University of Glasgow | College of Medical, Veterinary & Life Sciences

Failure of Passive Transfer and Colostrum Quality in Scottish Dairy Calves.
MVM(R) thesis.

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Abstract

Bovine neonates are born agammaglobulinaemic and therefore immunologically naïve. They are dependent on the ingestion and passive transfer of immunoglobulin G (IgG) from dams' colostrum to confer sufficient immunity to disease in the first few weeks of life. Failure of passive transfer (FPT) is defined as the failure to absorb colostral antibodies sufficient to achieve a serum IgG concentration of > 10mg/mL. FPT has well defined health, welfare, and economic implications for calves. IgG can be measured directly in calf serum using radial immunodiffusion (RID) or indirectly by measuring total protein (TP), total solids (Brix) or turbidity (ZST). Colostrum quality, in terms of IgG concentration and bacterial contamination, is one of the crucial factors in achieving adequate passive transfer.

The aims of this study were to determine the prevalence of FPT in Scottish dairy calves, to compare the diagnostic test performance of direct and indirect methods for determining serum IgG concentration and determine the risk factors associated with FPT and colostrum quality.

Between February and June 2019, 392 serum samples from calves aged between 1 – 7 days were collected from 38 farms. Two mixed veterinary practices were recruited as collaborators and farms were selected prospectively from their client base in the Stirlingshire, Lanarkshire and Dumfries and Galloway regions of Scotland. Farms were selected on a convenience basis according to willingness to participate and having routine reproduction visits which enable practitioners to attend each farm on a regular basis. The IgG concentrations of the serum samples were measured directly via RID to determine the FPT prevalence and indirectly via Brix, TP, ZST and Biuret methods. The IgG concentration (via Brix), total bacteria count (TBC) and total coliform count (TCC) of 252 colostrum samples collected at the point of feeding during this time were also measured. A questionnaire detailing calf and colostrum management at farm-level was completed at enrolment, prior to sample collection. Multivariable mixed effect logistic and linear regressions were carried out to determine significant risk factors ($p<0.05$) for FPT and colostrum quality.

FPT prevalence was estimated to be 14.2% (95% confidence interval (CI)= 10.8 – 18.2) based on RID testing. Only 39.4 % (99/252, 95% CI = 33.2 – 45.6%) of colostrum samples were of acceptable quality when assessed in terms of IgG concentration and bacterial contamination thresholds.
Risk factor analysis revealed an increased time spent in a bucket prior to feeding or storing was significantly associated with a TBC ≥100,000cfu/mL (p=0.01) and a TCC ≥10,000cfu/mL (*p*=0.03). Increasing volume of colostrum administered to neonatal calves at first feed was found to be significantly associated with reduced odds of FPT (*p*=0.05).

There was fair agreement between the reference (RID) and indirect tests (kappa= 0.28 for Brix, 0.34 for TP and 0.24 for ZST). Brix, TP and ZST testing underestimated IgG concentration, resulting in an overestimation of FPT prevalence (40.5%, 29.5%, 46.3% respectively). Overall analysis of indirect testing methods compared with RID, the direct reference test revealed no perfect test exists for the diagnosis of FPT. The performance of all three indirect screening tests was improved by lowering test cut points (to 5 g/dL for TP, 8.2% for Brix and 15 units for ZST) which improved test specificity and accuracy. However, TP and Brix offer cheap, reliable calf-side diagnostic capacity. Clinicians should be mindful of the clinical context in terms of expected FPT prevalence and overall calf health on farm when interpreting FPT results at a herd level.

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Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

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There are three published papers associated with this work. Dr Katie Denholm is listed as first author in two papers because I was on a maternity leave during this period.

Denholm, K., Haggerty, A., Mason, C., Ellis, K., 2021 Comparison of tests for failure of passive transfer in neonatal calf serum using total protein refractometry and the biuret method. *Preventive Veterinary Medicine*, 189, 167-172

Haggerty, A., Mason, C., Ellis, K., Denholm, K. 2021 Risk factors for poor colostrum quality and failure of passive transfer in Scottish dairy calves. *Journal of Dairy Research*, 88(3), 337-342

Denholm, K., Haggerty, A., Mason, C., Ellis, K., 2022 Comparison of testing for failure of passive transfer in calf serum using four different testing methods. *The Veterinary Journal,* 281

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Definitions/Abbreviations

BA Plots	Bland Altman Plots
Brix	Brix Refractometer
CFU/mL	Colony Forming Units/mL
ELISA	Enzyme-linked Immunosorbent Assay
FPT	Failure of Passive Transfer
GGT	Gamma glutamyl-transferase
Ig	Immunoglobulin
IgG	Immunoglobulin G
PVS	Private Veterinary Surgeon
RID	Radial Immunodiffusion
ROC Curve	Receiver Operator Characteristic Curve
TBC	Total Bacterial Counts
TCC	Total Coliform Counts
TEC	Total E.coli Counts
TIA	Turbidimetric Immunoassay
TP	Total Protein
ZST	Zinc Sulphate Turbidity Test

1 Literature review: Failure of Passive Transfer and colostrum in bovines

1.1 The importance of colostrum to bovine neonates

Bovine neonates are born agammaglobulinaemic because of the epitheliochorial anatomy of the bovine placenta. The foetal epithelium is in contact with the maternal endometrial epithelium, Figure 1-1, thus effectively separating the foetal and maternal blood supply preventing transplacental transmission of immunoglobulins and other immune factors *in utero* (Weaver *et al.*, 2000; Peter, 2013). Subsequently, bovine neonates depend on the quick consumption of colostrum of sufficient quality and quantity to provide sufficient mass of immunoglobulins to confer immunity to disease in early life.

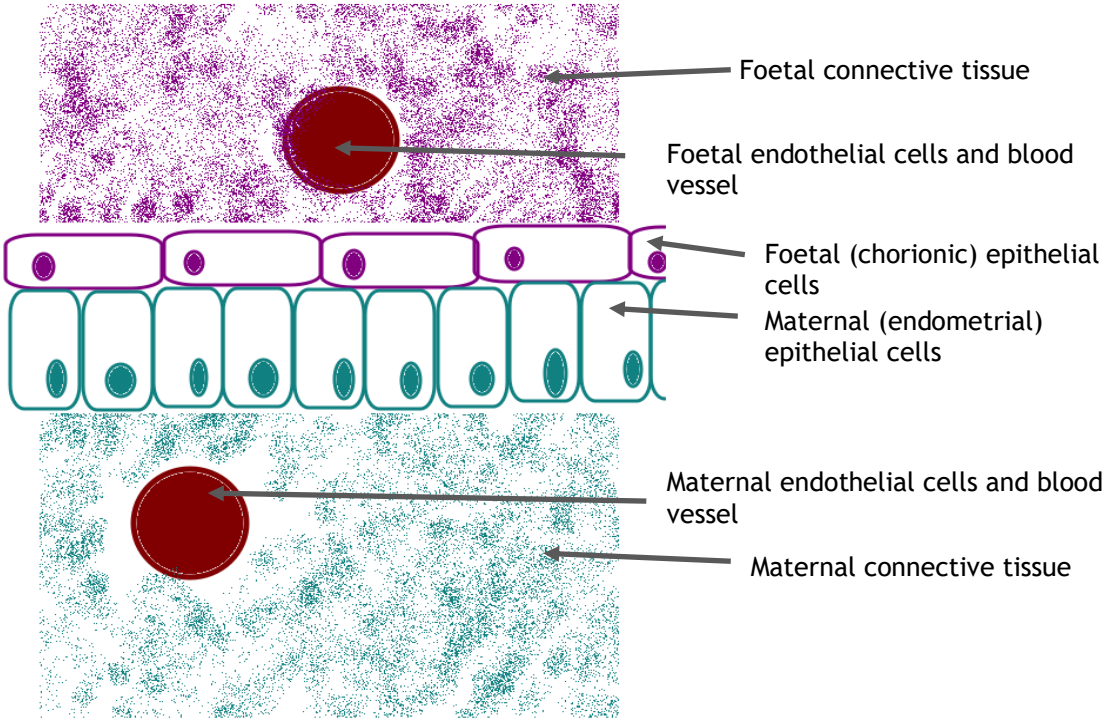


Figure 1-1 The epitheliochorial anatomy of the bovine placenta showing the contact between the foetal epithelium and maternal epithelium preventing transplacental transmission of immunoglobulins to the foetus.

During parturition the neonate leaves the sterile uterine environment and enters a highly contaminated farm environment (Barrington and Parish, 2001). A calf's immune response does not reach competence until approximately 2-3 weeks post parturition. Between 36 hours and three weeks of age, endogenous production of immunoglobulin, IgG1, is estimated to be around one gram per day (Devery *et al.*, 1979).

1.2 The mechanics of colostrogenesis in the udder and passive transfer

Colostrum is the secretion harvested from the mammary gland immediately post parturition (McGrath *et al.,* 2016). Colostrum is a mixture of lacteal secretions and constituents of maternal blood. Production starts several weeks before parturition, by a process of transcytosis, and stops around parturition (Godden *et al.,* 2019). More recent research has debated older concepts that colostrogenesis stops abruptly at parturition (Gross *et al.,* 2014; Baumrucker *et al.,* 2016; Kessler *et al.,* 2020). These three papers support the idea that transfer of IgG into colostrum continues and is potentially accelerated in the first few hours post parturition.

Colostrum contains the essential immunoglobulin G (IgG) for passive immunity as well as carbohydrates, fat, protein, leukocytes, growth factors, vitamins and minerals (Baumrucker *et al.,* 2010). These other components are essential to provide energy to the newborn and act as cofactors for enzymes in metabolic pathways (Morrill *et al.,* 2012). Of the total immunoglobulin fraction, IgG makes up around 85 - 90% with IgA and IgM making up 5% and 7% respectively (Godden *et al.,* 2019). The role of cytokines, interleukins, and maternal leukocytes are thought to revolve around immune modulation, homeostasis, induction, or amplification, of the neonate's immune response. These growth factors, maternal leukocytes and other non-nutritive factors clearly have benefits to the neonatal calf. However, research has not shown undisputable, specific benefits of these factors and is ongoing. Conducting research in this area is logistically challenging and expensive (Weaver *et al.,* 2000; Godden *et al.,* 2019).

Calves acquire colostral immunoglobulins (specifically IgG) from colostrum via the transfer of passive immunity. Transfer of passive immunity is the absorption, via pinocytosis by neonatal enterocytes, of IgG across the small intestine during

the first 24 hours after birth (Figure 1-2). Gut permeability to macromolecules, such as IgG, decreases in an increasing rate from 12 hours postpartum with gut closure occurring at approximately 24 hours postpartum (Stott *et al.*, 1979).

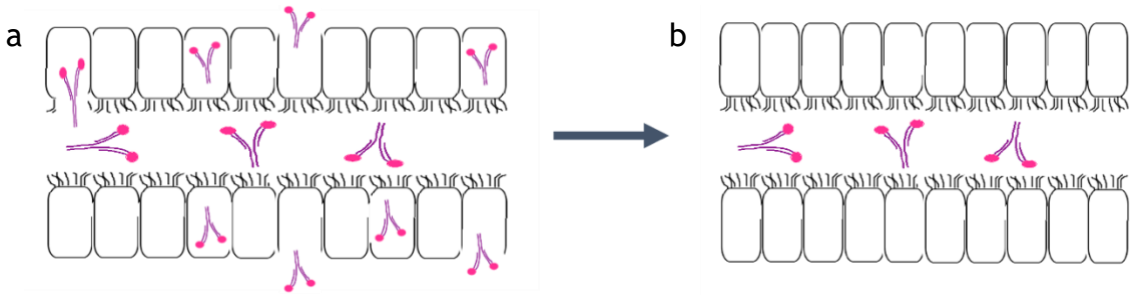


Figure 1-2 Mechanism of passive transfer a) Colostral antibodies crossing the neonatal enterocyte border in the gut via pinocytosis b) At 24 hours the enterocytes are no longer permeable to immunoglobulins therefore passive transfer cannot occur. Immunoglobulins remain in the gut lumen.

When passive transfer does not occur, the calf is said to be suffering from failure of passive transfer (FPT). It is well established and accepted across the literature that calves are defined as having FPT if the calf serum IgG concentration is less than 10 mg/mL when sampled aged between 24 hours and 7 days of age (Weaver *et al.*, 2000; McGuirk and Collins., 2004; Godden *et al.*, 2019). This cut point has been used based on the increased mortality risk below this threshold (Weaver *et al.*, 2000; Windeyer *et al.*, 2014).

1.3 Current colostrum feeding recommendations to guard against Failure of Passive Transfer

There is variation in the finer detail between different UK dairy industry bodies in terms of calf colostrum feeding recommendations but broadly speaking the recommendations are the same.

National Animal Disease Information Service (NADIS) recommends ensuring that calves receive: 'plenty of good-quality colostrum within the first 6 hours after birth' (Ohnstad, 2010). The Welfare for Farmed Animal Regulation (Department for Environment, Food and Rural Affairs) advises to ensure that each calf receives bovine colostrum as soon as possible after it is born and no more than 6 hours after birth (DEFRA, 2003). The Agriculture and Horticulture Development Board (AHDB) are more specific and recommends a volume of 3-4L of colostrum (approximately 10% of the neonatal calf's body weight) should be fed within 2 hours and no later than 6 hours of birth. This should contain >50 mg/mL

immunoglobulin with a Total Bacterial Counts (TBC) of <100,000 colony forming units (CFU)/mL (AHDB, 2018). Animal Health Ireland endorse 'colostrum 1-2-3': use colostrum from the first milking, give colostrum within two hours from the calf's birth and give at least three litres (Animal Health Ireland, 2021).

The mainstay of these recommendations come from international literature, particularly US literature, for colostrum quality and calf gut absorption efficiency. Review articles define good quality colostrum as a high immunoglobulin concentration (>50 mg/mL of IgG) and minimal bacterial contamination: TBC <100,000 CFU/mL and Total Coliform Count (TCC) < 10,000 CFU/mL (McGuirk and Collins, 2004; Godden *et al.*, 2019). The ability of the neonatal calf's gut to absorb IgG is maximal in the first six hours from parturition (Stott *et al.*, 1979). Feeding an adequate volume of colostrum and feeding colostrum promptly after birth will help safeguard against the risk of FPT (Weaver *et al.*, 2000; McGuirk and Collins, 2004; Godden *et al.*, 2019).

1.4 **Welfare and Failure of Passive Transfer**

Welfare has been traditionally defined using the five freedoms of animal welfare, Table 1-1, but welfare opinions have developed to include concepts such as 'naturalness' and a 'life worth living' (Mellor and Beausoleil, 2015). Multiple studies have linked FPT with poor welfare indicators such as increased calf morbidity and mortality and a reduction in calf growth rates (Tyler *et al.*, 1999; Faber *et al.*, 2005; Lora *et al.*, 2018).

A very recent study conducted a quantitative analysis of calf mortality in the cattle industry (dairy and beef) in Great Britain (Hyde *et al.*, 2020). Overall, on-farm calf mortality rate by 3 months was found to be 3.87%. Hyde *et al.* (2020) conclude that a relatively low mortality rate, < 2%, is both achievable and a reasonable target for neonatal calves aged 0-3 months of age. Looking specifically at the dairy sector, of the deaths that occurred before 24 months, 50% died within the first three months of life. It is likely that increased mortality in the first three months of life is linked to immune status of these calves.

Calf neonatal disease, primarily scour and pneumonia, are painful, cause distress and prevent expression of normal behaviours thus contravening the five freedoms. By improving FPT and potentially improving subsequent welfare

indicators, producers can ensure the rearing process of their calves is attaining good welfare concepts.

The Five Freedoms of Animal Welfare and their definition	
Freedom from hunger and thirst	Access to clean water and a well-balanced, appropriate diet
Freedom from discomfort	Access to a suitable environment in terms of shelter and housing
Freedom from pain, injury, and disease	Access to veterinary care when necessary
Freedom to express normal behaviour	Access to a suitable environment and exercise
Freedom from fear and distress	Appropriate handling and social grouping

Table 1-1 The Five Freedoms of Animal Welfare as formalised by the UK Farm Animal Welfare Committee.

Three studies have evaluated welfare indicators and FPT (Tyler *et al.,* 1999; Dewell *et al.,* 2006; Raboisson *et al.,* 2016). Tyler *et al.* (1999) carried out a cohort study on a population of 3,479 calves and established that 8.2% died before 16 weeks of age. The sister study established the FPT prevalence, defined as serum IgG concentration of <5.0 g/dL, in the 3,479 calves, regardless of mortality, to be 34 % (Tyler *et al.,* 1998). Tyler *et al.* (1999) established 39% of the observed mortality was attributed to inadequate passive transfer.

Dewell *et al.* (2006) studied a sample of 1,568 crossbred beef calves and found that a lower perinatal serum IgG concentration at calf level was associated with higher morbidity and mortality rates and lower average daily liveweight gains. This study, however, involves beef calves and therefore the external validity of the study in terms of the dairy calf population and any extrapolation should be considered with caution. A recent meta-analysis of the consequences of FPT including 10 studies of dairy calves from the USA, France and Canada found calves with FPT were twice as likely to die than calves without FPT between birth and 200 days of age (Relative Risk 2.12, 95% CI = 1.43 – 3.13) (Raboisson *et al.,* 2016). These three studies show poor welfare and production indicators such as high mortality rates and lower daily liveweight gains are associated with FPT and provide evidence that FPT is linked with poor welfare.

Further evidence of the link between FPT and poor welfare is provided by Furman-Fratczak *et al.* (2011). They conducted a prospective cohort study concluding morbidity and intensity of disease course were lowest in heifer calves

with serum IgG concentration exceeding 10 g/L at 30 to 60 h of life, compared with heifer calves with serum IgG concentration below 10g/L. They also concluded that heifers with serum IgG concentration >10 g/L after passive transfer showed better health status and achieved growth rate targets which allowed first insemination sooner (Furman-Fratczak *et al.,* 2011). Accepted industry growth rate is 0.7-0.9 kg/day, achieving 65% of adult bodyweight at bulling and 90% of adult bodyweight at calving (AHDB, 2021). The study design meant that calves were offered colostrum via a nipple drinker once they showed signs of standing. Consequently, the mean volume of colostrum suckled by each group was low: group 1 = 3.05% of bodyweight (SD 1.25%) through to group 4 = 4.8% of bodyweight (SD 1.09%). All study groups consumed less than 2L of colostrum at first feed. Under commercial conditions calves would have likely received a higher volume of colostrum. Palczynski *et al.* (2020) conducted questionnaire research of dairy industry stakeholders and found that most participants were aware of the volume of colostrum required to ensure adequate passive transfer. Feeding lower volumes of colostrum is a risk factor for FPT (Reschke *et al.,* 2017; Godden *et al.,* 2019). Therefore, the study design as conducted may have resulted in calves being predisposed to FPT. The definition of FPT as defined by Weaver *et al.* (2000) as < 10 g/L is used in this study and the FPT prevalence is calculated at 60 % (105/175 calves). This is much higher than comparable studies and could well be due to the low volumes of colostrum consumed. This may result in associations with higher IgG concentration at 30 to 60 hours of life and improved health status and growth appearing stronger than they actually are. Despite limitations of this study, its conclusions are reinforced by wider evidence from the literature to support the concept that ensuring adequate passive transfer leads to better welfare indicator status such as lower disease morbidity, lower mortality (<2%) and higher growth rates (> 0.7-0.9kg/day). If improving passive transfer improves welfare indicators it can be concluded overall calf welfare can be improved with ensuring adequate passive transfer.

1.5 Economics and Failure of Passive Transfer

As discussed, it is well documented in the literature that FPT improves welfare indicators such as disease morbidity and mortality. Therefore, it is intuitive that there is an economic cost to the farming enterprise associated with FPT as well

as the welfare implications. The most robust endorsement of the economic impact of FPT is an assessment by Raboisson *et al.* (2016). They calculated the total mean cost to be €60 per dairy calf with FPT. In a further article, Raboisson *et al.* (2018) investigated the profitability of colostrum management. They concluded farmers should spend 15 mins/calf on colostrum management as a minimum unless labour costs were high. Prior to these two studies there were limited data assessing the economic and profitability of managing FPT. Two older studies, Faber *et al.* (2005) and DeNise *et al.* (1989), are frequently cited to demonstrate the economic impact of FPT however their limitations are discussed below.

Faber *et al.* (2005) concluded that heifer calves fed four litres of high-quality colostrum within the first hour of life had lower veterinary costs, quantified as US $15 per calf, from birth until first calving compared with calves fed two litres of high-quality colostrum. The calves had greater average daily liveweight gain, and they produced an average of 1 kg more milk per day across two lactations compared with cohorts fed two litres of colostrum. Volume of colostrum fed at first feed is a known risk factor for FPT (Godden *et al.*, 2009). However, this study did not quantify FPT within the two study groups, only included 68 Brown Swiss heifers, measured colostrum quality with a colostrometer and feeding and rearing practices included feeding colostrum until 14 days. As discussed below (Section 1.9 Evaluating colostrum IgG concentration), the colostrometer has several limitations for reliably measuring colostrum quality, including fragility and temperature sensitivity (Bartens *et al.*, 2016; Buczinski and Vandeweerd., 2016). Commercial rearing practices in the UK do not usually include feeding colostrum to 14 days of age. Therefore, the external validity of this study is limited however the concept of economic impact of FPT is likely to hold true.

Inefficiencies in production due to FPT were investigated by DeNise *et al.* (1989). They found that cows in the calf group with the lowest IgG concentration were culled extensively for low production (undefined) and concluded that colostrum immunoglobulins acquired at birth may be an indicator of subsequent growth and production (DeNise *et al.* 1989). They correctly acknowledge that it is impossible to say from their data whether IgG concentration at birth is directly or indirectly linked to these factors. The cut point in the study for low serum IgG was 12mg/mL, which is higher than the more commonly used cut point of 10

mg/mL in the international literature. This cut-point was used as the DeNise *et al.* (1989) study was a continuation from a study by Robison et al (1988) in which the cut point 12mg/mL was used. Furthermore, DeNise *et al.* (1989) was published in 1989, and the dairy industry has changed dramatically in terms of intensification, genetics, calf management and labour in this time meaning that this study is likely superseded by Raboisson *et al.* (2016). However, both studies make similar conclusion about the economic impact of FPT on the farming enterprise. When evaluating the literature, it can be concluded that FPT has an economic impact on farming enterprise. What that impact is will depend on the system and extent of the problem.

1.6 Evaluating serum IgG concentration

To minimise on farm FPT prevalence and associated consequences it is necessary to correctly screen the calf population for the condition. This is achieved by measuring calf serum IgG concentrations either through direct or indirect methods. Direct measures directly measure serum IgG concentrations whereas indirect measures estimate serum IgG concentration by proxy from another measure. Methods that directly measure serum IgG concentration include Radial Immunodiffusion testing (RID) and Enzyme Linked Immunosorbent Assays (ELISA) testing. Several indirect methods are available to estimate concentrations of IgG. Examples are measuring serum total protein (TP) concentration using an optical refractometer or via the biuret method or measuring total solids using brix refractometry (Brix), the zinc sulphate turbidity test (ZST) and GGT. Indirect methods such as TP and Brix, are more widely used on a day-to-day basis by veterinary practitioners as a screening test because they are rapid, cost efficient and many can be performed under field settings (Weaver *et al.,* 2000; Elsohaby *et al.,* 2019).

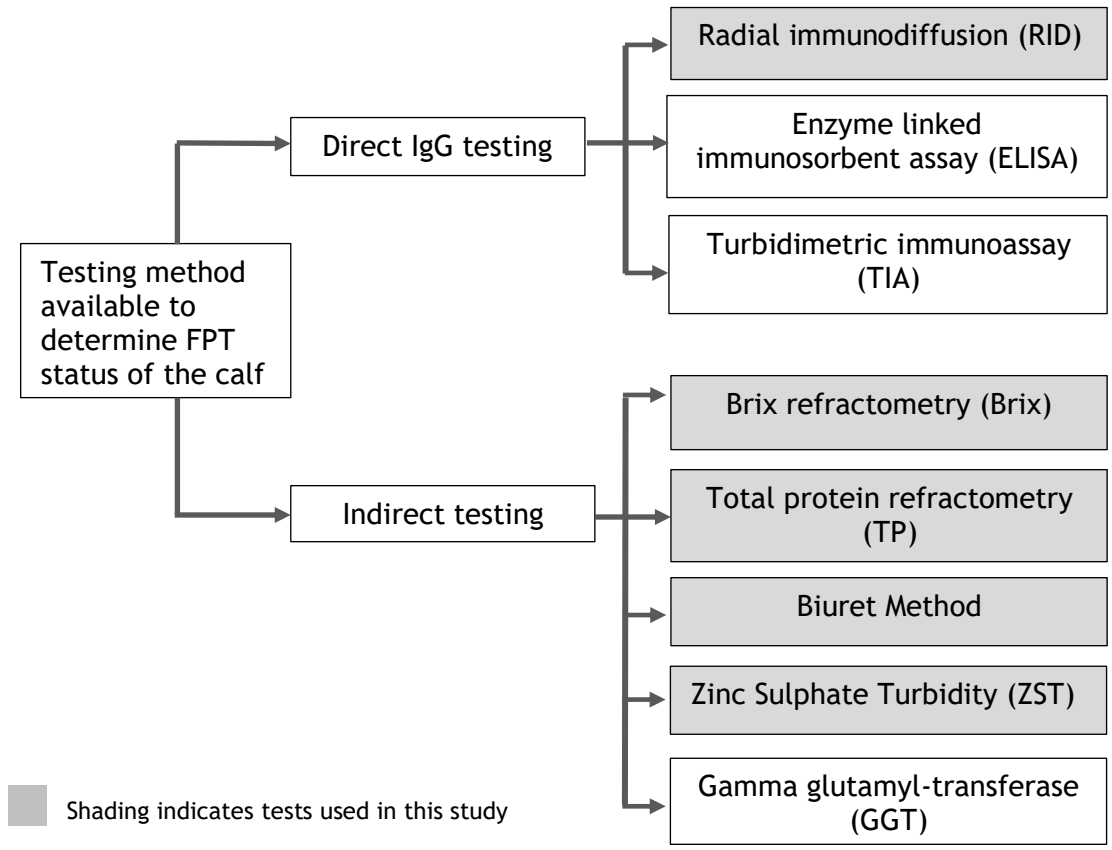


Figure 1-3 Branch diagram showing the testing strategies available to determine FPT status of the calf.

1.6.1 Evaluating Diagnostic Test Performance

When considering which test to use and how to use it is important to consider how the test performs, including sensitivity, specificity, negative predictive value and positive predictive value and accuracy. A clinician needs to understand if the test is being used in a screening capacity or a diagnostic capacity. Table 1-2 and Figure 1-4 reviews the definitions of these terms. These tables refer to the presence and absence of 'disease' because the broader concepts of test performance are being discussed. It is acknowledged that FPT is not a disease in the traditional definition of the word. The term 'disease' or 'no disease' is used to describe the presence or absence of FPT. Precedence has been set by previous peer reviewed literature to use the term 'disease' in this way (Banoo *et al.*, 2010, Baratloo *et al.*, 2015, Trevethan, 2017). Very often the purpose of screening or diagnostic tests are in detect the infection which subsequently leads to the development of pathology or disease.

Term	Definition	Reference (Year)
Diagnostic test	Provide definitive information regarding the presence or absence of disease	Trevethan. (2017)
Screening test	Detect the likelihood of the presence of disease where symptoms may or may not be present	Trevethan. (2017)
Sensitivity	The probability of correctly identifying, solely from among those who are known to have the disease, all those who do have the disease. True positives.	Trevethan. (2017)
Specificity	The probability of correctly identifying, solely from among those who are known not to have the disease, all those who do not have the disease. True negatives	Trevethan. (2017)
Negative Predictive Value	The probability that a negative result accurately indicates the absence of disease	Banoo *et al.* (2006)
Positive Predictive Value	The probability that a positive result accurately indicates the presence of disease	Banoo *et al.* (2006)
Prevalence	The proportion of a population with the disease at a given time	Banoo *et al.* (2006)
Accuracy	Ability of a test to differentiate healthy and diseased animals.	Baratloo *et al.* (2015)

Table 1-2 The definitions pertaining to describing a diagnostic or screening test's performance in a population.

		Gold Standard (Actual)		
	Total Population (= P + N)	Positive (PP)	Negative (PN)	
Test (Predicted)	Positive (P)	True positive (TP)	False positives (FP)	Sensitivity (= TP/P)
	Negative (N)	False negatives (FN)	True negatives (TN)	Specificity (= TN/N)
	Prevalence (= P/(P+N))	Positive Predicative Value (PPV = TP/PP)	Negative Predictive Value (NPV = TN/PN)	

Figure 1-4 Contingency Table describing the relationship between results obtained from gold standard testing method vs alternative testing methods and the prevalence, sensitivity, specificity, positive predictive value and negative predictive value.

A gold standard diagnostic test infers that the results provide definite and undisputable evidence as to the presence or absence of disease (Trevethan.,

2017). The performances of other diagnostic tests are compared to these gold standards. However, not all gold standard tests are perfect in terms of 100% sensitivity and specificity. They may only be the best estimate of a true results. Because of the concern regarding the validity of the 'gold standard' it is more accurate to refer to these tests as the reference test. The reference test for serum IgG concentration is the direct measure RID. When evaluating test performance clinicians must appreciate that the gold standard test in which indirect test are compared to may, in actual fact, be better described as a reference test.

Test performance can be further explored through Bland Altman Plots (BA Plots), Receiver Operator Characteristic Curves (ROC Curves), Youden's Index and Cohen's Kappa Statistic. These statistical methods of test performance analysis are defined in Table 1-3.

Statistical Method of Analysis	Purpose and Definition	Reference
Bland Altman Plot	Explore agreement between two methods by plotting the difference between the two outcomes determined by each method against their mean. Whether the limits of agreement are acceptable or not is a clinical decision. A BA plot will not only show the agreement between the two testing methods but any fixed or proportional bias present fixed bias is assessed by the mean difference between the two test methods. Proportional bias, that is bias that occurs dependent on the value of the measurement is visually assessed by the scatter of the observation points.	Altman and Bland. (1983)
Receiver Operator Characteristic Curve	Graphical representation of the sensitivity vs 1-specificity (probability of incorrect classification) with the area under the curve representing the diagnostic ability to differentiate between diseased and non-diseased (1 = perfect. 0.5 = chance)	Perkins and Schisterman. (2006)
Youden's Index	Summary statistic from ROC Curves that defines the maximum potential effectiveness of a biomarker. It enables the optimal cut point at which both sensitivity and specificity is maximised (Se + Sp -1)	Fluss *et al.* (2005)
Cohen's Kappa Statistic	Is a measure of interrater reliability. It ranges from 0 to 1. Where zero represents agreement from random choice and one represents perfect agreement.	McHugh. (2012)

Table 1-3 Description of statistical methods to compare the diagnostic performance of a test against a reference test.

1.6.2 **Radial immunodiffusion testing**

Radial immunodiffusion testing is considered the gold standard technique for the measurement of serum IgG in a diagnostic laboratory and research setting because it is a true and direct measure of IgG (Bielmann *et al.*, 2010). A gold standard test is a diagnostic test best available to unequivocally give the true diagnosis (Kumar, 2016). Elsohaby *et al.* (2019) estimated the sensitivity of RID to be 0.96 and the specificity to be 0.93. There are several RID test kits available commercially of which some have not been validated. These unvalidated tests may more accurately be described as a 'reference test' as in some cases the results may not adequately mirror the truth. RID works by specific antiserum being mixed uniformly in agar gel. The specimen to be tested is added to the well and forms an antibody/antigen complex that diffuses radially forming a concentric precipitin ring Figure 1-5.

Figure 1-5 An example of a radial immunodiffusion plate (Triple J Agar Plates, Bovine IgG RID Kit, Triple J Farms, Bellingham, WA) after incubation showing the concentric precipitin ring formed by diffusion of the antibody/antigen complex as indicated by black arrow.

The precipitin ring is physically measured to give a quantitative result (Fahey and McKelvey, 1965). RID is a laboratory procedure that is expensive; the commercial diagnostic laboratory, Biobest Laboratories, Edinburgh, charges the client around £32/sample. RID is also time consuming and requires skilled

technicians to perform and measure the zones of precipitation accurately; therefore, it is of limited practical application under field settings (Weaver *et al.,* 2000; Deelen *et al.,* 2014; Elsohaby *et al.,* 2019). One of the criticisms of the RID test is that they are time consuming to conduct. It would be an obvious advantage to be able to take accurate measurements earlier to reduce time (from 40 hours to 24 hours) and costs associated.

1.6.3 **Enzyme Linked Immunosorbent Assay testing**

ELISA testing offers advantages over RID in terms of cost, time and the capacity for running numerous samples at once (Lee *et al.,* 2008). The method involves antigens to the desired detected antibody stuck to a plastic surface, the test sample added, and any antibodies binding to the antigen. A second antibody and marker are added which binds to any antibody/antigen complex. A substrate is added that causes a colour change with the marker; this colour change represents a positive results (Gelsinger *et al.,* 2015). ELISA testing is not available commercially in the UK.

Lee *et al.* (2008) found the sensitivity to be 0.98 (95% CI = 87-100%) and the specificity to be 0.91 (95% CI = 82- 96%) at cut point 10 g/L. They also found there to be good agreement (94%) and good diagnostic performance when Bland Altman plots were used to compare ELISA testing with RID testing. Furthermore, Dunn *et al.* (2018) used Lin's concordance correlation and found strong agreement between RID and ELISA method, $R^2 = 0.97$; $p < 0.001$. Gelsinger *et al.* (2015) look at the correlation only between RID and ELISA and found a weaker relationship, $r = 0.59$; $p < 0.01$. Correlations only shows if a relationship exists or not and indicates the strength of this relationship. It does not show agreement. Agreement and bias are tested by statistical tests such as Bland-Altmann as carried out by Lee *et al.,* (2008). Overall, the literature appears to show the ELISA test has good correlation and agreement with RID.

