Address the Public Health and Food security Concerns of Babesiosis Through Molecular Detection of Babesia bovis in Suspected Carrier Cattle of Selected Localities in Sri Lanka

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Abstract

Emergence of babesiosis in both public health and food security concern in global perspectives cannot be ignored. The dairy farming plays an important role in food industry that, fulfilling the protein requirement through producing both milk and meat. The farming operations may get hampered due to non-infectious and infectious diseases. Infectious diseases also cause huge production losses in all farming operations in time to time. Babesiosis becomes a major concern in public health and animal production specialists in recent years both locally and internationally due to severity of out breaks, carrier animals and zoonotic nature. Babesiosis as an emerging zoonotic disease and also causing heavy production losses due to the both clinical diseases and carrier animals. Babesia parasites, mainly Babesia bovis and B. bigemina, are tick-borne hemoparasites inducing bovine babesiosis in cattle globally. Babesiosis is known to occur in tropical and subtropical regions of the world and it is one of the major constraints to the livestock industry which adversely affects economic return and food security by reducing milk, meat production and if not treated leads to the death of the animal. Babesia parasites are considered to be endemic in central Sri Lanka and the prevalence of babesiosis is high in other areas. Carrier cattle infected with babesiosis have low number of parasites in circulation. Babesiosis is difficult to detect and is a challenge to conventional diagnostic methods. However, diagnosis of carrier animals in herd is important for preventing outbreaks by transmission through vector ticks to healthy animals and for obtaining epidemiological data of the disease. Here, we have conducted nested PCR detection of Babesia bovis in carrier cattle (Jersey crosses, Friesian crosses and Australian milking zebu) of selected localities of Sri Lanka. For this study, 30 blood samples were collected from suspected carrier cattle and analyzed using light microscopy and nested PCR. Screening by light microscope indicated that 47% of the samples to be positive. PCR analysis of samples
diagnosed 80% as positive. Hence, 33% of the animals appeared to be healthy through routine light microscope diagnosis were in fact carriers posing a threat for the healthy herd population. The high prevalence of babesiosis in carrier cattle in Sri Lanka emphasized that island-wide control and prevention programs against bovine babesiosis are needed to minimize the financial burden caused by the parasites to reduce to production losses. This preliminary study on molecular detection of *Babesia bovis* in selected localities offers a rapid and efficient screening method for suspected carrier herds while providing new avenues to minimize losses incurred in milk production due to unidentified carrier animals.

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**Keywords:** *Babesia bovis*; PCR; bovine babesiosis; carrier animal; ticks

**Introduction**

The dairy farming plays an important role in food industry that, fulfilling the protein requirement through producing both milk and meat. The farming operations may get hampered due to non-infectious and infectious diseases. Among the non-infectious causes metabolic disorders are often associated with the low productivity of cattle. Infectious diseases also cause huge production losses in all farming operations in time to time. Bovine babesiosis is caused by microscopic parasites that infect red blood cells and are spread by certain ticks. Bovine babesiosis and other tick borne diseases are responsible for more than 50% losses in the crossbred cattle (Chaudhry *et al*., 2010). Babesiosis creating public health issues being zoonotic and plays a vital role in food security by impacting on milk and meat production (The Center for Food security and Public Health, 2008, Bovine Babesiosis, College of Veterinary Medicine, Iowa State University). Bovine babesiosis is caused by *Babesia bovis* and *Babesia bigemina*. They are widely distributed and of major importance in Africa, Asia, Australia, and Central and South America. *Babesia divergens* is economically important in some parts of Europe.

*B. bovis* infection lasts for at least four years in *Bos taurus* cattle and *B. bigemina* usually less than 6 months. Immunity to both parasites remains for at least four years regardless of the status of the infection (de Vos *et al*., 2004). Most cattle with a significant *Bos indicus* blood lose *B. bovis* infection within 2 years, but immunity to *B. bovis* lasts at least for 3 years (Johnston LA *et al*., 1978). No apparent clinical signs were noticed during this carrier state. Diagnosing this carrier cattle population with Babesiasis is a challenge to the conventional diagnostic procedures due to low number of parasites in peripheral blood. For instance *in vitro* culture methods have been used to demonstrate the presence of carrier infections of *Babesia spp.* (Holman *et al*., 1993). However, the minimum parasitaemia detectable by this method will depend, to a large extent, on the facilities available and the skills of the operator (Bose *et al*., 1995). On the other hand animal inoculation for confirmation of infection in a suspected carrier animal is also possible. But this method is cumbersome, expensive, and obviously not suitable for routine diagnostic use because of its’ complexity. Although serological tests have been described that detect the reactive antibodies with *Babesia spp.*, they do not consistently detect the infections in carrier animals, and their specificity is limited by the cross-reactivity of the antigens of other *Babesia spp.*
Nevertheless, diagnosis of low-level infections are important for epidemiological studies; i.e., carrier cattle act as a reservoir for the infection during the low transmission periods that maintain the infectious agent within the herd (Fahrimal et al., 1992). Therefore attempts have been made to come up with more sensitive tools for the diagnosis of the infection which can cover the asymptomatic carrier animals. Nucleic acid-based diagnostic assays are very sensitive particularly in detecting B. bovis and B. bigemina in carrier cattle (Buling et al., 2007; Costa-Junior et al., 2006; Criado-Fornelio, 2007). Among those methods, polymerase chain reaction based techniques are reported to be about 1000 times more sensitive than microscopic methods for detection of Babesia spp. Given the importance of livestock production in Sri Lankan economic landscape, the main objective of this study was to establish a nested PCR assay to specifically detect B. bovis, in suspected carrier cattle blood samples (n=30) collected from four different locations representing Central Province and North-Western Province of Sri Lanka.

