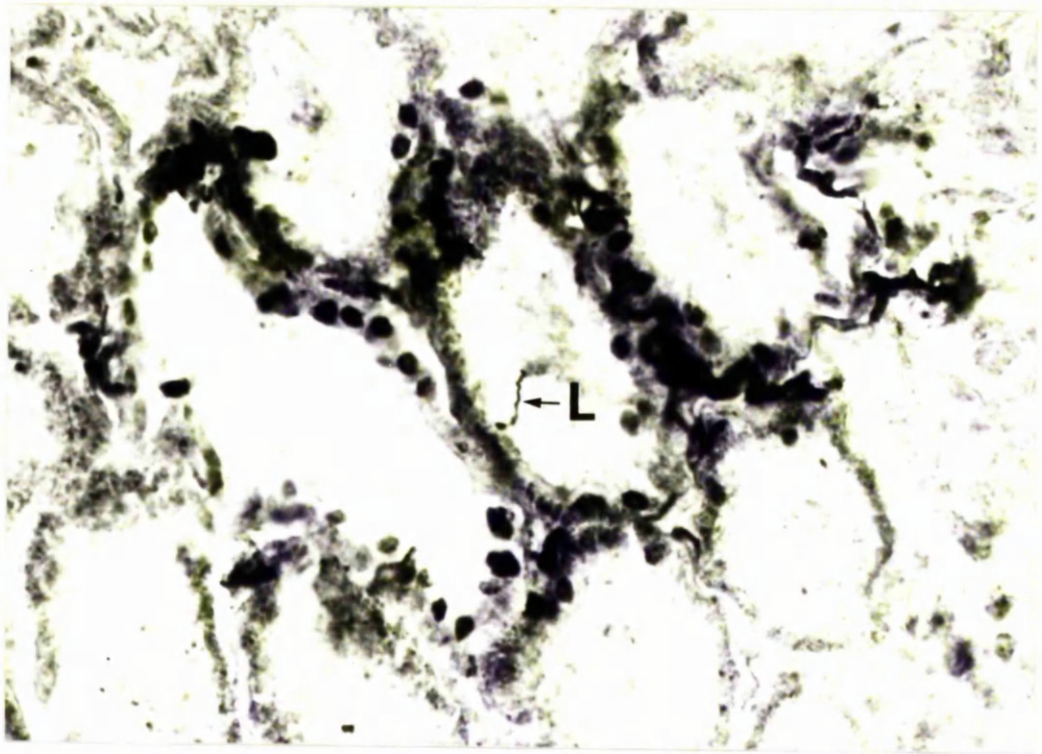

f
h

Fig. 20. Photomicrograph of a section of kidney from cow J10. A single leptospire (L) may be seen lying free in the lumen of a tubule.
Young's method x 640

Fig. 21. A higher magnification of Fig. 20 with only half of the leptospire in focus.
It shows the spiral morphology of the organism.
Young's method x 1,600


L

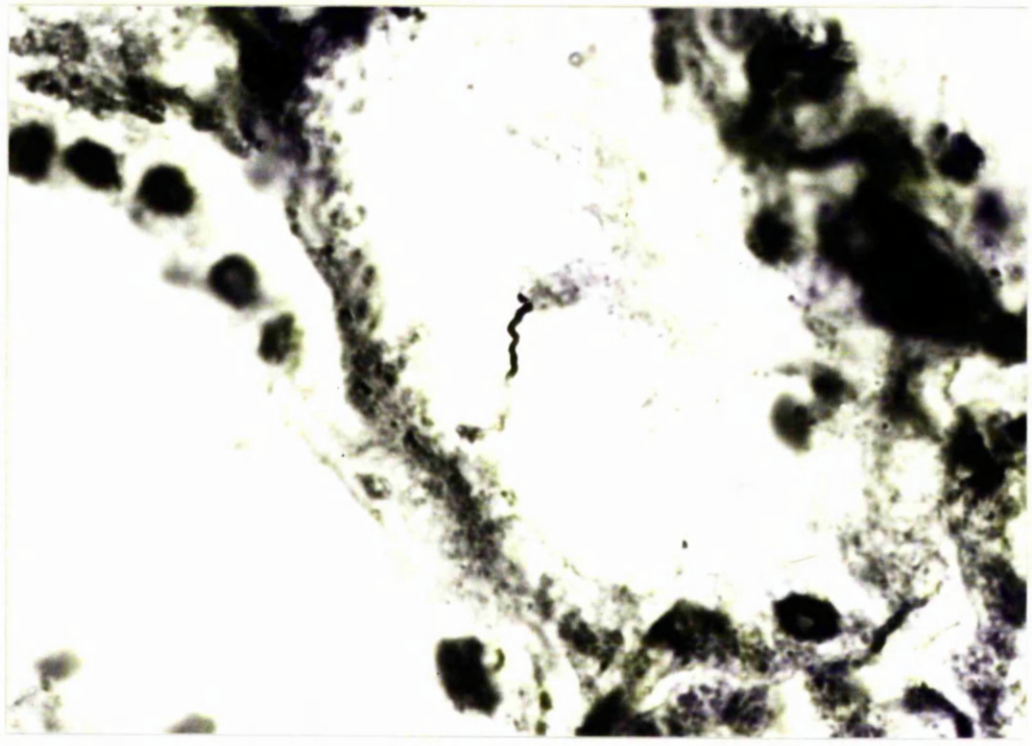
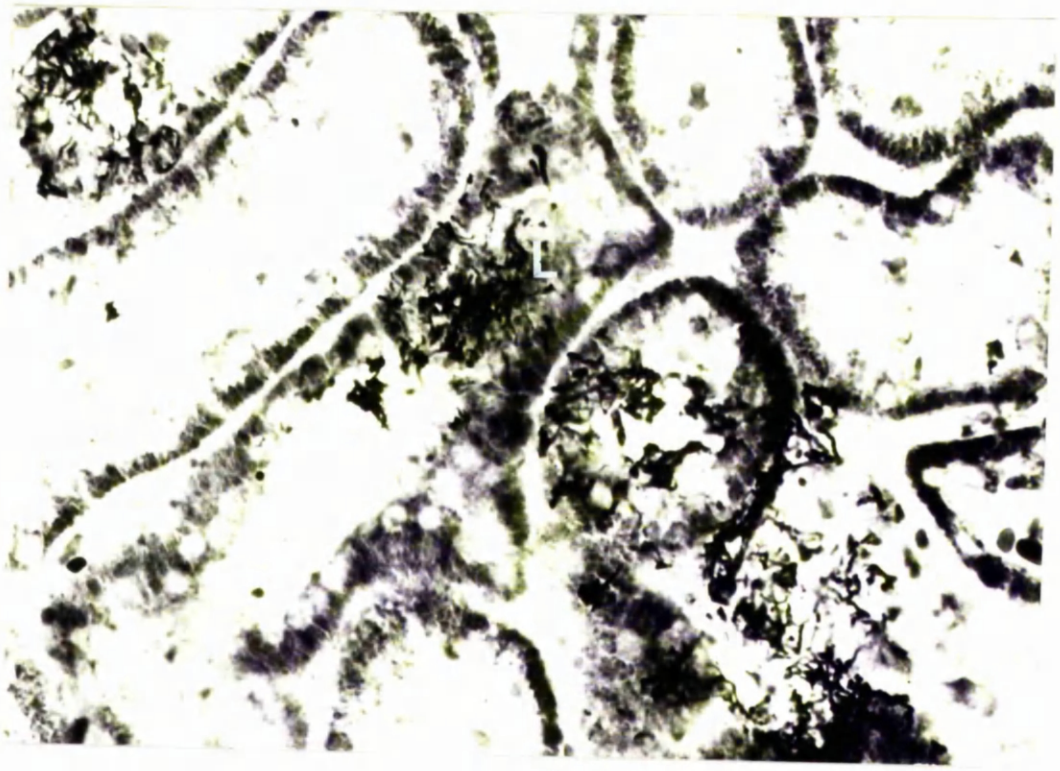


Fig. 22. Photomicrograph of a section of kidney from cow 98.
Note the large number of leptospires in the tubule lumen (L) and the focal nature of the leptospiral accumulations.
Young's method x 400

Fig. 23. A higher magnification of part of the area shown in Fig. 22.
Note the leptospires projecting from the tubule epithelium (a), between the epithelial cells (b) and in the degenerating tubule (d).
Young's method x 1,075


L

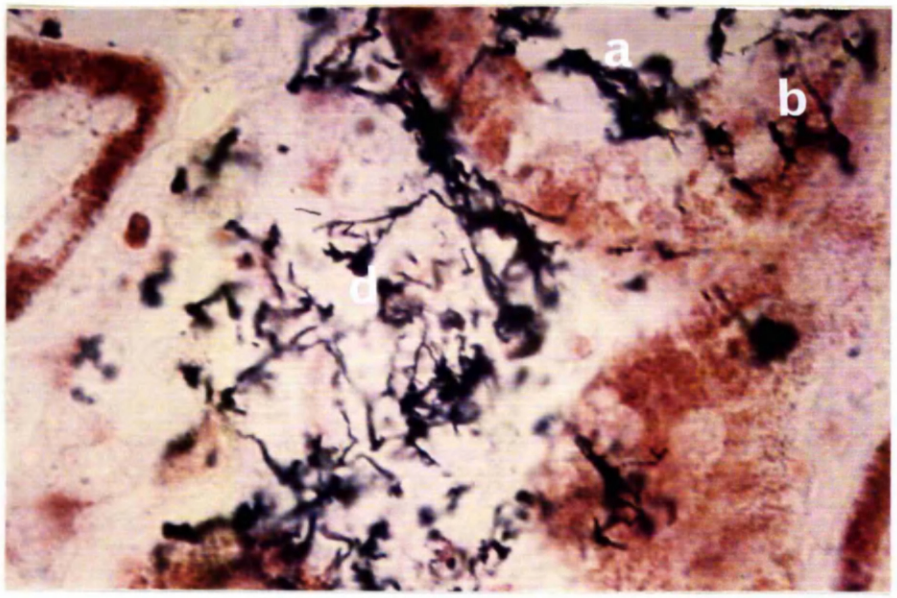

a
b
d

Fig. 24. Photomicrograph of a section of R9 kidney showing a large number of leptospires in and surrounding a large collecting duct.

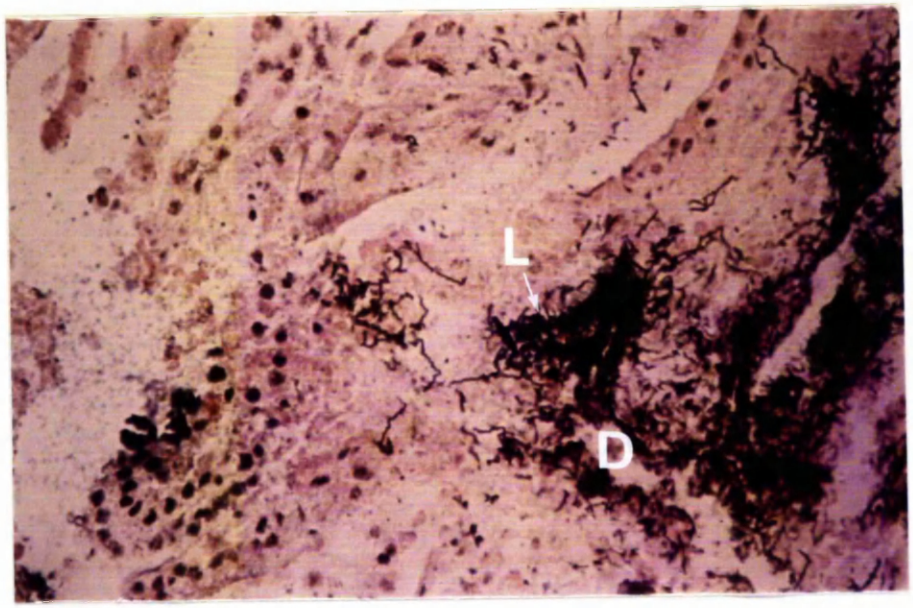
L = leptospires

D = collecting duct

Young's method x 430

Fig. 25. A higher magnification of Fig. 24 showing individual leptospires.

Young's method x 1,075


L
D

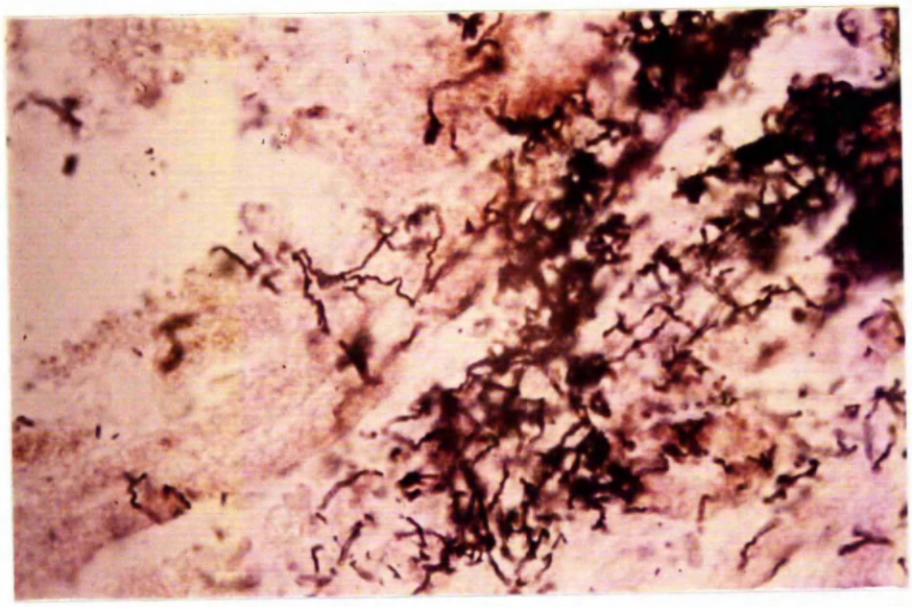


Fig. 26. Photomicrograph of a section of J10 kidney.

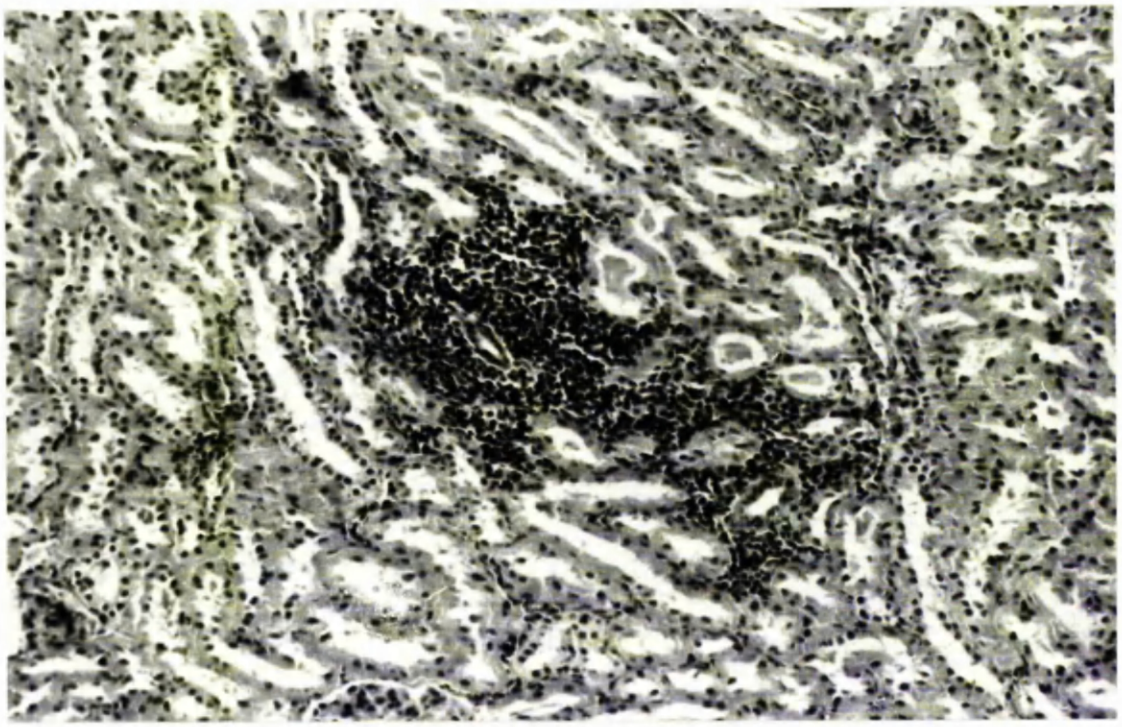
Note the discrete focal nature of the interstitial nephritis.

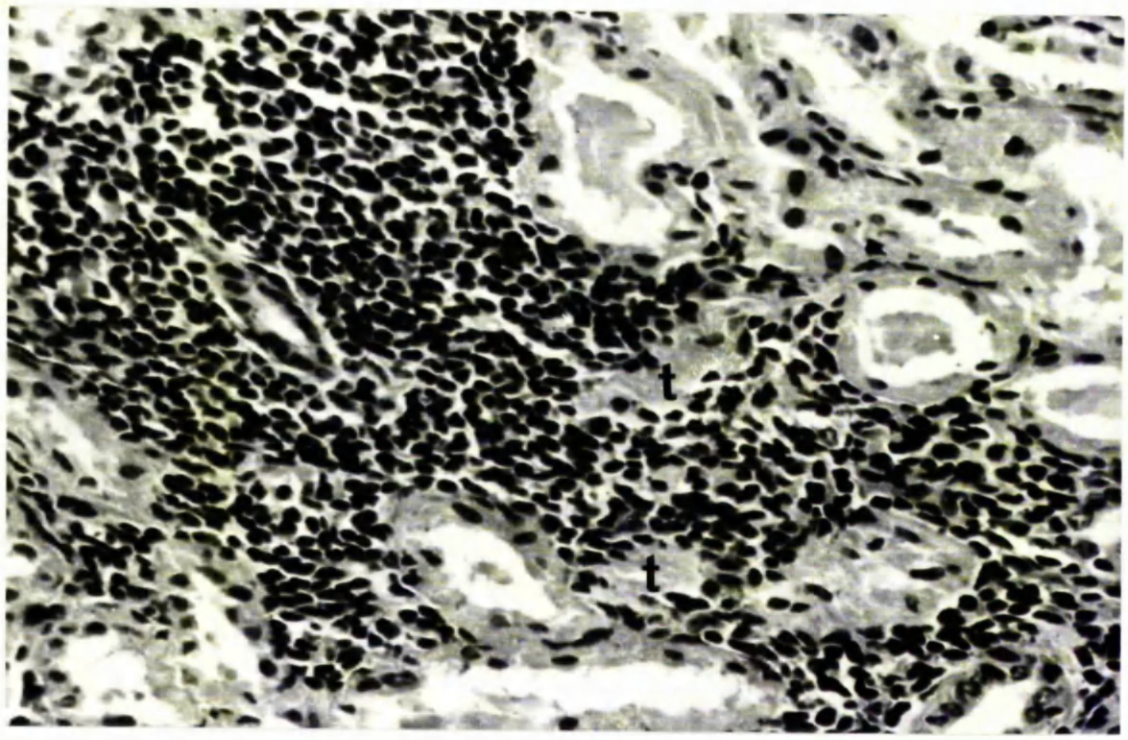
Faine's stain, counterstained with H & E. x 170

Fig. 27. A higher magnification of Fig. 26 showing the interstitial lymphocytic infiltration and tubular necrosis.

t = degenerating tubule

Faine and H & E. x 430




t
t

Discussion

The isolation of strain J10, a leptospire belonging to the hebdomadis serogroup, from the kidneys of a cow which had aborted, is the third such report in the United Kingdom. Previously, Michna and Campbell (1969) reported the isolation of sejroe and Michna et al. (1974) recorded the isolation of hardjo from cows with a similar history. Members of this group have also been recovered from voles (Broom and Coghlan, 1958; Michna and Campbell, 1970) and field mice (Turner, 1969).

The J10 strain is closely related antigenically to serotype hardjo which is also found in Europe, North America and Australia. Field evidence indicates that the disease picture in Britain, namely abortion, premature birth, mastitis (Chapter 3 Part A) and, perhaps breeding difficulties (Michna, 1971) associated with infection by the hebdomadis serogroup is similar to that observed in North America (Robertson et al., 1964; Sulzer et al., 1964; Hanson and Brodie, 1967; Stoenner, 1967) and in Australia (Corbould, 1971; Hoare and Claxton, 1972).

The isolation of leptospires provides irrefutable proof of infection. The results of the isolation attempts described above, however, indicate that culture is not reliable as a method of demonstrating infection. The micro-organism was isolated from the kidneys of only one of twelve cows showing serological evidence of infection, yet it was demonstrated histologically in the kidneys of four animals.

Factors which may influence the success of isolation include the following:

1) The presence of inhibitory substances in the inoculum may prevent growth of leptospires. Kenzy et al. (1958) noted that leptospires could not be isolated from bovine kidney tissue suspended at concentrations of 1:50 and 1:500 but were isolated when it was diluted to 1:5,000 and they

postulated that this may have been due to the greater concentration of antibodies in the lower dilutions. Yanagawa et al. (1963) showed that the same phenomenon occurred with cultures of guinea pig and mouse kidney and liver suspensions and that it was independent of specific antibodies. According to Stalheim (1965) this inhibitory factor was a lipid. Clearly, the dilution techniques used to overcome the effect of renal lipids will militate against the isolation of leptospires if the number present in the initial inoculum is small. In the technique employed here, graded amounts of tissue were used to inoculate each bottle of culture and this may explain why growth only occurred in two out of thirty bottles inoculated with kidney material from cow J10.

2) As leptospires were only found in discrete foci, it is possible that they were not present in the particular part of the kidney from which the inoculum was taken but were present in the parts taken for histological examination.

3) Despite care in the preparation of the culture media, variations between the quality of one batch of medium and another occurred. Consequently the medium used for culturing kidney material from cows 98, R3 and R9, in which leptospires were demonstrated histologically, may not have been as satisfactory as that used for cow J10.

4) The interval between the death of the animal and the inoculation of cultures may also have influenced the success of the isolation procedure.

Strains belonging to the hebdomadis serogroup which occur in cattle have proved to be very fastidious in their growth requirements. Alexander and Evans (1962) pointed out "that the repeated failure to isolate organisms from

bovine hebdomadis group reactors may well reflect the inability to readily cultivate the aetiological agent on conventional media". Roth and Galton (1960) succeeded in isolating hardjo from the urine of a yearling bull only after two blind passages in calves, while Robertson et al. (1964) recovered hardjo from the urine of a cow after nine serial passages in guinea pigs, after attempts to isolate from earlier passages had failed. Stoenner (1967) pointed out that whilst he was able to isolate pomona from cows' urine after one hamster passage, he failed to grow hardjo during the first two hamster passages using the same medium (Stuart's semi-solid) and only succeeded on the third passage by the inoculation of another semi-solid medium (Ellinghausen and McCullough, 1965). Even after its isolation, attempts to cultivate the organism in Stuart's, Fletcher's and Verwoort's media failed.

The long time lag between the inoculation of media and the detection of leptospiral growth which was experienced (5 weeks) has been noted by most workers. Three weeks has been the shortest time observed and Sulzer et al. (1964) reported an interval of 58 days in one instance.

It is necessary to be able to grow any isolate in liquid media for serotyping. Roth and Galton (1960), Sulzer et al. (1964) and Sullivan and Stallman (1969), all of whom recovered hardjo in semi-solid medium, reported great difficulty in growing their isolates in liquid media. Although J10 strain was isolated in liquid medium, maintenance of the culture has proved to be extremely difficult, and it has been impossible to obtain sufficient quantities for serological tests. This has hindered the final identification of the isolate.

The morphology of J10 strain, as revealed by studies of negatively stained preparations in the electron microscope was similar to that of pomona as described by Ritchie and Ellinghausen (1965).

The gross and histopathological findings in the kidneys described above were similar to those described by Michna and Campbell (1969). The most prominent feature was the very mild nature of the focal interstitial reaction

(Fig. 26) and the lack of cellular reaction adjacent to the foci of leptospires in those cases where leptospires were demonstrated. This minimal reaction to the presence of leptospires is almost certainly an important factor in the establishment of the biological equilibrium between cattle and this group of leptospires which allows the carrier state to develop and persist. Seibold et al. (1961), in a histopathological study of subclinical leptospirosis in 338 cattle noted that leptospires seen in carrier animals were invariably located within the lumina of certain undamaged kidney tubules in the region of the corticomedullary junction and that the lining epithelium was not appreciably damaged by the presence of the organisms.

The presence of large numbers of leptospires deep in the medulla of the kidney of cow R9 is unusual and may have been an important reason for failing to isolate the pathogen from this case, as inoculum was taken only from cortical material.

The methods of Faine (1965) and especially that of Young (1969) proved satisfactory for the demonstration of leptospires in kidney tissue. Both methods had their advantages and disadvantages. Young's method possessed the distinct advantage that it stained the organisms more sharply than did Faine's technique which tended to give the spirochaetes a rather beaded appearance. This difference is illustrated in the photo-micrographs in this thesis (e.g., Figs. 53 and 55). Young's method also had the advantage that it was simpler to perform because it did not require a dark room and this allowed the progress of the staining process to be judged visually. The major advantage of Faine's method was that sections could be satisfactorily counterstained with haematoxylin and eosin. In both methods the pH of the solutions used was critical, pH 3.6 to 3.7 being the optimum. Small increases in pH produced marked thickening of the spirochaetes. Leviditi's method proved unsatisfactory, probably due to the lack of control the operator has over any method involving block staining.

Frequently the foci of spirochaetes were so localised that the organism might be present in appreciable numbers in only a small number of sections, thus the technique of staining large numbers of serial sections was an important factor in the success of the silver impregnation techniques used.

Summary of Findings

1. Strain J10, a fastidious leptospire belonging to the hebdomadis serogroup of *Leptospira interrogans*, was isolated from the kidneys of a cow which had aborted five weeks prior to slaughter. The micro-organism was closely related antigenically to serotype *hardjo* which has been found in cattle in Scotland, Australia, North America and Europe.

2. Leptospires were demonstrated in the kidneys of four cows with antibody titres to *sejroe* ranging from 1:1,000 to 1:3,000. The kidneys of infected animals showed minimal inflammatory changes.

PART C. THE DEMONSTRATION OF LEPTOSPIRES IN NATURALLY ABORTED FOETUSES AND IN A PREMATURE CALF

Introduction

Abortion has often been the most consistently observed feature in natural infections of adult cattle by a number of Leptospira serotypes in various parts of the world. Gochenour and Yager (1953) and Bailey (1953), in the U.S.A., noted abortion associated with infection by serotype pomona in cattle which showed no other clinical signs. In Australia, abortion has been one of the major symptoms noted in herds infected with hardjo (Corbould, 1971; Hoare and Claxton, 1972), while in the United Kingdom, Michna (1971) reported a high incidence of infection by the hebdomadis serogroup in Scottish herds where abortions had occurred.

The diagnosis of leptospirosis in aborted bovine foetuses has depended on attempts to isolate the pathogen; on the demonstration of leptospires in tissues by silver impregnation methods; or on fluorescent antibody techniques.

The recovery of leptospires by cultural methods has been rare. In the U.S.A., Podgewaite et al. (1955) reported the isolation of pomona from the peritoneal and thoracic fluids of 3 out of 13 foetuses examined and Dacres and Kiesel (1958) cultured pomona from 1 of 20 foetuses examined. The isolation of pomona from the body fluids of two bovine foetuses and the isolation of canicola from a third was described by Roberts et al. (1961). Icterohaemorrhagiae was recovered from an aborted calf in Brazil (Santa Rosa et al., 1961) and also in Peru (Fernandez and Acosta, 1966).

Turner et al. (1958) reported the isolation of canicola from a day-old calf which had almost certainly been infected in utero.

Attempts to demonstrate leptospires by silver impregnation techniques have proved nearly as difficult as the isolation of the pathogen. Leptospires

were first seen in foetal membranes by Mathews (1946) during an outbreak of leptospirosis in which abortion was a feature, but he failed to demonstrate the organism in foetuses. During an investigation into abortion associated with pomona infection in New Zealand Te Punga and Bishop (1953) demonstrated leptospires in kidney and liver sections of three aborted foetuses using Leviditi's method. Subsequently Bridges (1958) used a modification of the Warthin-Starry technique to stain leptospires in five aborted calves and in the foetal membranes of another case (Bridges and Luna, 1957) and Fennestad and Borg-Peterson (1958a) demonstrated leptospires by Leviditi's method in two aborted foetuses during an epizootic of pomona infection.

A fluorescent antibody technique was successfully applied to demonstrate leptospires in various tissues from 7 out of 10 aborted bovine foetuses by Maestrone (1963). In a comparative study of a silver impregnation and a fluorescent antibody technique in the diagnosis of bovine leptospiral abortion Smith et al. (1967) demonstrated leptospires in 9 out of 57 foetuses by immuno-fluorescence but failed to demonstrate them by the Warthin-Starry silver impregnation method.

In a scientific letter describing hardjo infection in Tasmania, Corbould (1971) reported the detection of leptospires in histological sections of an aborted foetus, but presented no evidence to suggest that the serotype causing this abortion was in fact hardjo. Apart from this reference there are no reports of leptospires belonging to the hebdomadis serogroup having been demonstrated in naturally aborted bovine foetuses. It is the purpose of this section to record the demonstration of leptospires in two naturally infected aborted bovine foetuses and in a premature calf which died two days after birth. Evidence is presented which incriminates a member of the hebdomadis serogroup as the causative organism.

Materials and Methods

Sources of material

Attempts were made to demonstrate leptospires in foetuses from two herds included in the serological survey. The decision to select these two farms was based on three factors; firstly, the farmer's willingness to co-operate meant that abortions were reported quickly; secondly, both farms were within a twenty-five mile radius of Glasgow thus it was possible to get the foetuses to the laboratory as early as possible; thirdly, full records were kept on both farms and were available.

Farm A (LH 28). In the autumn of 1972 antibodies to sejroe, in dilutions of 1:10 to 1:3,000 were found in the sera of 102 out of 121 adult cows whilst all 27 heifers, kept on a separate outfarm, proved to be seronegative. The previous late winter and spring, 7 of the 24 heifers in the previous batch had aborted and were found to have antibodies to the hebdomadis serogroup. These heifers had been kept on the same outfarm as the next batch and the abortions occurred after their introduction to the home farm. In January 1973, a cow which was approximately six months pregnant aborted. The foetus (I) was collected about four hours after expulsion and a blood sample was taken from the mother. In April 1973 another cow produced a live 7-7½ months foetus (II) which was very small and weak and died on the third day. It was collected immediately after death and brought to the laboratory with a blood sample from its dam. In neither case did the cow show any other clinical signs of infection.

Farm B (LH 23). In addition to a sporadic abortion problem, this herd had a serious infertility problem for a number of years. In 1967, three strains of leptospires were isolated from the kidneys of cows in this herd which had aborted (Michna and Campbell, 1969). In October 1971, serum samples from the entire adult stock were tested for leptospiral antibodies and 169 out of 236 cows were found to have antibodies to serotype sejroe in titres from 1:10 to 1:10,000. In an eleven month period between 1st June, 1972 and

31st April, 1973 there were 23 near full term stillbirths, 2 abortions earlier in gestation and 1 premature live birth. Apart from these cases there were 29 instances of retained foetal membranes and/or metritis and there was a high mortality rate in calves under 3 days of age. The last cow in this group to produce a full term dead calf did so on 13th April, 1973, and this was brought to the laboratory within a few hours of expulsion. The cow showed no other clinical signs. No serum samples were available from this cow.

Serum samples were examined for leptospiral antibodies using the microscopic agglutination test. Strains representing the sixteen pathogenic Leptospira serogroups were used as antigens in these tests.

Laboratory procedures

The calves were opened for post-mortem examination and samples of fluid were taken for culture from the stomach and the thoracic and peritoneal cavities. Tissue was also taken for culture from brain (calf II), liver and kidney. The isolation of leptospires was attempted both by cultural means and by hamster inoculation. In addition, a variety of solid media were inoculated with the same material and incubated under aerobic and microaerophilic conditions in order to detect other bacterial causes of abortion. Mycological examination was also carried out.

Blocks from a number of tissues were fixed in 10% formal saline for histological examination and 5 μ sections were stained by the methods of Faine (1965) and Young (1969).

Cryostat sections of liver, lung and kidney which had been stored at -20°C and formalin-fixed sections of foetus III were stained by an immunofluorescence technique in an attempt to determine the infecting serotype as no serum sample was available from the dam.

Fluorescent antibody (FA) technique: the gamma-globulin fraction produced by the method of Nairn (1969) from a pooled hyperimmune serum was

conjugated with Celite* absorbed fluorescein isothiocyanate by the method of Rinderknecht (1962). The pooled hyperimmune serum was prepared by mixing equal parts of antisera produced in rabbits against two strains (J10 and 204) belonging to the hebdomadis serogroup which were isolated from cattle in the west of Scotland. The conjugate was purified by passage through a column (2.8 x 18 cm) of Sephadex G25** and absorbed with calf liver powder in the manner described by Nairn (1969). Sections were stained by the method of Maestrone (1963) except that 0.01% Evans Blue was used as a counterstain.

The specificity of the fluorescence in the positive tissues was verified using the blocking test described by Nairn. Positive and negative control smears, prepared from cultures of hardjo, canicola, icterohaemorrhagiae, ballum and bratislava, were also examined.

* Calbiochem Ltd., 10 Wyndham Place, London

** Pharmacia, Uppsala, Swedon

Results

Post-mortem examination of the calves showed little of note, except the presence of an excessive amount of dark red fluid in the thorax and abdomen of the two foetuses.

Attempts to isolate leptospires from the foetuses by direct culture and by hamster inoculation were unsuccessful. No other significant bacteria or fungi were isolated. The findings are summarised in Table 12.

Leptospires were demonstrated in sections of liver (Figs. 28 and 29) and kidney from foetus I by the silver impregnation techniques of Faine and Young. The dam had an antibody titre of 1:300 to sejroe.

In the premature calf (II), leptospires were demonstrated in silver impregnated sections of kidney (Fig. 30), liver (Fig. 31) and brain (Figs. 32 and 33). Leptospires were present in large numbers in the cerebrum and they had a marked perivascular distribution. There was a mild lymphocytic interstitial infiltration in some areas of the renal cortex (Fig. 34). The calf had a titre of 1:30 to sejroe, whilst the level of antibody in the serum of its mother was 1:300.

In foetus III, leptospires were demonstrated in kidney (Figs. 35 and 36) and liver (Fig. 37) by the methods of Faine and Young. Leptospires were also demonstrated in liver and kidney by the FA technique. The specificity of the staining was demonstrated using the blocking test. The fluorescent antibody stained the positive control, but there was only slight or no fluorescence of the other serotypes.

In the silver-stained sections from all three cases, foci of beaded argentrophilic material were seen in areas where obvious leptospires could not be distinguished. This phenomenon was much more noticeable in sections stained by Faine's method than in those stained by Young's method.

Table 12. The Demonstration of Leptospires in two Aborted Foetuses and a Premature Calf

Farm	Specimen	Cultural findings	Histological findings - silver impregnation			Fluorescent antibody	Antibodies to sejroe
			Kidney	Liver	Brain		
L/H28 A	6 mth foetus I	-	+	+	N.E.	N.E.	Dam ++1:300
	7-7½ mth premature calf - died 2 days after birth II	-	+	+	+	N.E.	Dam ++1:300 Calf ++1:30
L/H23 B	Full term dead foetus III	-	+	+	N.E.	Liver) +ve Kidney)	N.E.