1.6.4 **Turbidimetric Immunoassay**

Turbidimetric immunoassay (TIA) measures IgG directly in the serum by using antibodies directed against a specific antigen (i.e. bovine IgG) to form immunocomplexes. A quantitative result is obtained by measuring the turbidity of a sample using a calibrated spectrophotometer (Ferris and McCue, 2009). TIA

can be carried out in a laboratory setting but also as a cow-side, portable analyser (Alley *et al.*, 2012)

1.6.5 **Serum Total Protein**

Serum from a clotted blood sample consists of protein which comprises of albumin and globulin. The globulin component consists of immunoglobulin and non-immune globulin (Hogan *et al.* 2015). The serum total protein (STP) concentration of blood samples from calves can be suggestive of immunoglobulin concentrations because most of the protein content is made up of globulins and of that, the majority is immunoglobulin. Hogan *et al.* (2015) suggest the non-immunoglobulin component of the globulin concentration is about 1-1.5g/dL.

Ingestion and absorption of colostral immunoglobulins will raise STP levels in the blood (Hogan *et al.* 2015). It is well established in the literature that in calves aged between 1 and 7 days, STP is a good estimate of serum immunoglobulin concentration (Devery *et al.*, 1979; Hancock, 1985). At this age, endogenous IgG production is minimal; protein concentration and therefore IgG concentration can be attributable to passive transfer of immunity from colostrum (Weaver *et al.* 2000). Serum protein concentrations are preferred to plasma protein concentrations as the latter gives a poorer correlation with immunoglobulin concentrations when compared with a RID reference test (Hogan *et al.*, 2015; Elsohaby *et al.*, 2019).

Measuring STP has limitations. STP cannot be used in dehydrated or clinically sick animals due to the variations in protein concentrations attributable to the disease process which can lead to misclassification of FPT (Tyler *et al.*, 1999). Hajimohammadi et al. (2013) found a significant increase in acute phase proteins in calves clinically suffering from diarrhoea compared with healthy calves. Albumin concentrations have been reported to be variable in calves suffering from clinical disease (Thornton *et al.*, 1972, Hogan *et al.*, 2015).

1.6.5.1 Biuret method

The Biuret method is the reference test for measuring serum or plasma total proteins (Doumas *et al.*, 1981). The method is based on the formation of copper chelates when the cupric ions (Cu^{2+}) react with the peptide linkages of the

353 protein in a basic solution (Katsoulos *et al.,* 2017). The colour change, from blue
354 to purple, in a biuret test is proportional to the total protein concentration of
355 the solution. Test performance is described in Table 1-4

	Optimal Cut Point (g/dL)	Sensitivity	Specificity	AUROC	Kappa (K)	Correlation (r)	Ref Test
Biuret Method							
Cuttance *et al.*, (2017)	5.2	0.92	0.97	0.99	N/R	N/R	TIA
Zakian *et al.*, (2018)	5.3	0.95	0.89	0.96	0.65	0.93	ELISA

356 **Table 1-4 The literature evaluating the optimal cut point of TP as measured by Biuret**
357 **method in terms of sensitivity, specificity, AUROC, correlation (r) and Kappa in comparison**
358 **to the reference test (RID, ELISA or TIA).**

359 ### 1.6.5.2 Serum total protein refractometry

360 On farm, total protein refractometry (TP) provides an inexpensive, rapid and
361 accurate alternative for the Biuret method (Katsoulos *et al.,* 2017; Zakian *et al.,*
362 2018). A TP refractometer measures the amount of light refracted by the total
363 proteins in the serum sample (Wallace *et al.,* 2006).

364 Table 1-5 summarises the literature pertaining to TP refractometry test
365 performance as a proxy for IgG when compared to a direct reference test (RID,
366 ELISA or TIA). Generally speaking, the literature points to the optimum cut point
367 lying somewhere between 5.0 g/dl -5.8 g/dl. Chigerwe et al. (2015) conclude
368 greater than 5·8–6·3 g/dl as a cut point to indicate adequate passive immunity
369 which is higher than other studies. Ultimately, any cut point chosen should
370 reflect the goal of the monitoring programme and the intervention taken with
371 any FPT calves.

	Optimal Cut Point (g/dL)	Sensitivity	Specificity	AUROC	Kappa (K)	Correlation (r)	Ref Test
Optical TP Refractometer							
Elsohaby *et al.*, (2015)	5.5	0.8	0.81	0.88	N/R[1]	0.74	RID
Hernandez *et al.*,(2016)	5.3	1.00	80.4	0.95	N/R	0.82	RID, TIA[2]
Lee *et al.*, (2008)	5.8	0.83	0.73	N/R	0.78	N/R	RID
Hogan *et al.*, (2015)	5.2	1.00	0.56	N/R	0.59	N/R	ELISA
Calloway *et al.*, (2002)	5.0	0.8	0.91	N/R	N/R	N/R	RID
Digital TP Refractometer							
Zakian *et al.*, (2018)	5.2	1.00	0.96	0.99	0.87	0.95	ELISA

Table 1-5 The literature evaluating the optimal cut point of TP refractometry (optical and digital) in terms of sensitivity, specificity, AUROC, correlation (r) and Kappa in comparison to the reference test (RID, ELISA or TIA).

1.6.6 Brix refractometry

Figure 1-6 A Brix refractometer.

[1] N/R = Not recorded

[2] TIA = Turbidimetric Immuno Assay

The Brix refractometer measures the sucrose content in high sucrose liquids such as fruit juices, wine, and molasses. One percent Brix is the equivalent to the refractive index of a solution of 1g sucrose in 100g solution (Cuttance *et al.*, 2017). When used in non-sucrose containing liquids it approximates the total solid percentage of which, in calf serum, the majority are protein immunoglobulins (Quigley *et al.*, 2013). Table 1-6 summarizes the literature regarding test performance when compared to a reference test.

	Optimal Cut Point (%)	Sensitivity	Specificity	AUROC	Kappa (K)	Correlation (r)	Reference Test
Optical Brix Refractometer							
Thornhill et al., (2015)	10	1.00	N/R[3]	N/R	N/R	N/R	RID
Deelen et al., (2014)	8.4	0.89	0.89	N/R	N/R	0.93	RID
Digital Brix Refractometer							
Hernandez et al., (2016)	8.5	1.00	0.89	0.96	N/R	0.79	RID, TIA[4]
Zakian et al., (2018)	7.8	1.00	1.00	1	1	0.98	ELISA
Elsohaby et al., (2015)	8.3	0.86	0.83	0.83	0.89	0.79	RID
Cuttance et al.,(2017)	8.8	0.98	0.94	N/R	N/R	0.92	TIA

Table 1-6 The literature evaluating the optimal cut point of Brix refractometry (optical and digital) in terms of sensitivity, specificity, AUROC, Kappa and correlation (r) in comparison to the reference test (RID, ELISA or TIA).

Interestingly, the meta-analysis by Buczinski *et al.* (2018) could not draw any summary with respect to cut point sensitivity or specificity of Brix because of the low number of studies that used the same cut point. This study went on to compare the accuracy of TP refractometry and Brix refractometry as tools for assessment of FPT and went on to conclude there was no definitive accuracy of one refractor scale over another because they only identified three studies that evaluated both refractor scales. Both techniques, as previously stated, are cheap, easy and quick to carry out and remain a mainstay of testing protocols in commercial clinical veterinary practice (Weaver *et al.*, 2000; Hernandez *et al.*, 2016).

[3] N/R = Not Recorded

[4] TIA = Turbimetric Immunoassay

1.6.7 **Zinc sulphate turbidity testing (ZST)**

Zinc Sulphate Turbidity (ZST) test was first described by McEwan *et al.* (1970). The test works by measuring the density of the precipitate, caused by the precipitation of the metal salt and globulins, using a colorimeter, which is proportional to the IgG content. The test has a good sensitivity (100%) but poor specificity at around 52 - 67% (Tyler *et al.,* 1996; Zakian *et al.,* 2018) meaning that there is the potential for a high number of false positives i.e. calves being falsely diagnosed as having FPT when their serum immunoglobulin concentration is adequate. Hogan *et al.* (2016) found several factors to affect the results: haemolysis of the samples, time and ambient temperature of the reaction and carbon dioxide acting on the Zinc Sulphate solution as well as the wavelength of light used to read the turbidity (Hogan *et al.* 2016). It was also concluded that increasing the concentration of the zinc sulphate solution used from 250 to 350 mg/l improved the specificity without decreasing sensitivity. This, in combination with lowering the cut point to 12.5 ZST units instead of 20 ZST units reduced misclassification of calves and improved specificity (0.95) with minimal effect on sensitivity (0.96) (Hogan *et al.,* 2016).

	Optimal Cut Point	Sensitivity	Specificity	AUROC	Kappa (K)	Reference Test
ZST						
Hogan *et al.* (2015)	11 units	0.81	0.83	N/R[5]	0.66	ELISA
Todd *et al.* (2018)	12-19 units	N/R	N/R	N/R	N/R	ELISA
Zakian *et al.* (2018)	14.6	100	67.1	N/R	0.34	ELISA

Table 1-7 The literature evaluating the optimal cut point of ZST in terms of sensitivity, specificity, AUROC, Kappa and correlation (r) in comparison to the reference test (ELISA)

1.6.8 **Gamma glutamyl-transferase (GGT)**

Gamma glutamyl-transferase (GGT) is an enzyme that is excreted by the ductal epithelial cells in the mammary gland and therefore the concentration in colostrum is extremely high. Because the pinocytosis of IgG in the neonatal gut is non-selective (see Section 1.2), other macromolecules such as GGT are absorbed alongside IgG. Thompson *et al.* (2011) reported serum GGT activity > 60 times higher in calves that had consumed colostrum when compared with calves that had not consumed colostrum. When compared with the correlation

[5] N/R = Not Recorded

of TP and IgG, GGT decreases rapidly in the first week of life therefore the cut points used to define FPT need to be age-related (Cuttance *et al.,* 2019). Parish *et al.* (1997) developed a series of cut points based on age for diagnosis for FPT. This study only found a moderate association between GGT and IgG concentrations - r = 0.63 for calves up to 10 days of age and r = 0.54 for calves up to 17 days of age. Table 1-8 summarises comparisons of GGT test performance with direct reference test. Alongside the limitations of this indirect method of estimating IgG, GGT is not readily available as a commercial test for FPT diagnosis in Scotland. Therefore, GGT as an indirect test is not discussed further and not used in comparisons in this thesis.

Reference	Optimal Cut Point	Sensitivity	Specificity	AUROC	Kappa (K)	Reference Test
Perino *et al.* (1993)	200 IU/L (24 hours old)	0.80	0.97	N/R[6]	0.72	ELISA
Fecteau *et al.* (2013)[7]	179 IU/L (1-13 days of age)	0.97	0.27	N/R	N/R	RID
Hogan *et al.* (2015)	100 IU/L (<4days of age)	0.97	0.98	N/R	0.96	ELISA
Zakian *et al.* (2018)	815 IU/L (48 hours old)	0.75	0.95	0.90	0.67	ELISA

Table 1-8 The literature evaluating the performance of GGT in terms of sensitivity, specificity, AUROC, Kappa and correlation (r) in comparison to the reference test (RID and ELISA)

1.6.9 **Summary of serum IgG Testing**

There are various testing methods available to measure serum IgG to diagnose FPT. Direct testing methods are usually reserved for research due to cost, time, and laboratory expertise. Whereas indirect methods have practical applications in the field. Each testing strategy has its own benefits and drawbacks. It is apparent from the literature that there is little agreement in the amongst authors as to the precise optimal cut points especially with regards to Brix. This leads to the potential for considerable debate about how and when the testing strategies are use.

[6] N/R = Not Recorded

[7] This study used ill calves, as the purpose of the study was to evaluate assays in ill calves.

1.7 On farm monitoring of Failure of Passive Transfer

The current body of literature demonstrates it is pertinent for modern dairy producers to be actively evaluating their colostrum management program through routine testing for FPT within their calf population. (Godden *et al.*, 2019). What is deemed 'routine testing' will be farm specific however factors such as herd size, calving pattern, staffing, calf disease morbidity, calf mortality and FPT prevalence will all play a part.

McGuirk and Collins (2004) recommend a minimum sample size of 12 calves to achieve adequate confidence in the interpretation of test result and FPT prevalence at herd level. Although this review article was peer reviewed, a clear explanation as to how this number is arrived at is not given. Small sample sizes will lead to imprecise FPT prevalence estimates. However, in reality the number of serum samples that can be collected on a farm may be limited by the number of calves which fall into the appropriate age category especially in an all-year-round calving system. Hence, the regularity of monitoring is also important. Cuttance *et al.,* (2019) reviewed the sample size required to have confidence in the precision of the result at herd level yet be cost and time effective to sample. The sample size needed for precise herd level prevalence estimates depends on test sensitivity, specificity, accuracy, expected prevalence and number of animals in the at-risk population. They summarize that if only 12 calves are sampled there can only be 95% certainty that the herd prevalence lies < 20% if no calves test positive for FPT. If testing is carried out in a limited, *ad hoc* manner, it is difficult for the clinician to determine if any increased disease incidence is indeed due to inadequate immunity, or an increase in infection challenge because FPT prevalence estimates may be unreliable. Therefore, in a small herd, test results should be accumulated over time to ensure the precision of the FPT prevalence estimates. It is evident that practitioners should consider carefully whether enough samples have been taken to draw conclusions on what is happening at herd level with respect to FPT.

Calves from 24 hours of age to 7 days are considered eligible for FPT testing. Hancock (1985) concluded FPT could be accurately assessed by sampling calves in the first two weeks of life. In practice, it is acceptable to sample calves within the first seven days of life to determine FPT status because at this stage the protein content of the serum is largely, approximately 90%, exogenous IgG

from colostrum opposed to from endogenous production (Weaver *et al.,* 2000). For the indirect measures of Brix and TP refractometry calves should be clinically healthy and not dehydrated as previously discussed disease processes can alter the total protein and solids concentration of a serum sample. Consideration should also be given to the representativeness of a sample. A sample should be non-biased and based on a random sampling methodology of eligible calves. In an on-farm, clinical context, this usually entails sampling all eligible calves at a routine farm visit. Then repeating the sampling process at the next routine visit and so on, to build a bank of on-farm data. This furthers the knowledge regarding FPT prevalence on that farm. This is not random, as it is dictated by the day of the farm routine visit. However, it is pragmatic cost and time-effective compromise for both veterinarian and farmer. In a research context, a more formal randomisation process for enrolment of calves leads to a more robust methodology.

Determination of the test cut point depends on the planned application of test results, the prevalence of passive transfer in the study population, and the costs associated with false-positives (Tyler *et al*. 1996; Cuttance *et al*. 2019). Prevalence is defined as the number of new and ongoing cases at one point in time. Prevalence will be higher if the 'disease' (e.g. FPT) has a longer duration. The sensitivity and specificity of any testing method depends on the cut point used. A higher sensitivity in a test may be desirable when considering a high value animal, in which intervention such as plasma transfusion may be considered (Hogan *et al*. 2015). As a herd health screening tool, it would also be desirable to maximise sensitivity to ensure all calves with FPT are detected (true positives). Reduced specificity may be tolerable because the consequences of misdiagnosing calves with FPT would only lead to improvements in colostrum management to maximise serum IgG concentration. However, if the test is to be used as a monitoring tool, when management practices have been implemented to minimise FPT, a lower specificity would mean animals would be misclassified as having FPT and the progress in improving FPT on farm may not be clear through testing.

The proportion of calves falling below the acceptable cut point of serum IgG concentration determines the adequacy of on farm colostrum management. It is suggested an interference level of 20% is set as regards to concerns of FPT being

a problem within the herd (McGuirk and Collins, 2004). More recently it has been proposed that a successful colostrum management programme could deliver 90% of calves with a serum total protein of > 5.0 – 5.2 g/dL. Traditional FPT cut offs are based on mortality risk and do not take into account incremental lower morbidity seen at incremental higher serum IgG values (Godden *et al.*, 2019). As a result, a group of researchers and calf expert from the US and Canada proposed the herd level evaluation described in Table 1-9, which takes into account reduce morbidity associated with higher serum IgG concentrations.

Category	IgG (g/L)	Equivalent STP (g/dL)	Equivalent Brix (%)	Percentage of calves in each category (%)
Excellent	>25.0	>6.2	>9.4	>40
Good	18.0 – 24.9	5.8-6.1	8.9 – 9.3	~30
Fair	10.0 – 17.9	5.1-5.7	8.1 – 8.8	~20
Poor	<10.0	<5.1	<8.2	<10

Table 1-9 For herd level evaluation of FPT Godden *et al.* (2019) recommend the summarising results into the above categories: excellent, good, fair and poor. The IgG concentration along with equivalent STP and Brix measurements for each category is shown as well as the percentage of calves recommended in each category.

1.8 International and UK prevalence of Failure of Passive Transfer

Table 1-10 summarises the FPT prevalence studies in the literature including their country of origin, determined prevalence for the study sample and the method for testing serum IgG concentration. Worldwide prevalence estimates range from 15.6% in the US to 41.9% in Australia (Beam *et al.*, 2009; Abuelo *et al.*, 2019). Countries represented in the prevalence studies include the United States of America, New Zealand, Australia, United Kingdom, Norway, Canada, and the Czech Republic. The studies use both indirect and direct measurements of IgG to determine prevalence. Generally, when indirect testing strategies are used, IgG will be underestimated therefore FPT will be overestimated. Five of the nine prevalence studies conducted in the last ten years used indirect testing methods. Numbers of calves involved in the individual studies ranged from 156 – 3819 calves aged from 1 – 8 days of age. Data are lacking from the major dairy producing nations of the world such as Brazil, India, and China.

Reference (Year of Publication)	Study Details	Method	FPT Prevalence	Comment
Abuelo *et al.* (2019)	253 serum samples from calves aged 1 – 7 days from 23 farm across **Australia**	Radial Immunodiffusion (Direct) Cut Point:10g/L	41.9 %	Reference method used to determine IgG concentration
Beam *et al.*, (2009)	1816 serum samples from calves aged 1 – 7 days from 394 dairy farms across 17 states in the **US** milking >30 cows	Radial Immunodiffusion (Direct) Cut Point: 10g/L	19.2%	Reference method used to determine IgG concentration
Cuttance *et al.* (2017)	3819 serum samples from calves aged 1 – 8 days from 107 dairy farms from 9 regions across **New Zealand**	Biuret Method (Indirect) Cut Point: 52g/L	33 %	Method used has lower specificity therefore false positives are likely
Johnsen *et al.* (2019)	156 serum samples from calves aged 24-48 hours from 20 farms in **Norway**	Radial Immunodiffusion (Direct) Cut Point: 10g/L	30.8 %	Reference method used to determine IgG concentration
Lawrence *et al.* (2017)	230 serum samples from calves aged less than 7 days from 11 farms in the Manawatu region, **New Zealand**	Total Protein refractometry (indirect) Cut Point: ≤ 50g/L	24.8%	Method used has lower specificity therefore false positives are likely
MacFarlane *et al.* (2015)	444 serum samples from calves aged 1 - 7 days from 7 dairy farms in Cheshire and Wirral, **UK**	Total Protein refractometry (Indirect) Cut Point: 5.6 g/dL	26 %	Method used has lower specificity therefore false positives are likely
Stanislav *et al.* (2019)	1175 serum samples from calves aged 1-6 days from 33 dairy farms in the **Czech Republic**	Radial Immunodiffusion (Direct) Cut Point 10g/L	34.6%	Reference method used to determine IgG concentration
Trotz-Williams, *et al.* (2008)	422 serum samples from calves aged 1 – 7 days 119 farms in Southern Ontario, **Canada**	Total Protein refractometry (Indirect) Cut Point: 5.2 g/dL	37.1 %	Method used has lower specificity therefore false positives are likely
Urie *et al.* (2018)	2,498 serum samples from calves aged 1 – 7 days 104 farms across 13 states in the **US**	Radial Immunodiffusion (Direct) Cut Point: 10g/L	15.6%	Reference method used to determine IgG concentration
Vogels *et al.* (2013)	1018 serum samples from calves aged 1- 7 days from 100 farms in SW Victoria, **Australia**.	Total Protein refractometry (Indirect) Cut Point: 5.0 g/dL	38 %	Method used has lower specificity therefore false positives are likely

542 **Table 1-10 Summary of studies from a literature search detailing FPT prevalence worldwide**

1.9 Evaluating colostrum IgG concentration

Thorough investigations into FPT problems on farm should include an evaluation of the colostrum quality available. Good quality colostrum, in terms of IgG content, is defined in the literature as having greater than 50 g/L IgG content (Weaver *et al.*, 2000; Godden *et al.*, 2019). Colostral IgG concentrations can be measured directly or indirectly. Direct measurements, as with serum IgG, involve RID testing and similar limitations for this technique apply (Buczinski *et al.*, 2016). Indirect measures can be carried out on farm using a colostrometer or by Brix refractometry. Colostrum colour and consistency have been reported as an objective technique for colostrum quality assessment. However a lack of accuracy has been reported with this technique and a low sensitivity and specificity of 50% (Gross et al., 2014). This study reported no significant relationship between colour and IgG concentration (r = 0.0061, p=0.41) therefore this technique cannot be relied upon.

1.9.1 Colostrometer

Figure 1-7 A Colostrometer (hydrometer). The colour scale green – red gives the user an indication of good or poorer quality colostrum

A colostrometer is a hydrometer, which gives an estimation of immunoglobulin concentration by measuring specific gravity (Bartier *et al.*, 2015). However, there are several limitations of the colostrometer such as the effect of sample temperature on accuracy as well as fragility of the colostrometer meaning it is

an inferior indirect measure of colostrum quality when compared with Brix refractometry and direct measure RID testing (Bartens *et al.,* 2016; Buczinski and Vandeweerd, 2016).

Morin *et al.* (2001) conducted a study on a dairy herd in the USA where 1085 colostrum samples were tested from 608 cows. They found that breed, lactation number, and month and year of calving all influenced specific gravity as measured by the colostrometer. A further problem with the use of the hydrometer is that low and high immunoglobulin concentration colostrum samples have specific gravity distributions that overlap therefore there is a risk that colostrum of acceptable quality is discarded (Weaver *et al.,* 2000). Therefore, evidence would point to other methods, such as Brix refractometry, of estimating IgG concentration in colostrum are of more practical use.

1.9.2 **Brix Refractometer**

As previously discussed, the Brix refractometer approximates total solid percentages when used to measure non sucrose containing liquids (Deelen *et al.,* 2014). Bartens *et al.* (2016) conducted a study of 193 colostrum samples from multiparous cows from one farm to evaluate two hydrometers and two Brix refractometers compared with RID assessment of IgG concentrations. They concluded that the Brix refractometers provided the most accurate assessment of colostrum quality of the devices evaluated, and it demonstrated excellent precision in terms of repeatability. This was evaluated by each observer testing each sample twice, using the optical and digital Brix refractometers. The Intraclass Correlation Coefficient (ICC) for the measurements performed by two independent observers was 0.98 (95% CI=0.98–0.99).

The Brix refractometer does not measure IgG directly and therefore is only a proxy for antibody concentration. Bartier *et al.* (2015) determined Brix readings and IgG as determined by RID was moderately correlated (r = 64). This moderate correlation between Brix readings and RID measurements was also found by Enger *et al.* (2021); r = 0.52 for IgG_1, r = 0.57 for IgG_2. Colostrum composition in terms of fat and non-immunoglobulin protein content is also thought to interfere with Brix readings (Bielmann *et al.,* 2010; Morrill *et al.,* 2012).

Table 1-11 summarises the sensitivity and specificity of the Brix refractometers as reported in the literature. The differences are postulated by Buczinski *et al.* (2018) in their meta-analysis to be due to inter- or intra-study variability in design of the studies involved for example the different make of refractometers used.

Reference (Year of Publication)	Study Details	Sensitivity	Specificity	Cut Point (%)
Bartier *et al.* (2015)	569 colostrum samples from 13 US dairy farms. Optical Brix refractometry compared with RID	0.83	0.66	23
Bielmann *et al.* (2010)	288 colostrum samples from 3 US dairy farms. Digital and Optical Brix refractometry compared with RID.	0.92	0.81	22
Chigerwe *et al.* (2008)	171 colostrum samples for 1 US dairy farm. Digital Brix refractometry compared with RID.	0.78	0.75	22
Chamorro *et al.* 2015	1590 colostrum samples from 130 US dairy farms. Digital Brix refractometry compared with RID	0.78	0.84	22
Morrill *et al.* (2012)	827 colostrum samples from 67 US dairy farms. Optical Brix refractometry compared with RID	0.94	0.92	Refractive Index stated only
Morrill *et al.* (2015)	58 colostrum sample from Jersey cows only on one farm. Optical Brix refractometry compared with RID.	0.92	0.95	18
Quigley *et al.* (2013)	183 colostrum samples from 7 US dairy farms. Optical Brix refractometry compared with RID	0.93	0.66	21
Vandeputte *et al.* (2014)	396 colostrum samples from 92 Belgian suckler herds. Digital Brix refractometry compared with RID	0.94	0.86	22.5

Table 1-11 A description of studies and reported sensitivities, specificities and optimum cut point determined when investigating the diagnostic accuracy of Brix refractometry compared to RID (Gold Standard).

1.9.3 Summary of evaluating colostrum IgG concentration

As with diagnosis of FPT, there are different testing strategies to evaluate colostrum IgG concentration. Brix refractometry has clear advantages over RID and the colostrometer. Overall, the Brix refractometer is an inexpensive on farm tool for the rapid estimate of the IgG concentration of colostrum. It remains the mainstay of monitoring on farm colostrum quality despite disagreement in the literature about cut points, sensitivity, and specificity. It should be reiterated again that the colour and consistency of colostrum cannot be used to establish IgG concentration.

1.10 Evaluating colostrum bacterial contamination

Further to the IgG concentration of colostrum being an indicator of quality, TBC and TCC can also describe colostrum quality through indicating bacterial contamination (Weaver *et al.,* 2000; Godden *et al.,* 2019). The mechanisms by which bacteria interrupt the successful passive transfer of IgG in the newborn is discussed further in section 1.11.3 Bacterial contamination of colostrum.

Colostrum TBC can be evaluated by total plate counts. This is a method by which the colostrum sample is put onto a growth medium and incubated to allow the bacteria to multiply to measurable colonies. These colonies are then counted as CFU and the results gives a reference as to the bacterial contamination of the colostrum (Ginn *et al.,* 1984). As alluded to already, current literature advise that producers should be aiming to feed colostrum to newborn calves with a TBC <100,000 CFU/mL (Godden *et al.,* 2019).

Coliforms can be identified using various methods including the use of MacConkey agar and Petrifilms™ (Ginn et al., 1984). The colostrum samples are inoculated onto the growth medium, incubated and then colonies counted. Current literature advises that producers should be feeding colostrum to newborn calves with a TCC <10,000 CFU/mL (Godden *et al.,* 2019). Morin *et al.* (2021) conducted a study to validate the Petrifilm culture system. They found the area under the curve of the receiver operating characteristic curve of TBC and CC compared with the standard laboratory technique of plate counts were 0.83, and 0.95, respectively. They concluded that despite the Petrifilms and

plate counts failing to give identical results, the Petrifilm is an appropriate method to identify bacterial contamination in colostrum.

pH can be used as a proxy for bacterial contamination in the field where the practicality, time delay and expense means sending samples to the laboratory is not possible (Denholm *et al.,* 2017). Fresh colostrum has a pH between pH 5.6-6.6 (Stewart *et al.,* 2005; Cummins *et al.,* 2016). Cummins *et al.* (2016) found TBC to be negatively correlated with pH (Pearsons correlation, r = - 0.87) meaning a greater TBC was associated with a lower pH (P<0.01). In research work, plate counts and Petrifilms™ are the most common technique used to establish bacterial contamination of colostrum.

1.11 Calf level risk factors for Failure of Passive Transfer

The body of research from the literature suggests worldwide the prevalence of FPT varies from 15.6 – 41.9% and confirms the welfare and economic implications associated with FPT. It is therefore prudent for producers to minimise failure of passive transfer prevalence on farm. Ensuring each calf achieves adequate passive transfer is a function of three key factors – the quality of the colostrum fed (IgG concentration and bacterial contamination), the volume of colostrum fed, and the timing of colostrum feeding (McGuirk and Collins, 2004).

1.11.1 Colostral immunoglobulin mass administered

Chigerwe *et al.* (2008) conducted a study to determine the mass of colostral IgG required for adequate passive transfer in calves administered colostrum by use of oesophageal intubation. They concluded at least 150-200 g of colostral IgG is required for adequate passive transfer in calves. This broadly agrees with earlier literature that recommends over 100g of colostral IgG to achieve adequate passive transfer (Besser *et al.,* 1991). Recently, to achieve excellent rates of passive transfer it has been suggested this figure should be doubled to 300g of IgG shortly after birth (Godden *et al.,* 2019). When refining on farm colostrum management programmes, producers should be encouraged to feed a target mass as opposed to target volume. However, where poor colostral IgG concentration does exist, this can be overcome to some degree by feeding a larger volume. Care must be taken to avoid overloading with volume as this can

lead to distention of the viscera and pain, overflow and subsequent ruminal drinking syndrome.

Colostral Ig mass is dependent on the transcytosis of IgG during colostrogenesis. Baumrucker *et al.* (2010) determined the total mass of IgG_1 from 214 first milking colostrum samples. The mean mass was found to be 291.6g (range: 14 – 2223g). Furthermore, no relationship between the mass of IgG_1 and the mass of mammary parenchymal tissue showing that the mass of IgG_1 is not dependent on the mass of secretory tissue but likely due to endocrine regulation and genetic variation (Baumrucker *et al.*, 2010).

Multiple international studies have reported on IgG concentrations harvested from dairy cows. Morrill *et al.* (2012) surveyed 827 colostrum samples, via RID testing, from 67 farms over 12 US states and found up to 30% of samples had IgG concentrations less than 50g/L (Range = <1g/L – 200g/L). This study design sampled colostrum from fresh, frozen, refrigerated, individual and pooled samples, depending on farm management protocol. More recent work from Shivley *et al.* (2018) found that 22.6% of colostrum samples surveyed to have <50g/L IgG therefore agreeing with the earlier work done by Morrill *et al.* (2012).The study range was not given but the mean IgG concentration was 74.4 g/L. It should also be noted maternal and colostrum replacer were sampled. Work from the Waikato region of New Zealand found the mean Brix reading to be 18.7 % (21-22% Brix = 50g/L IgG) of 281 samples collected from 14 herds. Only 22.4 % (n = 63) exceed the Brix threshold of 22% for acceptable quality (Denholm *et al.*, 2017). This is lower than other international studies but in the methodology the authors described how it was not possible to determine if the cows had been suckled by the calves prior to colostrum sample collection or not.

UK information is again limited; however, in a six-month study of 444 calvings from seven UK dairy farms, MacFarlane *et al.* (2015) use the indirect method of Brix refractometry to measure colostrum quality. They found colostrum quality ranging from 10.3 – 34.7% Brix and 67% of samples were above the Brix cut point for acceptable quality of 22% therefore broadly agreeing with literature from the United States (MacFarlane *et al.*, 2015). In their work, Bartier *et al.* (2015) determined each 1% increase in the Brix scale compares to a 3g/L IgG concentration. From this worldwide body of evidence, it can be concluded that

the concentration of IgG in colostrum is extremely variable from maternal colostrum. If producers do not measure colostrum IgG concentration available to calves, they risk feeding colostrum, in terms of concentration and volume, that cannot achieve the necessary mass of IgG for adequate passive transfer.

The volume of colostrum harvested at first milking is also extremely variable with the range reported as 2.8 L to 26.5 L (Godden *et al.,* 2019). Some dairy herds report a seasonal reduction in the volume of colostrum harvested, over and above individual cow to cow or parity variation. The seasonal reduction is usually reported in the autumn and winter months (Gavin *et al.,* 2018). Recently this phenomenon was examined by Cabral *et al.* (2016), Gavin *et al.* (2018), and Soufleri *et al.* (2019). This body of research all conclude that photoperiod and genetic factors were likely to be involved. However, it is an area that needs more investigation to understand exact mechanisms and develop strategies to combat a deficiency of colostrum production during certain periods of the year.

To achieve the recommended consumption of 150g-200g of IgG to give serum concentration of IgG above 10 mg/mL it is recommended that neonates receive 10-15 % of bodyweight of colostrum which equates to 3 – 4 L (Chigerwe *et al.,* 2008; Godden *et al.,* 2019). Three papers in the body of literature make conclusions about the volume of colostrum producers should administer. Morin et al. (1997) concluded that administering a large volume (4 L) of high IgG colostrum within 3 h after birth significantly increased calf serum IgG concentration and did not reduce the efficiency of IgG1 absorption when compared with a small volume (2L) of high IgG colostrum. Chigerwe *et al.* (2008) recommends that calves are provided colostrum by use of an oesophageal tube receive 3 L of colostrum within 2 hours after birth. Godden *et al.* (2009) concluded from their study looking at interactions between feeding method and volume of colostrum to improve passive transfer, that producers should be encouraged to feed calves larger volumes (3L) of colostrum.

When discussing colostrum feeding, the method of administration is worth considering. Suckling should be strongly discouraged as this is the least efficient because of delayed suckling as well as loss of control of quality and volume administered (Patel *et al.,* 2014; Godden *et al.,* 2019). One study showed no difference between passive immunity rates and apparent efficiency of

absorption of IgG between calves fed via an oesophageal feeder and via nipple bottle (Chigerwe *et al.,* 2012). This finding is explained by an earlier study that found that although colostrum is delivered into the forestomachs via an oesophageal feeder, rapid flow into the abomasum and small intestine mean IgG is optimally absorbed (Lateur-Rowet and Breukink, 1983). Feeding colostrum via an oesophageal tube is a quick and easy method for producers. The hygiene of feeding equipment as well as its condition is also of paramount importance.

As discussed, Chigerwe *et al.* (2008) made key conclusions about the critical mass of IgG required to achieve adequate passive transfer. The IgG mass available is a function of colostral volume harvested and IgG concentration. The literature consistently demonstrates that both these factors are extremely variable in the modern dairy cow.

1.11.2 Timing of colostrum administration

Closure of the gut to macromolecules, such as colostral IgG, occurs spontaneously with age and at an increasing rate from 12 hours postpartum (Stott *et al.,* 1979). The efficiency of colostrum absorption across the gut epithelium and therefore passive transfer is optimum in the first four hours postpartum (Weaver *et al.,* 2000; Godden *et al.,* 2019). A further small scale study on 27 male Holstein calves, conducted by Fischer *et al.* (2018), found that delaying colostrum feeding by 6 or 12 h after birth decreased the passive transfer of IgG compared with feeding colostrum immediately after birth. All calves in the study were fed a single batch of pooled, heat-treated colostrum containing 62 g of IgG/L at 7.5% of birth weight. The weight of literature supports the recommendation of feeding colostrum as soon as possible after birth and within six hours as industry standard.

