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Review

Moving past serology: Diagnostic options without serum

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ABSTRACT

Detecting antibodies formed in serum in response to infection is the traditional function of serology. Diagnostic modalities have included complement fixation tests, agar gel immune-diffusion, radioimmunoassay, ELISA and immunofluorescence. More recent technology now allows for the direct detection of pathogens by PCR. This review details the options for diagnostic testing using specimen types other than serum, identifying the advantages and disadvantages of these options and providing evidence for more widespread use of these techniques and specimen types.

Introduction

A quest for clean serum specimens to detect immunological responses to the presence of important pathogens has characterised the history of disease testing in veterinary medicine. Serum has traditionally been preferred over whole blood to decrease non-specific reactions, and give more accurate and reliable results. Assays such as the complement fixation test, dating from the very beginning of the last century (Bialynicki-Birula, 2008), agar gel immune-diffusion test (Ouchterlony, 1948), radioimmunoassay (Yalow and Berson, 1960) and immunofluorescence antibody testing (Voller, 1964) were some of the first tests used. In more recent years, ELISA (Engvall and Perlmann, 1971; Van Weemen and Schuurs, 1971) in its various permutations (direct, indirect, competitive, sandwich, capture) has been able to detect either antibody or antigen, and is popular because it is simple, inexpensive and rapid.

Newer technologies such as PCR and quantitative PCR (Mullis and Faloona, 1987) are pathogen detection methods and could readily be applied to a variety of a specimen types containing genetic material. Extraction and amplification clean-up steps now make PCR less prone to interference than earlier versions that relied on observation to detect lines or agglutination. Recent further developments in PCR technology, eliminating the need for expensive thermocyclers, have the potential to further revolutionise field diagnostics (Thekisoe et al., 2007).

Specimen types other than serum have commonly been collected and tested in recent years, but difficulties of obtaining a clean and reliable signal to confirm a diagnosis have had to be overcome. Improvements in laboratory science and accessibility to good laboratory services and practices have improved diagnostic efficiency and decreased turnaround times. Additionally, efficient courier services make it practical to transport suitable specimens to a well-equipped laboratory.

In some parts of the world it is still difficult to get good quality specimens reliably to a diagnostic laboratory, either because the necessary transport infrastructure is absent or distances within the country or between countries to the laboratory infrastructure are too great. Similarly, convenience and cost-effectiveness can affect specimen collection, as obtaining a blood specimen is often the domain of veterinary or para-veterinary personnel, adding to the expense of diagnostic testing. Other readily obtainable body fluids, excretions or tissues could be obtained by less skilled personnel or animal owners to save on collection costs. In the human diagnostic field there is currently significant interest in exploring alternative specimen analysis such as dried blood spot testing for the diagnosis of hepatitis C (Coats and Dillon, 2015).

Recently, there has been a move away from centralised laboratory services, to 'in-house' or practice-based laboratory services for a variety of clinical disciplines, including serology. The robustness and reliability of the sampling technology has improved and has also started to venture out towards more novel applications, including point-of-care single specimen assays.

This review discusses a number of testing modalities with some examples from the authors' experience. Since this field is constantly evolving, the examples are not comprehensive, but will hopefully encourage others to explore more novel testing options.

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Milk

Milk can be a suitable medium for animal disease testing as it is generally easy to obtain (often without any specialised equipment), and in dairy cattle it is often available throughout the year. Using milk as a specimen, a wide range of animal diseases can be tested for in individual animals and in pooled specimens from herds. Tests for antibodies against the following pathogens are available: *Brucella abortus* (Nielsen and Gall, 2001), bovine viral diarrhoea virus (BVDV; Lanyon et al., 2014b), enzootic bovine leukosis (EBL) and bovine herpes virus 1 (BoHV1; Reber et al., 2012), *Neospora caninum* (Schares et al., 2004; Hall et al., 2006), liver fluke (*Fasciola hepatica*; Reichel et al., 2005), Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis*; MAP; Collins et al., 2005) and *Ostertagia ostertagi* (Charlier et al., 2005; Forbes et al., 2008) in cattle. Single animal testing can be performed and tank milk from herds of dairy cows is a ready-made pool for testing groups of animals. Tank milk presents a natural pool of animal biological specimens that, with adequate test analytical sensitivity, enables the tester to screen large numbers of animals for the presence or absence of disease. While testing for EBL by antibody ELISA, milk pools rarely exceeded 100–200 cows (Ridge and Galvin, 2005), but PCR testing for BVDV is now routinely performed on pools in excess of 400 because high test analytical sensitivity provides a very cost-effective way of screening large herds and, more broadly, whole dairy industry. Herds of >400 cows are typical for the New Zealand dairy industry (Hill et al., 2010).

Historically, milk testing for pathogens was used to test for *B. abortus* infection using the 'milk ring test' (Fleischhauer, 1955), where a drop of stained *B. abortus* antigen was added to a pooled milk specimen. If antibody to *B. abortus* was present, an antibody–antigen complex was formed, adhering to milk fat globules and rising to the surface of the milk as a coloured ring (Fleischhauer, 1955). False positive results for *B. abortus* antibody can occur in cattle vaccinated against *B. abortus* <4 months prior to testing, or in milk containing colostrum or from cows with mastitis. Infection with other pathogens, such as *Yersinia enterocolitica*, can also cause non-specific positive reactions (Kittelberger et al., 1997). Comparable results are obtainable with the fluorescence polarisation assay (Nielsen and Gall, 2001), and this newer test can be used in the field.

Viral pathogens such as bovine leukaemia virus (causative agent of EBL) and BoHV1 (causative agent of infectious bovine rhinotracheitis; Witte et al., 1989) can also be tested for in milk.

BVDV is widely tested for in milk specimens and testing can be simultaneously performed to detect virus (by PCR) and antibody (by ELISA), thereby providing a means of establishing both the presence and absence of the virus and measuring herd immunity. Antibodies formed against BVDV are excreted into milk and correlate well with serum antibody titres. Pooled testing provides a quantitative ELISA assessment strongly correlated with within-herd prevalence (Lanyon et al., 2014b). High levels of antibody in the tank milk suggest sufficient exposure to virus to reduce the need for vaccination, while low tank milk antibody levels suggest the need for biosecurity measures or vaccination to prevent infection. The ability to monitor the relative changes in pooled antibody levels, and therefore, within-herd prevalence is particularly valuable. A sudden increase may be indicative of a recent incursion of infection, a change that can be difficult and expensive to detect when relying on individual animal testing, and would suggest the need for further investigation, beginning with bulk milk PCR testing.

