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Review

Detection of Animal Pathogens by Using the Polymerase Chain Reaction (PCR)

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SUMMARY

The polymerase chain reaction (PCR) is a nucleic acid-based technique that enables the rapid and sensitive detection of specific micro-organisms. Although this technique is widely used in veterinary research, it has not yet found applications in routine microbiological analysis of veterinary clinical samples. However, advances in sample preparation together with the increasing availability of specific gene sequences will probably lead to the more widespread diagnostic use of PCR in the future. PCR is likely to have a strong impact in the epidemiology, treatment and prevention of animal infectious diseases.

KEYWORDS: PCR; diagnosis; virus; bacteria; parasites.

INTRODUCTION

The development of polymerase chain reaction (PCR) has revolutionized the field of molecular biology. The technique consists basically of the enzymatic synthesis of millions of copies of a target DNA sequence (Saiki *et al.*, 1985). Using a thermostable DNA polymerase (Saiki *et al.*, 1988), and a succession of cycles that includes denaturation of the template DNA, hybridization of specific DNA primers to the template and extension of the primers, it is possible to generate multiple copies of the target region enzymatically. Thus, PCR provides a method for obtaining large quantities of specific DNA sequences from small amounts of DNA, including degraded DNA samples. The technology has been extensively reviewed (see for example, Ehrlich, 1989; Innis *et al.*, 1990; Griffin & Griffin, 1994). Although PCR is widely used in an increasing number of applications, those in the area of microbiology and diagnosis of infectious diseases have undergone outstanding advances in recent years.

PCR IN VETERINARY MICROBIOLOGY

Traditionally, strategies for identifying most microbial pathogens involve isolation on selective agar media or cell cultures, and the use of phenotypical tests but these techniques are usually slow and laborious. The important cost that animal infectious diseases can have on national economies has therefore stimulated the search for faster, more sensitive and more specific methods to identify microbial pathogens. Many useful nucleic acid probes and immunological assays have been developed for diagnostic purposes, but these techniques also have some deficiencies (Jones & Bej, 1994). The emergence of PCR, however, offers the potential to improve the laboratory-based diagnosis of pathogens (Mahbubani & Bej, 1994). Although PCR has some shortcomings, such as the problems caused by contaminants and inhibitors or the lack of suitable sequences for designing specific primers, the outstanding research effort focused on this technique, together with the remarkable development of molecular biology have minimized the deficiencies and allowed its increased general use as a diagnostic tool.

VIRUSES

Ruminants

Foot-and-mouth disease virus (FMDV). FMDV is

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one of the most dangerous viruses of ruminants. Its speed of spread and ability to change its antigenic identity makes FMDV very threatening to the beef and dairy industries of many countries. Fast and accurate detection of FMD outbreaks is needed to limit spread of the disease. The virus consists of 60 copies of each of the four proteins VP1 to 4, of which VP1 is the main protein determining antigenic identity. PCR systems for detecting FMDV have been developed by different laboratories (Meyer *et al.*, 1991; Laor *et al.*, 1991, 1992). There are also reports on the use of PCR to determine FMDV serotype (Rodríguez *et al.*, 1992; Stram *et al.*, 1993, 1995).

Rinderpest (RPV). Animal diseases greatly influence animal production and trade. Diagnosis should enable fast implementation of control measures to minimize losses. This is particularly important in the case of highly-contagious pathogens such as RPV and peste des petit ruminants (PPRV) viruses. They are, at present, confined to developing countries where they remain a constant threat to livestock. RPV may infect all artiodactyls, with cattle and buffaloes being the most susceptible species, while PPRV specifically causes disease in small ruminants. Field diagnosis of classical rinderpest, with its many indicative clinical signs, is easy but those signs are not always clearly seen, particularly in countries where the disease is endemic (Diallo *et al.*, 1995). Moreover, some mild strains can fail to produce clinical signs unless the infected animals are stressed. The situation is more complicated in small ruminants because they can be infected with RPV and/or PPRV, and the disease produced by both viruses is similar. The laboratory tests commonly used are either expensive (animal inoculation), slow (virus isolation or neutralization) or insensitive (agar gel immunodiffusion). PCR can drastically improve the diagnosis. Chamberlain *et al.* (1993) grouped different isolates of RPV combining PCR with sequencing studies. Using the same procedure, Barrett *et al.* (1993) identified two different RPV strains in the same clinical sample and Warmwayi *et al.* (1995) showed that there was co-circulation of two different lineages of RPV in Nigeria during the epizootics of the 1980s.

Bovine viral diarrhoea virus (BVDV). BVDV is another important pathogen of cattle, causing considerable economic losses throughout the world. Three syndromes caused by BVDV have been described: an acute gastroenteritis with severe diarrhoea, mucosal disease, and chronic infections of several weeks' duration in calves up to 1-year-old. Persistently infected animals are the main source of infection to herdmates because they continually shed large quantities of virus in body secretions and excretions. Due to the obvious impact of BVDV infections, screening of animals must be carefully made and current methods of detection, such as virus isolation or immunoassays, either lack optimal sensitivity or rapidity for consistent and large scale testing in animal specimens (Radwan *et al.*, 1995). PCR, however, can readily detect BVDV (Belak & Ballagi-Pordany, 1991; Brock, 1991; Hertig *et al.*, 1991; Ward & Misra, 1991; Hoft van Iddekinge *et al.*, 1992; Gruber *et al.*, 1994), and PCR analysis of bulk tank milk samples has provided a rapid and sensitive method to screen herds for the presence of the virus. Sensitive studies using reference strains of BVDV from persistently infected carriers have shown that reverse transcription (RT)-PCR has greater sensitivity than other tests, including enzyme-linked immunosorbent assay (ELISA) (Horner *et al.*, 1995); unfortunately, cost currently makes this technique unsuitable for large-scale testing but it should be valuable as a confirmatory test in cases where ELISA results are in the 'suspicious range' or where the viral titre is low, such as in batches of foetal bovine serum. Additionally, it is possible to discriminate among different BVDV strains using PCR (Tajima *et al.*, 1995) and PCR-restriction fragment length polymorphism (RFLP) tests have demonstrated that 13 BVDV isolates from ruminants on four different farms in Sweden were herd-specific rather than species-specific, and that the virus is readily transmitted between cattle and sheep (Paton *et al.*, 1995).

Bluetongue (BT). BT is an arthropod-borne viral infection of ruminants caused by bluetongue virus (BTV). Clinically, BT varies depending on factors such as population density and competence of the *Cullicoides* sp. vector, the distribution of susceptible hosts and the virulence of the different serotypes of BTV. Among ruminants, only sheep are clinically affected while cattle are usually asymptomatic reservoirs. PCR-based procedures have been developed for the diagnosis of BTV (Gould *et al.*, 1989; Dangler *et al.*, 1990; Wade-Evans *et al.*, 1991; McColl & Gould, 1991; Akita *et al.*, 1993; Parsonson *et al.*, 1994). Whole blood seems to be the most convenient clinical sample, but fractions of blood have also been successfully used for PCR detection of BTV infection in sheep (McColl & Gould, 1994). In a comparison of methods for isolation of BTV in infected calves, virus was detected in embrionating chicken eggs for 2–8 weeks, whereas PCR detected BTV nucleic acid for 16–20 weeks (MacLachlan *et al.*, 1994). The sensitivity of the technique means that it can be adapted to detected BTV in *Cullicoides* sp. samples (Wilson & Chase, 1993).

Epizootic haemorrhagic disease virus (EHDV). EHDV is an orbivirus related to BTV that causes fatal haemorrhagic disease in domestic and wild ruminants. Clinical signs and pathological changes caused by EHDV and BTV are indistinguishable. Aradaib *et al.* (1995) compared the value of PCR with virus isolation for the detection of EHDV in clinical samples taken from naturally-infected deer, and concluded that PCR assays for EDHV can provide a diagnostic alternative superior to the current cumbersome and time-consuming virus isolation procedures.

Bovine immunodeficiency virus (BIV). BIV is structurally and genetically related to human immunodeficiency virus (HIV). BIV causes lymphoproliferative changes and enlargement of subcutaneous lymphatic nodules in cattle. Although infection by BIV is widely prevalent in beef and dairy cattle, there is no accurate diagnostic test for the virus. Using PCR, Nash *et al.* (1995) detected BIV-infected leucocytes in the blood and milk of BIV-seropositive cows. These data confirmed the presence of BIV in milk and highlighted the potential for lactogenic transmission of the virus. Suarez *et al.* (1995) examined blood samples from BIV-experimentally infected calves by virus isolation, protein immunoblot and nested PCR and showed that the nested PCR test is more sensitive than any other method for the detection of BIV infection in cattle.

Bovine herpesvirus-1 virus (BHV-1). BHV-1 causes infectious bovine rhinotracheitis (IBR), an economically important disease of cattle characterized by acute respiratory infection and reproductive problems such as abortion, infertility, vulvovaginitis and balanoposthitis. Latently infected animals can be reservoirs of BHV-1 in the herd. Virus detection is often requested for the laboratory diagnosis of most cases of respiratory and reproductive problems in cattle. Several reports have described the PCR of different BHV-1 genes from tissue cultures (Vilcek, 1993; Kibenge *et al.*, 1994; Yason *et al.*, 1995) and bovine semen (Wiedman *et al.*, 1993; Xia *et al.*, 1995).

Louping-ill, Turkish sheep encephalitis (TSE) and Spanish sheep encephalitis (SSE). All three viruses belong to the tick-borne encephalitis virus group, within the genus Flavivirus. These viruses produce a similar clinical syndrome, and the histological changes that they produce in the brains of affected sheep are indistinguishable. Moreover, animals from affected flocks have antibodies that cross-react with the other viruses (Gonzalez *et al.*, 1987). Sequencing of PCR products obtained from cDNA of SSE have permitted the location of specific genetic markers for this flavovirus (Marin *et al.*, 1995). PCR has also enabled the construction of recombinant vaccinia virus expressing PrM and E glycoproteins of louping-ill virus (Venugopal *et al.*, 1994).

Caprine arthritis encephalitis (CAE). CAE is a worldwide multisystemic disease of domestic goats, characterized by progressive arthritis, leucoencephalomyelitis and mastitis. Although the virus persists for life, infection of goats with CAE is often subclinical. Isolation of CAE is not attempted routinely as a diagnostic tool but PCR has recently been adapted for the detection of proviral DNA in CAEV-infected cells from clinical specimens (Clavijo & Thorsen, 1995). The technique has a sensitivity which is several orders of magnitude higher than direct hybridization, and may represent an important alternative procedure for identification of persistently infected animals.

Other ruminants viruses for which PCR protocols have been successfully developed include bovine leukaemia virus (Naif *et al.*, 1990, 1992; Murtaugh *et al.*, 1991; Ballagi-Pordany *et al.*, 1992; Sherman *et al.*, 1992; Agresti *et al.*, 1993; Kelly *et al.*, 1993), bovine coronavirus (Verbeek & Tijssen, 1990), rotavirus (Xu *et al.*, 1990), and Maedi-visna virus (Staskus *et al.*, 1991; Zanoni *et al.*, 1992).

Pigs

Porcine parvo virus (PPV). The role of PPV in inducing swine reproductive failure characterized by embryo and fetal deaths has been extensively described, often when other clinical signs of disease are lacking. Sources of PPV include contaminated semen, the female reproductive tract or exposure during gamete/embryo manipulation. Molitor *et al.* (1991) developed a PCR amplification test for the detection of PPV thereby minimizing the risk of transmission of PPV to seronegatives recipients through embryo transfer (Gradil *et al.*, 1994).

Swine influenza. Swine influenza induces high morbidity and low mortality in pig populations throughout the world. Although the disease usually resolves, infected pigs represent a substantial source of economical loss to the producer because of their weight loss and poor weight gain. The results obtained by Schorr *et al.* (1994) proved that RT-PCR from nasal swabs specimens of pigs is significantly more sensitive than the techniques currently used such as the infectivity assay in embrionating chicken eggs.

Porcine reproductive and respiratory syndrome (PRRS). The disease complex known as PRRS has become an economically important health problem throughout Europe and North America. PCR has been used to confirm the presence of PRRS genes in infected monolayers (Katz *et al.*, 1995), thus providing the first steps for the development of a PCR test to analyze PRRS virus in clinical samples.

