

*Full Length Research Paper*

## Diagnostic approaches for tick-borne haemoparasitic diseases in livestock

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Received 3 November, 2014; Accepted 17 December, 2014

Tick-borne diseases (TBDs) are a major economic constraint to livestock production affecting the productivity of livestock worldwide. Identification of these haemoprotozoan and rickettsial infections is essential in understanding the epidemiology and it is important to distinguish between species and subspecies involved. Conventional techniques including serological and microscopic examinations do not always meet these requirements. Clinical diagnostic and surveillance tools, such as the complement fixation test (CFT), the indirect fluorescent antibody test (IFAT) and the enzyme linked immunosorbent assay (ELISA) have been successfully used over decades. In addition, DNA-based tests for diagnosis, differentiation and characterisation of different haemoparasites have been developed. Molecular diagnostic techniques, such as DNA hybridization and polymerase chain reaction (PCR), allow detection of parasites in blood, tissue or ticks with high levels of sensitivity, specificity and reliability. In addition, some techniques can identify multiple pathogens in the same samples. Furthermore, these techniques can also be exploited to identify unambiguous species and subspecies. Under the precondition that these tests are correctly designed and validated, they provide a powerful tool for epidemiology, with greater advantages of affordability and amenability to standardization. The implementation of these techniques for studying TBDs worldwide will be invaluable. Thus, the aim of this study is to put together the details of the techniques in the form of small review consultation of the practitioners and researchers.

**Key words:** Diagnosis, livestock, molecular, haemoparasites, tick-borne diseases.

### INTRODUCTION

'Diagnosis' is an art of knowing about the cause of a particular disease (Dia = through, gnosis = knowledge). Diagnosis of parasitic infections has changed remarkably as technical skills and knowledge have expanded. Ticks rank first as arthropod vectors of protozoa, rickettsiae, bacteria and viruses in nonhuman vertebrates and rank

second only to mosquitoes as vectors of pathogens to humans (Zhou et al., 2009). Though tick borne diseases (TBDs) differ among ecological regions, their impact on animal production is more important in tropical and subtropical regions of the world as they pose major threat to the health and management of livestock in these regions

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(Jongejan and Uilenberg, 1994). These diseases are ranked very high in terms of their impact on the livelihood of resource poor farming communities in developing countries (Perry et al., 2002; Minjaw and McLeod, 2003; Bishop et al., 2008). The most important tick-borne haemoparasitic disease in veterinary medicine include the protozoan parasite of *Theileria* species (e.g. *Theileria annulata*, *Theileria parva*, *Theileria lestoquardi*), *Babesia* species (e.g. *Babesia bovis*, *Babesia bigemina*, *Babesia ovis*) and Rickettsial species (e.g. *Cowdria ruminantium*, *Anaplasma marginale*, *Anaplasma centrale*) (Uilenberg, 2006). In addition, several novel *Babesia* and *Theileria* (*Theileria uilenbergi* and *Theileria luwenshuni*) species were isolated from naturally infected sheep in China, where it cause severe and often lethal disease (Yin et al., 2007; Guan et al., 2010).

The direct impacts of these tick-borne pathogens are reflected in reduction of production, loss of weight and death of substantial proportion of the affected animals. By reducing the losses due to tick-borne diseases there would be a better chance to increase livestock production. The effective management of tick-borne haemoparasitic diseases requires rapid, reliable and highly sensitive diagnostic tests, which can also serve to monitor the effectiveness of the therapeutic and prophylactic measures. The common conventional method (history, blood or tissue smears examination, clinical signs, postmortem lesions and conventional serology) provide the basic needs for diagnosis, but have the disadvantage of sensitivity, specificity, involvement of expertise, labour intensiveness, etc., so do not meet complete requirements. The serodiagnosis is not different between current and past infection as the animal may already have cleared the pathogen, but remain seropositive. Nucleic acid based diagnostics, particularly, a wide range of DNA based techniques have been developed and validated for identification, characterization and pathogenic studies for various pathogens. The polymerase chain reaction (PCR) based assays permit identification of parasite at levels far below the detection limit of the commonly used parasitological techniques. Several techniques have been developed separately for detection for each species tick-borne haemoprotozoa (Figueroa and Buening, 1995) (Table 1). In this review, a detailed discussion on diagnostic procedures from classical to molecular approaches including clinical, parasitological, serological and molecular techniques is made.