N.E. = not examined

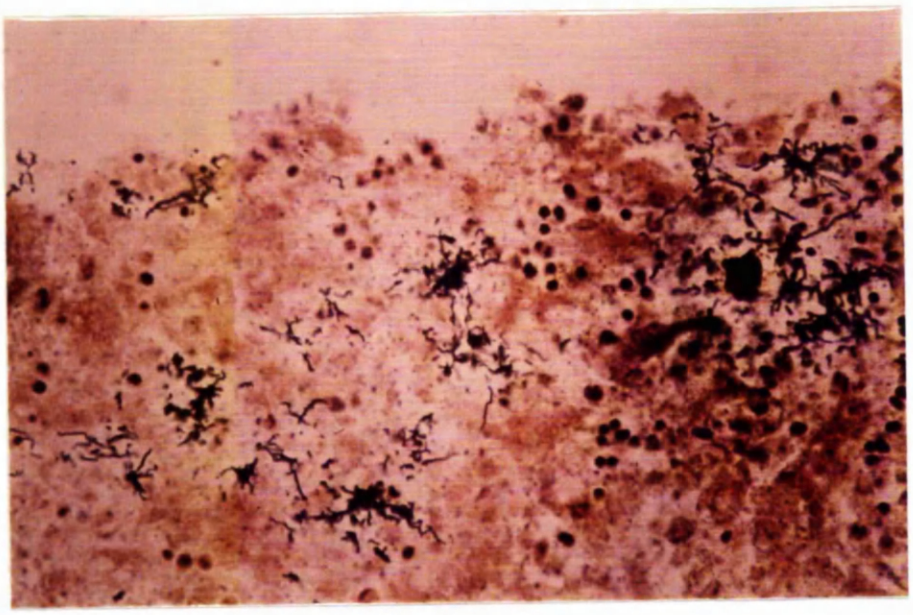
Fig. 28. Photomicrograph of a section of liver from foetus I showing numerous leptospires.

Young's method x 430

Fig. 29. A higher magnification of liver section from foetus I.

L = leptospires

Young's method x 1,600



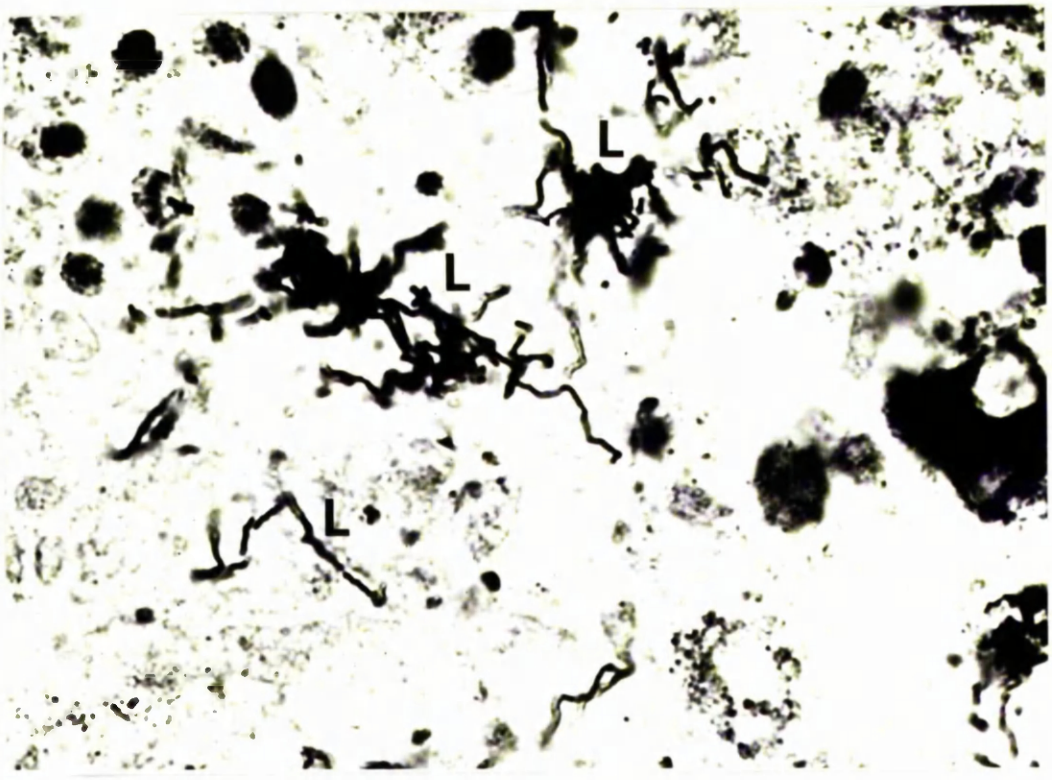
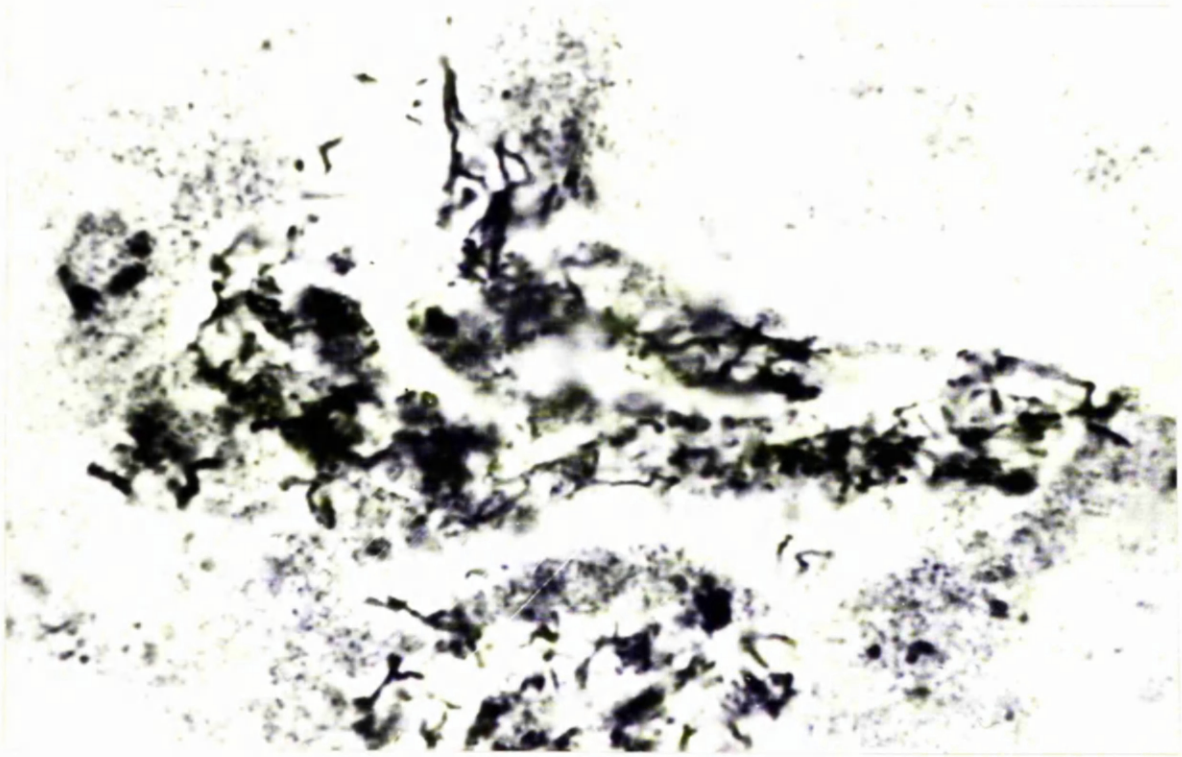

L
L
L

Fig. 30. Photomicrograph of a kidney section from premature calf II showing a clump of leptospires.

Young's method x 1,725

Fig. 31. Photomicrograph showing leptospires in the liver of premature calf II.

Young's method x 1,725



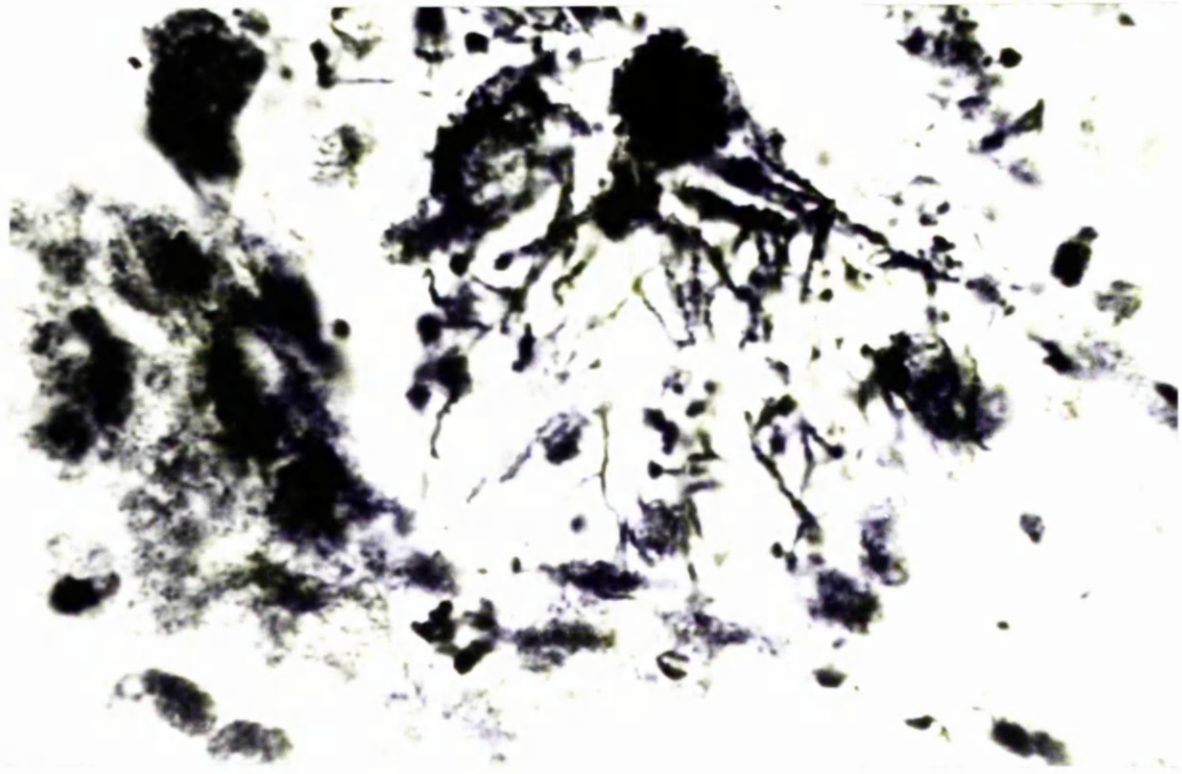


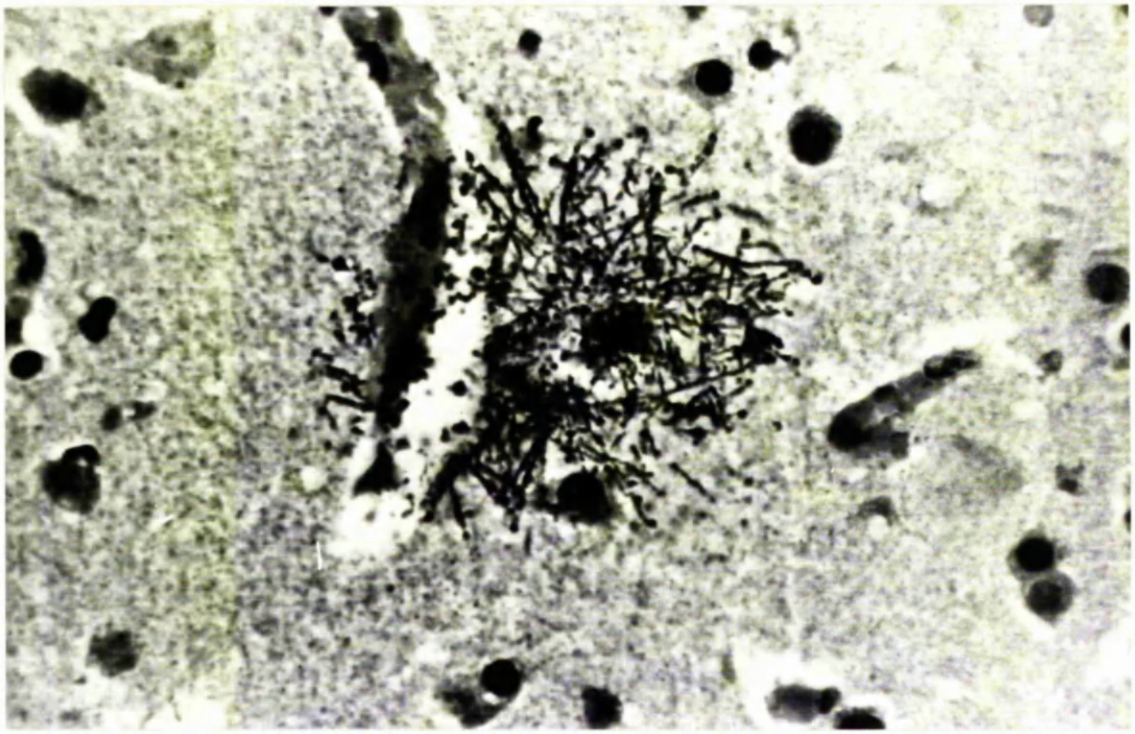
Fig. 32. A photomicrograph showing large numbers of leptospires in the brain of the premature calf (II).

Faine's method and H & E. x 685

Fig. 33. A photomicrograph showing leptospires in the brain of the premature calf (II). Note the marked perivascular distribution of the leptospires.

C = capillary L = leptospires

Faine's method and H & E x 685



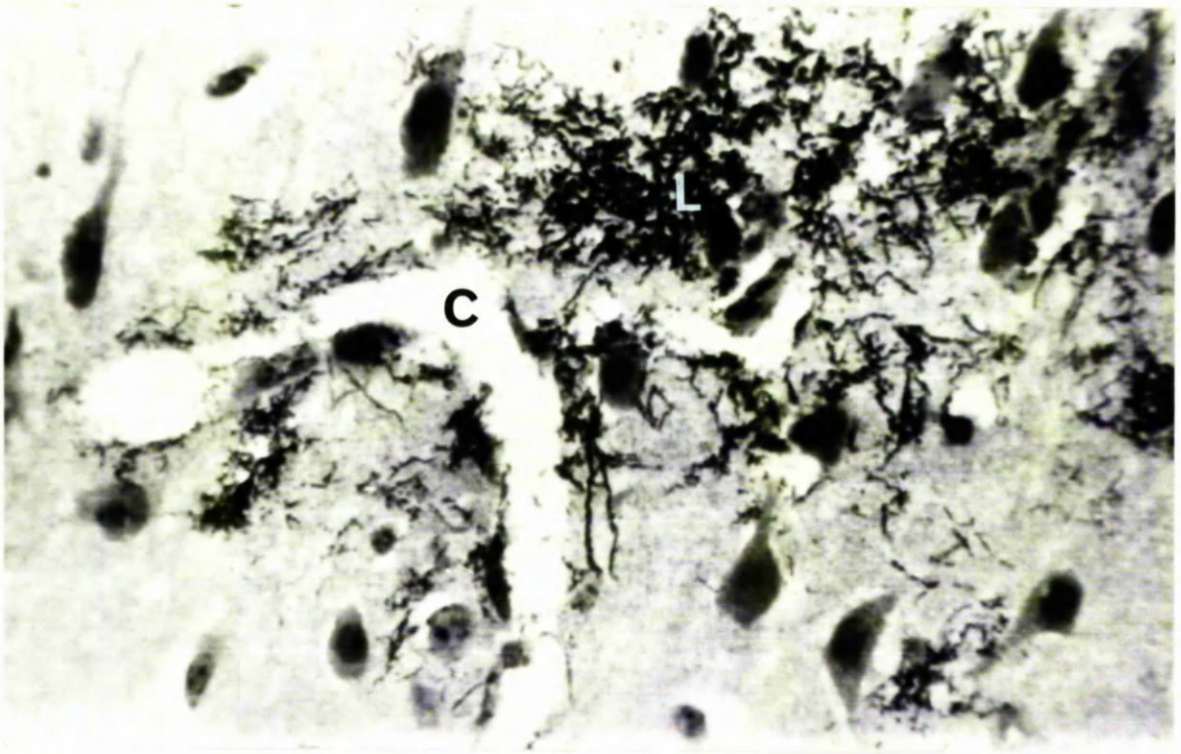

L
C

Fig. 34. A photomicrograph of a section of kidney from the premature calf II showing a focus of early interstitial lymphocytic infiltration.

Faine's method and H & E. x 170

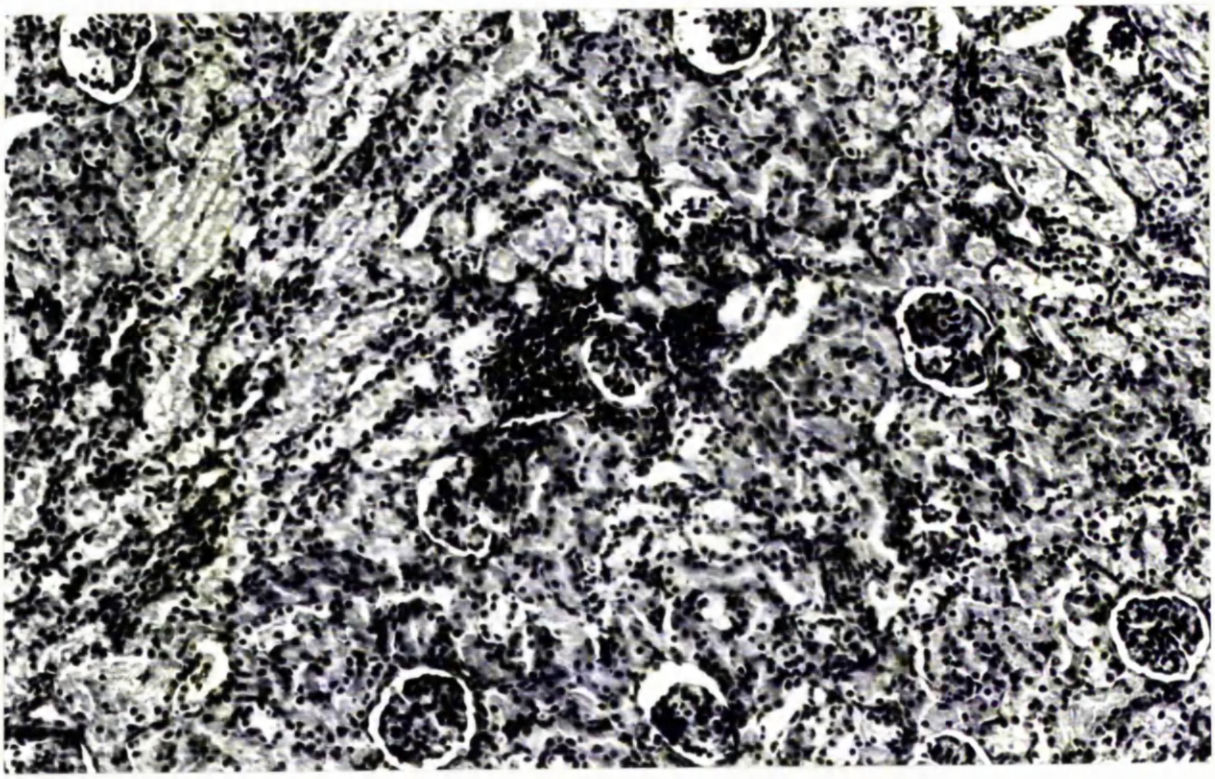
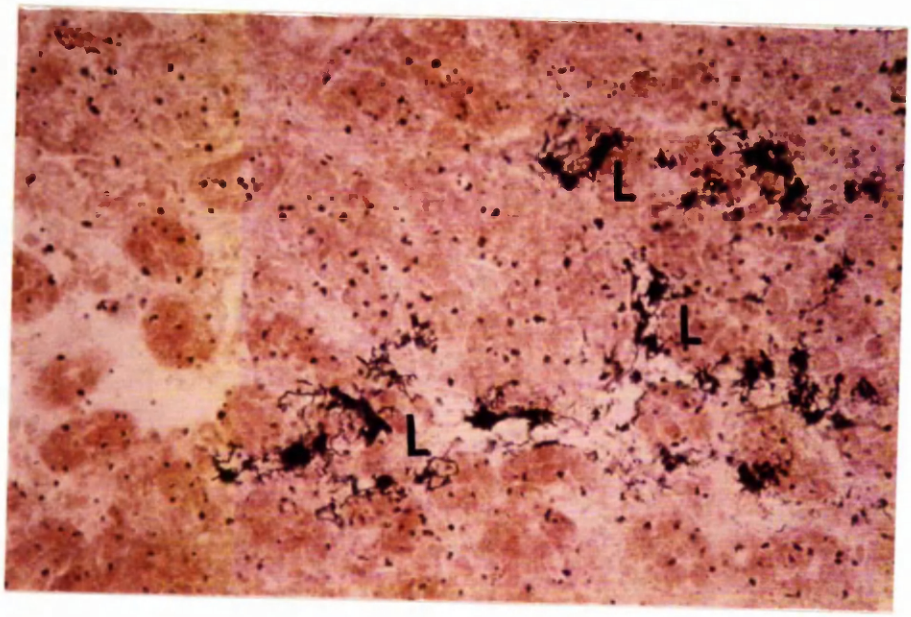


Fig. 35. Photomicrograph of a section of kidney from the stillborn foetus (III) showing leptospires (L).

Young's method x 275

Fig. 36. A higher magnification of an area from Fig. 35 showing the morphology of individual leptospires.

Young's method x 1,075


L
L
L

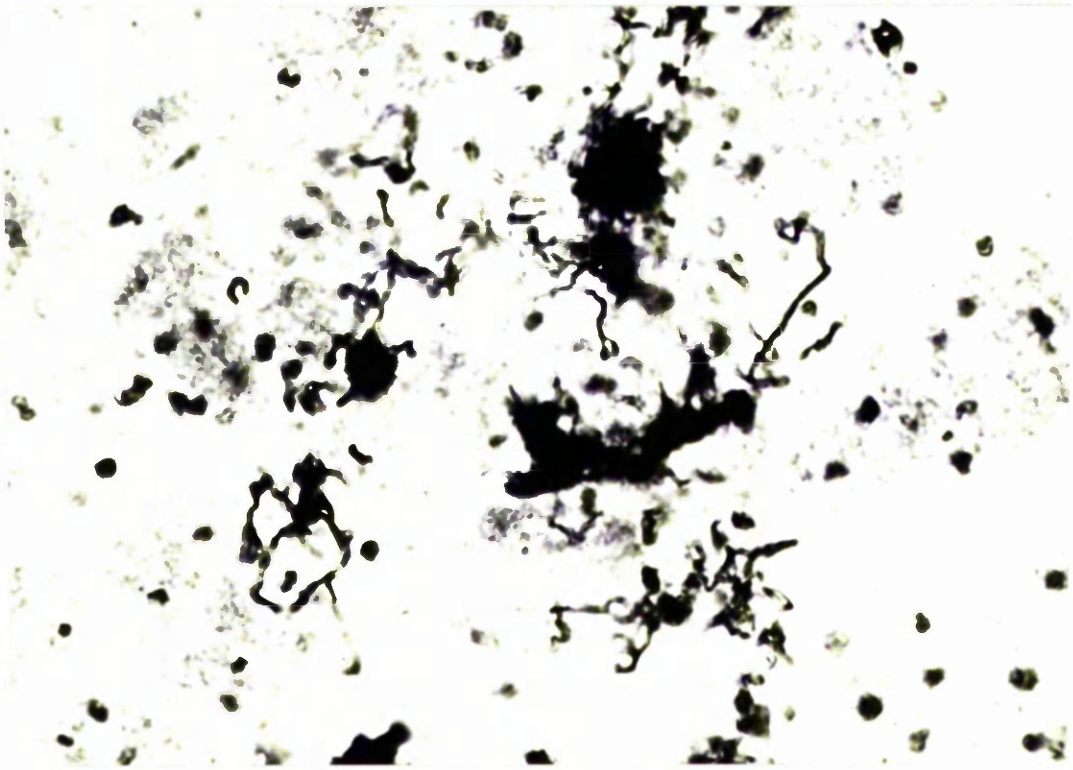
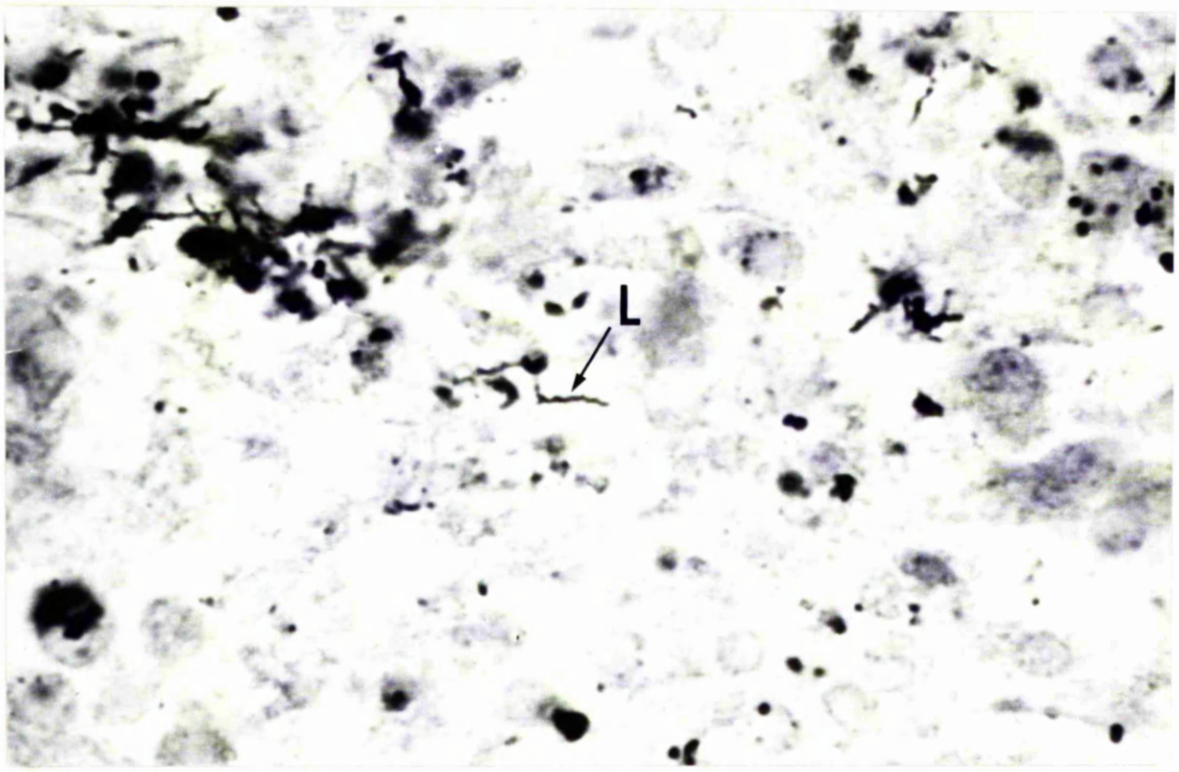


Fig. 37. Photomicrograph of a section of liver from the stillborn foetus III. There is a particularly well defined leptospire in the centre of the plate.

L = leptospire

Young's method x 685


L

Discussion

It has been generally assumed that the autolysis which occurs after the death of the foetus kills the organisms and renders them uncultivable (Smith *et al.*, 1967). This assumption has been based on the following observations:

(1) Dacres and Kiesel (1958) and Roberts *et al.* (1961) who, although they succeeded in isolating *pomona* from some foetuses, failed to recover the pathogen from others in which leptospires were demonstrated by silver impregnation techniques.

(2) Roberts *et al.* (1961) cultured *pomona* from foetuses which were brought direct to the laboratory by the farmer but failed to isolate leptospires from samples brought in by a commercial carrier (which took more than a day to reach the laboratory) although they demonstrated leptospires in a number of them by silver impregnation techniques.

(3) Dacres and Kiesel (1958), Roberts *et al.* (1961) and Podgewaite *et al.* (1955) isolated leptospires from body fluids (usually thoracic and peritoneal fluid) and not from organs.

(4) The experimental findings of Fennestad and Borg-Peterson (1958b), suggested that the time interval between foetal death from leptospirosis and expulsion of the foetus in cows aborting in the last trimester of pregnancy is usually about the same as the maximum survival time of leptospires in dead foetuses *in utero*, i.e., somewhere between 24 and 48 hours.

This assumption has been strengthened by the work of Murphy and Jensen (1969). In a study of the pathogenesis of *pomona* infection in pregnant heifers, in which the animals were killed at fixed intervals after infection, *pomona* was isolated from five viable foetuses but was not recovered from one aborted foetus or from another five that were dead *in utero*.

In those cases described in this section, there was the added difficulty of a much more fastidious serotype being involved than in the pomona cases discussed. While the ultimate proof that strains belonging to the hebdomadis serogroup have an abortifacient role in cattle will come from the isolation of the pathogen from aborted foetuses, the literature quoted indicates that the chances of such an isolation are slim.

The demonstration of leptospires in foetuses by silver impregnation techniques has not proved quite as difficult as the recovery of the pathogen. This can be explained on the grounds that although the organisms seen may have become non-viable they may not have lost their characteristic morphology and their ability to retain silver stains. It may well be that the foci of beaded argentophilic material observed in silver stained sections, especially those stained by Faine's method, are due to the staining of leptospiral fragments.

Fluorescent antibody techniques have the advantage that leptospiral fragments can be identified with certainty and so this technique is less dependent on having fresh material than are isolation and silver impregnation techniques, indeed, Smith et al. (1967) demonstrated leptospires in nine bovine foetuses showing moderate to marked autolysis in which they failed to demonstrate leptospires by a silver impregnation method. The main disadvantage associated with fluorescent antibody techniques has been that they were only genus specific. The conjugate used in this study appeared to be reasonably serogroup specific. This may be due to the fact that from the results of other serological tests, the hebdomadis serogroup appears to be antigenically distinctive and shows few cross reactions with other serogroups.

The presence of large numbers of leptospires in the liver and kidney of both aborted foetuses suggests that these foetuses died in the acute phase of foetal leptospirosis and the assumption of any other cause appears superfluous. The presence of antibodies to sejroe alone in the dam indicates that a member of the hebdomadis serogroup was the agent responsible in foetus I and

the specific staining of the leptospires in the liver and kidney of the other aborted foetus III by a fluorescein isothiocyanate conjugated serum produced against two strains belonging to the hebdomadis serogroup indicates that this case was also caused by a strain belonging to the hebdomadis serogroup.

The presence of large numbers of spirochaetes in the liver, kidney and brain of the two day old premature calf suggests that it died during the leptospiraemic phase of infection; as the minimum incubation time for experimentally induced infection in calves is much longer than the interval between the birth and death of this calf,(see chapter 4), this infection must have been acquired in utero.

The demonstration of leptospires in aborted foetuses and in a premature calf associated with infection by the hebdomadis serogroup confirms the clinical observations made in herds with antibodies to this serogroup.

Summary of Findings

Leptospires were demonstrated in two aborted bovine foetuses and in a premature calf. Evidence is presented to indicate that a member of the hebdomadis serogroup was the causative agent. This is believed to be the first authenticated report of the demonstration of leptospires belonging to the hebdomadis serogroup in either aborted bovine foetuses or in premature calves.

Chapter 4

EXPERIMENTAL INFECTION OF PREGNANT HEIFERS AND YOUNG CALVES WITH A FIELD ISOLATE (STRAIN J10) BELONGING TO THE HEBDOMADIS SEROGROUP

INTRODUCTION AND REVIEW OF THE LITERATURE

A number of experimental studies have been carried out to establish the role of leptospires as aetiological agents in bovine disease and to enable a detailed study to be made of the different phases of the disease in cattle of various ages.

Serotypes used

The first experimental study of bovine leptospirosis was carried out by Semskow (1941) in the U.S.S.R. using a strain which he called Leptospira icterohaemoglobinuriae. This strain, serologically related to grippotyphosa (Fennestad, 1963), had been isolated from the blood of a calf which showed jaundice and haemoglobinuria. The four calves used by Semskow died six to nine days after infection by which time three of them had developed icterus and haemoglobinuria.

In Israel, Bernkopf et al. (1947, 1948) infected 34 young calves with cultures recovered from a bovine and a human case of jaundice. They successfully reproduced an acute fatal illness associated with jaundice in 13 calves but in the other 21 calves the condition was mild or subclinical. Their strains were serologically related to a Swiss strain of grippotyphosa; the authors used the latter strain to infect a further eight calves and found it to be less pathogenic, producing fatal infection in only one calf, while the other seven suffered only a mild or subclinical infection. Wolff and Bohlander (1952) and van der Hoeden (1953) concluded that the serological differences between the Israeli strains and grippotyphosa strains were not great enough to differentiate the Israeli strains from this serotype.

Baker and Little (1948) isolated leptospires from the milk of cows with atypical mastitis in the U.S.A. and used them to infect calves and lactating

cows. Gochenour et al. (1950) showed these strains to be serotype pomona.

Later experimental studies of bovine leptospirosis have employed strains belonging to the pomona, grippotyphosa, javanica (poi), icterohaemorrhagiae, ballum, australis (australis and bratislava), canicola, hebdomadis (hebdomadis, sejroe, saxkoebing, hardjo) serogroups, however, pomona is the only serotype to have been used extensively. Many different isolates of this serotype have been employed to infect a considerable number of calves and mature cattle, not only to investigate the pathogenesis and pathology of the condition but also to evaluate the efficiency of pomona vaccines.

Fennestad (1963) reviewed the literature on experimental infection of calves and mature cattle. Since his article went to print a number of reports of experimental infection with pomona have been published. The effect of pomona infection on 6 bulls and the possibility that leptospires might be transmitted either by natural service or by artificial insemination was investigated by Sleight and Williams (1961) and Sleight et al. (1964). Sleight and Langham (1962) reported the effects of large doses of pomona haemolysin on 13 pregnant cows. The disease caused by three Australian strains of pomona in 26 calves was studied by Spradbrow and Seawright (1963). Doherty (1967b, c) reported pomona infection in a group of inoculated cattle and in an experimentally initiated outbreak of pomona infection in a herd of 44 animals kept under simulated natural conditions. In an attempt to elucidate the pathogenesis of abortion due to pomona, Murphy and Jensen (1969) carried out sequential slaughtering of 27 pregnant heifers between the tenth and twentieth days after infection. There have also been a number of reports of experimental pomona infections in vaccination trials.

The pathogenicity of several serotypes belonging to the hebdomadis serogroup has been investigated in a number of countries. In Australia, hardjo was used for the experimental infection of eight-month old bullocks

(Sullivan, 1970a) and pregnant heifers (Sullivan 1970b, 1972) and in Italy it was used to infect pregnant cows and calves (Farina et al., 1972).