As a logical follow-on after eradication of BVDV from the national dairy herd in Switzerland, on-going monitoring for continued freedom from BVDV infection is now based on regular bulk milk surveillance in that country (Presi et al., 2011). Surveillance testing for recently emerging animal diseases, such as bluetongue and Schmallenberg viruses, has also been based on bulk milk testing by ELISA (Balmer et al., 2014).

Pooled milk testing has also been successfully applied for EBL detection. Screening of all dairy herds in New Zealand by testing pools of milk from groups of 20 dairy cows using EBL ELISA found no evidence of infection by 2011 (Voges, 2011). While primary testing was on pooled milk specimens, further testing was required for any suspicious or positive results using individual serum antibody ELISA and PCR tests. By contrast, the EBL status of the beef industry in New Zealand remains unknown.

In Switzerland, a bulk tank milk specimen to test for BoHV1 reported significant cost savings by using milk instead of serum to detect antibodies. If the expenditure was identical, testing bulk milk yielded significant increases in test sensitivity (Reber et al., 2012), thus improving diagnostic outcomes.

Diagnostic outcomes have also been improved through the use of *N. caninum* bulk milk ELISA testing to predict the prevalence of infection in dairy herds in Australia (Hall et al., 2006). The stage of lactation affected the accuracy of the comparison between serum and milk (Schares et al., 2004), and milk testing was more sensitive.

Excellent accuracy has also been demonstrated when testing individual milk specimens for antibodies against *F. hepatica*, with very high sensitivity and specificity close to 100%. However, when bulk tank milk specimens were tested there was a decrease in sensitivity, so only dairy herds where the prevalence of *F. hepatica* was >60% could be identified (Reichel et al., 2005).

Serological tests for Johne's disease have low sensitivity but reasonable specificity. Testing of individual milk specimens yielded a sensitivity of 28% (Collins et al., 2005), slightly higher than serum, and sensitivity increased with age of animal tested (Nielsen et al., 2013). PCR can also be used to test for the presence of MAP DNA in milk (Buergelt and Williams, 2004), as can the peptide-mediated magnetic separation-phage (PMS-phage) assay (Foddai et al., 2011). However, advances in PCR testing for MAP in faeces could negate the need to use antibody based tests.

Antibody based tests (ELISA) are available to measure antibodies in bulk tank milk to the abomasal parasite *O. ostertagi* (Forbes et al., 2008). Only an association between ELISA values and milk yield can be made using these test results, rather than confirming true positive nematode infections in the herds, so additional diagnostic testing is required to establish the parasite status of the herd.

In sheep, Q-fever (*Coxiella burnetii*; Klaasen et al., 2014), *Brucella melintensis* (Hamidi et al., 2015) and *Mycoplasma agalactiae* (Poumarat et al., 2012) can be tested for using milk; in goats, milk specimens can be used to test for caprine arthritis and encephalitis (Nagel-Alne et al., 2015). Q-fever outbreaks in humans are associated with *C. burnetii* infection in small milking ruminants in Africa (Klaasen et al., 2014). Shedding of the organism is intermittent, thus infection was not always detected by PCR and serological tests might also be required. In contrast, PCR testing of milk for *B. melintensis* detected was more sensitive than serology in one study (Hamidi et al., 2015). Accurate serological classification of the *M. agalactiae* status of sheep is difficult and PCR testing of milk specimens with two PCRs should be used to confirm the presence of the organism. The resultant PCR results also require cross checking with a dot-immunobinding technique (Poumarat et al., 1991).

Milk testing can be utilised for detection of non-infectious conditions. For example, lateral flow devices to test for progesterone concentrations in milk present opportunities to define the oestrus cycle and pregnancy status of cows (Waldmann and Raud, 2016) and technological modifications may allow for testing to occur during milking (Dobson, 2016).

Colostrum

Colostrum is another medium that can be used for animal disease testing instead of milk. Its availability is restricted to a shorter time period, but provides other testing and diagnostic advantages. Testing

colostrum for antibodies (as an alternative or add-on to milk testing) is potentially useful because of the higher concentration of immunoglobulins in colostrum compared to milk. The concentration of IgG is estimated to be up to 100 times higher than milk in the first few days after parturition (Korhonen et al., 2000). Recent work in cattle suggests that testing colostrum increases analytical and diagnostic sensitivity compared to milk. This could be most useful for on-going surveillance of animal diseases requiring less frequent checks, as colostrum is only available during the perinatal period (Jenvey et al., 2012, 2015; Cockcroft et al., 2014).

The drawback of colostrum testing is the small window of availability and the difficulty of collection in some species. In dairy cattle, sampling of colostrum might be most usefully applied for diseases where diagnostic tests are hampered by low analytical and/or diagnostic sensitivity (such as is the case with Johne's disease; Reichel et al., 1999). Colostrum has also been used successfully for testing for rotavirus and mycoplasma infections (Corthier and Franz, 1981; Zimmermann et al., 1986; Rautiainen, 1998).

Hair and ear notch skin specimens

Hair specimens can be easily obtained, even by animal owners, and submitted for testing. Meanwhile, ear notches may be the by-product of routine farming procedures such as ear-tagging, thereby reducing the amount of additional handling and restraint required for animal disease testing.

Hair and ear notch specimens have been used successfully to detect BVDV persistently infected animals (Hill et al., 2007; Lanyon et al., 2014c) and formed the basis of the recent successful Swiss BVDV eradication campaign (Presi and Heim, 2010). In this campaign, detection of BVDV antigen in skin was the specimen of choice to identify persistently infected calves, and was preferred over serum. After the ingestion of colostrum, maternal anti-BVDV antibodies can bind to BVDV antigen and prevent its successful detection in the routinely used antigen-capture ELISA (Fux and Wolf, 2012). Using ear notch skin specimens reduces this complication, as there are fewer antibodies in ear notch tissue. Heating of serum specimens under specific conditions to break up antibody-antigen complexes can overcome the interference of maternal antibody and allow successful serum testing. This adds extra steps to the procedure, but has been used effectively in BVDV testing (Lanyon and Reichel, 2016) and in heartworm serology (Little et al., 2014a, 2014b; Velasquez et al., 2014).