## CONVENTIONAL DIAGNOSTIC TECHNIQUES

The microscopic techniques for diagnosis of tick-borne diseases are still considered as the “gold standard” technique. Microscopic examination shows *Theileria* schizonts in the lymph node smears and piroplasms alone or along with schizonts in blood smears (Figure 1).

However, the detection of piroplasms in blood smears in the absence of clinical assessment and lymph node biopsy are difficult to interpret, since piroplasms of *T. annulata*, *T. parva*, *Theileria mutans* and *T. lestoquardi* can be found in the blood smears (Norval et al., 1992) which are difficult to differentiate based on the morphology. It is also quite difficult, if not impossible, to demonstrate parasites in carrier animals as the numbers of parasites in such animals fall below detectable levels soon after the acute stages of the disease (de Waal, 2012).

Babesiosis is diagnosed by examination of blood or organ smears stained with Romanowsky stain (Callow et al., 1993; Bose et al., 1995). The direct method involves identifying the parasite in the stained blood smears; however, this technique shows a low sensitivity in subclinical and chronic phase of the infection (Terkawi et al., 2011). Blood film examination requires very much expertise to differentiate between *Babesia* species from one or more animal species which look similar under stained preparation (Figure 2). Quantitative buffy coat (QBC), an acridine orange based improved technique, which concentrates and stains parasitized blood in one step (Levine et al., 1989) which may prove more valuable in low parasitaemic cases (Figure 3).

The most commonly used method for diagnosis of *Anaplasma* infection is the microscopic examination of Giemsa stained thin blood smears especially in clinical acute form of disease (Figure 4). The sensitivity of this method is  $10^6$  infected erythrocytes per milliliter of blood (Gale et al., 1996), but due to the low parasitemia in carrier cattle and difficulty to differentiate *Anaplasma* from other structures even by an experienced hand, this method is not recommended for the characterization of persistently infected cattle (Carelli et al., 2007). Sub-inoculation of *A. marginale* infected erythrocytes into susceptible splenectomized calves has been considered as the ‘gold standard’ for detection of such cattle, but it is not practical for routine testing (Luther et al., 1980).

Owing to the rapid development of heartwater disease (Cowdriosis), the frequent lack of characteristic clinical signs, and sometimes even a total absence of lesions, the diagnosis of the disease in live animal is difficult (Camus et al., 1996). Tentative diagnosis relies on some clinical, epidemiological and macroscopic appearance of some organs. Differential diagnosis should consider diseases that cause central nervous system “CNS” manifestations. In dead animals, tentative diagnosis could be made by the presence of transudates in the pericardium and thorax at postmortem, but definitive diagnosis requires the demonstration of *C. ruminantium* in brain crush smears. This has been established much earlier and by the application of specific serological and molecular techniques in live animals.

The conventional microscopy for diagnosis is simple and does not require the purchase and maintenance of expensive equipments. However, limitations of conventional microscopy method include: (i) it is extremely

**Table 1.** Developmental progression of some of the molecular techniques commonly used in the diagnosis of tick-borne haematozoa.

Assay	Haematozoa	Reference
ELISA	<i>T. annulata</i> ; <i>T. parva</i> ; <i>C. ruminantium</i>	Kachani et al.(1996), Boulter et al. (1998), Ilhan et al. (1998), Bakheit et al. (2004), Renneker et al. (2008), Gray et al. (1980), Mboloi et al. (1999)
PCR	<i>T. annulata</i> , <i>T. parva</i> ; <i>B. bovis</i> , <i>B. bigemina</i>	d'Oliveria et al.(1995), Bishop et al. (1993), Calder et al. (1996), Figueroa et al. (1992)
Multiplex PCR	<i>T. evansi</i> , <i>B. bigemina</i>	Figueroa et al. (1993),
Real-time PCR	<i>T. parva</i> , <i>A. marginale</i> , <i>Ehrlichia risticii</i> , <i>T. evansi</i>	Chaisi et al. (2013), Schotthoefler et al. (2013), Pusterla et al. (2000), Sharma et al. (2012)
DNA probe	<i>A. marginale</i> , <i>A. centrale</i>	Visser and Ambrosio (1987)
RLB	<i>Theileria</i> spp., <i>Babesia</i> spp., <i>Anaplasma</i> , <i>Ehrlichia</i>	Gubbels et al. (1999), Cornelis et al. (2002)
LAMP	<i>T. annulata</i> , <i>T. parva</i> , <i>T. lestoquardi</i> , <i>Babesia canis</i>	Salih et al. (2008), Lui et al. (2012), Thekisoe et al. (2010), Salih et al. (2012), Muller et al. (2010)

labour intensive particularly when a large number of samples are to be examined in a short period; (ii) trained technicians are required for accurate diagnosis; (iii) not of much use in cases, when haemoparasites are morphologically similar or too small to be missed or infection is very low; (iv) in some cases culture of the parasite is needed, which may require specialized media, which is again time consuming.