In the U.S.A. hardjo has been used to infect pregnant cows (Hanson and Brodie, 1967) and calves (Roth and Galton, 1960; Hanson and Brodie, 1967) and Ristic et al. (1957) and Hoag (1957) used sejroe to infect calves. Danish strains of sejroe and saxkoebing have been used to infect calves, foetuses and pregnant cows (Fennestad and Borg-Petersen, 1956, 1958b, 1962 and Fennestad 1963). Iwata (1961) reported infection of six calves using a Japanese strain of hebdomadis.

Data on experimental infection by strains belonging to other serogroups is very limited, but will be mentioned here for the sake of completeness. Field and Sellers (1950), in England, injected six calves with icterohaemorrhagiae. In Denmark, Fennestad and Borg-Petersen (1956) infected pregnant cows with icterohaemorrhagiae and poi while Fennestad (1963) reported the experimental infection of calves with Danish and Israeli strains of grippotyphosa. Infection of calves with strains belonging to the australis serogroup was carried out by Spradbrow (1965) and Fennestad et al. (1967). Strains of canicola were used to infect six calves in the U.S.A. (Imbabi et al., 1967) and one calf in Israel (van der Hoeden, 1955). Hoag (1957) infected a calf with serotype ballum.

Origin and History of Strains Used

The strains used in pomona and hardjo experiments have usually been strains of bovine origin which were maintained either, by passage in laboratory animals, embryonated eggs or, artificial media for variable periods of time. For experiments with other serotypes, strains of either human or small wild mammal origin have been used.

Most workers have found difficulty in reproducing bovine leptospirosis experimentally, and often, only freshly isolated field strains have proved suitable for establishing experimental infections. Spradbrow and Seawright (1963), for example, infected groups of calves with three strains of pomona

of bovine origin. One strain which had been maintained in a sealed culture for several years and had undergone twenty-eight guinea-pig passages and another one which had undergone only seven guinea-pig passages produced only mild infection in calves while a third, freshly isolated strain, produced a more severe clinical syndrome. Reinhard (1951) succeeded in infecting five calves, three to six months old, using a pomona strain (NYA) which in the period from isolation to inoculation had undergone 18-26 consecutive passages in guinea pigs or embryonating eggs. The same strain (pomona, NYA) was employed to infect four calves after it had been cultured in vitro for about three years, or after 100 serial passages in embryonating eggs and was found to be considerably less pathogenic (Reinhard and Hadlow, 1954). Hamdy and Ferguson (1957) claimed that a pomona strain (Hardacre) was more pathogenic for cattle after only seven passages in hamsters than after 23 or more passages in the same species.

Bernkopf et al. (1948) infected 18 calves of less than ten days of age with a recently isolated Israeli strain of grippotyphosa as well as eight calves of a similar age with an established Swiss strain of grippotyphosa (Andwill strain). The Swiss strain produced a much milder syndrome than did the Israeli strain. Wolff and Bohlander (1952) and van der Hoeden (1953) suggested that the reason for this difference might well be the long period of in vitro culture before it was used to infect the calves. There are, however, marked differences in pathogenicity between strains of a given serotype from one geographical location and strains of the same serotype from other places. Fennestad (1963), for example, found that two freshly isolated Danish strains of grippotyphosa were less pathogenic for calves than a recently isolated Israeli strain of the same serotype.

Inoculum and Route of Inoculation

A wide range of inocula have been used to establish infection successfully in experimental cattle. Examples are:- blood from infected guinea pigs (Baker and Little, 1948); allantoic fluid from infected chick

embryos (Reinhard and Hadlow, 1954); tissue suspensions from infected guinea pigs and hamsters (Fennestad and Borg-Petersen, 1956; Ferguson et al., 1957); blood from cattle (Bernkopf et al., 1947); urine from a calf (Ringen et al., 1955) and culture (Bernkopf et al., 1948).

Cattle have been infected experimentally by a number of routes. Sullivan (1970a) established infection via the intranasal, conjunctival and intramuscular routes but failed to do so in three eight-month-old bullocks given 5 ml of infected guinea pig tissue suspension per os, however De Lay et al. (1955) produced a fatal disease in a calf by dosing it orally with 25 ml of pomona culture. The intraperitoneal, intravenous and subcutaneous routes have been commonly used. Morter and Morse (1956) reported the infection of a cow which had been placed in a pen for 30 hours with a calf which died of acute leptospirosis. Sloight and Williams (1961) infected a cow by natural service using a bull with a marked leptospiruria, and another heifer was infected after artificial insemination with semen from this bull. Ringen and Bracken (1956) infected two of six, twelve-month-old Hereford bulls by placing an abraded foot of each in a bucket of diluted urine containing pomona for a maximum of two minutes. Foetal infection has been achieved by inoculating intracervically (Hanson and Brodie, 1967) or by injecting culture into a placentome via a laparotomy incision (Fennestad and Borg-Petersen 1958b, 1962).

Susceptibility of the Experimental Cattle

McDonald and Rudge (1957) showed that the presence of circulating antibodies at the time of inoculation could influence the course of the disease. They reported that 24 out of 25 calves which received colostral antibodies from dams vaccinated with pomona, were protected against infection by this serotype during the first month of life. By comparison, 19 out of 20 control calves apparently became infected with that serotype. Other studies have shown that colostral antibodies can persist for up to five months (Fennestad and Borg-Petersen, 1956). Fennestad (1963) maintained that

in some of the early experimental work on bovine leptospirosis the animals used did not appear to have been examined for leptospiral antibodies.

Stalheim (1971) showed that none of twenty steers vaccinated with an avirulent pomona vaccine developed either pyrexia or became renal carriers when challenged with a virulent pomona strain 14 months after vaccination by which time antibody titres had fallen to less than 1:10. Six out of seven controls did become renal carriers. Researchers in experimental bovine leptospirosis have assumed that a seronegative animal is susceptible and, as such, is suitable as a research tool. No attention has been paid to the status of the herds of origin of the experimental animals. Stalheim's results cast doubts on the susceptibility of many of the animals which have been used.

Sullivan (1970b) suggested that the presence of antibodies to pomona and hyos in heifers which he infected with a hardjo strain may have enhanced non-specific resistance to hardjo infection, although previously Kemenes (1964) demonstrated no cross-immunity between pomona and sejroe in guinea pig experiments, though the latter is closely related serologically to hardjo.

The Course of Acute Infection

Incubation Period

In his review of earlier experimental work Fennestad (1963) noted that the period between inoculation and the first rise in temperature in both calves and mature cattle was most often 3-7 days. In a few cases, however, fever appeared as early as 1-2 days after infection while in other cases, the fever appeared as late as sixteen days afterwards. The results of work published since then fall within those parameters.

Clinical Signs

Fever (up to 107°F) usually lasting for two or three days, has been the most consistent feature of experimental infection. In some cases, however, where animals have later developed other signs of infection, pyrexia has not been detected, while in others, fever has lasted for up to 12 days.

A wide variation in the clinical signs was observed even in experiments using the same strain to infect animals of approximately the same age group. Apart from pyrexia, clinical signs such as anaemia, depression, anorexia, diarrhoea, conjunctival discharge, increase in the respiratory rate, jaundice, haemoglobinuria, haematuria and death have been recorded in descriptions of severe, acute infections. Fatalities commonly occurred 6-16 days post infection. Leptospires could usually be isolated from, and demonstrated histologically in, the internal organs, especially the livers and kidneys, of those animals. In less severe infections, transitory depression, anorexia and stiffness have been recorded, while some experimentally infected animals have shown only fever and in others, infection has been asymptomatic.

Few observations of experimental infection have been made in lactating cows, although Baker and Little (1948) found that the milk yield was halved during the febrile stages of infection while, at the same time, the milk had a colostrum-like appearance. Leptospires have been demonstrated in milk during the febrile phase by a number of authors (Baker and Little, 1948; Hengl et al., 1958; Gillespie and Kenzy, 1958).

Leptospiraemia

This usually developed during the first week of disease, coinciding with the pyrexic phase and lasting for 1 to 3 days, Fennestad (1963) however, demonstrated leptospiraemia in calves 1 to 2 days before the onset of pyrexia. It usually disappeared before agglutinating antibodies could be detected although an ususual finding was described by Morter and Morse (1956) who demonstrated leptospiraemia in a cow on the 19th day after exposure, twelve days after the onset of the initial pyrexia and four days after the appearance of detectable antibodies. Fennestad (1963) demonstrated leptospiraemia throughout a period of nine days in a new born calf infected with sejroe. Leptospiraemia has been demonstrated more easily and more consistently with pomona than with members of the hebdomadis serogroup; this presumably reflects the greater difficulty in culturing the latter and their lower pathogenicity for hamsters and guinea pigs.

Significance of the Age of the Experimental animals

The influence of age in determining the severity of clinical leptospirosis has not been studied sufficiently although it is apparent that age is an important factor. A survey of the literature shows that symptomatic pomona infection occurs more frequently and is more severe in calves than in mature cattle. Only one fatality has been recorded in experimentally infected mature cattle and that was in a one-year-old bull (Ferguson et al., 1957) whereas fatalities have occurred regularly in calves infected with pomona (Baker and Little, 1948; Morter and Morse, 1956; Spradbrow and Seawright, 1963, and others). In experimental infection of bovine foetuses, varying in age from 111 to 223 days, Fennestad and Borg-Petersen (1962) found saxkoebing infection to be more severe in younger than in older foetuses, and as a result of his experiments in calf leptospirosis Fennestad (1963) concluded that clinical manifestations as a whole became less pronounced with increasing age of the calves at the time of inoculation.

Variations Associated with Serotype in the Course of Acute Clinical Illness

Apart from the publications of Fennestad and Borg-Petersen (1956, 1958b) and Fennestad (1963) all the reports have been of experiments using strains of the same serotype.

Fennestad and Borg-Petersen (1956) found no great difference in the clinical course of acute infection in pregnant heifers infected with strains of five different serotypes. In contrast to this, the same authors (1958b), found a clear difference in the outcome of infection in seven-month-foetuses inoculated with strains of three different serotypes. Foetuses infected with pomona and sejroe died, whereas those infected with saxkoebing survived.

Fennestad (1963) found that a relationship existed between the serotype and the clinical response in calves infected with pomona and sejroe, but not in grippotyphosa infection, unless the origin of the strains was also taken into account: in that case Danish grippotyphosa strains evidently behaved like sejroe strains whereas infection by the Israeli grippotyphosa strain

resulted in a severe disease with jaundice and prolonged proteinuria.

If reports on strains of individual serotypes are compared it would appear that acute infection with Russian and Israeli strains of grippotyphosa caused an infection in calves, characterised by jaundice and haemoglobinuria, (Semskow, 1941; Bernkopf et al., 1947, 1948; Fennestad, 1963) which was fatal in a proportion of cases, while Danish strains caused only a mild illness.

Acute infection of calves with pomona caused haemoglobinuria but not jaundice (Baker and Little, 1948; Reinhard, 1951; Morter and Morse, 1956; McDonald and Rudge, 1957 and Spradbrow and Seawright, 1963).

Acute infection with sejroe and hardjo in calves caused a mild disease characterised by pyrexia, depression and slight anorexia (Ristic et al., 1957; Fennestad, 1963; Hanson and Brodie, 1967; Farina et al., 1972), however, Fennestad (1963) observed jaundice in a newborn calf infected with sejroe.

Bratislava was reported to cause a severe haemorrhagic nephritic syndrome with haematuria but without haemoglobinuria in seven young calves, three of which died during the acute phase of the disease (Fennestad et al., 1967).

Imbabi et al. (1967) reported a mild disease syndrome in calves infected with canicola similar to that observed in calves infected by sejroe or hardjo.

Significance of the Dose and Route of Inoculation

Both the method of inoculation and the dose of leptospires seem to be important in determining the length of time between inoculation and the development of pyrexia and leptospiraemia. Ristic et al. (1957) found that in those calves infected with culture, leptospiraemia was demonstrated from the second to the fifth day after infection while in those infected with blood, leptospiraemia occurred later (6-13 days post-infection). Ringen et al. (1955) reported an average incubation period of 13 days in heifers infected by conjunctival instillation, longer than most other authors observed using other routes.

There is a great disparity between the size of the dose which has been necessary to produce infection by various routes. Sullivan (1970a) successfully infected eight-month-old bullocks with 0.25 ml of a 10 per cent

suspension of infected guinea pig liver, kidney and spleen in saline, placed on the conjunctiva but failed to infect similar bullocks by giving them 5 ml of the same suspension by mouth. De Lay et al. (1955) successfully produced fatal leptospirosis in a calf by feeding it 25 ml of culture. Sullivan (1970a) concluded from his observations on the route of infection that the route of inoculation influences the minimum infective dose rather than the pathogenesis of the disease.

According to Doherty (1967b) and Sullivan and Callan (1970) urine from carrier cattle does not appear to be a suitable inoculum for the experimental production of the disease possibly because undiluted bovine urine is a poor survival medium for leptospires and the possible presence of antibodies in the urine.

Sequelae to Infection

Leptospiruria

Leptospires localise in the kidney tubules where they multiply and can persist for considerable lengths of time. Leptospires have been demonstrated in kidney material as early as the fifth day after inoculation (Fennestad, 1963) and in the urine as early as the sixth day (Semskow, 1941, Fennestad, 1963). The period for which leptospiruria persisted was highly variable; the longest period was recorded by Farina et al. (1972) who demonstrated leptospires in the urine of a cow at slaughter, 118 days after inoculation with serotype hardjo.

Fennestad (1963) showed that organisms not only persist but undergo a period of multiplication in the renal tubules. In five infected calves in which leptospiruria reached very high levels, leptospires were found in the urine for the first time on the sixth to the eleventh days. During the subsequent days an obvious rise in concentration of leptospires in the urine was observed, a maximum being reached between the fifteenth and twentieth day. The maximum concentration was present for only a few days, after which it gradually declined.

The development, maximum level and persistence of leptospiruria would appear to depend on the age of the animal when infected and the strain and serotype of the infecting leptospire. Doherty (1967c) found that the mean time between the occurrence of the maximum rectal temperature and the commencement of leptospiruria was significantly less ($P<0.001$) in calves than in heifers or steers (8 months old). He also found that the mean maximum level of leptospiruria was significantly greater ($P<0.01$) in calves than in heifers or 8-month-old steers, but there was no significant difference between heifers and steers. Doherty (1967c) also found that the mean duration of leptospiruria was significantly less ($P<0.01$) in heifers than in steers or calves, but that there was no significant difference between steers and calves.

The influence of serotype on the degree of leptospiruria was noted by Fennestad (1963) who observed that pomona strains caused a massive leptospiruria in three and five week old calves while infection by sejroe caused only slight leptospiruria. Other authors have also noted that experimental infection by the hebdomadis group caused only a slight leptospiruria (Sullivan, 1970 a & b). Ristic et al. (1957) could not demonstrate leptospiruria in nine calves infected by sejroe.

The strain of leptospire also plays a part in the degree of leptospiruria. Reinhard and Hadlow (1954) used two pomona strains of different virulence to inoculate calves and found leptospiruria only in the calves infected with the virulent strain. Similarly, Fennestad (1963) noted a much heavier leptospiruria in calves infected with an Israeli strain of grippotyphosa than in those infected with Danish strains.

Proteinuria

Proteinuria has been observed in a few infection experiments on calves. Reinhard and Hadlow (1954) found a slight or moderate proteinuria, as late as 25-60 days after inoculation, in calves infected with pomona. Fennestad (1963) noted a marked proteinuria in calves which had been experimentally infected with pomona strains. In one calf this was still present six months

after the onset of disease. The same author found a proteinuria of not more than two weeks duration in two of six calves infected with sejroe but in only one was it marked. Proteinuria which began on the 3rd or 4th day in calves infected with a bratislava strain, reached its peak 1 to 7 days later (300 mg% or more) (Fennestad et al., 1967). It decreased gradually in the five surviving calves and ceased in four of them during the 2nd, 3rd, 5th and 8th weeks, but there were still traces of protein in the urine of one calf when it was sacrificed 12 weeks after the onset of the disease.

Abortion

Abortion has been reported as a sequel to experimental infection of pregnant cattle by pomona in a number of studies (Ringen et al., 1955; Morse and McNutt, 1956; Ferguson et al., 1957; Murphy and Jensen, 1969). Fennestad and Borg-Petersen (1956) reported that 1 out of 4 heifers infected with icterohaemorrhagiae had aborted and a similar reaction was obtained when poi was used. Attempts to induce abortion by infecting pregnant cattle with sejroe and saxkoebing (Fennestad and Borg-Petersen, 1956) and hardjo (Sullivan, 1970b; Farina et al., 1972) failed. Sullivan (1972), however, observed that 1 out of 11 heifers aborted two months after infection with hardjo, but discounted the possibility of its resulting from hardjo infection because of the long time lag and his inability to carry out bacteriological and histopathological examinations of the aborted foetus.

Infection of the foetus by direct inoculation into a pregnant uterus has demonstrated that sejroe, saxkoebing (Fennestad and Borg-Petersen, 1958b, 1962) and hardjo (Hanson and Brodie, 1967) can cause foetal death and abortion if the pathogens enter the foetal circulation. Even when foetal leptospirosis was induced, abortion did not necessarily follow: Fennestad and Borg-Petersen (1962) showed that the foetus could overcome infection by leptospires of low pathogenicity and suggested that this ability increases with the age of the foetus.

Retention of foetal membranes following abortion by experimentally infected cows was observed by Fennestad and Borg-Petersen (1958b).

Serology

In the few early studies that provided detailed information on the antibody response (Reinhard and Hadlow, 1954; Fennestad and Borg-Petersen, 1956; Ristic et al., 1957) infected animals were found to produce antibodies against the infecting serotype. Antibodies were first detected in the second week after infection and a few days later a maximum titre was attained that remained unchanged for a period ranging from a few days to several weeks, after which there was a varying fall in titre.

In a few cases, a serological response could not be demonstrated in calves of less than 3 months of age when inoculated with pomona, although leptospires were found in the urine or kidneys of these calves (Reinhard, 1951; McDonald and Rudge, 1957). Fennestad (1963) suggested that in those cases it could have been due to antibody present at the time of inoculation.

Fennestad (1963) found that there was an apparent relationship between the serotype of the infecting strain and the serological response in 3 week-old calves infected with pomona and sejroe strains. In pomona infection the serological response was characterised by a protracted period of rising titre and a high maximum titre, whereas in sejroe infection the serological response was characterised by a short period of rising titre and a low or moderate maximum titre. Doherty (1967c) likewise observed a slow rise of antibody titre in cattle infected with pomona; the mean interval from first detection of serum antibody to the development of maximum titre was over three weeks. Doherty also noted that maximum serum antibody titres were most frequently reached after the end of leptospiruria. Both Fennestad (1963) and Doherty (1967c) were of the opinion that the continued presence of leptospires in the renal tubules was responsible for the high, slow-rising antibody titres.

Sullivan (1972) reported a biphasic pattern in antibody response in six of eight cows experimentally infected with hardjo. The secondary rise in antibody titre occurred three months after inoculation and did not correlate with alterations in the degree of leptospirurla. It may have been an anamnestic response to challenge by leptospires present in the environment.

Fennestad and Borg-Petersen (1962) demonstrated that foetuses which survived leptospiral infection produced antibodies to the infecting strain. Five of eight foetuses survived direct inoculation with saxkoebing and produced antibody titres of 1:1,000 or more against that serotype. The youngest foetus which survived was only 134 days old when infected.

Pathology

A variety of gross lesions have been described in acute fatal cases, in particular jaundice, haemoglobinuria, perirenal oedema, haemorrhages in various organs and enlargement of the liver and kidneys. In recovered cases the only gross lesions consistently observed were grey-white foci on the surface of the kidneys, often surrounded by a ring of hyperaemia.

Spradbrow and Seawright (1963) described two types of microscopic damage to the liver in fatal cases. The first type consisted of focal necrosis with foci of inflammatory cell infiltration in the portal tracts and was presumably due to localisation of the organisms in the liver. The second type was seen in animals which died as a result of severe anoxaemia. Central zonal degeneration and necrosis of the parenchyma were superimposed on the focal changes.

In animals slaughtered 9-11 days after infection with pomona, the prominent histopathological feature of the livers was cellular infiltration of the portal spaces (Reinhard and Hadlow, 1954). This was especially prominent around the bile ducts.

The most important changes reported in the kidneys in fatal cases were cloudy swelling of the tubular epithelium especially that of the convoluted tubules, desquamation of the epithelium, and the presence of casts of

cellular accumulations, largely of lymphocytes were also present in some cases. In recovered cases focal interstitial nephritis was the outstanding lesion. In these foci lymphocytes were the predominant cell type present. Tubular collapse and varying degrees of fibrosis were also found.

In fatal cases degenerative changes have also been recorded in heart and skeletal muscle and in the central nervous system (Avrorov, 1941).

Reinhard and Hadlow (1954) reported slight mononuclear cell cuffing in the midbrain and small areas of haemorrhage in the brain of a calf killed 11 days after infection with pomona, while in another calf killed 13 days post inoculation, perivascular accumulations of lymphocytes were found in several meningeal vessels in the sulci of the cerebrum.

Foci of predominantly lymphocytic infiltration in the testicular tissue of four out of six bulls experimentally infected with pomona were described by Sleight et al. (1964).

In cases of abortion, the prominent gross findings in the placenta were the presence of uniformly atonic, fawn-coloured cotyledons and oedema of the intercotyledonary areas (Fonnestad, 1956, 1962). Histologically, there was no cellular infiltration of the cotyledons, the smaller blood vessels were collapsed and there was necrosis of the villi. Murphy and Jensen (1969) reported mild polymorphonuclear leucocyte infiltration in the stalks of the caruncles in cows with foetuses which had died in utero in the course of an abortion pathogenesis experiment using pomona.

Aborted foetuses showed generalised oedema and the pleural, pericardial and peritoneal cavities contained large amounts of dark red coloured fluid. Microscopically, degenerative changes and small interstitial foci of cellular infiltration were seen in the kidneys.

The Present Investigation

Following the observation of abortion in Scottish herds infected by the hebdomadis serogroup, and the finding of a high incidence of infection, it was decided to investigate the pathogenicity of J10 strain for heifers at various stages of gestation. Seven young calves were also included in the study.

MATERIALS AND METHODS

Experimental Animals

Four groups of cattle were infected experimentally.

Group I - This group consisted of six Friesian-Ayrshire cross heifers (Nos. 1-6) all in the last month of gestation. All the heifers came from the same farm in Ayrshire. The herd had been free from leptospiral infection when tested previously against antigens of 16 serogroups. When the six heifers were brought to the experimental premises they were seronegative to sejroe and hebdomadis serotypes, although on subsequent re-examination with hardjo (204 strain) heifer 3 was found to have a titre of 1:30 to hardjo.

Group II - comprised six Friesian heifers (Nos. 7-12) which came from another Ayrshire farm with seronegative stock. The heifers were 6-7 months pregnant.

GroupIII - Eight Highland and Galloway cross Shorthorn heifers (Nos. 13-20) were purchased at Oban cattle sales after serum samples from them were found to be free from leptospiral antibodies. They were between 3½ and 5 months pregnant. On the day after they arrived at the experimental byre blood samples were taken from all of the animals for re-examination. The heifers were immediately infected with J10 strain before the results of the test were known. The second test of the pre-infection sera revealed antibody titres of 1:30 and 1:10 in heifers 13 and 18 respectively to hardjo (204 strain) whilst the serum from heifer 19 reacted at a dilution of 1:30,000 with hardjo. It would appear that heifer 19 became exposed to leptospiral infection at about the time of the first bleeding (14 days previously) or soon afterwards.

GroupIV - consisted of seven calves (CA21-CA27) between 4 and 33 days old. They were colostrum-fed calves born to seronegative dams. Leptospiral antibodies were not detected in their sera.

Management of the Experimental Animals

The heifers were kept tied up in individual stands in a byre with separate feeding troughs and watering bowls. After calving, moveable metal partitions were erected to form a pen around each animal to prevent cross suckling by their calves. The calves were kept with their dams until the termination of the experiment.

Production of Infection in Experimental Animals

Leptospira strain J10 was used to infect all four groups. This strain was isolated from the kidneys of a cow which had aborted. Details of its isolation are given in Chapter 3, Part B. Studies by Dr Dikken have shown that strain J10 belongs to the hebdomadis serogroup and is closely related to serotype hardjo. Work is contunuing on the final identification of this strain.

Inoculation Procedure

Each heifer was inoculated with 5 ml of a very sparsely growing culture; no attempt was made to quantitate the number of leptospires present in the inoculum. The calves were infected by either the inoculation of blood from another calf which was in the leptospiraemic phase of infection or with 2 ml of J10 culture which had gone through a variable number of calf and hamster passages. The details are summarised in Table 13.

The route of inoculation was always intramuscular.

Clinical, serological, bacteriological, biochemical and pathological examinations of the experimental animals were carried out at regular intervals.

A Clinical Examination, including the taking of rectal temperatures, was carried out twice daily on all experimental animals with the exception of the heifers in Group III, for four days prior to infection and on all animals following inoculation. Rectal temperatures of 103.0^{o}F or more were regarded

Table 13. A Summary of the Inoculation Procedure used in the Infection of the various Experimental Animals

Group	History of Inoculum	Dose	Route of Inoculation
I (Heifers 1-6)	J10: the seventh subculture since its isolation 3 months previously	5 ml	I.M.
II (Heifers 7-12)	ditto	5 ml	I.M.
III (Heifers 13-20)	3 week old culture obtained from the heart blood of a hamster inoculated with macerated liver of calf 21	5 ml	I.M.
IV (CA21-27)			
CA21	Culture recovered from the kidney of heifer 11 passaged in a hamster	2 ml	I.M.
CA22	Given (1) blood from CA21 on day 0	20 ml	I.M.
	(2) macerated liver from CA21 on day 1 challenged with	2 ml	I.M.
	(3) culture isolated from CA21 - day 20	2 ml	I.M.
CA23	Culture isolated from CA21, via a hamster	2 ml	I.M.
CA24	Culture isolated from heifer 16, via a hamster	2 ml	I.M.
CA25	Culture isolated from heifer 14, via a hamster	2 ml	I.M.
CA26	(1) Macerated liver and blood from a hamster inoculated with blood from CA25 - Failed. Repeated with	2 ml	I.M.
	(2) blood from CA27	20 ml	I.M.
CA27	2 ml of culture isolated from CA25	2 ml	I.M.

I.M. = intra-muscular

as indicative of pyrexia (Blood and Henderson, 1963). The incubation period was considered to be the time between inoculation and the onset of pyrexia.

Serological Examination

Blood samples for serological examinations were collected just prior to infection, on days 3, 7, 10 and 13 post-inoculation and thereafter at weekly intervals. They were also collected on the day of calving, three days later and at slaughter. Newborn calves were sampled before they received colostrum, if possible, and thereafter on the days on which their dams were bled.

Blood samples were left to clot overnight. The following day the sera were decanted, screened for leptospiral antibodies and then stored at -20°C.

Milk samples were collected immediately after calving, on the first and third days after calving and thereafter at weekly intervals. Each milk sample was centrifuged at 500g for 10 minutes and 10 ml of the defatted milk was removed from beneath the cream layer and two drops of rennet were added to it. This was allowed to stand overnight at room temperature or placed in an incubator at 37°C for two hours. The sample was then centrifuged at 1,000g for 10 minutes and the clear whey was removed and stored at -20°C.

At the end of the experiment all the serum and whey samples from a given animal were titrated against hebdomadis, sejroe and hardjo (204 strain) on the same day. This ensured that the same density of antigen was used and hence changes observed in the agglutinating antibody titre reflected actual changes in antibody titre to that strain.

When it was realised that J10 strain could not be grown to a density suitable for the microscopic agglutination test, hardjo (204 strain) was introduced to check the antibody response.

Bacteriological Examination

Attempts to demonstrate leptospiraemia were made on the fourth and sixth days after infection and during the period of subsequent pyrexia.

Urine samples were collected from the heifers on the fourteenth day post-inoculation and thereafter at weekly intervals. They were examined for leptospires by dark ground microscopy.

Isolation of leptospires was attempted from the kidneys of the infected heifers, their progeny and the experimental calves. The liver, lungs and foetal membranes of the aborted calf from heifer 7 and the liver and lungs of calf 21 were also cultured.

The liver, lungs, stomach contents and foetal membranes of the aborted foetus, and the internal organs of calf 21 were subjected to a comprehensive bacteriological examination, using a variety of solid media, incubated under aerobic and microaerophilic conditions. Mycological examination was also carried out on the aborted foetus.

Biochemical Examination

Blood samples for biochemical analysis were collected at the same time as those for serological and bacteriological examinations. The assays listed below (Table 14) were carried out on each sample.

Table 14. List of Biochemical Assays performed on Blood Samples from Experimental Animals

Assay	Method
Urea	Technicon Method* AA11-1
Inorganic Phosphate	Robinson et al. (1971)
Bilirubin	Technicon Method* AA11-18
Alkaline Phosphatase	Technicon Method* AA11-06
Serum Glutamic Oxaloacetic Transaminase	Technicon Method* AA11-10
Serum Glutamic Pyruvic Transaminase	Technicon Method* AA11-22
Total Protein	Technicon Method* AA11-14
Creatinine	Technicon Method* AA11-11

*Technicon Instrument Corporation, 511 Benedict Avenue, Tarrytown, New York.

Method of Slaughter

All experimental heifers and calves were killed at the termination of the experiment. The animals were stunned with a captive-bolt pistol and then exsanguinated.

Pathological Examination

The foetal membranes were examined macroscopically as soon as they were expelled and either selected cotyledons or the whole placentas were photographed. Complete cotyledons intended for histological examination were pinned to discs of filter paper and fixed in formal saline. Blocks of kidney, liver and, occasionally, lung and brain were taken from the calves at post-mortem while blocks of kidney only were obtained from the heifers.

Foetal membranes were also collected from four seronegative cows belonging to the Department of Veterinary Medicine and selected cotyledons were taken for comparative histological examination and processed by the method described above.

RESULTS

Clinical Findings

The clinical syndrome produced in the pregnant heifers was very mild or subclinical. A marked pyrexia was the major feature. One heifer aborted and another produced a live, premature calf. The clinical manifestations of the disease were more severe in the calves than in the adult cattle and one calf died nine days after infection. The main clinical findings are summarised in Tables 15, 16, 17 and 18 and graphs of the thermal response of individual animals are shown in Figure 74 (Appendix 2).

Incubation Period

The incubation period was usually three to five days, although two heifers (1 and 3) showed a mild pyrexia on the day following infection, while one heifer (15) failed to show a thermal response until the tenth day post-inoculation (P.I.) and calf 24 failed to develop pyrexia until the 13th day P.I.

Acute Infection

Pyrexia was the clinical feature most consistently observed. It was often marked (up to 106.2°F) and occurred within 13 days of inoculation in all the experimental animals with the exception of heifers 7 and 9 and calves 21 and 26. The initial pyrexic phase usually lasted 1-5 days, although heifer 19, which was assumed to have been infected naturally, remained pyrexic for 20 days. Calves 21 and 26 showed no significant increase in rectal temperature in response to infection, however, calf 21 developed hypothermia on days 8 (97.4°F) and 9 (95.5°F) P.I. prior to its death on day 9 P.I. The maximum temperatures recorded in calves were not as great (a maximum of 104.4°F in CA25) as in the adult cattle.

During the initial febrile period the heifers developed increased respiratory and pulse rates and ruminal movements decreased with some loss of appetite but, considering the degree of pyrexia, the associated symptoms were rather mild and would probably have gone unnoticed under field conditions.

Heifer 11 showed an increase in SGOT values which reached a maximum on day 11 (244 SF units) and then declined.

During the acute phase of the disease, a greater range of clinical signs was observed in the calves than in the heifers. Calf 21 was noted to be rather dull and uninterested in food on the afternoon of the 7th day P.I. and on the morning of day 8 it was found to be recumbent and unable to rise, opisthotonus developed and death followed on the afternoon of the 9th day. A urea level of 63 mg% was found in a blood sample taken from it on the 8th day. Calves 23, 24 and 27 appeared very dull and unwilling to move or eat during the initial febrile period. Similar, though more severe, clinical signs were noted in calf 25: it appeared to be very weak and had great difficulty in standing up during the pyrexic phase (days 6-8). In the remaining two calves (CA22 and 26) only mild transitory dullness and partial anorexia were observed.

Later Findings

Nineteen animals (16 heifers and 3 calves) had relapses of fever, most often commencing on the 8th to the 15th day P.I. Two or more recurrences of fever were observed in 17 animals (15 heifers and 2 calves): the second relapse usually began on the 15th to 25th day P.I. Pyrexia was often very marked during these secondary febrile periods, for example, a rectal temperature of 106.9°F was recorded in cow 8 on the 13th day P.I. Usually, no other clinical features were associated with these periods of pyrexia.

Heifer 5 produced a live, weak calf on day 16 P.I. This calf was always reluctant to move or suckle and it died one week later. During this period it was severely trampled on by its dam on a number of occasions.

Heifer 7 showed no evidence of pyrexia until the 49th day P.I. when it developed a fever which lasted for 5 days and reached a maximum of 105.2°F on day 50. This heifer aborted a near-term foetus on day 58. The abortion took place easily and unassisted and the foetus did not appear to have been dead long.

Heifer 9, which did not show any evidence of pyrexia until day 28 (104.0°F), also showed pyrexia during the period 49-53 days post-infection. The maximum temperature (104.7°F) was recorded on day 50. A live, weak premature calf (240-245 days gestation) was born on day 60. The calf had to be held up to suckle for the first two days of life but it survived for 7 days after which time it was slaughtered.

The remaining heifers produced live healthy calves, however, heifer 12 developed pyrexia on days 42 and 49 P.I. and heifer 16 looked as if it might calve on a number of occasions during the six weeks prior to calving. In all cases the foetal membranes were passed in less than six hours after parturition.

Reinfection of calf 22 on day 20 was followed by a thermal response on days 22, 23 and 25.

Table 15. A Summary of the Clinical Findings in the Experimental Heifers - (Group I)

Heifer No.	Stage of Gestation (months) at inoculation	Incubation Period (days)	Initial Period of Fever (>103.0°F)		Subsequent Periods of Pyrexia (>103.0°F) (days P.I.)	Max. Temp (°F) Recorded (day P.I.)	Clinical Features
			Days P.I.	Max. Temp °F			
1	8½	1	1	103.5	3, 6-8, 16, 21	104.4(16)	Very mild. Live calf produced.
2	8½	5	5-6	104.8	15, 35	104.8(5)	ditto
3	8	1	1	103.2	5-8, 10-13, 15-16, 21-22, 25-27	104.2(25)	ditto
4	8	5	5-6	105.0	12, 17, 22, 24, 27	105.0(6)	ditto
5	8½	4	4-8	106.0	10-11, 13, 15	106.0(4)	Very mild. Produced a weak live calf which died one week later and *E. coli* was isolated from a peritonitis.
6	8½	3	3	103.4	6-7, 11	103.4(3)	Very mild. Produced a live calf.