Pseudorabies virus (PRV). PRV is the aetiological agent of a major disease that has substantial economic impact in swine industry. The disease is fatal to young pigs but in adults the infection is less severe, and sometimes clinical signs are not apparent. Pigs surviving PRV infection remain latently infected for life. PCR has become the recommended method for evaluating PRV latency; reports from several laboratories have indicated that neuronal tissues, and especially the trigeminal ganglia, are the most reliable sources for detection of latent PRV genome (Belak *et al.*, 1989; Wheeler & Osorio, 1991; Volz *et al.*, 1992; Brockmeier *et al.*, 1993) but trigeminal ganglion assay can be performed only after death of the affected animal. Tonsil biopsy specimens can be obtained from live animals and used to amplify PRV sequences by PCR (Chung, 1995). PRV has also been detected in the semen of boars (Guérin *et al.*, 1995). The method is simple and allows the detection of around 370 viral DNA sequences per microlitre of sample. PRV infects cells of the lymphatic tissue and white blood cells of a variety of mammals. These cells are also present in sausages, and Schunk & Rziha (1994) established a PCR method specifically to detect PRV in artificially contaminated sausages and showed that PCR was less affected by extreme pH values than tissue culture techniques usually employed to recover the virus.

Other important swine virus that have been detected by PCR include hog cholera virus (Boye *et al.*, 1991; Liu *et al.*, 1991; Wirz *et al.*, 1993) and African swine fever virus (Steiger *et al.*, 1992).

Poultry

Intensive breeding of poultry means that high populations often live in confined spaces. Under such conditions, the entry of a virulent virus can cause high mortality and big economical losses. Rapid diagnostic tests are needed to minimize the consequences of viral outbreaks in these environments. When compared with virus isolation and other classic techniques, PCR is the method of choice for diagnosis of many poultry viruses including Marek's disease virus (Becker *et al.*, 1992, 1993; Silva, 1992; Zhu *et al.*, 1992; Davidson *et al.*, 1994; Zerbes *et al.*, 1994), reticuloendotheliosis virus (Aly *et al.*, 1993; Davidson *et al.*, 1994), avian leucosis virus (van Woensel *et al.*, 1992), infectious bronchitis virus (Andreasen *et al.*, 1991; Lin *et al.*, 1991; Jackwood *et al.*, 1992; Zwaagstra *et al.*, 1992; Kwon *et al.*, 1993a, b), Newcastle disease virus (Jestin & Jestin, 1991), lymphoproliferative disease virus (Sarid *et al.*, 1994) and infectious bursal disease virus (Lee *et al.*, 1992; Wu *et al.*, 1992a, b).

Horses

Equine viral arteritis (EVA). EVA is a ubiquitous disease present throughout mainland Europe. The variety and severity of clinical signs vary widely from inapparence to abortion and death. A proportion of seropositive stallions shed the causal organism, equine arteritis virus (EAV), in their semen, and play a primary role in its dissemination and perpetuation in the equine population. Therefore, when a stallion is identified as EAV positive, the first priority is to ascertain whether virus is being shed before the animal is allowed to cover mares. PCR is included among the three methods that may be used to establish the presence of virus in the semen (Chirnside & Spaan, 1990; Horserace Betting Levy Board, 1993).

Equine herpesvirus. PCR has been successfully applied to detect EHV 1 and 4 in aborted equine fetuses (Ballagi-Pordany *et al.*, 1990) and in nasopharyngeal swab specimens from horses with respiratory or neurological disease (Sharma *et al.*, 1992; Wagner *et al.*, 1992).

Other equine viral diseases which have been diagnosed by PCR include equine infectious anaemia (O'Rourke *et al.*, 1991) and African horse sickness (Zientara *et al.*, 1993; Stone-Marschat *et al.*, 1994).

Dogs

Rabies. Rabies is still one of the most life-threatening zoonosis in some regions of the world. Obviously, fast and accurate detection of infected animals is of vital importance. Research results have shown that PCR can play a remarkable role in the rapid, sensitive and specific detection of the rabies virus (Ermine *et al.*, 1990; Sacramento *et al.*, 1991; Shankar *et al.*, 1991; Kamolvarin *et al.*, 1993; McColl *et al.*, 1993) and the technique should spread among the reference laboratories located in regions at risk.

Canine parvovirus (CPV). CPV is the causative agent of haemorrhagic enteritis and myocarditis, and at present is one of the most common pathogenic viruses causing diarrhoea in dogs. CPV is not easily inactivated with the usual disinfectants, and can survive more than 3 months once a hospital or kennel is contaminated, often leading to secondary infections. As a result, it is important to have a rapid, specific and sensitive method to distinguish infected from uninfected dogs. PCR assays based on VP1 and VP2 genes have been used to detect CPV in paraffin-embedded tissues (Truyen *et al.*, 1994; Uwatoko *et al.*, 1995) and in faeces of diarrhoeic dogs (Hirasawa *et al.*, 1994). Additionally, PCR-RFLP analysis is a practical and reliable method for differentiating wild- and vaccine-type CPVs (Hirasawa *et al.*, 1995; Senda *et al.*, 1995).

Canine distemper virus (CDV). CDV induces a multifocal demyelinating disease in the central nervous system of dogs, in which virus persistence plays a key role. PCR has been an essential research tool to study the virus's nucleocapsid protein, and to provide a molecular basis for the observed differences in virus release and spread between attenuated and virulent CDV (Stettler & Zurbriggen, 1995).

Cats

Feline infectious peritonitis virus (FIPV). FIPV causes a severe, often fatal disease in domestic and wild cats. Despite considerable research, no routine diagnostic method is available. Detection of FIPV by nested PCR has been attempted (Egberink *et al.*, 1995) but the authors concluded that the value of PCR for the identification of sick animals and asymptomatic carriers needed to be further studied. In their work a positive PCR in healthy animals failed to provide an absolutely definitive diagnosis of FIP; equally, a negative PCR result from a sick animal did not completely exclude FIP.

Better results have been achieved in the PCR detection of active and latent feline herpesvirus 1 (Nunberg *et al.*, 1989; Reubel *et al.*, 1993) and feline immunodeficiency virus (Rimstad & Ueland, 1992).

Marine mammals

Morbillivirus infections in marine mammals were first reported in 1988, and are known to be distributed among a wide spectrum of seals and cetaceans in the Atlantic ocean and the Mediterranean sea. RT-PCR has revealed that there were no obvious links between the morbillivirus outbreak in marine seals in Northern Europe in 1988 and that which occurred in freshwater seals in Lake Baikal in 1987 (Visser *et al.*, 1990; Barrett *et al.*, 1992). Direct sequencing of PCR products that included the haemagluttinin protein gene of the Lake Baikal seals isolate (PDV-2) revealed that it was closely related to two isolates of CDV from Germany but different from CDV vaccines currently used in the Lake Baikal region (Mamaev *et al.*, 1995).

BACTERIA

Staphylococcus

Staphylococcal mastitis is an important problem in dairy farms. Several staphylococci, mainly *Staphylococcus aureus* strains, cause acute and chronic mastitis, and can lead to gangrenous mastitis. Human handling of the udder or the milking machine is a potential source of staphylococci, and contaminated milk can be the cause of food-borne intoxication in man. Rapid detection of staphylococci, including those killed by heat treatment, in suspected food could prevent foodborne staphylococcal gastroenteritis, and differentiation of *S. aureus* strains has been achieved by DNA amplification fingerprinting (Saurnier *et al.*, 1993; Van Belkum *et al.*, 1993).

Listeria monocytogenes

Although *Listeria monocytogenes* infection may produce clinical syndromes of abortion and neonatal septicaemia, encephalitis is most common in adult animals. The clinical diagnosis of listeric encephalitis in ruminants is difficult because of the existence of a broad spectrum of central nervous system diseases with similar clinical symptoms. In addition, listeria can only rarely be cultured from the cerebrospinal fluid (CSF) of affected animals. Because PCR is able to detect low numbers of bacteria, it may be a tool for increasing the sensitivity of listeria detection in CSF of ruminants (Peters *et al.*, 1995). It is also important to detect asymptomatic carriers because of the zoonotic nature of the infection. During the last decade several outbreaks and single cases of human listeriosis have demonstrated that the disease is often transmitted by contaminated food. Jaton *et al.* (1992) developed a sensitive nested PCR assay for the detection of *L. monocytogenes* in human CSF. Additionally, PCR has confirmed its usefulness to detect specific strains in the epidemiological investigations of listeriosis (Ericsson *et al.*, 1995).

Anthrax

Anthrax is a fatal infection of humans and livestock that is caused by the Gram-positive, endospore-forming bacterium *Bacillus anthracis*. Humans are infected primarily through contact with products derived from contaminated animals. There is a growing need for methods to detect *B. anthracis* spores and vegetative cells, not only to prevent large-scale livestock destruction, but also to protect humans that may come into contact with them. PCR amplification of some *B. anthracis* genes has already been reported (Carl *et al.*, 1992; Turnbull *et al.*, 1992; Hutson *et al.*, 1993; Johns *et al.*, 1994; Reif *et al.*, 1994), allowing the detection of even a single spore of *B. anthracis* (Reif *et al.*, 1994). Henderson *et al.* (1994) examined the variation among isolates of *B. anthracis* using restriction patterns and PCR and found that the *B. anthracis* profiles were unique when compared with those of closely related species, including *B. cereus*, *B. thuringiensis* and *B. mycoides*. Their results showed that isolates of *B. anthracis* are almost completely homogeneous and distinct from other members of the *B. cereus* group.

Clostridium botulinium

Botulism is a severe foodborne disease caused by *Clostridium botulinum* and is characterized by generalized flaccid paralysis. Botulinal neurotoxins, produced by seven distinct serological types of *C. botulinum* are among the most potent biological substances known and neurotoxins A, B, C, D, E and F have all been implicated as causes of human and/or animal disease. The mouse bioassay is the established method for the detection of neurotoxin but alternatives to the use of animals for diagnostic purpose are ethically desirable and should be encouraged. Some immunological methods have been proposed but the use of DNA-based techniques has not been extensively explored. However, some authors have confirmed that PCR has a great potential for the identification of botulism neurotoxin-producing strains (Szabo *et al.*, 1992, 1993; Fach *et al.*, 1995), and clearly demonstrated that PCR methods should be used for the development of highly sensitive and specific assays for organisms harbouring botulism-neurotoxin genes.

Clostridium perfringens

Clostridium perfringens enterotoxin genes have also been detected in stools without isolation of the organism (Saito *et al.*, 1992; Fach *et al.*, 1993). Although the isolates were from human food-poisoning outbreaks or sporadic diarrhoeal cases, *C. perfringens* is also a well-known animal pathogen, being the aetiological agent of haemorrhagic and necrotic enteritis. Thus, the application of PCR should be desirable and appropriate in veterinary laboratories. A PCR assay has in fact been developed recently for the rapid detection of genes encoding *C. perfringens* enterotoxins (Buogo *et al.*, 1995), and successfully applied in samples of small and large intestine from infected piglets.

Escherichia coli

Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrhoea in neonatal and post-weaned calves, lambs and piglets. Several fimbrial adhesins and enterotoxins are recognized as the virulence factors of ETEC. The sequencing of the enterotoxins and fimbrial genes have made possible the application of nucleic acid-based methods for their detection (Harel *et al.*, 1991; Woodward *et al.*, 1992). These methods have the advantage that they are readily applicable to a large number of isolates, in contrast to classic methods such as agglutination, infant mouse, ligated swine intestine and cell culture assays. PCR results obtained in Sweden by Kennan *et al.* (1995) showed that the gene for the major subunit of F107 fimbria was present on approximately half of the strains not expressing K88, K99, 987P and F41 fimbria isolated from piglets older than 1 week with diarrhoea. This suggested that F107 fimbria are of major importance among ETEC strains causing post-weaning diarrhoea. Ojeniyi *et al.* (1994) applied two different genotyping methods, colony hybridization and PCR, to detect enterotoxin, verotoxin and fimbrial genes in 708 *E. coli* strains from piglets with diarrhoea, and the results were compared with those obtained by phenotypic methods. The correlation between the genotypic and phenotypic results was 97.7–100%. Detection of fimbrial and enterotoxin genes detected more pathogenic strains than the serotyping using a set of rabbit OK antisera. Using such techniques, the verotoxin and the fimbrial F107 genes were found to be more frequent in post-weaning than in neonatal *E. coli* strains and genotypic tests are becoming valuable tools in the identification of pathogenic *E. coli.*

Together with staphylococcal mastitis, coliform mastitis is a major problem in dairy farms. Identification of *E. coli* strains from cows with clinical mastitis can be accomplished by PCR amplification using repetitive extragenic palindromic (REP) and enterobacterial repetitive intergenic consensus (ERIC) sequences. Such procedure has revealed that *E. coli* strains isolated from repeated episodes of clinical mastitis in the same cow have similar genotypes (Lipman *et al.*, 1995).