## INDIRECT DIAGNOSTIC METHODS

When parasites occur at densities below the sensitivity of direct method employed or cannot be directly demonstrated in a biological sample due to the life cycle in the host, in those cases indirect methods of diagnosis are used, which include serological tests either used for detection of antibodies or antigens. Among the various serological tests, most important once include complement fixation test (CFT), indirect fluorescent antibody technique (IFAT) and enzyme-linked immunosorbent assay (ELISA).

### CFT

CFT has been used for diagnosis of *Babesia*, *Theileria*,

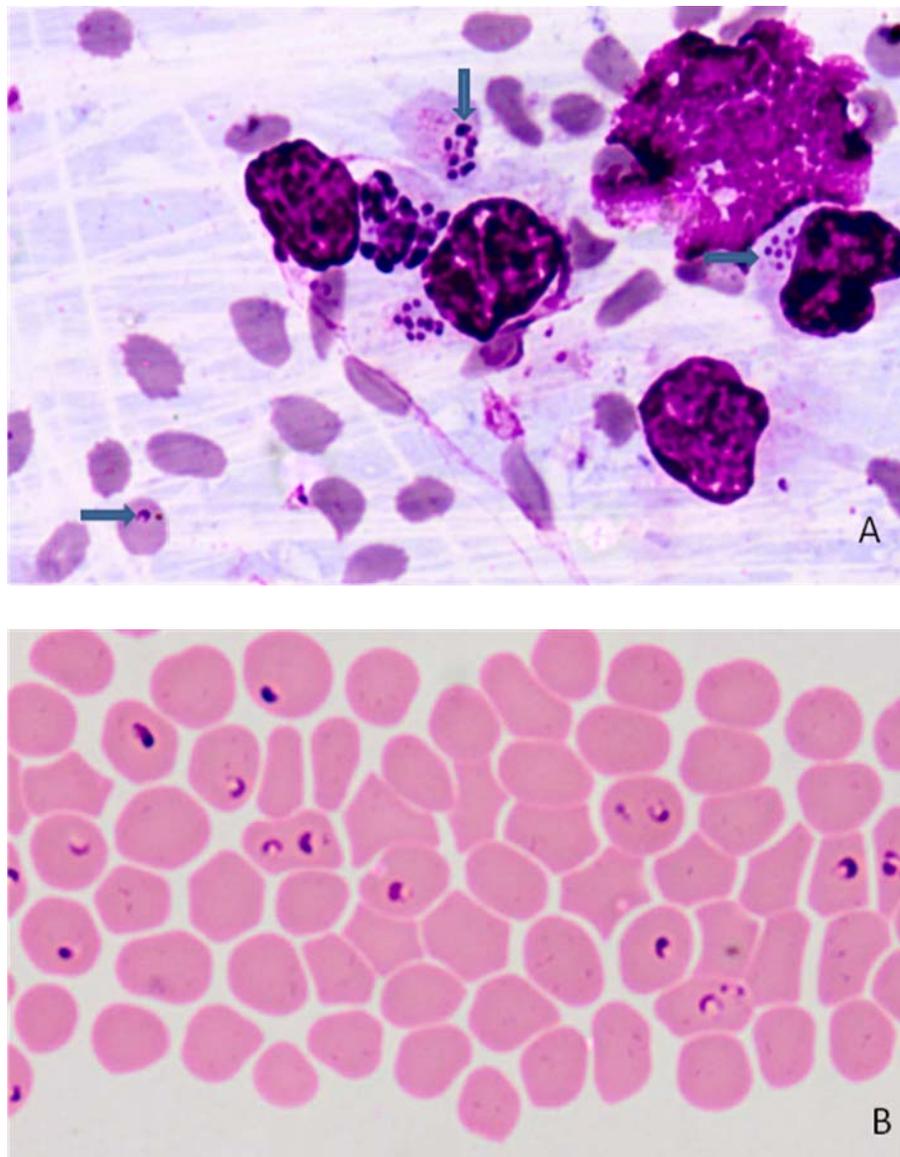
*Toxoplasma*, *Trypanosoma*, etc (Herr et al., 1985; Bose et al., 1995). Based on this test, commercial kit (COFEB Kit) has been developed for diagnosis of equine piroplasmosis (Sengupta, 2001).