P.I. = post inoculation

Table 16. A Summary of the Clinical Findings in the Experimental Heifers - (Group II)

Heifer No.	Stage of Gestation (months) at inoculation	Incubation Period (days)	Initial Period of Fever (>103.0°F)		Subsequent Periods of Pyrexia (>103.0°F) (days P.I.)	Max Temp (°F) Recorded (day P.I.)	Clinical Features
			Days P.I.	Max Temp °F			
7	6½	-	-	-	A period of marked pyrexia occurred 49-53 days P.I.	105.2(50)	Subclinical initial phase. Aborted a near term dead calf. 58 days P.I.
8	6½	4	4-7	105.9	12-15, 29	106.9(13)	Very mild. Produced a live calf.
9	6	-	-	-	28, 49-53	104.7(50)	Subclinical. Produced a live weak premature calf (240-245 days gestation) - 60 days P.I.
10	7	5	5-7	105.0	15-22, 24, 29	105.6(20)	Very mild. Produced a live calf.
11	7	8	8	103.0	17-18, 23, 26-31, 37	103.6(30)	ditto
12	7	5	5-6	105.6	10, 17-18, 27-28, 42 49	105.6(6)	ditto

P.I. = post inoculation

Table 17. A Summary of the Clinical Findings in the Experimental Heifers - (Group III)

Heifer No.	Stage of Gestation (months) at inoculation	Incubation Period (days)	Initial Period of Fever (>103.0°F)		Subsequent Periods of Pyrexia (> 103.0°F) (days P.I.)	Max Temp (°F) Recorded (day P.I.)	Clinical Features
			Days P.I.	Max Temp °F			
13	4½-5	7	7	103.2	10-13, 17	105.2 (11, 17)	Subclinical
14	3½	5	5-6	104.4	10-12	104.5(11)	Subclinical. Had an elevated SGOT (244 SF units) on day 11.
15	4½	10	10	103.2	12-13, 17, 32	103.4(17)	Subclinical
16	4½-5	5	5-7	106.2		106.2(6)	Very mild. On several occasions during the 6 weeks before calving it appeared about to calve but did not. Monophasic thermal response.
17	3½	3	3-5	103.6		103.6(5)	Monophasic thermal response.
18	5	3	3-4	103.5	6, 12, 32	103.5(3)	Subclinical
19	6½	Presumed naturally infected			Continuous pyrexia days 1-20 with subsequent relapses	105.0(17)	Subclinical
20	4-4½	11	11-16	105.7	19, 25, 28-29, 31 43-44	105.7(14)	Subclinical

Table 18. A Summary of the Clinical Findings in the Experimental Calves -(Group IV)

Calf No.	Age when infected (days)	Incubation Period (days)	Initial Period of Fever (>103.0°F)		Subsequent Periods of Pyrexia (>103.0°F) (days P.I.)	Max Temp (°F) recorded (day P.I.)	Clinical Features
			Days P.I.	Max Temp °F			
CA21	4	-	-	-	Temperature subclinical on days 8 and 9	-	Calf died 9 days P.I. No pyrexia.
CA22	13 & 33	1	1	103.2	3-5, 8-12	103.7(9)	Very mild. Reinfected on day 20 and a secondary thermal response was elicited (103.0°F - days 22, 23 and 25).
CA23	23	7	7	103.5	14, 16	104.0 (14, 16)	Very dull and stiff on days 5-7 when it was anorexic.
CA24	5	13	13	103.4	28	103.4 (13, 28)	Very dull and stiff on days 13 and 14 when it was anorexic.
CA25	13	6	6-8	104.4		104.4(7)	Very weak, dull and had difficulty in rising on days 6-8. Monophasic thermal response.
CA26	29	-	-	-			Mild. No thermal response.
CA27	24	5	5-6	104.0		104.0(6)	Dull - days 5 & 6. Monophasic thermal response.

Bacteriological Findings

Leptospiraemia

Leptospiraemia was demonstrated in 10 of the 20 heifers and in all 7 experimental calves during the first week after inoculation (Table 19). The results do not give a complete picture of the duration of leptospiraemia since tests for this were not made daily during the period, however, leptospires were isolated from the blood of calf 25 on 4 days (4, 5, 6 and 8) over a five day period. The occasions on which leptospiraemia was demonstrated relative to the occurrence of pyrexia and the appearance of circulating antibodies are shown in Figure 38. The results show that leptospiraemia may occur as early as four days prior to the initial fever (heifer 11) but commonly occurred 1-2 days before the initial rise of temperature (heifers 2, 10, 12, 16, 17 and calves 25 and 27). Leptospiraemia was also present during the initial pyrexic phase (2, 5, 8, 10, CA25, CA27) and in the absence of a thermal response (CA21 and CA26), yet it was never demonstrated during the secondary febrile phases; however, failure to recover leptospires does not exclude the possibility of their presence in the blood at that time, it merely indicates that there was less than one guinea pig or hamster infective dose per ml of blood.

Leptospiraemia was still present in heifers 2 and 8 on the first day on which antibody was detected but it soon disappeared.

Of the six heifers which either had traces of antibody present at the time of inoculation (3, 13, 18 and 19) or in which the pattern of antibody response suggested an anamnestic response (15 and 20), leptospiraemia was only demonstrated in one (heifer 3, day 6, titre 1:300) whereas it was detected in 16 of the other 21 experimental animals.

Hamsters were a much more sensitive monitor of leptospiraemia than were guinea pigs. Infection usually proved lethal for hamsters (Fig. 39) within 8-12 days of inoculation with infected blood and leptospires could be demonstrated in their livers (Fig. 40) and kidneys. All the guinea pigs survived similar challenge.

Table 19. The Demonstration of Leptospiraemia in Experimental Animals (Groups I, II, III and IV)

Number of animal	Day leptospiraemia detected	Maximum rectal temperature** on day of attempted detection	Titre* when infected (hardjo)	Titre at** time of attempt to demonstrate leptospiraemia
Heifer No.				
1	-	102.8, 103.6	0	0, 10
2	4, 6	102.4, 103.5	0	0, 10
3	6	102.4, 103.1	30	30, 300
4	-	102.8, 105.0	0	0, 30
5	4, 6	106.0, 103.9	0	0, 0
6	-	102.6, 103.0	0	0, 0
7	-	102.0, 101.7	0	0, 10
8	4, 6	103.2, 105.9	0	0, 10
9	-	101.8, 101.5	0	0, 0
10	4, 6	102.2, 105.0	0	0, 0
11	4	101.8, 102.0	0	0, 10
12	4	102.6, 105.6	0	0, 10
13	-	101.8, 101.8	30	30, 30
14	4	102.4, 103.2	0	0, 0
15	-	102.2, 102.6	0	10, 30
16	4	102.6, 106.2	0	0, 10
17	4	102.4, 102.4	0	0, 30
18	-	103.0, 103.0	10	10, 10
19	-	103.4, 103.4	30,000	30,000 100,000
20	-	102.6, 102.9	0	10, 10
Calf No.				
CA21	6	101.2	0	0, 0, 0
CA22	3, 5	103.5, 103.3	0	0, 0
CA23	5, 7	102.3, 102.6, 103.5	0	0, 0, 0
CA24	5	102.3, 102.5	0	0, 0
CA25	4,5,6,8	102.4, 102.0, 103.8, 104.4, 104.0	0	0, 0, 0, 0, 0
CA26	5	102.2, 102.6, 102.4	0	0, 0, 0
CA27	4,5,6	101.2, 103.4, 102.8	0	0, 0, 0

*Titre expressed as the reciprocal of the actual titre.

**Values for temperature and titre underlined are the values for the days on which leptospiraemia was demonstrated.

Fig. 38. The demonstration of leptospiraemia relative to the occurrence of pyrexia and the appearance of circulating antibody.

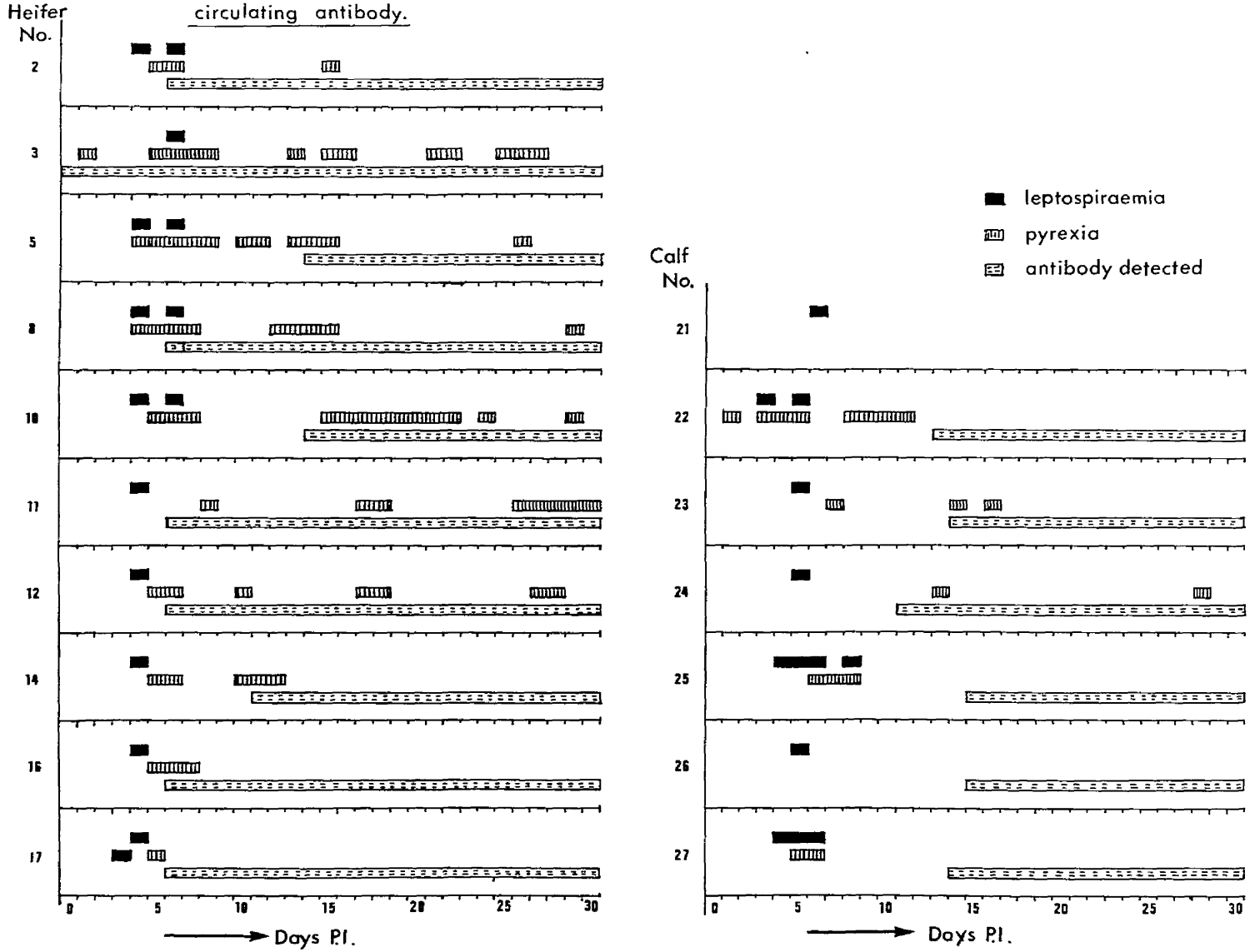
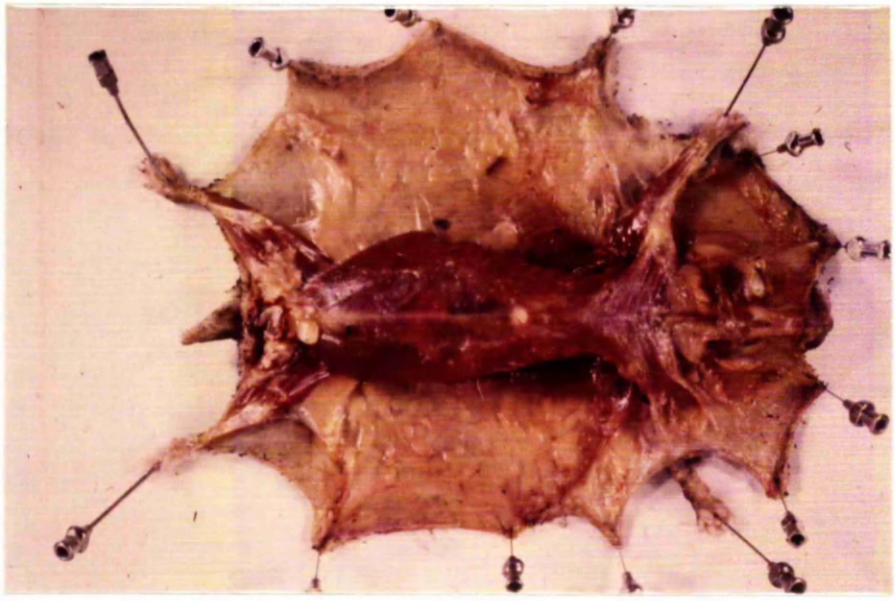
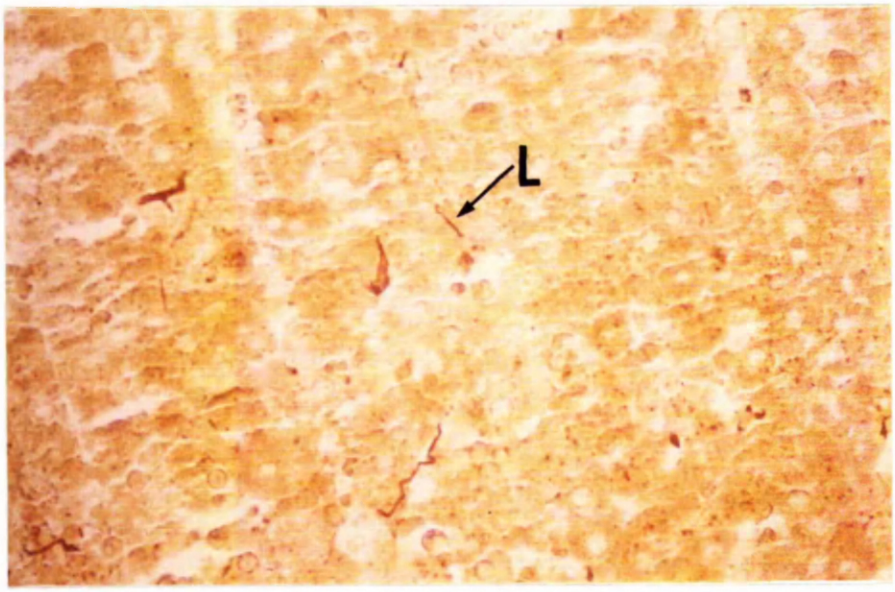


Fig. 39. A hamster which died 9 days after inoculation with blood taken from a cow during the leptospiraemic stage.
Note the jaundiced appearance of the subcutaneous tissue.

Fig. 40. Photomicrograph of a section of liver from the hamster shown in Fig. 39. Note the leptospire (L)
Leviditi x 430




L

The Presence of Leptospires in the Placentas

The demonstration and distribution of leptospires in the placentas are summarised in Table 20. Leptospires were isolated by guinea pig inoculation and subsequently demonstrated by histological techniques (Fig. 41) from the placenta of heifer 1 which delivered a live calf 14 days P.I. and were demonstrated histologically in the placentas of 5 heifers (1, 4, 5, 6 and 12) Figs. 45, 46, 47, 48, 49, 50 and 51) which produced live full term calves 14-55 days P.I. Leptospires were also found in the placenta of heifer 7 (Figs. 42 and 43) which aborted 58 days P.I. and in that of heifer 9 which produced a premature calf on day 60 (Fig. 44).

Leptospires were only demonstrated in the placentas of heifers infected later than six months gestation. They were not demonstrated in the placentas of any of the heifers which had either antibody to hardjo (3, 13, 18, 19) when infected or in those in which the pattern of antibody response suggested an anamnestic response (15, 20).

Leptospires usually occurred on the surface of the trophoblast layer or were seen interdigitating between the trophoblast cells either singly or in small clumps (Figs. 45, 46 and 47). In three cases, heifers 7, 9 and 12, leptospires were found in necrotic trophoblast material (Figs. 44 and 48) and in two instances (7 and 12) they were found in the mesenchymal layer (Figs. 42, 43 and 49).

Bacteriological Findings in the Calf (CA21)which died 9 days P.I.

Leptospires were isolated from a mixed inoculum of blood and liver from calf 21 by means of hamster and calf (CA22) inoculations. They were also demonstrated in histological sections of its kidneys.

Bacteriological Findings in the Aborted Foetus (CA7), the Premature Calf (CA9) and Calf 5

Attempts to isolate leptospires from the liver, kidneys and lungs of the aborted foetus (CA7) failed, as did attempts to recover other bacteria or fungi. Histological examination of sections of the liver (Figs. 52 and 53)

Table 20. The Demonstration and Distribution of Leptospires in the Placentas

	Heifer No.						
	1	6	5	4	12	7	9
1. Stage of gestation when inoculated (months)	$8\frac{1}{2}$	$8\frac{1}{2}$	$8-8\frac{1}{2}$	8	7	$6\frac{1}{2}$	6
2. Titre of antibody to *hardjo** at calving	1,000	3,000	10,000	3,000	1,000	100	100
3. Leptospires demonstrated in the placenta (days P.I.)	14**	15	16	32	55	58	60
4. Position of leptospires in cotyledon:							
a. On the surface of the trophoblast	+	+	+	+	+	+	-
b. Interdigiting between trophoblast cells	+	+	+	+	+	+	-
c. In necrotic trophoblast material	-	-	-	-	+	+	+
d. In mesenchyme	-	-	-	-	+	+	-
5. Outcome of pregnancy	Healthy live calf	Healthy live calf	Weak calf died one week later	Healthy live calf	Healthy live calf	Aborted dead calf. Leptospires in foetal liver, kidney and lung	Premature live calf - had an antibody titre of 1:10 to *hardjo*

* Titre expressed as the reciprocal of the actual titre.

** A guinea-pig inoculated with macerated placenta from *heifer 1* developed antibody titre to *hardjo* and leptospires were demonstrated in its kidney (Fig. 41).

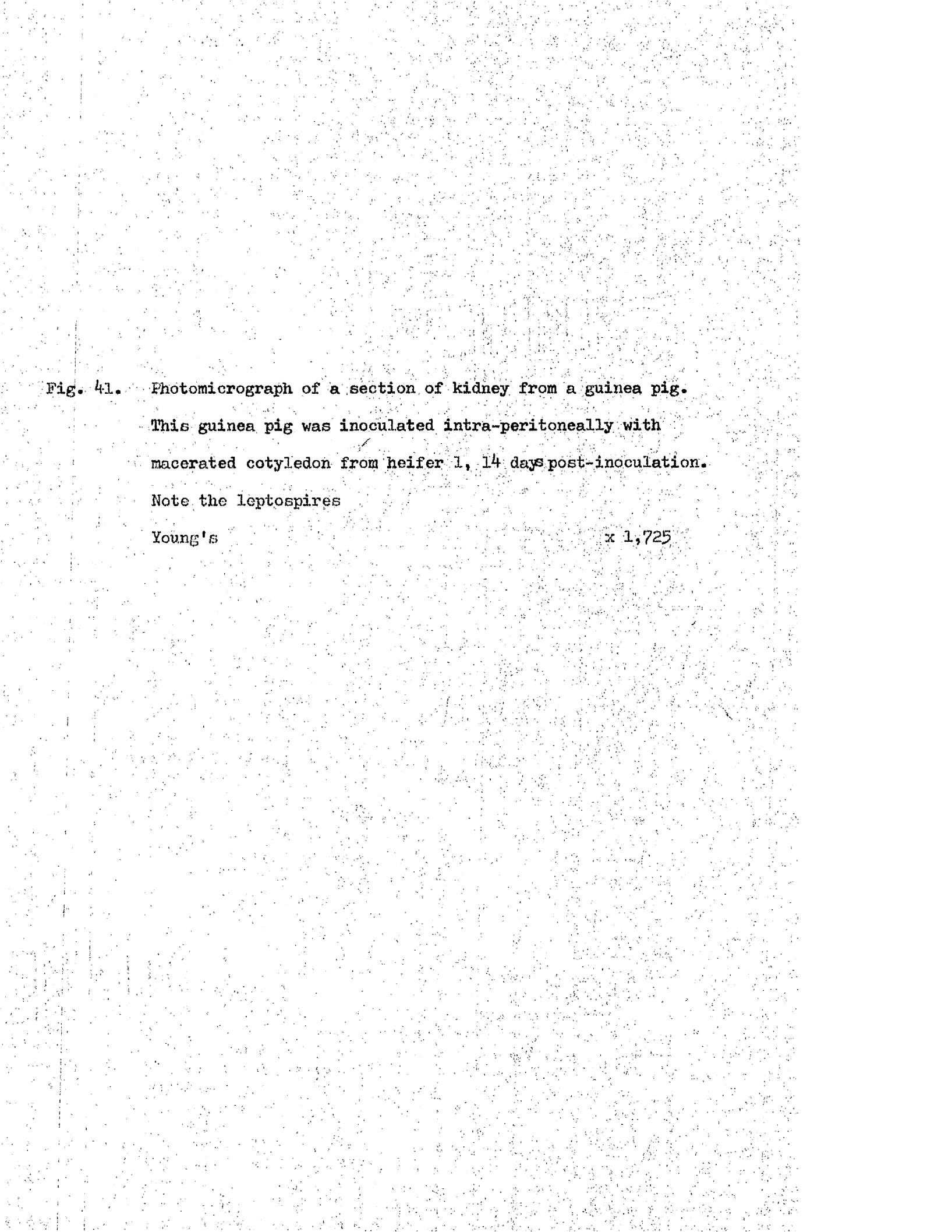


Fig. 41. Photomicrograph of a section of kidney from a guinea pig. This guinea pig was inoculated intra-peritoneally with macerated cotyledon from heifer 1, 14 days post-inoculation. Note the leptospires

Young's x 1,725

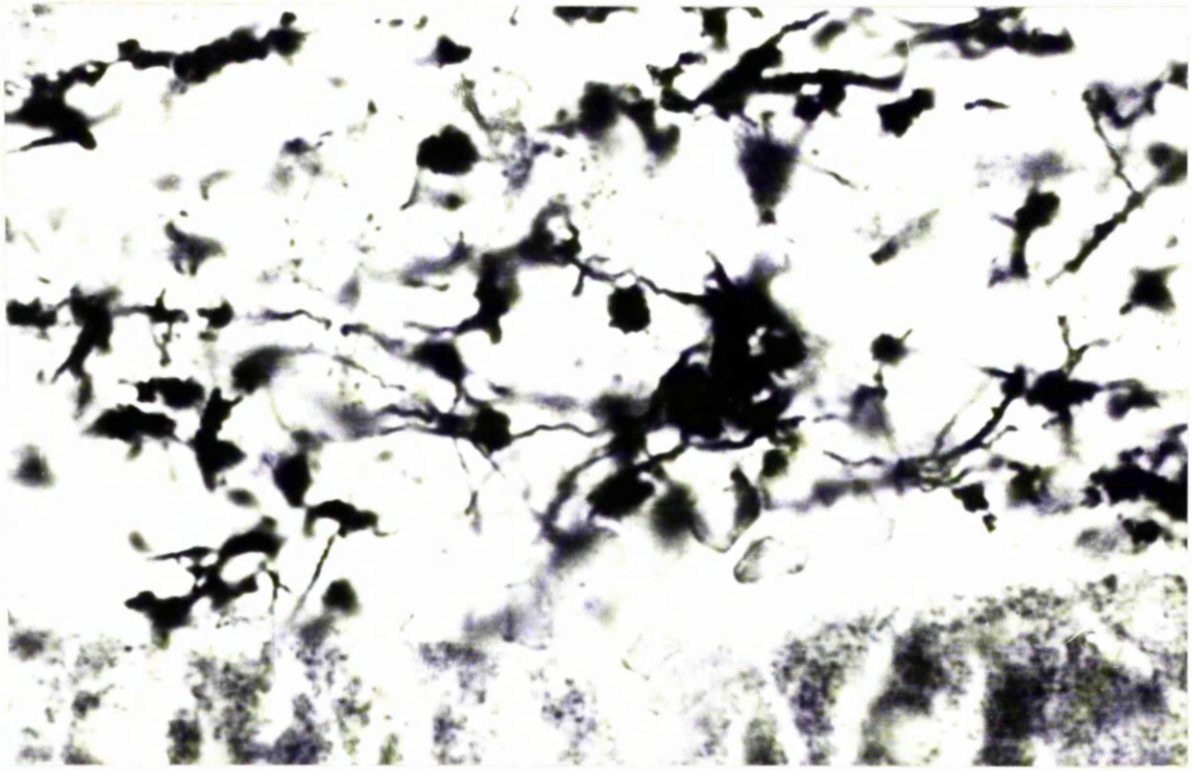
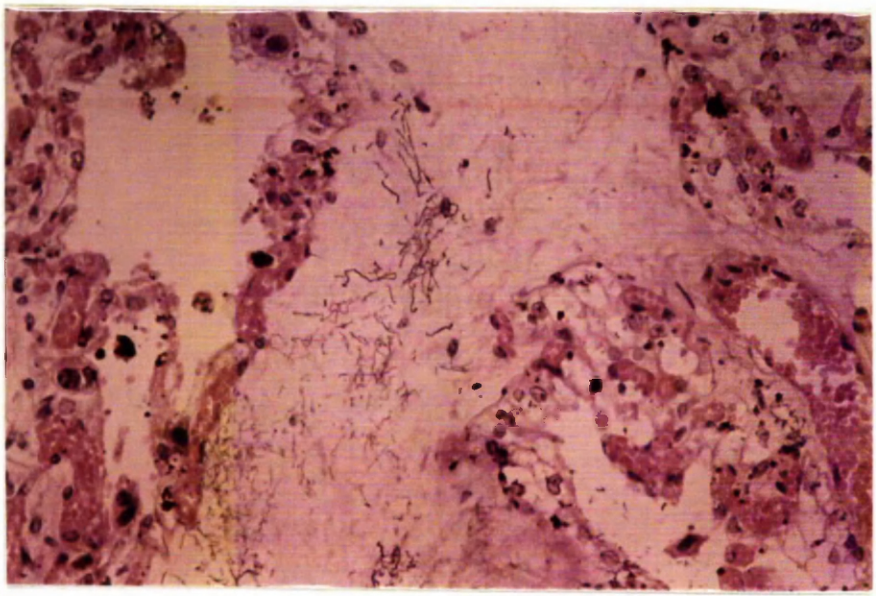


Fig. 42. Photomicrograph of a section of cotyledon from heifer 7, at abortion 58 days post-inoculation. There are numerous leptospires in the mesenchymal connective tissue.

Faine and H & E x 275

Fig. 43. A higher magnification of an area shown in Fig. 42. The morphology of individual leptospires is shown.

Faine and H & E x 1,600



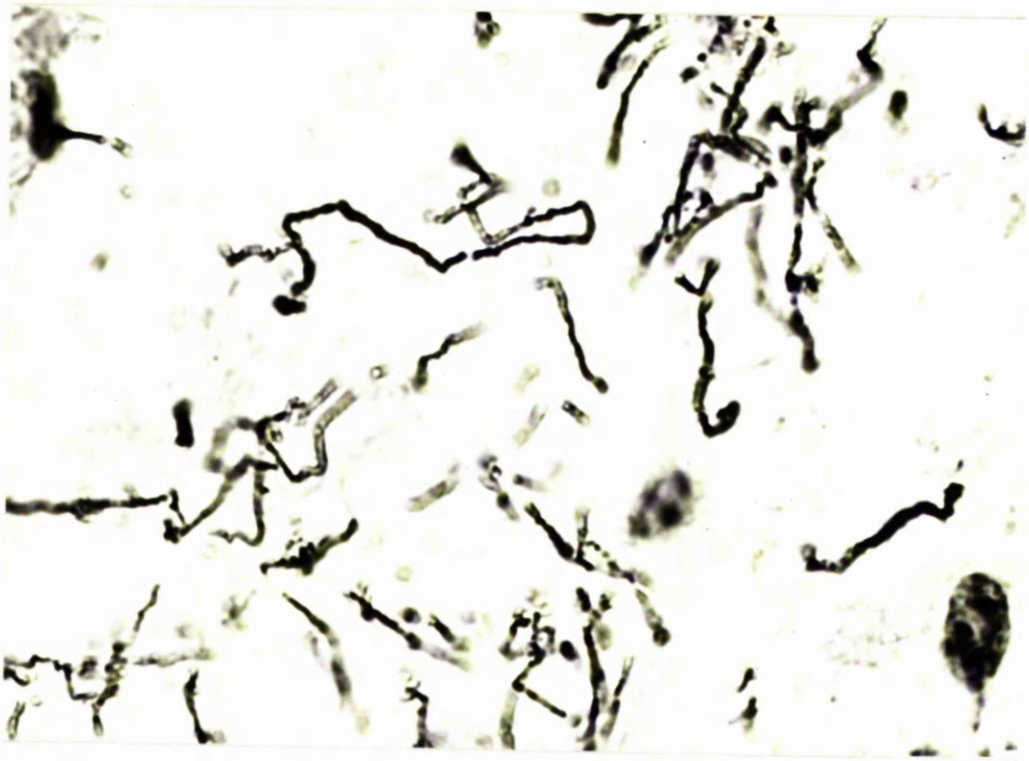
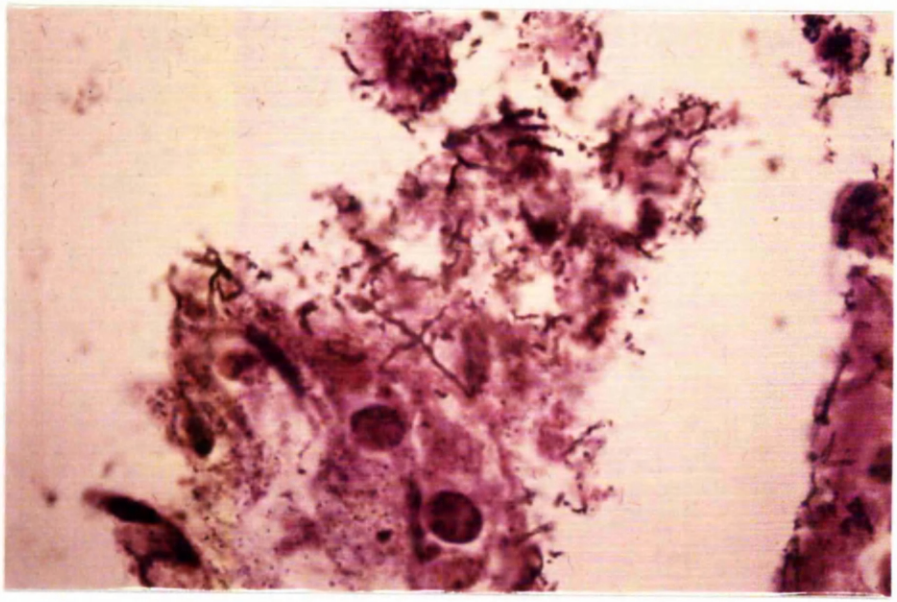


Fig. 44. Photomicrograph of a section of cotyledon from heifer 9, at premature calving 60 days post-inoculation.
Leptospires can be distinguished in the necrotic material.
Faine and H & E x 1,075

Fig. 45. Photomicrograph of a section of cotyledon from heifer 12, at calving 55 days post-inoculation.
Clumps of leptospires (L) may be seen in the trophoblast layer.
Faine and H & E x 275



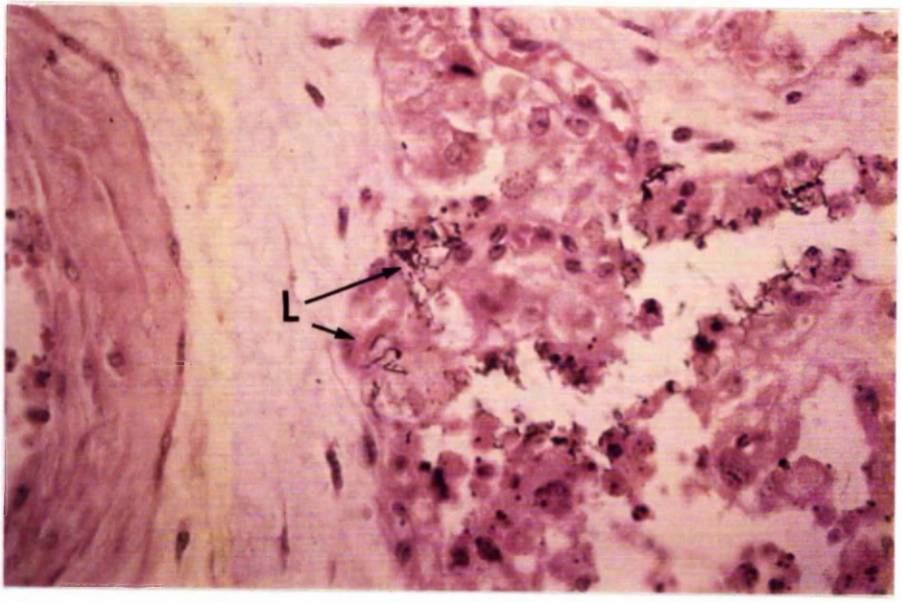
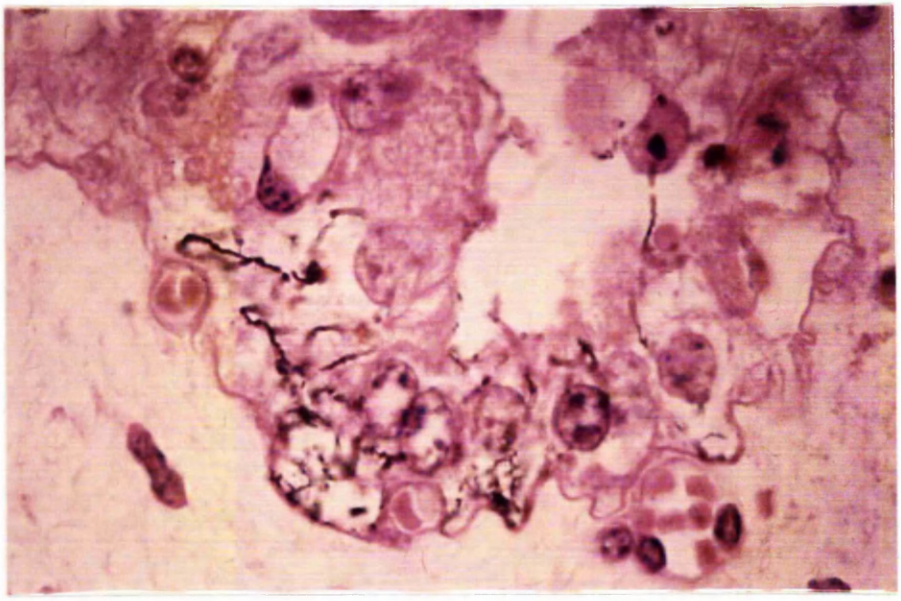

L

Fig. 46. Photomicrograph of a clump of leptospires in the trophoblast layer of a section of cotyledon from heifer 12.
Faine and H & E x 1,075

Fig. 47. Photomicrograph of a section of cotyledon from heifer 12 showing a single leptospire (L) interdigitating between trophoblast cells.
Faine and H & E x 1,600