Methodology

Blood sampling

The blood samples were collected from 30 suspected carrier cattle which had already recovered from tick fever within last 3 years from Central and North-Western Provinces of Sri Lanka. One milliliter (1 ml) of blood was collected from each animal into EDTA-coated tubes by jugular or coccygeal venipuncture and stored at -20°C prior to processing. Clinical history of the cows was also obtained.

Microscopic blood smear examination

Thin and thick blood smears were prepared by taking a blood drop from tail tip or ear vein. The dried blood smears were fixed in absolute methyl alcohol for one minute. Staining was performed using Giemsa as described by Benjamin (1986). Thick and thin blood smears were stained with one fourth dilution of commercially available Giemsa stain for four minutes and the smear was observed with the help of oil immersion lens (100X) to detect the presence of B. bovis. Identification was conducted using standard keys (Hazem et al., 2014).

DNA isolation

The molecular detection was conducted at Genetech Molecular Diagnostics, Colombo, Sri Lanka. Genomic DNA was extracted from 300μl of blood using Promega Wizard Genomic Extraction kit according to the manufacturer's guidelines. Extracted DNA was dissolved in 100μl of rehydration buffer and stored at −20°C until further analysis.

PCR amplification and Data analysis

The method described by Figueroa et al., (1993), was slightly modified to establish the PCR assay. The sizes of the amplified products were the 360bp for the first round PCR and 298bp for the nested PCR. The PCR reaction mixture contained 2.5 μl of template DNA, 1x Taq PCR green buffer (Promega go Taq) 1.5 mM MgCl2, 1.25 U of Taq DNA polymerase, deoxynucleoside triphosphates (0.35 mM each; Geneshun Biotech) and 0.6 μM concentration of the primer pair (Macrogen) in final volume of 25 μl. The amplification profile consisted of 3 minutes of initial denaturation at 95°C followed by 35 cycles of 30S at 95°C, 45S at 55°C, 1 min at 72°C, and final extension at 72°C for 10 minutes in a thermal cycler (Bioradmycycler). 2μl of first round PCR products were used for the second round (nested) PCR mixtures
comprising of similar composition of reagents as the first round PCR, except that the external primers were replaced with the nested PCR primers. PCR mixtures were cycled as described above. The PCR products obtained were visualized by UV transillumination after electrophoresis of 12μl aliquots of the reaction mixtures through 2.0 % agaroseethidium bromide gels.

Results and Discussion

Out of 30 blood sample collected from suspected carrier cows, 14 were positive for either of the species by microscopic examination (46.67%). Parasitemia of infected animals can range from 0.0001% to 1% of the red blood cells (Criado-Fornelio, 2007). *Babesia bigemina* is larger in size and having a paired structure at an acute angle to each other. *Babesia bovis* is smaller in size and having paired form at an obtuse angel to each other. However, it is hard to differentiate the two species by microscopic examination (Fig. 1) alone under our laboratory conditions.

In contrast to the 14 positive babesiosis samples which were detected from microscopic examination, 24 positives were obtained by Nested PCR including both localities (Table 1). All the suspected carrier cows were animals which recovered from acute disease and according to our result that 80% of them were positive, despite their clinical conditions (Fig. 2). European breeds of cattle are very susceptible and mortality after infection with *Babesia* species can be high. *Bos indicus* breeds such as Brahman, Sahiwal are affected to a lesser extent, crossbred cattle show resistance to the disease. Despite this low susceptibility, nearly 1 in 5 outbreaks of tick fever involves in cross bred animals (Veterinary Epidemiological Bulletin Sri Lanka, 2012). In our findings it is indicated almost 80% of suspected crossbred cows acting as carriers were of European origin. This observation is in agreement with previous findings of Chaudhry et al. (2010).