Ear notch specimens are increasingly being used for parentage testing because of the ease with which they can be obtained[1]. Hair has also been used (Singh et al., 2011) to identify persistently BVDV infected animals, and in parentage testing[2] and DNA profiling.

Swabs from mucosal surfaces

Swabbing of readily accessible animal surfaces is an option for diagnostic testing in animals that could otherwise be difficult to restrain for more invasive blood sampling. Swabs from mucosal surfaces, including the mucous membranes of the eyes, vagina, prepuce, and oral cavity can yield antibody and antigen in sufficient quantities for testing to produce meaningful results (Lanyon et al., 2014c). Swabs can also be used for parentage (DNA) testing and have been used to detect the following pathogens: *Bordetella bronchiseptica*, *Leishmania*, feline leukaemia virus (FeLV), feline herpes virus 1 (FHV-1), *Chlamydia felis*, feline calicivirus (FCV), BoHV1, bat lyssavirus, *Mycoplasma felis* and rinderpest virus (Mushi and Wafula, 1984; Lutz and Jarrett, 1987; Echevarria et al., 2001; Strauss-Ayali et al., 2004; Di Muccio et al., 2012; Hernandez et al., 2015; Litster et al., 2015).

The characteristics of specific disease states can influence the usefulness of these tests. One study found that 96% of latently infected cats did not shed FeLV RNA in saliva and were not detected by PCR (Gomes-Keller et al., 2006a), but virus shedding was a consistent feature in viraemic cats (Gomes-Keller et al., 2006b). A conjunctival swab tested by PCR had the highest sensitivity compared to immunofluorescent antibody and lymph node microscopy for the diagnosis of Leishmania, but still only detected 74% of infected dogs (Di Muccio et al., 2012). Screening bats for lyssavirus with PCR using oropharyngeal swabs was more accurate than PCR of fresh brain (Echevarria et al., 2001), and could be performed antemortem. Combination PCR testing of oropharyngeal swabs and either conjunctival or nasal swabs were needed to identify all cats infected with *B. bronchiseptica*, FHV-1, *C. felis*, FCV, and *M. felis* in one study (Litster et al., 2015), but a single PCR test could be performed on both specimens. One-step multiplex PCR testing of nasal swabs and bronchoalveolar lavage specimens from cattle for BoHV1, bovine respiratory syncytial virus and bovine parainfluenza virus 3 was more sensitive than virus isolation and had a sensitivity of 97% in another study (Thonur et al., 2012).

Preputial and vaginal swabs can be tested in cattle for the presence of *Campylobacter fetus* spp. *venerealis* (McMillen et al., 2006) using a 5' *Taq* PCR assay. Subsequent studies found there was cross reaction between *C. fetus* spp. *venerealis* and *Campylobacter hyointestinalis* (Spence et al., 2011), illustrating the need for additional confirmatory testing of any PCR positive *C. fetus* spp. *venerealis* isolates. Improved sensitivity in detecting *Tritrichomonas fetus* in smegma and vaginal mucous was demonstrated using PCR (McMillen and Lew, 2006) compared to culture. Quantitative PCR testing for *Ureaplasma diversum* was 100-fold more sensitive than conventional PCR (Marques et al., 2013). In addition, buccal swabs can also be collected to test for a range of inherited diseases including collie eye anomaly (Chang et al., 2010), hereditary cataract (Mellersh et al., 2006), and ceroid lipofuscinosis in dogs (Karli et al., 2014). Collie eye anomaly can be diagnosed by examination of the retinas in 5–10 week old puppies, but after 12 weeks this is impossible as the lesions become covered with pigment. Saliva collected onto filter paper from Collie-related breeds of dog of any age can rapidly be tested for the anomaly with the cost effective SYBR green method (Chang et al., 2010) allowing for large scale screening of populations. Saliva can also be collected for real time PCR testing for ceroid lipofuscinosis in dogs (Mizukami et al., 2011), providing early opportunities for diagnosis rather than waiting for the onset of clinical signs and confirmation by histopathology.

Urine

Urine specimens are relatively difficult to obtain in animals, requiring direct capture of voided specimens with risks of contamination. Alternatively, urinary catherisation or cystocentesis of individual animals is needed if a clearly identifiable uncontaminated specimen is required. Urine has been used as a medium for testing animal diseases and is particularly useful to test for Leptospira infection. Increasingly, PCR is used as the method of choice for the detection of the organism (Chappel et al., 1985; Fang et al., 2014; Hamond et al., 2014). Urine testing is also used to detect if performance enhancing drugs have been used in horses (and humans athletes; Thevis et al., 2016).

[1] See: http://www.genomnz.co.nz/ear-punch-sampling-new-method-for-genomnz/ (accessed 18 April 2016).

[2] See: http://www.genomnz.co.nz/how-to-take-dna-samples/hair-samples/ (accessed 18 April 2016).

Semen

Semen of male animals is frequently tested to screen for infection, mostly reproductive pathogens such as *Brucella ovis* in rams, *N. caninum,* BoHV1 and *T. fetus* in cattle, but also viruses such as equine infectious arteritis (Campero et al., 1990; Ramina et al., 1999; Ortega-Mora et al., 2003; Serrano-Martinez et al., 2007; Wang et al., 2007; Rana et al., 2011; Ridler et al., 2014). Specimens can be difficult to obtain in pastured animals and may require sedation, specialised equipment and collection procedures. However, diagnostic testing of semen is particularly valuable for ensuring semen collected from bulls at semen collection centres is free from *C. fetus* spp. *venerealis* (Eaglesome et al., 1995), BVDV (Rikula et al., 2008) and BoHV1 (Alegre et al., 2001) to ensure venereal infection is not transmitted via natural or artificial breeding.