### IFAT

Indirect fluorescent antibody technique has been used since long for the diagnosis of parasites like *Babesia* spp. (Morzaria et al., 1977; Anderson et al., 1980), *Theileria* (Morzaria et al., 1977; Darghouth et al., 2004). IFA test has also been recommended by OIE as one of the diagnostic test for theileriosis.

### ELISA

ELISA is increasingly being used for detection of parasite-specific antibodies, antigens and immune complexes (Kachani et al., 1992). ELISA based on schizont antigen functioned well in the case that cellular fraction was enriched from the soluble fraction (Manuja et al., 2000). On the other hand, ELISA for the diagnosis of *Theileria* spp. infection in sheep using piroplasm antigen obtained from experimentally infected sheep with parasitemia reaching 30% was developed (Gao et al., 2002).

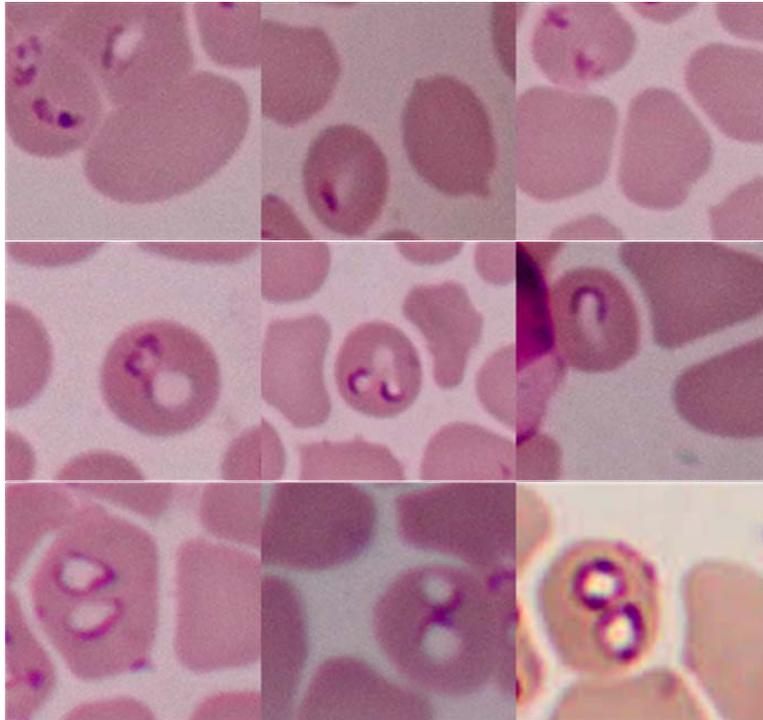


**Figure 1.** Photomicrograph of GSTBS revealing (A) Schizonts and piroplasm of *Theileria* spp., (B) Pleomorphic piroplasms of *Theileria* spp.

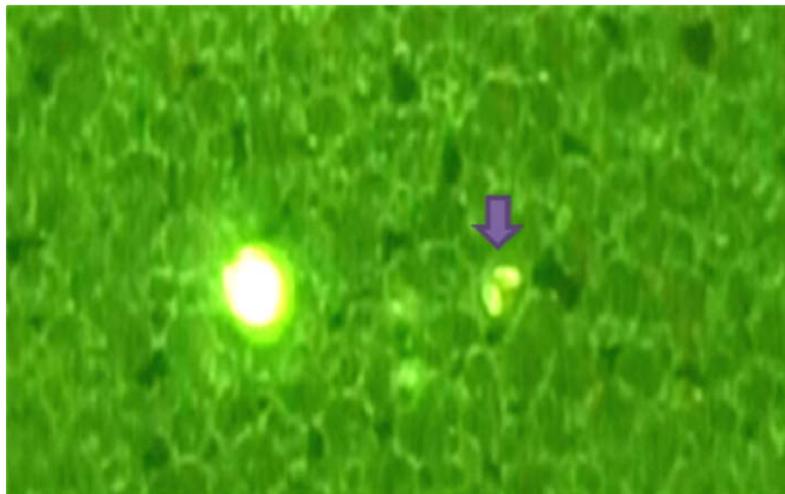
However, cross-reaction was found with *B. ovis*. This result may be questionable, because it could not be excluded that these sheep were already infected with both *Theileria* spp. and *B. ovis*.