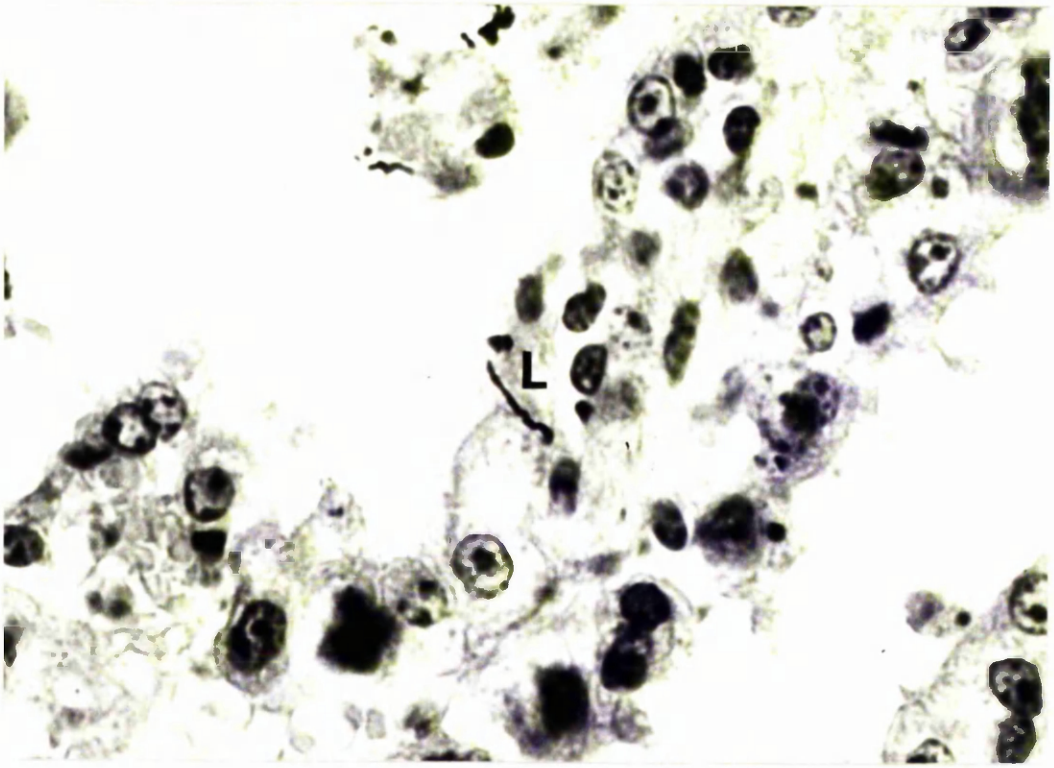
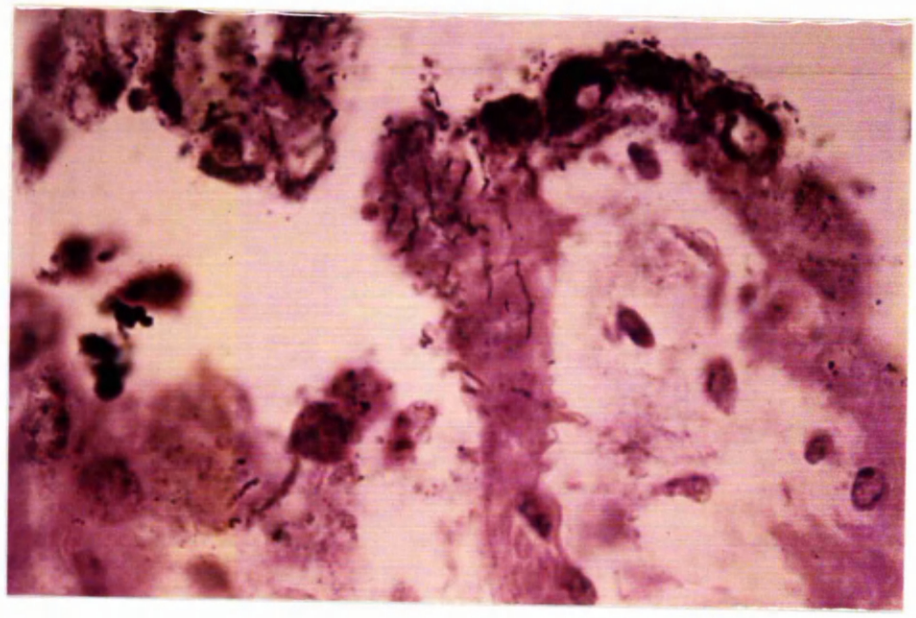

L

Fig. 48. Photomicrograph of a section of cotyledon from heifer 12 showing leptospires in degenerating trophoblast layer.
Faine and H & E x 1,075

Fig. 49. Photomicrograph of a section of cotyledon from heifer 12 showing leptospires in mesenchymal tissue.
Faine and H & E x 1,600



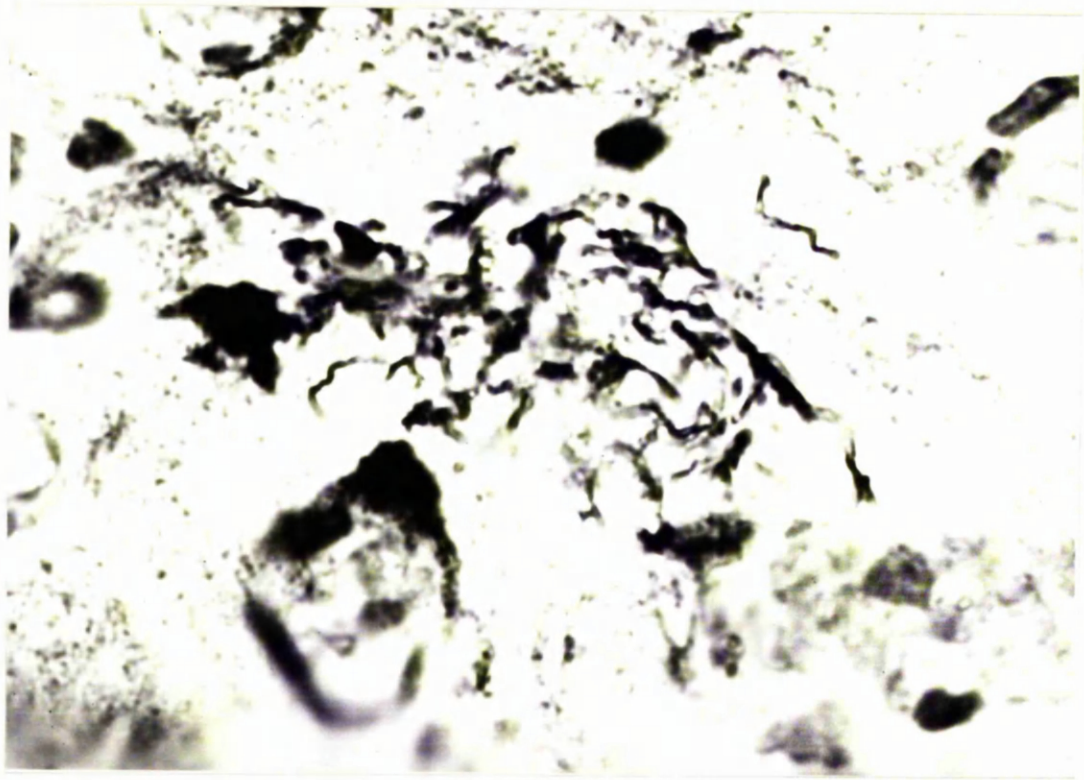
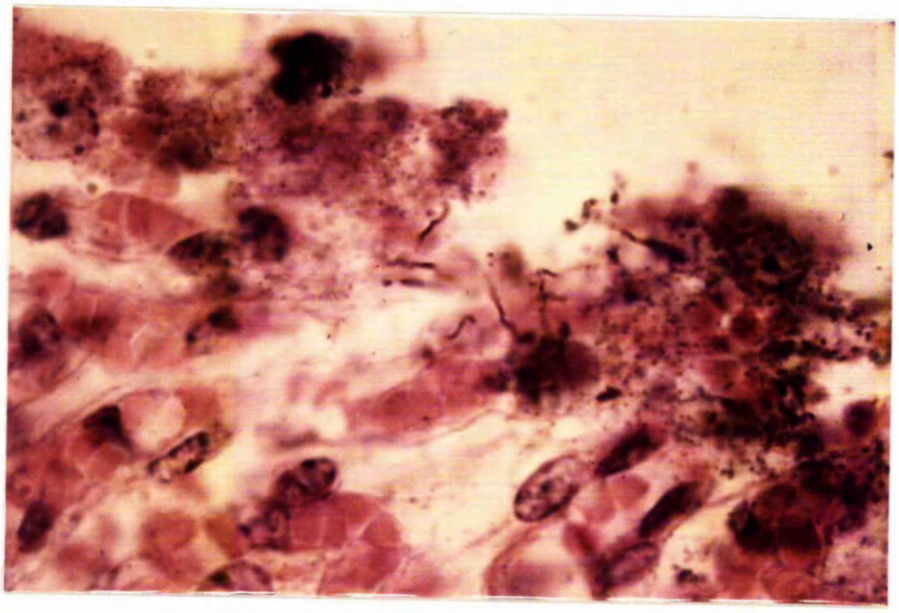


Fig. 50. Photomicrograph of leptospires in sections of cotyledon from heifer 6 at calving 15 days post-inoculation.
Faine and H & E x 1,075

Fig. 51. Photomicrograph of the same area shown in Fig. 50 taken in a different plane of focus to illustrate the difficulty encountered in photographing leptospires in tissue sections.
Faine and H & E x 1,075



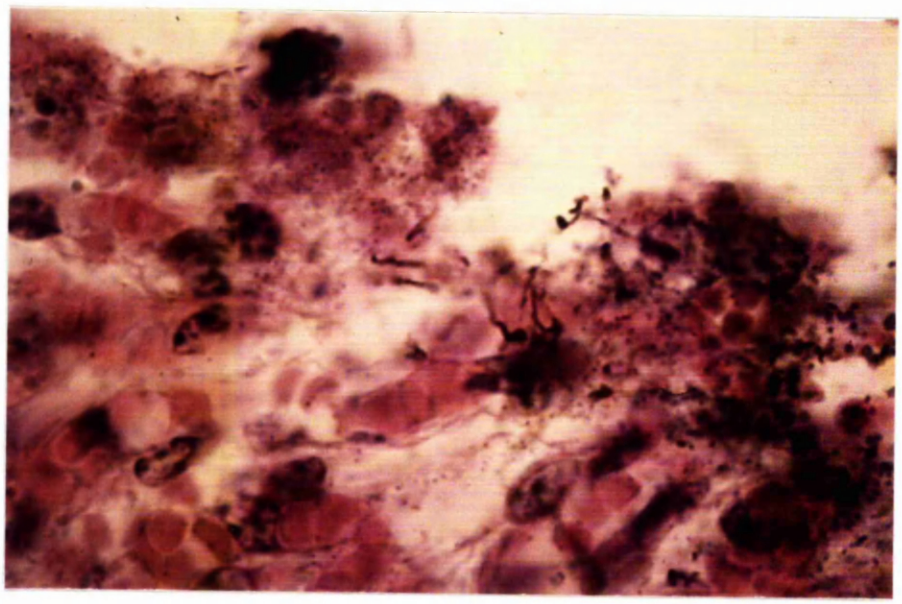
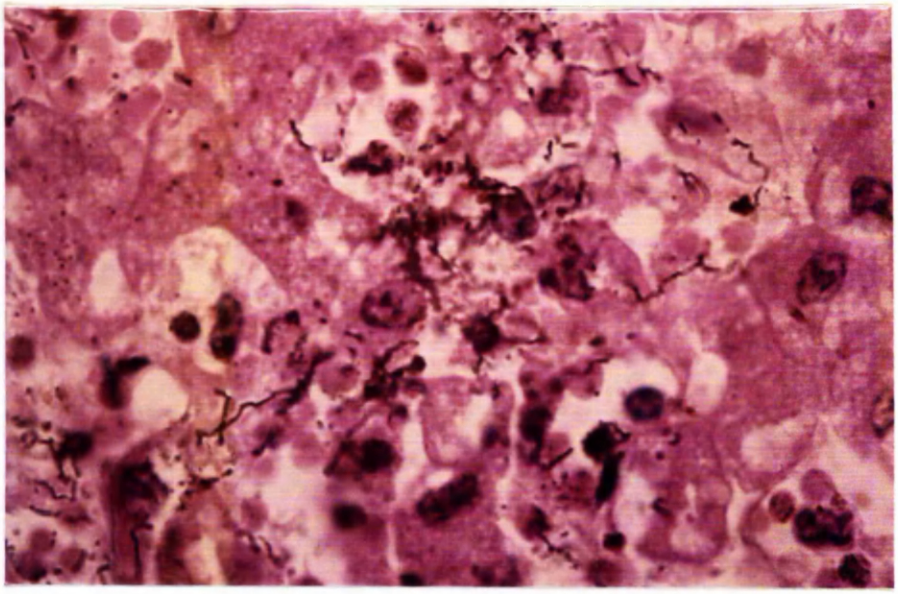


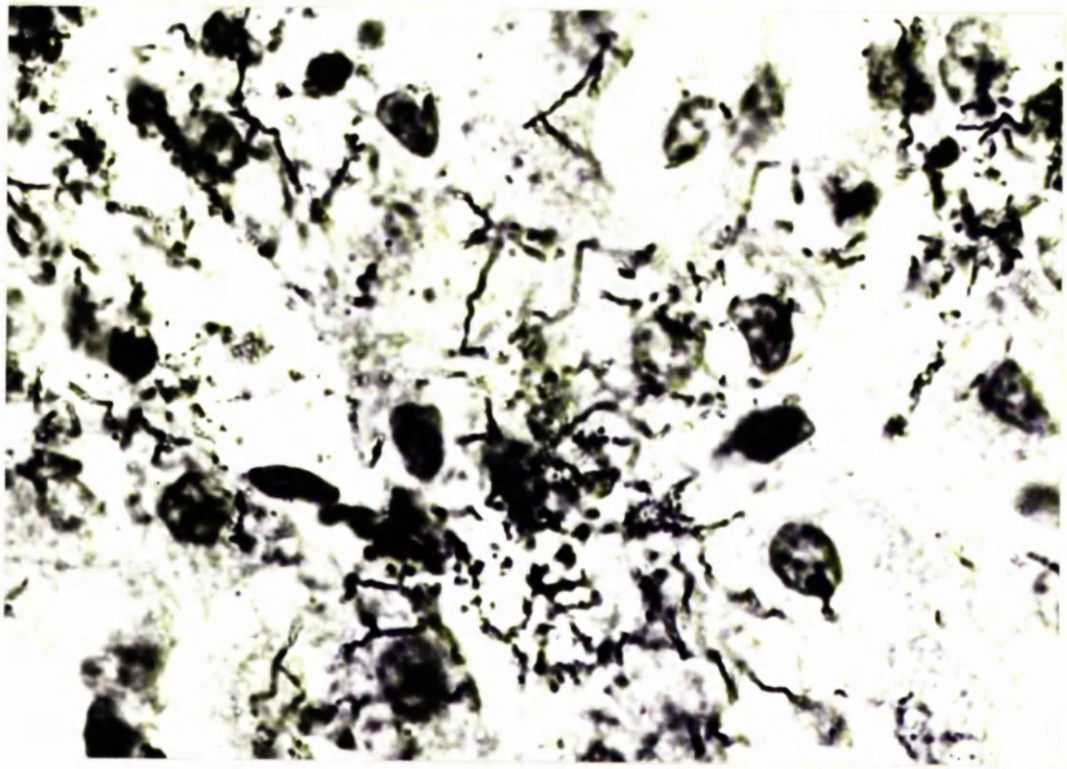
Fig. 52. Photomicrograph of a section of liver from calf 7, aborted 58 days post-inoculation, showing the presence of numerous leptospires.

Faine and H & E x 1,075

Fig. 53. Photomicrograph of another area from the liver of calf 7. Note the large numbers of leptospires.

Faine and H & E x 1,600





and kidneys (Figs. 54 and 55) revealed large numbers of leptospires. A few leptospires were also detected in lung sections. Neither cultural nor histological examinations demonstrated the presence of leptospires in the premature calf (CA9) nor were they isolated from calf 5. E. coli was recovered in pure culture from the peritoneal fluid of calf 5.

Leptospiruria

Intermittent leptospiruria was demonstrated in seven of the twenty heifers (Table 21). It was first demonstrated on the 22nd day P.I. and the latest day on which it was seen was the 70th day P.I. The urine of the experimental calves was not examined routinely for the presence of leptospires although leptospiruria was readily demonstrated in calf 27 at slaughter (Fig. 56).

Leptospires in Kidneys

The presence of leptospires was demonstrated in the kidneys of ten out of the twenty heifers (Table 21). Leptospires were cultured from the kidneys of one of the three animals slaughtered at 174 days P.I., which indicated that infection could persist for at least 174 days P.I. in some cases. The three animals in whose kidneys leptospires were detected longest, namely heifers 16 (153 days), 17 (174 days) and 18 (153 days), were kept in adjacent stalls.

Both cultural and histological methods proved successful in demonstrating leptospires in the kidneys of six heifers and three calves, whilst in two heifers and two calves isolation alone was successful and in two other heifers and two calves only histological methods were successful.

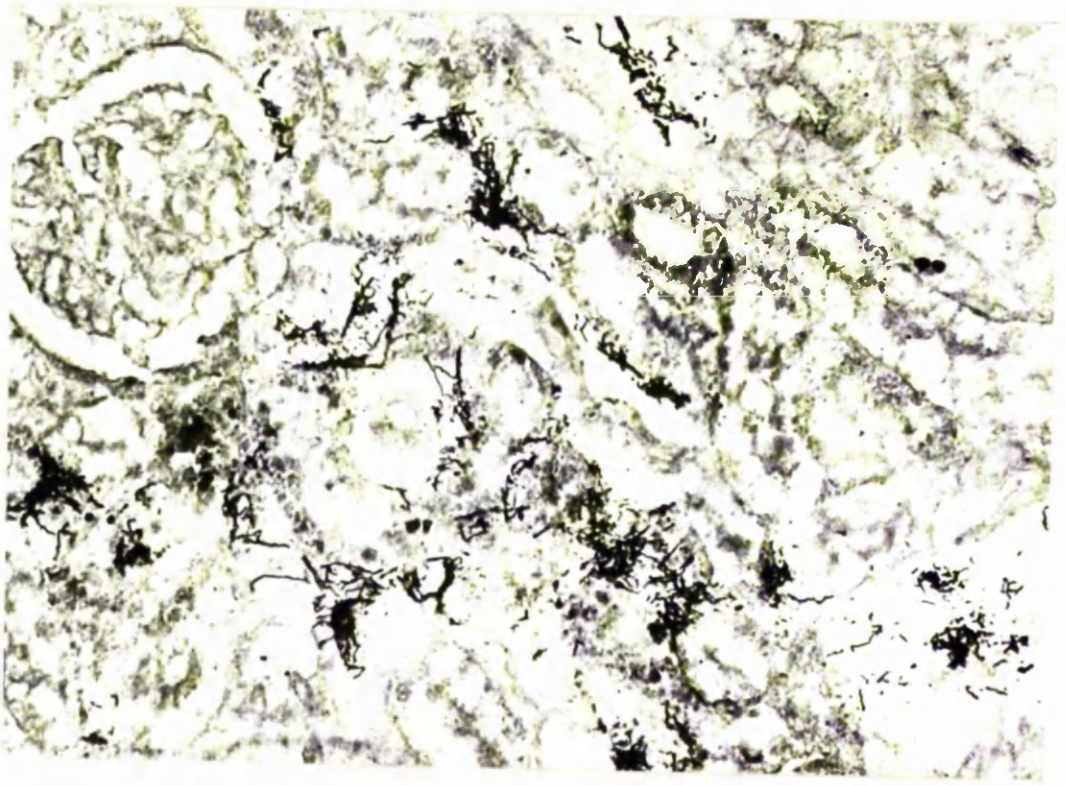
The kidneys of live calves born to infected dams did not yield leptospires on culture when this was carried out at slaughter between 7 and 56 days after birth. An interesting finding was the demonstration of large numbers of leptospires in sections of kidney from calf 19 slaughtered 21 days after birth (Figs. 57 and 58).

Fig. 54. Photomicrograph of a section of kidney from aborted calf 7, showing numerous leptospires scattered throughout the section.

Young's x 400

Fig. 55. Photomicrograph of organisms from an area shown in Fig. 54 to show the characteristic morphology of the leptospires.

Young's x 2,000



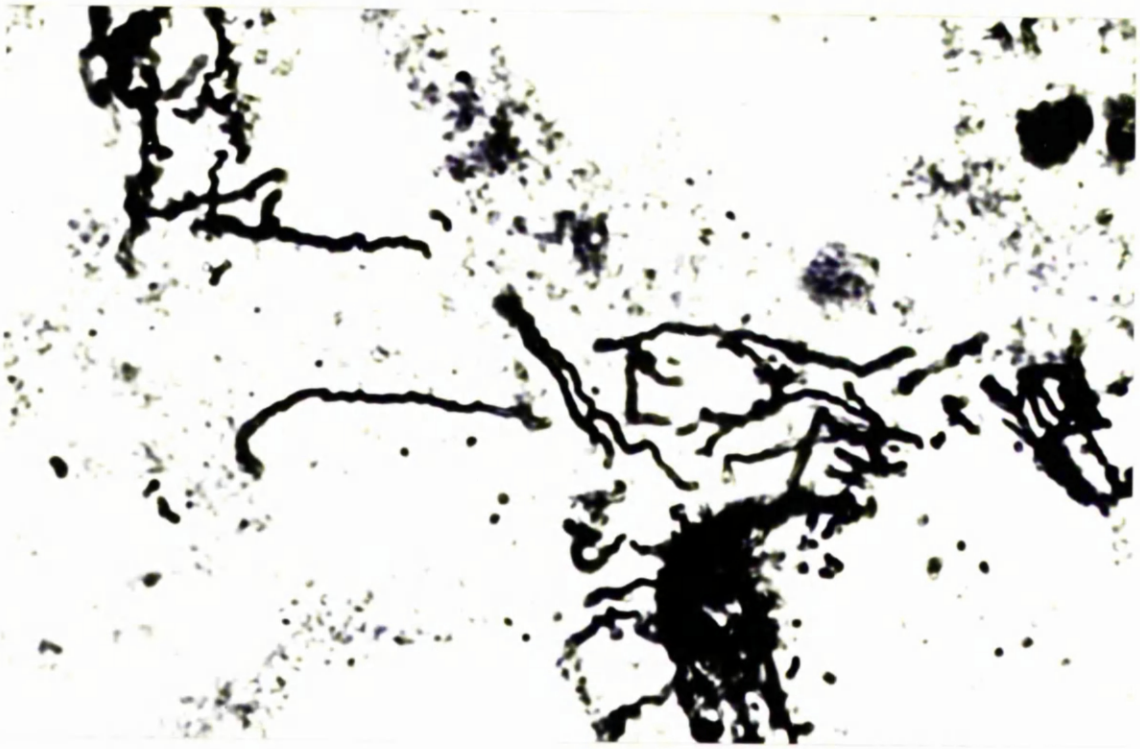


Table 21. The Demonstration of Leptospires in the Urine and Kidneys of the Experimental Animals (Groups I, II, III & IV)

Animal No.	Antibody titre at slaughter (hardjo)*	Demonstration of Leptospires			Presence of interstitial nephritis	Day P.I. slaughtered **
		Urine	Kidney			
			Culture	Histo-logically		
Heifer No.						
1	100	+ (d36)	-	-	+	72
2	300	+ (d22)	+	+	+	58
3	100	+ (d36)	-	-	+	58
4	300	-	-	-	+	65
5	1,000	-	-	-	+	65
6	100	+ (d36)	-	-	+	58
7	100	-	+	+	+	65
8	1,000	+ (d36)	+	+	+	93
9	100	+ (d36)	-	+	+	72
10	300	-	+	+	+	65
11	100	-	+	+	+	93
12	300	-	+	+	+	72
13	10	-	-	-	+	174
14	1,000	+ (d70)	-	-	+	174
15	30	-	-	-	+	153
16	10,000	-	-	+	+	153
17	1,000	-	+	-	+	174
18	3,000	-	+	-	+	153
19	10,000	-	-	-	+	91
20	0	-	-	-	+	153
Calf No.						
CA21	-	N.A.		+	+	9
CA22	30,000	N.A.	+	-	+	70
CA23	3,000	N.A.	+	+	+	50
CA24	1,000	N.A.	-	+	+	45
CA25	10,000	N.A.	+	+	+	32
CA26	10,000	N.A.	+	-	+	26
CA27	3,000	+ (d32)	+	+	+	32

* Titre expressed as the reciprocal of the actual titre.

N.A. = not attempted

** Underlined numbers represent animals in which leptospires were demonstrated.

Fig. 56. Leptospires in a formalised urine sample from calf 27 viewed by dark ground microscopy.

x 1,600

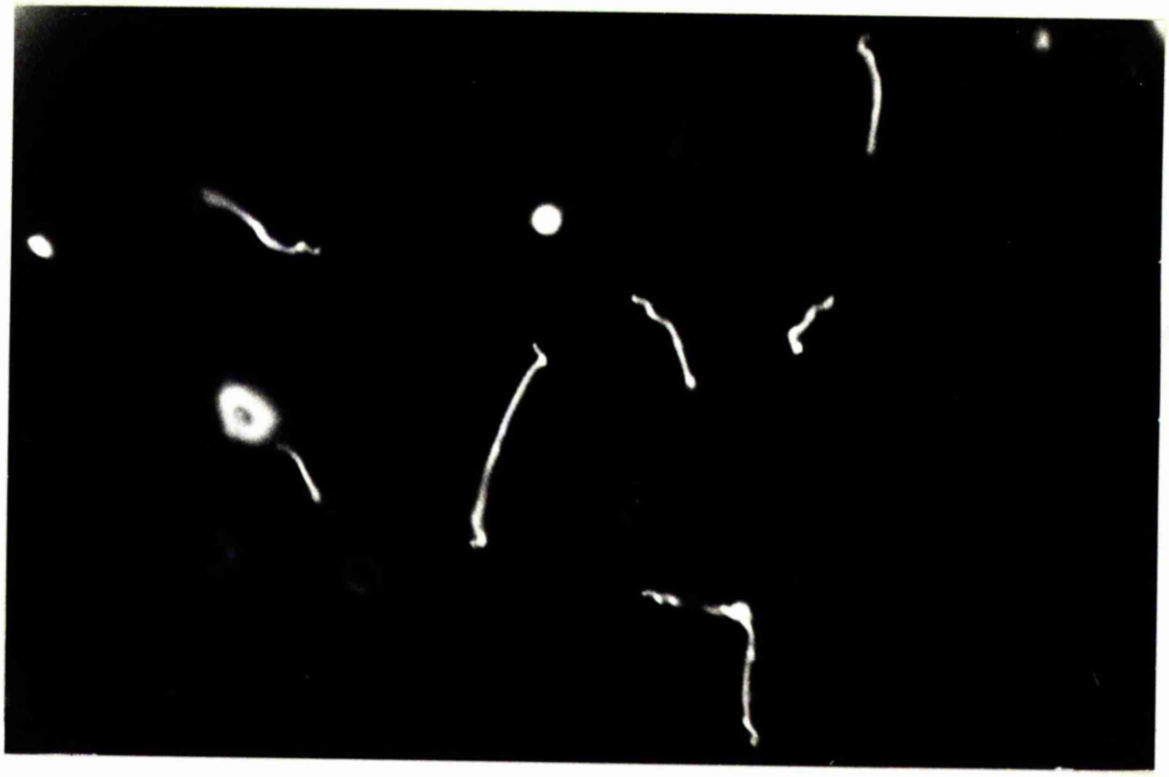
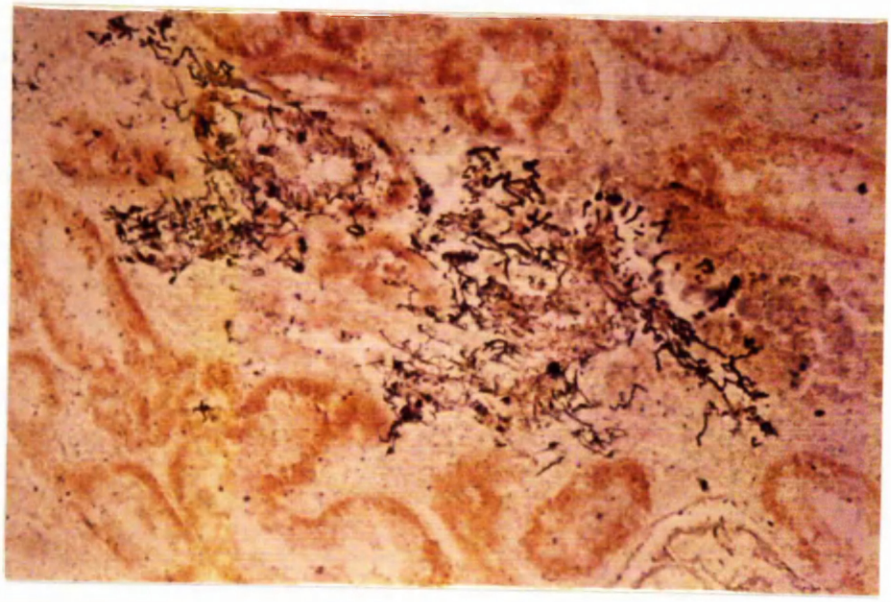
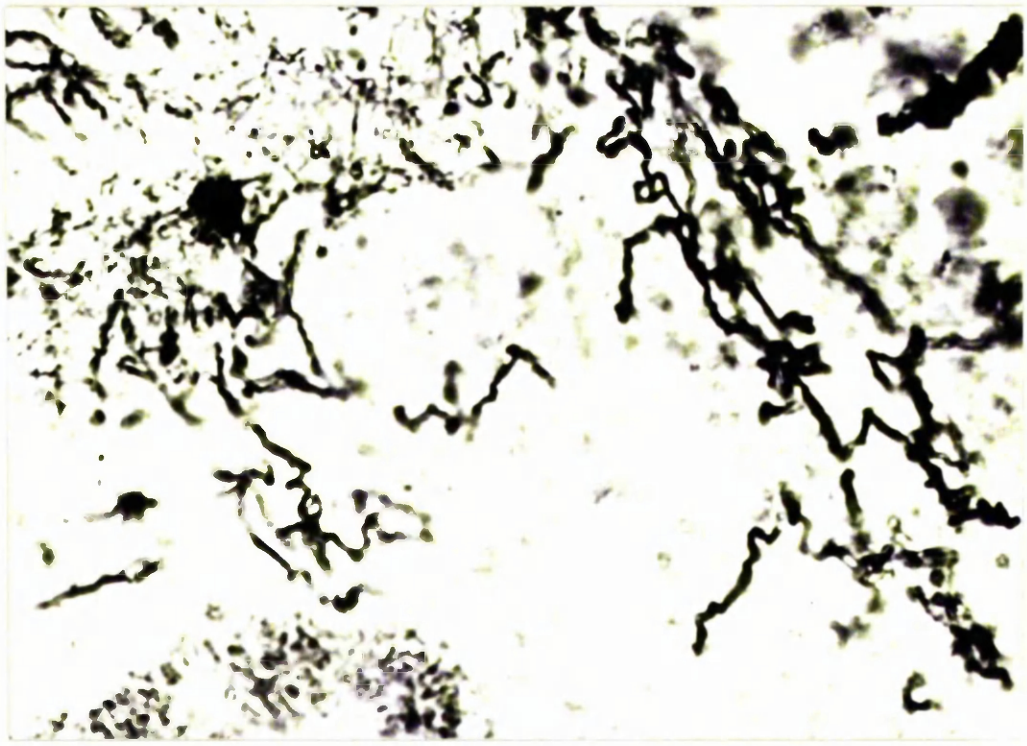


Fig. 57. Photomicrograph of a section from the kidney of calf 19 at 21 days of age, showing a focus of leptospires.
Young's method x 430

Fig. 58. Photomicrograph of an area of the focus of leptospires shown in Fig. 57 to show the leptospires in more detail.
Young's method x 1,600





Distribution of the Leptospires in the Kidneys

Leptospires were mostly found in the cortex, in the region of the cortico-medullary junction, in both proximal and distal convoluted tubules and occasionally in collecting ducts in the medulla. They were found attached to the luminal surface of the epithelial cells, in the tubular lumen, between the tubular epithelial cells and between epithelial cells and the basement membrane (Figs. 59, 60, 61 and 62).

Fig. 59. Photomicrograph of a histological section from the kidney of heifer 7 showing leptospires within the tubular epithelium.

L = leptospires

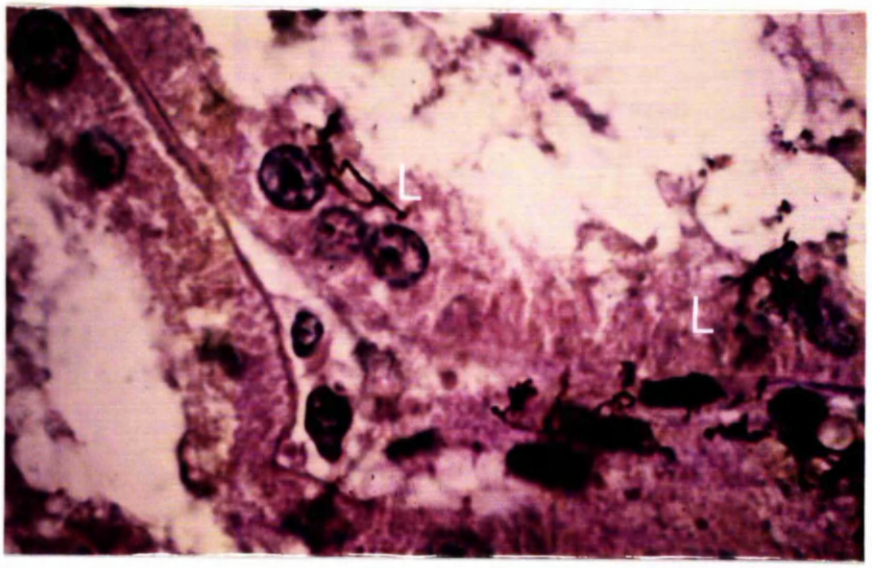
Faine and H & E x 1,075

Fig. 60. Photomicrograph of a histological section of a kidney from heifer 7 in which leptospires can be seen both in the lumen and epithelial cells of the proximal tubule.