Faeces

Testing for the presence of pathogens in faeces is a preferred option for diseases that are spread via excretion in the faeces, and has certain advantages over serological testing. For example, for *F. hepatica*, serological tests perform very well, demonstrating high diagnostic sensitivity and specificity (Reichel, 2002) and the detection of antibodies confirms previous exposure to the parasite with high accuracy. However, antibodies may persist for weeks after an infection has been eliminated by effective treatment with a flukicide. In order to detect patent infections (with shedding), faecal (copro)-antigen ELISAs have successfully been deployed to detect active, patent infections with a high degree of diagnostic sensitivity (and perfect specificity; Palmer et al., 2014), thus giving an indication of current infection. Similarly, the application of diagnostic assays to detect the presence of nematode larvae and eggs in faeces presents new options for investigation of the parasite infection status of sheep and cattle, using a combined microscopy-molecular technique where parasite eggs are separated from faeces by a salt flotation technique before PCR testing to identify the species present (Bott et al., 2009; Hoglund et al., 2013). This avoids the need for time-consuming larval culture and identification.

Testing for antibodies to Johne's disease is hampered by the late onset immunological (particularly humoral) response, limiting the diagnostic sensitivity of serological tests (Reichel et al., 1999). Culture is similar to serological testing in its ability to detect infected animals, although it was considered the reference standard for testing. Faecal culture enhanced by PCR testing has improved diagnostic sensitivity and is now the new reference standard (Whittington et al., 2000; Marsh and Whittington, 2001).

Faecal ELISA testing can be used to detect rotavirus in foals, (Browning et al., 1991), *Giardia duodenalis* in cats (Mircean et al., 2011) and *Cryptosporidium parvum* (Anusz et al., 1990), *Escherichia coli* K99 and coronavirus in cattle (Cho et al., 2012). Faecal PCR testing enables identification of enterotoxigenic *E. coli* from diarrhoeic piglets (Byun et al., 2012) and *T. fetus* infection in cats (Gookin et al., 2002).

Point of care test devices

Very recently, the diagnostic test devices themselves have been shown to be suitable as specimens, as foot-and-mouth disease virus was recovered successfully from lateral flow devices (Fowler et al., 2014), further expanding the possible range of sampling modalities.

The concept of specimen pooling

Pooling of specimens can be used to provide information about immunological reactions to specific pathogens, or to identify pathogens or reagents of interest. Often this method is used for economic reasons; testing a large number of pooled specimens with one test is generally far cheaper than testing each animal individually. This is the case where the additional individual diagnostic test adds considerable cost, but the specimen is relatively cheap and readily available (e.g. milk). Pooled specimen testing is particularly useful as a screening test for the absence of disease or where disease prevalence is low. While pooling offers a method of investigating the disease status of a large population quickly (Lanyon et al., 2014a), there are two points important to note. Firstly, when testing for the presence of a condition/factor or pathogen, pooling only makes sense if it makes testing more cost-effective or less time-consuming than individual testing. If the prevalence of the factor of interest is higher than the dilution factor caters for, all pools will be positive, and nothing has been gained. For example, if you pool 20 animals/pool for BVDV virus testing and the disease prevalence in the population is 1–2%, 80% of pools are likely to screen negative, saving time and money. However, if you test 100 specimens/pool from the same population, all pools are likely to be positive, and you are no closer to identifying which animals are infected. By contrast, a run of 100 specimens is within the analytical capability of a PCR assay. Secondly, the diagnostic test also needs to possess sufficient analytical sensitivity to detect the antibody or antigen at the dilution factor determined by the degree of pooling. Usually an additional safety factor is added to ensure this. When testing for antibodies to EBL, the greatest dilution allowed for pooling is 10 times less than the highest dilution of a standard positive that can be detected e.g. if the standard can be detected diluted 1:100, pools of 10 animals each can be tested (Reichel et al., 1998).

One of the issues with pooling is the loss of the ability to account for the influences of individuals with very strong relative contributions, such as an animal with a very high antibody titre, distorting the overall picture. Hence, pooling generally indicates the level of infection or immunity at the herd, flock or unit level. For example, when testing for BVDV antibodies by ELISA, a single strong positive individual can result in a positive result in a pool of up to 128 individuals, while even a weak positive individual can cause a positive result in a pool of eight (Lanyon et al., 2014a). On the other hand, pooling provides a very cost-effective snapshot of that herd, flock or unit and can, if interpreted appropriately and used in conjunction with antibody and pathogen testing, can provide a very effective ongoing surveillance tool to augment biosecurity.

Conclusions

In addition to serum, a variety of animal excretions, secretions and tissues can successfully be used for the detection of an immune response to an agent, or for detection of the agent itself. Improving detection technologies present exciting future options for disease diagnosis and control. Drivers for exploring different test media have, in some cases, been the quest for more cost-effective testing modalities or an effort to make use of specimens that are more readily available and can easily obtained by the farmers themselves.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with people or organisations that could inappropriately influence or bias the content of the paper.

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References

Alegre, M., Nanni, M., Fondevila, N., 2001. Development of a multiplex polymerase chain reaction for the differentiation of bovine herpesvirus-1 and -5. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health 48, 613–621.

Anusz, K.Z., Mason, P.H., Riggs, M.W., Perryman, L.E., 1990. Detection of Cryptosporidium parvum oocysts in bovine feces by monoclonal antibody capture enzyme-linked immunosorbent assay. Journal of Clinical Microbiology 28, 2770–2774.

Balmer, S., Vogtlin, A., Thur, B., Buchi, M., Abril, C., Houmard, M., Danuser, J., Schwermer, H., 2014. Serosurveillance of Schmallenberg virus in Switzerland using bulk tank milk samples. Preventive Veterinary Medicine 116, 370–379.

Bialynicki-Birula, R., 2008. The 100th anniversary of Wassermann-Neisser-Bruck reaction. Clinics in Dermatology 26, 79–88.

Bott, N.J., Campbell, B.E., Beveridge, I., Chilton, N.B., Rees, D., Hunt, P.W., Gasser, R.B., 2009. A combined microscopic-molecular method for the diagnosis of strongylid infections in sheep. International Journal for Parasitology 39, 1277–1287.

Browning, G.F., Chalmers, R.M., Snodgrass, D.R., Batt, R.M., Hart, C.A., Ormarod, S.E., Leadon, D., Stoneham, S.J., Rossdale, P.D., 1991. The prevalence of enteric pathogens in diarrhoeic thoroughbred foals in Britain and Ireland. Equine Veterinary Journal 23, 405–409.