In spite of relatively good performances of crude antigens, the disadvantages of this approach include the requirement for experimental animals for piroplasm antigen production and the batch-to-batch variation, beside the need to standardize protocols to obtain antigen from crude parasite material (Gubbels, 2000). These problems have been circumvented by use of several recombinant parasite antigens in ELISA. Two ELISAs based on recombinant proteins have been developed. Firstly, the sporozoite antigen (SPAG-1) has been

demonstrated to detect exposure to *T. annulata*, but sensitivity and specificity of this ELISA has not been evaluated (Boulter et al., 1998). Secondly, a merozoite surface antigen, Tams-1 has been tested as a candidate antigen for a diagnostic ELISA (Ilhan et al., 1998). Moreover, an ELISA for detection of *T. annulata* infection was established and validated and applied for epidemiological studies in the field (Schnittger et al., 2002; Bakheit et al., 2004; Salih et al., 2005, 2007; Seitzer et al., 2007). An advance in serological diagnosis was achieved with the development of a competitive ELISA applying the TaSP antigen and using a monoclonal antibody (1C7) that was found to bind to TaSP antigen (Renneker et al., 2008). ELISA is widely used as the basis for epidemiological surveys



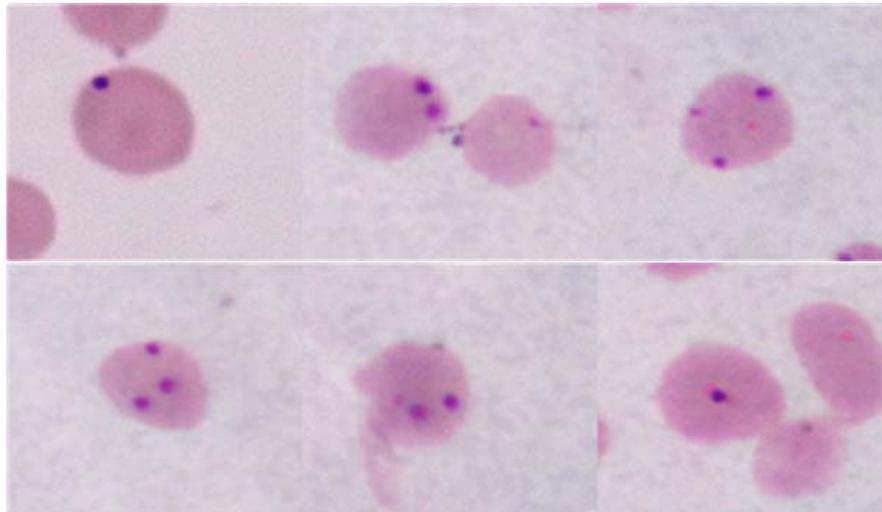
**Figure 2.** Photomicrograph of different shapes of *Babesia* spp. difficult to differentiate based on morphology.



**Figure 3.** Photomicrograph of *Babesia* spp. in acridine orange stained blood smear showing pyriform bodies (arrow).

(Passos et al., 1998) and for evaluation of vaccination programme (Guglielmo et al., 1997). Serological tests have the disadvantage of relying on the presence of specific antibodies, which may take days or weeks to develop in an infected animal or may persist for months after the infection has been cleared (Mosqueda et al., 2012).

Mboloi et al. (1999) investigated the major antigenic protein 1 fragment B (MAP1-B) in ELISA for the diagnosis of *C. ruminantium* infection. They concluded that the MAP1-B ELISA is a useful test for the diagnosis of *C. ruminantium* infection in small ruminants. However, this test is known to be less sensitive in cattle than in small



**Figure 4.** Photomicrograph of Giemsa stained blood smear showing *Anaplasma marginale*.

ruminant (Knopf et al., 2002).

## NUCLEIC ACID-BASED TECHNIQUES

Molecular nucleic acid-based diagnostic techniques have been developed for a number of haemoparasites. They have been proved to be sensitive, easy to use, can analyze large number of samples and can detect the parasites directly in clinical and environmental samples without culture (Weiss, 1995; Dey and Singh, 2009). The first introduced diagnostics was nucleic acid hybridization technique, however, in the recent years, PCR and allied techniques along with genomic sequencing have over taken it, and have become a driving force for the development of rapid, sensitive and specific assays capable of genomic detection. They are widely used because of being highly sensitive and can be performed rapidly in a cost effective manner. Various molecular diagnostic techniques that have been developed for diagnosis of parasites include conventional PCR, RAPD-PCR, RFLP-PCR, multiplex-PCR, real-time PCR, reverse transcriptase PCR, PCR-ELISA, micro-arrays, loop-mediated isothermal amplification (LAMP), etc.