Calves from immune mothers receive temporary protection (maternal antibody) from the colostrum, which prevents clinical disease in the short term. However, the calves may be infected by this time. This protection lasts about three months and in most cases it is followed by an age resistance that lasts until the animals are about nine months old. Calves that are exposed to infection when the maternal antibodies are present or those who have high age resistance, rarely show clinical symptoms but develop a solid, long-lasting immunity. In our study, 18 heifer cows aged from 6 months to 2.5 years were identified as suspected carrier animals by disease history from both localities of Central Province and North-Western Province. Further, out of 18 heifer cows, 17 were positive for Babesiosis (94.44%).

Prevalence of *Babesia bovis* is strongly based on the prevalence of ticksunder the farming practices observed. Strict quarantine and management practices are required to eliminate or reduce this high prevalence of *Babesia spp.* in suspected carrier cattle throughout Central and North-Western Provinces of Sri Lanka. Isolation of carrier animals from the herd and early detection will be the best options to reduce the occurrence of babesiosis in entire herd. In addition, routine disease diagnostic and clean environment which can eliminate the tick infestation plays vital role in eliminating the *Babesia spp.* in a herd.

Control of bovine babesiosis can be either by tick management, immunization, anti-babesia drugs or by a combination of these approaches. Chemotherapy of babesiosis is important for controlling the disease either to treat field cases or to controlartificially induced infections. A large number of chemical compounds have been reported to be effective against bovine *Babesia* parasites. Imidocarb (3,3’-bis (2-imidazolin-2-yl)-carbanalidae) is the principal babesiacide used in animals, the only one that consistently clears the host of parasites and has been used in the treatment and prophylaxis of babesiosis (Masquedaet al., 2012). Imidocarbdipropionate salt (Imizol®) is effective at a dose 1mg/ kg Body Weight (BW). Imidocarb
has been successfully used as a chemoprophylactic at a high dose rate of 3mg/ kg BW that will prevent clinical infection for as long as 2 months but allow mild subclinical infection to occur as the drug level wanes resulting in premunition and immunity. The use of imidocarb can disrupt the animal’s immunity to tick fever. Diminazeneaceturate (Berenil®) is widely used and given in a 7 % aqueous solution by deep intra muscular injection; It does not sterilize the diseased animal completely (El Sawalhy, A. A. 1999). Further, the use of imidocarb in food producing animals has caused some concern because its’ poor elimination abilities. Clinical disease of all selected localities of current study was treated with Diminazeneaceturate (4,4’(azoamino) dibenzamidine) is anaromatic diamidine, derived from Surfen (bis-2-methyl-4-aminquinolyl-6-carbamide) Berenil.

This preliminary study based on Friesian crossers, Jersey crossers, and Australian Friesian Sahiwal breeds in Sri Lanka in selected localities namely, Central Province and North-Western Province, indicated that all these breeds can act as carrier animals to Babesia bovis. By direct observation of cattle rearing practices and analyzing clinical history of individual cows, it was clear that there are deficiencies in optimal management practices, well formulated disease diagnostics, treatment and follow-up programmes, which were causing high prevalence of Babesia bovis among the herds investigated. Prevalence of Babesia spp. in a herd creates the biggest threat to the herd by remaining as a reservoir of the Babesia spp. which leads to rapid transmission to other animals in the herd. This study confirmed that the PCR assay is more sensitive compared to the microscopic method in detecting carrier animals. Early detection by PCR screening will help in identifying the carrier animals which will facilitate preventive measures through isolation of carriers from the herd during routine diagnostic and screening practices.

**Conclusion**

This preliminary study on molecular detection of Babesia bovis in selected localities have shown rapid and efficient screening method to detect carrier herds.

**Table 1: Prevalence of B.bovis**

<table>
<thead>
<tr>
<th>Location</th>
<th>Breed/Breeding status</th>
<th>Prevalence of B.bovis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Province</td>
<td>Friesian/Friesian cross</td>
<td>69.23%</td>
</tr>
<tr>
<td></td>
<td>Heifers</td>
<td>87.5%</td>
</tr>
<tr>
<td></td>
<td>Cows in lactation</td>
<td>42.85%</td>
</tr>
<tr>
<td>North-Western Province</td>
<td>Jersey cross</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Heifers</td>
<td>100%</td>
</tr>
</tbody>
</table>
Figure 1: Giemsa stained microplate; Arrow shows *Babesia spp.* inside the bovine erythrocytes.
Figure 2: Representative gel showing the specificity nested PCR for the efficient detection of bovine babesiosis.
Lane 1,3,11 and 14; negative sample for *B. bovis*, Lane 2,4,5,6,7,8,9,10,12,13,15,16,17,18 and 19; positive sample for *B. bovis* at 298bp, Lane L; 50bp DNA ladder marker.

References