Faine and H & E x 1,075

Ll = leptospires in the lumen

Le = " " " epithelium


L
L

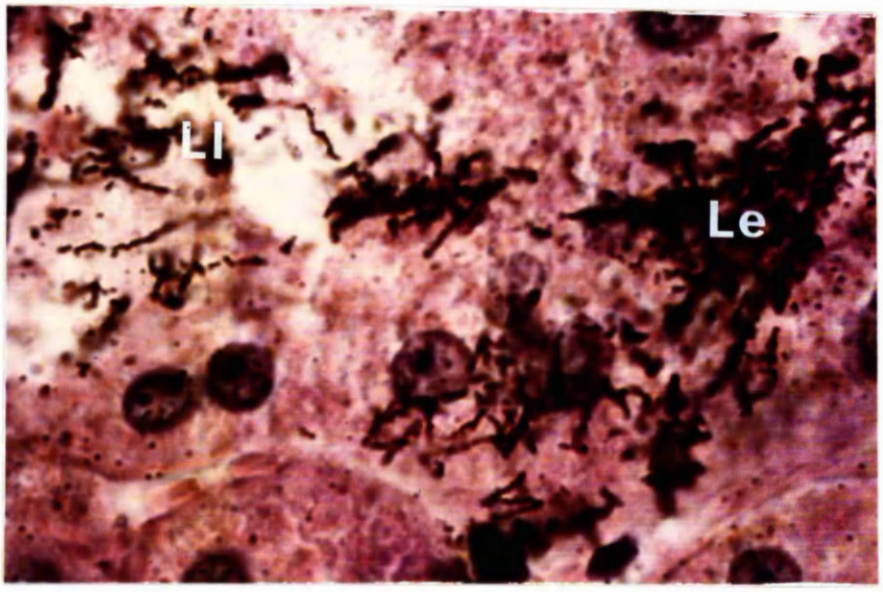
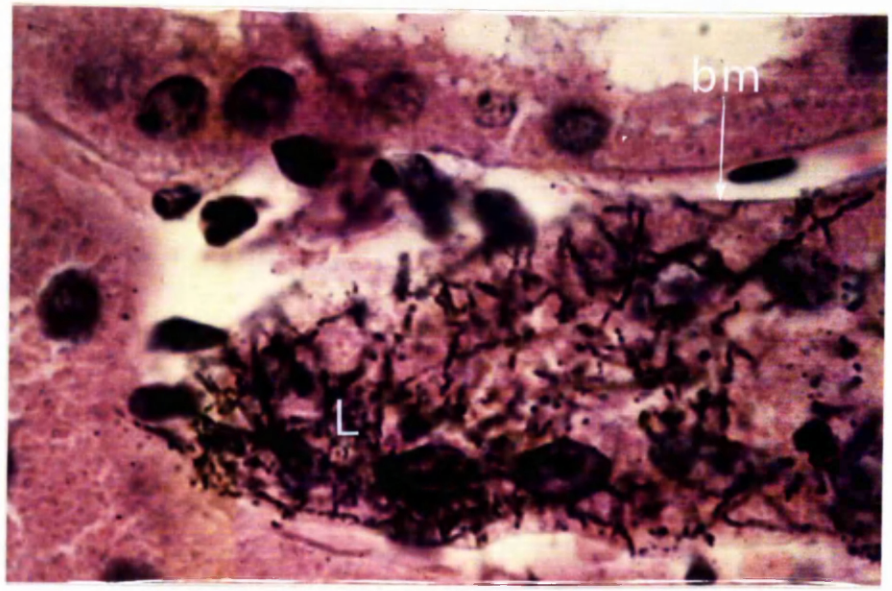
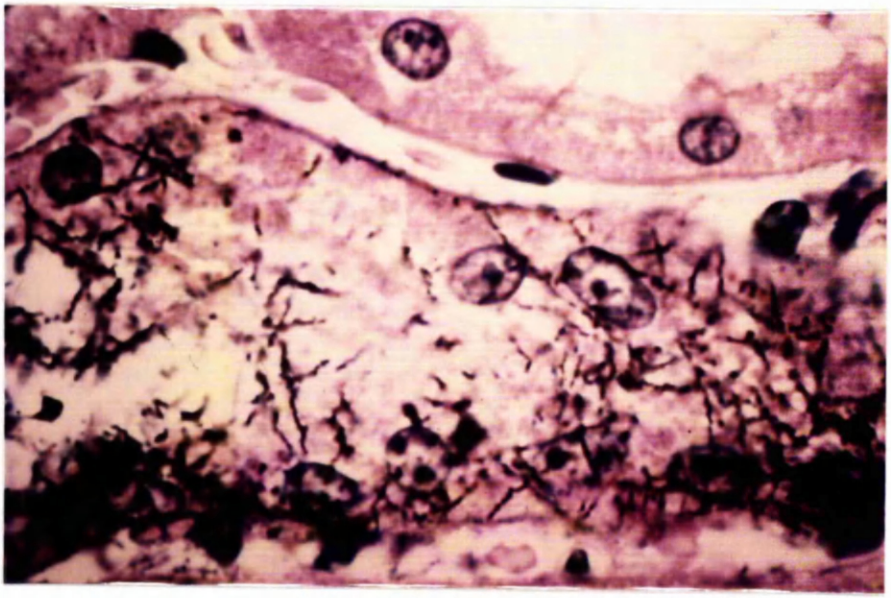

Ll
Le

Fig. 61. Photomicrograph of a section taken from a kidney of calf 23. Large numbers of leptospires (L) can be seen within a tubule and are limited only by the basement membrane (bm).
Faine and H & E x 1,075

Fig. 62. Photomicrograph showing an area adjacent to that shown in Fig. 61. Note the limitation of the leptospires to the one tubule.
Faine and H & E x 1,075


bm
L



Serological Findings

Table 22 summarises the major features of the serological response in the experimental heifers and calves as assessed by the microscopic agglutination test (M.A.T.) using hardjo (204 strain) as the antigen.

The pattern of the serological reactions of individual heifers and calves to hardjo (204), sejroe and hebdomadis serotypes is shown in Figs. 75 and 76 (Appendix 3).

Microscopic Agglutination Titres to Hardjo

Antibodies appeared in serum dilutions of 1:10 or more in two cows (15 and 20) on day 4 P.I. although leptospires could not be detected in either the blood, urine or kidneys of these two heifers - suggesting that this was an anamnestic response. Antibodies were detected in a further ten heifers on day 6 P.I. and in the remaining heifers and calves by day 15. Antibodies were not demonstrated using the M.A.T. in calf 21 which died in the leptospiraemic stage of infection.

After detection the titres rose sharply and in most cases reached maximum levels of 1:1,000 to 1:100,000 between 11 and 21 days P.I. The exceptions were heifers 15, 19 and 20 and calf 24. In heifers 15 and 20, maximum titres of only 1:300 and 1:30 respectively were recorded. In heifer 19 the antibody titre reached a maximum on the fourth day. The period over which the antibody titre rose was slightly longer in calf 24 (Fig. 76) and the titre did not reach its maximum until 28 days P.I.

Peak titres persisted for 3-21 days after which time they began to decline. In most cases they continued to fall gradually until the end of the experiment and were still detectable at slaughter 26 to 174 days P.I. in all animals except heifer 20. In this animal antibodies were only detectable for six weeks.

Secondary rises in antibody titres were detected (Fig. 75) in heifers 14 (10-12 weeks P.I.), 16 (14-16 weeks P.I.) 17 (20-21 weeks P.I.) and 18 (11-12 and 18-19 weeks P.I.). The antibody titres of serum samples

Table 22. A summary of the Serological findings in the Experimental Heifers and Calves

(v. hardjo)

Animal No.	Initial titre	First detection of antibody		Period of rising titre (days)	Maximum titre		Approximate persistence of maximum titre (days)	Titre at slaughter	
		Titre	Day P.I.		Titre	Day P.I.		Titre	Day P.I.
1	0	10	6	8	1,000	14	14	100	72
2	0	10	6	5	3,000	11	10	300	58
3	30	30	0	14	10,000	14	≤ 7	100	58
4	0	10	6	8	30,000	14	≤ 7	300	65
5	0	10,000	>9 & ≤ 14	≤ 5	10,000	14	21	1,000	65
6	0	10	6	10	3,000	16	≤ 5	100	58
7	0	10	6	8	1,000	14	7	100	65
8	0	10	6	15	10,000	21	7	1,000	93
9	0	1,000	14	≤ 7	1,000	14	7	100	72
10	0	3,000	14	≤ 7	3,000	14	21	300	65
11	0	10	6	8	3,000	14	7	100	93
12	0	10	6	8	30,000	14	≤ 7	300	72
13	30	30	0	11	1,000	11	≤ 7	10	174
14	0	100,000	>6 & ≤ 11	-	100,000	11	≤ 7	1,000	174
15	0	10	4	10	300	14	≤ 7	30	153
16	0	10	6	8	100,000	14	≤ 7	10,000	153
17	0	30	6	5	3,000	11	17	1,000	174
18	10	10	0	14	10,000	14	≤ 7	3,000	153
19	30,000	30,000	0	4	100,000	4	7	10,000	91
20	0	10	4	7	30	11	3	0	153
CA21	0	0		-	0		-	0	Died on day 9
CA22	0	10	13	7	30,000	20	≤ 7	30,000	70
CA23	0	10,000	14	≤ 7	10,000	14	>2 & ≤ 10	3,000	50
CA24	0	100	11	17	1,000	28	17	1,000	45
CA25	0	10,000	14	≤ 7	10,000	14	>18	10,000	32
CA26	0	10,000	15	≤ 8	10,000	15	>11	10,000	26
CA27	0	10,000	14	≤ 7	10,000	14	≤ 14	3,000	32

All the titres are expressed as the reciprocals of the actual titres.

collected at slaughter from heifers 16 (154 days), 17 (173 days) and 18 (153 days P.I.) were only one log dilution, a $\frac{1}{2}$ $\log_{10}$ dilution and a $\frac{1}{2}$ log dilution respectively, lower than their maximum recorded titres. Secondary rises in antibody titres were not noted following relapses of fever.

Table 23 records the results of the microscopic agglutination test using hardjo as antigen which were carried out on the serum and milk whey samples collected from the experimental heifers at calving and on the serum samples taken from their calves before and after being fed colostrum. Fig. 63 shows the pattern of antibody response to hardjo in the serum and whey of heifer 18 and the antibody titre of its calf after being fed colostrum.

The maximum whey antibody titres detected in the heifers were found in the samples collected immediately after parturition. They ranged from a $\log_{10}$ dilution less to a $\log_{10}$ dilution more than the serum antibody titre at that time. Whey antibody titres fell rapidly over the first 3 days of lactation but persisted at a low level for some time; more than 32 days in the case of heifer 5 (Table 23). The persistence of whey antibodies appeared to depend on the height of the initial titre.

Serum samples were collected from eleven calves born to infected heifers and were taken before they obtained colostrum. Antibodies were not detected at serum dilutions of 1:10 or more in ten of the eleven calves. Calf 9, which was born five weeks premature, had an antibody titre of 1:10 to hardjo at birth, although the aborted foetus (CA7) was seronegative.

The maximum antibody titres to hardjo in the sera of calves fed colostrum were found within three days of birth and ranged from a $\log_{10}$ dilution less to a $\frac{1}{2}$ $\log_{10}$ dilution more than that found in the dam's milk. They persisted for 21 to more than 56 days; calf 1 had a titre of 1:10 to hardjo 56 days after birth.

Microscopic Agglutination Titres to Heterologous Strains

The pattern of the serological response in individual animals to two other members of the hebdomadis serogroup, namely sejroe and hebdomadis is shown in Figs. 75 and 76 (Appendix 3).

Table 23. A summary of the maximum whey antibody titres to hardjo and their persistence in experimentally infected heifers and the pre- and post-colostrum serum titres of their calves

Calf No.	Serum antibody titre* of dam at calving (hardjo)	Maximum whey antibody titre* (hardjo)	Persistence of dam's whey antibody titre* - days ***	Pre-colost. antibody titre* in calf	Max. titre* acquired by calf	Persistence of acquired titre (days)
CA1	1,000	1,000	22	0	300	≥56
CA2	3,000	300	10	0	300	35
CA3	1,000	300	4	NK	300	35
CA4	3,000	3,000	≥24 (100)	NK	1,000	35
CA5	10,000	10,000	≥32 (30)	0	1,000	Died
CA6	3,000	30	12	0	300	35
CA7	100	-	-	0	-	Aborted
CA8	1,000	3,000	NK	0	30	NK
CA9	100	100	NK	10**	30	21 (premature live calf)
CA10	1,000	1,000	NK	NK	1,000	≥9
CA11	300	300	NK	NK	100	35
CA12	1,000	10,000	NK	0	3,000	≥4
CA13	10	10	1	0	30	21
CA14	3,000	30,000	≥10 (1,000)	NK	30,000	≥10
CA15	30	100	1	NK	300	≥10
CA16	100,000	100,000	≥26 (100)	NK	30,000	≥28
CA17	1,000	100	≥3 (30)	NK	100	≥4
CA18	300	3,000	≥31 (30)	0	300	≥52
CA19	10,000	100,000	≥21 (30)	NK	10,000	≥21
CA20	0	0	0	0	0	0

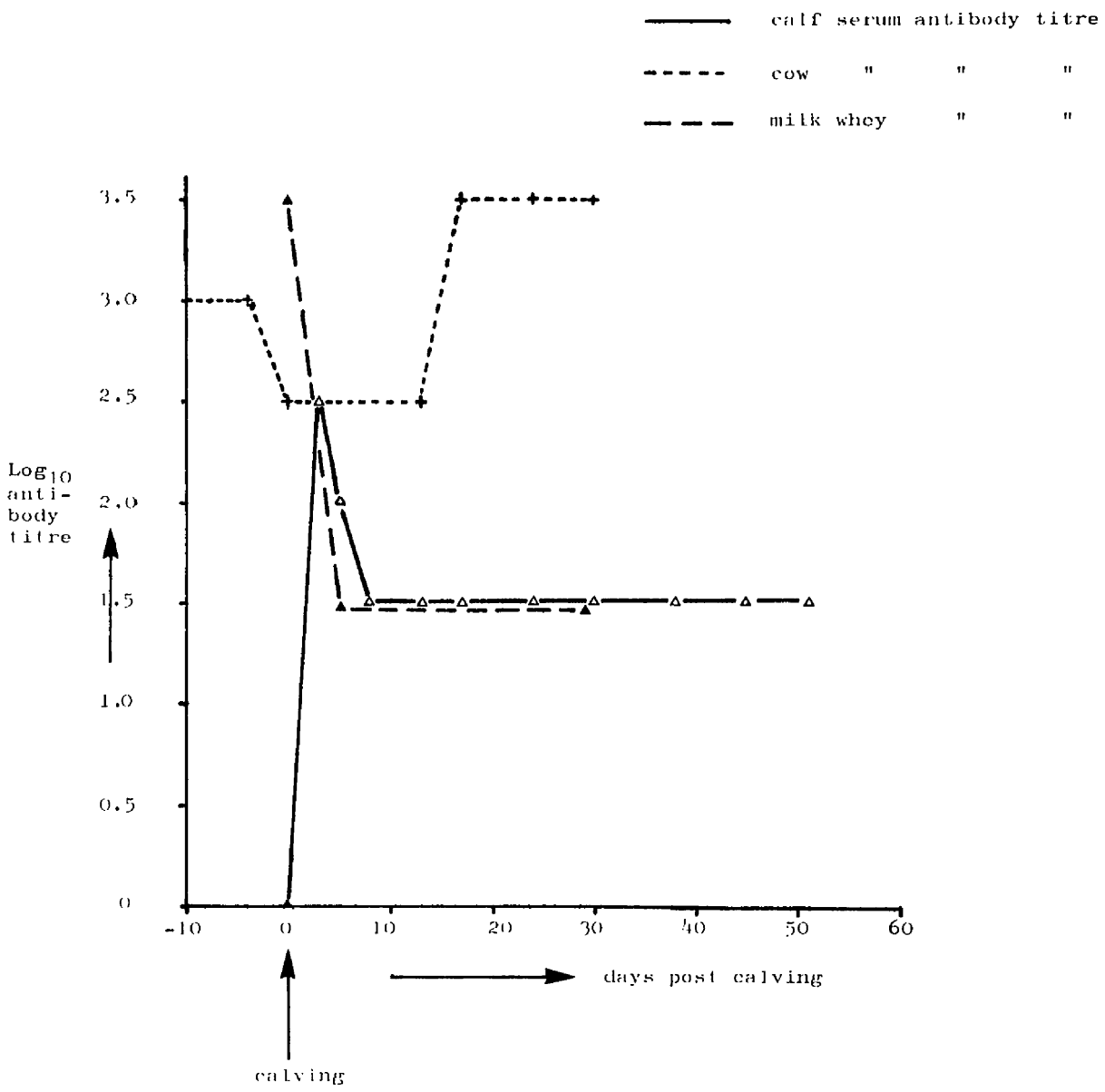
NK = Not known

* All titres are expressed as reciprocals of the actual titres.

** Note presence of antibody titre of ++++ 1:10 to hardjo in the premature (240-245 day) calf born to heifer 9.

*** Figures in brackets are the reciprocal whey antibody titres on the day of slaughter.

Fig. 63. The serological response to hardjo in heifer 18 and her calf (CA 18) from parturition to slaughter.



Sejroe titres were usually a $\frac{1}{2}$ $\log_{10}$ dilution to a $\log_{10}$ dilution lower than hardjo titres, while hebdomadis titres were a further $\frac{1}{2}$ $\log_{10}$ to a $\log_{10}$ dilution lower still. Sejroe and hebdomadis titres fell in step with hardjo titres.

In a number of instances, antibodies to hebdomadis had disappeared before the conclusion of the experiment, while antibodies to sejroe and hardjo persisted at detectable levels. In some cases, antibodies to hebdomadis and sejroe had fallen to a level where they could not be detected by methods other than the agglutination test using live antigens or would not have been considered significant. Heifer 7, which aborted, and heifer 9, which produced a premature calf, had antibody titres of 1:30 to sejroe and 1:10 to hebdomadis at the time of abortion or calving.

Heterologous reactions with serotypes belonging to other serogroups were of no significance. Traces of antibody to ballum, javanica and bataviae were detected for a few weeks in several heifers.

Table 24 shows the length of time taken for antibody titres to sejroe and hardjo to fall below 1:300 and 1:100. By 9 weeks P.I. the titre to sejroe had fallen to 1:100 or less in fifteen out of twenty heifers and to 1:30 or less in ten of the heifers (Table 25). The titre to hardjo had fallen to 1:100 or less in eight of the twenty heifers and to 1:30 or less in one of the heifers by 9 weeks P.I.

Differences in the Serological Response of Heifers and Calves

The serological reaction in calves was much more specific than it was in the heifers (Figs. 75 and 76, Appendix 3). Four calves (CA23, 24, 26 and 27) did not develop detectable levels of antibody to hebdomadis (Fig. 76), although they developed maximum antibody titres to hardjo of 1:10,000, 1:1,000, 1:10,000 and 1:10,000 respectively. Antibodies to sejroe developed in all six surviving calves but were always at least a $\log_{10}$ to 3 $\log_{10}$ dilutions less than the hardjo antibody titres.

Table 24. The time* taken for Antibody Titres** to Sejroe and Hardjo to fall below 1:300 and 1:100

Heifer No.	Time* (wks) taken for sejroe titre to fall to		Time (wks) taken for hardjo titre to fall to	
	<300	<100	<300	<100
1	5	7	7	> 10
2	4	7	> 10	> 10
3	5	6	7	> 10
4	5	9	> 10	> 10
5	> 10	> 10	> 10	> 10
6	4	6	6	> 8
7	4	7	7	> 9
8	8	> 10	> 10	> 10
9	3	5	5	> 10
10	> 10	> 10	> 10	> 10
11	4	9	> 10	> 10
12	8	> 10	> 10	> 10
13	<1	4	4	10
14	> 10	> 10	> 10	> 10
15	4	10	4	> 10
16	> 10	> 10	> 10	> 10
17	10	> 10	> 10	> 10
18	7	> 10	> 10	> 10
19	> 10	> 10	> 10	> 10
20	<1	<1	<1	<1

Table 25. The Number of Heifers whose Antibody Titre to Sejroe and Hardjo had fallen to <1:300 and <1:100 by 9 wks P.I.

sejroe		hardjo	
<300	<100	<300	<100
15/20	10/20	8/20	1/20

*Time was measured from date of inoculation.

**Antibody titres expressed as reciprocals of actual titres.

Pathological Findings

Post-mortem Findings

Pale yellowish foci, up to 3 mm in diameter, often surrounded by a ring of hyperaemia could be seen on the surface of the kidneys from all the experimentally infected heifers (Figs. 64 and 65) and from those calves which survived infection (Fig. 66).

The placentas of heifer 7 and heifer 9 showed oedema of the intercotyledonary area. Numerous fawn atonic cotyledons were present in the placenta of heifer 7, although some cotyledons appeared relatively normal (Figs. 70 and 71). The cotyledons of heifer 9 were not so severely affected: many had fawn atonic areas but few cotyledons were entirely affected (Figs. 72 and 73). Similar but less noticeable areas were found in the cotyledons of some of the other experimental animals and in the four control placentas.

The aborted foetus (CA7) did not appear to have been dead long. There were no obvious gross pathological features.

In the experimentally infected calf which died (CA21) the outstanding feature was the presence of large areas of haemorrhage in the meninges. Congestion of the kidney was noted.

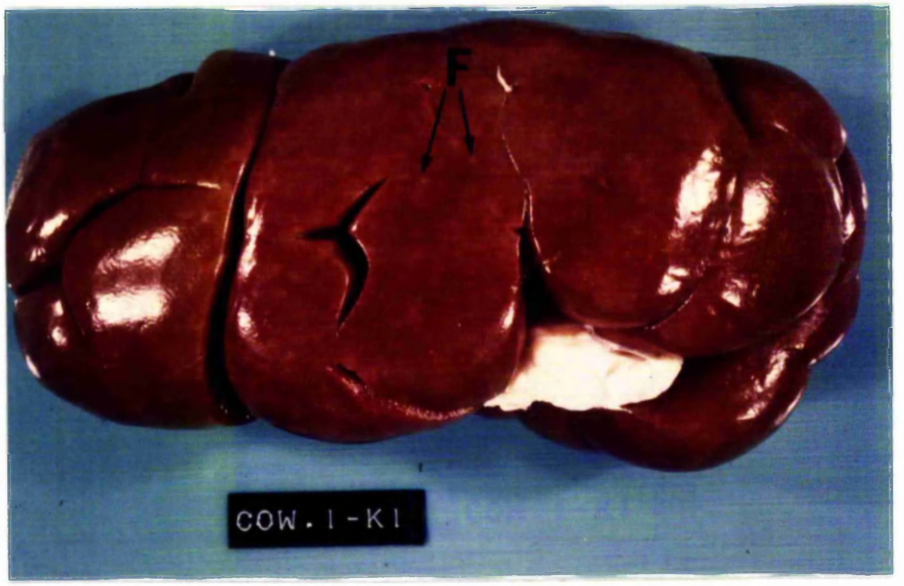
Histological Findings

A mild focal interstitial nephritis was present in all kidneys from the experimental heifers (Fig. 67). The interstitial lesion was more pronounced in the kidneys of the experimentally infected calves. The interstitial cellular infiltrate consisted largely of lymphocytes with occasional plasma cells. There appeared to be no inflammatory changes directly associated with the foci of leptospires (Fig. 68).

Histological examination revealed areas of hydropic degeneration and focal necrosis of the villi in all the placentas from experimental heifers and the control placentas. The degree of these changes varied between cotyledons in the same placenta. Necrosis of the villi was much more marked in the placentas of heifers 7 and 9, especially that of heifer 7.

Fig. 64. Photograph of a kidney from cow 1 showing the characteristic appearance of the surface of the cortex.
Note the yellow foci (F).

Fig. 65. Photograph of a kidney from cow 3 showing areas of hyperaemia around the yellow foci.
The areas of hyperaemia are marked (H).


F
COW. 1 - K1

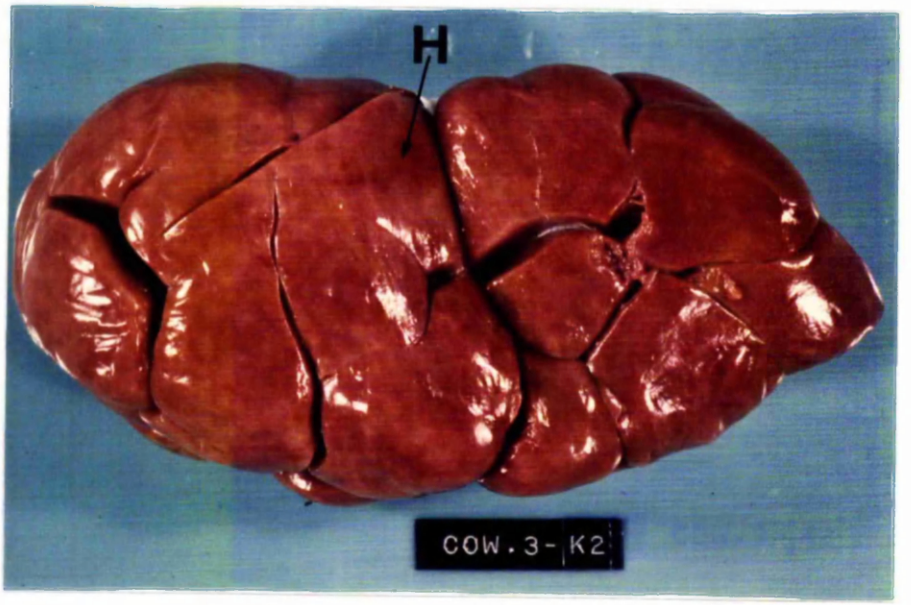
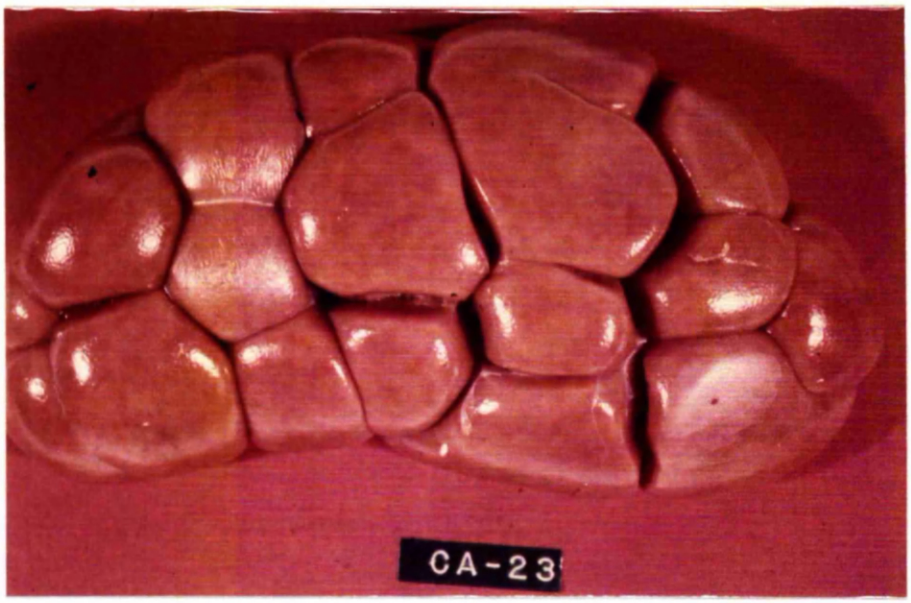

H
COW. 3 - K2

Fig. 66. Photograph of a kidney from calf CA23 which had a more diffuse type of lesion.

Fig. 67. Photomicrograph of a section of kidney from heifer 3 showing an area of interstitial nephritis.

Faine and H & E x 275


CA-23

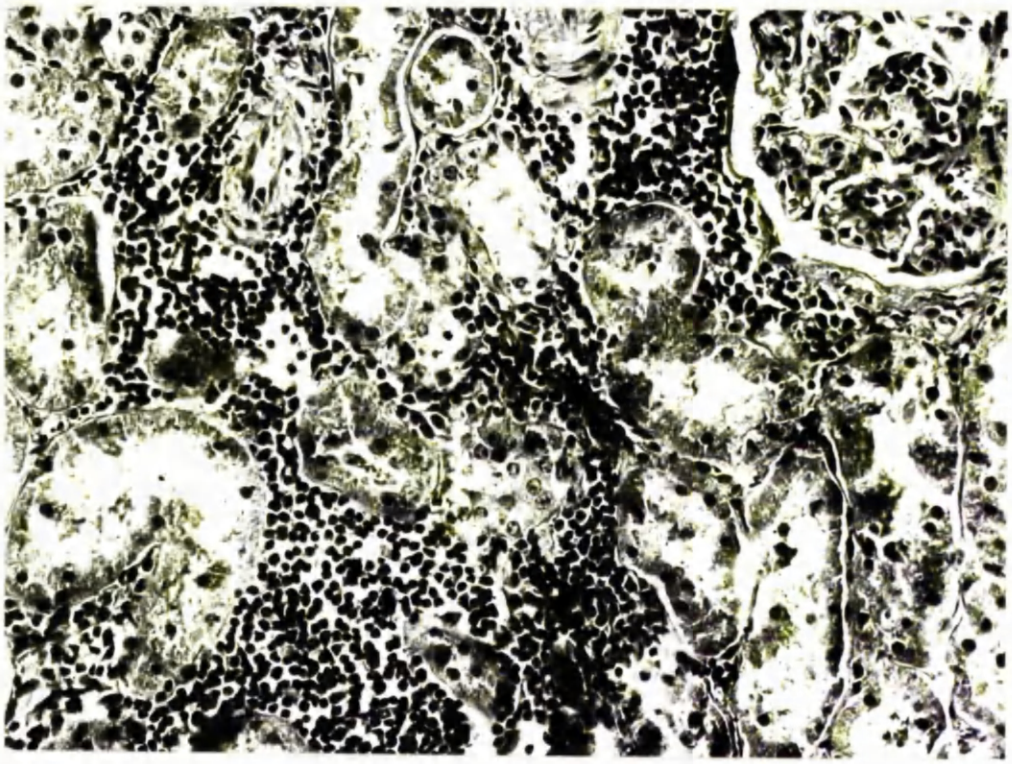
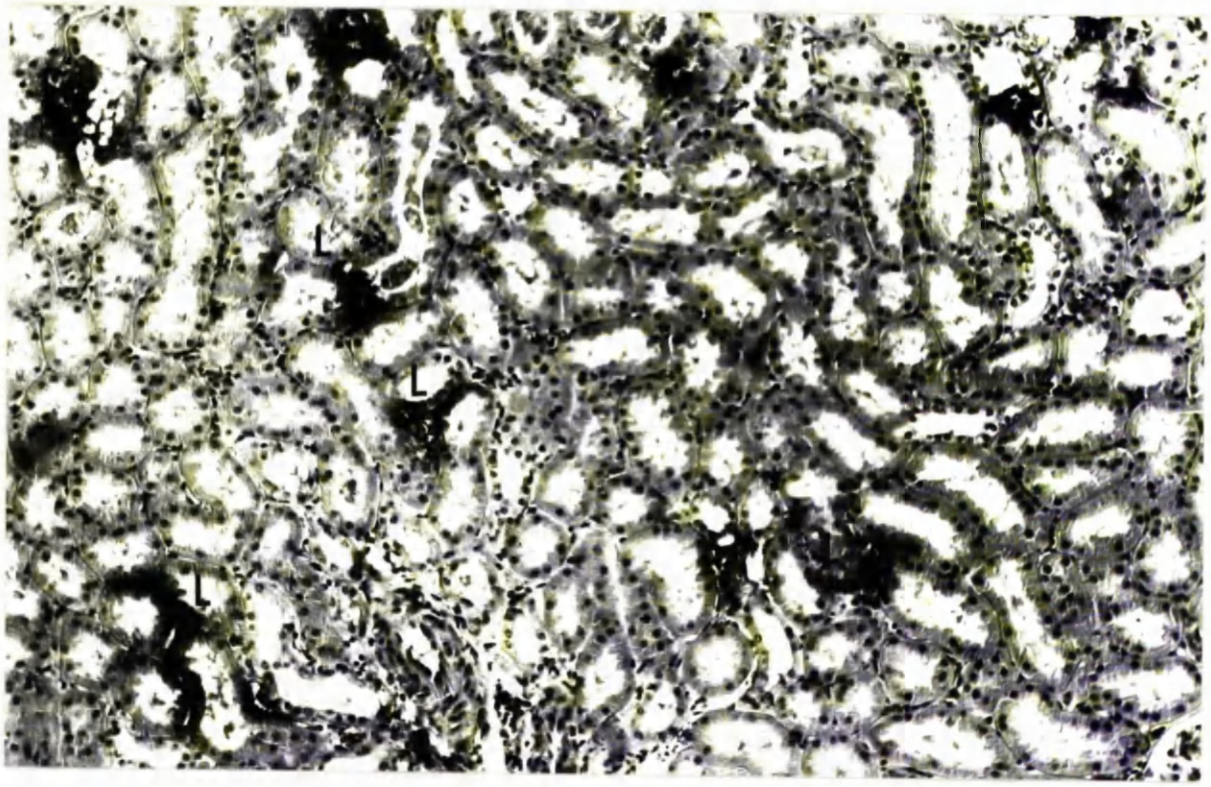


Fig. 68. Photomicrograph of a section of kidney from heifer 7 taken 65 days P.I. There appears to be no inflammatory changes directly associated with the chronic foci of leptospires. Areas of argentrophil material containing leptospires are labelled L.

Faine and H & E x 150

Fig. 69. Photomicrograph of a section of kidney from the foetus aborted 58 days P.I. (calf 7) showing a focus of interstitial nephritis and associated tubular necrosis (n) in the renal cortex.

Faine and H & E x 430


L
L
L

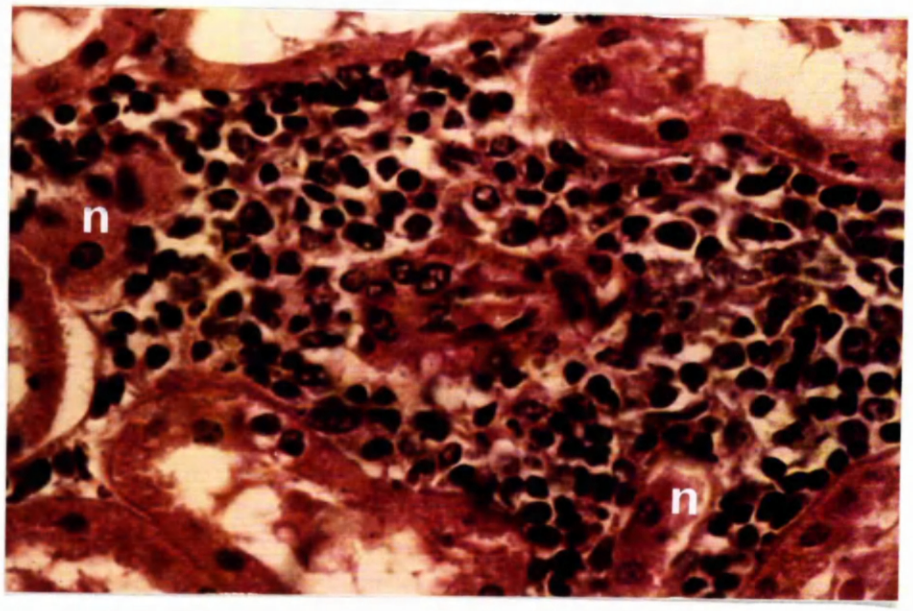
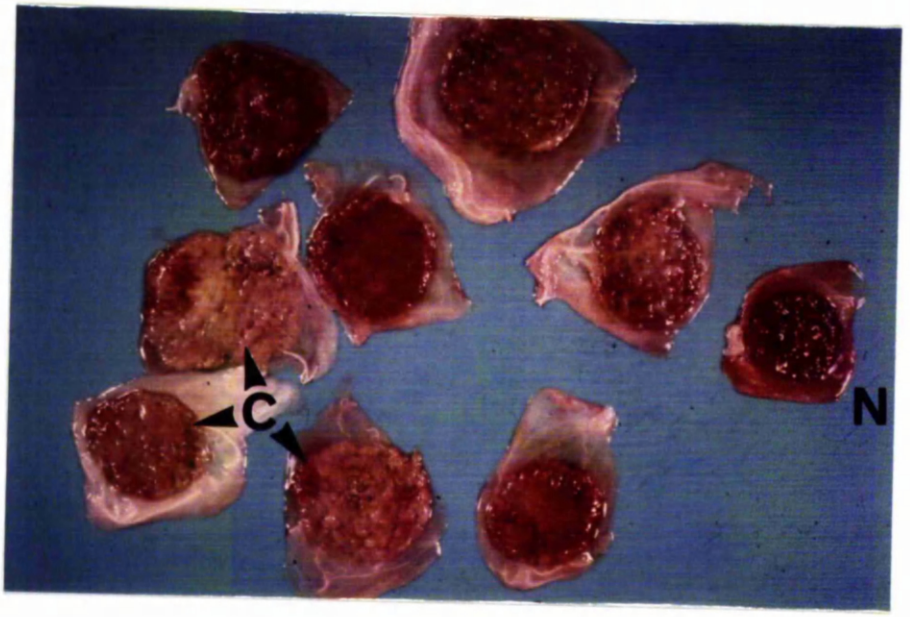

n
n

Fig. 70. Photograph of a number of selected cotyledons from the placenta of heifer 7 showing a range of cotyledons varying in appearance from relatively normal to those showing marked necrosis.