## PCR

Many authors discussed the use of molecular tools for the study of *Theileria* parasites, which affect ruminants, mostly bovines. Tanaka et al. (1993) utilized a probe derived from a gene encoding a 32 kDa intra-erythrocytic piroplasm surface protein of *Theileria sergenti* (*Theileria orientalis*). Their method was sufficiently sensitive to detect four parasites per microlitre of blood with a 10  $\mu$ l sample. Detection of *T. annulata* in blood samples of

carrier cattle using polymerase chain reaction (PCR) was reported (d'Oliveria et al., 1995). The assay employed primers specific for the gene encoding the 30 kDa major merozoite surface antigen of this species. This PCR assay was found to be highly specific and sensitive (three parasites per microlitre of blood). Allsop et al. (1993) developed another technique based on PCR using oligonucleotide probes, which detected small subunit ribosomal RNA sequences (srRNA). These probes were efficient in discriminating between six different tick-borne parasites harboured by cattle. Bishop et al. (1993) were able to further increase the accuracy of the identification of isolates or strains by using random amplified polymorphic deoxyribonucleic acid 'DNA' (RAPD). Moreover, several real-time PCR assay has been developed for diagnosis and quantitation of many tick-borne parasites (Dong et al., 2013; Schotthoefer et al., 2013; Bloch et al., 2013).

The sensitivity and specificity of molecular methods is very high and over the years a number of different approaches have been developed to detect *Babesia* spp. in the hosts and vectors. Deoxyribonucleic acid (DNA) probing was the first developed method, which was used to detect babesial DNA from parasitized blood (Buening et al., 1990). Figueroa et al. (1994) set up a PCR-based diagnostic assay to detect *B. bovis* in chronically infected cattle. The target sequence was a gene encoding a 60 kDa merozoite surface protein. The level of sensitivity was high as the PCR product was detected in blood samples containing approximately 20  $\mu$ l of packed cell with a parasitemia of 0.000001%. Nested PCR (nPCR) has been effective for the detection of carrier animals infected with *B. bigemina*; the sensitivity was reported to be as low as one infected erythrocyte in 108 cells (Figueroa et al., 1992). Carson et al. (1994) used RAPD

to reveal markers of species and isolates for *B. bovis* and *B. bigemina*. As mentioned earlier, RLB technique has proved highly sensitive and specific for the detection of these parasites (Gubbels et al., 1999). A hot-start PCR (semi-nested) to detect *B. bovis*, *B. bigemina* based on aspartic proteinase babesipain gene was developed and successfully applied (Martin et al., 2008; Awad et al., 2011). Multiplex PCR (Figuroa et al., 1993), real-time PCR (Buling et al., 2007) and multiplex LAMP (Iseki et al., 2007) are in process of development and validation for identification and characterization of *B. bigemina*.

Probes specific for *A. marginale* and *A. centrale* have been isolated (Visser and Ambrosio, 1987). These probes could detect 127 and 8 ng DNA of *A. centrale* and *A. marginale*, respectively. This probe could also be used on blood from field samples to detect parasitemia in cattle. On the other hand, the *A. marginale* probes described by Goff et al. (1988) could detect 0.01 ng DNA, which is equivalent to a parasitemia of 0.000025% (Eriks et al., 1989). This probe could also detect the presence of the parasite in the infected tick vector. Presently, knowledge about carrier state of anaplasmosis is so difficult to know the epidemiological status in enzootic regions. PCR based on *msp4* gene for *A. marginale* and *Anaplasma ovis* (de la Fuente et al., 2001, 2003) have also been developed. Sequencing of *gltA* and *ompA* genes, identification of *Rickettsia* species based on the sizes of highly variable intergenic spacers, namely, *dksA-xerC*, *mppA-purC*, and *rpmE-tRNA<sup>fMet</sup>* was carried out. Application of multiplex PCR for simultaneous amplification of 3 spacers combined with capillary electrophoresis separation technique is simple, accurate, and high-throughput fragment sizing with considerable time and cost savings (Nakaoa et al., 2013). PCR base tests including PCR ELISA and duplex PCR have been developed and applied successfully with high sensitivity and specificity to differentiate tick borne haematzoan diseases (Galle et al., 1996; Ala and Wayne, 2005; Torina et al., 2008; Ashuma et al., 2013; Sharma et al 2013). Further, nested PCR devised for detection of *A. marginale* in cattle shows increased specificity and sensitivity (Ybanez et al., 2013). Recently, semi-quantitative multiplexed-tandem PCR for the detection and differentiation of four *T. orientalis* genotypes in cattle has been applied (Pereraa et al., 2014).