Some studies have found an association between the first and second feed interval and the risk of FPT. Morin *et al.* (1997) found that in calves fed colostrum with low IgG concentration (23.9 mg/mL IgG) doubling the volume did not improve the serum IgG status at 48 hours, however an additional feed at six hours post birth alongside the feed at birth did. However this finding is not consistent across the literature. Reschke *et al.* (2017) did not find a significant association between timing of the second colostrum feed with respect to the first ($p = 0.9$, 95 % CI 0.5 – 3.5). The inconsistencies in the true association are

further highlighted by another recent study which showed calves had higher serum IgG concentrations when they were fed a second feed of colostrum or colostrum/milk mixture at 12 hours post birth compared to just milk (Pletts *et al.* 2018). Although the variant in this study was feed type opposed to interval, more work is required in this area to determine the true association and impact.

1.11.3 Bacterial contamination of colostrum

Bacteria, specifically coliforms, can reduce the amount of IgG absorbed by the newborn calf (Johnson *et al.,* 2007; Godden *et al.,* 2019). This negative relationship between colostral bacterial counts and passive transfer of IgG is shown by the study by Godden *et al.* (2012). There are several mechanisms by which this is postulated to occur: Firstly, the physical binding of free IgG molecules by microbes within the gut lumen prevents uptake across the intestinal wall. Secondly, bacteria directly block uptake and transportation across the enterocytes. Thirdly, when these pathogenic bacteria damage intestinal cells there is enhanced renewal of the epithelium thereby accelerating gut closure (Corley *et al.*, 1977; James et al., 1981; Staley and Bush., 1985).

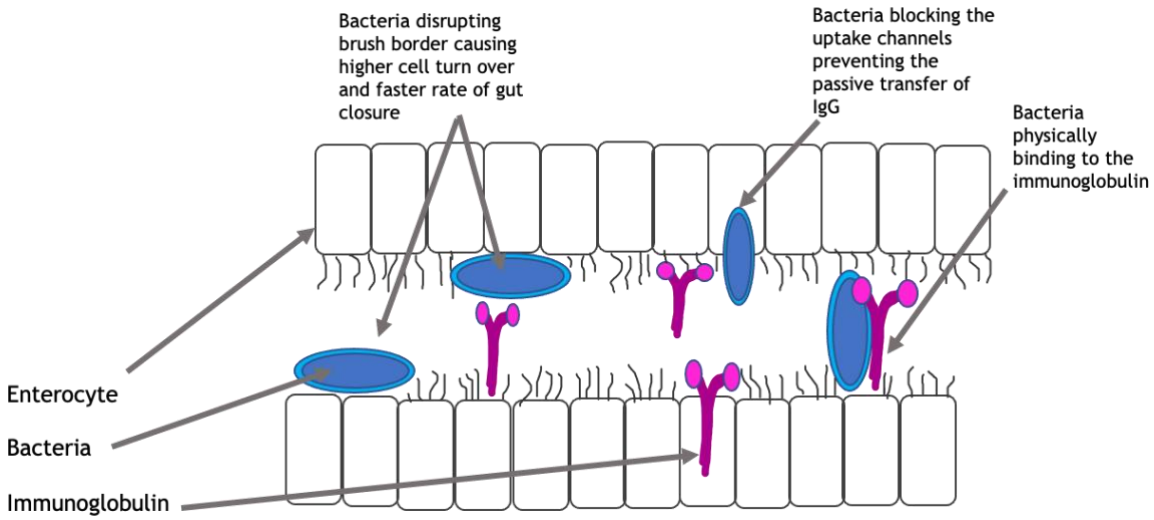


Figure 1-8 Schematic showing the mechanism by which bacteria can disrupt the mechanisms of passive transfer of colostral IgG across the neonatal gut enterocytes.

Several international studies have investigated the bacterial contamination of colostrum. Morrill *et al.* (2012) found up to 60% of US samples did not meet industry TBC standards of <100,000 CFU/mL. In Australia, Abuelo *et al.* (2019), 41.6% and 21.6% of samples did not meet industry standards for TBCs and TCCs, respectively. Denholm *et al.* (2017) conducted a survey of colostrum from New

Zealand dairy farms and found only 8.6 % (n= 23/268) samples had bacterial counts below recommended TBC thresholds. These studies provide evidence that bacterial contamination of colostrum is a problem on dairy farms worldwide and therefore a significant risk factor to FPT. It should be noted that UK data is currently scarce. Only recently, Hyde *et al.* (2020) published a quantitative analysis of colostrum bacteriology on British dairy farms and found that samples collected from feeding equipment has a mean TBC of 439,438 CFU/mL and that 29.6% of all samples collected exceeded the recommended TBC threshold.

Colostrum can become contaminated during the collection, handling and storage process and farm specific factors are likely to be at play (Fecteau *et al.*, 2002). Pathogens such as *Mycobacterium avium* spp. *paratuberculosis*, *Salmonella* spp., *Mycoplasma* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Mycobacterium bovis*, and *Escherichia coli* may contaminate colostrum either by direct shedding from the mammary gland or, more significantly, post-harvest contamination (Stewart *et al.*, 2005). Stewart *et al.*(2005) demonstrated that the colostrum harvest process is a significant critical control point for bacterial contamination of first-milking colostrum. Current industry standards from US data recommend TBC <100,000 CFU/mL and TCC <10,000 coliforms/mL as an indication of acceptable concentration of bacterial contamination (Godden *et al.*, 2019). The negative linear relationship between total coliform count and serum IgG concentration observed by Godden *et al.* (2012) suggest that there is no optimal cut point, it is simply the lower the better.

1.12 Management risk factors for poor quality colostrum

The colostrum management protocol on farm is of utmost importance to maximise colostrum quality available and guard against FPT to ensure maximal calf welfare and productivity (Beam *et al.*, 2009; Godden *et al.*, 2019). The two measures of colostrum quality are immunoglobulin concentration and bacterial contamination. Factors affecting these two indicators are discussed below.

1.12.1 Timing of collection of first milk colostrum from dam

There is a body of evidence that substantiates the longer the time between parturition and colostrum harvesting, the lower the IgG concentration. Moore *et al.* (2005) found that colostrum collected 6, 10, and 14 hours after calving had

significantly lower IgG concentrations than colostrum collected two hours after calving. Morin *et al.* (2010) used regression analysis to investigate associations between colostral IgG concentrations and the interval between calving and first milking. They concluded that colostral IgG concentration drops by 3.7% during each subsequent hour after calving. A further study by Quigley *et al.* (2013) found that time after calving when colostrum was collected was linked to IgG concentration and that IgG concentration was lower in later time points of collection after calving. Prompt harvesting of colostrum after parturition will ensure maximise the IgG content of colostrum. (Moore *et al.,* 2005; Morin *et al.,* 2010; Quigley *et al.,* 2013). Reschke *et al.* (2017) found that a lag time of greater than six hours between parturition and first milking was a risk factor for poor colostrum quality.

Whilst this body of research signposts that every effort should be made to harvest colostrum from freshly calved cows promptly after parturition to maximise the immunoglobulin concentration, there is more recent evidence that colostrogenesis may continue beyond parturition. Gross *et al.* (2014) look at the time of parturition and hormonal changes and the effect on colostrum yield and quality and concluded that the transcytosis of IgG continues beyond parturition. This finding that colostrogenesis doesn't stop abruptly at parturition was further explored by Kessler *et al.* (2020) who concluded that each mammary quarter is independent and the processes of colostrogenesis and lactogenesis are not firmly set at parturition.

1.12.2 Pooling of Colostrum

Pooling of colostrum will reduce the IgG concentration as demonstrated by the example given in the review of passive transfer by Weaver *et al.* (2000). Cow A produces 15 kg of colostrum containing 20 g/L of IgG and cow B produces 5 kg of colostrum containing 40 g/L of IgG. This pooled colostrum will contain 25 g/L of IgG ([(20)(15) + (40)(5)]/20). Low-immunoglobulin, high-volume colostrum will be overrepresented in any pooled colostrum.

A New Zealand study by Denholm *et al.* (2018) found that 90% of pooled colostrum samples had a Brix reading of < 22 % (inadequate quality) providing further evidence that pooling reduces colostrum quality. Not only will pooling reduced to concentration of IgG in the colostrum, it also acts as a mechanism for

spreading colostrum borne pathogens more widely, e.g. *Mycobacterium avium* spp. *paratuberculosis* and *Mycoplasma bovis* (Godden *et al.,* 2019). For both these reasons, the pooling of colostrum should be strongly discouraged to maximise colostrum quality available on farm.

1.12.3 Pasteurising Colostrum

Pasteurisation can improve colostrum quality by reducing the bacterial contamination and increasing the apparent efficiency of absorption (AEA) of IgG. Three papers, Elizondo-Salazar and Heinrichs (2009), Gelsinger *et al.* (2014), and Gelsinger *et al.* (2015), were fundamental in determining that lower bacteria populations lead to the higher serum IgG concentration in calves opposed to any other mechanisms. Gelsinger *et al.* (2015) compared feeding heated and unheated colostrum of either high or low bacterial counts and provided conclusive evidence for the benefits of minimising bacterial contamination. Armengol and Fraile (2016) demonstrated that pasteurisation of colostrum and milk significantly improves calf health status and reduces morbidity and mortality during the first three weeks of life.

Two key papers from Godden *et al.* (2006) and Elizondo-Salazar *et al.* (2010) determined heating at 60°C for 30 or 60 min is sufficient to maintain IgG concentrations whilst reducing bacteria concentrations. Heating at higher temperatures will denature the colostral proteins. Johnson *et al.* (2007) found that calves fed pasteurised colostrum had significantly higher mean serum IgG concentration at 24 hours of age when compared with calves fed raw colostrum. The pasteurised colostrum had a mean total bacterial count of 813 CFU/mL, and the raw colostrum had a mean total bacterial count of 40,738 CFU/mL.

The body of evidence supports pasteurisation as a method to improve colostrum management in certain circumstances. But, it should not be considered the silver bullet and heat treatment will not increase IgG concentration or the AEA when colostrum IgG is high or cleanliness is good (Heinrichs *et al.,* 2019). Furthermore, pasteurisation will not reduce bacterial counts to zero.

1.12.4 Storing and preserving of colostrum.

On commercial dairy units, the main methods of storing colostrum are by refrigeration, freezing and via addition of preservatives. The effects of these methods have been reviewed extensively in the literature. Freezing has been found to maximise the retention of IgG concentration and nutrients compared with other storage methods (Holloway *et al.*, 2001; McGuirk and Collins, 2004). Although Holloway *et al.* (2001) only used colostrum frozen for two days and in reality colostrum is stored for longer periods of time on farm. Alrabadi (2015), froze 30 raw milk samples for eight weeks and tested TBC and TCC weekly, concluded that bacterial counts decrease significantly as the freezing time increases. If the colostrum undergoes multiple freeze-thaw cycles IgG concentration is likely to decline as measured by RID; however further research is warranted to quantify this (Morrill *et al.*, 2015).

Cummins *et al.* (2016) investigated the serum IgG concentration, bacterial contamination and health parameters in calves fed colostrum stored using a range of conditions. They found that storage ≤4°C for two days was sufficient to reduce bacterial growth, thereby ensuring adequate passive transfer when colostrum cannot be pasteurized before feeding or when it cannot be fed to calves immediately. Interestingly, they also found no effect of storage on colostrum IgG concentration for up to 72 hours. This is contradicted by Morrill *et al.* (2012) who concluded that the storage of colostrum had a significant impact on bacterial contamination therefore colostrum should be fed fresh or frozen immediately and not stored in a refrigerator.

The use of preservative, specifically potassium sorbate, was described by Stewart *et al.* (2005) and Denholm *et al.* (2018). Stewart *et al.* (2005) described the effect of refrigeration and the use of potassium sorbate preservative in controlling the total bacterial count and total coliform count in stored colostrum. They concluded storing untreated colostrum in warm ambient temperatures resulted in rapid increase in bacterial counts. The most effective treatment was the use of potassium sorbate preservative combined with refrigeration, in which TBC and TCC dropped significantly and then remained constant during the 96-hour storage period. More recent work by Denholm *et al.* (2018) agreed; preservation with potassium sorbate resulted in little or no decline in Brix percentage and limited bacterial proliferation in pooled

colostrum. These findings were applicable to colostrum stored in ambient temperature for up to seven days.

It has been demonstrated that proper colostrum storage and preservation is vital to minimise bacterial growth and maximise IgG absorption efficiency, however the effect of storage on IgG concentration is not as clear cut. It can be deduced from the literature that correct management protocols pertaining to the storage and preservation of colostrum will maximise colostrum quality available on farm.

1.13 Cow level risk factors for poor colostrum quality

1.13.1 Breed

Morrill *et al.* (2012) surveyed 827 colostrum samples from US dairy farms and found that breed did not affect IgG concentration. The mean results were similar for Holstein cows (74.2 mg/mL) and Jersey cows (65.8 mg/mL). This finding contradicts older literature from Muller and Ellinger (1981) but agrees with a more recent study by Denholm *et al.* (2018). Muller and Ellinger (1981) found that cows of the Ayrshire and Jersey breeds had significantly higher immunoglobulin concentrations than the Holstein breed and Tyler *et al.* (1999) found significant differences in IgG concentration between the Guernsey and Holstein breeds. Both these study population were small, only 72 and 99 cows respectively, and therefore this limits their reliability. In a more recent New Zealand study by Denholm *et al.* (2018), no associations between breed and colostrum quality was found.

Morin *et al.* (2010) surveyed 1085 first milking colostrum samples from 608 cows and found there were differences in colostrum specific gravity between breeds. Their study strength was that multiple breeds were housed on the same farm therefore underwent the same management practices. However, the colostrum samples were tested using a colostrometer and there are limitations of this techniques have been discussed earlier (Bartens *et al.*, 2016; Buczinski and Vandeweerd, 2016).

Overall, no definite conclusions can be drawn regarding breed and colostrum quality. It is likely that other factors (such as timing of harvesting post calving,

pooling of colostrum and bacterial load) have a more significant impact on colostrum quality in real terms in any colostrum management programme.

1.13.2 Parity and Lactation Number

Shivley *et al.* (2018) found that colostrum samples from dams in third or greater lactation had the highest quality, which is consistent with older literature reported by Tyler *et al.* (1999). As cows progress in lactation number, they are exposed to more farm-specific pathogens, which may increase the IgG concentration in the colostrum (Godden *et al.,* 2019). Shivley *et al.* (2018) further concluded that there was no significant difference in colostrum quality between first and second lactation dams. In a practical context, the relevance of this finding is that any management practices that involve categorically discarding colostrum from first lactation heifers based on presumed poorer quality are inadvisable. Instead, producers should test colostrum quality of all colostrum fed.

1.13.3 Mammary Gland Size

Research into the effect of mammary gland size and relationship with colostral IgG concentration is limited. A literature search revealed only Baumrucker *et al.* (2010) had investigated and found that mammary gland mass had no correlation with mass of IgG in a first milking colostrum sample. Realistically, this is not a factor that can be easily controlled and managed on the commercial dairy farm therefore focus is on other management strategies.

1.13.4 Prepartum Diet

Studies have shown that prepartum diet does not affect colostrum quality in dairy cattle (McGuirk and Collins, 2004; Godden *et al.,* 2019). Dunn *et al.* (2017) evaluated factors, including prepartum energy level, that affected colostrum quality and concluded that there was no relationship between prepartum diet energy level and IgG colostrum concentration which is in agreement with further literature from Mann *et al.* (2016).

These two studies concentrate on prepartum energy levels, a recent study by Edinburgh University proposed a theory regarding insufficient Effective Degradable Rumen Protein (EDRP) supply specifically as a risk factor for

colostrum quality and FPT in beef calves (Corbishley *et al.,* 2017). They reported two case studies of spring calving suckler herds with FPT and neonatal disease problems. Metabolic profiling of dry cows found blood urea-N <1.7 mmol/l in most cows sampled. Blood urea-N <1.7 mmol/l are indicative of insufficient ERDP intake. They reported blood urea-N results obtained during routine metabolic profiling of dry suckler cows were generally lower than those of dry dairy cows (1.86 mmol/l vs. 2.27 mmol/l). They suggest that insufficient ERDP supply in late gestation is a potential risk factor for FPT in beef suckler cows.

Earlier work by Blecha *et al.* (1981) found no correlation between IgM and IgG in cow sera and colostrum and the crude protein intake in the dam. Limitations of this study include the lack of use of metabolic profiling to determine protein status and examining crude protein opposed to specifically ERDP. However they did identify a positive correlation between IgG in the calf sera and maternal crude protein intake which is in agreement with the more recent work from Corbishley *et al.* (2017).

In general, the literature is conflicting regarding the impact of late gestation diet and colostrum IgG. There are differences between nutritional management on commercial dairy and beef farms. Dairy farms generally have higher levels of input from veterinary and nutrition advisors with more in-depth analysis of forage and diet formulations compared with their beef counterparts. Therefore, nutritional deficiencies may be present on beef farms and not identified, meaning that diet related colostrum risk factor may be more significant on beef farms compared with dairies.

1.13.5 Mastitis

Maunsell *et al.* (1998) investigated the effects of mastitis during the dry period on colostral volume, concentrations, and total yields of IgG. They found that colostral volume from persistently infected glands was lower compared to colostral volume from uninfected glands; however, infection did not affect the IgG concentration in the colostrum. This finding is confirmed by more recent work that the presence of an intramammary infection did not have a clear impact on the IgG concentration in colostrum (Enger *et al.,* 2021). However, the practice of feeding colostrum (or milk) to calves from cows with clinical mastitis

should be discouraged. Mastitic colostrum/milk has variable quality, will contain high numbers of bacteria and potentially antibiotic residues from treated cows (Selim and Cullor 1997).

1.13.6 Dry period length

Shoshani *et al.* (2014) compared the colostrum quality between cows subjected to a 40-day dry period and 60-day dry period and found no significant difference. This agrees with earlier data from Annen *et al.* (2004) and Watters *et al.* (2008) who compared the colostrum quality between cows subjected to a 40-day or 60-day dry period and a 55-day and a 34-day dry period respectively. Both studies found no significant difference in colostrum quality between the two treatments. Annen *et al.* (2004) further qualified this as their data suggested that while shortening of the dry period did not affect colostrum quality, complete omission of a dry period likely would reduce colostrum quality. This agrees with a later study conducted by Mayasari *et al.* (2015) who found when comparing three dry periods of 0 days, 30 days and 60 days, colostrum production and IgG concentration in colostrum were lower for cows with a 0-d dry period than a 60-d dry period. The conclusions drawn from the literature and subsequent advice to producers would be skipping the dry period would not be recommended to safeguard colostrum quality.

1.14 Summary

The worldwide prevalence of failure of passive transfer has been well demonstrated in the literature. Moreover, FPT impacts calf health and welfare as well as productivity. However, there is no perfect test available to diagnose FPT calf side in the field and debate exists about thresholds as well as test sensitivity and specificity. There are limited data under UK conditions and farming systems with respects to FPT prevalence and test performance. Furthermore, there are limited UK data pertaining to FPT and colostrum quality risk factors. This led to a need for further research to develop clear guidance with respect to testing strategies and risk factor management for the UK dairy industry.

1.15 Aims and objectives

This study has four aims:

1. To estimate the prevalence of FPT within the Scottish dairy calf population
2. To assess agreement between serum testing methods for FPT
3. To investigate colostrum quality on Scottish dairy farms
4. To determine risk factors associated with FPT and poor colostrum quality on Scottish dairy farms

2 Material and methods

2.1 Ethics

This study was approved by the University of Glasgow Veterinary School Ethics committee (under ethics licence number 13a18). Participation in the study was voluntary. Farms and calves were enrolled with written consent from the farmer (Appendix 1).

2.2 Study design

This study was a prospective, observational study over a period of 5 months (February – June 2019) across a convenience sample of farms recruited from the client base of two veterinary practice on Scotland. The geographical area in the study represented the area covered by the private veterinary practices. Farms were enrolled on a voluntary basis from a list of convenience from the two private veterinary practices. Serum samples were taken at routine farm visits from all eligible calves at the time of the visit. Calves of any breed or sex were deemed eligible if they were aged between 1-7 days, healthy and had no history of being treated for a disease process. Health was judged by visual inspection by the sampling vet and verbal history from the calf rearer. This meant that all calves on all enrolled farms born in the study period were potentially eligible for enrolment; however, they were not necessarily captured because the sampling was limited to the days of routine farm visits.

It was estimated that a sample size of 388 calves was required to estimate test agreement, with a desired discordance rate of 0.01 and tolerance probability of 0.90 (Liao, 2010). It was aimed to sample at least 12 calves per farm which balanced the financial constraints of the study and was based on peer reviewed work from McGuirk and Collins (2004) and Cuttance *et al.* (2019).

Colostrum samples were taken by farm staff during the study period, February – June 2019. All colostrum fed to newborn calves on their first feed was eligible for sampling. Whether samples were collected or not was governed by farm staff motivation for participation in the study.

2.3 Farm enrolment and questionnaire data collection

Thirty-eight commercial dairy farms in the Stirlingshire, Lanarkshire and Dumfries and Galloway regions of Scotland between February and June 2019. At enrolment, all farmers were asked to complete a questionnaire concerning neonatal calf and colostrum management practices, Table 2-1. The questionnaire was constructed after the literature review was carried out to identify risk factors for failure of passive transfer (FPT) and colostrum quality. It was beta tested, *a priori*, by five veterinary surgeons to ensure ease of understanding and answering. Questionnaire data were collected face-to-face by four private veterinary surgeons (PVS) prior to sample collection. The PVS had a thorough working knowledge of the farm and a trusted relationship with enrolled farmers. This aimed to limit recall and interview biases. The respondents were asked to answer the questions as to what happens to the majority of calves on farm. This was because it is appreciated that on farm the time of day, staffing, and other ongoing tasks means, despite best efforts, not all protocols are consistently carried out from calf to calf.

Questions	Responses				
When are newborn calves actively first fed after birth?	< 2 hours after birth	2.5 to 6 hours after birth	6.5 to 12 hours after birth	12.5 – 24 hours after birth	
What volume of colostrum is fed to newborn calves at first feed?	< 2 litres	2.5 – 3 litres	3.5 – 4 litres	4.5 – 5 litres	> 5 litres
When is the colostrum collected from a newly calved cow?	Less than 2 hours after calving	2.5 – 6 hours after calving	6.5 – 12 hours after calving	12.5 – 24 hours after calving	
Does colostrum from a newly calved cow get collected into a bucket in the milking parlour?	Yes	No			
If NO, what do you collect the fresh colostrum into?					
Does the colostrum sit in a bucket before feeding to calves[8]?	Yes	No			
If YES, for how long?	< 6 hours	> 6 hours			
If YES, is the bucket or container covered with a lid?	Yes	No			
Where does the colostrum go after collection?[9]	Into another container	Straight into calf feeder			

[8] This explores further the opportunity for bacterial contamination and multiplication. Colostrum should be either fed straight away after collection or stored correctly (refrigeration, frozen, chemical preservation).

[9] This question captures more information regarding opportunity for bacterial contamination of colostrum.

Do you clean your test buckets and calf feeding equipment regularly?	Yes	No			
Method of feeding used for feeding first feed	Oesophageal Tube	Teat Feeder	Bucket	Other	
What is the interval between first and second feed of newborn calves?	< 6 hours	6.5 – 12 hours	12.5 – 18 hours	18.5 – 24 hours	> 24 hours
Are newborn calves fed first milking colostrum only?	Yes	No			
Are newborn calves fed a mixture of first milking and later milking colostrum mixed?	Yes	No			
Are newborn calves fed fresh colostrum?	Yes	No			
Are newborn calves fed stored colostrum?[10]	Yes	No			
How is colostrum stored?	Freezer	Fridge	Other		
If you store colostrum, do you have a temperature gauge on your fridge or freezer?	Yes	No			

Table 2-1 The questionnaire posed to farmers to explore colostrum management protocols on farm at to enrolment in the study.

[10] Stored = refrigerated, frozen, chemical preservation.

2.4 Serum sample collection and analysis

Blood samples were collected by jugular venepuncture using a 20-gauge, 1-inch needle. Two sterile 5mL vacutainers without anticoagulant were filled. Blood samples were allowed to clot and then chilled immediately after collection. Samples were separated by the PVS using a centrifuge at the practice within six hours of collection and two aliquots of serum were frozen at -20°C. All samples were transported on ice to the University of Glasgow laboratory and stored at -20°C until they were batch defrosted for testing. The second aliquot of serum was then transported on ice, by hand, to Scottish Rural Colleges, Auchencruive laboratory for ZST testing to be completed. Figure 2-1 shows a flow diagram of the number of samples collected and the number analysed by each testing method. Bovine serum total protein (STP) concentration was noted to decline at 1.2 g/L/month of storage at -20°C for a four month period (Villarroel *et al.*, 2015). Therefore, it was aimed to test samples as soon as possible after receipt into the laboratory. The last recorded date samples were received into the lab was June 2019 and the last sample testing also occurred in June 2019. Clinical information and reference test results were not available to the technicians performing the indirect testing and *vice versa*.

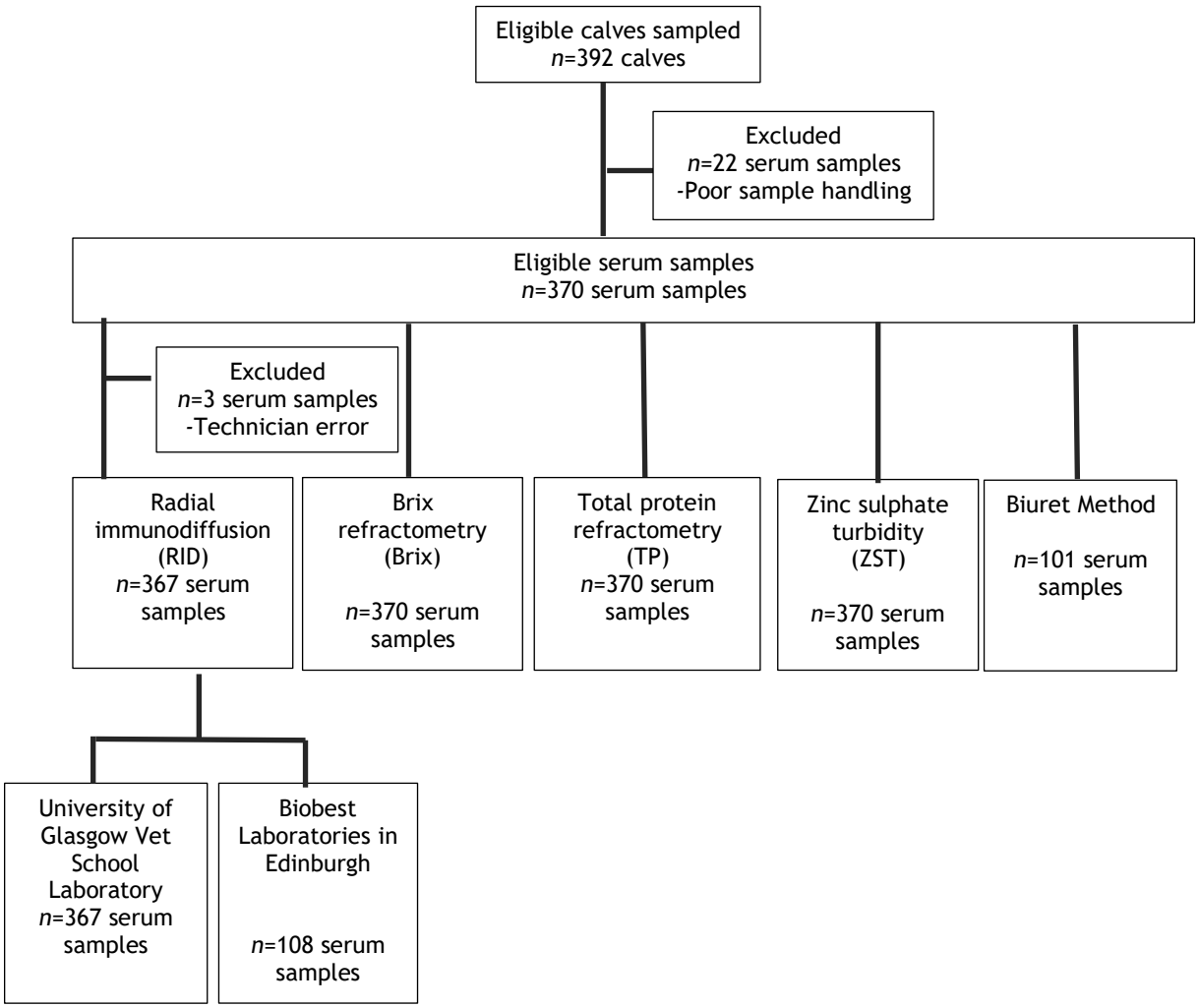


Figure 2-1 Flow diagram showing the number of samples collected and the number of samples undergoing each sampling technique

2.4.1 **Radial Immunodiffusion Testing**

Radial immunodiffusion was carried out using Triple J Agar Plates (Bovine IgG RID Kit, Triple J Farms, Bellingham, WA). This reference test and methodology was used due to commercial availability and precedence in previous peer reviewed literature. RID testing using Triple J Agar Plates was carried out according to the manufacturer's instructions on all serum samples at the study lab, University of Glasgow. Three standard solutions of bovine serum (196mg/dL, 1402mg/dL and 2748mg/dL of IgG) provided with the test kits were included on each plate. These standard solutions were used to create standard curves for each test plate from which the IgG concentration of the test samples could be determined. A calf serum sample of known IgG concentration (1000mg/dL IgG) was included in each assay as a positive control. The plates were incubated at room temperature on a flat surface for 40 hours. The results were read at 24 and

40 hours by placing the plate on a light box and measuring the precipitin rings using a ruler (Saikin-Kagukel Institute Co Ltd) with a precision of 0.1mm. The diameter of the precipitin ring was compared with a standard curve created by the standard solutions to determine IgG concentration in mg/mL. The timings of reading were selected according to manufacturer's recommendations and available laboratory time.

A subset of 125 samples were also transported frozen on ice to a commercial laboratory (Biobest Laboratories Ltd, 6 Charles Darwin House, The Edinburgh Technopole, Edinburgh, EH26 0PY) for comparison of the RID technique carried out in the internal study laboratory at The University of Glasgow. The 125 samples were purposively selected to represent low (<10g/L), medium (10-25g/L) and high IgG(≥25g/L) concentrations and ensure FPT prevalence in each data set were similar. The RID test conducted in the internal study lab at the University of Glasgow was referred to as RID(Glasgow) and the RID test conducted at the commercial external laboratory was referred to as RID(Biobest).

2.4.2 **Total Protein and Brix Refractometry Testing**

Serum samples were thawed at room temperature and inverted three times prior to analysis. They were analysed using total protein (TP) refractometry (RHCN-200ATC, Gain Express Holdings Ltd, London W7 3SA, UK) and Brix refractometry (RSA-BR32T Refractometer w ATC, 0-32%, Brix, Cole-Parmer, Cambridgeshire PE19 8YX, UK) following the methodology described by Elsohaby *et al*. (2015). Briefly, the refractometers were calibrated every thirty samples and where laboratory temperature fluctuated by 5°C. They were cleaned using 70% ethanol before each use. Then one to two drops of the sample were placed onto the prism of the TP refractometer and Brix refractometer. The refractometer was then held up to a light source and the reading noted (in % for Brix and in g/dL for TP). Between each sample the prism was cleaned using alcohol wipes to prevent residue accumulation.

2.4.3 **Biuret Method Testing**

The biuret method, also used to measure TP, was carried out. A subset of 101 samples from 29 farms were purposively selected as previously described to

include high, medium, and low IgG concentrations. Testing was carried out using an automated biochemistry analyser (Dimension clinical chemistry system, Siemens Healthcare Diagnostic, 500 GBC Drive, Newark. USA) at the University of Glasgow laboratory.

2.4.4 Zinc Sulphate Turbidity Testing

Serum samples were analysed using the ZST method as described by McEwan *et al. (1*970). Briefly, 6mL of distilled water and 100µl of test serum (aliquot A) and 6mL of zinc sulphate solution (0.208g/litre) and 100µl of test serum (aliquot B) were prepared in separate test tubes from each sample. The test tubes were inverted three times to mix and then labelled accordingly. Control samples, of known ZST values, of foetal and newborn calf serum were setup in the same way. All samples were incubated at room temperature for one hour. A colorimeter (Hitachi Fluorescence Photometer, 4020) was calibrated with distilled water. Aliquots A and B for each serum sample were measured and the difference calculated to produce the result.

2.4.5 Descriptive Statistics: Comparison of indirect FPT testing diagnostic tests

Descriptive statistics for RID, TP, ZST, Brix and Biuret testing methods were calculated. The overall prevalence of FPT in the study population was calculated from the reference test RID(Glasgow). FPT prevalence was then calculated for each geographical region involved in the study, again from the reference test RID(Glasgow). Prevalence of FPT was also calculated from the indirect testing methods TP, Brix, ZST, and Biuret. The cut points used for each test are shown in Table 2-2. Cut points were chosen after a review of the peer reviewed literature and with reference to cut points used in laboratories offering commercial testing (SRUC Diagnostic Services and Biobest Laboratories).

Test	Cut Point	Reference (Year of Publication)
RID	10 mg/mL	Weaver *et al.* (2000), Godden *et al.* (2019)
TP Refractometry	5.2 g/dl	Elsohaby *et al.* (2015)
Biuret	5.2 g/dl	Zakian *et al.* (2018)
Brix	8.4%	Deelen *et al.* (2014)
ZST	20	McEwan *et al.* (1970) and SRUC Diagnostic Services

Table 2-2 Testing method and cut point used to determine FPT status of 370 serum samples from dairy bred calves from 38 Scottish dairy farms between February - June 2019.

2.4.6 Comparison of test performance for the diagnosis of FPT

The data were analysed for normality by plotting frequency histograms and using the Shapiro-Wilk test. A null hypothesis of the data being normally distributed within the population was proposed. A $p \geq 0.05$ in the Shapiro-Wilk test leads to the null hypothesis being accepted and normal distribution assumed. Scatter graphs were constructed using Pearson's correlation (r) and 95% confidence intervals were calculated to determine the association between the reference test RID(Glasgow) and indirect methods TP, Brix and ZST. Univariable models were constructed with each indirect testing method as the predictor variable and the reference test, RID(Glasgow), as the response variable in order to calculate the r^2 value. The r^2 value is an indicator of the proportion of the variance in the response variable that can be explained by the predictor variables in the regression model.