In Western countries, enterohaemorrhagic *E. coli* (EHEC), especially serotype O157:H7, have become a major concern for human health. EHEC strains produce verocytotoxins, and have been identified as causative agents of human diarrhoea, haemorrhagic colitis (HC), haemolytic-uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP). Cattle seem to be the most important reservoir of EHEC, and although EHEC can produce haemorrhagic colitis in calves, many healthy animals are carriers. The high levels of EHEC carriage among young animals is of concern as meat may be a significant source of transmission from bovines to humans. Because verocytotoxin genes can be detected by PCR (Smith *et al.*, 1988; Tyler *et al.*, 1991), this technique has become useful to determine the prevalence and clinical significance of EHEC isolated from cattle herds with and without calf diarrhoea. Burnens *et al.* (1995) found a 20% level of EHEC carriage among cows, but it was reassuring that no EHEC were detected in milk samples.

Salmonella

Enteric disease caused by infection with *Salmonella* is an important cause of morbidity in animals. *S. enteritidis* in particular is associated with human food-borne illness resulting from the consumption of contaminated poultry eggs or meat. Salmonellas are generally identified by microbiological culture of faeces, tissue or body fluids. Although ELISAs may be used to identify salmonellas, full identification still requires culture. Amplification of salmonella genes offers a specific and direct means of detection (Rahn *et al.*, 1992; Widjojoatmodjo *et al.*, 1992; Aabo *et al.*, 1993; Cohen *et al.*, 1993; Way *et al.*, 1993; Nguyen *et al.*, 1994; Wood *et al.*, 1994). Booster PCR methods for the genus-specific detection of salmonellas in equine and chicken faeces have been developed (Cohen *et al.*, 1994a, b) with detection possible within 10–12 h from the time of submission of samples. Although booster PCR is highly sensitive, its cost is about twice that of a simple PCR reaction. Cohen *et al.* (1995) described an alternative method using enrichment followed by a simple PCR reaction that enabled *Salmonella* to be detected in faeces within 24 h of submission of samples. A quantitative method using a known quantity of competitor DNA to quantify the numbers of salmonellas in chicken faeces has also been developed (Mahon & Lax, 1995), but some problems with inhibitory substances have been reported. Comparison of PCR and microbiological cultures for the detection of salmonellas in drag-swabs from poultry houses have revealed that PCR is significantly more sensitive than culture for environmental monitoring (Cohen *et al.*, 1994c).

Yersinia enterocolitica

Y. enterocolitica also causes food-borne human gastroenteritis, with pigs implicated as the major reservoir for the pathogenic serotypes O:3, O:8 and O:9. Detection of *Y. enterocolitica* often includes enrichment and biochemical confirmation but the whole process can take up to 3 weeks. PCR can be successfully used for recognition of pathogenic *Y. enterocolitica* (Kapperud *et al.*, 1993; Koeppel *et al.*, 1993; Rasmussen *et al.*, 1994), and the best results are achieved if the bacteria are concentrated by immunomagnetic separation (IMS) before PCR. This approach has been used to detect *Y. enterocolitica* O:3 in faecal samples and tonsil swabs from pigs (Rasmussen *et al.*, 1995) and the authors concluded that IMS-PCR was a reliable method when used on pre-enriched medium, enabling the detection of positive samples which are not recognized by traditional methods.

Helicobacter pylori

H. pyrlori is a microaerophilic, Gram-negative spiral organism that has received great attention for its association with human gastritis, peptic ulcers and even gastric cancer. Other species of the genus have been isolated from the gastric mucosa of animals and mostly associated with gastritis of the host. Because it has been suggested that some strains of *Helicobacter canis* are capable of zoonotic transmission, sensitive methods for their detection are needed, and PCR has already been shown to be useful (Stanley *et al.*, 1993).

Brucella

Bacteria of the genus *Brucella* are well-known as intracellular pathogens that cause animal and human infections. Rapid and sensitive PCR detection of brucellas with or without extraction of DNA has been accomplished (Fekete *et al.*, 1990a, 1990b; Ouahrani *et al.*, 1993).

Mycobacterium

Mycobacterium bovis, the causative agent of tuberculosis in cattle, is a member of the tuberculosis complex, a group of related species that includes *Mycobacterium tuberculosis*, the major cause of human tuberculosis. Histological examinations enable rapid decisions to be made on suspect carcasses during meat inspection. However, agents other than *M. bovis* can induce similar lesions, and additionally, the microscopic detection of acid-fast organisms can only detect bacteria in great concentrations. Laboratory culture of *M. bovis* is sensitive but requires viable bacteria, and the growth of this organism may take 6–8 weeks. Species identification procedures extend the reporting time even further. Tests based on PCR have been shown to be very promising for mycobacterial detection in clinical samples (Cousins *et al.*, 1991; Buck *et al.*, 1992; Yule *et al.*, 1994; Wards *et al.*, 1995).

Mycobacterium paratuberculosis causes Johne's disease, a commonly diagnosed disease of sheep, goats and other ruminants. The organisms can be detected by PCR from intestinal and lymph node tissue of infected animals (Ridge *et al.*, 1995).

Ovine foot rot

Ovine foot rot is a highly contagious, economically serious disease of sheep with worldwide distribution, especially in temperate farming areas. Although foot rot results from a mixed bacterial infection, *Dichelobacter nodosus* has been shown to be the essential pathogen for the initiation and establishment of the disease. Clinical diagnostic methods currently available for foot rot are subjective and lack precision. Consequently, there is a demand for rapid and precise tests to differentiate virulent strains. The use of PCR based on specific regions of 16S rRNA constitutes a competent assay for foot rot (La Fontaine *et al.*, 1993). PCR assays employing virulent- and benign-specific primers are capable of specific and sensitive differentiation of strains causing virulent, intermediate or benign foot rot (Liu & Webber, 1995).

Leptospirosis

Leptospirosis is probably one of the world's most widespread zoonoses. Rapid diagnosis of leptospirosis is important in view of the need for adequate early treatment. Clinically, it is sufficient to know whether or not a patient is infected with pathogenic leptospires but, epidemiologically, it would be of considerable value if the causative leptospira can be identified at the strain level. Serology does not contribute to early diagnosis as antibodies become detectable on approximately the seventh day of infection. Conventional methods to detect leptospires in blood are either unreliable or too slow to give early results. PCR is a promising tool for early detection of leptospires in blood, urine or CSF in the period between the first appearance of clinical symptoms and the time when antibodies become detectable (Van Eys *et al.*, 1989; Gerritsen *et al.*, 1991; Hookey, 1992; Merien *et al.*, 1992; Gravekamp *et al.*, 1993).

Borrelia

The genus *Borrelia* contains several human and animal pathogens. The aetiological agent of Lyme disease is *Borrelia burgdorferi*, which is primarily transmitted by *Ixodes* ticks. Several authors have successfully employed PCR for diagnosis of Lyme disease (Rosa & Schwan, 1989; Marconi & Garon, 1992; Kawabata *et al.*, 1993). It is well-known that ticks feed on deer species, and using PCR, Kimura *et al.* (1995) demonstrated the presence of *B. burgdorferi* in the skin of naturally infected wild sika deer, thus confirming the potential of deer as a source of transmission. PCR data also support the notion that birds are partly responsible for the heterogeneous distribution of Lyme disease *Borrelia* spirochetes in Europe (Olsén *et al.*, 1995). Zingg and LeFebvre (1994) have developed a high-sensitive PCR assay for *Borrelia coriaceae* that does not cross-react with any other closely related spirochetes.

Mycoplasma

Mycoplasmas are known to produce a wide spectrum of animal diseases. Cattle infected with *Mycoplasma mycoides* subsp. *mycoides* infection can either remain apparently healthy or develop contagious bovine pleuropneumonia (CBPP), a disease characterized by respiratory problems. *Post mortem* findings should be followed by bacteriological culture of the organism from affected tissue which can take up to 2 weeks to complete. The serological detection of antibodies is highly specific but asymptomatic animals in the early stages of infection and chronically-infected animals may not have detectable levels of antibodies. Bashiruddin *et al.* (1994) described the use of PCR to detect specific DNA in clinical material and isolates from outbreaks of CBPP in cattle and buffaloes in Italy. These data showed that PCR can identify the aetiological agent within 2 days of extraction of clinical material, and the specificity of the PCR test to distinguish *M.* subsp. *mycoides* from other subspecies was confirmed.

Mycoplasma hyopneumoniae has been identified as the causative agent of mycoplasmal pneumonia in pigs. Because an effective vaccine is not currently available, efforts to control the disease have focused on the elimination of sick animals. Unfortunately, efforts have been hampered by difficulties in differentiating *M. hyopneumoniae* from cross-reacting *Mycoplasma flocculare* and *Mycoplasma hyorhinis*. Stemke *et al.* (1994) developed a method for differentiation of those three species on the basis of amplification of a 16S rRNA gene sequence. PCR methodology for detection of *Mycoplasma gallisepticum* have also been reported (Nascimento *et al.*, 1991; Kempf *et al.*, 1993, 1994). The organism is the cause of chronic respiratory disease in chickens which results in reduced egg production and significant downgrading of carcasses at slaughter.

Chlamydia psittaci

Chlamydia psittaci includes a heterogeneous group of mammalian and avian isolates but, at present, there is no generally accepted and accessible method for typing these. The major outer-membrane protein (MOMP) is the most important antigen at the cell surface of chlamydia. Recently, PCR-RFLP analysis of the MOMP encoding gene has been used for typing of *C. psittaci* strains (Denamur *et al.*, 1991; Kaltenboeck *et al.*, 1992; Sayada *et al.*, 1994).

Coxiella burnetii

Coxiella burnetii, a zoonotic organism, is the aetiological agent of Q fever. In humans, Q fever occurs as a influenza-like illness, pneumonia, granulomatous hepatitis or chronic endocarditis. In animals, coxiella can reach high concentrations in the female reproductive system and infection can be followed by abortion or infertility. Although the infection of cattle is usually latent, *C. burnetii* may be shed via milk by infected cows for one or several lactation periods. The organism can survive, in low numbers, for a long time in dairy products made from non-pasteurized milk of infected cows and detection in milk requires a high-sensitive method. A PCR approach with primers based on repetitive transposon-like sequences have been established for the highly-sensitive and specific detection of *C. burnetii* in cow's milk (Willems *et al.*, 1994).

PARASITES

Leishmania

Leishmaniasis is a group of infestations of the viscera, skin and mucous membranes caused by protozoa of the genus *Leishmania*. Multicopy 16S rRNA has been the basis of some PCR assays that specifically detects *Leishmania* sp. (Guevara *et al.*, 1992; Van Eys *et al.*, 1992). Kinetoplast DNA (kDNA) is a target of interest because both maxi- and minicircles are present in each cell in multiple copies. However, it has proved to be difficult to select species-specific kDNA sequences for diagnosis by PCR (Smyth *et al.*, 1992; López *et al.*, 1993), and it is important to investigate only small regions of minicircles to find species-specific sequences conserved among strains of the same species. PCR has been used to detect leishmanias in conjunctival biopsies (Roze, 1995), showing that a number of cases of ocular inflammation can be attributed to this parasite.

Trypanosoma

In some tropical countries, the protozoan parasites of the genus *Trypanosoma* are responsible for life-threatening diseases in animals and humans, and PCR is now being used to evaluate the vectorial ability of *Glossina longipalpis* in Western Africa (Solano *et al.*, 1994; Weiss, 1995).

Toxoplasma

The cyst-forming apicomplexan parasite *Toxoplasma gondii* infects a broad spectrum of vertebrates. Domestic and feral cats are the definitive hosts but humans and other animal species can be infested by ingestion of oocysts or tissue cysts. Overwhelming infestations, especially in immunosuppressed individuals, may be fatal. Application of PCR can quickly and accurately detect *T. gondii* in a variety of clinical specimens including formalin-fixed and paraffin embedded tissues (MacPherson & Gajadhar, 1993; Wastling *et al.*, 1993; Hyman *et al.*, 1995).