There are certain reservations to the routine use of such technique. First of all, contamination of the laboratory environment has to be rigorously controlled and this implies numerous controls for quality diagnosis. Suggestions for avoiding contamination have been presented by Altwegg (1995) and Carino and Lee (1995). The first suggestion relates to the organization of the laboratory space so as different stages of diagnosis are carried out in separate areas. The second suggestion relates to the use of Uracil DNA Glycosylase (UDG) for preamplification sterilization of the PCR product (Longo et al., 1990). For the detection of contamination, negative

control must be realized at each step of the PCR preparation (Comes et al., 1995). PCR may also fail due to the inhibition of specific amplification (false negative). Thus, the use of positive control is necessary to increase confidence in negative PCR results. The cost of diagnosis is the second drawback of PCR. Although PCR identification costs are being progressively reduced, they remain higher than those of parasitological and immunological techniques. The third drawback is the absence of quantification. Only limited attempt of using PCR has been conducted to date to estimate the intensity of parasitemia due tick-borne parasitic diseases.

### RAPD-PCR

Random Amplification of Polymorphic DNA-PCR also known as AP-PCR (arbitrary primed PCR), in which primers of arbitrary sequences are used to amplify fragments of the genome. This technique is very simple, fast and does not require either prior knowledge of the DNA sequence or DNA hybridization. This technique has been employed to differentiate species of *Leishmania* and also to study polymorphisms of *Plasmodium*, *Trypanosoma*, etc (Hajjaran et al., 2004).

### PCR-RFLP

PCR-Restriction Fragment Length Polymorphism is used for diagnosis of species and genotypes of parasites. It is performed by digesting the PCR products obtained from parasitic gene amplification, by restriction enzymes or endonucleases. These enzymes cut DNA into fragments of certain sizes, whose analysis on agarose or polyacrylamide gel results in different patterns of fragment sizes, enabling the identification. Zaeemi et al. (2011) were able to differentiate among *T. lestoquardi*, *Theileria ovis*, and *T. annulata* in case of sheep. Recently, semi nested PCR-RFLP was used for detection of persistent anaplasmosis in tick infested cattle (Jaswal et al., 2014).

### Multiplex PCR

Multiplex polymerase chain reaction is a modification of conventional polymerase chain reaction in order to rapidly detect deletions or duplications in a large gene in a single reaction. It is a variant of PCR which offers a significant advantage over single-plex PCR as this two or more target loci from one or more organisms are amplified using mixture of locus-specific primer pairs in a single reaction (Edwards and Gibbs, 1994; Markoulatos et al., 2002). Multiplex PCR had been employed in detection of concurrent infections in field for the detection of haemoprotozoans (Figuroa et al., 1998). Various duplex PCR for the detection of concurrent infections of

economically important haemoprotozoans have also already been standardized in our laboratory with convenient large scale field application (Sharma et al, 2013; Kaur et al., 2012).

### Real-time PCR (RT-PCR)

This technique involves the analysis of genome using fluorogenic probes that release fluorescent signals during amplification. The advantages of real time PCR assay over conventional PCR are that it is relatively rapid and convenient because there is no need to perform gel electrophoresis to visualize the PCR products. Real-time PCR is a simple, fast, closed and automatized amplification system responsible for decreasing the risk of cross contamination. This technique has been used for the detection of a number of parasites including *Leishmania*, *Plasmodium*, *Trypanosoma* (Bell and Ranford-Cartwright, 2002; Gasser, 2006; Sharma et al., 2012) in various regions of the world. Real-time PCR has engendered wider acceptance of PCR due to its improved rapidity, sensitivity, reproducibility and the reduced risk of carryover contamination (Mackay, 2004). Jeong et al. (2003) applied real-time PCR for diagnosis and quantification of *T. sergenti* using specific primer for 33 kDa gene. A pan-*Theileria* FRET-qPCR that can detect all recognized *Theileria* spp. of ruminants in a single reaction has also been developed (Yang et al., 2014).