Buergelt, C.D., Williams, J.E., 2004. Nested PCR on blood and milk for the detection of *Mycobacterium avium* subsp *paratuberculosis* DNA in clinical and subclinical bovine paratuberculosis. Australian Veterinary Journal 82, 497–503.

Byun, J.W., Jung, B.Y., Kim, H.Y., Fairbrother, J.M., Lee, W.K., 2012. Real-time PCR for differentiation of F4 (K88) variants (F4ab, F4ac, F4ad) of enterotoxigenic *Escherichia coli* from diarrhoeic piglets. The Veterinary Journal 193, 593–594.

Campero, C.M., Hirst, R.G., Ladds, P.W., Vaughan, J.A., Emery, D.L., Watson, D.L., 1990. Measurement of antibody in serum and genital fluids of bulls by ELISA after vaccination and challenge with *Tritrichomonas foetus*. Australian Veterinary Journal 67, 175–178.

Chang, H.S., Mizukami, K., Yabuki, A., Hossain, M.A., Rahman, M.M., Uddin, M.M., Arai, T., Yamato, O., 2010. A novel rapid genotyping technique for Collie eye anomaly: SYBR Green-based real-time polymerase chain reaction method applicable to blood and saliva specimens on Flinders Technology Associates filter paper. Journal of Veterinary Diagnostic Investigation 22, 708–715.

Chappel, R.J., Adler, B., Ballard, S.A., Faine, S., Jones, R.T., Millar, B.D., Swainger, J.A., 1985. Enzymatic radioimmunoassay for detecting *Leptospira interrogans* serovar *pomona* in the urine of experimentally-infected pigs. Veterinary Microbiology 10, 279–286.

Charlier, J., Claerebout, E., De Muelenaere, E., Vercruysse, J., 2005. Associations between dairy herd management factors and bulk tank milk antibody levels against *Ostertagia ostertagi*. Veterinary Parasitology 133, 91–100.

Cho, Y.I., Sun, D., Cooper, V., Dewell, G., Schwartz, K., Yoon, K.J., 2012. Evaluation of a commercial rapid test kit for detecting bovine enteric pathogens in feces. Journal of Veterinary Diagnostic Investigation 24, 559–562.

Coats, J.T., Dillon, J.F., 2015. The effect of introducing point-of-care or dried blood spot analysis on the uptake of hepatitis C virus testing in high-risk populations: A systematic review of the literature. International Journal on Drug Policy 26, 1050–1055.

Cockcroft, P.D., Jenvey, C., Reichel, M.P., 2014. Role for colostrum and whey in testing for bovine TB and Johne's disease? Veterinary Record 175, 597.

Collins, M.T., Wells, S.J., Petrini, K.R., Collins, J.E., Schultz, R.D., Whitlock, R.H., 2005. Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. Clinical and Diagnostic Laboratory Immunology 12, 685–692.

Corthier, G., Franz, J., 1981. Detection of antirotavirus immunoglobulins A, G, and M in swine colostrum, milk, and feces by enzyme-linked immunosorbent assay. Infection and Immunity 31, 833–836.

Di Muccio, T., Veronesi, F., Antognoni, M.T., Onofri, A., Fioretti, D.P., Gramiccia, M., 2012. Diagnostic value of conjunctival swab sampling associated with nested PCR for different categories of dogs naturally exposed to *Leishmania infantum* infection. Journal of Clinical Microbiology 50, 2651–2659.

Dobson, H., 2016. Measuring progesterone from the milk line. Veterinary Record 178, 258–259.

Eaglesome, M.D., Sampath, M.I., Garcia, M.M., 1995. A detection assay for Campylobacter fetus in bovine semen by restriction analysis of PCR amplified DNA. Veterinary Research Communications 19, 253–263.

Echevarria, J.E., Avellon, A., Juste, J., Vera, M., Ibanez, C., 2001. Screening of active lyssavirus infection in wild bat populations by viral RNA detection on oropharyngeal swabs. Journal of Clinical Microbiology 39, 3678–3683.

Engvall, E., Perlmann, P., 1971. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry 8, 871–874.

Fang, F., Collins-Emerson, J.M., Heuer, C., Hill, F.I., Tisdall, D.J., Wilson, P.R., Benschop, J., 2014. Interlaboratory and between-specimen comparisons of diagnostic tests for leptospirosis in sheep and cattle. Journal of Veterinary Diagnostic Investigation 26, 734–747.

Fleischhauer, G., 1955. [The ring test in Brucella and Salmonella infections]. Deutsche Medizinische Wochenschrift 80, 390–391.

Foddai, A., Strain, S., Whitlock, R.H., Elliott, C.T., Grant, I.R., 2011. Application of a peptide-mediated magnetic separation-phage assay for detection of viable *Mycobacterium avium* subsp. *paratuberculosis* to bovine bulk tank milk and feces samples. Journal of Clinical Microbiology 49, 2017–2019.

Forbes, A.B., Vercruysse, J., Charlier, J., 2008. A survey of the exposure to Ostertagia ostertagi in dairy cow herds in Europe through the measurement of antibodies in milk samples from the bulk tank. Veterinary Parasitology 157, 100–107.

Fowler, V.L., Bankowski, B.M., Armson, B., Di Nardo, A., Valdazo-Gonzalez, B., Reid, S.M., Barnett, P.V., Wadsworth, J., Ferris, N.P., Mioulet, V., et al., 2014. Recovery of viral RNA and infectious foot-and-mouth disease virus from positive lateral-flow devices. PLoS ONE 9, e109322.

Fux, R., Wolf, G., 2012. Transient elimination of circulating bovine viral diarrhoea virus by colostral antibodies in persistently infected calves: A pitfall for BVDV-eradication programs? Veterinary Microbiology 161, 13–19.

Gomes-Keller, M.A., Gonczi, E., Tandon, R., Riondato, F., Hofmann-Lehmann, R., Meli, M.L., Lutz, H., 2006a. Detection of feline leukemia virus RNA in saliva from naturally infected cats and correlation of PCR results with those of current diagnostic methods. Journal of Clinical Microbiology 44, 916–922.