Cryptosporidium

Cryptosporidiosis is now recognized as an important cause of human and animal diarrhoea. PCR amplification combined with chemiluminescence can specifically detect *Cryptosporidium parvum* DNA present in fixed paraffin-embedded tissues (Laxer *et al.*, 1991, 1992). Species and strain differentiation of domestic fowl coccidia of the genus *Eimeria* has also been achieved by PCR (Procunier *et al.*, 1993).

Echinococcus

Echinococcosis is a disease caused by larval stages of different cestode species of the genus *Echinococcus*, especially *Echinococcus granulosus* and *Echinococcus multilocularis*. These species are widely prevalent and may cause severe disease in animals and humans. A PCR study including several independent *E. multilocularis* isolates and various other cestodes revealed that the PCR product was obtained from genomic DNA of all *E. multilocularis* isolates but not from DNA of other cestode species (Gottstein & Mowatt, 1991). The sensitivity of the *E. granulosus* PCR was evaluated experimentally and approached 2.5 pg of template DNA, which corresponds to the DNA content of a single echinococcus egg (Rishi & McManus, 1987). A random amplified polymorphic DNA (RAPD) method has permitted a detailed genetic analysis of Swiss and Spanish isolates of *E. granulosus* (Siles-Lucas *et al.*, 1994). The application of PCR to detect echinococci can allow the identification of biopsy material obtained from liver lesions of unknown aetiology and the demonstration of adult-stage parasite tissue or eggs in samples derived from faeces, small intestines or anal swabs of definitive carnivore hosts (Gottstein, 1992).

Taenia

Tapeworms of the genus *Taenia* can cause human and animal taeniasis and cysticercosis. Although the eggs from *Taenia solium* and *Taenia saginata* cannot be differentiated morphologically, a 500 bp sequence that hybridize specifically to a single-copy gene sequence of *T. solium* and not to *T. saginata* DNA may be available in the future for rapid PCR differentiation (Rishi & McManus, 1988).

Dictyocaulus

Lungworms are common parasites of ruminants, and to a lesser extent, horses. In cattle, they cause considerable economic losses due to weight loss and deaths. RAPD-PCR has proved to be a valuable tool to examine genome differences among *Dictyocaulus* species from cattle, sheep and fallow deer (Epe *et al.*, 1995).

Trichinella

The nematode *Trichinella spiralis* can infect nearly all meat-eating animals. Trichinellosis is transmitted within two cycles that can interact; a sylvatic cycle in wild animals and a domestic cycle in pigs which is the major source of human infestation. Two different sets of primers have been developed specifically to discriminate domestic from sylvatic isolates (Dupouy-Camet *et al.*, 1991; Dick *et al.*, 1992). PCR has been able to detect, *in situ*, a single excysted larva, as well as a single encysted larva, in infected mouse muscle following boiling (Dick *et al.*, 1992). RAPD-PCR has also been useful for the identification of Trichinella species (Bandi *et al.*, 1993; Dupouy-Camet *et al.*, 1993).

CONCLUSION

PCR has already played an important role in studies of the epidemiology, taxonomy and pathogenesis of micro-organism infections in animals but is not yet used routinely for the diagnosis of any animal infectious disease. In fact, PCR has become a routine tool only in research laboratories. However, infectious diseases will remain among the major areas for application of PCR detection and genotyping, offering the potential to analyse most micro-organisms of veterinary importance by a single technique. Although many systems have been developed, few have proceeded towards field trials or large-scale clinical evaluation, and PCR application to the routine analysis of biological samples is still a major diagnostic challenge. Most of the assays to detect micro-organisms have high sensitivity with purified DNA samples, but advances in sample preparation and detection of amplified products under field or clinical laboratory conditions are needed in order to achieve high sensitivity with animal specimens.

Diagnosis of viral diseases should be a major target for PCR application because laboratory tests for identification of viruses are either slow, expensive or insensitive. The technique has found large-scale application for the routine detection of human pathogens such as HIV and hepatitis viruses. Among animal viral diseases, pseudorabies, equine viral arteritis, bovine leukaemia and bovine viral diarrhoea are good candidates for early development. The approach should also be focused on viral diseases that have a deep socio-economic impact in endemic regions, such as African Swine Fever or rinderpest. Eradication programmes must include the diagnosis of sick animals, asymptomatic carriers and vectors, and often involve the rapid screening of a large number of samples for which PCR would be very useful.

In relation to bacterial diseases, PCR can be used for the rapid detection of those pathogens whose *in vitro* cultivation is difficult, time-consuming or unavailable. RFLP patterns using PCR-amplified DNA is an excellent method for bacterial typing and has already been used for the identification of the bacterial strains involved in human foodborne outbreaks (Hill, 1996). Parasitic infestations will probably be the last field of veterinary clinical diagnosis to incorporate PCR techniques, partly because of the relative scarcity of important parasitic diseases in the main countries where PCR research is being developed (Weiss, 1995).

In conclusion, PCR will most likely become the standard diagnostic test in situations where either the micro-organism level is low, differentiation between morphologically identical organisms is required, or whether the immune response to the infection is uninformative. As happened with the progressive introduction of enzyme-linked immunosorbent assays (ELISA) as routine diagnostic tools, the existence of a strong demand for improved diagnosis methods will surely lead, in the next decades, to the development of PCR-based test kits suitable for field application.

REFERENCES

Aabo, S., Rasmussen, O. F., Rossen, L., Sorensen, P. D. & Olsen, J. E. (1993). *Salmonella* identification by the polymerase chain reaction. *Molecular and Cellular Probes* **7**, 171–8.

Agresti, A., Ponti, W., Rocchi, M., Meneveri, R., Marozzi, A., Cavalleri, D., Peri, E., Poli, G. & Ginelli, E. (1993). Use of polymerase chain reaction to diagnose bovine leukemia-virus infection in calves at birth. *American Journal of Veterinary Research* **54**, 373–8.

Akita, G. Y., Glenn, J., Castro, A. E. & Osburn, B. I. (1993). Detection of bluetongue virus in clinical samples by polymerase chain reaction. *Journal of Veterinary Diagnostic Investigation* **5**, 154–8.

Aly, M. M., Smith, E. J. & Fadly, A. M. (1993). Detection of reticuloendotheliosis virus infection using the polymerase chain reaction. *Avian Pathology* **22**, 543–54.

Andreasen, J. R., Jackwood, M. W. & Hilt, D. A. (1991). Polymerase chain reaction amplification of the genome of infectious bronchitis virus. *Avian Diseases* **35**, 216–20.

Aradaib, I. E., Akita, G. Y., Pearson, J. E. & Osburn, B. I. (1993). Comparison of polymerase chain reaction and virus isolation for detection of epizootic hemorrhagic disease in clinical samples from naturally infected deer. *Journal of Veterinary Diagnostic Investigation* **7**, 196–200.

Ballagy-Pordany, A., Klingeborn, B., Flensburg, J. & Belak, S. (1990). Equine herpesvirus type 1: detection of viral DNA sequences in aborted fetuses with the polymerase chain reaction. *Veterinary Microbiology* **22**, 373–81.

Ballagy-Pordany, A., Klintevall, K., Merza, M., Klingeborn, G. & Belak, S. (1992). Direct detection of bovine leukemia virus infection: practical applicability of the polymerase chain reaction. *Journal of Veterinary Medicine B* **39**, 69–77.

Bandi, C., La Rosa, G., Comincini, S., Damiani, G. & Pozio, E. (1993). Random amplified polymorphic DNA technique for the identification of *Trichinella* species. *Parasitology* **107**, 419–24.

Barrett, T., Crowther, J., Osterhaus, A. D. M. E., Subbarao, S. M., Groen, J., Haas, L., Mamaev, L. V., Titenko, A. M., Grachev, M. A., Visser, I. K. G. & Bostock,

C. J. (1992). Molecular and serological studies on the recent seal virus epizooties in Europe and Siberia. *Science of the Total Environment* **115**, 117–32.

Barrett, T., Amarel-Doel, C., Kitching, R. P. & Gusev, A. (1993). Use of polymerase chain reaction in differentiating rinderpest field virus and vaccine virus in the same animals. *Revue Scientifique et Technique. Office International des Epizooties* **12**, 865–72.

Bashiruddin, J. B., Nicholas, R. A. J., Santin, F. G., Ready, R. A., Woodward, M. J. & Taylor, T. K. (1994). Use of polymerase chain reaction to detect mycoplasma DNA in cattle with contagious bovine pleuropneumonia. *Veterinary Record* **134**, 240–1.

Becker, Y., Asher, Y., Tabor, E., Davidson, I., Malkinson, M. & Weisman, Y. (1992). Polymerase chain reaction for differentiation between pathogenic and nonpathogenetic serotype 1 Marek's disease virus (MDV) and vaccine viruses of MDV-serotypes 2 and 3. *Journal of Virological Methods* **40**, 307–22.

Becker, Y., Tabor, E., Asher, Y., Davidson, I., Malkinson, M. & Witter, R. L. (1993). PCR detection of amplified 132 bp repeats in Marek's disease virus type 1 (MDV-1) DNA can serve as an indicator of initial genomic rearrangement leading to the attenuation of virus virulence. *Virus Genes* **7**, 277–87.

Belak, S. & Ballagy-Pordany, A. (1991). Bovine viral diarrhea virus infection: rapid diagnosis by the polymerase chain reaction. *Archives of Virology* **3**, (Suppl.) 181–90.

Belak, S., Ballagy-Pordany, A., Flensburg, A. & Virtanen, J. (1989). Detection of pseudorabies virus DNA sequences by the polymerase chain reaction. *Archives of Virology* **108**, 279–86.

Boye, M., Kamstrup, S. & Dalsgaard, K. (1991). Specific sequence amplification of bovine viral diarrhea virus (BVDV) and hog cholera virus and sequencing of BVDV nucleic acid. *Veterinary Microbiology* **29**, 1–13.

Brock, K. V. (1991). Detection of persistent bovine viral diarrhea virus infections by DNA hybridization and polymerase chain reaction assay. *Archives of Virology* Suppl. **3**, 199–208.

Brockmeier, S. L., Lager, K. M. & Mengeling, W. L. (1993). Comparison of *in vitro* reactivation and polymerase chain reaction for detection of latent pseudorabies virus infection in swine. *Journal of Veterinary Diagnostic Investigation* **5**, 505–9.

Buck, G. E., O'Hara, L. C. & Summersgill, J. T. (1992). Rapid, simple method for treating clinical samples containing *Mycobacterium tuberculosis* to remove DNA for polymerase chain reaction. *Journal of Clinical Microbiology* **30**, 1331–4.

Buogo, C., Capaul, S., Hani, H., Frey, J. & Nicolet, J. (1995). Diagnosis of *Clostridium perfringens* type C enteritis in pigs using a DNA amplification technique (PCR). *Journal of Veterinary Medicine B* **42**, 51–8.

Burnens, A. P., Frey, A., Lior, H. & Nicolet, J. (1995). Prevalence and clinical significance of vero-cytotoxin-producing *Escherichia coli* (VTEC) isolates from cattle in herds with and without calf diarrhea. *Journal of Veterinary Medicine B* **42**, 311–8.

Carl, M., Hawkins, S. R., Coulson, N., Lowe, J., Robertson, D. L., Nelson, W. M., Titball, R. W. & Woody, J. N. (1992). Detection of spores of *Bacillus anthracis* using the polymerase chain reaction. *Journal of Infectious Diseases* **165**, 1145–8.

Chamberlain, R. W., Wamwayi, H. M., Hockley, E., Shaila, M. S., Goatley, L., Knowlesn, J. C. & Barrett, T. (1993). Evidence for different lineages of rinderpest virus reflecting their geographic isolation. *Journal of General Virology* **74**, 2775–80.

Cheung, A. K. (1995). Investigation of pseudorabies virus DNA and RNA in trigeminal ganglia and tonsil tissues of latently infected swine. *American Journal of Veterinary Research* **56**, 45–50.

Chirnside, E. D. & Spaan, W. J. M. (1990). Reverse transcription and cDNA amplification by the polymerase chain reaction of equine arteritis virus (EAV). *Journal of Virological Methods* **30**, 133–40.