### PCR-ELISA

By PCR ELISA, the sensitivity of ELISA and specificity of PCR are combined and used for the detection of parasitic genome. The PCR products are hybridized to an immobilized capture probe. The assay thus measures sequences internal to the PCR product and is a less expensive assay and can be an alternative to real time PCR. This ELISA is useful for detecting and differentiating between multiple targets. This technique has been used in detection and quantification of *Trypanosoma evansi* in animals and vectors (Chansiri et al., 2002). The sensitivity limit of PCR-ELISA was 0.01 pg, which corresponded to one parasite/ml of blood. No cross-reactivity of the assay was observed against *B. bovis*, *B. bigemina*, *A. marginale*, *Theileria* spp. and host DNA (Chansiri et al., 2002).

### RLB

Two integrated approaches were developed to detect several *Theileria* or *Babesia* spp. in one assay (Figueroa et al., 1993; Allsop et al., 1993). Using these approaches, multiple species can be detected in one assay without performing independent PCR reactions for each parasite

(Gubbels et al., 1999). One of such techniques, reverse line blot (RLB) hybridization, combines a genus specific PCR with hybridization to membrane bound type/species-specific oligonucleotide for differential detection. This technique can differentiate all known *Theileria* and *Babesia* spp. of importance in cattle in the sub-tropics on the basis of their differences in 18S subunit rRNA gene sequences (Gubbels et al., 1999). The specificity of the techniques result from the fact that amplified conserved domains of the 18 srRNA genes of the parasites are hybridized to species specific oligonucleotide immobilized on a solid membrane.

### LAMP

Recently, a rapid, simple, and sensitive technique, loop mediated isothermal amplification (LAMP), was developed (Notomi et al., 2000). This is a novel strategy for gene amplification which relies on the auto-cycling strand displacement synthesis of target deoxyribonucleic acid (DNA) by *Bst* DNA polymerase under isothermal conditions. Further improvement of the technique has been achieved by the use of additional loop primers, which increased its efficiency and rapidity (Nagamine et al., 2002). The LAMP technique allows visual detection of amplified products through the addition of fluorescent dyes such as SYBR Green (Poon et al., 2006) and measurement of turbidity (Mori et al., 2001). Unlike PCR, LAMP is carried out at a temperature range of 60 to 65°C eliminating the need of a thermal cycler. In addition, the reaction can be carried out without the need of DNA extraction. The method has been successfully developed for the detection of several TBDs (Salih et al., 2008; Liu et al., 2008; Muller et al., 2010; Thekisoe et al., 2010; Salih et al., 2012).

### DNA Microarrays

The technology commonly known as gene chip, DNA chip, or biochip was originally developed for mapping of genes being used to detect a wide variety of pathogens through multi-gene detection. The microarrays consist of solid supports like glass slide or silicon chip or nylon membrane, onto which the nucleic acid sequences from thousands of different genes are attached at fixed locations. The main advantage of this technique is that it combines DNA amplification strategies with subsequent hybridization to oligonucleotide probes specific for multiple target sequences. Secondly, it allows for the simultaneous analysis of a larger number of genetic features in a single trial. However, the technique is costly. This technique has been used in detection and genotyping of vector-borne parasites of medical importance like *Leishmania*, *Plasmodium*, *Toxoplasma*, and *Trypanosoma* (Duncan, 2004).

## CONCLUSION

Diagnosis of haemo-parasitic infection has been largely based on clinical symptoms and identifying/demonstrating the causative agent by parasitological techniques. Although, microscopy is still considered as a gold standard in the diagnosis of many parasitic diseases, it cannot be applied to all situations particularly where the diagnostic requirements demand defining the carrier status. Although, the use of various serological methods provide definite clues about the parasitic infection in general, but these tests have some limitations. In this direction, the recently introduced molecular biological techniques will certainly be of help, though presently their use on large scale is mainly limited to large parasitology laboratories. The use of DNA hybridization probes, although developed several years ago, has never been developed to suite regular diagnostic laboratories and has now been super ceded by polymerase chain reaction. The ability of PCR to detect very small quantities of a target material and the absence of the need to use radioactive elements are two of the advantages of PCR compared with hybridization techniques. However, more accurate identification of a PCR product may require the use of specific nucleic acid probes. But, it is not evident, with exception of RLB which is now being commercially produced, that the use of the technique will spread as a routine diagnostic tool in the laboratories. The use of molecular biology tools based on nucleic acid for tick-borne diseases will therefore, for sometime continue to be used in research activities rather than for day-to-day diagnosis in the laboratories. However, recombinant antigens based ELISAs may be available for routine diagnosis in the field.

## Conflict of Interest

Authors have no conflict of interest.

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