Gomes-Keller, M.A., Tandon, R., Gonczi, E., Meli, M.L., Hofmann-Lehmann, R., Lutz, H., 2006b. Shedding of feline leukemia virus RNA in saliva is a consistent feature in viremic cats. Veterinary Microbiology 112, 11–21.

Gookin, J.L., Birkenheuer, A.J., Breitschwerdt, E.B., Levy, M.G., 2002. Single-tube nested PCR for detection of *Tritrichomonas foetus* in feline feces. Journal of Clinical Microbiology 40, 4126–4130.

Hall, C.A., Reichel, M.P., Ellis, J.T., 2006. Prevalence of *Neospora caninum* infection in Australian (NSW) dairy cattle estimated by a newly validated ELISA for milk. Veterinary Parasitology 142, 173–178.

Hamidi, A., Mayer-Scholl, A., Dreshaj, S., Robaj, A., Sylejmani, D., Ramadani, N., Al Dahouk, S., Nockler, K., 2015. Isolation and identification of *Brucella melitensis* biovar 3 from vaccinated small ruminants: A public health threat in Kosovo. Transboundary and Emerging Diseases doi:10.1111/tbed.12336 (E-published ahead of print).

Hamond, C., Martins, G., Loureiro, A.P., Pestana, C., Lawson-Ferreira, R., Medeiros, M.A., Lilenbaum, W., 2014. Urinary PCR as an increasingly useful tool for an accurate diagnosis of leptospirosis in livestock. Veterinary Research Communications 38, 81–85.

Hernandez, L., Montoya, A., Checa, R., Dado, D., Galvez, R., Otranto, D., Latrofa, M.S., Baneth, G., Miro, G., 2015. Course of experimental infection of canine leishmaniosis: Follow-up and utility of noninvasive diagnostic techniques. Veterinary Parasitology 207, 149–155.

Hill, F.I., Reichel, M.P., McCoy, R.J., Tisdall, D.J., 2007. Evaluation of two commercial enzyme-linked immunosorbent assays for detection of bovine viral diarrhoea virus in serum and skin biopsies of cattle. New Zealand Veterinary Journal 55, 45–48.

Hill, F.I., Reichel, M.P., Tisdall, D.J., 2010. Use of molecular and milk production information for the cost-effective diagnosis of bovine viral diarrhoea infection in New Zealand dairy cattle. Veterinary Microbiology 142, 87–89.

Hoglund, J., Engstrom, A., von Samson-Himmelstjerna, G., Demeler, J., Tyden, E., 2013. Real-time PCR detection for quantification of infection levels with *Ostertagia ostertagi* and *Cooperia oncophora* in cattle faeces. Veterinary Parasitology 197, 251–257.

Jenvey, C., Caraguel, C., Howarth, G.B., Riley, C.B., 2012. Identification of periparturient mare and foal associated predictors of post parturient immunoglobulin a concentrations in Thoroughbred foals. Equine Veterinary Journal. Supplement 73–77.

Jenvey, C.J., Reichel, M.P., Cockcroft, P.D., 2015. *Erysipelothrix rhusiopathiae* and *Mycoplasma hyopneumoniae*: The sensitivities of enzyme-linked immunosorbent assays for detecting vaccinated sows of unknown disease status using serum and colostrum, and the correlation of the results for sow serum, colostrum, and piglet serum. Journal of Veterinary Diagnostic Investigation 27, 211–216.

Karli, P., Karol, A., Oevermann, A., Drogemuller, C., Gorgas, D., Henke, D., 2014. [The canine neuronal ceroid-lipofuscinosis: A review]. Schweizer Archiv für Tierheilkunde 156, 417–423.

Kittelberger, R., Reichel, M.P., Joyce, M.A., Staak, C., 1997. Serological crossreactivity between *Brucella abortus* and *Yersinia enterocolitica* 0:9. III. Specificity of the in vitro antigen-specific gamma interferon test for bovine brucellosis diagnosis in experimentally *Yersinia enterocolitica* 0:9-infected cattle. Veterinary Microbiology 57, 361–371.

Klaasen, M., Roest, H.J., van der Hoek, W., Goossens, B., Secka, A., Stegeman, A., 2014. Coxiella burnetii seroprevalence in small ruminants in The Gambia. PLoS ONE 9, e85424.

Korhonen, H., Marnila, P., Gill, H.S., 2000. Milk immunoglobulins and complement factors. British Journal of Nutrition 84, S75–S80.

Lanyon, S.R., Reichel, M.P., 2016. Pre-treatment of serum samples to reduce interference of colostrum-derived specific antibodies with detection of Bovine viral diarrhea virus antigen by ELISA in young calves. Journal of Veterinary Diagnostic Investigation (E-published ahead of print). doi:10.1177/1040638716633496.

Lanyon, S.R., Anderson, M.L., Reichel, M.P., 2014a. Pooling serum to identify cohorts of nonmilking cattle likely to be infected with Bovine viral diarrhea virus by testing for specific antibodies. Journal of Veterinary Diagnostic Investigation 26, 346–353.

Lanyon, S.R., McCoy, R., Bergman, E., Reichel, M.P., 2014b. Milk as a diagnostic sample for a commercially available ELISA to identify bovine viral diarrhoea (BVD) antibodies in dairy herds. Australian Veterinary Journal 92, 269–273.

Lanyon, S.R., Sims, S.K., Cockcroft, P.D., Reichel, M.P., 2014c. Comparison of serum, ear notches, and nasal and saliva swabs for Bovine viral diarrhea virus antigen detection in colostrum-fed persistently infected (PI) calves and non-PI calves. Journal of Veterinary Diagnostic Investigation 26, 783–787.