Normal cotyledon - N
Necrotic cotyledon - C

Fig. 71. Photograph showing the surface of a necrotic cotyledon from heifer 7. Note the colour and loss of detail.


C
N

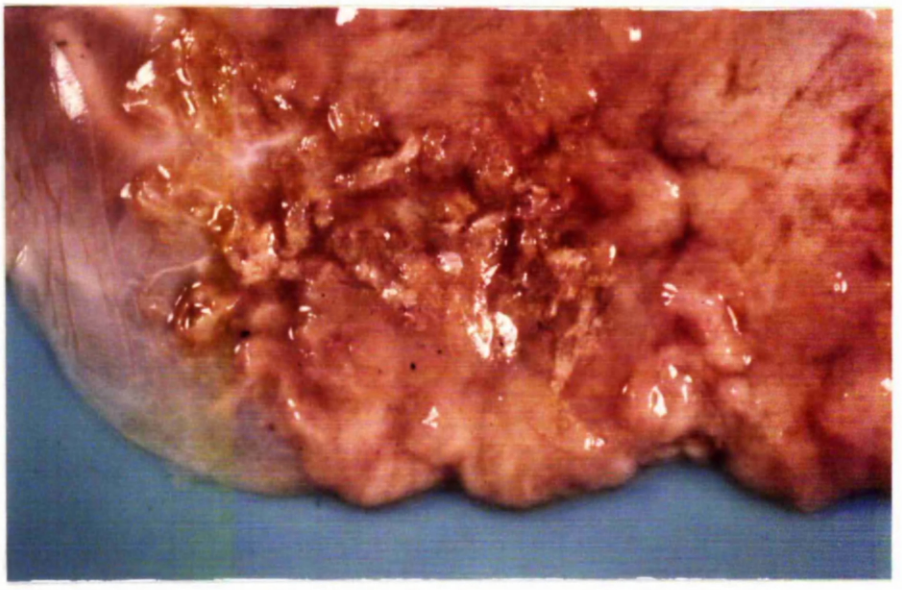
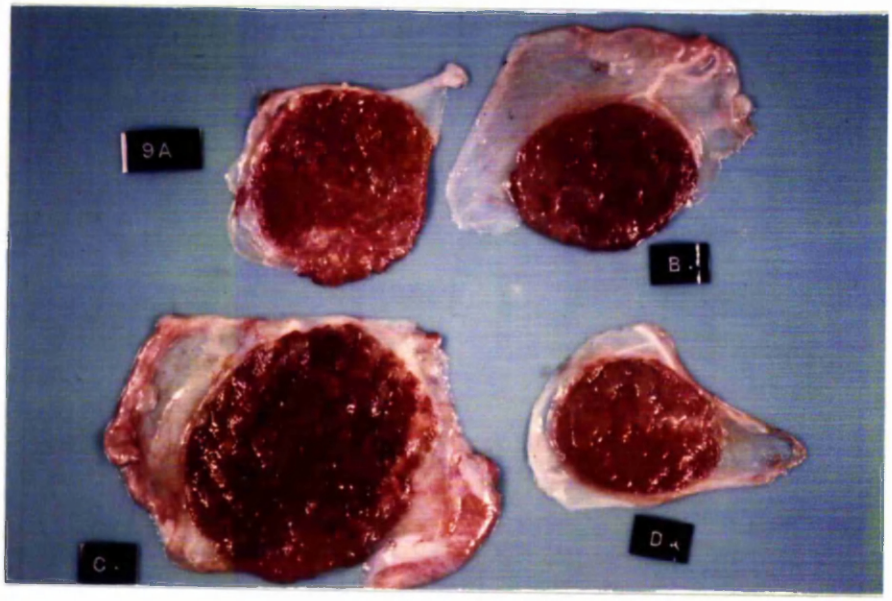
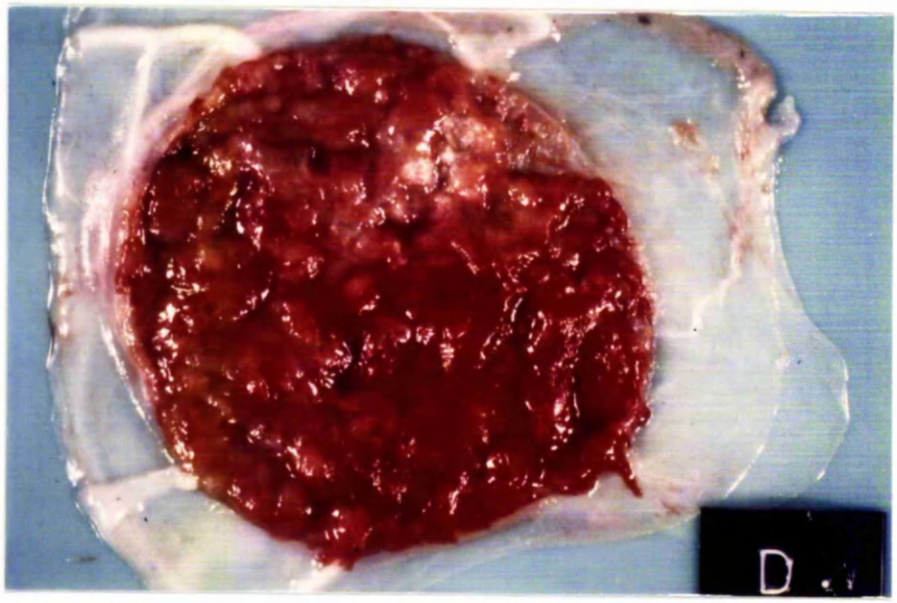


Fig. 72. Photograph of selected cotyledons from heifer 9. Some areas of necrosis may be seen but these are not as noticeable as those in the placenta of heifer 7 (Figs. 70 & 71).

Fig. 73. Photograph of a cotyledon from heifer 9 showing details of the surface.


9A
B
C
D


D

Occasional foci of tubular necrosis and associated early focal interstitial nephritis were found in the renal cortices of the aborted foetus (CA7) (Fig. 69). Its liver showed generalised degenerative changes consistent with post-mortem change.

The kidneys of calf 21 were congested and a very early interstitial reaction consisting of plasma cells with a few lymphocytes, polymorphonuclear leucocytes and macrophages was present in the renal cortices. Some areas of renal tubular necrosis were noted, especially deep in the cortex. In the medulla there was widespread necrosis of the collecting tubules with some macrophage and polymorphonuclear leucocyte infiltration and haemorrhage. Mitotic figures were present in some tubular cells.

The most striking lesion in the liver of calf 21 was a diffuse, severe "cloudy swelling" of the hepatocytes which in some areas merged into early hepatic coagulative necrosis. In some areas the whole lobule was involved while in other lobules the centrilobular part was spared and the lesion had a mid zonal and/or periportal distribution. There was moderate hypertrophy and hyperplasia of the von Kuppfer cells. Inflammatory cells, mainly macrophages and lymphocytes with a few polymorphonuclear leucocytes were present in the hepatic blood vessels especially the central veins and a diffuse infiltrate of plasma cells and lymphocytes was present in the portal triads.

DISCUSSION

Acute Clinical Disease

As pointed out in the introduction to this chapter, earlier experiments have shown marked variations in the clinical picture of acute experimental leptospirosis related to the age of the animal, the serotype involved, the origin of the serotype used and its virulence. When comparing the results of the present study with those obtained by other workers it is necessary to take into account not only these factors but also the immunological status of the animals and the stage of gestation at which pregnant heifers were infected.

The mild nature of the acute phase of the disease in adult cattle described above resembles that described by other workers (Fennestad and Borg-Petersen, 1956; Sullivan 1970b, 1972; Farina et al., 1972) who have carried out experimental infection of adult cattle using strains belonging to the hebdomadis serogroup. Similarly, the more severe nature of the disease in young calves described above is consistent with the observation that the severity of leptospirosis decreases with increasing age of the animal (Fennestad, 1963). A fatal infection has not been recorded previously in calves infected with members of the hebdomadis serogroup, neither have opisthotonus and meningeal haemorrhages. Nervous signs and meningitis have been reported in natural cases of pomona infection in adult cattle (Hoag and Bell, 1954a; Gerlach, 1956; Stoenner et al., 1963) and meningitis accompanied by severe headaches is frequently seen in human cases of leptospirosis.

Febrile relapses have been observed previously in experimentally infected cattle by Fennestad (1963), Doherty (1967c) and Sullivan (1970a), however, only Fennestad (1963) observed the occurrence of numerous relapses (up to 56 days P.I.) of the type which were observed in this experiment. Febrile relapses commonly occur in human leptospirosis (Galton et al., 1962) and are often associated with a marked rise in antibody titre and drop in urinary excretion of leptospires (van Thiel, 1948). Doherty (1967c) made a similar observation in two calves, although Sullivan (1970a) found no such

relationship between the occurrence of a secondary febrile reaction, antibody titre and leptospiruria.

The cause of these febrile relapses is unknown. Doherty (1967c) suggested that this recrudescence of fever was a function of rejection of renal leptospirosis by the host. Although no one has demonstrated leptospiraemia during these phases the possibility of a secondary leptospiraemia occurring cannot be ruled out. Failure to demonstrate leptospires in the blood at that time merely indicates that there was less than one guinea pig or hamster infective dose present per inoculum. The presence of a high antibody titre at the time of these febrile relapses may not preclude the possibility of a secondary leptospiraemia as Taylor et al. (1970) demonstrated a secondary leptospiraemia in two dogs infected with canicola, 25 to 28 days previously, although antibodies (1:10,000 and 1:100,000 respectively) were detected in their sera on these days.

The very late pyrexic phases noticed in heifer 7, five to nine days prior to abortion and in heifer 9, seven to eleven days before a premature calving, have not been noted in cattle by other authors. The results show no direct evidence that this late thermal response was indeed due to leptospiral infection, however, the time interval between the onset of pyrexia and abortion and premature birth would suggest that foetal infection may have taken place about this time.

Localisation of Leptospires in the Kidneys

Renal localisation of J10 strain and the subsequent shedding of the organism in the urine confirms the observations of other workers using closely related strains. The light and intermittent nature of the leptospiruria was similar to that observed by Sullivan (1970a & b, 1972) in steers and heifers infected by hardjo. The isolation of leptospires from the kidneys long after leptospiruria had apparently ceased indicated that the cows continued to excrete leptospires in their urine but that the organisms were not present in detectable numbers on the days on which urine was examined. Alexander (1970)

pointed out that the success of dark-ground examination for leptospiruria depends on the number of leptospires in the specimen: to detect one leptospire per high power field probably requires a concentration of 10,000-20,000 leptospires per ml. While centrifugation increases the concentration of leptospires per ml of urine examined, even with a twenty fold concentration of leptospires in the sample 500-1,000 leptospires must be present in each ml of the original sample if one leptospire is to be detected per high power field. Also the centrifugation method used in this experiment damages the leptospires to a varying degree and created difficulties in interpreting the results.

The proglonged period for which leptospires were excreted by some of the cows and the mild histological changes in the kidneys are consistent with the conclusion in Chapter 3, Part A, that cattle act as a maintenance host for leptospires of the hebdomadis serogroup found in Scotland.

Localisation of Leptospires in the Placenta

Localisation of leptospires in the placenta of pregnant cattle has been shown in cases of abortion and in cattle killed serially after experimental infection (up to 20 days - Murphy and Jensen, 1969) but have not been demonstrated in the placenta of cows giving birth to live healthy calves or to premature calves as was found in this study nor have they been found so long after infection of the dam (60 days P.I. in heifer 9).

It would appear from the results obtained here that leptospires of the hebdomadis serogroup localise in and persist on and between the trophoblast cell layer in a similar manner to the way in which they localise in the proximal renal tubular epithelium. Certain similarities exist between these two sites which may facilitate the persistence of leptospires there. Firstly, both the proximal tubular epithelium and the trophoblast consist of a single layer of metabolically active cells involved in the active transfer of metabolites. The luminal surface of the tubular epithelial cells and the surface of the trophoblast cells which oppose the maternal epithelium have

numerous microvilli. Secondly, leptospires on the luminal surface of the tubular epithelium and between the maternal uterine epithelium and trophoblast are separated from circulating antibody by three cell layers, namely capillary endothelium, connective tissue layer and tubular epithelium or maternal uterine epithelium and so are largely protected from the effects of circulating antibody.

Foetal Infection and Abortion

The experimental reproduction of abortion and premature live birth for the first time in pregnant heifers by inoculation with a strain belonging to the hebdomadis serogroup was the most important feature of this experiment. As mentioned in the introduction, Fennestad and Borg-Petersen (1958b, 1962) and Hanson and Brodie (1967) have shown that members of this serogroup could cause abortion if they entered the foetus, but that attempts to produce abortion by infecting the dam (Fennestad and Borg-Petersen, 1956; Sullivan, 1970b; Farina et al., 1972) failed. Sullivan (1970b) noted that two experimentally infected heifers produced small, live, weak calves but did not mention whether they were premature.

The failure to isolate leptospires from the aborted foetus is in accord with the conclusions drawn in Chapter 3, Part C, however, the presence of large numbers of leptospires in sections of liver, kidney and lung stained by the methods of Faine (1965) and Young (1969) indicates that the foetus died during acute leptospirosis.

The prolonged interval (58 days) between infection of the dam and abortion was much longer than that observed in pomona experiments, however, it is consistent with the experimental observation of Sullivan (1972) and the field evidence of Hoare and Claxton (1972) of a longer interval between infection and abortion in cases of hardjo infection. Hoare and Claxton (1972) noted that abortions resulting from infection with serotype hardjo occurred 6-12 weeks after clinical illness in the cow, during a study of hardjo infection in herds in New South Wales.

The reasons for the long time lag between maternal leptospiraemia and the time that leptospires enter the foetal circulation in sufficient numbers to produce a fatal leptospirosis is unknown; Murphy and Jensen (1969) have suggested that either the organisms penetrate the placental barrier in such small numbers that additional time is required for sufficient multiplication to produce fatal leptospirosis in the foetus or that the foetal environment is not conducive to rapid multiplication of the organism. Such arguments may give a plausible explanation in pomona infection where the time interval between maternal leptospiraemia and foetal death is only 2-3 weeks but would not explain the long delay observed in this experiment and in the field cases of hardjo infection reported by Hoare and Claxton (1972). It would appear from the results of this experiment that leptospires of the hebdomadis serogroup can persist in the placenta for up to 2 months in much the same way as they do in the kidney and that at some point they may succeed in passing through the placenta and on entering the foetal circulation set up a fatal foetal leptospirosis. This delay may not be due to the time taken for fatal foetal leptospirosis to develop after infection of the foetus but to the time which elapses between localisation of the leptospires in the trophoblast layer and their being able to penetrate the placenta and enter the foetal circulation.

The reason why leptospires should cross the placenta later rather than sooner is unknown but several factors may be of relevance. Firstly, the late thermal response noted in heifer 7 which aborted, in the heifer 9 which produced the premature calf and in the other heifer (12) in which leptospires were seen in layers of the placenta other than the trophoblast may be important. This pyrexia may possibly have been due to the fall in maternal circulating antibody titre allowing a sudden increase in the numbers of leptospires and causing a secondary maternal leptospiraemia sufficient to cause invasion of the foetus. This is unlikely as there was no anamnestic serological response by the dams. Alternatively, this pyrexia may have been

due to some other pyrogen, either exogenous or endogenous, such as tissue pyrogen released as the leptospires penetrated the placenta. The time interval (5-9 days) between the pyrexia and abortion was similar to the time taken for death to occur in the experimentally-infected newborn calf (CA21) without the production of detectable levels of circulating antibody. The time interval (7-11 days) between pyrexia and the occurrence of the premature live birth was long enough to allow detectable levels of circulating antibody to appear. The time interval (6 days) between the onset of pyrexia and parturition in heifer 12 was too short for detectable levels of circulating antibody to develop in the affected calf before birth. Secondly, Murphy and Jensen (1969) suggested another factor which may be important, namely, that the degenerative changes and increased permeability which occur with aging of the placenta may allow leptospires from affected areas to penetrate the foetal placenta.

The newborn calf receives all its passive immunity through the colostrum (Brambell, 1970), therefore antibodies detected in a foetus or calf before it is fed colostrum must be produced by the foetus in response to antigenic stimulus *in utero*. IgM and IgG have been observed in foetal calf sera as early as 130 days and 145 days respectively (Shultz *et al.*, 1971; Sawyer *et al.*, 1973) and Fennestad and Borg-Petersen (1962) have demonstrated that foetuses which survive leptospiral infection produce antibodies to the infecting strain; the youngest foetus to do so was only 134 days old when infected. Hence the finding of antibodies to *hardjo* in the sera of the premature calf (CA9) prior to its receiving colostrum and the presence of a mild focal interstitial nephritis at slaughter may indicate that the calf had been infected *in utero* and had survived infection. Furthermore, had this calf not been held up to suckle it would not have been able to obtain adequate colostrum and colostrum derived immunoglobulins are necessary to prevent neonatal coliseptic aemia and colibacillosis (McEwan *et al.*, 1970).

Calf 5. The role played by leptospiral infection in the production of the weekly calf (CA5) which died of an intercurrent infection (E. coli) must be a matter of conjecture, however, leptospires were demonstrated histologically in the foetal membranes at calving. The role of leptospires and other abortifacient agents in the production of hypogammaglobinaemic calves especially in suckler herds is something which merits investigation.

Serological Findings

The appearance of antibodies from the sixth day onwards, the subsequent sharp rise in titres, which reached a peak 11 to 21 days P.I. and the ensuing gradual decline in titre observed in most of the experimental animals was similar to that observed by others (Sullivan 1970b, 1972; Farina et al., 1972) in cattle infected by strains belonging to the hebdomadis serogroup. The long period of rising titre observed by Fennestad (1963) and Doherty (1967c) in cattle infected with serotype pomona was not observed in this study.

The secondary antibody response noted in heifers 14, 16, 17 and 18, ten to twenty weeks P.I. was similar to that observed by Sullivan (1972), in 6 of 8 cows, three months after inoculation. Sullivan pointed out that this might represent an anamnestic response to challenge by leptospires contaminating the environment as the cows were being kept at pasture at that time. This argument also applies to the heifers mentioned above as three of them (16, 17 and 18) were kept in adjacent stalls and still had leptospires in their kidneys 153, 174 and 153 days P.I. respectively. The other heifer (14) was still excreting leptospires in its urine and contaminating its environment at the time of the secondary serological response. Another possibility is that this secondary response in the heifers was not due to challenge by leptospires which had been voided into the environment but was due to invasion of the body by leptospires from foci of infection in the kidney tubules.

The observation that abortion takes place while the dam's antibody titre is falling is consistent with the field observation of Hoare and Claxton (1972) who followed the serological response of cows during a natural outbreak of hardjo infection in Australia.

The results of this experiment illustrate the advantages and disadvantages of using arbitrary significant titres. The fact that titres to sejroe had fallen to 1:100 or less in 15 of the 20 heifers by 9 weeks post inoculation vindicates the use of an arbitrary titre of 1:300 or more as a guide to recent exposure to infection in interpreting the results of the field survey. Conversely, the low levels of antibody present in heifer 7 (1:100 to hardjo and 1:30 to sejroe) at the time of abortion and similarly in heifer 9 at the time it delivered a premature calf would have been dismissed if presented for diagnosis as a possible leptospiral abortion case to a laboratory where an arbitrary titre of 1:300 or more was accepted as diagnostic in the interpretation of the results of the M.A.T. Kirkbride et al. (1973) in a recent survey of bovine abortion and stillbirth diagnosed Leptospira-induced abortion on the basis of a titre of 1:1,000 or greater in the dam's serum. The abortion in heifer 7 and premature birth in heifer 9 would not have been diagnosed correctly if these criteria had been used.

Fennestad and Borg-Petersen (1956) reported a similar fall in the whey antibody titre to that observed in this experiment. They noted that the level of the colostral whey antibody was generally 3 to 10 times higher than that in the dam's serum but 24 hours later it had fallen to from 1/10th to 1/100th of the titre at parturition.

The considerable variation in the length of time for which passively acquired antibodies persist noted in this study (3-8 weeks) has been observed in other reports. Farina et al. (1972) found that they persisted at a detectable level for 2 to 2½ months while Fennestad and Borg-Petersen (1956) reported that a calf with a titre of 1:300 two days after birth still had a titre of 1:30 five months later.

Pathological Findings

Apart from the absence of jaundice and the presence of meningeal haemorrhages, the pathological findings in calf 21 resembled those reported by Fennestad (1963) in fatal cases of grippotyphosa and pomona infections in

calves. The histopathological changes in the liver and kidney resembled changes seen in calves dying from other septicaemias. While losses in calves due to hardjo or related serotypes have not, as yet, been reported in the literature, such cases may have been mistaken for one of the more common calf septicaemias on the basis of the histopathological findings.

The mild focal interstitial nephritis observed in the experimental heifers was similar to that observed in the natural cases (Chapter 3, Part B) while the relatively more severe lesions in the calf kidneys were consistent with the observation by Fennestad (1963) that the younger the animal the more severe the kidney lesions.

The gross and histological changes in the placenta were similar to those reported by Murphy and Jensen (1969).

Origin of the Organism

No appreciable difference was noted between the acute clinical signs of infection of pregnant adult cattle with strain J10, and those recorded by other authors who have investigated the pathogenicity of hardjo or related serotypes for pregnant cattle in spite of the fact that J10 was isolated from a cow which aborted. It is possible that the origin of the J10 strain contributed to the successful reproduction of the most important sequel to acute infection, i.e., the production of abortion and premature birth. Other investigators who have failed to produce abortion have used strains which came from either small rodents (Fennestad and Borg-Petersen, 1956) or from the urine of infected cows (Sullivan, 1970b, Farina et al., 1972) but not from a cow with a history of abortion as was the case with J10 strain.

Virulence of the Organism

The use of an organism which, although growing poorly, had undergone only a small number of sub-cultures, would appear to have overcome the problem of loss of virulence. Virulence, as judged by pathogenicity for hamsters on primary re-isolation from experimental animals, appeared to be maintained throughout the experiment. It is impossible to make a comparison of the virulence of J10 strain with the hardjo strains used by Sullivan (1970b and

1972) and Farina et al. (1972) since they relied solely on guinea pigs for the biological isolation of leptospires. Sullivan (1970b, 1972), Sullivan and Callan (1970) and Farina et al. (1972) have noted the low pathogenicity of Australian and Italian hardjo strains for guinea pigs, similar to that observed in this experiment.

Influence of Animal Susceptibility on the Experimental Disease

Stalheim (1971) has shown that cattle which have been exposed previously to infection may remain refractory to reinfection for long periods even when their circulating antibody titre has fallen to less than 1:10. It is impossible to evaluate what influence the susceptibility of the individual animals had on the role of J10 strain as an abortifacient agent, as most of the animals of doubtful susceptibility had not reached the stage of gestation where foetal infection would be expected to take place (heifers 13, 15, 18, 20). The converse is also true, i.e., it is impossible to say whether the results of this experiment concur with the commonly held view that leptospiral abortion occurs in the last trimester (Fennestad and Borg-Petersen, 1958b, Stoenner, 1968) as the susceptibility of four (13, 15, 18, 20) of the seven heifers infected before they were six months pregnant, must be doubtful.

The susceptibility of the individual animal at the time of inoculation may well have influenced the course of the acute disease, as leptospiraemia was only demonstrated in one of the heifers which had either traces of antibody present at the time of inoculation (3, 13, 18, 19) or where the pattern of antibody response suggested an anamnestic response (15, 20) whereas leptospiraemia was detected in 16 of the other 21 animals.

The results indicate the much greater reliability of purchasing experimental animals from herds whose immunological status is known rather than buying apparently susceptible animals of unknown origin.

Relationship of the Experimental Disease to Field Observations

While the two main features of the experimental disease namely the very mild or subclinical nature of the acute infection and abortion were similar to

what was observed in the field investigation the abortion rate was lower than that observed in some naturally infected groups of susceptible animals, e.g., LH28 where 7 out of 24 heifers aborted. There were a number of important differences between the way the experiment was carried out and what must happen in natural cases, the affect of which is very difficult to determine.

Firstly, there were the differences in the environmental conditions under which the animals were kept and their level of nutrition. While the experimental heifers were kept tied up in a warm,dry byre and maintained on a good level of nutrition throughout the course of the experiment many replacement heifer stocks on Scottish farms are kept in wet paddocks or exposed covered yards on a low nutritional plan during the winter time when they are most likely to be exposed to infection by leptospires of the hebdomadis serogroup. This difference in stress may well cause differences in the severity of infection. Doherty (1967c) considered the death of 3 undernourished steers in an experimentally induced outbreak in a herd kept under natural conditions to be due to the additive affects of leptospirosis and nutritional stress. A similar "ill-thrift" syndrome has been emphasised by Sutherland et al. (1949) and Stoenner et al. (1956).

Secondly, the route and dose of inoculum used (a single 5 ml dose of culture given intramuscularly) was very different to what must happen in the field where infection is thought to occur through the mucosa of the eye or nose, or through moist abraded skin, or per os, and where the animal is likely to be subjected to challenge not just once but repeatedly. Not only might this alter the clinical disease and the serological response of naturally infected animals but other things such as leptospiruria. Sullivan (1970a) observed a light intermittent leptospiruria in steers experimentally infected with hardjo but two steers which were naturally infected by contact with experimentally infected animals at grass developed a much more marked leptospiruria than was observed in the experimentally infected steers.

SUMMARY OF FINDINGS

Experimental infection of pregnant heifers with J10 strain confirmed the field observation that infection by strains belonging to the hebdomadis serogroup could cause a mild or subclinical infection which occasionally resulted in abortion or premature live births. The major findings are outlined below.

Following experimental infection there was an incubation period, usually of 3 to 5 days, after which a marked pyrexia (up to 106.2°F) developed in most of the animals. This pyrexia lasted from 1 to 5 days. During this period the heifers developed increased respiratory and pulse rates, ruminal movements decreased and there was some loss of appetite. The clinical signs observed in the experimentally infected calves were more severe than those seen in the heifers. Calf 21 died 9 days post infection after having been recumbent for a day during which it showed opisthotonus. Four of the remaining calves were very dull and unwilling to eat during the initial febrile period.

Relapses of fever occurred in 19 animals between the 8th to 15th day P.I. and further relapses occurred later. These relapses were frequently very marked (up to 106.9°F). No other clinical signs were associated with these periods of pyrexia.

One heifer aborted a near-term foetus on day 58 P.I. and another produced a live, weak, premature calf (240-24 days gestation) on day 60 P.I. This is the first report of the occurrence of these syndromes following the experimental infection of pregnant cows with strains belonging to the hebdomadis serogroup.

A leptospiraemia of 1 to 5 days duration was demonstrated in 10 of the 20 infected heifers and in the 7 experimentally infected calves during the first week of infection. It was usually associated with the period of initial pyrexia and disappeared with the appearance of circulating antibody. Leptospiraemia was not demonstrated during the secondary pyrexic phases.

Leptospires were demonstrated in the kidneys of the experimental animals from day 9 P.I. (calf 21) to day 174 P.I. (heifer 17).

Leptospires were demonstrated in the urine of seven heifers from day 22 (heifer 4) to day 70 (heifer 14). Leptospiruria was light and intermittent.

Localisation of leptospires also occurred on and between the cells of the trophoblast layer of the placentas of some of the experimental heifers. They were demonstrated in the placentas of five heifers (1, 6, 5, 4 & 12) which produced live full-term calves 14, 15, 16, 32 and 55 days after infection respectively.

Leptospires were demonstrated in the liver, kidney and lungs of the aborted foetus and in its placenta. No leptospires were found in the internal organs of the premature live calf when it was slaughtered three weeks after birth, however, they were demonstrated in histological sections of the cotyledons taken at calving.

Leptospires were isolated from a mixed inoculum of blood and liver from calf 21 which died and they were demonstrated in histological sections of its kidneys.

Antibodies appeared in the serum of susceptible, experimentally infected cattle from the 6th day P.I. onwards and maximum titres (up to 1:100,000 to hardjo) were reached between 11 and 21 days P.I. These maximum titres persisted for 3 to 21 days after which time they began to decline. In most animals they continued to fall gradually until the end of the experiment, although they were still detectable at slaughter 26 to 174 days P.I.

Secondary rises in antibody levels were detected in four heifers between 10 and 21 weeks P.I.

Antibodies were detected in the milk of infected heifers. The highest titres (up to 1:100,000 to hardjo) were found in samples collected immediately after parturition. Milk whey titres declined rapidly over the first 3 days of lactation but persisted at a low level for some time; more than 32 days in the case of heifer 5.

An antibody titre of 1:10 to hardjo was found in a sample of serum collected from the premature, live calf (CA9) immediately after birth which indicated that infection of the calf had taken place in utero.

Calves born to infected dams acquired passive antibody titres of up to 1:30,000 to hardjo within three days of birth. These titres persisted from 21 to more than 56 days in these calves.

All the experimental animals developed a mild interstitial nephritis. The cotyledons from the placenta of the cow which aborted showed marked necrosis.

Chapter 5

DISCUSSION

While it was not the specific purpose of this study to investigate the pathogenesis of leptospiral abortion in cattle, the diagnosis of leptospiral abortion in cattle, the control of bovine leptospirosis or any aspects of possible zoonoses, the results of the field and experimental studies do have a bearing on these topics.

Pathogenesis of Abortion

Opinions differ on the pathogenesis of leptospiral abortion, although a number of features of the disease are accepted by most investigators:

(1) Abortion is not a constant sequel to leptospirosis in pregnant cattle.

(2) Abortion due to leptospirosis occurs in the latter half of pregnancy. There have been no reports of abortion resulting from infection of the dam before five months gestation, except those of (a) Hanson and Brodie (1967) who observed abortion in a cow following intra-uterine inoculation with hardjo on the 80th day of gestation and (b) Fennestad and Borg-Petersen (1962) who induced abortion by infecting a foetus directly with saxkoebing on the 111th day of gestation.

(3) There is an appreciable time interval between inoculation of the dam and abortion. This has varied from 18 (Murphy and Jensen, 1969) to 47 days (Ferguson et al., 1957).

(4) In most cases the dam is free of clinical signs and is serologically positive at the time of abortion, though occasionally the titre may be low.

(5) Abortion is often the only clinical sign of leptospirosis.

(6) Aborted foetuses usually appear to have been dead 24 hours or more before expulsion.

(7) The cotyledons are uniformly atonic and fawnish in colour.

Fennestad and Borg-Petersen (1960) presented the hypothesis that leptospiral abortion is the expulsion of a foetus that has died from foetal leptospirosis developed after leptospires have crossed the placental barrier from maternal to foetal circulation during maternal leptospiraemia. This hypothesis was based on the demonstration of leptospires in aborted foetuses using silver impregnation methods (Fennestad and Borg-Petersen, 1958a; Te Punga and Bishop, 1953; Dacres and Kiesel, 1958 and Bridges, 1958).

The opponents of this view have argued that silver impregnation techniques only demonstrate "leptospira-like" structures and do not provide a definite diagnosis of leptospirosis. This argument is supported by the rare isolation of leptospires from aborted foetuses.

The hypothesis based on foetal leptospirosis was supported by the results which Fennestad and Borg-Petersen (1958b, 1962) obtained by infecting bovine foetuses in passively immunised dams. Infection by pomona and sejroe led to intra-uterine death in the leptospiraemic stage followed by expulsion of the foetus, while foetuses of a similar age infected with saxkoebing, survived the infection and were apparently normal at birth. The changes in the aborted foetuses were similar to those found in foetuses aborted after experimentally produced maternal leptospirosis and in naturally occurring leptospiral abortions.

The concept of foetal leptospirosis was further promoted by the demonstration of leptospires in aborted foetuses by immunofluorescence (Smith et al., 1967) and by the isolation of leptospires from placenta as well as from live foetuses long after maternal leptospiraemia had ceased (Murphy and Jensen, 1969). The latter authors believed that haemolysin released from leptospires in the foetal circulation destroyed foetal erythrocytes and the resultant anoxia killed the foetus.

Ferguson et al. (1957), however, proposed that the foetus was not invaded by leptospires, but by a toxic substance released from leptospires which have been destroyed in the dam by developing antibodies. This substance crosses

this hypothesis: firstly, Ferguson et al. (1957) and other investigators (Morse and McNutt, 1956) who failed to isolate leptospires from aborted foetuses did not attempt to demonstrate leptospires by silver impregnation techniques, and secondly, it fails to explain the interval of 2-3 weeks usually observed between maternal leptospiraemia and the occurrence of abortion.

The third hypothesis that has been proposed is that suggested by Morter et al. (1958). After examining aborted foetuses and foetal membranes from experimentally infected cows killed at intervals after infection they concluded that leptospiral infection of the dam produced histopathological changes which interfered with the normal physiological function of the placenta and resulted in foetal death.

Sleight and Langham (1962) induced abortion in two of eleven cows by the intravenous administration of large volumes of pomona haemolysin. Both animals had severe haemolytic crises prior to abortion. The authors maintained that maternal erythrolysis by haemolysin resulted in a decrease in the oxygen carrying capacity of the blood, causing disruption of placental cellular metabolism and subsequent death of placental cells. This interfered with the transfer of essential nutrients and caused foetal death. The placental changes they found were similar to those described by Morter et al. (1958).

The argument against abortion being due to histopathological changes in the placenta has been based on (1) the observation of similar changes to those described by Morter et al. (1958) and Sleight and Langham (1962) in normal bovine placenta during the last trimester (Bjorkman, 1954) and (2) the histopathological findings of Murphy and Jensen (1969) that there were no differences between the placentomes of infected and control heifers with the exception of cases in which the foetus had died. The changes in these latter placentas were thought to be concomitant with foetal death.