To assess diagnostic performance of the indirect testing methods the sensitivity, specificity, positive predictive value and negative predictive values were calculated using RID (Glasgow) as the reference test. Receiver operator characteristic (ROC) curves plot the sensitivity (true positives) against 1-specificity (false positives) of a test at all cut points to determine the test's diagnostic ability. ROC curves show a graphical representation of trade-off between sensitivity and specificity at each cut point. These were constructed for each indirect testing method to establish the cut point at which both the sensitivity and specificity were maximised. The area under the curve (AUROC) was calculated along with the Youden index to assess the performance of a diagnostic test in its ability to discriminate between calves with and without FPT. The Youden Index gives equal weighting to false positives and false negatives. As discussed in Chapter 1, sensitivity and specificity may be prioritised in different circumstances, for example as a herd health screening

tool, it would also be desirable to maximise sensitivity to minimise false negatives results.

Bland Altmann (BA) plots were constructed to test limits of agreement and identify variability and bias in the measurements between RID(Biobest) and RID(Glasgow) and the indirect testing methods TP, Brix and ZST. The mean of each pair of measurements were plotted versus the difference between the two measurements. BA plots are only suitable for checking agreement between tests that use the same measurement scale. Because the test measures bias through plotting the difference of the means from zero, tests that measure ultimately the same phenomenon but on different scales cannot be examined for bias. To get round this, the measurements were standardised. To check agreement between RID(Glasgow) and TP, Brix and ZST testing strategies general linear modules were constructed to give actual and predicted IgG concentrations and it was from these values that BA plots were constructed. Percentage agreements were calculated from the BA plots. (Percentage agreement = number within limits of agreements/total number x 100). The limits of agreement (2SD of the difference of the two IgG concentration estimates) were assessed. *A priori* it was clinically acceptable for ≤ 5mg/mL of a difference to exist (Cuttance *et al.*, 2017). Fixed bias was assessed by looking at the mean difference between the two measurements. Proportional bias was visually assessed by the pattern of the scatter of points.

The continuous variable outcomes were dichotomized as adequate passive transfer (1) or FPT (0) using the respective test cut points for FPT. The agreement between RID(Glasgow), RID(Biobest), TP, Brix and ZST was further explored, using these dichotomized results, through use of the Cohen's Kappa statistic. A Kappa value of 1 implies perfect agreement, whereas a Kappa value of 0 implies agreement due to chance. A limitation of kappa is that it is affected by the prevalence of the finding under observation.

2.5 Colostrum sample collection and analysis

Two hundred and fifty-two colostrum samples were collected by trained farm staff at the point of feeding to the neonatal calves. At the point of feeding was defined as colostrum taken from the feeding container (bottle or stomach tube) just before it was fed to the calf. Colostrum samples were frozen on farm and collected by the PVS at regular intervals. All samples were transported on ice to the University of Glasgow laboratory and stored at -20°C until Brix refractometry and bacterial contamination testing. Colostrum samples were then batch thawed at room temperature prior to analysis. Once fully thawed each colostrum sample was vortexed (Vortex Genie 2, Scientific Industries Inc. 80 Orville Drive, Suite 102, Bohemia, New York 11716 USA) for approximately 10 seconds before testing.

Farmer Colostrum Collection Protocol
1. Only sample first feed colostrum to be fed to newborn calves
2. Put on gloves
3. Pour the colostrum into the feeder and mix it thoroughly
4. Open the sample container without touching the inside of the lid or the inside of container
5. Do not place the lid of the sample container down, hold it carefully without touching the inside of the lid.
6. Fill the sample container from the colostrum in the feeder (20-30 mLs)
7. Put the lid on the container and label with sample number, date, and the calf number to which the colostrum was to be fed.
8. Put the colostrum sample in the freezer and keep frozen at -20 °C until collected by the vet

Table 2-3 Colostrum collection protocol farm staff were trained in prior to collection of 252 colostrum samples from 38 Scottish dairy farms.

2.5.1 Brix Refractometry

Brix refractometry (RSA-BR32T Refractometer w ATC, 0-32%, Brix, Cole-Parmer, Cambridgeshire PE19 8YX, UK) was used to estimate the IgG concentration for each colostrum sample using the methodology described previously (Elsohaby *et al.*, 2015).

2.5.2 **Total Bacterial Counts**

Total bacterial counts (TBC) were also measured for each colostrum sample. Two dilutions of each sample were prepared (1:10 and 1:100) and 0.1mL of each dilution was pipetted onto 5% sheep blood agar plate (E + O Laboratories Limited, Burnhouse, Bonnybridge, Scotland) using a calibrated pipette (Gilson 74395 Pipetman Single Channel Pipette P1000). The agar plates were then incubated for 24 hours at 37°C (LTE Scientific Swallow Incubator, LTE Scientific Ltd, Greenbridge Lane, Oldham OL3 7EN). Colonies were counted using a colony counter (Stuart Scientific, Cole Palmer, UK). If colonies were too numerous to count, the procedure was repeated at dilutions 1:1000 and then 1:10000 until counts could be obtained.

2.5.3 **Total Coliform and *E.coli* Counts**

Total coliform and *Escherichia coli* (*E.coli*) counts (TCC and TEC respectively) were measured for each colostrum sample. Petrifilms (3M Health Care, St Paul, MN 55144, USA) were used as validated by Morin *et al.* (2021). Briefly, 1 mL of each undiluted sample was pipetted onto Petrifilm and the films were then incubated for 24 hours at 37°C (LTE Scientific Swallow Incubator, LTE Scientific Ltd, Greenbridge Lane, Oldham OL3 7EN). Coliform and *E.coli* colonies were counted using a colony counter (Stuart Scientific, Cole Palmer, UK). The procedure was repeated for highly contaminated samples (with too numerous colonies to count) at dilutions 1:1000 and then 1:10000 until a count could be obtained.

2.5.4 **Descriptive statistics**

Descriptive statistics were calculated for each measure of colostrum quality - Brix, TBC, TCC, TEC. Each quality indicator, apart from TEC, has an accepted satisfactory threshold that has been well defined and used in previous peer reviewed science (Weaver *et al.,* 2000; McGuirk and Collins., 2004; Godden *et al.,* 2019). The percentage of colostrum samples failing each quality measure was then reported for the study population as a whole and from the specific geographic regions – Stirlingshire, Lanarkshire and Dumfries and Galloway.

Measure of Colostrum Quality	Threshold	Reference (Year of Publication)
Brix	>22 %	Bielmann *et al.* (2010)
Total Bacterial Count (TBC)	<100,000 CFU/mL	Godden *et al.* (2019)
Total Coliform Count (TCC)	<10,000 CFU/mL	Godden *et al.* (2019)
Total *E.coli* Count (TEC)	None cited, arbitrary cut point <20 CFU/mL used	Fecteau *et al.* (2002) and Stewart *et al.* (2005)

Table 2-4 The measures of colostrum quality, the cut points used to define industry thresholds and the references. Colostrum quality outcomes were dichotomised into two categories: 0 = unacceptable as defined by industry standard and 1 = acceptable as defined by industry standards

2.6 Risk factors analysis

All data were stored on a relational database (Microsoft Access 2016) and spreadsheets were constructed using Excel (Microsoft 2016). Statistical analysis was carried out using statistical software (Stata/IC 15.0, StataCorp LP, College Station, TX, USA).

Only questionnaire variables that could have a biologically plausible effect on the outcome (FPT or colostrum quality) under investigation were examined. Spearman Rank Correlation was carried out on risk factors predictor variables to confirm independence. Where any variables were highly correlated ($r > 0.8$) only one of the variables was included in further multivariable model construction to prevent over parameterisation of the models.

2.6.1 Failure of Passive Transfer Risk Factors

The seven categorical risk factor variables for FPT investigated were the interval between birth and first colostrum feed, volume of first feed, time between calving and first harvesting colostrum, the method of administering the first feed, the interval between the first and second feed, feeding of first milk colostrum only, and the feeding of fresh or stored colostrum. Risk factors response categories for FPT were condensed based on their distribution if required. The FPT outcome was dichotomised where 1= FPT (i.e. IgG = <10g/L) and 0 = no FPT (i.e. IgG = >10g/L).

2.6.2 Colostrum Quality Risk Factors

Six categorical risk factor variables were investigated for the impact on colostrum quality: interval between birth and first feed, the method of

administering the first feed, time of harvesting colostrum after calving, colostrum sat in a bucket post harvesting prior to feeding, colostrum buckets covered with a lid, colostrum stored on farm. Risk factors response categories for colostrum quality were condensed based on their distribution if required. Colostrum quality outcomes (Brix, TBC and TCC) were dichotomised as shown in Table 2-5.

Colostrum Quality Measure	Cut point	Dichotomised Categories	
		0	1
Brix	22%	Unacceptable IgG (< 22%)	Acceptable IgG (>22%)
TBC	100,000 CFU/mL	Acceptable TBC (<100,000 CFU/mL)	Unacceptable TBC (>100,000 CFU/mL)
TCC	10,000 CFU/mL	Acceptable TCC (<10,000 CFU/mL)	Unacceptable TCC (>10,000 CFU/mL)

Table 2-5 The Colostrum quality measure alongside the cut point and dichotomised values used for logistic regression analysis.

2.6.3 Variance, interaction, and confounding

Clustering at the farm level was calculated using intraclass correlation coefficients to establish the amount of variance in the outcome variable attributable to farm. Farm was included in multivariable model analysis as a random effect based on these calculations

All biologically plausible interaction terms were explored. Two independent variables interact if the effect of one of the variables differs depending on the level of the other variable. It was important that all biologically plausible interactions were explored because an interaction term could change the co-efficient of the relationship between the predictor and outcome variable causing a spurious relationship.

Confounding terms were explored. A confounding variable is a variable related to predictor variable and outcome variable under investigation. They can cause an under- or overestimate of the causal relationship between variables and is therefore important to investigate and include in the final multivariable models if appropriate.

2.6.4 **Univariable and Multivariable Logistic Regression Analysis**

Univariable logistic regression for all risk factor variables for FPT and colostrum quality (Brix, TBC, TCC) were carried out with statistical significance declared at $p \leq 0.2$. This level of statistical significance was used to ensure that all possible relationships were captured and to determine predictor variables to include in the multivariable analysis.

Finally, multivariable logistic regression was carried out and risk factors were excluded from the multivariable model using a backwards, stepwise elimination process and the likelihood ratio test was used to compare the models to determine significant risk factors. Statistical significance was assigned at $p \leq 0.05$. Postestimation and model diagnostics were performed using the predict function in Stata for all multilevel logistic regression modelling. Model residuals were plotted and examined.

3 Descriptive results: Failure of passive transfer (FPT), serum testing and colostrum quality

3.1 Geographical distribution of study population

The regions in which the study farms were located (Dumfries and Galloway, Stirlingshire, and Lanarkshire) were representative of the areas of Scotland where the majority of dairy farming occurs according the Scottish Agriculture Census (Scottish Government, 2020). The Scottish Dairy Council state that there are 843 dairy herds in Scotland, 38 were enrolled in this study (4.5% of farms).

Figure 3-1 Outline map of Scotland showing the geographical location and distribution of the 38 dairy farms enrolled in the study between February 2019 - June 2019. Farms were in Stirlingshire, Lanarkshire and Dumfries and Galloway.

3.2 **Missing data**

From 38 farms, 392 samples were obtained from dairy bred calves aged between 24 hours and seven days. The branch diagram in Figure 3-2 explains missing data and the samples available for testing and statistical analysis.

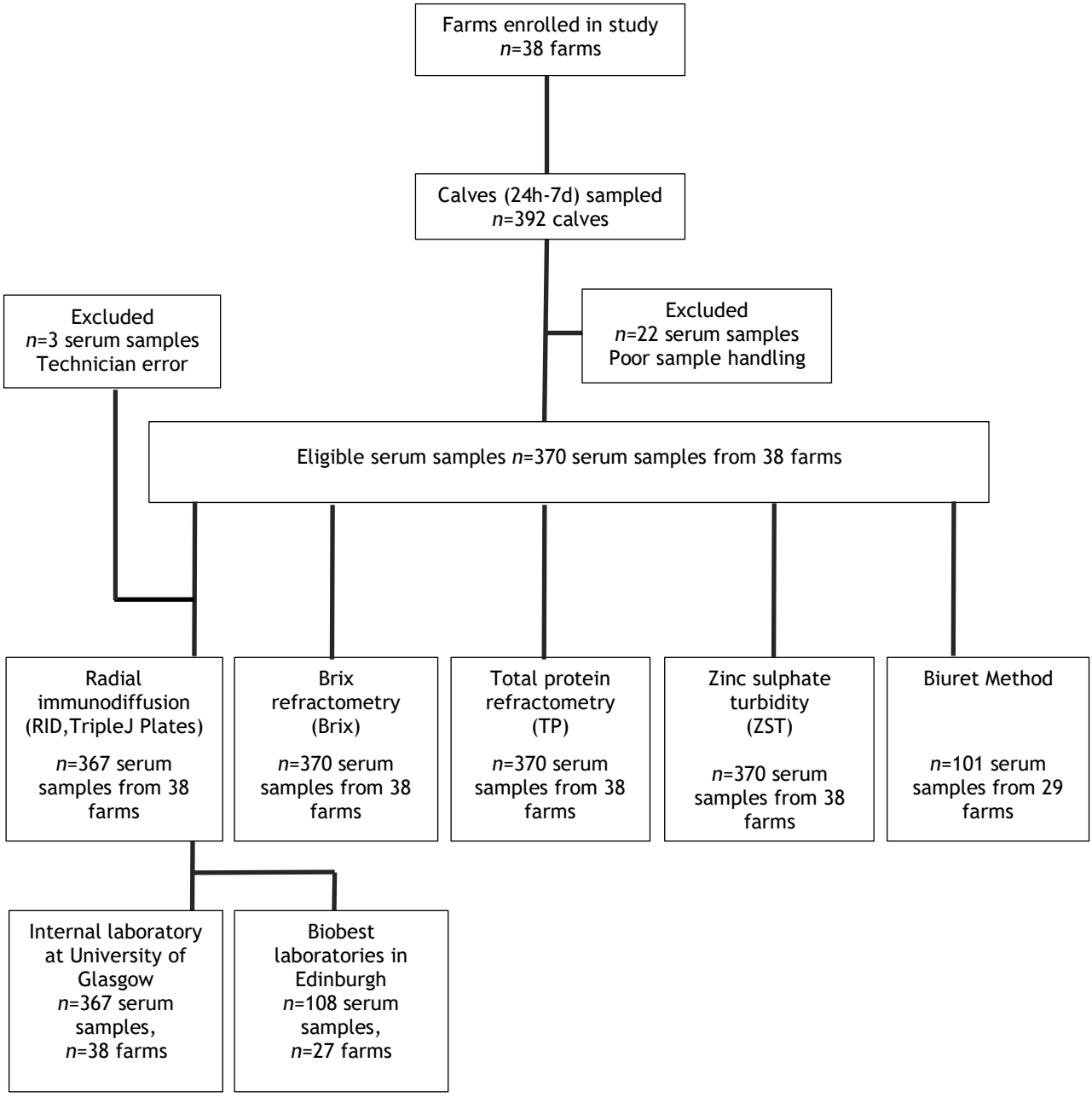


Figure 3-2 Branch diagram explaining the missing data from the original 392 serum samples collected and the samples available for statistical analysis

All 252 colostrum samples enrolled in the study were fit for testing. Table 3-1 shows how many serum and colostrum samples eligible for testing were obtained per region.

Region	Serum	Colostrum
Dumfries and Galloway	102	93
Stirlingshire	170	83
Lanarkshire	98	76
Samples collected by not suitable for analysis	22	0
Total	392	252

Table 3-1 The serum (n = 392) and colostrum (n=252) samples collected between June and February 2019 and eligible to undergo testing from each geographical region in Scotland – Dumfries and Galloway, Stirlingshire and Lanarkshire.

3.3 Baseline demographic of study population

Figure 3-3 shows the age distribution of the 370 calves enrolled in the study between February and June 2019. The mean and median age was four days, SD ± 1.74. When assessed visually the distribution appears approximately normal. The *p* value of the Shapiro Wilk test was > 0.05 therefore the null hypothesis was accepted that distribution was normal. The breed and sex of the calves sampled were not recorded.

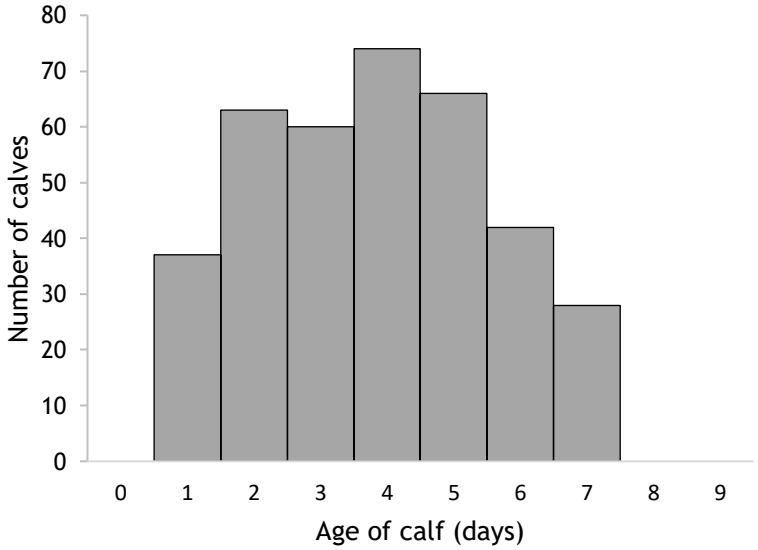


Figure 3-3 Histogram showing the normal distribution of age of the 370 calves blood sampled for serum samples to undergo IgG testing in the study between February - June 2019.

3.4 Descriptive statistics of serum sample IgG testing

The descriptive statistics of the serum IgG testing carried out on the 370 calf serum samples obtained during the study are shown in Table 3-2.

Test	Total (n)	Mean	Median	SD	Range
TP Refractometer (g/dL)	370	5.7	5.6	1.1	2.2-12.0
TP Biuret (g/dL)	101	6.3	5.9	1.2	4.2-12.0
Brix (%)	370	8.7	8.6	1.4	4.0-16.3
ZST (unit)	370	21.3	20	11.2	0-61
RID(Glasgow) (mg/mL)	367	20.6	21.3	9.4	0.5-48
RID(Biobest) (mg/mL)	108	22.3	24	8.4	2-60.0

Table 3-2 Descriptive statistics of the five testing strategies that directly or indirectly measure IgG concentration in 370 serum samples from calves aged 1- 7 days old from 38 Scottish dairy farms sampled between February and June 2019.

The descriptive statistics of serum IgG testing for each Scottish geographical region involved in the study were assessed (Table 3-3). The TP as measured by Biuret and the RID values measured at the commercial lab were not included due to the small subset of sample size per region. The purpose of the regional breakdown was to examine the data for any outliers within the region at may affect the results as at study population level.

Region	Test	Total (n)	Mean	Median	SD	Range
Dumfries and Galloway	TP (g/dL)	102	5.7	5.6	1.4	2.2-11.8
	BRIX (%)	102	8.8	8.6	1.6	4-16
	ZST (units)	102	22.6	21	11.3	0-54
	RID(Glasgow) (mg/mL)	101	20.3	20.5	9.8	0-48
Stirlingshire	TP (g/dL)	170	5.6	5.6	0.9	2.4-8.8
	BRIX (%)	170	8.7	8.7	1.2	4.8-12.8
	ZST (units)	170	21.4	20.5	11.1	1-61
	RID(Glasgow) (mg/mL)	170	20.8	21.3	8.6	2.50-43.50
Lanarkshire	TP (g/dL)	98	5.5	5.4	1.1	3.4-12
	BRIX (%)	98	8.4	8.4	1.16	5.6-11.4
	ZST (units)	98	19.7	19.5	11.2	0-50
	RID(Glasgow) (mg/mL)	96	20.0	21.6	10.8	0-47

Table 3-3 Descriptive statistics, by geographical region, for the five testing strategies that directly or indirectly measure IgG concentration in serum samples from dairy bred calves involved in the study from February – June 2019. Please refer to Figure 3-2 to explain missing data from RID(Glasgow) in each geographical region.

The frequency distributions of the results for all five direct and indirect serum IgG testing methods are show in Figure 3-4. The smaller subsets for TP Biuret and RID(Biobest) contained 101 samples and 108 samples respectively. The distributions were visually assessed to be normal.

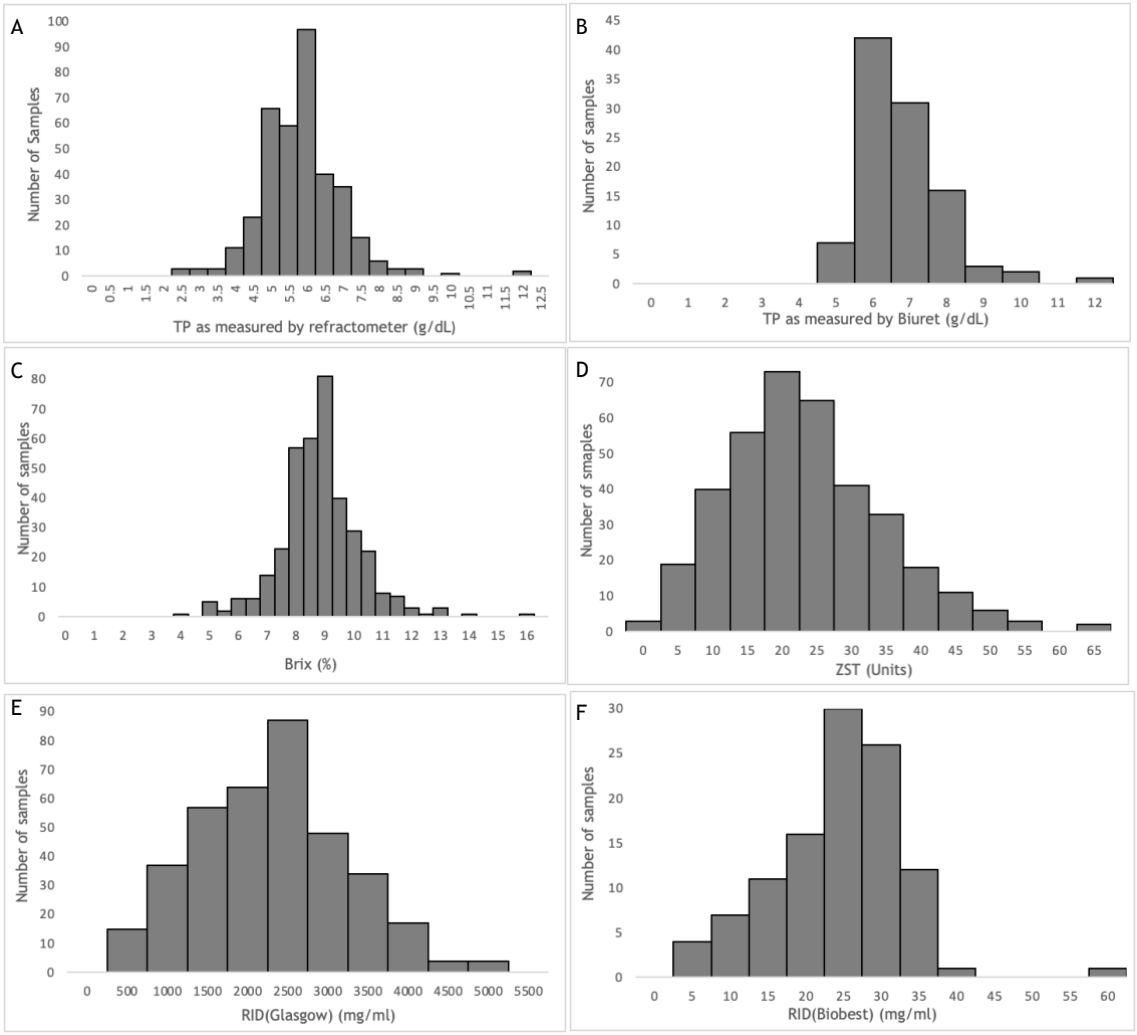


Figure 3-4 A-F. Frequency distribution histograms of the calf serum samples sampled between February – June 2019 showing the distribution for each direct and indirect testing strategy (ZST, Brix, TP (Biuret method and refractometer) and RID (Glasgow and Biobest)). Sample size for ZST, Brix and TP were 370, for RID(Glasgow) 367, and for TP Biuret and RID(Biobest) 101 and 104 respectively.

3.5 Prevalence of Failure of Passive Transfer in the studied dairy calf population

The flow diagram in Figure 3-5 shows the results of the reference and indirect testing in context of missing data. Table 3-4 shows the overall prevalence of FPT in the studied dairy calves as determined by each testing strategy at the given cut points. The cut points were decided *a priori* after a literature review, as discussed in Chapter 2 Material and Methods.

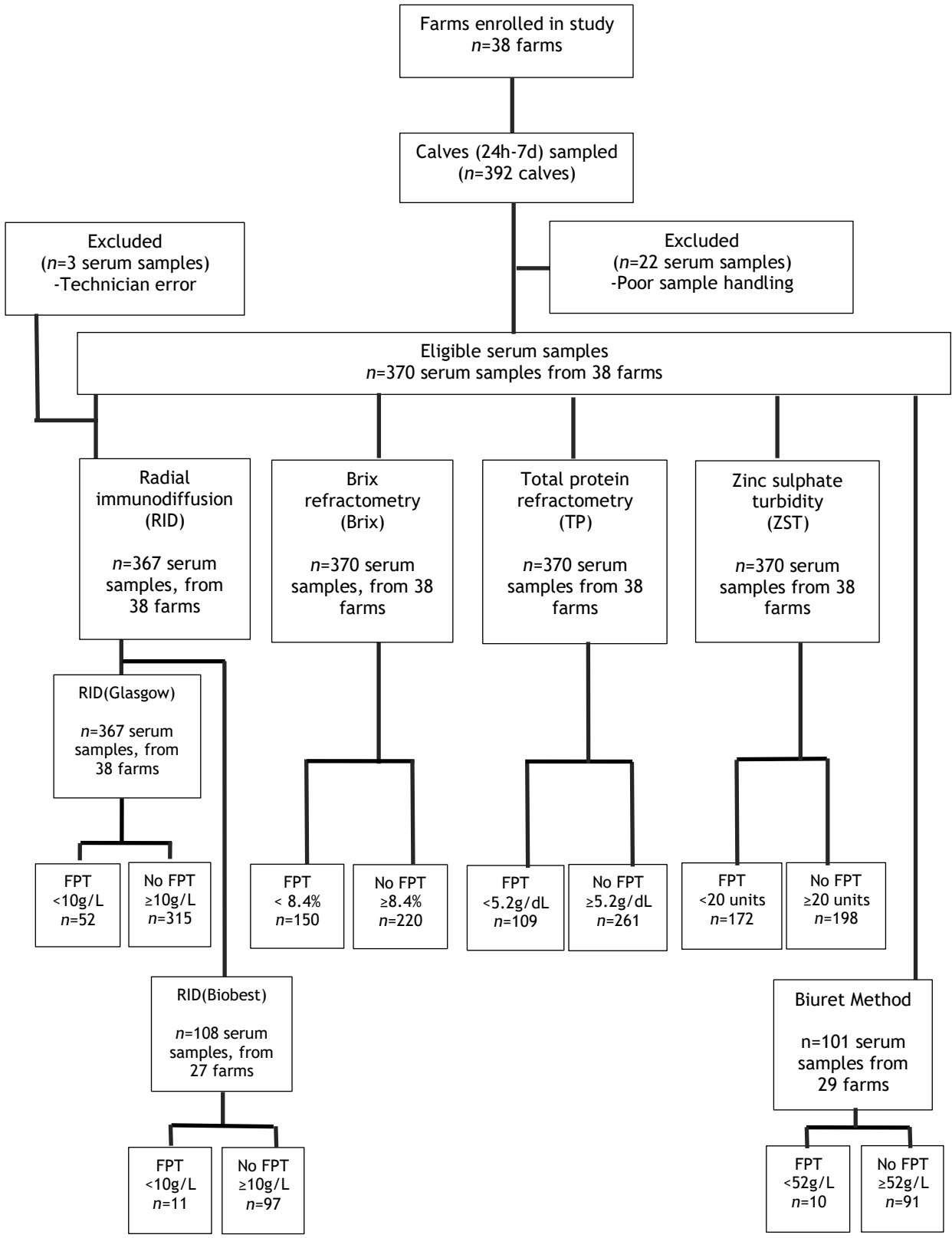


Figure 3-5 Flow diagram showing the results of serum sample testing using reference test (RID) and indirect tests (Brix, TP, ZST)

Test	Total (n)	Cut Point	Prevalence of FPT (%)	95% Confidence Intervals	Number with FPT (n)
TP	370	5.2 g/dL	29.5	24.8-34.0	109
TP Biuret	101	5.2 g/dL	9.9	4.9 – 17.5	10
Brix	370	8.4 %	40.5	35.5 - 45.0	150
ZST	370	20 units	46.5	41.4 – 50.0	172
RID(Glasgow)	367	10 mg/mL	14.2	10.8 – 18.2	52
RID(Biobest)	108	10 mg/mL	10.2	5.2 – 17.5	11

Table 3-4 Prevalence of FPT in studied dairy calves on 38 commercial dairy farms as determined by each direct and indirect testing strategy (TP (Biuret and refractometer), Brix, ZST, RID(Glasgow and Biobest)) at given cut point.

3.6 Prevalence of Failure of Passive Transfer by reference test RID(Glasgow) and RID(Biobest)

The FPT prevalence was determined by the reference test RID(Glasgow). The overall prevalence of FPT in the sampled dairy calf population was found to be 14.2 % (n=52/370). From the subset of samples sent to the commercial laboratory Biobest for validation of the RID technique, the FPT prevalence was determined to be 10.2 % (n=11/108).

3.7 Prevalence of Failure of Passive Transfer on individual farms

Figure 3-6 shows the individual farm prevalence of FPT amongst dairy calves of the 38 farms enrolled in the study. Eleven farms out of the 38 enrolled had 0% FPT prevalence as determined by RID(Glasgow) testing. However, the mean number of calves sampled per farm was 9.7 therefore these results should be interpreted with caution. Only 8/38 farms achieved the 12 calves to be sampled as stipulated in the methodology.

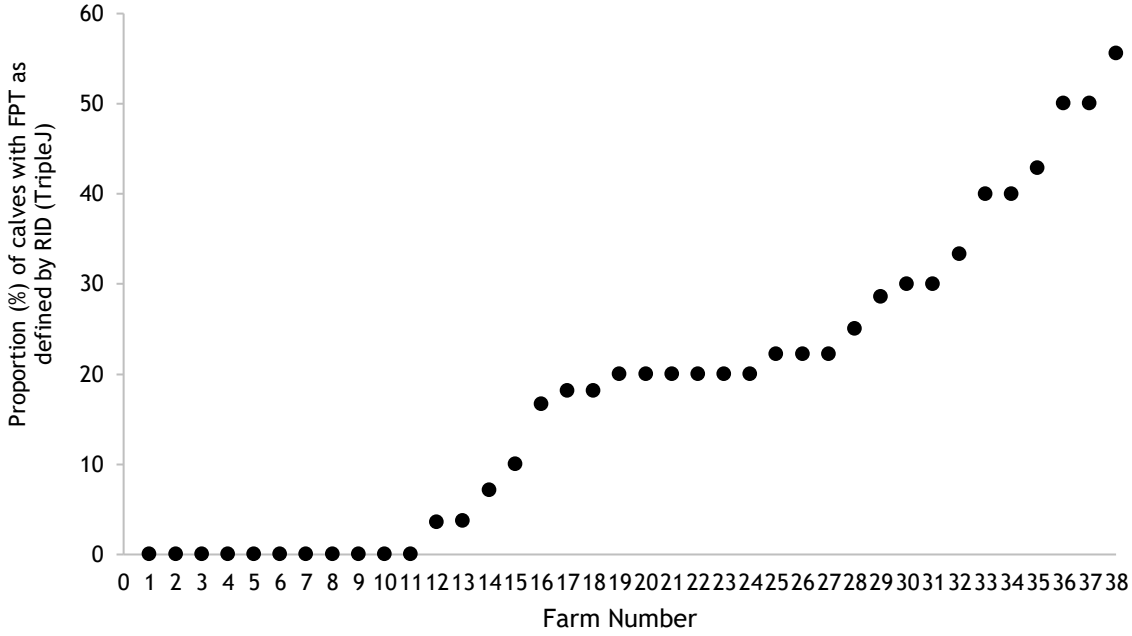


Figure 3-6 Scatter graph showing the proportion of calves (%) with FPT, as determined by RID(Glasgow) on an individual farm, numbered 1- 38 (arbitrary), from samples collected between February 2019 – June 2019.

3.8 Descriptive statistics of colostrum quality testing

Table 3-5 shows the descriptive statistics of the colostrum quality indicators: IgG concentration (Brix %) and bacterial contamination (TBC, TCC and TEC CFU/ml).

Measure of Quality	Number	Mean	Median	SD	Range
IgG concentration Brix (%)	252	22	22	4.31	11-30
Total Bacterial Counts (CFU/mL)	252	$0.046x10^8$	21,500	$0.27x10^8$	0.000001 – $0.026x10^8$
E. coli (CFU/mL)	252	4,860	4.5	53,800	$0\text{-}0.08x10^8$
Coliform (CFU/mL)	252	68,000	415	274,000	0 – $0.02x10^8$

Table 3-5 Descriptive Statistics of colostrum quality indicators: IgG concentration (Brix (%)) and bacterial load (TBC TCC and TEC (CFU/mL)) of 252 colostrum samples collected from 34 Scottish dairy farms between February – June 2019.

Colostrum quality measure results are shown in Table 3-6 for each geographical region studied.