Clavijo, A. & Thorsen, J. (1995). Chemiluminescent detection of caprine arthritis encephalitis virus with a PCR-generated single stranded nonradiolabelled probe. *Veterinary Microbiology* **43**, 295–305.

Cohen, N. D., McGruder, E. D., Neibergs H. L., Behle, R. W., Wallis, D. E. & Hargis, B. M. (1994a). Detection of *Salmonella enteritidis* in feces from poultry using booster polymerase chain reaction and oligonucleotide primers specific for all members of the genus *Salmonella*. *Poultry Science* **73**, 354–7.

Cohen, N. D., Neibergs, H. L., Wallis, D. E., Simpson, R. B. & McGruder, E. D. (1994b). Genus-specific detection of salmonellae in equine feces by use of the polymerase chain reaction. *American Journal of Veterinary Research* **55**, 1049–54.

Cohen, N. D., Wallis, D. E., Neibergs, H. L., McElroy, A., McGruder, E. D., De Loach, J. R., Corrier, D. E. & Hargis, B. M. (1994c). Comparison of the polymerase chain reaction using genus-specific oligonucleotide primers and microbiologic cultures for the detection of *Salmonella* in drag-swabs from poultry houses. *Poultry Science* **73**, 1276–81.

Cohen, N. D., Wallis, D. E., Neibergs, H. L. & Hargis, B. M. (1995). Detection of *Salmonella enteritidis* in equine feces using the polymerase chain reaction and genus-specific oligonucleotide primers. *Journal of Veterinary Diagnostic Investigation* **7**, 219–22.

Cousins, D. V., Wilton, S. D. & Francis, B. R. (1991). Use of DNA amplification for the rapid identification of *Mycobacterium bovis*. *Veterinary Microbiology* **27**, 187–195.

Dangler, C. A., Demattos, C. A., Demattos, C. C. & Osburn, B. I. (1990). Identifying bluetongue virus ribonucleic acid sequences by the polymerase chain reaction. *Journal of Virological Methods* **28**, 281–92.

Davidson, I., Borovskaya, A., Perl, S. & Malkinson, M. (1995). Use of polymerase chain reaction for the diagnosis of natural infection of chickens and turkey with Marek's disease virus and reticuloendotheliosis virus. *Avian Pathology* **24**, 69–94.

Denamur, E., Sayada, C., Souriau, A., Orfila, J., Rodolakis, A. & Elion, J. (1991). Restriction pattern of the major outer membrane gene provides evidence for a homogeneous invasive group among ruminant isolates of *Chlamydia psittaci*. *Journal of Clinical Microbiology* **137**, 2525–30.

Diallo, A., Libeau, G., Couacy-Hymann, E. & Barbron, M. (1995). Recent developments in the diagnosis of rinderpest and peste des ruminants. *Veterinary Microbiology* **44,** 307–17.

Dick, T. A., Lu, M., De Vos, T. & Ma, K. (1992). The use of polymerase chain reaction to identify porcine isolates of *Trichinella*. *Journal of Parasitology* **78,** 145–8.

Dupouy-Camet, J., Soule, C., Guillou, J. P., Rover, E., De Souza, S. L., Ancelle, T. & Benarous, R. (1991). Detection of repetitive sequences of *Trichell spiralis* by the polymerase chain reaction in experimentally infected mice. *Parasitology Research* **77,** 180–2.

Dupouy-Camet, J., Robert, F. & Soule, C. (1993). RAPD in the genus *Trichinella*. *Parasitology Today* **9,** 463–4.

Egberink, H. F., Herrewegh, A. P. M., Schuurman, N. P. M., Van Der Linde-Sipman, J. S., Horzinek, M. C. & De Groot, R. J. (1995). FIP, easy to diagnose? *The Veterinary Quarterly* **17,** S24–5.

Epe, C., Bienioschek, S., Rehbein, S. & Schnieder, T. (1995). Comparative RAPD-PCR analysis of lungworms (*Dictyocaulidae*) from fallow deer, cattle, sheep and horses. *Journal of Veterinary Medicine B* **42,** 187–91.

Ericsson, H., Stalhandske, P., Danielsson-Tham, M. L., Bannerman, E., Bille, J., Jacquet, C., Rocourt, J. & Tham, W. (1995). Division of *Listeria monocytogenes* serovar 4b strains into two groups by PCR and restriction enzyme analysis. *Applied and Environmental Microbiology* **61,** 3872–4.

Erlich, H. (1989). *PCR Technology: Principles and Applications for DNA Amplification*. New York: Stockton Press.

Ermine, A., Larzul, D., Ceccaldi, P. E., Guesdon, J. L. & Tsiang, H. (1990). Polymerase chain reaction amplification of rabies virus nucleic acids from total mouse brain RNA. *Molecular and Cellular Probes* **4,** 189–91.

Fach, P., Delbart, M. O., Schlachter, A., Poumeyrol, M. & Popoff, M. R. (1993). Apport de la technique d'amplification génique (PCR) au diagnostic des toxi-infections alimentaires a *Clostridium perfringens*. *Médicine du Maladies Infectioses* **23,** 70–7.

Fach, P., Gilbert, M., Griffais, R., Guillou, J. P. & Popoff, M. R. (1995). PCR and gene probe identification of botulinum neurotoxin A-, B-, E-, F-, and G-producing *Clostridium* spp. and evaluation in food samples. *Applied and Environmental Microbiology* **61,** 389–92.

Fekete, A., Bantle, J. A., Halling, S. & Sanborn, M. R. (1990a). Preliminary development of a diagnostic test for *Brucella* using polymerase chain reaction. *Journal of Applied Bacteriology* **69,** 216–27.

Fekete, A., Bantle, J. A., Halling, S. & Sanborn, M. R. (1990b). Rapid, sensitive detection of *Brucella abortus* by polymerase chain reaction without extraction of DNA. *Biotechnology Techniques* **4,** 31–4.

Gerritsen, M. J., Olyhoek, T., Smits, M. A. & Bokhout, B. A. (1991). Sample preparation method from polymerase chain reaction-based semiquantitative detection of *Leptospira interrogans* serovar *hardjo* serotype Hardjobovis in bovine urine. *Journal of Clinical Microbiology* **29,** 2805–8.

Gonzalez, L., Reid, H. W., Pow, I. & Gilmour, J. S. (1987). A disease resembling louping-ill in sheep in the Basque region of Spain. *Veterinary Record* **121,** 12–3.

Gottstein, B. (1992). Molecular and immunological diagnosis of echinococcosis. *Clinical Microbiology Review* **5,** 248–61.

Gottstein, B. & Mowatt, M. R. (1991). Sequencing and characterization of an *Echinococcus multilocularis* DNA probe and its use in the polymerase chain reaction (PCR). *Molecular and Biochemical Parasitology* **44,** 183–94.

Gould, A. R., Hyatt, A. D., Eaton, B. T., White, J. R., Hooper, P. T., Blacksell, S. D. & Leblanc Smith, P. M. (1989). Current techniques in rapid bluetongue diagnosis. *Australian Veterinary Journal* **66,** 450–4.

Gradil, C. M., Harding, M. J. & Lewis, K. (1994). Use of polymerase chain reaction to detect porcine parvovirus associated with swine embryos. *American Journal of Veterinary Research* **55,** 344–7.

Gravekamp, C., Van De Kemp, H., Franzen, M., Carrington, D., Schoone, G. J., Vaneys, G. J. J. M., Everard, C. O. R., Hartskeerl, R. A. & Terpstra, W. J. (1993). Detection of seven species of pathogenic leptospires by PCR using two sets of primers. *Journal of General Microbiology* **139,** 1691–1700.

Griffin, H. G. & Griffin, A. M. (1994). *PCR Technology. Current Innovations*. Boca Raton: CRC Press.

Gruber, A. D., Moenning, V., Hewicker-Trautwein, M. & Trautwein, G. (1994). Effect of formalin fixation and long-term storage on the detectability of bovine viral diarrhea virus (BVDV) RNA in archival brain tissue using polymerase chain reaction. *Journal of Veterinary Medicine B* **41,** 654–61.

Guerin, C., Allieta, M., Guerin, B. & Thibier, M. (1995). Utilisation de la technique d'amplification de gène pour la détection du virus de la maladie d'Aujeszky dans le sperme de verrat. *Veterinary Research* **26,** 140–4.

Guevara, P., Alonso, G., Da Silveira, J. F., De Mello, M., Scorza, J. V., Anez, N. & Ramirez, J. L. (1992). Identification of a new world *Leishmania* using ribosomal gene spacer probes. *Molecular and Biochemical Parasitology* **56,** 15–26.

Harel, J., Lapointe, H., Fallara, A., Lortie, L. A., Bigras-Poulin, M., Lariviere, S. & Fairbrother, J. M. (1991). Detection of genes for fimbrial antigens and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with diarrhea. *Journal of Clinical Microbiology* **29,** 745–52.

Henderson, I., Duggleby, C. J. & Turnbull, P. C. B. (1994). Differentiation of *Bacillus anthracis* from other *Bacillus cereus* group bacteria with the PCR. *International Journal of Systematic Bacteriology* **44,** 99–105.

Hertig, C., Pauli, V., Zanoni, R. & Peterhans, E. (1991). Detection of bovine viral diarrhea (BVD) virus using the polymerase chain reaction. *Veterinary Microbiology* **26,** 65–76.

Hill, W. E. (1996). The polymerase chain reaction: applications for the detection of foodborne pathogens. *Critical Reviews in Food Science and Nutrition* **36,** 123–173.

Hirasawa, T., Kaneshige, T. & Mikazuki, K. (1994). Sensitive detection of canine parvovirus DNA by the nested polymerase chain reaction. *Veterinary Microbiology* **41,** 135–45.

Hirasawa, T., Yono, K. & Mikazuki, K. (1995). Differentiation of wild- and vaccine-type canine parvoviruses by PCR and restriction-enzyme analysis. *Journal of Veterinary Medicine B* **42**, 601–10.

HOFT VAN IODEKINGE, B. J. L., VAN WAMEL, J. L. V. B., VAN GENNIP, H. G. P. & MOORMAN, R. J. M. (1992). Application of polymerase chain reaction to the detection of bovine viral diarrhea virus infections in cattle. *Veterinary Microbiology* **30**, 21–34.

HOOKEY, J. V. (1992). Detection of *Leptospiraceae* by amplification of 16S ribosomal DNA. *FEMS Microbiological Letters* **90**, 267–74.

HORNER, G. W., THAM, K. M., ORR, D., RALSTON, J., ROWE, S. & HOUGHTON, T. (1995). Comparison of an antigen capture enzyme-linked assay with reverse transcription-polymerase chain reaction and cell culture immunoperoxidase tests for the diagnosis of ruminant pestivirus infections. *Veterinary Microbiology* **43**, 75–84.

HORSERACE BETTING LEVY BOARD (1993). The Horserace Betting Levy Board's code of practice for equine viral arteritis for the 1994 breeding season. *Veterinary Record* **134**, 512–4.

HUTSON, R. A., DUGGLEBY, C. J., LOWE, J. R., MANCHEE, R. J. & TURNBULL, P. C. B. (1993). The development and assessment of DNA and oligonucleotides for the specific detection of *Bacillus anthracis*. *Journal of Applied Bacteriology* **75**, 463–72.

HYMAN, J. A., JOHNSON, L. K., TSAI, M. M. & O'LEARY, T. J. (1995). Specificity of polymerase chain reaction identification of *Toxoplasma gondii* in paraffin-embedded animal tissues. *Journal of Veterinary Diagnostic Investigation* **7**, 275–8.

INNIS, M. A., GELFAND, D. H., SNINSKY, J. J. & WHITE, T. J. (1990). *PCR Protocols: A Guide to Methods and Applications.* San Diego: Academic Press.

JACKWOOD, M. W., KWON, H. M. & HILT, D. A. (1992). Infectious bronchitis virus detection in allantoic fluid using the polymerase chain reaction and DNA probes. *Avian Diseases* **36**, 403–9.

JATON, K., SAHLI, R. & BILLE, J. (1992). Development of polymerase chain reaction assays for detection of *Listeria monocytogenes* in clinical cerebrospinal fluid samples. *Journal of Clinical Microbiology* **30**, 1931–6.

JESTIN, V. & JESTIN, A. (1991). Detection of Newcastle disease virus RNA in infected allantoic fluids by *in vitro* enzymatic amplification (PCR). *Archives of Virology* **118**, 151–61.