The results of this investigation (experimental and field) indicate that abortion due to hardjo (or related strain) results from foetal leptospirosis because leptospires were demonstrated in large numbers in cases of natural and experimentally induced abortion. So far a haemolysin has not been demonstrated for hardjo; it is unlikely therefore that anoxia resulting from haemolysis of foetal erythrocytes was the cause of foetal death and the placental changes observed. The suggestion by Fennestad and Borg-Petersen (1960) that the placental changes were due to lysis following foetal death does not explain the pathogenesis of live premature birth associated with foetal leptospirosis seen in this investigation. It would seem probable that premature live birth results from damage caused to the placenta during foetal leptospirosis either by the leptospire or some cytotoxic substance produced by it.

The Diagnosis of Leptospiral Abortion

The diagnosis of leptospiral abortion is very difficult. There are a number of reasons for this.

1) The isolation of leptospires from aborted foetuses is almost impossible for the reasons given in Chapter 3, Part C.
2) Most cows which abort will be seropositive at the time of abortion and if a second sample is taken 14 days later its titre will usually be either the same or less than the titre of first sample (Stoenner, 1968).
3) Many seropositive animals have not aborted and many cows which abort for other reasons may be seropositive, therefore, the presence of Leptospira antibodies in an individual cow is of little value in determining the cause of an abortion.

The results of this investigation suggest that for herds in which no other cause of abortion can be found, it is useful to test many or all of the animals in a herd for antibody to Leptospira. If 1) there is a mixed population of recently infected cattle (antibody titres of 1:300 or more) and

susceptible pregnant cattle, abortions occur before and after testing and the animals which abort are seropositive or 2) there is a very high incidence of infection and abortions only occur before testing, then leptospirosis is likely to be the cause of abortion.

If freshly aborted foetuses can be obtained, properly applied silver impregnation and fluorescent antibody techniques can be very useful in reaching a diagnosis.

The Control of Bovine Leptospirosis

In countries such as Australia, the U.S.A. and the U.S.S.R. where bovine leptospirosis is a recognised problem, control of clinical disease is by vaccination. Vaccines to hardjo or related strains are not available commercially in the United Kingdom and so an alternative method of control may be necessary. On the basis of observations made in this study certain recommendations on control can be made.

Firstly, in herds where hardjo infection is absent it is unlikely that there will be a reservoir of infection in the wild rodent population and control measures must be aimed at preventing the introduction of infection to the herd. Therefore, a) the introduction of cattle should be avoided if possible but if introduction is necessary then the animals should be treated with dihydrostreptomycin as this eliminates leptospires from the kidneys of carrier cattle (Stalheim, 1969); b) stock should not be grazed on other farms which may be infected.

Secondly, in herds where infection is already present, control measures should utilise the self-limiting nature of the disease, and a form of natural vaccination should be practised. All heifer stock should be exposed to infection before their first gestation by mixing them with the adult herd, especially in the autumn and winter.

Zoonoses

Since Sakula and Moore (1969) first reported the infection of four cowmen with strains belonging to the hebdomadis serogroup, infection in man by members of this serogroup have been recognised with increasing frequency

in Britain. In 1973 infection by this serogroup was the most commonly diagnosed form of human leptospirosis in Britain (Anon, 1974). Most of the cases have been in agricultural workers and the source of infection has been thought to be infected bovine urine. The finding of leptospires localised in the placentas of experimentally infected cows in this study suggests that this tissue may also be a possible source of human infection as many calvings are manually assisted.

Future Research

This study has established that -

1) infection by the hebdomadis serogroup is widespread in Scottish cattle - 41.8 per cent of 3,600 cattle had antibodies to sejroe;
2) natural and experimental infection of pregnant cattle by strains belonging to the hebdomadis serogroup can cause abortion and premature birth.

The close correlation between the incidence of infection and abortion in the twenty-nine herds studied suggests that on individual farms leptospiral abortion may be a serious problem. This study has not produced any evidence of the importance of leptospirosis as a cause of bovine abortion in general. Future studies must investigate this point.

It is possible that abortion is not the most important aspect of the disease but is only the most obvious result of infection. Other clinical syndromes may be more important, e.g., mastitis, the birth of weakly live calves, predisposition to infection by other infectious agents.

Infection by the hebdomadis serogroup has been associated with mastitis and drop in milk yield in North America (Robertson et al., 1964; Sulzer, 1964), Australia (Sullivan and Callan, 1970) and New Zealand (Lake, 1973). Howell et al. (1969) described an outbreak in the south of England and a similar mastitis was reported in one infected herd (LH26) in this study. Despite the field evidence for leptospiral mastitis there are no reports of the infection of lactating cattle with strains belonging to the hebdomadis serogroup. There

is a need to establish experimentally whether mastitis and/or a drop in milk yield are associated with infection by leptospires of this serogroup in the United Kingdom. If so, the financial importance of the loss of milk resulting from enzootic and epizootic infections should be assessed.

Having established the incidence of leptospiral abortion and the possible economic losses resulting from abortion, mastitis and premature weakly calves a decision could be made as to the need for a hardjo vaccine in the United Kingdom.

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APPENDIX 1 - TABLE 26

THE SEROLOGICAL AND CLINICAL SURVEY OF 29 HERDS

SUMMARY OF RESULTS

Table 26. Serological and Clinical Survey of 29 Herds (2,687 Cows and Heifers) - Summary of Results

No.	Herd No.	No. of cattle tested	% of cattle with antibodies to sejroe		No. of abortions in 18 mth period			Comments
			1:10 or more	1:300 or more	9 mths before testing	9 mths after testing	%	
1	LH2	62	0	0	0	0	0	
2	LH3	33	0	0	0	0	0	Closed beef herd.
3	LH15	52	0	0	0	0	0	Closed beef herd.
4	LH5	55	1.8	0	0	0	0	Closed beef herd.
5	LH8	251	2.0	0	0	0	0	
6	LH16	83	2.4	0	0	1	1.2	Beef herd. The two animals with antibody titres were both bought-in cows.
7	LH6	64	4.7	3.1	0	0	0	Closed dairy herd. The three animals with antibodies to sejroe were 2 year-old heifers. Six animals had antibodies to panama (titres ranged from 1:30 to 1:1,000).
8	LH22	97	8.2	0	0	0	0	Beef herd. Virtually closed (only a few bought-in animals).

The herds have been listed in order of ascending values for the incidence of sejroe antibodies (++ 1:10 and greater) in the sera.

Herd Survey Summary (continued)

No.	Herd No.	No. of cattle tested	% of cattle with antibodies to sejroe		No. of abortions in 18 mth period			Comments
			1:10 or more	1:300 or more	9 mths before testing	9 mths after testing	%	
9	LH10	106	14.2	0.9	Not Known	Not Known	?	It proved impossible to obtain a detailed record of this herd.
10	LH18	27	14.8	0	0	0	0	Beef herd. The four animals with antibodies to sejroe were bought-in animals.
11	LH19	60	21.7	0	0	0	0	Dairy herd. Virtually closed - only 3 bought-in animals.
12	LH21	68	22.1	7.4	0	0	0	(Nothing of note).
13	LH7	96	37.5	10.4	N.K.	N.K.	?	No history.
14	LH17	77	37.7	9.1	N.K.	N.K.	?	Not known. From Westmoreland.
15	LH25	60 cows 15 heifers	43.3	10.0	2	2	5.3	The two animals which aborted subsequently were a 5 year-old cow and a heifer which were negative when the herd test was carried out. No sample was received from the cow but the heifer at the time of abortion had a titre of 1:100 to sejroe. The heifers were running with the bull.

Herd Survey Summary (continued)

No.	Herd No.	No. of cattle tested	% of cattle with antibodies to sejroe		No. of abortions in 18 mth period			Comments
			1:10 or more	1:300 or more	9 mths before testing	9 mths after testing	%	
16	LH27	85	44.7	5.9	1	0	1.2	Beef herd. Fair proportion of bought-in animals. Only two heifer samples tested.
17	LH11	39 cows 9 heifers	51.3 10.0	5.1 66.7	1	0	2.1	Heifers were kept with the cows.
18	LH9	237	51.5	37.6	Not Known	Not Known	?	No details of the exact number of abortions were obtained, however, it is known that abortions occurred both before and after testing. Human cases of hebdomadis group infection occurred on this farm.
19	LH29	22	54.5	27.3	2	2	18.2	Dairy herd. Had a problem with retained foetal membranes and chronic infertility. Leptospires were demonstrated histologically in the kidneys of 2 out of 3 cows examined.
20	LH12	89	58.2	18.0	3	2	5.6	The cases of abortion both before and after testing occurred in the early winter following a period of close confinement in a small severely "poached" paddock prior to being brought in for the winter.

Herd Survey Summary (continued)

No.	Herd No.	No. of cattle tested	% of cattle with antibodies to sejroe		No. of abortions in 18 mth period			Comments
			1:10 or more	1:300 or more	9 mths before testing	9 mths after testing	%	
21	LH14	39 cows 22 heifers	61.5 4.5	15.4 0	3	0	4.9	Dairy herd.
22	LH23	236 cows 22 heifers	69.1 31.8	30.9 18.2	10	13	8.9	Dairy herd. Quite a number of bought-in cows. Abortions continue at a high rate - in an 11 mth period subsequent to this period there were 23 stillbirths (11 in heifers) and 2 abortions earlier in gestation and one premature birth occurred. Apart from these cows 29 others had retained foetal membranes and/or endometritis. Has a high calf mortality in the first three days of life. Heifers are mixed with cows when they are in the latter half of gestation. Included amongst the 236 cows are 25 two year-old animals which had antibodies to sejroe. Leptospires were demonstrated in a foetus. Previously 4 strains of leptospires belonging to the hebdomadis serogroup had been isolated from cows and a bull hebdomadis infection diagnosed in an employee.

Herd Survey Summary (continued)

No.	Herd No.	No. of cattle tested	% of cattle with antibodies to sejroe		No. of abortions in 18 mth period			Comments
			1:10 or more	1:300 or more	9 mths before testing	9 mths after testing	%	
23	LH20	55 cows 19 heifers	69.1 78.9	29.1 42.1	1	0	1.4	Recent active infection in heifers and adult cows. The heifers were 2-3 mths pregnant and were infected before susceptible phase.
24	LH1	86 cows	70.9	29.1	12	?		Failed to isolate from the kidneys of three cows from this herd. Most of the abortions occurred in cows in their second gestation.
		28 heifers (year 71-72)	0	0		exact number not known	>10	Heifers were kept on a separate farm.
		29 heifers (year 72-73)	10.3	10.3				Abortions died out in the latter part of 1971 but started again in the autumn of 1972.
25	LH13	90 cows	72.2	21.1	4	0	3.3	A member of the hebdomadis serogroup (strain J10) was isolated from the kidneys of a cow which had aborted in this herd.

Herd Survey Summary (continued)

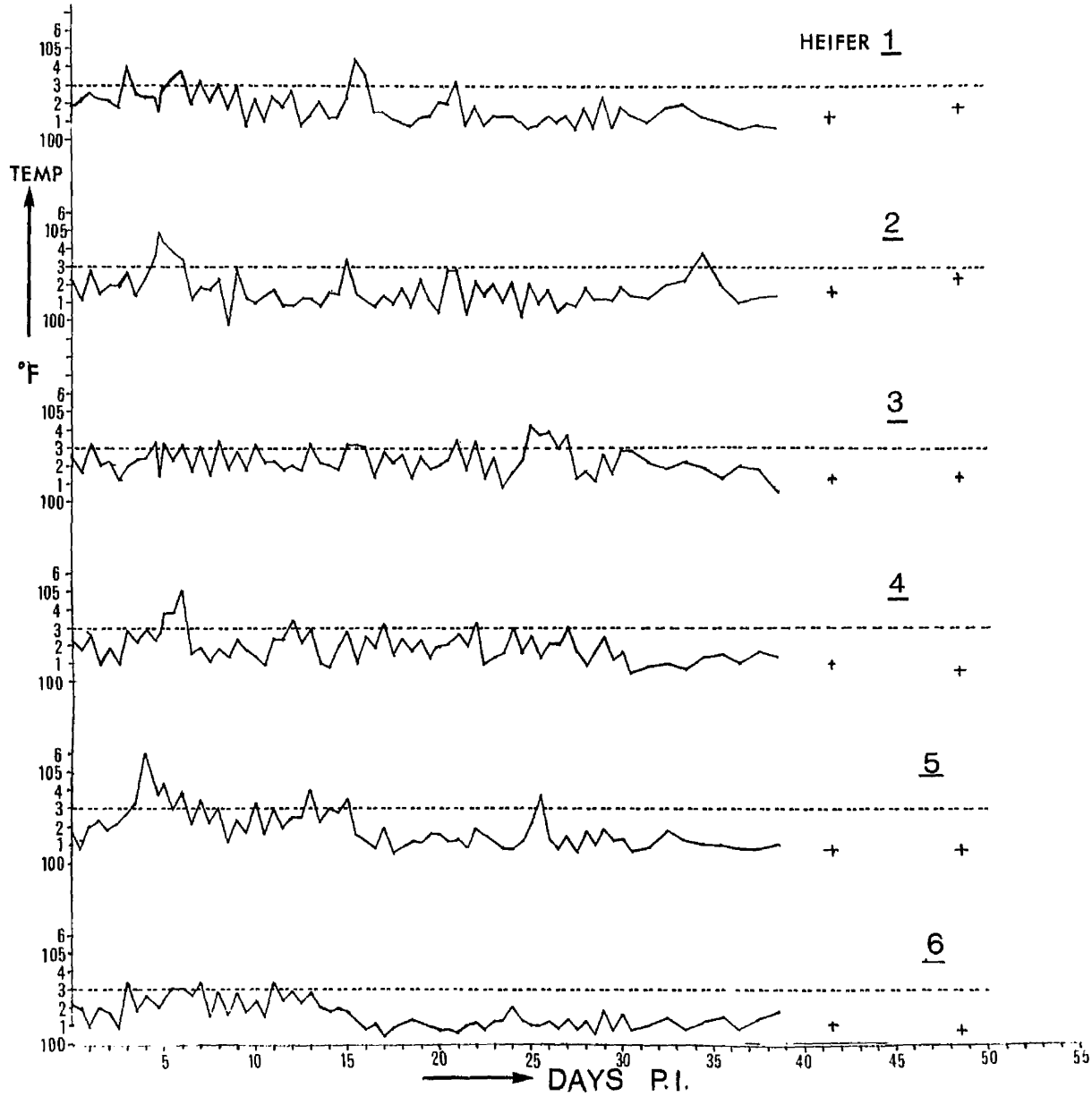
No.	Herd No.	No. of cattle tested	% of cattle with antibodies to sejroe		No. of abortions in 18 mth period			Comments
			1:10 or more	1:300 or more	9 mths before testing	9 mths after testing	%	
26	LH26	52 cows 18 heifers	82.7 88.9	19.2 50.0	7	0	10	A lot of "poached" pasture and surface water. Farmer had noticed several groups of cows affected by mastitis in all 4 quarters which responded readily to penicillin therapy. Abortion did not occur in any one age group of animals.
27	LH28	121 cows 24 heifers	84.3 0	37.2 0	8	1 and 1 premature birth	6.8	Heifers were kept on separate farm. Seven out of 24 heifers in the previous year's batch aborted after their return to the main farm, where they were kept on a small wet area which became very poached. They fed on a self-feed silo. A small stream which flowed through the paddock provided the heifers with drinking water. The effluent from the covered yard containing the milking herd flowed into this stream. Leptospires were demonstrated in a foetus and a premature calf from this herd.
28	LH24	48	89.6	33.3	3	0	6.3	Beef herd - a lot of bought-in cows.
29	LH4	52 cows 26 heifers	96.2 100	40.4 73.1	0	1 premature birth	0	Dairy herd. Cows and replacement stock grazed together. A lot of poached land and surface water.

APPENDIX 2 - FIGURE 74

CHARTS SHOWING THE TEMPERATURE RESPONSE OF EXPERIMENTALLY

INFECTED HEIFERS

Fig.74a Temperature charts of the heifers (group 1) experimentally infected with J.10 strain.



+ = individual temperature readings taken after routine clinical examination had finished

Fig.74b Temperature charts of the heifers (group 11) experimentally infected with J.10 strain.

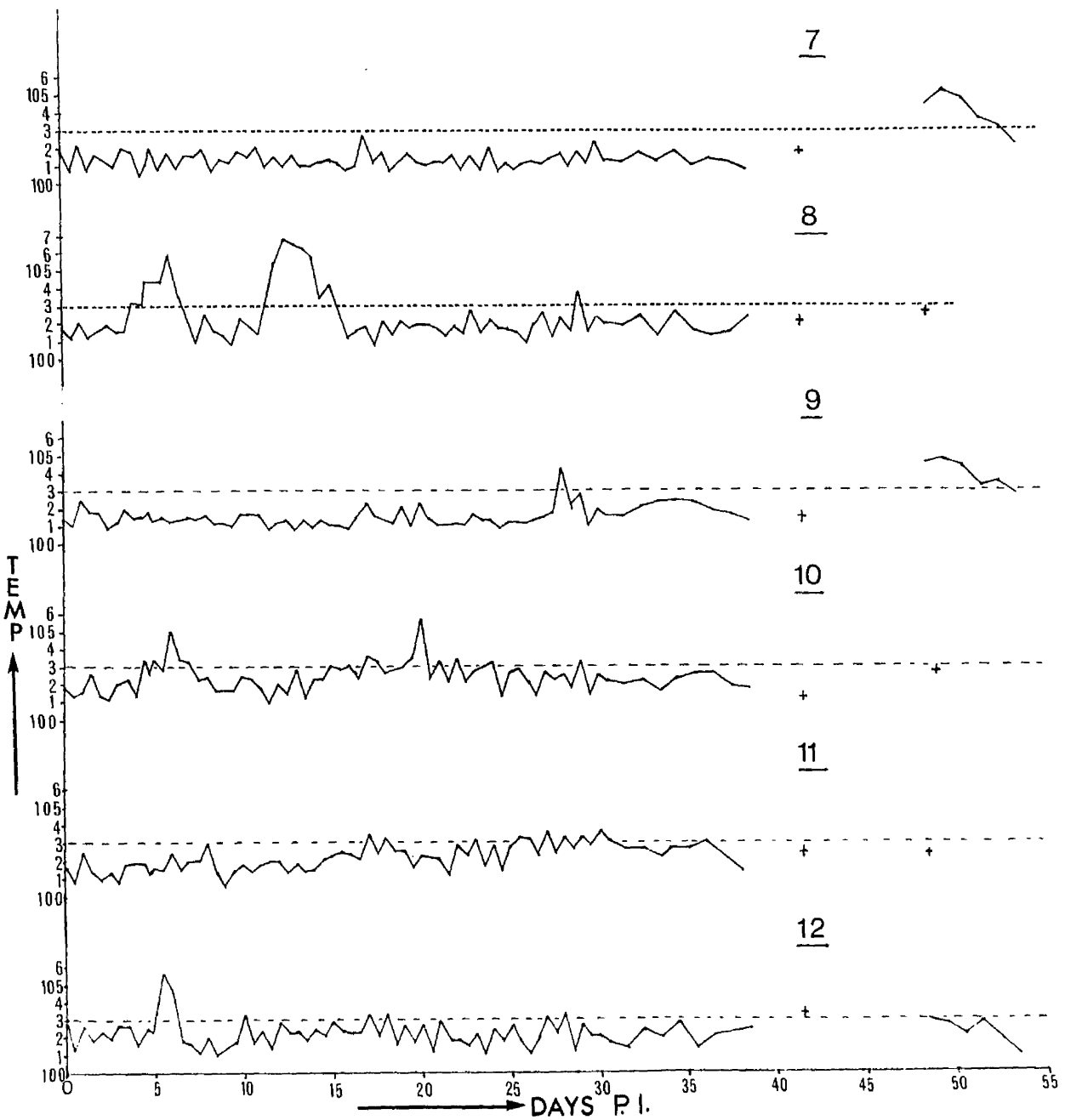
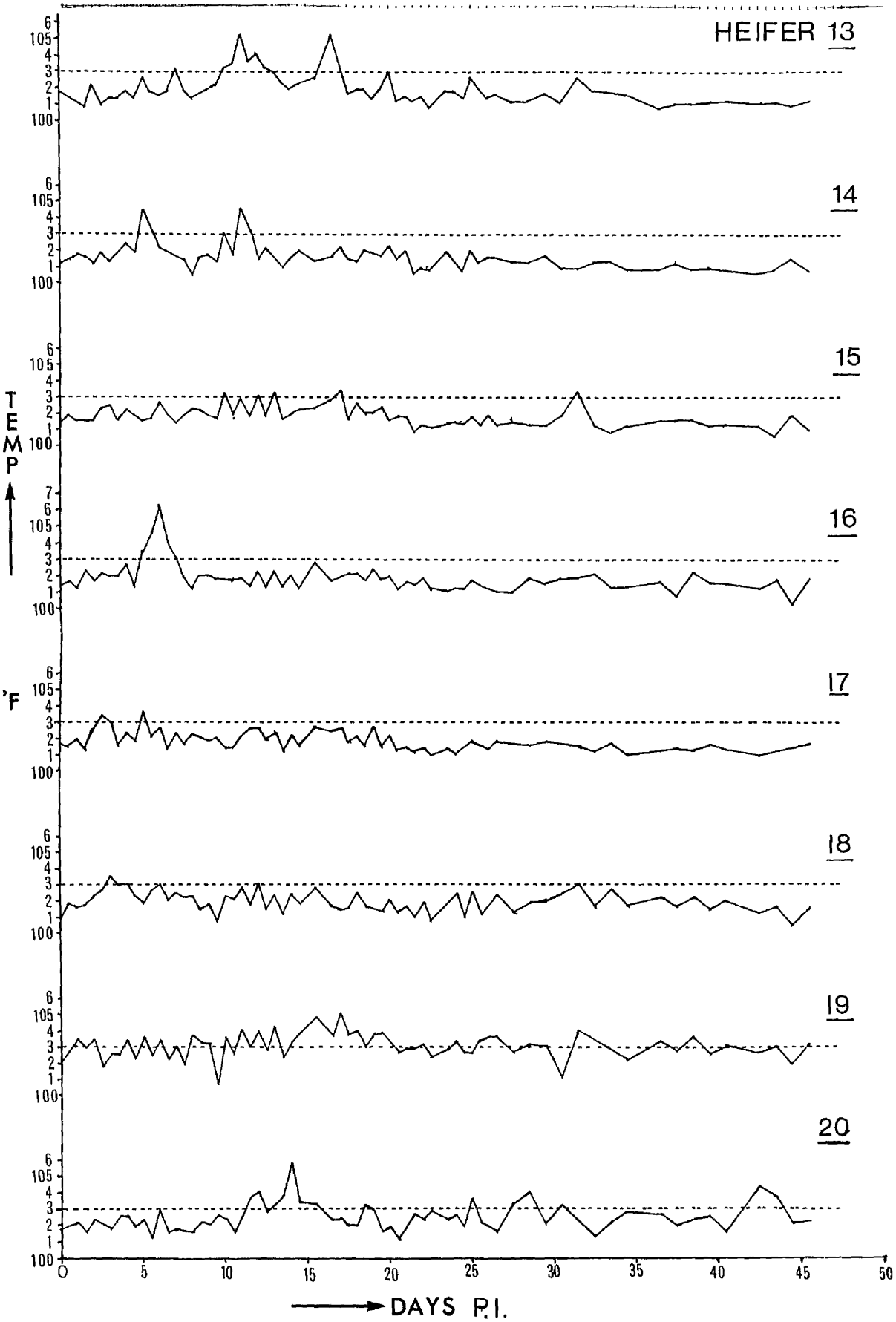


Fig.74c Temperature charts of the heifers (group III) experimentally infected with J.10 strain.



APPENDIX 3 - FIGURES 75 AND 76

CHARTS OF THE ANTIBODY RESPONSE OF HEIFERS AND CALVES TO INFECTION WITH J10 STRAIN. THREE MEMBERS OF THE HEBDOMADIS SEROGROUP WERE USED AS ANTIGENS, NAMELY HEBDOMADIS, SEJROE AND HARDJO (204 STRAIN).

Fig. 75a The antibody response in heifers (groups 1 & II) experimentally infected with J.10 strain

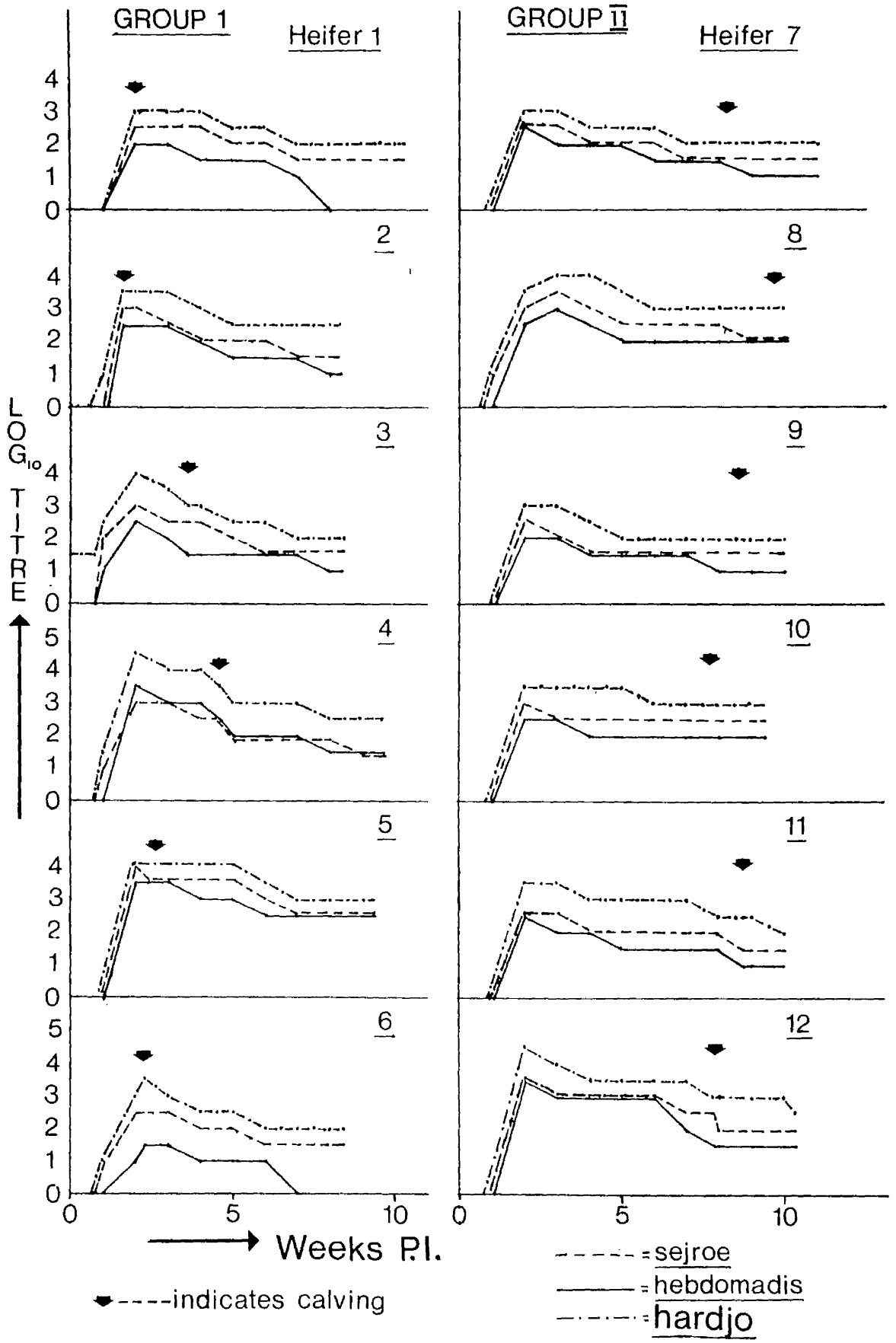


Fig. 75b The antibody response in heifers (group III) experimentally infected with J.10 strain

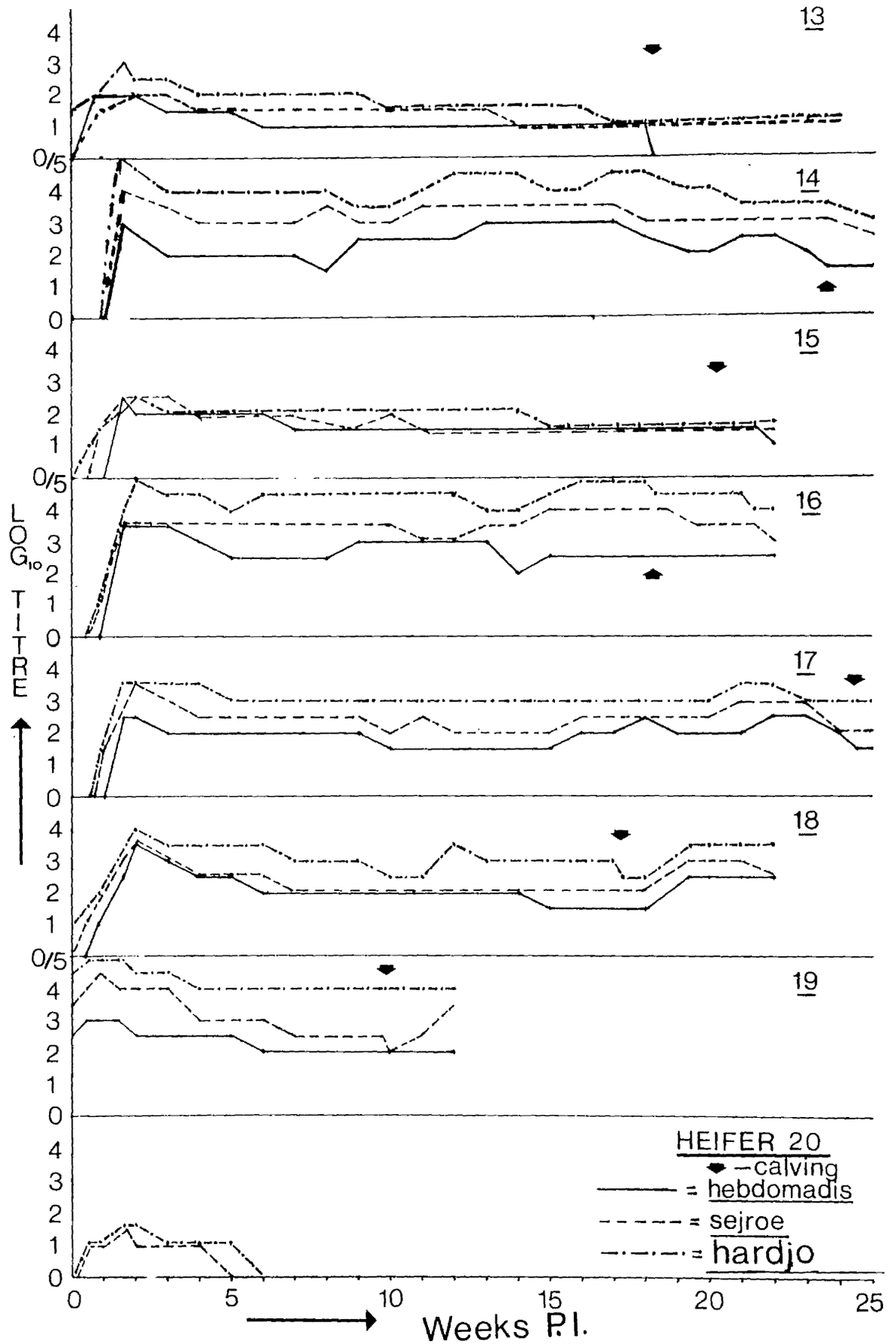
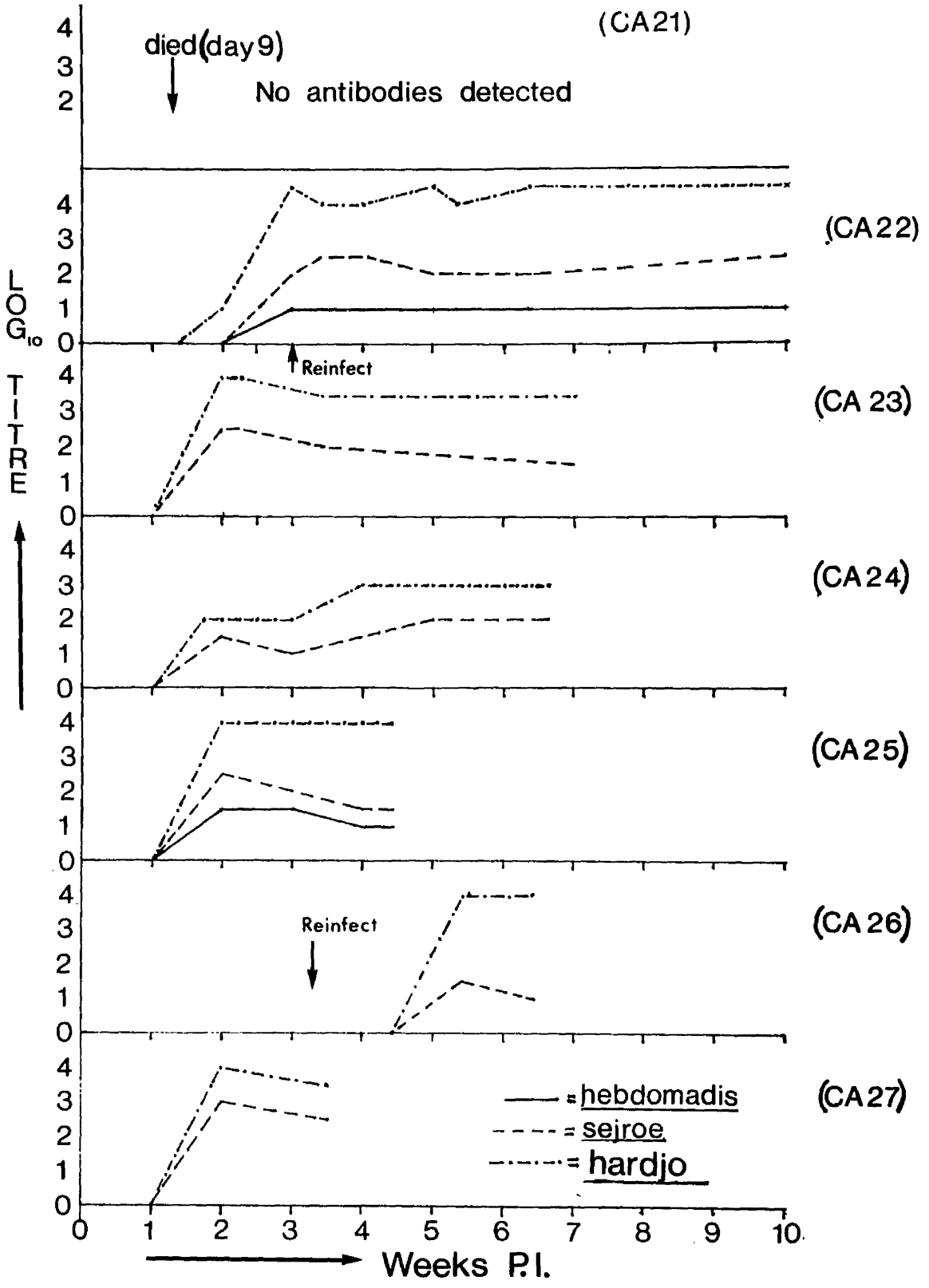


Fig. 76 The antibody response in calves experimentally infected with J.10 strain



Calves CA 23, 24, 26 and 27 did not develop detectable levels of antibody to serotype hebdomadis.