Region	Measure of Quality	Number	Mean	Median	SD	Range
Dumfries and Galloway	**Brix** (%)	93	21.29	21	3.82	11 - 30
	Total Bacterial Counts (CFU/ml)	93	$0.8x10^8$	80000	$0.4x10^8$	2000 – $2.6x10^8$
	E.coli (CFU/ml)	93	11,900	10	87,900	0-800,000
	Coliform (CFU/ml)	93	102,000	1050	326,000	8-$0.02x10^8$
Stirlingshire	**Brix** (%)	83	24.14	25	3.64	16-30
	Total Bacterial Counts (CFU/ml)	83	$0.033x10^8$	8000	$0.25x10^8$	100-$2.3x10^8$
	E.coli (CFU/ml)	83	1,200	1	10,500	0-99,000
	Coliform (CFU/ml)	83	53,700	80	243,000	0-$0.02x10^8$
Lanarkshire	**Brix** (%)	76	20.55	21	4.68	11 - 30
	Total Bacterial Counts (CFU/ml)	76	$0.013x10^8$	22000	$0.06x10^8$	1000 – $0.36x10^8$
	E.coli (CFU/ml)	76	240.46	2	1376.43	0-8800
	Coliform (CFU/ml)	76	42,300	600	23,200	10-$0.02x10^8$
Total		252				

Table 3-6 Descriptive Statistics of colostrum quality indicators: IgG concentration (Brix (%)) and bacterial contamination (TBC TCC and TEC (CFU/mL)) by geographical region (Dumfries and Galloway, Stirlingshire and Lanarkshire) from 252 colostrum samples from 34 Scottish dairy farms collected between February and June 2019.

The frequency distributions of the Brix %, TBC, TEC and TCC are shown in Figure 3-7. The frequency distribution of Brix % appears approximately normally distributed when assessed visually; however, the distribution of TBC, TEC and TCC are not. In this study the means for TBC, TEC and TCC are skewed due to outlying extremes which is not uncommon for count data. The standard deviation for these colostrum quality measures indicates a wide spread of values.

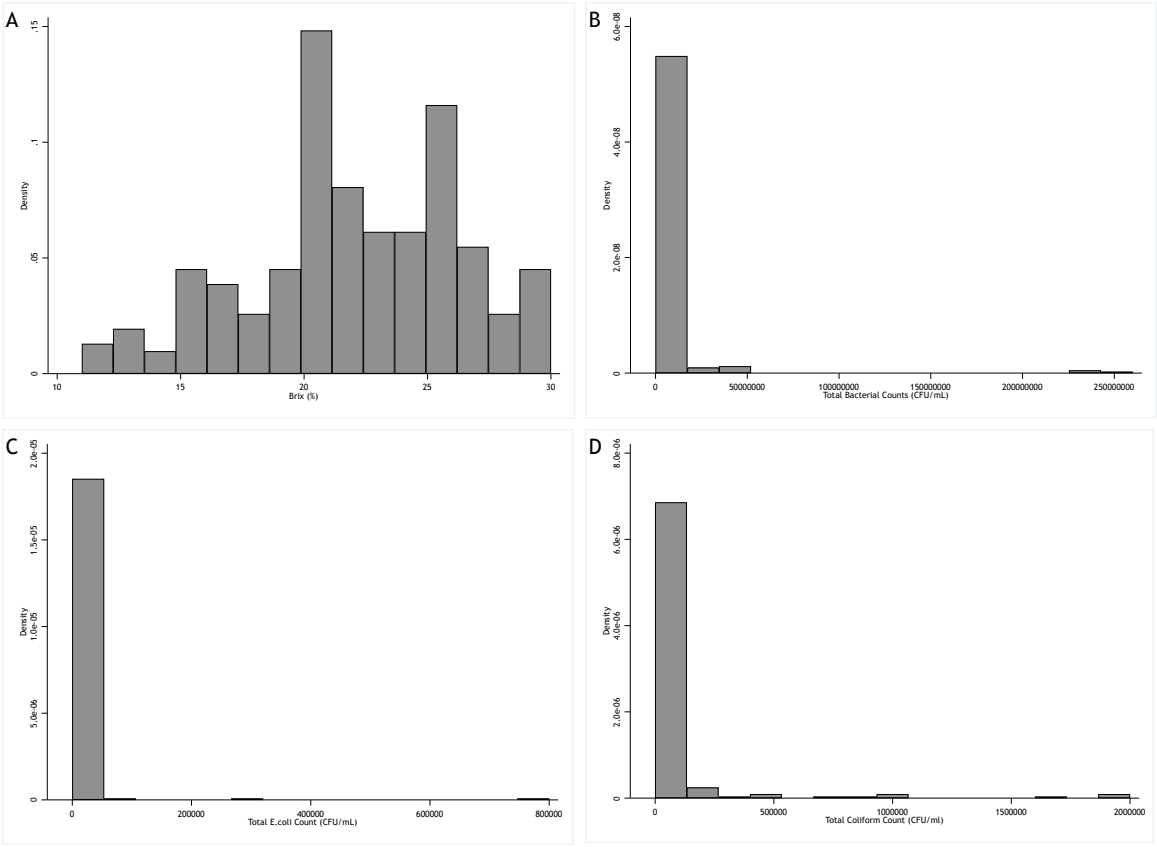


Figure 3-7 Frequency distribution histograms showing the colostrum quality indicators: A. Brix, B. TBC C. TEC and D. TCC of 252 colostrum samples taken from 34 Scottish dairy farms between February and June 2019

The results for the 252 colostrum samples were analysed with respect to standard industry threshold for acceptable IgG concentration and bacterial contamination of colostrum. Industry thresholds are shown in Table 3-7. Whilst there is no standard industry threshold for *E.coli* contamination, any *E.coli* contamination is undesirable, therefore the arbitrary cut point of 20 CFU/mL was used (Fecteau *et al.*, 2002, Stewart *et al.*, 2005). Table 3-8 shows the proportion of samples meeting industry thresholds for good quality colostrum. When industry recommendations for Brix, TBC and TCC in colostrum at the point of feeding, were considered, only 39.28 % (n = 99/252) met all three criteria and were therefore considered good quality colostrum.

Measure of Colostrum Quality	Industry Threshold	Reference (Year of Publication
IgG Concentration	50 mg/mL	Godden *et al.* (2019)
Brix (Indirect IgG Concentration)	22 %	Godden *et al.* (2019)
Total Bacterial Count	100,000 CFU/mL	Godden *et al.* (2019)
Total Coliform Count	10,000 CFU/mL	Godden *et al.* (2019)
Total *E.coli* Count	None available	None available

Table 3-7 Industry standard threshold for acceptable IgG concentration as measured by RID (reference standard) and Brix bacterial contamination (TBC, TCC and TEC) of colostrum as determined by pervious published literature.

Colostrum Quality Measure	Percentage of Samples [95% CI] (n)
Brix threshold (>22% Brix)	55.9 % [49.6-62.2] (141/252)
TBC threshold (<100,000 CFU/mL)	69.4 % [63.4-95.1] (175/252)
TCC threshold (<10,000 CFU/mL)	80.2 % [74.7-84.9] (202/252)
Good quality colostrum: >22% Brix, TBC <100,000 CFU/mL TCC <10,000 CFU/mL	39.3 % [33.2 – 45.6] (99/252)

Table 3-8 The measure of colostrum quality and industry threshold to indicate good quality colostrum alongside the percentage of the 252 colostrum samples collected from 34 dairy farms between February – June 2019 that achieved the industry standards.

The scatter graphs shown in Figure 3-8 show the proportion of samples failing each colostrum quality indicator threshold at individual farm level.

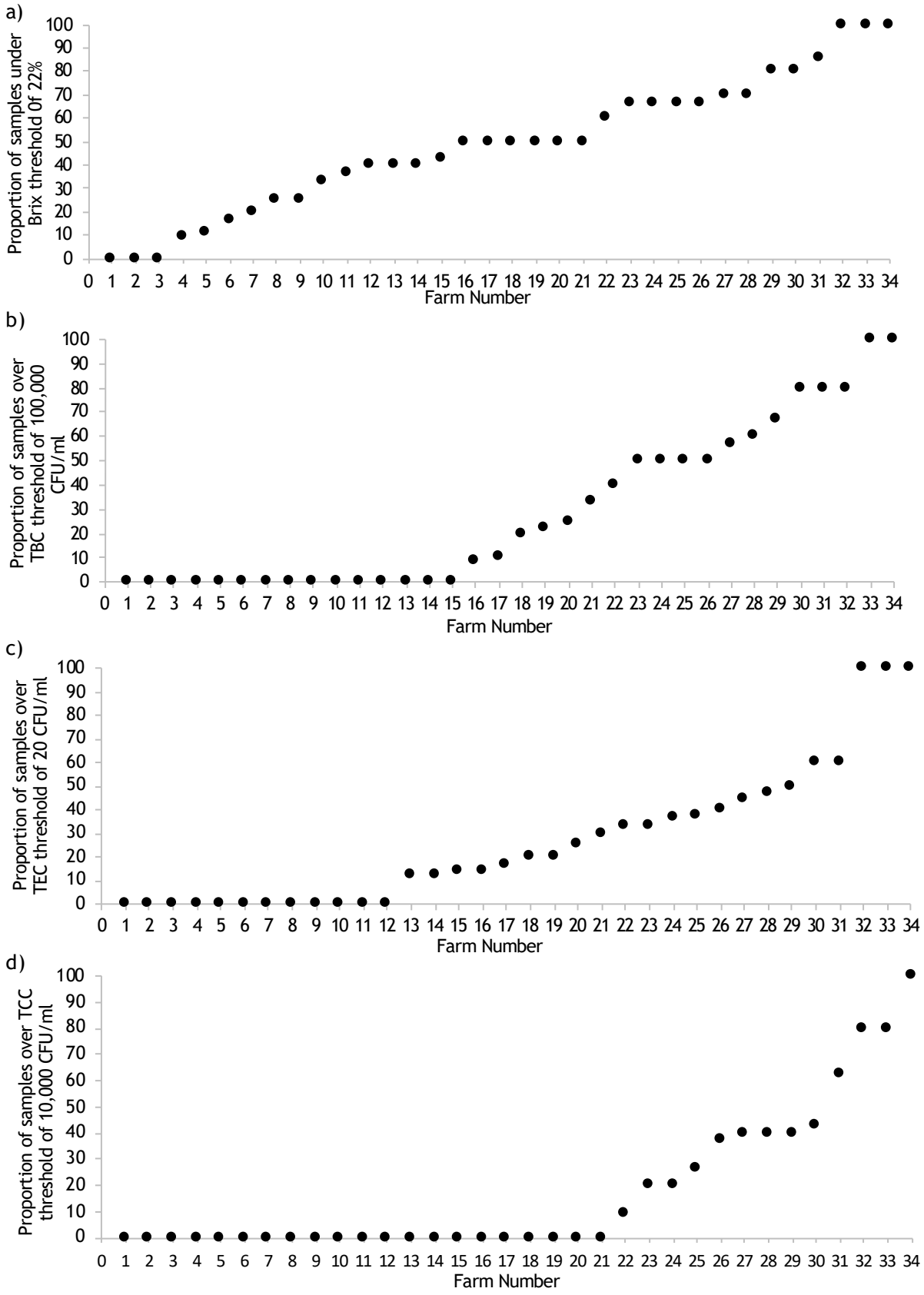


Figure 3-8 Scatter graphs showing the proportion of colostrum samples from each of the 34 dairy farms (arbitrarily numbered from 1-34) enrolled in the study between February-June 2019 failing to meet colostrum quality thresholds a)>22 Brix (%) b)<100,000 TBC (CFU/mL) c) <20 TEC CFU/mL d) <10,000 TCC/mL. Each farm is not represented by the same farm number in each graph. Proportions of each colostrum quality indicator are in ascending order to ease interpretation by the reader.

3.9 Discussion: serum testing

3.9.1 Study design

3.9.1.1 Internal and external validity.

The trial was conducted on a purely voluntary basis and farms were enrolled conveniently from the client lists of two commercial veterinary practices. This means that the data captured in this study is potentially from more progressive farmers who may have more inclination to be involved in this type of research work. Their progressive nature may be reflected in their farm businesses such that management protocols are in place to maximise efficiency and animal health and welfare (i.e. calves may be less likely to have FPT). Whilst the farms involved may represent a more progressive cohort, all calves born on the farm in the study period had the potential to be eligible for enrolment regardless of breed or sex. However, only a sample of calves born in the study period were sampled. This was because eligible calves were only sampled by commercial, private veterinarians (PVS) at routine farm visits. In this type of field-based research, a balance must be struck between ensuring accuracy and precision of results and ease of conducting the study methodology on commercial farms.

The farms enrolled in the study were from three geographical areas of Scotland. These areas are representative of the areas in which the majority of dairying occurs in Scotland (Figure 3-1). One criticism could be that Ayrshire was not included however it is in the author's opinion every effort was made to enrol a range of farms, herd sizes (Table 3-1) and cover an area sufficient to ensure external validity of the study and the results. However, care should be taken when extrapolating results to avoid over interpretation.

The methodology of the study was to sample 12 calves per farm. Previous literature suggests sampling a minimum of 12 calves per farm to give appropriate confidence in results although other studies have published herd prevalence results with a maximum of 10 calves sampled per herd (McGuirk and Collins., 2004; Beam *et al.,* 2009). However, from these two peer reviewed papers it is not clear how these numbers have been determined. The mean number of calves sampled per farm was 9.7. Only 8/38 (21.05%) farms achieved sampling of the desired 12 calves. Sample size is crucial to determine FPT at a

herd level; too few and they can be unrepresentative, too many and time and money are wasted (Cuttance *et al.*, 2019). The variable number of calves sampled per farms was likely to be due to calving patterns, availability of eligible calves when the PVS visited the farms and motivation from farmer and PVS.

From a project run in parallel with this study it was established that clinicians in Scotland are not sampling the recommended representative sample size before making clinical assumptions based on indirect testing (Denholm and Morrison., 2021). At the herd level, the proportion of calves testing positive for FPT is important, opposed to mean serum IgG concentration. McGuirk and Collins. (2004) recommended that the on-farm intervention cut point for FPT is set at 20% prevalence when an appropriate sample of calves, usually greater than 10 – 12 and aged between 1- 7 days, have been tested using one of the testing strategies described. As previously discussed, the sample size calculation for numbers of 10-12 calves is not clear in the peer reviewed literature. Practitioners should be mindful that adequate numbers of calves have been sampled, on multiple occasions during the herd's calving pattern, to ensure confidence in the conclusions drawn regarding FPT.

Lombard *et al.* (2020) proposed changes to intervention levels that potentially make the situation more complicated to farmers but maximise the benefits of lower morbidity seen at higher concentrations of IgG. Sample size and intervention cut points are discussed further in Chapter 5 and 6. Twenty out of the thirty-eight (47.37 %) farms enrolled had an FPT prevalence of 20% or higher. However, this result should be interpreted with caution as the number of calves enrolled from each farm ranged from 1 – 28, with the mean being 9.7 calves. Additionally, serum samples were only obtained from February to June 2019 therefore there could be bias as annual variations in FPT due to environmental and management conditions at different times of the year were not represented. Having said that the study period captured the end of the winter period in Scotland, Spring and the start of Summer.

3.9.1.2 Baseline demographics of the study population

Only calves aged between 1 – 7 days of age were enrolled in the study. This was in line with previous FPT prevalence studies and literature recommendations

(McGuirk and Collins., 2004; MacFarlane *et al.,* 2015; Abuelo *et al.,* 2019). Hancock. (1985) investigated the correlation between serum IgG concentrations in the first to fifth week of life and concluded that the efficiency of passive transfer within a herd may be accurately assessed by sampling calves in the first two weeks of life. The median age in this study was 4 days. Burton *et al.* (1989) found concentrations of immunoglobulin peaked within the 24 – 36 hours of life. Finally, between 36 hours and 3 weeks of age, the calf's own production of IgG is estimated to be approximately 1g/day (Devery *et al.,* 1979). Therefore, sampling calves between 1- 7 days of age means a balance is struck between allowing IgG to be absorbed and before endogenous IgG production is significant.

3.9.1.3 Sample Handling

Multiple freeze-thaw cycles have been shown to degrade serum proteins (Morrill *et al.* 2015). Furthermore, bovine serum total protein (STP) concentration was noted to decline at 1.2 g/L/month of storage at -20°C for a four month period (Villarroel *et al.,* 2015). Considering sample handling in the methodology was important to ensure accurate results. At the time of sample collection, two samples were taken thereby ensuring the samples only underwent one freeze-thaw cycle between collection and testing via RID(Glasgow), TP, Brix and ZST. Only the subset of samples that underwent RID(Biobest) and Biuret testing underwent a second freeze thaw cycling. All samples were transported on ice by hand or by post to study laboratories.

3.9.2 Failure of Passive Transfer prevalence

Exploring FPT in the Scottish dairy calf population was unprecedented work. The prevalence in this study, 14.2%, was lower when compared to other UK prevalence data. Johnson *et al.* (2017) surveyed 492 dairy heifer calves from 11 dairy farms in South-east England and found the FPT prevalence to be 20.7% as measured by RID using the same cut point < 10g/L.

MacFarlane *et al.* (2015) surveyed seven dairy farms in Cheshire and the Wirral found an FPT prevalence of 26%, which is also higher than determined in this current study and by Johnson *et al.* (2007). MacFarlane *et al.* (2015) used TP refractometry as a proxy for serum IgG concentration. This indirect method has reduced specificity which leads to false positives and an overestimation of the

prevalence of FPT (Tyler *et al.,* 1996; Deelen *et al.,* 2014). Prevalence from this study as determined by TP refractometry was 29.5%. When compared with that of MacFarlane *et al.* (2015) also determined by TP refractometry, the two estimates are similar.

The two prevalence estimates are similar despite different cut points used. MacFarlane *et al.* (2015) used 5.6 g/dl as the cut point to determine FPT status. Whereas in this study, a cut point of 5.2 g/dl was used based on work carried out by Calloway *et al.* (2002) who found that cut points of 5.2 g/dl and 5.0 g/dl gave accurate results and cut points higher or lower tended to decreases the accuracy of the test. This was corroborated by later work carried out by Chigerwe *et al.* (2008) that states 5.2 g/dl is the equivalent to 10mg/mL which is the cut point used for RID testing in this study. RID is a direct measure of IgG and considered the reference test for determining the true serum IgG concentration therefore this measure was used to determine the prevalence of FPT in this study (Weaver *et al.,* 2000). The use of the cut point of < 10mg/mL was to determine FPT status has been well established in previous studies (Bielmann *et al.,* 2010; Buczinski *et al.,* 2016).

When comparing the prevalence from this study with international literature, the prevalence is broadly speaking similar to results from US studies. Beam *et al.* (2009) found the prevalence to be 19.2 % and a more recent study by Urie *et al.* (2018) found the prevalence to be 15.6%. Both studies used RID testing methodology.

However, higher rates are reported from other countries ranging from 24.8% in New Zealand and 41.9% in Australia (Lawrence *et al.,* 2017; Johnsen *et al.,* 2019). Worldwide prevalences are reviewed in more detail in Table 1.9, Chapter 1:Literature Review. Methodologies across literature were not uniform and some studies used indirect methods which will overestimate FPT rates through lower specificity. Overall, a prevalence of 14.86%, indicated that the Scottish dairy industry was broadly in line with the US industry. Furthermore the Scottish dairy industry was achieving lower rates of falure of passive transfer when compared other countries. However, one in seven calves will still suffer from FPT and the increased risk of the associated negative consequences. These estimates are likely to be conservative due to the likelihood of a more progressive population

of dairy farmers would have been captured in this study. Enrolment in this trial was on a voluntary basis from a list of convenience. This aspect of study design could have introduced bias by capturing a more progressive sample population, therefore any estimates of FPT are likely to be conservative. Atkinson *et al*. (2017) reported that the simple act of benchmarking and reporting, encouraged changes that improved rates of FPT. Results were reported to participants once data collection was complete to reduce any potential for bias.

3.10 Discussion: colostrum quality

3.10.1 Study design

3.10.1.1 Internal and external validity

This study is the first of its kind to describe the quality of colostrum, in terms of IgG concentration and bacterial contamination, available to neonatal calves on commercial dairy farms throughout Scotland. There are several limitations in the study design to acknowledge. Because the study only took place from February – June 2019 the colostrum available to this study is not necessarily representative of colostrum available all year round on Scottish dairy farms. It is perfectly feasible that bacterial proliferation would be higher in the warmer weather of July or August. Also, collection of colostrum samples by farm personnel means was potential for inconsistency in sampling technique however, a standard operating procedure was given to minimise this. Any inconsistencies arising were likely to be random. As per the serum sample collection, enrolment in this study was voluntary and therefore this sample is potentially representative of more progressive dairy farmers as they may be more likely to engage in a research project. The strength of this study lies in the fact is more in depth than previous UK studies. 252 samples from 34 commercial dairy farms across the three regions of Scotland were available for analysis. MacFarlane *et al*. (2015) looked at colostrum quality and collected 406 samples from only seven English dairy farms. This study is more in line with recent research from Hyde *et al*. (2020) which collected 328 samples from 56 farms in Scotland, England, and Wales; however, only 151 of these were from the point of feeding to calves so many of these samples are not representative of the colostrum actually fed to neonatal calves.

3.10.1.2 Sample Handling

Once collected samples were frozen and then transported to the study laboratory on ice by hand or post. Alrabadi (2015) froze 30 raw milk samples for eight weeks, defrosted and tested TBC and TCC weekly, and concluded that bacterial counts decrease significantly as the freezing time increases. Sample testing was carried out on a weekly basis from the start of the study period. Samples were only thawed to carry out Brix and initial TBC, TEC and TCC testing were carried out. Repeated freeze/thaw cycles should be avoided as they potentially will decrease the IgG content (Morrill *et al.,* 2015). Morrill *et al*. (2015) did not evaluate the consequence of repeated freeze/thaw cycles on bacterial counts.

3.10.2 Colostrum IgG concentration

Brix is an indirect measurement of colostrum IgG concentration. As with serum IgG measurements, the direct measure RID is considered the reference test for determining colostrum IgG. Numerous studies show that Brix is an accurate test for rapid, inexpensive, on farm measurement of colostrum quality (Bartens *et al.,* 2016; Buczinski *et al.,* 2016; Elsohaby *et al.,* 2016).

The literature reports various recommendations for the Brix cut point to use as an indicator of acceptable IgG concentration. Quigley *et al*. (2017) found that a cut point of 21% Brix had the highest accuracy of IgG determination at 88.5% (95% CI = 83.9 – 93.1%) when compared to reference test RID and, at this cut point, sensitivity was 92% and specificity was 65%. Bielmann *et al*. (2010) recommended 22% Brix as a cut point, whilst Bartier et al. (2015) recommends 23% Brix as a cut point. In this study, 22% Brix was used and this is generally agreed to represent 50 mg/mL IgG as measured by RID (McGuirk and Collins., 2004; Godden *et al.,* 2019).

As reported, in the current study, the mean Brix % was 22% (range = 11-30) with 44.1% of samples falling below the industry cut point of 22% for good quality colostrum. This was similar to that previously reported by a survey of UK dairy farms which had a mean Brix percentage of 21.9% (range = 10.3–34.7%) (MacFarlane *et al.,* 2015). The percentage of samples falling below the cut point in this study is marginally higher than MacFarlane *et al*. (2015) who found only

37 % of samples fell below the standard cut point of 22% Brix. The present study included a larger number of farms, 38, from three regions across Scotland compared with the MacFarlane *et al.* (2015) study where only seven farms were enrolled from one region of England, Cheshire and the Wirral. Furthermore, the present study sampled colostrum at the point of feeding to newborns whereas, MacFarlane *et al.* (2015) sampled colostrum at the point of harvesting. Bacterial contamination and storage (refrigeration, freezing and preservation) can affect the IgG concentration of colostrum (Denholm *et al.*, 2018; Godden *et al.*, 2019). Study design, when the colostrum is collected and potential for bacterial contamination and storage, could account for the increased percentage of samples falling below the Brix % cut point of good quality colostrum found.

In terms of international literature, Morrill *et al.* (2012) found that 29.4 % of the 593 colostrum samples taken from US dairy farms between June and October 2010 fell below the standard cut point for Brix %. Again, this is lower than found in this study. RID testing, which is a direct measure of IgG concentration, was used by Morrill *et al.* (2012). RID is the reference test and therefore has a higher sensitivity and specificity compared with the indirect measure of Brix used in this study (Buczinski and Vandeweerd., 2016). An Australian study by Phipps *et al.* (2016) found that 53.3% of samples failed to meet the cut point of 22% Brix. The higher failure rate could again be attributed to study design and collection methodology: Phipps *et al.* (2016) study population was mainly composed of block calving herds and after collection colostrum was only stored at -4°C prior to analysis. Furthermore, 20% of samples from Phipps et al., 2016 were from a pooled source, whereas no samples were from a pooled source in the present study. It was well established in the literature that when colostrum is pooled the poorer quality colostrum, in terms of IgG concentration, is overrepresented. (Weaver *et al.*, 2000).

3.10.3 Bacterial contamination (TBC, TCC, TEC)

As previously discussed, bacteria can reduce the absorption efficiency of IgG by the intestine by binding directly to IgG, blocking uptake, or damaging neonatal enterocytes, directly limiting their permeability and accelerating closure (McGuirk and Collins., 2004; Johnson *et al.*, 2007; Godden *et al.*, 2009). Colostrum samples in this study were taken at the point of feeding, meaning any

detected bacterial contamination could have come from the harvesting, storing, or feeding processes. It is the standard across the peer reviewed literature to use 100,000 CFU/mL as the threshold for TBC and 10,000 CFU/mL as the threshold for TCC therefore some comparisons between other research and this study can be drawn (Godden *et al.,* 2019).

This study found that 30.6% of samples exceeded the TBC threshold, 100,000 CFU/mL. This is in broad agreement with a recently published UK paper that found 29.6 % of colostrum samples exceeded the TBC threshold (Hyde *et al.,* 2020). From the international literature, direct comparisons can be drawn between a Canadian study, Fecteau *et al.* (2002), and this study because methodologies are similar – all samples were taken from the point of feeding. Fecteau *et al.,* (2002) found that 35.9% of samples exceeded the TBC threshold which was again like findings in this study.

A slighter higher prevalence of contamination was found in the US by Morrill *et al.* (2012) and Australia by Phipps *et al.* (2016), where both studies found 42% of samples exceeded the TBC threshold. Several study design aspects could explain this increase. Phipps et al. (2016) surveyed pooled samples from block calving herds and stored samples at -4°C. Most other peer reviewed literature exploring bacterial contamination stored colostrum samples prior to testing at – 18 - 20°C (Fecteau *et al.,* 2002; Morrill *et al.,* 2012) Morrill et al. (2012) did not necessarily take samples at the point of feeding and sampled a mixed of individual, pooled, fresh, refrigerated, and frozen samples. A New Zealand study found that 91.4% of samples exceeded the TBC threshold; however, the samples studied were pooled samples from grass based, seasonal calving herds (Denholm *et al.,* 2017). Additionally, samples were stored at 4°C after collection and during transportation to the laboratory for analysis. Cummins *et al.* (2016) found that regardless of storage at room temperature or 4°C, TBC increased by 5% in the first six hours after harvesting. Direct comparison to Scottish results should be made with caution.

The samples from Dumfries and Galloway were more contaminated, mean TBC = $0.8x10^8$ CFU/mL, than those from the Stirlingshire and Lanarkshire areas of Scotland, mean TBC = $0.03x10^8$ CFU/mL and $0.013 x10^8$ CFU/mL respectively. It is postulated that this could be due to larger herd size and high number of staff

on these farms responsible for delivering different aspects of colostrum management aspects and therefore lack of accountability. Because of the colostrum collection protocol described in Chapter 2: Material and Methods, the collection and storage method did not vary between regions. Table 3-9 shows the number of herds and average size in each region involved in the study.

Region	Number of Herds	Average Herd Size
Dumfries and Galloway	331	287
Stirlingshire	33	205
Lanarkshire	89	89

Table 3-9 Showing the regions involved in the study and the total number of dairy herds attributed to each with average herd size (Source: Scottish Dairy Cattle Association)

In this study, 20 % of samples were found to have a TCC greater than the threshold 10,000 CFU /mL. This is higher compared with data from Hyde *et al.* (2020) who found 7.6% of samples exceed the TCC threshold. However, some of their colostrum samples were taken at harvesting directly from the udder. Samples taken directly from the udder usually have minimal bacterial contaminated (Stewart *et al.*, 2005).

When compared with international literature, the percentage exceeding the TCC threshold in this study is also higher. Morrill *et al.* (2012) found that all TCCs were well below industry standards. Phipps *et al.* (2016) found that only 6% (n = 16) of samples from their Australian survey of colostrum quality exceed that coliform threshold. Samples collected by Phipps *et al.* (2016) were sampled from feeding apparatus at the point of feeding newborn calves; however, the laboratory method used to establish the TCC were not expanded on. Morrill *et al.* (2012) do not specify when samples were taken between harvesting and feeding but did use the same laboratory technique as this study to detect TCC. The main sources of coliform contamination in colostrum are from faecal and environmental sources (Stewart *et al.*, 2005). They are important neonatal disease causing pathogens and should be at the lowest levels possible in colostrum (Elizondo-Salazar *et al.*, 2010). The results in this study show that one in five colostrum samples from the study dairy farms exceeds industry recommendations for coliform contamination, therefore putting newborn calves at increased risk of FPT and neonatal disease. The study's methodology meant that samples were frozen after collection until analysis meaning samples only

went through one freeze thaw cycle. Freezing can negatively affects some bacterial species (e.g. *E.coli*) and will reduce bacterial counts as the freezing period increases (Alrabadi., 2015). Therefore, some samples in this study may well have been more contaminated than results show and estimates should be considered conservative.

TBC and TCC are highly correlated; however, it has been proposed that TCC are a better predictor of negative health events in calves. While results of TCC were dichotomized as acceptable or not acceptable, in reality TCC is a continuous biological scale. The negative relationship that exists between TCC and serum IgG suggests that the lower then TCC the better (Godden *et al.*, 2012; Hyde *et al.*, 2020).

The specific coliform, *E.coli*, was identified in this study using an *E.coli* specific Petrifilm™. *E.coli* can be responsible for intestinal disease such as neonatal diarrhoea and extra-intestinal disease such as meningitis and septicaemias, and can have low infectious dose (Stewart *et al.*, 2005). When *E.coli* causes a bacteraemia, it can rapidly lead to death due to the various virulence factors on the bacteria such as lipopolysaccharides, O antigen, K1 antigen and cytotoxins (Fecteau *et al.*, 2001). One other colostrum study that specifically identified *E.coli* contamination of colostrum samples in their methodology used the arbitrary cut point of 1000 CFU/mL (Fecteau *et al.*, 2002). From the literature surrounding *E.coli* and its clinical effects, it is prudent that any *E.coli* present is undesirable. In this study, the arbitrary cut point of 20 CFU/mL was used and 27% of samples exceed this threshold. Meaning approximately 1 in 4 samples are contaminated to a degree that could increase the risk of FPT and expose calves to a pathogen that can cause severe clinical disease and increase risk of mortality.

Ninety-nine (39.3 %) of the 252 samples analysed met all three quality criteria in terms of IgG concentration (Brix), TBC and TCC. In the international literature, Phipps *et al.* (2016) found that only 23% of colostrum samples (n=55) met all recommendations and Morrill *et al.* (2012) found 40% (n = 326) met all recommendations. There is clearly scope for improving the quality of colostrum available to neonates on dairy farms and this will be discussed in further detail

in Chapter 4: Questionnaire Data and Risk Factor Analysis for Failure of Passive Transfer (FPT) and Colostrum Quality.

3.11 **Summary**

It can be concluded that this sample of Scottish dairy bred calves on commercial dairy farms had an FPT prevalence of 14.2% as determined by RID testing. This means that approximately 1 in 7 calves are at risk of the associated negative consequences in terms of health, welfare, and productivity. Furthermore, 61 % of colostrum samples failed in terms of industry quality thresholds.

The results from this study demonstrate that there is a proportion of the Scottish dairy calf population is at risk of FPT and quality of colostrum available, in terms of IgG concentration and bacterial contamination, is an issue. This study shows it is pertinent and relevant for clinicians to investigate and monitor FPT as part of proactive involvement in calf management. Furthermore, colostrum quality investigations should form part of this preventative approach.

4 Questionnaire data and risk factor analysis for Failure of Passive Transfer and colostrum quality

4.1 Colostrum management questionnaire results

The colostrum management questionnaire responses are summarized in Table 4-1. Risk factor response categories were condensed as shown in Figure 4-1 prior to regression analysis to make sure assumptions were not based on one or two responses.

4.2 Missing data

A total of 331/367 (90 %) radial immunodiffusion (RID) (Bovine IgG RID Kit, Triple J Farms, Bellingham, WA) Triple J outcomes were available for FPT risk factor analysis. Thirty-nine RID(Glasgow) values were discounted for risk factor analysis because four farms did not return questionnaires and technician error in sample testing (See Figure 3-2, Section3.2 Missing Data). Colostrum quality measurements (Brix, TBC and TCC) were available from 245/252 (97 %) of colostrum samples. Seven colostrum samples were excluded because the farms of origin failed to return questionnaire data.