Litster, A., Wu, C.C., Leutenegger, C.M., 2015. Detection of feline upper respiratory tract disease pathogens using a commercially available real-time PCR test. The Veterinary Journal 206, 149–153.
Little, S.E., Munzing, C., Heise, S.R., Allen, K.E., Starkey, L.A., Johnson, E.M., Meinkoth, J., Reichard, M.V., 2014a. Pre-treatment with heat facilitates detection of antigen of *Dirofilaria immitis* in canine samples. Veterinary Parasitology 203, 250–252.
Little, S.E., Raymond, M.R., Thomas, J.E., Gruntmeir, J., Hostetler, J.A., Meinkoth, J.H., Blagburn, B.L., 2014b. Heat treatment prior to testing allows detection of antigen of *Dirofilaria immitis* in feline serum. Parasites and Vectors 7, 1.
Lutz, H., Jarrett, O., 1987. Detection of feline leukemia virus infection in saliva. Journal of Clinical Microbiology 25, 827–831.
Marques, L.M., Amorim, A.T., Martins, H.B., Rezende, I.S., Barbosa, M.S., Lobao, T.N., Campos, G.B., Timenetsky, J., 2013. A quantitative TaqMan PCR assay for the detection of *Ureaplasma diversum*. Veterinary Microbiology 167, 670–674.
Marsh, I.B., Whittington, R.J., 2001. Progress towards a rapid polymerase chain reaction diagnostic test for the identification of *Mycobacterium avium* subsp. *paratuberculosis* in faeces. Molecular and Cellular Probes 15, 105–118.
McMillen, L., Lew, A.E., 2006. Improved detection of *Tritrichomonas foetus* in bovine diagnostic specimens using a novel probe-based real time PCR assay. Veterinary Parasitology 141, 204–215.
McMillen, L., Fordyce, G., Doogan, V.J., Lew, A.E., 2006. Comparison of culture and a novel 5' Taq nuclease assay for direct detection of *Campylobacter fetus* subsp. *venerealis* in clinical specimens from cattle. Journal of Clinical Microbiology 44, 938–945.
Mellersh, C.S., Pettitt, L., Forman, O.P., Vaudin, M., Barnett, K.C., 2006. Identification of mutations in HSF4 in dogs of three different breeds with hereditary cataracts. Veterinary Ophthalmology 9, 369–378.
Mircean, V., Gyorke, A., Jarca, A., Cozma, V., 2011. Prevalence of Giardia species in stool samples by ELISA in household cats from Romania and risk factors. Journal of Feline Medicine and Surgery 13, 479–482.
Mizukami, K., Chang, H.S., Yabuki, A., Kawamichi, T., Kawahara, N., Hayashi, D., Hossain, M.A., Rahman, M.M., Uddin, M.M., Yamato, O., 2011. Novel rapid genotyping assays for neuronal ceroid lipofuscinosis in Border collie dogs and high frequency of the mutant allele in Japan. Journal of Veterinary Diagnostic Investigation 23, 1131–1139.
Mullis, K.B., Faloona, F.A., 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods in Enzymology 155, 335–350.
Mushi, E.Z., Wafula, J.S., 1984. The shedding of a virulent Kabete O strain of rinderpest virus by cattle. Veterinary Research Communications 8, 173–179.
Nagel-Alne, G.E., Valle, P.S., Krontveit, R., Solverod, L.S., 2015. Caprine arthritis encephalitis and caseous lymphadenitis in goats: Use of bulk tank milk ELISAs for herd-level surveillance. Veterinary Record 176, 173.
Nielsen, K., Gall, D., 2001. Fluorescence polarization assay for the diagnosis of brucellosis: A review. Journal of Immunoassay and Immunochemistry 22, 183–201.
Nielsen, S.S., Toft, N., Okura, H., 2013. Dynamics of specific anti-*Mycobacterium avium* subsp. *paratuberculosis* antibody response through age. PLoS ONE 8, e63009.
Ortega-Mora, L.M., Ferre, I., del-Pozo, I., Caetano-da-Silva, A., Collantes-Fernandez, E., Regidor-Cerrillo, J., Ugarte-Garagalza, C., Aduriz, G., 2003. Detection of *Neospora caninum* in semen of bulls. Veterinary Parasitology 117, 301–308.
Ouchterlony, O., 1948. In vitro method for testing the toxin-producing capacity of diphtheria bacteria. Acta Pathologica et Microbiologica Scandinavica 25, 186–191.
Palmer, D.G., Lyon, J., Palmer, M.A., Forshaw, D., 2014. Evaluation of a copro-antigen ELISA to detect *Fasciola hepatica* infection in sheep, cattle and horses. Australian Veterinary Journal 92, 357–361.
Poumarat, F., Perrin, B., Longchambon, D., 1991. Identification of ruminant mycoplasmas by dot immunobinding on membrane filtration (MF dot). Veterinary Microbiology 29, 329–338.
Poumarat, F., Le Grand, D., Gaurivaud, P., Gay, E., Chazel, M., Game, Y., Bergonier, D., 2012. Comparative assessment of two commonly used commercial ELISA tests for the serological diagnosis of contagious agalactia of small ruminants caused by *Mycoplasma agalactiae*. BMC Veterinary Research 8, 109.
Presi, P., Heim, D., 2010. BVD eradication in Switzerland – a new approach. Veterinary Microbiology 142, 137–142.
Presi, P., Struchen, R., Knight-Jones, T., Scholl, S., Heim, D., 2011. Bovine viral diarrhea (BVD) eradication in Switzerland – experiences of the first two years. Preventive Veterinary Medicine 99, 112–121.
Ramina, A., Dalla Valle, L., De Mas, S., Tisato, E., Zuin, A., Renier, M., Cuteri, V., Valente, C., Cancellotti, F.M., 1999. Detection of equine arteritis virus in semen by reverse transcriptase polymerase chain reaction-ELISA. Comparative Immunology, Microbiology and Infectious Diseases 22, 187–197.
Rana, S.K., Kota, S.N., Samayam, P.N., Rajan, S., Srinivasan, V.A., 2011. Use of real-time polymerase chain reaction to detect bovine herpesvirus 1 in frozen cattle and buffalo semen in India. Veterinaria Italiana 47, 313–322.
Rautiainen, E., 1998. The prevalence of *Mycoplasma hyopneumoniae* in pig herds in Western Finland based on the demonstration of antibodies in colostrum by ELISA. Acta Veterinaria Scandinavica 39, 325–330.
Reber, A., Reist, M., Schwermer, H., 2012. Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhinotracheitis and bovine enzootic leucosis in Switzerland. Schweizer Archiv für Tierheilkunde 154, 189–197.