JOHNS, M., HARRINGTON, L., TITBALL, R. W. & LESLIE, D. L. (1994). Improved methods for the detection of *Bacillus anthracis* spores by the polymerase chain reaction. *Letters in Applied Microbiology* **18**, 236–8.

JONES, D. D. & BEJ, A. K. (1994). Detection of foodborne microbial pathogens using polymerase chain reaction methods. In *PCR Technology. Current Innovations*, ed. H. G. Griffin & A. M. Griffin. Boca Raton: CRC Press.

KALTENBOECK, B., KOUSOULAS, K. G. & STORZ, J. (1992). Two-step polymerase chain reaction and restriction endonuclease analyses detection and differentiation of *ompA* DNA of *Chlamydia* spp. *Journal of Clinical Microbiology* **30**, 1098–1104.

KAMOLVARIN, N., TIRAWATNPONG, T., RATTANASIWAMOKE, R., TIRAWATNPONG, S., PAPANICH, T. & HEMACHUDHA, T. (1993). Diagnosis of rabies by polymerase chain reaction with nested primers. *Journal of Infectious Diseases* **167**, 207–10.

KAPPERUD, G., VARDUND, T., SKJERVE, E., HORNES, E. & MICHAELSEN, T. E. (1993). Detection of pathogenic *Yersinia entercolitica* in foods and water by immunomagnetic separation, nested polymerase chain reactions, and colorimetric detection of amplified DNA. *Applied and Environmental Microbiology* **59**, 2938–44.

KATZ, J. B., SHAFER, A. L., EERNISSE, K. A., LANDGRAF, J. G. & NELSON, E. A. (1995). Antigenic differences between European and American isolates of porcine reproductive and respiratory syndrome virus (PRRSV) are encoded by the carboxyterminal portion of viral open reading frame 3. *Veterinary Microbiology* **44**, 65–76.

KAWABATA, H. H., TASHIBO, H., YAMADA, K., MASUZAWA, T. & YANAGIHARA, Y. (1993). Polymerase chain reaction analysis of *Borrelia* species isolated in Japan. *Microbiology and Immunology* **38**, 591–8.

KELLY, E. J., KACKSON, M. J., MARSOLAIS, G., MORREY, J. D. & CALLAN, R. J. (1993). Early detection of bovine leukemia virus in cattle by use of the polymerase chain reaction. *American Journal of Veterinary Research* **54**, 205–9.

KEMPF, I., BLANCHARD, A., GESBERT, F., GUITTET, M. & BENNEJEAN, G. (1993). The polymerase chain reaction for the detection of *Mycoplasma gallisepticum. Avian Pathology* **22**, 739–50.

KEMPF, I., GESBERT, F., GUITTET, M. & BENNEJEAN, G. (1994). *Mycoplasma gallisepticum* infection in drug-treated chickens: comparison of diagnosis methods including polymerase chain reaction. *Journal of Veterinary Medicine B* **41**, 597–602.

KENNAN, R., SODERLIND, O. & CONWAY, P. (1995). Presence of F107, 2134P and Av24 fimbriae on strains of *Escherichia coli* isolated from Swedish piglets with diarrhoea. *Veterinary Microbiology* **43**, 123–9.

KIMURA, K., ISOGAI, E., ISOGAI, H., KAMEWAKA, Y., NISHIKAWA, T., ISHII, N. & FUJII, N. (1995). Detection of Lyme disease spirochetes in the skin of naturally infected wild sika deer (*Cervus nippon yesoensis*) by PCR. *Applied and Environmental Microbiology* **61**, 1641–2.

KINENGE, F. S. B., HARRIS, L. M., MCKENNA, P. K., WADOWSKA, D. & YASON, C. V. (1994). Amplification of strains of bovine herpesvirus 1 by use of polymerase chain reaction with primers in the thymidine kinase region. *American Journal of Veterinary Research* **55**, 1206–12.

KOEPEL, E., MEYER, R., LUETHY, J. & CANDRIAN, U. (1993). Recognition of pathogenic *Yersinia enterocolitica* by crystal violet binding and polymerase chain reaction. *Letters in Applied Microbiology* **17**, 231–4.

KWON, H. M., JACKWOOD, M. W., BROWN, T. P. & HILT, D. A. (1993a). Polymerase chain reaction and a biotin-labeled DNA probe for detection of infectious bronchitis virus in chickens. *Avian Diseases* **37**, 149–56.

KWON, H. M., JACKWOOD, M. W. & GELB, J. (1993b). Differentiation of infectious bronchitis virus serotyping using polymerase chain reaction and restriction fragment length polymorphism analysis. *Avian Diseases* **37**, 194–207.

LA FONTAINE, S., EGERTON, J. R. & ROOD, J. I. (1993). Detection of *Dichelobacter nodosus* using species-specific oligonucleotides as PCR primers. *Veterinary Microbiology* **35,** 101–17.

LAOR, O., TORGERSEN, H., YADIN, H. & BECKER, Y. (1991). Detection of FMDV RNA amplified by the polymerase chain reaction (PCR). *Journal of Virological Methods* **36,** 197–208.

LAOR, O., YADIN, H., CHAI, D. & BECKER, Y. (1991). Detection of foot and mouth disease virus in diagnostic material using the PCR method on viral genomic poly-A isolated with oligo dT on magnetic beads. *Israel Journal of Veterinary Medicine* **46,** 127–33.

LAXER, M. A., TIMBLIN, B. K. & PATEL, R. J. (1991). DNA sequences for the specific detection of *Cryptosporidium parvum* by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* **45,** 688–94.

LAXER, M. A., D'NICUOLA, M. E. & PATEL, R. J. (1992). Detection of *Cryptosporidium parvum* DNA in fixed paraffin-embedded tissue by polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* **47,** 450–5.

LEE, L. H., YU, S. L. & SHIEH, H. K. (1992). Detection of infectious bursal disease virus infection using the polymerase chain reaction. *Journal of Virological Methods* **40,** 243–54.

LIN, Z., KATO, A., KUDOU, Y. & UEDA, S. (1991). A new typing method for the avian infectious bronchitis virus using polymerase chain reaction and restriction fragment length polymorphism. *Archives of Virology* **116,** 19–31.

LIPMAN, L. J. A., DE NIJS, A., LAM, T. J. G. M. & GAASTRA, W. (1995). Identification of *Escherichia coli* strains from cows with clinical mastitis by serotyping and DNA polymorphism patterns with REP and ERIC primers. *Veterinary Microbiology* **43,** 13–9.

LIU, D. & WEBBER, J. (1995). A polymerase chain reaction assay for improved detection of virulence of *Dichelobacter nodosus,* the specific causative pathogen of ovine footrot. *Veterinary Microbiology* **43,** 197–207.

LIU, S. T., LI, S. N., WANG, D. C., CHANG, S. F., CHIANG, S. C. HO, W. C., CHANG, Y. S. & LAI, S. S. (1991). Rapid detection of hog cholera virus in tissues by polymerase chain reaction. *Journal of Virological Methods* **35,** 227–36.

LOPEZ, M., INGA, R., CANGALAYA, M., ECHEVARRIA, J., LLANOS-CUENTAS, A., ORREGO, C. & AREVALO, J. (1993). Diagnosis of *Leishmania* using the polymerase chain reaction: a simplified procedure for field work. *American Journal of Tropical Medical and Hygiene* **49,** 348–56.

MACLACHLAN, N. J., NUNAMAKER, R. A. & KATZ, J. B. (1994). Detection of bluetongue virus in the blood of inoculated calves: comparison of virus isolation, PCR assay, and in vitro feeding of *Cullicoides variipennis. Archives of Virology* **136,** 1–8.

MACPHERSON, J. M. & GAJADHAR, A. A. (1993). Sensitive and specific polymerase chain reaction detection of *Toxoplasma gondii* for veterinary and medical diagnosis. *Canadian Journal of Veterinary Research* **57,** 45–8.

MAHBUBANI, M. H. & BEJ, A. K. (1994). Application of polymerase chain reaction methodology in clinical diagnosis. In *PCR Technology. Current Innovations,* ed. H. G. Griffin & A. M. Griffin. Boca Raton: CRC Press.

MAHON, J. & LAX, A. J. (1993). A quantitative polymerase chain reaction method for the detection in avian feces of salmonellas carrying the *spvR* gene. *Epidemiology and Infection* **111,** 455–64.

MAMAEV, L. V., DENIKINA, N. N., BELIKOV, S. I., VOLCHKOV, V. E., VISSER, I. K. G., FLEMING, M., KAI, C., HARDER, T. C., LIESS, B., OSTERHAUS, A. D. M. E. & BARRETT, T. (1995). Characterisation of morbilliviruses isolated from Lake Baikal seals (*Phoca sibirica*). *Veterinary Microbiology* **44,** 251–9.

MARCONI, R. T. & GARON, C. F. (1992). Development of polymerase chain reaction primers sets for diagnosis of Lyme disease and for species-specific identification of Lyme disease isolates by 16S rRNA signature nucleotide analysis. *Journal of Clinical Microbiology* **30,** 2830–4.

MARIN, M. S., MCKENZIE, J., GAO, G. F., REID, H. W., ANTONIADIS, A. & GOULD, E. A. (1995). The virus causing encephalomyelitis in sheep in Spain: a new member of the tick-borne encephalitis group. *Research in Veterinary Science* **58,** 11–3.

MCCOLL, K. A. & GOULD, A. R. (1991). Detection and characterization of bluetongue virus using the polymerase chain reaction. *Virus Research* **21,** 19–34.

MCCOLL, K. A. & GOULD, A. R. (1994). Bluetongue virus infection in sheep: haematological changes and detection by polymerase chain reaction. *Australian Veterinary Journal* **71,** 97–101.

MCCOLL, K. A., GOULD, A. R., SELLECK, P. W., HOOPER, P. T., WESTBURY, H. A. & SMITH, J. S. (1993). Polymerase chain reaction and other laboratory techniques in the diagnosis of long incubation rabies in Australia. *Australian Veterinary Journal* **70,** 84–9.

MERIEN, F., AMOURIOUX, P., PEROLAT, P., BARANTON, G. & SAINT GIRONS, I. (1992). Polymerase chain reaction for the detection of *Leptospira* spp. in clinical samples. *Journal of Clinical Microbiology* **30,** 2219–24.

MEYER, R. F., BROWN, C., HOUSE, J. A. & MILTOR, T. W. (1991). Rapid and sensitive detection of foot and mouth disease virus in tissues by enzymatic RNA amplification of the polymerase gene. *Journal of Virological Methods* **34,** 161–72.

MOLITOR, T. W., ORAVEERAKUL, K., ZHANG, Q. Q., CHOI, C. S. & LUDEMANN, L. R. (1991). Polymerase chain reaction (PCR) amplification for the detection of porcine parvovirus. *Journal of Virological Methods* **32,** 201–11.

MURTAUGH, M. P., LIN, G. F., HAGGARD, D. L., WEBBER, A. F. & MEISKE, J. C. (1991). Detection of bovine leukemia virus in cattle by the polymerase chain reaction. *Journal of Virological Methods* **33,** 73–85.

NAIF, H. M., BRANDON, R. B., DANIEL, R. C. W. & LAVIN, M. F. (1990). Bovine leukaemia provirae DNA detection in cattle by the polymerase chain reaction. *Veterinary Microbiology* **25,** 117–29.

NAIF, H. M., DANIEL, R. C. W., COUGLE, W. G. & LAVIN, M. F. (1992). Early detection of bovine leukaemia virus by using an enzyme-linked assay for polymerase chain reaction-amplified proviral DNA in experimentally infected cattle. *Journal of Clinical Microbiology* **30,** 675–9.

NASCIMENTO, E. R., YAMAMOTO, R., HERRICK, K. R. & TAIT, R. C. (1991). Polymerase chain reaction for the detection of *Mycoplasma gallisepticum. Avian Diseases* **35,** 62–9.

NASH, J. W., HANSON, L. A. & COATS, K. S. C. (1995). Detection of bovine immunodeficiency virus in blood and milk-derived leukocytes by use of polymerase chain reaction. *American Journal of Veterinary Research* **56,** 445–9.

NGUYEN, A. V., KHAN, M. I. & LU, Z. (1994). Amplification of salmonella chromosomal DNA using the polymerase chain reaction. *Avian Diseases* **38,** 119–26.