Questions	**Response** ***Number of responses (%)***				
When are newborn calves actively first fed after birth?	< 2 hours after birth *2 (5.88)*	2.5 to 6 hours after birth *21 (61.77)*	6.5 to 12 hours after birth *11 (32.35)*	12.5 – 24 hours after birth *0*	
What volume of colostrum is fed to newborn calves at first feed?	< 2 litres *1 (2.94)*	2.5 – 3 litres *14 (41.18)*	3.5 – 4 litres *15 (44.12)*	4.5 – 5 litres *2 (5.88)*	>5 litres *2 (5.88)*
When is the colostrum collected from a newly calved cow?	< 2 hours after calving *3 (8.82)*	2.5 – 6 hours after calving *17 (50)*	6.5 – 12 hours after calving *11 (32.35)*	12.5 – 24 hours after calving *3 (8.82)*	
Does colostrum from a newly calved cow get collected into a test bucket in the milking parlour?	Yes *33 (97.06)*	No *1 (2.94)*			
If NO, what do you collect the fresh colostrum into?	Robotic milking -robot bucket *(1)*				
Does the colostrum sit in a bucket before feeding to calves?	Yes *28 (82.35)*	No *6 (17.65)*			
If YES, for how long?	< 6 hours *26 (92.86)*	> 6 hours *2 (7.14)*			
If YES, is the bucket or container covered with a lid?	Yes *12 (42.86)*	No *16 (57.14)*			
Where does the colostrum go after collection?	Into another container *13 (38.24)*	Straight into calf feeder *21 (61.76)*			
Do you clean your test buckets and calf feeding equipment regularly?	Yes *34 (100)*	No 0			
Method of feeding used for feeding first feed?	Oesophageal Tube *17 (50)*	Teat Feeder *17 (50)*	Bucket *0*	Other *0*	

Questions	Response				
	Number of responses (%)				
What is the interval between first and second feed of newborn calves?	< 6 hours *6 (17.65)*	6.5 – 12 hours *21 (61.76)*	12.5 – 18 hours *7 (20.59)*	18.5 – 24 hours *0*	> 24 hours *0*
Are newborn calves fed first milking colostrum only at first feed?	Yes *31 (91.18)*	No 3 *(8.82)*			
Are newborn calves fed a mixture of first milking and later milking colostrum mixed at subsequent feeds?	Yes *4 (11.76)*	No *30 (88.24)*			
Are newborn calves fed fresh colostrum?	Yes *32 (94.12)*	No *2 (5.88)*			
Are newborn calves fed stored colostrum?	Yes *23 (67.65)*	No *11 (32.35)*			
How is colostrum stored?	Freezer *21 (75)*	Fridge *6 (21.43)*	Other *0*		
If you store colostrum, do you have a temperature gauge on your fridge or freezer?	Yes *9 (26.47)*	No *25 (73.53)*			

Table 4-1 Colostrum management questionnaire and responses from 34 farms enrolled in the study between January - June 2019

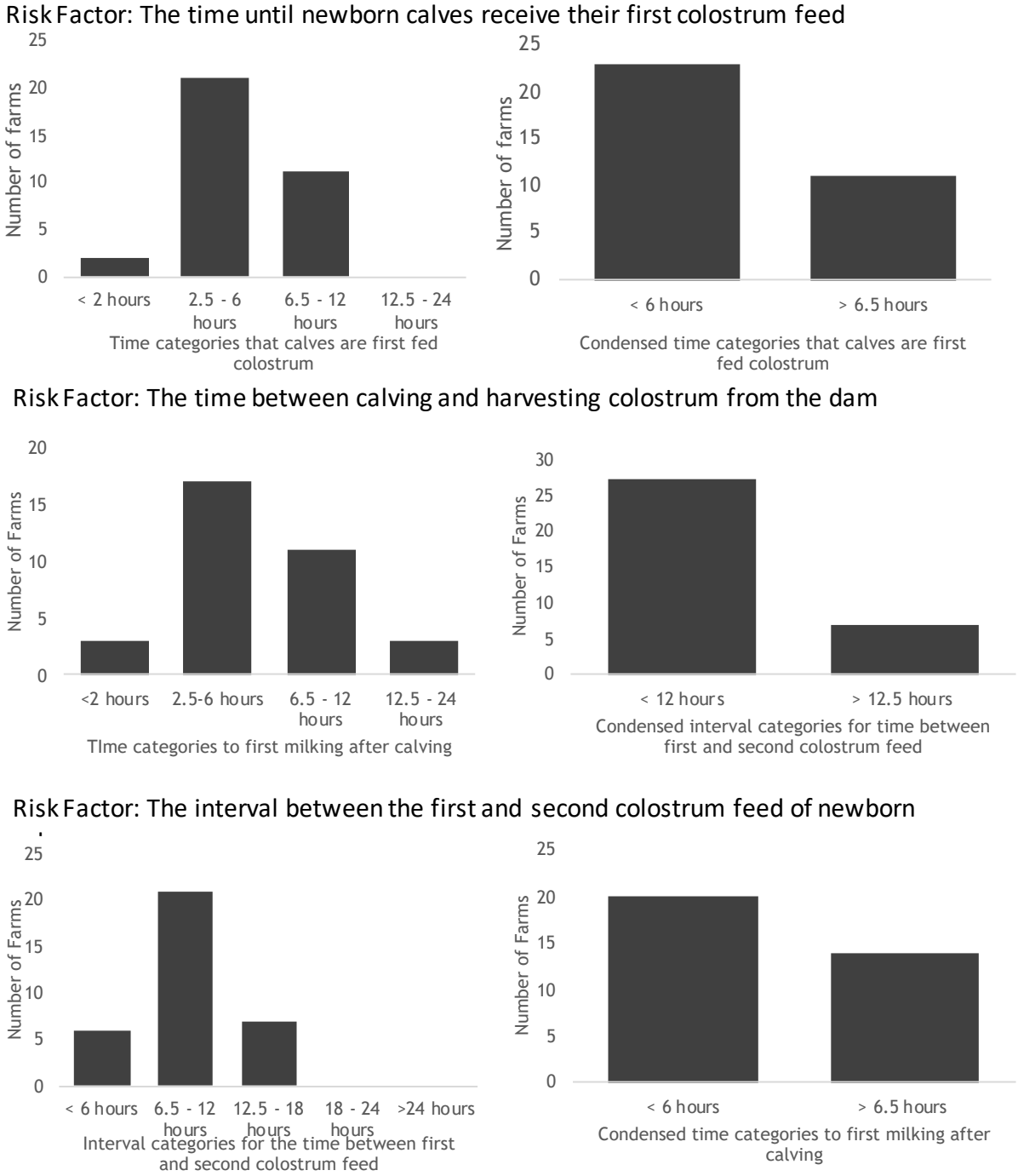


Figure 4-1 Showing the risk factors response categories for FPT and Colostrum Quality that were condensed based on their distribution.

4.3 Farm level risk factors associated with FPT: logistic regression analysis

The intraclass correlation (ICC) is shown in Table 4-2 and was found to be 0.153, indicating that the effect of clustering of calves due to the farm of origin did not have a significant impact on the outcome variable of FPT. Results of initial univariable analysis are shown in Table 4-3. Significance, at the univariable

level, was declared at $p \leq 0.2$ to ensure all potential risk factors were captured. The volume of colostrum fed at first feed was found to be significant and included in multivariable analysis.

	Intraclass Correlation	Standard Error	95% CI
Farm Name (FPT)	0.153	0.057	0.071 – 0.299

Table 4-2 The intraclass correlation and 95% Confidence Interval for FPT risk factor analysis to account for the impact of farm clustering on the FPT outcome variable of 331 serum samples from calves aged between 1-7 days of age from 34 dairy farms from the Lanarkshire, Stirlingshire and Dumfries and Galloway regions of Scotland between January and June 2019.

Outcome	Farm Level Risk Factor	Category of Risk Factor	Co-efficient	95% CI	p-value
FPT	Age (days) at sampling	2	-0.28	-1.56 – 1.0	0.67
		3	-0.20	-1.47 – 1.08	0.76
		4	-0.54	-1.80 – 0.72	0.40
		5	-0.48	-1.78 – 0.82	0.47
		6	-0.86	-2.47 – 0.74	0.29
		7	-0.47	-2.02 – 1.09	0.56
	When are newborn calves first fed after birth?	< 6 hours	*ref*	*ref*	*ref*
		> 6 hours	-0.14	-1.21 – 0.93	0.81
	What volume of colostrum is fed to newborn calves at first feed?	**<2 litres**	***ref***	***ref***	***ref***
		2.5 – 3 litres	**-2.22**	**-4.43 – -0.01**	**0.05**
		3.5 – 4 litres	**-2.12**	**-4.28 – 0.03**	**0.05**
		4.5. – 5 litres	**-3.76**	**-6.89 – -0.62**	**0.02**
	Timing of harvesting of colostrum after calving	< 6 hours	*ref*	*ref*	*ref*
		>6 hours	-0.17	-1.19 – 0.86	0.75
	How are calves fed?	Stomach Tube	*ref*	*ref*	*ref*
		Teat Feeder	0.26	-0.76 – 1.27	0.62
	What is the interval between first and second feed of newborn calves?	< 6 hours	*ref*	*ref*	*ref*
		6.5 – 12 hours	-0.66	-1.96 – 0.64	0.32
		12.5 – 24 hours	-0.97	-2.59 – 0.65	0.24
	Are newborn calves fed first milking colostrum only?	Yes	*ref*	*ref*	*ref*
		No	0.82	-1.31 – 2.94	0.45
	Are newborn calves fed fresh colostrum?	Yes	*ref*	*ref*	*ref*
		No	-0.90	-2.84 – 1.04	0.36

Table 4-3 Univariable logistic regression analysis of farm level risk factors associated with FPT in 331 serum samples from dairy calves, aged 1 – 7 days sampled from 34 dairy farms from the Lanarkshire, Stirlingshire and Dumfries and Galloway regions of Scotland between January and June 2019. Variables significant at the univariable level are shown in bold.

All biological interactions were explored, and confounding was tested between risk factors. Variables were declared to confound each other if the coefficient changed by ≥20% and interaction terms were included if $p<0.05$. Confounding was found to occur between the volume of colostrum fed and the interval between first and second colostrum feed, as well as the volume of colostrum fed, and the method used to feed the calves. By way of explanation of the biological plausibility of this confounding: the interval between first and second

feeds was likely to be longer if the initial volume fed was larger, keeping the calf 'fuller' for longer. The volume administered by stomach tube could also be larger than suckling from a bottle, as administration tends to be easier and quicker.

	Variables to go forward to the multivariable model
FPT	Volume of colostrum fed to newborn calves at first feed
	Method of feeding
	The time interval between first and second feed

Table 4-4 FPT and associated outcome variables significant at the univariable level or had interaction or confounding going forward into the multivariable model

The final multivariable model was constructed by a backwards, stepwise elimination approach and is summarized in Table 4-5. Increasing volume of colostrum administered to neonatal calves at first feed was found to be significantly associated with reduced odds of FPT (p=0.05). Postestimation and model diagnostics found residuals were found to lie within 2 standard deviations of the mean in all cases.

FPT Quality Indicator	Risk Factor	Category of Risk Factor	Co-efficient (95% CI)	OR (95% CI)	P value
FPT	What volume of colostrum is fed to newborn calves at first feed?	< 2 litres	*ref*	*ref*	*ref*
		2.5 – 3 litres	-2.22 (-4.43 - -0.01)	0.11 (0.01-0.99)	0.05
		3.5 - 4 litres	-2.12 (-4.28 – 0.03)	0.12 (0.14-1.39)	0.05
		4.5 - 5 litres	-3.76 (-6.89 - -0.62)	0.02 (0.001-0.54)	0.02

Table 4-5 Final model in multivariable regression for FPT risk factor analysis showing risk factor, co-efficient, odds ratios (95% confidence levels) and *p-value* demonstrating the impact of the risk factor variable on FPT in 331 dairy calves sampled in 34 Scottish dairy farms from January – June 2019.

4.4 Farm level risk factors associated with colostrum quality: logistic regression analysis

The ICC is shown in Table 4-6 and is low for all four outcome variables indicating that the farm clustering effect was not a significant influence on the outcome variable.

	Intraclass Correlation	Standard Error	95% CI
Farm Name (Brix%)	0.275	0.076	0.151 – 0.445
Farm Name (TBC)	0.127	0.054	0.053 – 0.274
Farm Name (TCC)	0.35	0.083	0.207-0.525

Table 4-6 The Intraclass Correlation and 95% Confidence Interval to account for the impact of farm clustering on 245 colostrum samples quality measure collected, at the point of feeding, from 34 dairy farms from the Lanarkshire, Stirlingshire and Dumfries and Galloway regions of Scotland between January and June 2019. Variables significant at the univariable level are shown in bold.

Table 4-7 to Table 4-9 summarise the results of the univariable analysis. Risk factors were carried to the multivariable model if $p \leq 0.2$ at the univariable level. All biologically plausible interaction terms were tested as described previously, and confounding was explored. Confounding was found in the case of the TCC outcome variable between the time of first colostrum feed and whether the colostrum sat in a bucket after collection, as well as the time of first colostrum feed and how long the colostrum sits in the bucket. By way of explanation: if the time to first colostrum feed is longer, then it is likely that the colostrum has sat in a bucket for longer. The variables that went forward to the multivariable model for each colostrum quality outcome are shown in Table 4-10.

Colostrum Quality Indicator	Risk Factor	Category of Risk Factor	Co-efficient	95% CI	p-value
Brix					
	When are newborn calves first fed after birth?	< 6 hours	*ref*	*ref*	*ref*
		≥ 6 hours	-0.20	-1.19 – 0.78	0.70
	Timing of harvesting of colostrum after calving	**< 6 hours**	***ref***	***ref***	***ref***
		≥ 6 hours	**-0.77**	**-1.65 – 0.12**	**0.09**
	Does the colostrum sit in a bucket after harvesting?	Yes	*ref*	*ref*	*ref*
		No	0.22	-0.93 -1.38	0.71
	How long does the colostrum sit in collection bucket after harvesting?	< 6 hours	*ref*	*ref*	*ref*
		≥ 6 hours	0.13	-0.79 – 1.05	0.78
	Is there a lid on the colostrum bucket?	Yes	*ref*	*ref*	*ref*
		No	0.28	-0.65 – 1.21	0.55
	Are newborn calves fed stored colostrum?	Yes	*ref*	*ref*	*ref*
		No	-0.71	-1.83 – 0.40	0.21
	If colostrum is stored, is there a temperature gauge on fridge/freezer?	Yes	*ref*	*ref*	*ref*
		No	-0.58	-1.54 – 0.38	0.23
	Is the temperature gauge checked?	Yes	ref	ref	ref
		No	-0.66	-1.72 – 0.41	0.23

Table 4-7 Univariable logistic analysis of farm level risk factors for adequate Brix% of colostrum showing co-efficient, odds ratio with 95% CI and *p*-value from 245 colostrum samples sampled at the point of feeding for 34 dairy farms from the Lanarkshire, Stirlingshire and Dumfries and Galloway regions of Scotland between January – June 2019. Variables significant at the univariable level are shown in bold.

Colostrum Quality Indicator	Risk Factor	Category of Risk Factor	Co-efficient	95% CI	p-value
TBC					
	When are newborn calves first fed after birth?	< 6 hours	*ref*	*ref*	*ref*
		≥ 6 hours	-0.86	-2.62 – 0.91	0.34
	Timing of harvesting of colostrum after calving	< 6 hours	*ref*	*ref*	*ref*
		≥ 6 hours	0.42	-1.21 – 2.05	0.62
	Does the colostrum sit in a bucket after harvesting?	**Yes**	***ref***	***ref***	***ref***
		No	**3.34**	**0.67 – 6.01**	**0.014**
	How long does the colostrum sit in collection bucket after harvesting?	**< 6 hours**	***ref***	***ref***	***ref***
		≥ 6 hours	**2.02**	**-0.20 – 3.48**	**0.03**
	Is there a lid on the colostrum bucket?	Yes	*ref*	*ref*	*ref*
		No	0.92	-0.72 – 2.56	0.27
	Are newborn calves fed stored colostrum?	Yes	*ref*	*ref*	*ref*
		No	-0.73	-2.78 – 1.32	0.48
	If colostrum is stored, is there a temperature gauge on fridge/freezer?	**Yes**	***ref***	***ref***	***ref***
		No	**-1.78**	**-3.62 – 0.06**	**0.09**
	Is the temperature gauge checked?	**Yes**	***ref***	***ref***	***ref***
		No	**-1.74**	**-3.95 – 0.48**	**0.12**

Table 4-8 Univariable logistic analysis of farm level risk factors for adequate TBC of colostrum showing co-efficient, odds ratio with 95% CI and *p*-value from 245 colostrum samples sampled at the point of feeding for 34 dairy farms from the Lanarkshire, Stirlingshire and Dumfries and Galloway regions of Scotland between January – June 2019. Variables significant at the univariable level are shown in bold.

Colostrum Quality Indicator	Risk Factor	Category of Risk Factor	Co-efficient	95% CI	p-value
TCC					
	When are newborn calves first fed after birth?	< 6 hours	*ref*	*ref*	*ref*
		≥ 6 hours	-0.20	-2.43 – 2.02	0.86
	Timing of harvesting of colostrum after calving	**< 6 hours**	***ref***	***ref***	***ref***
		≥ 6 hours	**1.38**	**-0.67 – 3.43**	**0.19**
	Does the colostrum sit in a bucket after harvesting?	**Yes**	***ref***	***ref***	***ref***
		No	**2.66**	**-0.77 – 6.08**	**0.13**
	How long does the colostrum sit in collection bucket after harvesting?	**< 6 hours**	***ref***	***ref***	***ref***
		≥ 6 hours	**2.23**	**-0.17 – 4.62**	**0.07**
	Is there a lid on the colostrum bucket?	Yes	*ref*	*ref*	*ref*
		No	1.14	-0.98 – 3.27	0.29
	Are newborn calves fed stored colostrum?	Yes	*ref*	*ref*	*ref*
		No	-1.08	-3.62 – 1.46	0.40
	If colostrum is stored, is there a temperature gauge on fridge/freezer?	Yes	*ref*	*ref*	*ref*
		No	-0.79	-3.07– 1.48	0.49
	Is the temperature gauge checked?	Yes	*ref*	*ref*	*ref*
		No	-0.23	-2.80 – 2.34	0.86

Table 4-9 Univariable logistic analysis of farm level risk factors for adequate TCC of colostrum showing co-efficient, odds ratio with 95% CI and *p*-value from 245 colostrum samples sampled at the point of feeding for 34 dairy farms from the Lanarkshire, Stirlingshire and Dumfries and Galloway regions of Scotland between January – June 2019. Variables significant at the univariable level are shown in bold.

Colostrum Quality Indicator	Variables to go forward to the multivariable model
Brix	Timing of harvesting of colostrum after calving
TBC	Does the colostrum sit in a bucket after harvesting?
	How long does the colostrum sit in collection bucket after harvesting?
	If colostrum is stored, is there a temperature gauge on fridge/freezer?
	Is the temperature gauge checked?
TCC	Timing of harvesting of colostrum after calving
	Does the colostrum sit in a bucket after harvesting?
	How long does the colostrum sit in collection bucket after harvesting?
	When are newborn calves first fed after birth?

Table 4-10 The colostrum quality indicators and associated outcome variables significant at the univariable level, interaction or confounding going forward into the multivariable model

A backwards, stepwise elimination approach was used to determine the final multivariable models for each colostrum quality outcome summarize in Table 4-11. Postestimation and model diagnostics found residuals were found to lie within 2 standard deviations of the mean in all cases.

Colostrum harvested from dams more than six hours after calving was half as likely to meet the Brix threshold of 22% (reflective of adequate IgG concentration). Furthermore, colostrum that was left in the collection bucket, as opposed to being stored, or fed immediately post-harvest, was found to be 28 times more likely to exceed TBC thresholds of 100,000 CFU/mL compared with colostrum that did not sit in a collection bucket post-harvest. And finally, with respect to TCC, colostrum that was left in the collection bucket for more than six hours was found to be eleven times more likely to exceed the threshold of 10,000 CFU/mL when compared with colostrum that was stored or fed immediately post-harvest.

Colostrum Quality Indicator	Risk Factor	Category of Risk Factor	Co-efficient (95% CI)	OR (95% CI)	P value
Brix					
	Timing of harvesting of colostrum after calving	< 6 hours	*ref*	*ref*	*ref*
		≥ 6 hours	-0.77 (-1.65 – 0.12)	0.45	0.09[NS‡‡‡]
TBC					
	Does the colostrum sit in collection bucket after harvesting?	No	*ref*	*ref*	*ref*
		Yes	3.33 (0.66 – 6.00)	28.09	0.01
TCC					
	How long does the colostrum sit in collection bucket after harvesting?	< 6 hours	*ref*	*ref*	*ref*
		≥ 6 hours	2.44 (0.18 – 4.70)	11.46	0.03

Table 4-11 Final multivariable logistic regression models for 3 colostrum quality measurements (Brix %, TBC and TCC) from 252 colostrum samples collected from 34 dairy farms from the Lanarkshire, Stirlingshire and Dumfries and Galloway regions of Scotland between January – June 2019.

‡‡‡ NS = Not significant (p ≥ 0.05)

4.5 Discussion

Questionnaire data was key in this research to generate risk factor categories to go forward for multivariable analysis. Questionnaire research can be associated with bias for several reasons (Greenhalgh, 2014). Bias can arise due to question design and communication barriers between researcher and respondents. Efforts were made to minimise any associated bias with questionnaire design by beta testing the questionnaire with five veterinary surgeons prior to conducting the research. Furthermore, only four separate private veterinary surgeons completed the questionnaire with enrolled farmers. These veterinary surgeons had a thorough working knowledge of the farms and a trusted relationship with the farmers. All data were collected prospectively to sample collection. Reporting of colostrum and serum sample results to study participants was done after all sample collection and analysis was complete.

4.5.1 Failure of Passive Transfer risk factors

Sixty-eight percent (n=23/34) of respondents routinely actively fed newborn calves within six hours of calving. Current recommendations are to give newborn calves a colostrum feed within six hours of birth. This is to coincide with maximum absorption efficiency of the neonatal gastrointestinal (GI) tract to IgG (Morin *et al.*, 1997; McGuirk and Collins, 2004; Godden *et al.*, 2019). Producers were asked when calves were actively fed to differentiate between allowing calves to suckle dams themselves. Producers were not asked about snatch calving and including this in the questionnaire could given greater clarity to responses. However, streamlining the questionnaire to make it as user friendly as possible was important. Data from the present study shows nearly a third of respondents (32 %, n = 11) leave the first colostrum feed of newborn calves to greater than six hours after birth. This means they are feeding the first feed of colostrum when the efficiency of absorption of the neonatal gut is declining, thus leaving these calves at increased risk of FPT (defined as serum IgG concentrations <10mg/mL).

The volume of colostrum fed to newborn calves at their first colostrum feed has been well-documented as a risk factor for FPT. In the current study, the odds ratio for feeding 3.5-4 litres of colostrum compared with feeding <2 litres of colostrum was 0.12. Calves receiving 3.5-4 litres are 88% less likely to have FPT.

Furthermore, the odds ratio for feeding 4.5 – 5 litres was 0.02 meaning that calves that received 4.5-5 litres of colostrum were 98% less likely to have FPT when compared to calves that received <2 litres. Current recommendations in terms of volume of colostrum administered are to feed 10-15 % of bodyweight of the calf (Patel *et al.*, 2014). Only 44% (n=15/34) of respondents fed between 3.5 – 4 litres routinely and 44% (n=15/34) fed less than three litres. The average Holstein Friesian calf will weigh between 35-45 kgs. From their research, Chigerwe *et al.* (2008), concluded that feeding greater than three litres of colostrum via oesophageal tube is recommended. By routinely feeding less than 10-15% of bodyweight (< 3.5 – 4.5 L) producers are leaving their calves at risk of FPT.

The findings of this study agree with previous research exploring FPT risk factors. Morin *et al.* (1997) found that giving of a larger volume (4L) of a high IgG concentration colostrum within 3 hours after birth compared with just 2L of a high IgG concentration colostrum increased serum IgG concentration, did not reduce the efficiency of IgG1 absorption, and resulted in no apparent discomfort from mechanical distention of the abomasum or disease. Faber *et al.* (2005) found that Brown Swiss calves fed 4L of colostrum at birth as opposed to 2L of colostrum had less veterinary input, higher daily bodyweight gains (1.03 kg ± 0.03 vs 0.80 ± 0.02; $p<0.001$) and improved lactation performance. Although a major limitation of this study was that serum IgG of the calves was not measured, the inference was that high volumes of good quality colostrum lead to better calf health. Godden *et al.* (2009) summarised that to promote adequate passive transfer, producers should feed larger volumes of colostrum regardless of the feeding method used. On balance, the evidence from these three studies supports the feeding of higher volumes of colostrum to neonatal calves, which aligns with conclusions drawn in this study.

A recent questionnaire survey conducted in the UK by Palczynski *et al.* (2020) found that producers, veterinarians and industry advisors were generally aware of the association between colostrum volume and FPT with many advocating providing between 2-4L of colostrum within six hours of birth. This is encouraging, as knowledge from the literature is being communicated through to the grass roots where it can be applied to the benefit of calves. However, FPT prevalence in the UK and globally is still high. Prevalence in this population of

Scottish calves was found to be 14.86% (95% CI 11.1-18.47%) and international estimates range from 15.6 % in the US to 41.9% in Australia (Urie *et al.*, 2018; Abuelo *et al.*, 2019). This shows a disconnect between knowledge of what should be happening and, what is actually happening.

The volume of colostrum required to give adequate passive transfer is linked to colostrum IgG concentration. If low IgG concentration colostrum is an issue on farm, producers may try to overcome this problem by feeding a higher volume to achieve the desired mass of IgG for adequate passive transfer. It appears that the effect of increasing colostrum volume fed on FPT prevalence is not linear. This is shown by the current study because feeding 2.5 – 3L of colostrum compared with feeding <2L of colostrum was found to be 89% protective of FPT but when feeding 4.5 – 5L of colostrum compared with feeding <2L the protective nature is 98%. This finding agrees with a study conducted by Sakai *et al.* (2012) who determined that the apparent efficiency of absorption of IgG and serum IgG concentration at 48 hours were similar in calves fed 3L versus 4 L of colostrum with similar colostral IgG concentrations via oesophageal tube. The median IgG concentration of the 3L feeding group was 51.6 g/L and of the 4L feeding group was 52.9 g/L. No statistically significant difference in IgG concentration (p>0.05) was found between the two groups. Jaster (2005) found serum IgG concentrations were higher in Jersey calves fed 2L at birth and 2L at 12 hour post birth opposed to those fed 4 L at birth only. Conneely *et al.* (2014) concluded that calves fed 8.5% of bodyweight in colostrum within two hours of birth achieved a greater serum IgG concentration in the first three days of life than calves fed either 7 or 10% of bodyweight. They postulated that this was due to decreased apparent absorption efficiency at 10% bodyweight due to abomasal distention and reduction in emptying. It should be noted that the mean colostrum IgG concentration was 110.8g/L (SD = 41.4g/L) which may explain why their recommendations seem to deviate from the current industry recommendations of 10 – 15 % of bodyweight. While there is a body of evidence that agrees a higher volume of colostrum fed reduces FPT incidence, these further studies that provide evidence that the picture is more complex. Too high a volume of colostrum can overwhelm the gastrointestinal (GI) capacity and reduce the absorption efficiency of colostrum. Indeed, overwhelming GI capacity or delivering colostrum to the incorrect part of the GI tract can contribute to disease syndromes such as ruminal drinking, abomasal ulceration and bloat

(Lorenz and Gentile. 2014). Too low a volume risks insufficient IgG absorption and FPT. Practitioners should take a detailed and holistic approach when investigating on farm FPT problems and investigate all risk potential risk factors and feeding practices.

Fifty percent (n=17/34) of respondents fed calves via oesophageal tube and 50% (n=17/34) fed calves with a teat feeder. The benefit of feeding a calf via a teat feeder promotes the closure of the oesophageal groove therefore allowing the colostrum to enter the abomasum where absorption takes place (Hegland *et al.,* 1957). When an oesophageal tube is used, the oesophageal groove reflex is not triggered and the milk enters the rumen (Tamate *et al.,* 1962). Because of concerns regarding insufficient intake of colostrum from the dam by the calf if left to suckle naturally in the modern dairy setup it is recommended that colostrum is artificially fed by farm staff (Patel *et al.,* 2014). There are a variety of reasons that colostrum intake may be insufficient if suckling of colostrum is left to happen naturally: heifers may avoid their calves, communal calving facilities may mean calves may go to the wrong dam or bullying of dams, difficult calving leading to weak calves, difficulty in finding the teat and achieving teat attachment, and unhygienic teats leading to bacterial contamination. The literature is not completely clear cut as to whether or not oesophageal tubing or teat feeding, and activation of the oesophageal groove reflex has benefits. Godden *et al.* (2009) looked at the interaction between the method of feeding and volume of colostrum of the efficiency of absorption of IgG. That study found that for calves fed a low volume of colostrum (1.5L) via a teat feeder the absorption efficiency was great than for those calves fed via an oesophageal feeder, but at larger volumes of colostrum, there was no significant difference. Kaske *et al.* (2005) concluded that proper use of the oesophageal tube is a useful method to supply adequate colostrum and the failure of oesophageal groove closure appears to be of no clinical consequence. These findings are supported by an earlier study that colostrum flows out of the forestomachs into the small intestine in a timely fashion, less than three hours, where it is then absorbed (Lateur-Rowet and Breukink, 1983). Chigerwe *et al.* (2012) found no difference in absorption efficiency and passive transfer prevalence between calves fed via a teat feeder and oesophageal tube. The focus of advice to producers should therefore be getting the correct volume of first milk colostrum into the calves in a timely, hygienic fashion. The use of a

stomach tube takes any guess work out of timing of delivery and volume consumed.

As discussed, the timing of the first colostrum feed is of vital importance to achieve adequate passive transfer. Debate exists in the literature about the significance of the interval between the first and second colostrum feed with respect to ensuring calves have adequate passive transfer. Morin *et al.*(1997) found the timing between the first and second colostrum feed to be a significant risk factor to FPT; whereas a later study by Reschke *et al.* (2017) did not. Pletts *et al.* (2018) found that calves fed a second colostrum feed, or a colostrum/milk feed at 12 hours had higher serum IgG concentrations. It is a legal requirement in the UK to feed calves on a liquid milk diet at least twice a day (Defra., 2003). Looking at the questionnaire data 21% (n=7/34) of respondents left 12.5 - 18 hours between 1st and 2nd feeds in this survey. Whereas 80% (n=27/34) of respondents fed calves a second feed within 12 hours of the first. In agreement with Reschke *et al.* (2017), this study found the interval between first and second milk feed not to be a significant risk factor for FPT.

4.5.2 **Colostrum quality risk factors**

Fifty-nine percent (n = 20/34) of respondents routinely collected colostrum from newly calved cows less than six hours after calving. It is these producers that are routinely maximising the IgG concentration in the colostrum available to their calves. Of the remaining respondents, 41 % (n =14), collected colostrum from newly calved cows more than 6 hours after calving. The review by Godden *et al.* (2019) suggested aiming to harvest colostrum within six hours of parturition. This present study identified that a Brix % above the threshold of 22% was associated with a time from calving to harvesting of less than six hours. This inverse relationship, although found to only be approaching significance in the current study, is well supported by previous literature. The reduction in IgG in the udder post calving was quantified by Morin *et al.* (2010) as a 3.7% decrease during each hour subsequent to calving. Reschke *et al.* (2017) found that leaving the harvesting of colostrum more than six hours after calving was associated with a lower IgG concentration of colostrum. Considering previous literature on the subject, the relationship detected in this study may well have been strengthened to achieve significance if all 38 farms had completed the

questionnaire. Study design aimed to preserve IgG concentration as much as possible prior to analysis. Multiple freeze thaw cycles were found by Morrill *et al.* (2015), to decrease the IgG concentration as detected by RID. The study methodology stipulated that all samples were frozen immediately after collection until analysis therefore not undergoing multiple freeze thaw cycles to preserve IgG.

The declining IgG concentration in the udder post parturition was well documented in previous literature (Morin *et al.,* 2010; Quigley *et al.,* 2013; Reschke *et al.* 2017). However, there is a body of literature that suggests the picture may not be as clear cut. The process of change over from colostrogenesis to lactogenesis is under complex control at an endocrine and local individual gland level. Colostrogenesis appears to continue beyond parturition (Gross *et al.,* 2014; Kessler *et al.,* 2020). Whilst considering this research it should be recognised that more research is required to clarify the situation. On a practical day-to-day level, the advice to producers is clear - in the event of an uncomplicated calving, harvesting colostrum from dams within six hours of calving will positively impact IgG concentration of the colostrum harvested.

The IgG concentration in colostrum produced by the modern dairy cow is variable irrespective of the decline at parturition. Morrill *et al.* (2012) found colostrum quality as low as <1g/L and as high as 200 g/L in their US study. MacFarlane *et al.* (2015) found in their UK colostrum study that the quality varied from 10 – 34 % Brix. Chigerwe *et al.* (2008) proposed that at least 150 – 200g of IgG are required to achieve adequate passive transfer in dairy calves. Newer research has proposed 300g of IgG are required to achieve excellent rates of passive transfer that associated with lower disease morbidity alongside mortality (Godden *et al.,* 2019; Lombard *et al.,* 2020). In the current study, the mean Brix concentration was just 22% (SD ± 4.31), with 44% (111/252) of samples falling below the industry threshold for acceptable colostral IgG concentration (See Chapter 3: Descriptive Statistics).

The clinical relevance of IgG concentration being variable and declining at a point after parturition is that producers should be encouraged measure the IgG concentration of the colostrum harvested opposed to solely relying on measuring volume fed. This will give confidence that all other factors being equal, the

desired mass of IgG is being delivered to the calf to achieve adequate passive transfer. Colostrum IgG can be estimated on farm through the use of Brix refractometry reliably and cheaply (Bielmann *et al.,* 2010). In retrospect, it would have been useful to survey study participants to see how many were actively doing this and how. However, as discussed previously, streamlining the questionnaire to specific risk factors was considered important.

Farmers were asked about colostrum management after harvesting and practices that could potentially affect the opportunity for bacterial contamination of the colostrum. Excessive bacterial contamination of colostrum reduces the absorption efficiency of the neonatal gut for IgG, thereby putting calves at risk of FPT (Johnson *et al.,* 2007; Godden *et al.,* 2012, 2019). This was discussed in detail in Chapter 1: Literature Review. Twenty-eight/thirty-four respondents (82%) left colostrum to sit in a bucket post collection opposed to storing (refrigeration, freezing or chemical preservatives) or feeding the colostrum straight away. Of these, only 2/28 (7 %) left colostrum > 6 hours in a bucket; however, 16/28 (57 %) did not put a lid on the bucket. Thirteen/thirty-four (38%) transferred the harvested colostrum into another container prior to the colostrum going into feeding equipment. By allowing the colostrum to sit at ambient temperature before feeding or storing and not sealing the container to prevent faecal contamination producers are allowing opportunity for bacterial contamination and multiplication (Stewart *et al.,* 2005; Cuttance *et al.,* 2018).