Reichel, M.P., 2002. Performance characteristics of an enzyme-linked immunosorbent assay for the detection of liver fluke (*Fasciola hepatica*) infection in sheep and cattle. Veterinary Parasitology 107, 65–72.
Reichel, M.P., Tham, K.M., Barnes, S., Kittelberger, R., 1998. Evaluation of alternative methods for the detection of bovine leukaemia virus in cattle. New Zealand Veterinary Journal 46, 140–146.
Reichel, M.P., Kittelberger, R., Penrose, M.E., Meynell, R.M., Cousins, D., Ellis, T., Mutharia, L.M., Sugden, E.A., Johns, A.H., de Lisle, G.W., 1999. Comparison of serological tests and faecal culture for the detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle and analysis of the antigens involved. Veterinary Microbiology 66, 135–150.
Reichel, M.P., Vanhoff, K., Baxter, B., 2005. Performance characteristics of an enzyme-linked immunosorbent assay performed in milk for the detection of liver fluke (*Fasciola hepatica*) infection in cattle. Veterinary Parasitology 129, 61–66.
Ridge, S.E., Galvin, J.W., 2005. A comparison of two ELISAs for the detection of antibodies to bovine leucosis virus in bulk-milk. Australian Veterinary Journal 83, 431–434.
Ridler, A.L., Smith, S.L., West, D.M., 2014. Seroconversion and semen shedding in rams experimentally infected with *Brucella ovis*. New Zealand Veterinary Journal 62, 47–50.
Rikula, U., Nuotio, L., Laamanen, U.I., Sihvonen, L., 2008. Transmission of bovine viral diarrhoea virus through the semen of acutely infected bulls under field conditions. Veterinary Record 162, 79–82.
Schares, G., Barwald, A., Staubach, C., Wurm, R., Rauser, M., Conraths, F.J., Schroeder, C., 2004. Adaptation of a commercial ELISA for the detection of antibodies against *Neospora caninum* in bovine milk. Veterinary Parasitology 120, 55–63.
Serrano-Martinez, E., Ferre, I., Martinez, A., Osoro, K., Mateos-Sanz, A., Del-Pozo, I., Aduriz, G., Tamargo, C., Hidalgo, C.O., Ortega-Mora, L.M., 2007. Experimental neosporosis in bulls: Parasite detection in semen and blood and specific antibody and interferon-gamma responses. Theriogenology 67, 1175–1184.
Singh, K., Miller, M.M., Kohrt, L.J., Scherba, G., Garrett, E.F., Fredrickson, R.L., 2011. Development of a novel diagnostic test for detection of bovine viral diarrhea persistently infected animals using hair. Journal of Veterinary Science 12, 295.
Spence, R.P., Bruce, I.R., McFadden, A.M., Hill, F.I., Tisdall, D., Humphrey, S., van der Graaf, L., van Bergen, M.A., Wagenaar, J.A., 2011. Cross-reaction of a *Campylobacter fetus* subspecies *venerealis* real-time PCR. Veterinary Record 168, 131.
Strauss-Ayali, D., Jaffe, C.L., Burshtain, O., Gonen, L., Baneth, G., 2004. Polymerase chain reaction using noninvasively obtained samples, for the detection of *Leishmania infantum* DNA in dogs. Journal of Infectious Diseases 189, 1729–1733.
Thekisoe, O.M., Kuboki, N., Nambota, A., Fujisaki, K., Sugimoto, C., Igarashi, I., Yasuda, J., Inoue, N., 2007. Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis. Acta Tropica 102, 182–189.
Thevis, M., Machnik, M., Schenk, I., Krug, O., Piper, T., Schanzer, W., Due, M., Bondesson, U., Hedeland, M., 2016. Nickel in equine sports drug testing – pilot study results on urinary nickel concentrations. Rapid Communications in Mass Spectrometry 30, 982–984.
Thonur, L., Maley, M., Gilray, J., Crook, T., Laming, E., Turnbull, D., Nath, M., Willoughby, K., 2012. One-step multiplex real time RT-PCR for the detection of bovine respiratory syncytial virus, bovine herpesvirus 1 and bovine parainfluenza virus 3. BMC Veterinary Research 8, 37.
Van Weemen, B.K., Schuurs, A.H., 1971. Immunoassay using antigen-enzyme conjugates. FEBS Letters 15, 232–236.
Velasquez, L., Blagburn, B.L., Duncan-Decoq, R., Johnson, E.M., Allen, K.E., Meinkoth, J., Gruntmeir, J., Little, S.E., 2014. Increased prevalence of *Dirofilaria immitis* antigen in canine samples after heat treatment. Veterinary Parasitology 206, 67–70.
Voges, H., 2011. Reports from industry surveillance and disease control programmes: New Zealand dairy enzootic bovine leukosis (EBL) control scheme. Surveillance 38, 37–38.
Voller, A., 1964. Fluorescent antibody methods and their use in malaria research. Bulletin of the World Health Organization 30, 343–354.
Waldmann, A., Raud, A., 2016. Comparison of a lateral flow milk progesterone test with enzyme immunoassay as an aid for reproductive status determination in cows. Veterinary Record 178, 260.
Wang, J., O'Keefe, J., Orr, D., Loth, L., Banks, M., Wakeley, P., West, D., Card, R., Ibata, G., Van Maanen, K., et al., 2007. Validation of a real-time PCR assay for the detection of bovine herpesvirus 1 in bovine semen. Journal of Virological Methods 144, 103–108.
Whittington, R.J., Fell, S., Walker, D., McAllister, S., Marsh, I., Sergeant, E., Taragel, C.A., Marshall, D.J., Links, I.J., 2000. Use of pooled fecal culture for sensitive and economic detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in flocks of sheep. Journal of Clinical Microbiology 38, 2550–2556.
Witte, K.H., Hannemann, P., Dopatka, H.D., Giesendorf, B., 1989. Technical improvements of a commercial ELISA to detect antibodies against bovine herpesvirus 1. Medical Microbiology and Immunology 178, 9–20.
Yalow, R.S., Berson, S.A., 1960. Plasma insulin concentrations in nondiabetic and early diabetic subjects. Determinations by a new sensitive immuno-assay technic. Diabetes 9, 254–260.
Zimmermann, W., Tschudi, P., Nicolet, J., 1986. [ELISA serology of the blood and colostral milk: A possibility for monitoring enzootic pneumonia (EP) in swine herds]. Schweizer Archiv für Tierheilkunde 128, 299–306.