NUNBERG, J. H., WRIGHT, D. K., COLE, G. E., PETROVSKI, E. A., POST, L. E., COMPTON, T. & GILBERT, J. H. (1989). Identification of the thymidine kinase gene of feline herpesvirus: use of degenerate oligonucleotides in the polymerase chain reaction to isolate herpesvirus gene homologies. *Journal of Virology* **63,** 3240–9.

OJENIYI, B., AHRENS, P. & MEYLING, A. (1994). Detection of fimbrial and toxin genes in *Escherichia coli* and their prevalence in piglets with diarrhea. The application of colony hybridization assay, polymerase chain reaction and phenotypic assays. *Journal of Veterinary Medicine B* **41,** 49–59.

OLSEN, B., JAENSON, T. G. T. & BERGSTROM, S. (1995). Prevalence of *Borrelia burgdorferi* sensu lato-infected ticks on migrating birds. *Applied and Environmental Microbiology* **61,** 3082–7.

O'ROURKE, K. I., BESOLA, M. L. & MCGUIRE, T. C. (1991). Proviral sequences detected by the polymerase chain reaction in peripheral blood cells of horses with equine infectious anemia lentivirus. *Archives of Virology* **117,** 109–19.

OUAHRANI, S., MICHAUX, S., WIDADA, J. S., BOURG, G., TOURNEBIZE, R., RAMUZ, M. & LIAUTARD, J. P. (1993). Identification and sequence analysis of IS*6501*, an insertion sequence in *Brucella* spp.: relationship between genomic structure and the number of IS*6501* copies. *Journal of General Microbiology* **139,** 3265–73.

PARSONSON, I. M., THOMPSON, L. H. & WALTON, T. E. (1994). Experimentally induced infection with bluetongue virus serotype 11 in cows. *American Journal of Veterinary Research* **55,** 1529–34.

PATON, D. J., CARLSSON, U., LOWINGS, J. P., SANDS, J. J., VILCEK, S. & ALENIUS, S. (1995). Identification of herd-specific bovine viral diarrhea virus isolates from infected cattle and sheep. *Veterinary Microbiology* **43,** 283–94.

PETERS, M., POHLENZ, J., JATON, K., NINET, B. & BILLE, J. (1995). Studies of the detection of *Listeria monocytogenes* by culture and PCR in cerebrospinal fluid samples from ruminants with listeric encephalitis. *Journal of Veterinary Medicine B* **42,** 84–8.

PROCUNIER, J. D., FERNANDO, M. A. & BARTA, J. R. (1993). Species and strain differentiation of *Eimeria* spp. of the domestic fowl using DNA polymorphisms amplified by arbitrary primers. *Parasitology Research* **79,** 98–102.

RADWAN, G. S., BROCK, K. V., HOGAN, J. S. & SMITH, K. L. (1995). Development of a PCR amplification assay as a screening test using bulk milk samples for identifying dairy herds infected with bovine viral diarrhea virus. *Veterinary Microbiology* **44,** 77–92.

RAHN, K. A., DE GRANDIS, A., CLARKE, R. C., MCEWEN, S. A., GALAN, J. E., GINOCCHIO, C., CURTISS, R. & GYLES, C. L. (1992). Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular and Cellular Probes* **6,** 271–9.

RASMUSSEN, H. N., RASMUSSEN, O. F., ANDERSEN, J. K. & OLSEN, J. E. (1994). Specific detection of pathogenic *Yersinia enterocolitica* by two-step PCR using hot-start and DMSO. *Molecular and Cellular Probes* **8,** 99–108.

RASMUSSEN, H. N., RASMUSSEN, O. F., CHRISTENSEN, H. & OLSEN, J. E. (1995). Detection of *Yersinia enterocolitica* O:3 in faecal samples and tonsil swabs from pigs using IMS and PCR. *Journal of Applied Bacteriology* **78,** 563–8.

REIF, T. C., JOHNS, M., PILLAI, S. & CARL, M. (1994). Identification of capsule-forming *Bacillus anthracis* spores with the PCR and a novel dual probe hybridization format. *Applied and Environmental Microbiology* **60,** 1622–5.

REUBEL, G. H., RAMOS, R. A., HICKMAN, M. A., RIMSTAD, E., HOFFMANN, D. E. & PEDERSEN, N. C. (1993). Detection of active and latent feline herpesvirus 1 infections using the polymerase chain reaction. *Archives of Virology* **132,** 409–20.

RIDGE, S. E., HARKIN, J. T., BADMAN, R. T., MELLOR, A. M. & LARSEN, J. W. A. (1995). Johne's disease in alpacas (*Lama pacos*) in Australia. *Australian Veterinary Journal* **72,** 150–3.

RIMSTAD, G. H. & UELAND, K. (1992). Detection of feline immunodeficiency virus by a nested polymerase chain reaction. *Journal of Virological Methods* **36,** 239–48.

RISHI, A. K. & MCMANUS, D. P. (1987). Genomic cloning of human *Echinococcus granulosus* DNA isolation of recombinant plasmids and their use as genetic markers in strain characterization. *Parasitology* **94,** 369–83.

RISHI, A. K. & MCMANUS, D. P. (1988). Molecular cloning of *Taenia solium* genomic DNA and characterization of taeniid cestodes by DNA analysis. *Parasitology* **97,** 161–76.

RODRIGUEZ, A., MARTINEZ-SALAS, E., DOPAZO, J., DAVILA, M., SAIZ, J. C. & SOBRINO, F. (1992). Primer design for specific diagnosis by PCR of highly variable RNA viruses: typing of foot-and-mouth disease virus. *Virology* **189,** 363–7.

ROSA, P. A. & SCHWAN, T. G. (1989). A specific and sensitive assay for the Lyme disease spirochete *Borrelia burgdorferi* using the polymerase chain reaction. *Journal of Infectious Diseases* **160,** 1018–29.

ROZE, M. (1995). Polymerase chain reaction: a revolution in diagnosis of ocular leishmaniosis? *Veterinary Quarterly* **17,** 547–8.

SACRAMENTO, D., BOURHY, H. & TORDO, N. (1991). PCR technique as an alternative method for diagnosis and molecular epidemiology of rabies virus. *Molecular and Cellular Probes* **5,** 229–40.

SAIKI, R. K., SCHARF, S., FALOONA, F., MULLIS, K. B., HORN, G. T., ERLICH, H. A. & ARNHEIM, N. (1985). Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230,** 1350–4.

SAIKI, R. K., GELFAND, D. H., STOFFEL, S., SCHARF, S., HIGUCHI, R., HORN, G. T., MULLIS, K. B. & ERLICH, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239,** 487–91.

SAITO, M., MATSUMOTO, M. & FUNABASHI, M. (1992). Detection of *Clostridium perfringens* enterotoxin gene by the polymerase chain reaction amplification procedure. *International Journal of Food Microbiology* **17,** 47–55.

SARID, R., CHAJUT, A., MALKINSON, M., TRONICK, S. R., GAZIT, A. & YANIV, A. (1994). Diagnostic test for lymphoproliferative disease virus infection of turkeys, using the polymerase chain reaction. *American Journal of Veterinary Research* **55,** 769–72.

SAURNIER, P., BOURNEUX, C., PREVOST, G. & ANDREMONT, A. (1993). RAPD assay is less discriminant than pulse-field gel electrophoresis for typing strains of methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology* **31,** 982–5.

SAYADA, C., ANDERSEN, A., RODRIGUEZ, P., EB, F., MILON, A., ELION, J. & DENAMUR, E. (1994). Homogeneity of the major outer membrane protein gene of feline *Chlamydia psittaci*. *Research in Veterinary Science* **56,** 116–8.

SCHORR, E., WENTWORTH, D. & HINSHAW, V. S. (1994). Use of polymerase chain reaction to detect swine influenza virus in nasal swab specimens. *American Journal of Veterinary Research* **55,** 952–6.

SCHUNCK, B. & RZIHA, H. J. (1994). Detection of foodborne viruses by the polymerase chain reaction. *Revue du Médicine Vétérinaire* **145,** 215–6.

SENDA, M., PARRISH, C. R., HARASAWA, R., GAMOH, K., MURAMATSU, M., HIRAYAMA, N. & ITHO, O. (1995). Detection by PCR of wild-type canine parvovirus which contaminates dog vaccines. *Journal of Clinical Microbiology* **33,** 110–3.

SHANKAR, V., DIETZSCHOLD, B. & KOPROWSKI, H. (1991). Direct entry of rabies virus into the central nervous system without prior local replication. *Journal of Virology* **65,** 2736–8.

SHARMA, P. C., CULLINANE, A. A., ONIONS, D. E. & NICOLSON, L. (1992). Diagnosis of equid herpesvirus 1 and 4 by polymerase chain reaction. *Equine Veterinary Journal* **24,** 20–5.

SHERMAN, M., EHRLICH, G. D., FERRER, J. F., SNINSKY, J. J., ZANDOMENI, R., DOCK, N. L. & POIESZ, B. (1992). Amplification and analysis of specific DNA and RNA sequences of bovine leukemia virus from infected cows by polymerase chain reaction. *Journal of Clinical Microbiology* **30,** 185–91.

SILES-LUCAS, M., FELLEISEN, L., CUESTA-BANDERA, C., GOTTSTEIN, B. & ECKERT, J. (1994). Comparative genetic analysis of Swiss and Spanish isolates of *Echinococcus granulosus* by Southern hybridization and randomly amplified polymorphic DNA technique. *Applied Parasitology* **35,** 107–17.

SILVA, R. F. (1992). Differentiation of pathogenic and non-pathogenic serotype 1 Marek's disease viruses (MDVs) by the polymerase chain reaction amplification of the tandem direct repeats within the MDV genome. *Avian Diseases* **36,** 521–8.

SMITH, H. R., SCOTLAND, S. M., WILLSHAW, G. A., WRAY, C., MCLAREN, I. M., CHEASTY, T. & ROWE, B. (1988). Verocytotoxin production and presence of VT genes in *Escherichia coli* strains of animal origin. *Journal of General Microbiology* **134,** 829–34.

SMYTH, A. J., GHOSH, A., HASSAN, M. Q., BASSU, D., DE BRUIJN, S., ADHYA, S., MALLICK, K. K. & BARKER, D. C. (1992). Rapid and sensitive detection of *Leishmania* kenetoplast DNA from spleen and blood samples of kala-azar patients. *Parasitology* **105,** 183–92.

SOLANO, P., ARGIRO, L., REIFENBERG, J. M. & DUVALLET, G. (1994). Utilisation de l'hybridisation moléculaire et de l'amplification en chaîne par polymérase pour la charactérisation des trypanosomes et l'epidemiologie des trypanosomes en Afrique de l'Quest. *Veterinary Research* **25,** 588–9.

STANLEY, J., LINTON, D., BURNENS, A. P., DEWHIRST, F. E., OWEN, R. J., PORTER, A., ON, S. L. W. & COSTAS, M. (1993). *Helicobacter canis*, sp. nov, a new species from dogs: an integrated study of phenotype and genotype. *Journal of General Microbiology* **139,** 2495–2504.

STASKUS, K. A., COUCH, L., BITTERMAN, P., RETZEL, E. F., ZUPANCIC, M., LIST, J. & HAASE, A. T. (1991). In situ amplification of visna virus DNA in tissue sections reveals a reservoir of latently infected cells. *Microbial Pathogenesis* **11,** 67–76.

STEIGER, Y., ACKERMANN, M., METTRAUX, C. & KIHM, U. (1992). Rapid and biologically safe diagnosis of african swine fever by using polymerase chain reaction. *Journal of Clinical Microbiology* **30,** 1–8.

STEMKE, G. W., PHAN, R., YOUNG, T. F. & ROSS, R. F. (1994). Differentiation of *Mycoplasma hyopneumoniae*, *Mycoplasma flocculare*, and *Mycoplasma hyorhinis* on the basis of amplification of a 16S rRNA gene sequence. *American Journal of Veterinary Research* **55,** 81–4.

STETTLER, M. & ZURBRIGGEN, A. (1995). Nucleotide and deduced amino acid sequence of the nucleocapsid protein of the virulent A75/17-CDV strain of canine distemper virus. *Veterinary Microbiology* **44,** 211–7.