This study found an association between colostrum sitting in a bucket without prompt, correct storage or feeding, and bacterial contamination exceeding industry thresholds (TBC >100,000 CFU/mL and TCC >10,000 CFU/mL). These findings concur with Stewart *et al.* (2005), Morrill *et al.* (2012) and Phipps *et al.* (2016). Stewart *et al.* (2005) found that when colostrum is left sitting in a bucket post-harvest, especially at ambient temperature, there is increased opportunity for bacterial contamination and proliferation. The average ambient temperature reported for the Stewart *et al.* (2005) work was 23℃, range = 19.6℃ – 26.8℃. Morrill *et al.* (2012) concluded that the storage of colostrum had a significant impact on bacterial contamination, therefore colostrum should be fed fresh, or frozen (-20℃) immediately after harvest. A more recent study by Phipps *et al.* (2016) carried out multilevel logistic regression analysis to examine the association between TBC and TCC and features of colostrum harvesting,

storing and feeding. Their findings - that excess colostrum should be refrigerated (4°C) as soon as possible (<1 hour) after collection - also supports this study's finding that colostrum left to sit in a bucket after harvest is more likely to exceed TBC thresholds. Whilst this study does not specify at what temperature colostrum should be refrigerated, it is generally considered this should be 4°C (Cummins *et al.,* 2016). This body of evidence shows colostrum should not be left sitting in a bucket after harvesting to minimise the opportunity for bacterial contamination and proliferation.

Thirty-four (100%) of respondents said that they cleaned their harvesting and feeding equipment regularly. Looking at bacterial contamination data in this study, 30.56% (n = 77/252) of samples exceeded the industry standard TBC threshold of 100,000 CFU/mL. As these samples were collected at the point of feeding, samples could have been contaminated at harvesting, storage or feeding. It suggests that cleaning protocols of udders, storing and feeding equipment on farm is not robust and sufficient despite farmer belief that all harvesting and feeding equipment is cleaned regularly. Stewart *et al.* (2005) found that TBC in colostrum was very low or nil when stripped directly from the mammary gland however significant contamination occurred during the process of milking into the bucket. The questionnaire did not explore further the exact cleaning protocol used on farm or what farmers considered 'regularly'. It would have been useful to enquire the exact cleaning of equipment, what detergents or disinfectants were used and specifically, how often cleaning took place. It is clear from questionnaire data, risk factor analysis and descriptive statistics of colostrum bacterial contamination it is pertinent for producers to review their hygiene protocols around colostrum management. This should include thorough cleaning with hot water and detergent to remove colostrum residue from buckets and feeding equipment.

After harvesting, colostrum should be safeguarded from further risk of bacterial contamination from, for example, faecal matter. Questionnaire data from this study indicated that only 43% (12/34) of producers covered buckets with a lid after harvesting. The covering of the collection bucket with a lid was not found to be a significant risk factor at the univariable level of analysis which is in agreement with Phipps *et al.* (2016) (Table 4-8 and Table 4-9). In practice this could be a simple, cheap way to prevent faecal matter from falling into the

stored colostrum, but the data and literature suggest other risk factors maybe more influential on colostrum cleanliness. In their analysis, Phipps *et al.* (2016) discussed bacterial contamination in the context of covering stored colostrum with a lid. They found 70% of samples without a lid were below TBC thresholds compared with only 46% of samples with a lid. This surprising result could be explained by the fact that the stored colostrum with a lid was stored not only by freezing or refrigeration, but also at ambient temperature. These were not identified as causative relationships but associations. Samples stored at ambient temperature allows for more bacterial proliferation.

Finally, the questionnaire explored the colostrum storage protocols in place on farm. Twenty-one/thirty-four (62 %) respondents froze colostrum, 9/34 (26 %) refrigerated colostrum and 1/34 (3 %) used another method although the questionnaire did not expand on what this was. Storing colostrum allows producers to preserve colostrum in times of excess to use in times of scarcity. For example, some herds experience a reduction in colostrum yield during the winter period due to reduced daylength. Although more research is required, it is postulated that this effect is linked to prolactin production (Gavin *et al.* 2018b). Control strategies to mitigate such effects are unclear, therefore simple measures like the storage of colostrum is a vital tool for producers to ensure a consistency of supply in times of shortage. However, improperly stored colostrum can be highly contaminated with bacteria and IgG levels can decline (Cuttance *et al.* 2018). The questionnaire and risk factor analysis clearly highlight this is an area of on farm colostrum management that could put calves at risk of FPT. Refrigeration at 4°C should be considered for short term storage (Godden *et al.,* 2019; Robbers *et al.,* 2021). Stewart *et al.* (2005) found that TBCs became unsatisfactorily high (>100,000 CFU/mL) after 2 days of refrigeration therefore short-term storage is really considered less than this. This is in contrast to some of the UK industry messaging that promotes refrigeration for up to a week (AHDB, 2014). Findings in Morrill *et al.* (2012) somewhat contradicted other literature. They concluded that the storage of colostrum had a significant impact on bacterial contamination therefore colostrum should be fed fresh or frozen immediately and not stored in a refrigerator. Cummins *et al.,* (2016) found no difference in IgG concentration in colostrum stored at ambient temperature vs refrigeration.

For longer term storage, colostrum can be stored in the freezer at-18 - -20 ℃ for up to 6-12 months (Godden *et al.,* 2019; Robbers *et al.,* 2021). Freezing has been found to maximise the retention of IgG concentration and nutrients compared with other storage methods (Holloway *et al.,* 2001; McGuirk and Collins, 2004). Alrabadi (2015), froze 30 raw milk samples for eight weeks and tested TBC and TCC weekly, and concluded that bacterial counts decrease significantly as the freezing time increases. When considering frozen colostrum, it is important to consider the method of thawing as this can affect the IgG content of the colostrum. Repeated freeze/thaw cycles should be avoided as they potentially will decrease the IgG content (Morrill *et al.,* 2015). Morrill *et al.* (2015) acknowledge the need for further research in this area as the effect of freeze/thaw on other immune components in colostrum is unknown. Only 9/34 (27%) of respondents had a temperature gauge on their fridge or freezer. It is clear stored colostrum is at risk from bacterial contamination, multiplication, and reduced IgG concentration through improper storage.

Chemical preservation can be done by adding potassium sodium (0.5% weight/volume) and has been described in previous peer review literature (Stewart *et al.,* 2005; Denholm *et al.,* 2017). Stewart *et al.* (2005) found potassium sorbate was most effective at reducing bacterial proliferation when used in combination with refrigeration. More recent work by Denholm *et al.* (2018) agreed, preservation with potassium sorbate resulted in little or no decline in Brix percentage and limited bacterial proliferation in pooled colostrum.

4.6 **Summary**

The volume of colostrum fed at first feed was identified as a significant risk factor for FPT. Farmers should be encouraged to feed 10-15% of the newborn calf's bodyweight in first feed colostrum within the first six hours of life to mitigate this risk. Protocols such as snatch calving and providing colostrum through a bottle or oesophageal tube can make this practically achievable.

Advising producers to minimise the time between parturition and colostrum harvesting will ensure the maximal IgG concentration in the colostrum available. Monitoring IgG concentration on farm can be done cheaply and reliably through Brix refractometry and should be encouraged as part of a proactive approach to

colostrum management. Minimising the time that harvested colostrum sits in a bucket prior to feeding to protect against bacterial contamination will also improve the colostrum quality available and allow maximal absorption efficiency of the calf's gut. Minimising time the colostrum sits in a bucket can be done simply and inexpensively by ensuring correct, prompt storage or hygienic feeding to calves immediately after harvesting. The bulk of these recommendation do not require significant financial investment rather attention to detail and cheap improvements to daily protocol.

5 Comparison of the Diagnostic Accuracy of Testing Methods for the Diagnosis of Failure of Passive Transfer.

Correct diagnosis of FPT is important to instil any management changes required for improvement in FPT status and for monitoring to ensure any changes have the desired effect. This study explored the testing methods available for diagnosis in terms of correlation and agreement and performance at given cut points.

5.1 Results

5.1.1 Agreement of radial immunodiffusion (RID) reference test carried out at two different laboratories

As discussed previously, Triple J Agar Plates (Bovine IgG RID Kit, Triple J Farms, Bellingham, WA) have not been validated by the manufacturer therefore this test is considered a reference test as opposed to gold standard. However their use is justified as a reference test as the precedent has been set by previous peer reviewed literature (Hogan *et al.,* 2015; Dunn *et al.,* 2018; Elsohaby *et al.,* 2019) To explore the variation and agreement of this RID test plate a subset of 108 samples were sent to an external commercial laboratory (Biobest Laboratories Ltd, 6 Charles Darwin House, The Edinburgh Technopole, Edinburgh, EH26 0PY). The 108 samples were purposively selected to represent low (<10g/L), medium (10-25g/L) and high IgG(≥25g/L) concentrations and ensure FPT prevalence in each data set were similar. The prevalence of FPT as measured by RID(Glasgow) and RID(Biobest) was 14.2% and 10.2% respectively. As previously described, the RID test conducted in the internal study lab at the University of Glasgow will be referred to as RID(Glasgow) and the RID test conducted in the commercial external laboratory will be referred to as RID(Biobest).

A scatter graph was constructed to show the correlation between RID(Glasgow) and RID(Biobest), shown in **Error! Reference source not found.**. It demonstrated there was a fair positive, linear correlation (r= 0.44). A Bland-Altman (BA) plot was constructed to assess agreement and consider any bias. The mean of the differences between RID(Biobest) and RID(Glasgow) was 1.54

mg/mL (95% CI = -0.36 to 3.43 mg/mL, SD = 9.84 mg/mL) meaning that on average compared with the RID(Biobest), RID(Glasgow) overestimated the concentration of IgG in the sample by 1.54 mg/mL. No proportional bias was apparent when the scatter was visually assessed. Ninety seven percent (n=105/108) of observations lay within the 95% limits of agreement (upper limit = 21.21 mg/mL and lower limit = -18.14 mg/mL). However, these limits of agreement were large and therefore agreement between the two techniques was poorer than expected. As previously mentioned, *a priori,* it was agreed in a clinical setting a difference of ≤ 5 mg/mL would be acceptable between the two IgG measurements. Cohen's Kappa statistic was measured at 0.33 (95 % CI = 0.09 – 0.57) indicating slight to fair agreement.

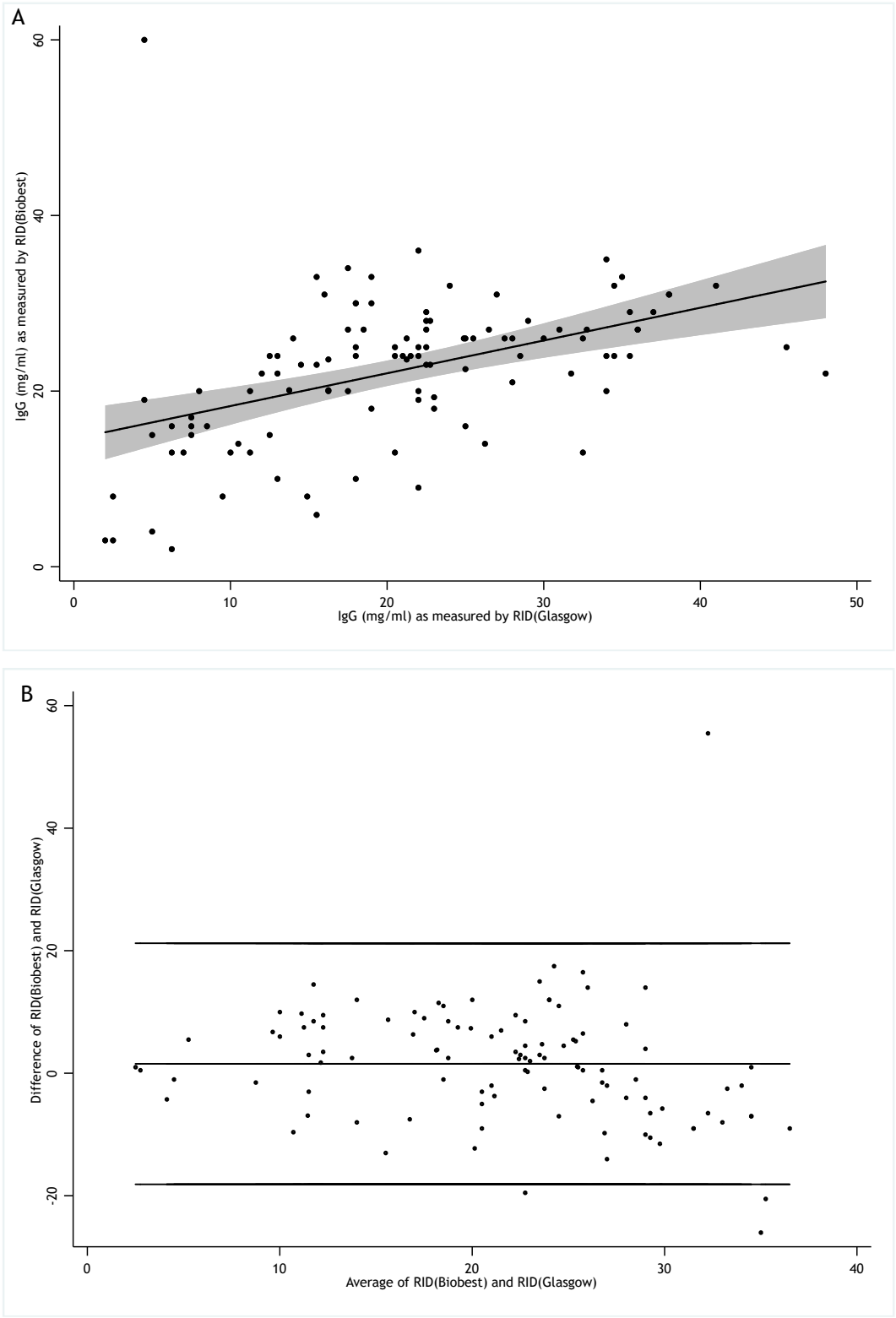


Figure 5-1 A. Scatter plot of the relationship between IgG concentration as measured by RID(Glasgow) and IgG concentration as measured by RID(Biobest) in 108 dairy bred calves. Pearson Correlation Co-efficient is shown (r) alongside 95% CI. B Bland Altmann plotting the average of RID(Biobest) and RID(Glasgow) vs the difference of RID(Biobest) and RID(Glasgow). The mean difference between the measurements and 95 % limits of agreement are shown.

Test	Agreement (%)	Expected Agreement (%)	Kappa (κ) (95% CI)	Standard Error
RID(Biobest)	*Ref*	*Ref*	*Ref*	*Ref*
RID(Glasgow)	84.26	76.54	0.33 (0.09-0.57)	0.09

Table 5-1 Agreement, Expected Agreement and Cohen's Kappa Statistic for readings of RID(Biobest) and RID(Glasgow) of 108 selected serum samples from dairy bred calves aged between 1-7 days.

	Interpretation of Cohen's Kappa Statistic					
	Poor	Slight	Fair	Moderate	Substantial	Almost perfect
Kappa	0.0	0.20	0.40	0.60	0.80	1.0

Figure 5-2 The interpretation of Cohen's Kappa Statistic of interrater reliability

5.1.2 Investigation into timings of reading RID zonal diffusion plates

One of the criticisms of the RID test is that they are time consuming to conduct. It would be an obvious advantage to be able to take accurate measurements earlier to reduce time (from 40 hours to 24 hours) and costs associated. The precipitin rings from RID(Glasgow) test plates were measured in the internal laboratory at the University of Glasgow after 24 and 40 hours of incubation to check for correlation and agreement between the different timings of measurements. A very strong, positive correlation of r = 0.98 was found to exist with a narrow 95% confidence interval (Figure 5-3).

Agreement was explored through the construction of a Bland Altman (BA) plot. The observations centred around a mean difference of only 1.05 mg/mL (95 % CI = 0.80 mg/mL – 1.31 mg/mL) meaning that on average the readings taken at 40 hours were only 1.05 mg higher than readings taken at 24 hours (Table 5-2). There is proportional bias evident from the scatter of observations at higher measurements of IgG (approximately >35 mg/mL). However, the current cut point for FPT diagnosis is 10 mg/mL, therefore this has limited clinical significance. When assessed in clinical terms, the limits of agreement were narrower than the limit of ± 5mg/mL agreed *a priori*. It was concluded that there was good agreement between the readings at 24 hours and 40 hours.

	Mean difference (mg/mL)	Limits of agreements (mg/mL)	Agreement (%)
RID Glasgow 24 vs 40 hour readings.	1.05	-3.84 – 5.95	96.5

Table 5-2 The results of the Bland Altman plot comparing RID(Glasgow) read at 24 hours and at 40 hours

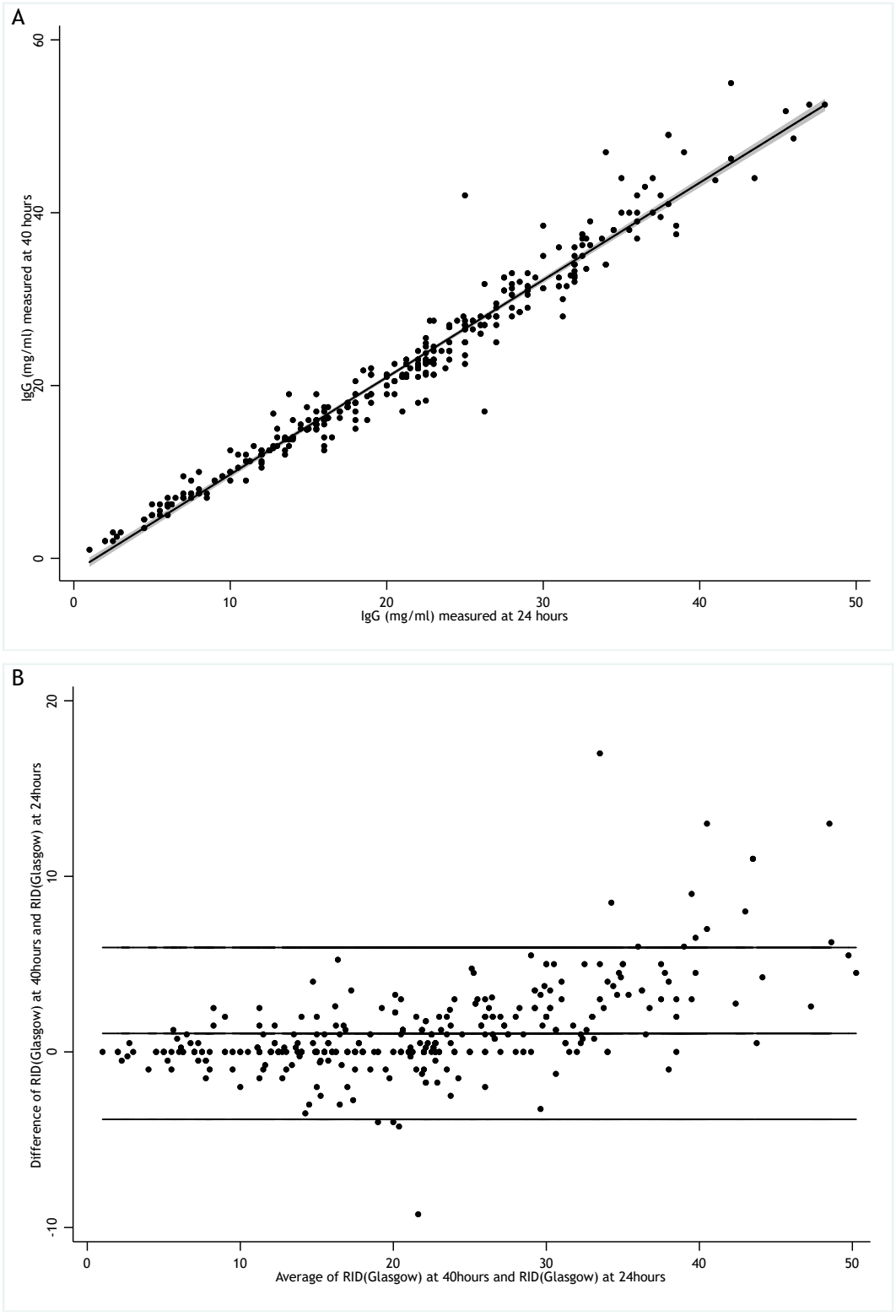


Figure 5-3 Scatter plot of the relationship between IgG concentration as measured by RID(Glasgow) read at 24 hours and 40 hours in 367 dairy bred calves. Pearson Correlation Co-efficient is shown (r) alongside 95% CI. B Bland Altmann plotting the average of RID(Glasgow) read at 24 hours and RID(Glasgow) read at 40 hours vs the difference of RID(Glasgow) read at 24 hours and RID(Glasgow) read at 40 hours. The mean difference between the measurements and 95 % limits of agreement are shown.

5.1.3 Correlation and agreement of indirect testing methods with reference test RID(Glasgow)

The correlation (r) between RID(Glasgow) and the indirect testing methods TP, Brix and ZST were 0.33, 0.36 and 0.6 respectively are shown in Figure 5-4Figure 5-5Figure 5-6.

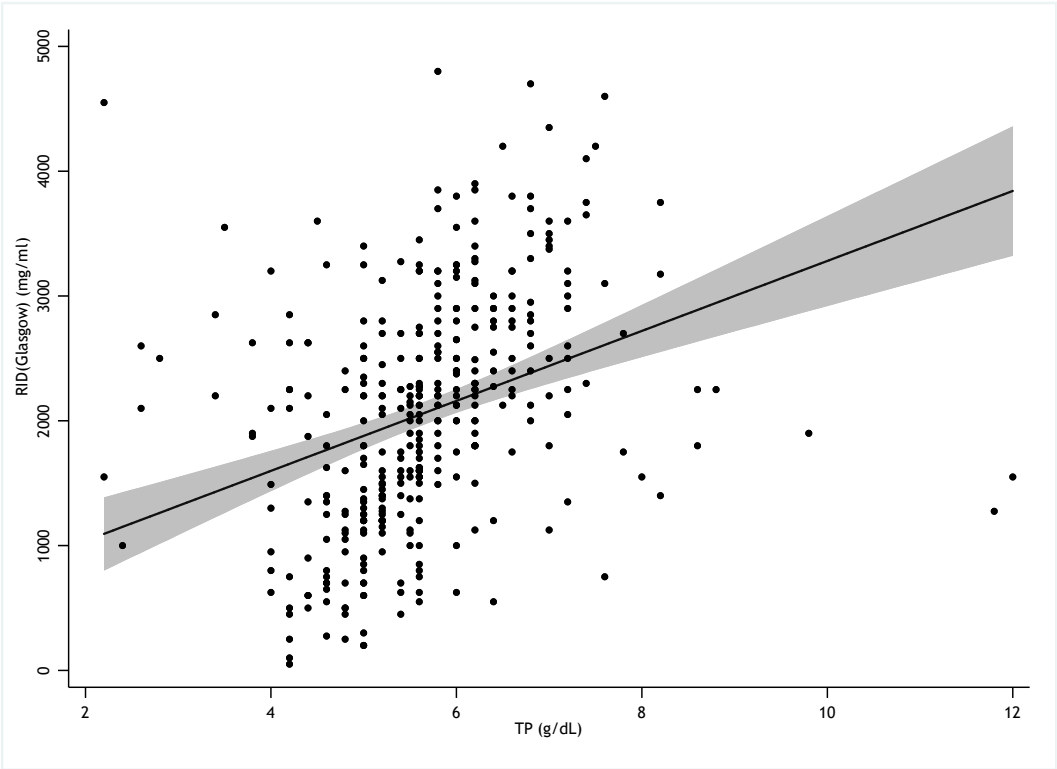
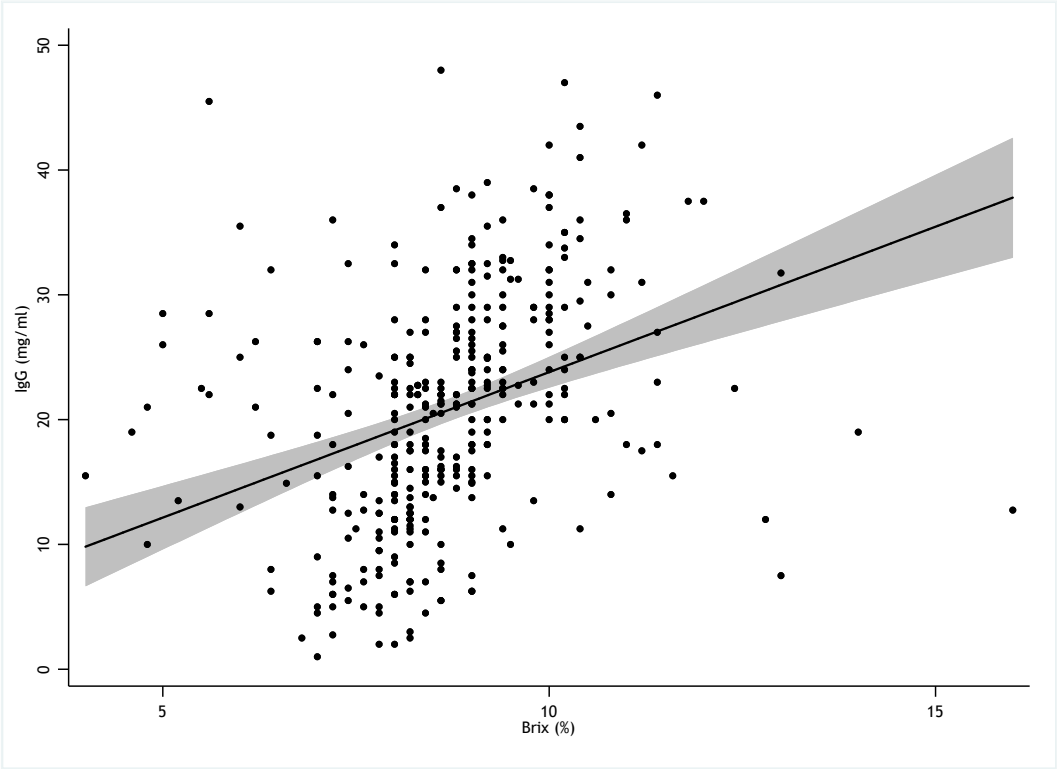
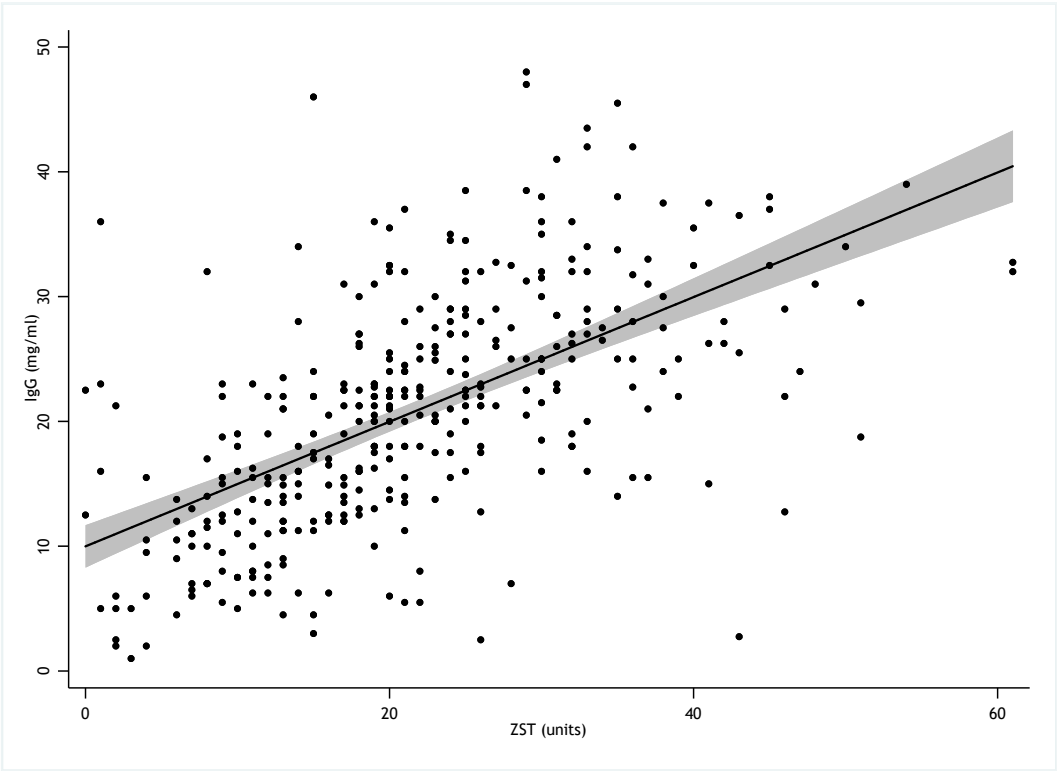


Figure 5-4 Scatter plot of the relationship between TP concentrations as measured by TP refractometry and IgG concentration as measured by RID(Glasgow) in 367 dairy bred calves.



2415

2416 **Figure 5-5 Scatter plot of the relationship between Total Solids (%) as measured by Brix**
2417 **refractometry and IgG concentration as measured by RID(Glasgow) in 367 dairy bred calves**



2418

2419 **Figure 5-6 Scatter plot of the relationship between turbidity (units) as measured by ZST**
2420 **testing and IgG concentration as measured by RID(Glasgow) in 367 dairy bred calves.**

The association between the tests was further explored by calculating r^2 between the RID(Glasgow) and the indirect testing methods, TP, Brix and ZST and were 0.11, 0.12 and 0.35 respectively (Figure 5-7Figure 5-8Figure 5-9). The percentage of samples that had predicted IgG values within ± 5 g/L of the true values (as defined by reference test RID(Glasgow) were 44.68%, 54.50%, and 42.78% for TP, Brix and ZST, respectively.

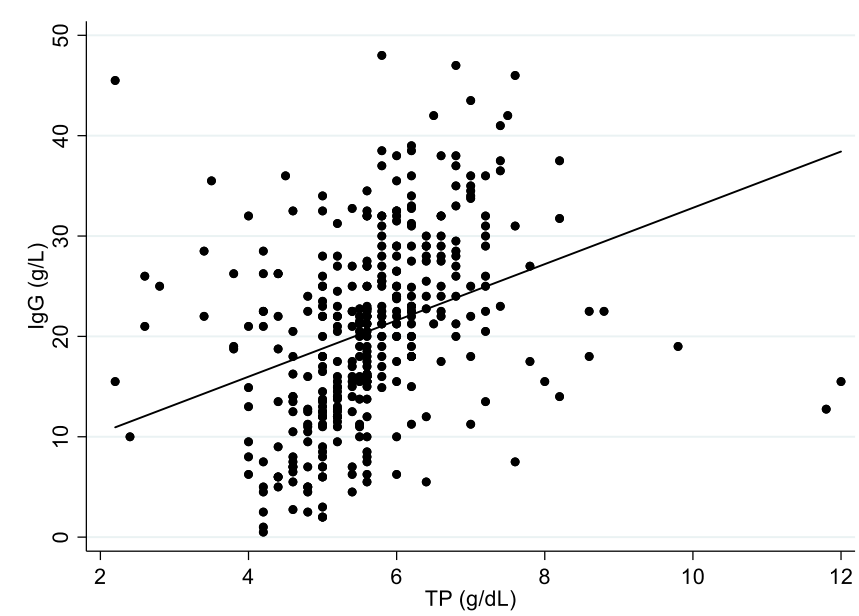


Figure 5-7 Scatter plot showing the relationship by r^2 between TP (g/dL) and RID (Glasgow) (mg/mL) in 367 dairy bred calves.

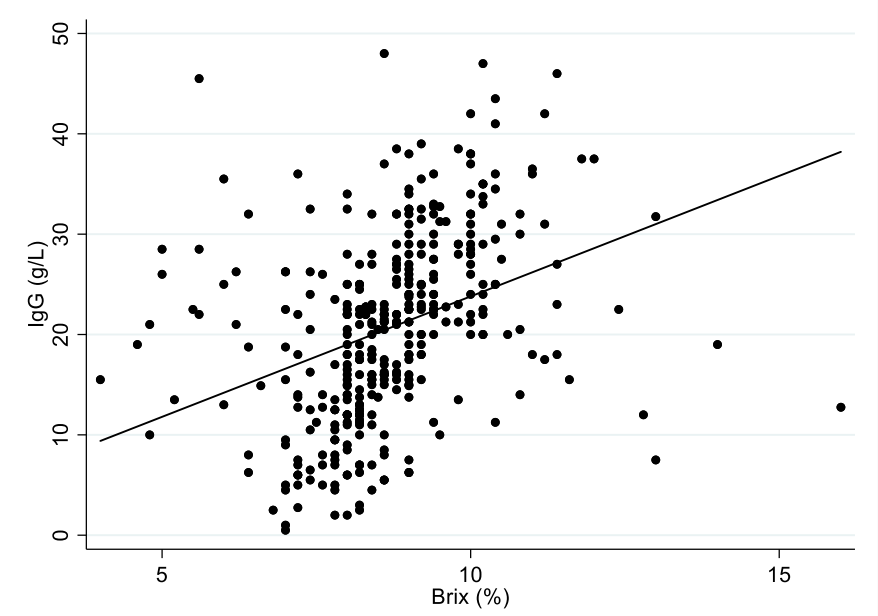


Figure 5-8 Scatter plot showing the relationship by r^2 between Brix (%) and RID(Glasgow) (mg/mL) in 367 dairy bred calves

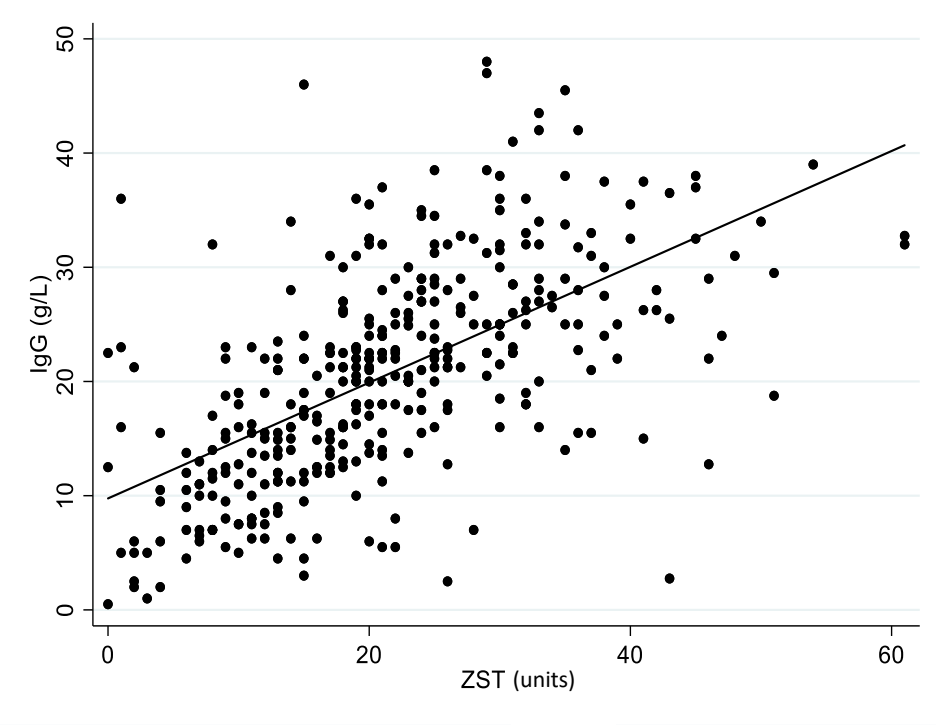


Figure 5-9 Scatter plot showing the relationship by r^2 between ZST (units) and RID(Glasgow) (mg/mL) in 367 dairy bred calves

Kappa statistic was used to further explore agreement. The κ values for the indirect tests are shown in Table 5-3 and the interpretation is shown in Figure 5-10. TP testing strategy had the best agreement (77.11%) and Kappa value (0.34) of all indirect testing strategies.

Test	Agreement (%)	Expected Agreement (%)	Kappa (κ) (95% CI)	Standard Error
RID(Glasgow)	*Ref*	*Ref*	*Ref*	*Ref*
TP	77.11	65.13	0.34 (0.24 – 0.45)	0.05
Brix	74.11	64.16	0.28 (0.18 – 0.38)	0.05
ZST	64.31	52.83	0.24 (0.17 – 0.32)	0.04

Table 5-3. Agreement, Expected Agreement and The Cohen's Kappa Statistic for indirect testing strategies, TP, Brix, ZST with RID(Glasgow) reference test from 367 serum samples from dairy bred calves aged 1-7 days of age.

	Interpretation of Cohen's Kappa Statistic					
	Poor	Slight	Fair	Moderate	Substantial	Almost perfect
Kappa	0.0	0.20	0.40	0.60	0.80	1.0

Figure 5-10 The interpretation of Cohen's Kappa Statistic of interrater reliability

5.1.4 **Cut point and test performance**

Optimal cut points for each diagnostic test were explored by constructing Receiver Operating Characteristic (ROC) Curves, Figure 5-11 to Figure 5-13. The Youden Index (*J*) and the Area Under the ROC (AUROC) summarised the diagnostic accuracy of the cut point and are shown in Table 5-4. A Youden value of one indicates that there is complete separation between FPT and no FPT whereas complete overlap gives a Youden Index of zero. AUROC of 0.5 indicates the test is no better than chance whereas AUROC of one indicates perfect distinction between FPT and not FPT.

Test	Optimum cut point	Youden Index (J)	AUROC Curve at cut point
RID(Glasgow)	*Ref*	*Ref*	*Ref*
TP	5.0 g/dl	0.5	0.75
Brix	8.2 %	0.4	0.72
ZST	15 units	0.6	0.80

Table 5-4 The ROC curve analysis of the three indirect testing methods (TP, Brix and ZST) with RID(Glasgow) measured at 24 hours as the reference test showing Youden Index, Optimal cut point, and Area Under the ROC (AUROC) Curve at cut point.

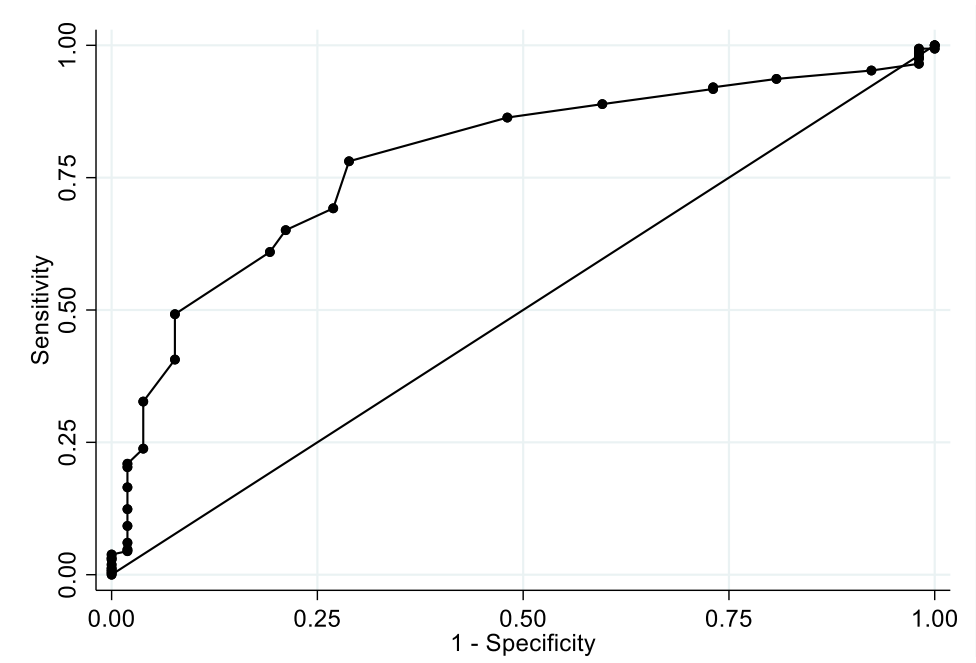


Figure 5-11 Receiver Operator Characteristic (ROC) curve of TP refractometry for diagnosing FPT in 367 dairy bred calves sampled between February and June 2019. The AUROC = 0.75 and optimal cut point = 5.0 g/dL

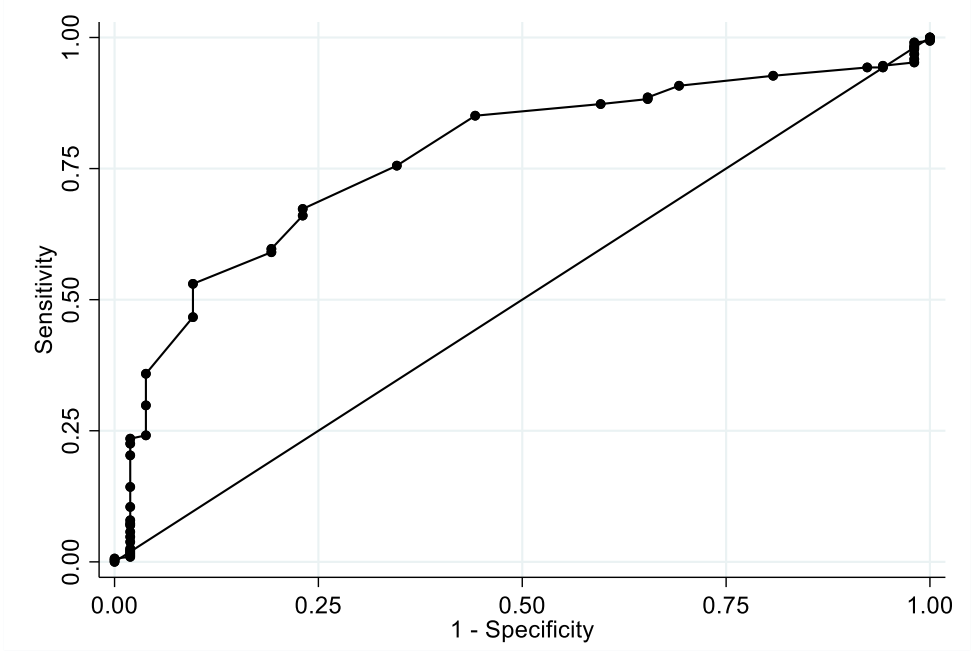


Figure 5-12 Receiver Operator Characteristic (ROC) curve of Brix refractometry for diagnosing FPT in 367 dairy bred calves sampled between February and June 2019. The AUROC = 0.72 and optimal cut point = 8.2%

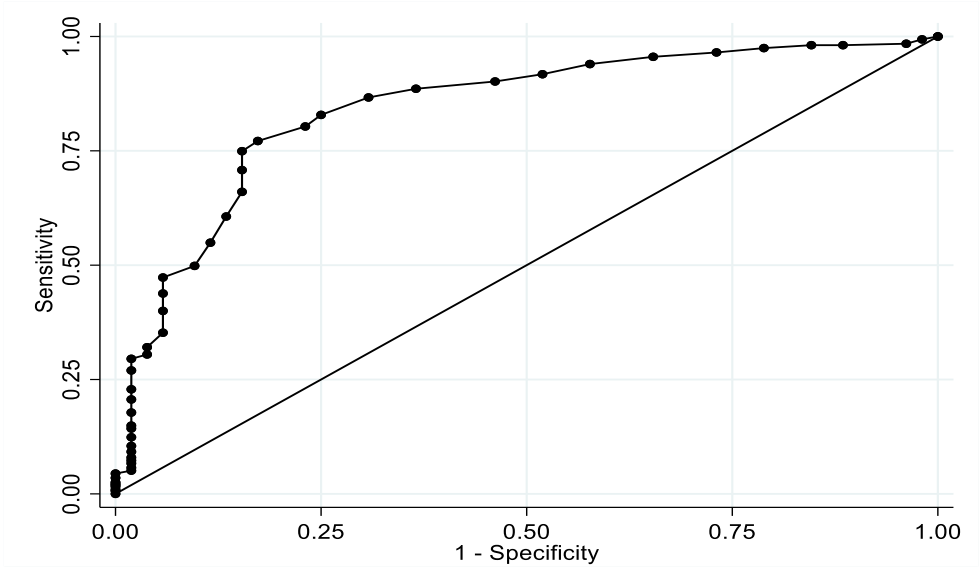


Figure 5-13 Receiver Operator Characteristic (ROC) curve of ZST Testing for diagnosing FPT in 367 dairy bred calves sampled between February and June 2019. The AUROC = 0.80 and optimal cut point = 15 units

ROC curves were used to redefine cut points for each of the indirect measures of serum IgG for this population of Scottish neonatal dairy calves. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for these new cut points (ROC) are shown in Table 5-5 along with those at cut points used in the study, as previous defined by published literature, for comparison. Accuracy was improved across all three testing strategies at the newly defined cut points. The specificity of each testing strategy was also improved at the newly defined cut points.

	Cut Point	Sensitivity (n) [95% CI]	Specificity (n) [95% CI]	PPV (n) [95% CI]	NPV(n) [95% CI]	Accuracy (n) [95% CI]
TP						
ref	5.2 g/dL	0.71 (37/52) [0.57-0.83]	0.78 (246/315) 0.73-0.83	0.35 (37/106) 0.26-0.45	0.94 (246/261) [0.91-0.97]	0.71 (283/367) [0.72-0.81]
ROC	5.0 g/dL	0.52 (27/52) [0.38-0.66]	0.86 (272/315) [0.82-0.90]	0.39 (27/70) [0.27-0.51]	0.92 (272/297) [0.89-0.94]	0.80 (294/367) [0.76-0.84]
Brix						
ref	8.4 %	0.78 (40/52) [0.63-0.87]	0.66 (208/315) [0.61-0.71]	0.27 (40/147) [0.20-0.35]	0.95 (208/220) [0.91-0.97]	0.68(248/367) [0.63-0.72]
ROC	8.2 %	0.65 (34/52) [0.51-0.78]	0.76 (238/315) [0.70-0.80]	0.31 (34/111) [0.22-0.40]	0.93 (238/256) [0.89-0.96]	0.73 (269/367) [0.68-0.77]
ZST						
ref	20 units	0.87 (45/52) [0.74-0.94]	0.61 (191/315) [0.55-0.66]	0.27 (45/169) [0.20-0.33]	0.97 (191/197) [0.93-0.99]	0.64 (236/367) [0.59-0.69]
ROC	15 units	0.77 (40/52) [0.63-0.87]	0.80 (253/315) [0.75-0.85]	0.39 (40/102) [0.30-0.49]	0.95 (253/265) [0.92-0.98]	0.80 (293/367) [0.75-0.84]

Table 5-5 Test results for the three indirect tests used to predict failure of passive transfer in dairy calves (defined as concentrations of IgG in serum ≤10.0 g/L). The indirect tests used were TP refractometry (g/dL) and Brix refractometry (%) and ZST (Units) in 367 calves aged 24 hours to 7 days. Cut points were derived from published data (ref) and shown alongside those which were optimised based on receiver operating characteristic curve analysis (ROC).

5.2 Discussion

5.2.1 Variation and agreement of RID plates between laboratories and the investigation into timing of readings.

RID has been long established in the literature as the gold standard or reference test for measuring serum IgG concentration (Weaver et al., 2000; Godden et al., 2019). There are now multiple RID assays available commercially. Correlation and agreement between RID(Glasgow) and RID(Biobest) in this study population were not perfect. The experienced technicians at the Biobest laboratories had refined their technique, such as using callipers to measure ring diameter as opposed to the supplied ruler when using this particular test plate. Some of the poorer agreement was attributed to the superior technique and experience at Biobest compared with internal university laboratory technicians who had not used the TripleJ test kits before. RID immunoassays where measurement of precipitin rings is required does not lend itself to automation therefore technician skill comes into play (Ameri and Wilkerson, 2008). In the literature, imperfections in RID assays have been noted previously and are attributed to inconsistencies in the reference standards (Ameri and Wilkerson, 2008) On balance, it was decided that RID(Glasgow) and RID(Biobest) testing strategies sufficiently agreed to justify using RID(Glasgow) methodology as the reference test in further analysis of the indirect testing strategies in this study. The limitations were acknowledged in the evaluation of results.

As discussed, the RID test method is time consuming. Indeed, the turnaround of testing at Biobest laboratories is quoted in their price list as up to seven days. The manufacturer (Bovine IgG RID Kit, Triple J Farms, Bellingham, WA) of the zonal diffusion RID plates recommends that end point readings of the test are taken > 24 hours. In this study, it was found that results could be accurately read at 24 hours instead of 40 hours. To reduce the reading time from 24 to 40 hours has obvious advantageous with respect to turnaround time and associated costs.

5.2.2 Comparison of indirect diagnostic tests with RID

To the author’s knowledge this is the first study to examine the diagnostic performance of testing methods for FPT in dairy calves in Scotland. Of the

indirect testing methods, correlation analysis showed ZST had the strongest relationship (r=0.6) with RID compared with TP and Brix. The correlation found between reference test and ZST is similar to that found by Zakian et al. (2018) (0.74) where the reference test used in that study was ELISA. Correlations between RID and TP and Brix have been described as stronger in other published literature ranging from 0.74 – 0.95 and 0.79 – 0.93 respectively (Chapter 1, Section 1.6) There were several outliers, which were maintained in the data set in the present study, which were likely to reduce the apparent correlation between testing methodologies. Wilm *et al.*, (2018) found that whilst IgG and serum TP were highly correlated in calves aged 4 days, in older calves this relationship was more variable. The mean age of calves in this study was 3.83 days (SD ± 1.74) and the outliers were aged between 2-5 days therefore the apparent poorer correlation is unlikely to be due to inappropriate age at sampling. Furthermore, the age of the calves was tested in the statistical models (Chapter 4, Section 1.3 and 1.4) and no effect was found on any measurements. When analysed, 3 of the outliers have high TP with a low IgG; it is possible that these calves were ill at time of sampling. Serum total proteins cannot be used as a proxy for FPT status in dehydrated or sick animals due to the variations in protein concentrations attributable to the disease process which can lead to misclassification of FPT (Tyler *et al.*, 1999). Data within the peer reviewed literature pertaining to r^2 values between RID and TP, Brix and ZST is limited, however Hernandez *et al.* (2016) found r^2 to be higher between RID and TP and Brix, 0.68 and 0.63 respectively, than found in this study.

However, a correlation does not necessarily show test agreement. Performance cannot be judged on this criterion alone (Altman and Bland, 1983). Agreement in this study was examined using Cohen's Kappa statistic. Slight to fair agreement was found to exist between serum TP and serum IgG and Brix and serum IgG, 0.34 and 0.28 respectively. Only slight agreement (0.24) was found to exist between RID and ZST testing methods. Therefore, despite having the strongest correlation (r=0.6), ZST has the weakest Kappa statistic when compared with RID(Glasgow).

Results were dichotomized using the threshold of 10mg/mL to indicate FPT or no FPT. Since Kappa is calculated on dichotomized FPT results, equal weighting is

given to a ‘near miss’ and a ‘far miss’ between tests. Therefore, some sensitivity is lost compared with if results were run on a continuous scale.

Furthermore, Kappa is affected by prevalence of disease. The prevalence of FPT was found to be 14.2% FPT in this study. The prevalence index is high meaning the prevalence of a ‘positive rating’ (i.e. having FPT) is low which means chance agreement is high and therefore Kappa is lower than the actual agreement present (Byrt et al., 1993). Both Lee et al. (2008) and Hogan et al. (2015) found a higher Cohen’s Kappa statistic for serum TP and IgG, 0.78 and 0.72 respectively. The prevalence of FPT in both these studies were higher than in this study at 35.65% and 58% respectively. Zakian et al. (2018) found a similar FPT prevalence to this study, as determined by ELISA, of 13% and Kappa of ZST to be 0.34. No previous studies have reported the Kappa statistic for optical Brix refractometry.

When compared with previous peer reviewed literature the indirect tests did not perform as well in the present study. The precedent of using this test had been set in many other peer reviewed papers (Hogan et al., 2015, McCracken et al., 2017, Dunn et al., 2018). The RID test was considered a reference test as opposed to a gold standard. Imperfect sensitivity and specificity could compound variation in all three indirect testing methods (Toft et al. 2005). However, indirect tests do not directly measure serum IgG and use measurements (e.g. total protein, total solids and turbidity) as proxy for IgG. Despite the imperfect performance of indirect testing, these methodologies remain clinically relevant given their inexpensive and rapid nature.

In the clinical context of FPT testing and on farm monitoring it is widely accepted that RID is not appropriate because of the delay in results, laboratory analysis and cost. Biobest Laboratories, Edinburgh, charges the client around £32/sample with a turnaround time of up to seven days. Despite the less than perfect correlation and agreement with the reference test, TP and Brix refractometry remain useful tools to monitor FPT on farm. These tests have the advantage that they can be performed calf side or in a practice laboratory and are much cheaper to carry out. In addition the Brix refractometer is a dual purpose instrument which can be used to measure colostrum quality; a key part of the holistic approach necessary for FPT investigations (Bartier et al. 2015). As

an indirect method, ZST testing requires laboratory facilities and demonstrated no clear advantage over calf side indirect tests such as TP and Brix in terms of test practicalities. Furthermore, test agreement when examined via Kappa was the poorest of the three indirect methods.

It has been discussed previously (Chapter 1, Section 1.6) that the most desired test characteristic for diagnosing FPT would be a higher sensitivity (true positives) and specificity can be compromised as the consequence of false positives is not harmful. All three indirect methods, TP Brix and ZST, overestimated the prevalence of FPT, 29.46%, 40.54% and 46.49% respectively compared with RID(Glasgow), 14.2%. Sensitivity and specificity of the individual test will not change depending on prevalence of disease in the population of interest but the positive predictive value (PPV) and negative predictive value (NPV) and accuracy will be affected by prevalence (Parikh et al. 2008). The NPV of all three indirect tests are high, therefore high confidence is placed in a negative result which is a desirable characteristic of a screening test. The low-moderate PPV of the indirect tests are associated with a higher false positive rate. However, false positives are tolerable when screening for FPT because the risk of harm from follow up management changes, for example improved colostrum management are minimal. In fact, potentially this may be even more beneficial to calves because, as discussed below, higher serum IgG concentrations are associated with not only reduced mortality but morbidity of disease as well (Chigerwe et al., 2015; Bragg et al., 2020). As the prevalence increases, the positive predictive value increase, leading to a decrease in false positives for every true positive, meaning the difference between the ability of the test to distinguish between true positives and false positives is less evident. The moderate PPV value associated with all three indirect testing methods is acceptable.

Analysis of ROC Curves revealed that test performance, in terms of accuracy, could be improved by lowering cut points to 5.0 g/dL, 8.2% and 15 units for TP, Brix and ZST respectively. Findings that the indirect tests perform better at lower cut points are in agreement with other peer reviewed science. The TP optimal cut point determined in this study is in line with previous findings by Calloway et al. (2002). However, they determined the sensitivity and specificity, at this cut point, to be 0.8 and 0.91 respectively which is much improved

compared to the estimated sensitivity and specificity of this study, 0.52 and 0.86 respectively. Elsohaby et al. (2015) found that a cut point of 8.2% Brix the sensitivity and specificity were 0.76 and 0.86 respectively. The sensitivity in that study was higher than found in this study, 0.65. Findings that the ZST test performs better at a lower cut point is in line with work carried out by Hogan et al. (2016). They recommend a cut point of 12.5 units. A test will perform differently in different populations and depends on the prevalence of disease in the study population (Parikh *et al*. 2008). The PPV and NPV of the tests on the whole remain unchanged at the lower cut points which was considered acceptable as they suited desired testing characteristics. ROC Curve Analysis and the Youden Index work to maximise both specificity and sensitivity. Mathematical statistical tests that assess test performance do not take into consideration influences such as calf health records, nutrition and housing which are all pertinent to clinical diagnosis of FPT at farm level. In the case of the diagnosis of FPT, decreasing serum IgG concentrations are associated with disease (i.e. FPT). It has been discussed previously, for initial screening tests, sensitivity may be prioritised over specificity. Lowering the cut points of the three indirect testing methods improved overall test accuracy at the expense of the sensitivity in all three cases. A screening test for FPT aims to identify all possible cases to aid herd level investigation and management decisions to maximise calf health. Highly sensitive tests will correctly identify all the calves that have FPT (true positives). It could be argued that tolerating some false positives is acceptable because overestimating the number of calves with FPT is of lesser signification than the implications of underestimation. There is a real risk of mortality if calves are subject to FPT. However the sensitivity characteristic of the test can also be compromised to some extent depending on how prevalent FPT is thought to be within the herd – i.e. the pre-test probability of the disease (Tompson and McNeil., 2000). Therefore, in a clinical setting, there is still a place for indirect testing methods with imperfect sensitivity and specificity. Ultimately, the performance of a diagnostic test will depend on the study population and more specifically the prevalence of disease within that population (Florkowski., 2008). Furthermore, as proactive calf health programmes, of which FPT is a crucial part, progress on farm the emphasis on test characteristic change to ensure the impact of management improvements are detected. Specificity becomes important to minimise false positives so that

any improvements due to management changes are detected and staff are not demoralised.

Furthermore, to give a calf 'FPT' or 'No FPT' status a decision cut point is required to dichotomise a continuous biomarker scale (serum IgG). Whilst dichotomisation allows for estimations of diagnostic test sensitivity and specificity, as described previously, this process assumes that a calf just below the cut point is very different from a calf just above the cut point. Numerous recent studies from both the beef and dairy sectors go beyond distinguishing calves with serum IgG concentration above and below a single cut point, usually 10 mg/mL (Furman-Fratczak *et al.* 2011, Bragg *et al.* 2020). They demonstrate that higher IgG concentrations are associated both with lower disease morbidity and mortality. In a recent discussion, Lombard et al. (2020) also reported higher IgG concentrations were associated with not only reduced mortality, but reduced disease morbidity. Subsequently, they have proposed a redefinition of the traditional dichotomized FPT outcome. Table 5-6 shows these redefined categories with the results of this study for interest. The traditional cut point of 10mg/mL is based on reducing mortality only and these redefined categories aim to reduce mortality and morbidity of disease (Lombard *et al.* 2020).

Category	Serum IgG (mg/mL)	Serum TP (g/dL)	Serum Brix (%)	Percentage of calves (%)	Percentage of calves (%) from this study (n)
Excellent	≥ 25.0	≥ 6.2	≥8.4	≥40	32.16 (119/370)
Good	18.0 – 24.9	5.8 – 6.1	8.9-9.3	~30	28.65 (106/370)
Fair	10.0 – 17.9	5.1 – 5.7	8.1-8.8	~20	25.51 (94/370)
Poor	<10.0	<5.1	≤8.1	<10	13/78 (51/370)

Table 5-6 The new proposed on farm categorising system for FPT monitoring with the aim to minimise morbidity of disease as well as mortality shown alongside results from this study for interest (Lombard et al., 2020).

It could be argued that these redefined categories complicate a scenario which is currently simple for practitioners to explain to farming clients. However, by oversimplifying the standards, the opportunity to exploit the lower risk of disease associated with higher serum IgG concentration is missed. There has to be a balance between the benefits of serum IgG >18mg/mL and the cost in terms of labour and time. Raboisson et al. (2018) found that farmers should be spending at least 15 minutes on colostrum management/ calf. It is difficult to quantify this in an on-farm situation. Further research is needed to clarify how

this time is spent to achieve higher serum IgG concentrations – more time per calf or more time on colostrum management or a combination. Hyde et al. (2020) recently looked at which risk factors would have the *largest effect* on colostrum hygiene on the *largest number* of farms through bootstrapping techniques. They concluded hygiene protocols around collection, storage and feeding of colostrum were important.

5.3 **Summary**

Indirect tests are imperfect and performed relatively poorly in this study; however, the reference test RID is not a gold standard and is inconvenient because of its time consuming, expensive nature, making it unsuitable for routine use in practice. Indirect testing for on-farm monitoring of FPT status in dairy calves remains clinically relevant to modern dairy farming. Of the indirect tests available, TP and Brix are preferred methods because both are inexpensive, provide rapid results and showed better agreement when examined via Kappa statistic. ZST showed no advantage over TP or Brix because it involves laboratory processing despite arguably better performance, particularly in terms of accuracy, at the suggested revised cut points.

6 General discussion

Failure of passive transfer (FPT) is prevalent worldwide. This study found that approximately 1 in 7 calves in this Scottish population is subject to FPT. The effects of FPT have been well established in terms of the consequences for disease morbidity and mortality, calf welfare, production losses and further economic losses for the farming enterprise (Tyler *et al.*, 1999; Raboisson *et al.*, 2016). High calf mortality rates in the early rearing period have been normalised by producers within the dairy industry (Hyde *et al.*, 2020). It the current climate, where there is a drive for efficient food production from healthy animals, it is relevant for dairy producers to work with their veterinary surgeon to minimised FPT prevalence on farm. The increased morbidity and mortality of calves with FPT will directly increase the number of animals treated with antimicrobials. This directly impacts the drive to refine and reduce antibiotic treatments in food producing animals. The investigation and monitoring of FPT can lead to management and husbandry changes that directly create healthier livestock that require less medication.

In order to diagnose FPT, clinicians have available to them a range of direct and indirect testing methods. When considering which test to use, clinicians should understand test performance in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Other test characteristics such as ease of carrying out the test, cost, and turnaround time are also important considerations. Yet it is these other test characteristics that can dominate decision making in a clinical practice setting rather than test performance. Denholm and Morrison, (2021) found that 48.5% of Scottish clinicians surveyed based their choice of FPT test on cost. Test performance can be affected by cut point used and it may be relevant to adjust the cut point depending on the predicted FPT prevalence in the calf population. In a herd health context, it is important to identify all calves with FPT therefore sensitivity should be maximised. Specificity can be forgone to some extent as the consqeuences of false positives would only be to strengthen colostrum management protocols and achieve higher IgG concentrations. Prior to this work, little work had been done previously in a UK and Scottish context to examine FPT diagnostic test performance within the calf population.

When all test characteristics are considered, this study determined that TP and Brix refractometry are the most useful indirect test for clinicians in the field. Brix refractometry has the further benefit of being able to be used to reliably and cheaply to analyse colostrum quality on farm. Whilst TP and Brix testing methods are cheap and easy to carry out, their performance is imperfect when compared to RID, the reference test. Indeed, in this study their performance was poorer than ZST. The available reference tests are not gold standard with imperfect sensitivity and specificity therefore any comparisons on correlation and agreement are less easy to interpret. This is because comparisons to determine test performance (in terms of sensitivity, specificity, PPV, NPV, and accuracy) are being made against less than 100% accurate outcomes. Any perceived imperfections in the indirect testing method may be due to imperfections in the reference standard to which it is compared.

When investigating FPT prevalence on farm, clinicians must endeavour to obtain a sample size to ensure precision of the prevalence estimate. McGuirk and Collins (2004) recommend a minimum sample size of 12 calves per farm. Work from New Zealand further explored sample size to include confidence levels in their sample size estimates which McGuirk and Collins (2004) do not include in their recommendations. Cuttance *et al*. (2017) concluded that if only 12 calves are sampled, there is a 95 % certainty that the herd level prevalence is < 20% only if no calves test positive for FPT. A parallel project to the current study identified that only 17% of Scottish clinicians are sampling an adequate number of calves in routine surveillance (Denholm and Morrison, 2021). The sample size that ensures precision depends very much on the predicted FPT prevalence and the population at risk. On some smaller farms, multiple visits over a period of time may be required to achieve this level of confidence. This highlights, once again, the importance of FPT monitoring as part of an on-going approach to calf health on farm oppose to relying on single point in time sampling.

In reality, farm-level assumptions regarding FPT are being made on imperfect testing methods and small samples sizes. The risk is assumptions are being made on small sample sizes and imperfect tests that FPT is not present, when in fact it is. FPT test results should not be taken in isolation but interpreted holistically along with farm hygiene standards and calf health records. In this study, test performance was assessed on results from a single animal sample. In clinical

circumstances FPT testing should ideally be undertaken as part of an ongoing approach to preventative calf health as the longitudinal nature of these test results is important. The emphasis of the difference test characteristics in terms of sensitivity and specificity changes after initial investigation into on farm prevalence. As a screening test maximising sensitivity is important to ensure all calves with FPT are identified. Specificity can be foregone to some extent as false positives would only lead to interventions to remedy FPT which would lead to higher serum IgG concentrations, and these have been associated with lower disease morbidity and mortality. As bespoke control strategies are implemented on farm and FPT is monitored over time, the specificity becomes important. This ensures false positives are minimised and there is not over estimation of FPT so that any improvements are detected, and staff are not demoralised. This study has identified that indirect testing methods underestimated IgG concentration therefore overestimate FPT.

The traditional cut points that dichotomise results into 'FPT' or 'no FPT' have been the precedent for FPT testing. The cut point of 10 mg/mL serum IgG is based around mortality only (Wells *et al.,* 1996; Weaver *et al.,* 2000). The conventional binary manner, whilst simple for clinicians to explain to producers, does not fully capture the association of lower morbidity and mortality associated with higher serum IgG concentrations. Lombard *et al.* (2020) proposed a multi-level categorising system as discussed in Chapters 1 and 5. The concept of multiple levels of IgG categories is not new. The concept was presented in 2015 by Chigerwe et al., who hypothesised that the current cut point of 10 mg/mL was too low to indicate adequate transfer of passive immunity. Urie et al., (2018) found a negative association between serum IgG concentration and morbidity. Furthermore, in their study of 175 dairy heifer calves, Furman-Fratczak et al. (2011) divided the calves into groups according to their serum IgG concentration at the point of sampling: <5g/L, 5-10 g/L, 10-15g/L and >15 g/L. They found that calves with >15 g/L serum IgG avoided respiratory tract infections. Bragg et al. (2020) found that a serum IgG concentration of <24 g/L was associated with increased morbidity and mortality in beef calves. This finding was in line with earlier work where a lower morbidity and mortality during the pre-weaning period in beef calves was associated with a serum IgG concentration of >24g/L (Dewell et al., 2006; Waldner and Rosengren., 2009). Whilst extrapolation between beef and dairy systems should

be done with caution, for example colostrum management is very different between the two systems, it is clear there is additional benefit to higher concentrations of serum IgG. In the current climate, where the public are increasingly interested in the welfare of production animals and antimicrobial resistance is a concern, focus should be given to producing a robust, resilient neonatal calf and to minimise both disease mortality and morbidity.

Colostrum is a crucial component to producing a robust and resilient calf. It provides a source of IgG for initial humoral immunity in the first few weeks of life, other important immune factors and nutrients (Godden *et al.*, 2019). Colostrum quality, in terms of IgG concentration and bacterial contamination, is intrinsically linked to FPT prevalence. To prevent FPT and its consequences neonatal calves must consume 10-15 % of bodyweight of good quality (IgG > 50g/L or 22 % Brix) clean (TBC < 100,000 CFU/mL, TCC< 10,000 CFU/mL) colostrum (Godden *et al.*, 2019). This study found the volume of colostrum consumed at first feed was a significant risk factor for the development of FPT in line with other published literature. Furthermore, this study revealed only 40% of colostrum samples tested met all three quality indicators (Brix >22%, TBC = <100,000 CFU/mL and TCC = < 10,000CFU/mL). Calf health and FPT prevalence can be improved when detailed attention is given to colostrum management programmes to improve the IgG concentration and reduce bacterial contamination. Further risk factor analysis revealed the more time that colostrum is left sitting in a bucket post-harvest, the greater the opportunity for contamination with faecal and environmental bacteria and bacterial multiplication.

Hygiene is a crucial part of any colostrum management protocol. Anecdotally, colostrum hygiene investigations are underutilized by clinicians in practice. Investigations through bacterial counts and Brix refractometry are cheap and easy to carry out. They should form part of every holistic investigation and monitoring programme of preventative calf health care.

Whilst this study has successfully established the prevalence of FPT in this Scottish calf population, analysed test performance available for FPT diagnosis and identified risk factors for colostrum quality and FPT, there still exists a disparity between what is known in the literature, what is known by producers

and what is actually happening at calf level. This is evident through FPT prevalence estimates and continued high neonatal mortality (MacFarlane *et al.*, 2015; Hyde *et al.*, 2020). As always, the focus of applied clinical research should be communication at farm level to make a tangible impact on, in this case, calf health and welfare. The veterinary profession and farming sector still have work to do but ensuring good colostrum management will bring rewards.

Appendix 1

University of Glasgow | College of Medical, Veterinary & Life Sciences

Consent from to permit information and samples gathered from your farm to be used in research and to state your understanding of the project outline to be conducted at you farm.

By signing this form, you are agreeing that:

1. The University of Glasgow can use any samples and information gathered in the course of visits and follow up communications for research and teaching purposes. This data will only be used anonymously, and in this form may be shared with groups which collaborate with the University of Glasgow for research purposes
2. The research project that will be conducted at your farm has been appropriately explained and you understand the implications that it has on your farm.

SIGNED_____(DATE)_____

PRINTED NAME

ADDRESS

On behalf of the University of Glasgow: I have discussed and explained the meaning of this form.

SIGNED_____(DATE)_____

PRINTED NAME

VETERINARY PRACTICE:

ENROLMENT DATE

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