STONE-MARSCHAT, M., CARVILLE, A., SKOWRONEK, A. & LAEGREID, W. W. (1994). Detection of African horse sickness virus by reverse transcription-PCR. *Journal of Clinical Microbiology* **32,** 697–700.

STRAM, Y., YADIN, H., CHAI, D., MOLAD, T., GELMAN, B., LAOR, O. & BECKER, Y. (1993). Identification of foot and mouth disease virus (FMDV) serotypes using the polymerase chain reaction. *Israel Journal of Veterinary Medicine* **48,** 57–60.

STRAM, Y., MOLAD, T., CHAI, D., GELMAN, B. & YADIN, H. (1995). Detection and subtyping of foot-and-mouth disease virus in infected cattle by polymerase chain reaction and amplified VP1 sequencing. *Journal of Veterinary Diagnostic Investigation* **7,** 52–55.

SUAREZ, D. L., VAN DER MAATEN, M. J. & WHETSTONE, C. A. (1995). Improved early and long-term detection of bovine lentivirus by a nested polymerase chain reaction test in experimentally infected calves. *American Journal of Veterinary Research* **56,** 579–86.

SZABO, E. A., PEMBERTON, J. M. & DESMARCHELIER, P. M. (1992). Specific detection of *Clostridium botulinum* type B by using the polymerase chain reaction. *Applied and Environmental Microbiology* **58,** 418–20.

SZABO, E. A., PEMBERTON, J. M. & DESMARCHELIER, P. M. (1993). Detection of the genes encoding botulinum neurotoxin types A to E by the polymerase chain reaction. *Applied and Environmental Microbiology* **59,** 3011–20.

TAJIMA, M., KIRISAWA, R., TAGUCHI, M., IWAI, H., KAWAKAMI, Y., HAGIWARA, K., OHTSUKA, H., SENTSUI, H. & TAKAHASHI, K. (1995). Attempt to discriminate between bovine viral diarrhea virus strains using polymerase chain reaction. *Journal of Veterinary Medicine B* **42,** 257–65.

TRUYEN, U., PLATZER, G., PARRISH, C. R., HANICHEN, T., HERMANNS, W. & KAADEN, O. R. (1994). Detection of canine parvovirus DNA in paraffin-embedded tissues by polymerase chain reaction. *Journal of Veterinary Medicine* **41,** 148–52.

TURNBULL, P. C. B., HUSTON, R. A., WARD, M. J., JONES, M. N., QUINN, C. P., FINNIE, N. J., DUGGLEBY, C. J., KRAMER, J. M. & MELLING, J. (1992). *Bacillus anthracis* but not always anthrax. *Journal of Applied Bacteriology* **72,** 21–8.

TYLER, S. D., JOHNSON, W. M., LIOR, H., WANG, G. & ROZEE, K. R. (1991). Identification of verotoxin type 2 variant B subunit genes in *Escherichia coli* by the polymerase chain reaction and restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology* **29,** 1339–43.

UWATOKO, K., SUNAIRI, M., NAKAJIMA, M. & YAMAURA, K. (1995). Rapid method utilizing polymerase chain reaction for detection of canine parvovirus in feces of diarrheic dogs. *Veterinary Microbiology* **43,** 315–23.

VAN BELKUM, A., BAX, R., PEERBOOMS, P., GOESSENS, W., VAN LEEUWEN, N. & QUINT, W. G. V. (1993). Comparison of phage typing and DNA fingerprinting by PCR for discrimination of methicillin-resistant *Staphylococcus aureus* strains. *Journal of Clinical Microbiology* **31,** 798–803.

VAN EYS, G. J. J. M., GRAVEKAMP, C., GERRITSEN, M. J., QUINT, W., CORNELISSEN, M. T. E., TER SCHEGGET, J. & TERPSTRA, W. J. (1989). Detection of leptospires in urine by polymerase chain reaction. *Journal of Clinical Microbiology* **27,** 2258–62.

VAN EYS, G. J. J. M., SCHOONE, G. J., KROON, N. C. M. & EBELLING, S. B. (1992). Sequence analysis of small subunit ribosomal RNA genes and its use for detection and identification of *Leishmania* parasites. *Molecular and Biochemical Parasitology* **51,** 133–42.

VAN WOENSEL, P. A. M., VAN BLAADEREN, A., MOORMANN, R. J. M. & DE BOER, G. F. (1992). Detection of proviral DNA and viral RNA in various tissues early after avian leukosis infection. *Leukemia* **6,** 135S–7S.

VENUGOPAL, K., SHIU, S. Y. W. & GOULD, E. A. (1994). Recombinant vaccinia virus expressing PrM and E glycoproteins of louping-ill virus: induction of partial homologous and heterologous protection in mice. *Research in Veterinary Science* **57,** 188–93.

VERBEEK, A. & TIJSSEN, P. (1990). Polymerase chain reaction for probe synthesis and for direct amplification in detection of bovine coronavirus. *Journal of Virological Methods* **29,** 243–56.

VILCEK, S. (1993). Detection of bovine herpesvirus-1 (BHV-1) genome by PCR. *Journal of Virological Methods* **41,** 245–8.

VISSER, I. K. G., KUMAREV, V. P., ORVELL, C., VRIES, P. D., BROEDERS, H. W. J., BILDT, M. W. G. V., GROEN, J. & TEPPEMA, J. S. (1990). Comparison of two morbilliviruses isolated from seals during outbreaks of distemper in North West Europe and Siberia. *Archives of Virology* **111,** 149–64.

VOLZ, D. M., LAGER, K. M. & MENGELING, W. L. (1992). Latency of a thymidine kinase-negative pseudorabies vaccine virus detected by the polymerase chain reaction. *Archives of Virology* **122,** 341–8.

WADE-EVANS, A. M., MERTENS, P. P. & BOSTOCK, C. J. (1991). Development of the polymerase chain reaction for the detection of bluetongue virus in tissue samples. *Journal of Virological Methods* **30,** 15–24.

WAGNER, W. N., BOGDAN, J., HAINES, D., TOWNSEND, H. G. G. & MISRA, V. (1992). Detection of equine herpesvirus and differentiation of equine herpesvirus type 1 and type 4 by the polymerase chain reaction. *Canadian Journal of Microbiology* **38,** 1193–6.

WAMWAYI, H. M., FLEMING, M. & BARRETT, T. (1995). Characterisation of African isolates of rindepest virus. *Veterinary Microbiology* **44,** 151–63.

WARD, P. & MISRA, V. (1991). Detection of bovine viral diarrhea virus using degenerate oligonucleotides primers and the polymerase chain reaction. *American Journal of Veterinary Research* **52,** 1231–6.

WARDS, B. J., COLLINS, D. M. & DE LISLE, G. W. (1995). Detection of *Mycobacterium bovis* in tissues by polymerase chain reaction. *Veterinary Microbiology* **43,** 227–40.

WASTLING, J. M., NICOLLS, S. & BUXTON, D. (1993). Comparation of two gene amplification methods for the detection of *Toxoplasma gondii* in experimentally infected sheep. *Journal of Medical Microbiology* **38,** 360–5.

WAY, J. J., JOSEPHSON, K. L., PILLAI, S. D., ABBASZADEGAN, M., GERBA, C. P. & PEPPER, I. L. (1993). Specific detection of *Salmonella* spp. by multiplex polymerase chain reaction. *Applied and Environmental Microbiology* **59,** 1473–9.

WEISS, J. B. (1995). DNA probes and PCR for diagnosis of parasitic infections. *Clinical Microbiology Reviews* **8,** 113–30.

WHEELER, J. G. & OSORIO, F. A. (1991). Investigation of sites of pseudorabies virus latency, using polymerase chain reaction. *American Journal of Veterinary Research* **52,** 1799–1803.

WIDJOJOATMODJO, M. N., FLUIT, A. C., TORENSMA, R., VERDONK, G. P. H. T. & VERHOEF, J. (1992). The magnetic immuno-polymerase chain reaction assay for direct detection of salmonellae in fecal samples. *Journal of Clinical Microbiology* **30,** 3195–9.

WIEDMAN, M., BRANDON, R., WAGNER, P., DUBOVI, E. & BATT, C. A. (1993). Detection of bovine herpesvirus-1 in bovine semen by a nested PCR. *Journal of Virological Methods* **44,** 129–40.

WILLEMS, H., THIELE, D., FROLICH-RITTER, R. & KRAUSS, H. (1994). Detection of *Coxiella burnetti* in cow's milk using the polymerase chain reaction. *Journal of Veterinary Medicine B* **41,** 580–7.

WILSON W. C. & CHASE, C. C. (1993). Nested multiplex polymerase chain reactions for the identification of bluetongue virus infection in the biting midge *Culicoides variipennis*. *Journal of Virological Methods* **45,** 39–47.

WIRZ, B., TRATSCHIN, J., MULLER, H. K. & MITCHELL, D. B. (1993). Detection of hog cholera virus and differentiation from other pestiviruses by polymerase chain reaction. *Journal of Clinical Microbiology* **31,** 1148–54.

WOOD, M. W., MAHON, J. & LAX, A. J. (1994). Development of a probe and PCR primers specific to the virulence plasmid of *Salmonella enteritidis*. *Molecular and Cellular Probes* **8,** 473–9.

WOODWARD, M. J., CARROLL, P. J. & WRAY, C. (1992). Detection of entero- and verocyto-toxin genes in *Esch-*

erichia coli from diarrhoeal disease in animals using the polymerase chain reaction. *Veterinary Microbiology* **31,** 251–61.

Wu, C. C. & Lin, T. L. (1992). Detection of infectious bursal disease virus in digested formalin-fixed paraffin embedded tissue sections by polymerase chain reaction. *Journal of Veterinary Diagnostic Investigation* **4,** 452–5.

Wu, C. C., Lin, T. L., Zhang, H. G., Davis, V. S. & Boyle, J. A. (1992). Molecular detection of infectious bursal disease virus by polymerase chain reaction. *Avian Diseases* **36,** 221–6.

Xia, J. Q., Yason, C. V. & Kibenge, F. S. B. (1995). Comparison of dot-blot hybridization, polymerase chain reaction, and virus isolation for detection of bovine herpesvirus-1 (BHV-1) in artificially-infected bovine semen. *Canadian Journal of Veterinary Research* **59,** 102–9.

Xu, L., Harbour, D. & McCrae, M. A. (1990). The application of polymerase chain reaction to the detection of rotaviruses in faeces. *Journal of Virological Methods* **27,** 29–38.

Yason, C. V., Harris, L. M., McKenna, P. K., Wadowska, D. & Kibenge, F. S. B. (1995). Establishment of conditions for the detection of bovine herpesvirus-1 by polymerase chain reaction using primers in the thymidine kinase region. *Canadian Journal of Veterinary Research* **59,** 94–101.

Yule, A. (1994). Amplification-based diagnostics target TB. *Biotechnology* **12,** 1335–7.

Zanoni, R. G., Nauta, I. M., Kuhnert, P., Pauli, U., Pohl, B. & Peterhans, E. (1992). Genomic heterogeneity of small ruminant lentiviruses detected by PCR. *Veterinary Microbiology* **33,** 241–51.

Zerbes, M., Tannock, G. A., Jenner, R. J. & Young, P. L. (1994). Some characteristics of a recent virus isolate of Marek's disease virus. *Australian Veterinary Journal* **71,** 21–2.

Zhu, G. S., Ojima, T., Hirowaka, T., Ihara, T., Mizukoshi, N., Kato, A., Ueda, S. & Hirai, K. (1992). Differentiation of oncogenic and nononcogenic strains of Marek's disease virus type 1 by using polymerase chain reaction DNA amplification. *Avian Diseases* **36,** 637–45.

Zientara, S., Sailleau, C., Moulay, S. & Cruciere, C. (1993). Diagnosis of the African horse sickness virus serotype 4 by a one-tube, one manipulation RT-PCR reaction from infected organs. *Journal of Virological Methods* **46,** 133–43.

Zingg, B. C. & Lefebvre, R. B. (1994). Polymerase chain reaction for detection of *Borrelia coriaceae*, putative agent of epizootic bovine abortion. *American Journal of Veterinary Research* **55,** 1509–15.

Zwaagstra, K. A., Van Der Zeijst, B. A. M. & Kusters, J. G. (1992). Rapid detection and identification of avian infectious bronchitis virus. *Journal of Clinical Microbiology* **30,** 79–84.

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