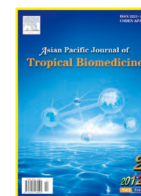
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(11)60202-5 © 2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Detection of *Babesia bovis* in blood samples and its effect on the hematological and serum biochemical profile in large ruminants from Southern Punjab

Samreen Zulfiqar[1], Sadia Shahnawaz[1], Muhammad Ali[2], Arif Mahmood Bhutta[1], Shahid Iqbal[1], Sikandar Hayat[2], Shazia Qadir[1], Muhammad Latif[1], Nazia Kiran[1], Ali Saeed[3], Muhammad Ali[3], Furhan Iqbal[1*]

[1]*Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakariya University, Multan 60800, Pakistan*
[2]*Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan*
[3]*Institute of Biotechnology, Bahauddin Zakariya University, Multan 60800, Pakistan*

ARTICLE INFO

Article history:
Received 13 July 2011
Received in revised form 5 August 2011
Accepted 21 August 2011
Available online 28 February 2012

Keywords:
Babesia bovis
PCR amplification
Cattle
Hematology
Serum biochemistry
Ruminant
Babesiosis
Serological profile
Cattle

ABSTRACT

Objective: To determine the presence of *Babesia bovis* (*B. bovis*) in large ruminants in southern Punjab and its effect on hematological and serum biochemical profile of host animals. **Methods:** Blood samples were collected from 144 large ruminants, including 105 cattle and 39 buffaloes, from six districts in southern Punjab including Multan, Layyah, Muzaffar Garh, Bhakar, Bahawalnagar and Vehari. Data on the characteristics of animals and herds were collected through questionnaires. Different blood (hemoglobin, glucose) and serum (ALT, AST, LDH, cholesterol) parameters of calves and cattle were measured and compared between parasite positive and negative samples to demonstrate the effect of *B. bovis* on the blood and serological profile of infected animals. **Results:** 27 out of 144 animals, from 5 out of 6 sampling districts, produced the 541-bp fragment specific for *B. bovis*. Age of animals (P=0.02), presence of ticks on animals (P=0.04) and presence of ticks on dogs associated with herds (P=0.5) were among the major risk factors involved in the spread of bovine babesiosis in the study area. ALT concentrations were the only serum biochemical values that significantly varied between parasite positive and negative cattle. **Conclusions**: This study has reported for the first time the presence of *B. bovis* in large ruminant and the results can lead to the prevention of babesiosis in the region to increase the livestock output.

1. Introduction

The role of livestock in Pakistan's rural economy may be realized from the fact that 30-35 million rural population is engaged in livestock raising which helps them to derive 30-40 percent of their income from it[1]. Tick infestation in cattle is one of the major constraints to the livestock industry in developing countries which adversely affects economic performance, mainly by transmission of serious pathogens of animals[2-5]. Bovine babesiosis is, transmitted by *Ixodid* ticks, caused by intraerythrocytic protozoan parasite, *Babesia bovis* (*B. bovis*) (Family: Babesiidae) that infects a wide range of domestic animals and causes progressive haemolytic anaemia[6,7]. A marked rise in body temperature, reaching (40-41) ℃, loss of appetite, cessation of rumination, labored breathing, hemoglobinurea, weakness and a reluctance to move are the symptoms developed especially in more protracted cases[8-11]. The fever during infections may cause pregnant cattle to abort and bulls to show reduced fertility lasting six to eight weeks[12,13].

The diagnosis of ruminant piroplasmosis is generally based upon the microscopic examination of Giemsa stained blood smears and by clinical symptoms in acute cases. After acute infections, recovered animals frequently sustain sub clinical infections, which are microscopically undetectable[14-16]. They can be considered as a source of infection for the potential vector causing natural transmission of the disease. Serological methods are

*Corresponding author: Dr. Furhan Iqbal, Ph.D., Department of Zoology, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan.
Tel: 0092-61-9210053
Fax: 0092-61-9210098
E-mail: furhan.iqbal@bzu.edu.pk
Foundation Project: This work was financially supported by the Directorate of Research and External Linkages, Bahauddin Zakariya University, Multan, Pakistan (grant No. DR & EL/D-40 dated 05-04-2010).

frequently employed in determining sub clinical infections. However, serology for detecting carrier state lacks specificity and sensitivity, especially for infection status[17-21]. Therefore, DNA amplification methods, which are more sensitive and specific than other conventional methods may facilitate and be used as a powerful tool for the diagnosis of babesiosis[22-30].

The aim of the present study was to establish a specific, reliable and sensitive molecular tool, the polymerase chain reaction (PCR), for the detection of *B. bovis* in cattle and buffaloes from southern Punjab. Two different diagnostic methods, blood smear screening and PCR, were also compared for the efficient parasite detection. Furthermore, the present study provided a baseline data regarding the presence of *B. bovis* and risk factors involved in the spread of tropical babesiosis in large ruminants and we have also compared various hematological and serum biochemical parameters between parasite positive and negative cattle and calves in order to demonstrate the effect of babesiosis on blood profile, if any, of the host.

2. Materials and methods

2.1. Sample and data collection

Blood samples were collected from 144 clinically healthy large ruminants (105 cattle and 39 buffaloes) from randomly selected herds located in the important livestock production regions of southern Punjab including Multan, Layyah, Muzaffar Garh, Bhakar, Bahawalnagar and Vehari districts during January to August 2010. Blood was collected from the jugular vein of the animals and immediately preserved in 10 mL Eppendorf tubes by adding 400 μL of 0.5 M EDTA. Data regarding the characteristics of animals (species, gender, age, presence of ticks) and herd (location, size, species of animals, dogs associated with the herds, presence of ticks on dogs associated with the herds) were collected through questionnaires completed by investigators on sampling sites in order to calculate the risk factors involved in the spread of bovine babesiosis.

2.2. Blood film formation

Blood films were prepared, fixed with methanol, stained with Giemsa and microscopically observed for the detection of *Babesia* sp. in blood.

2.3. DNA extraction

Inorganic method of DNA extraction was used following Shaikh *et al*[31]. The quality of the DNA extract in regard to purity and integrity was assessed with optical density counts at 260/280 nm and submerged gel electrophoresis.

2.4. PCR amplification

A set of oligonucleotide primers was used to amplify the 541 bp fragment of small subunit (SSU) rRNA gene sequences of *B. bovis* as previously described by Durrani and Kamal[6]. The nucleotide sequence of the primer-pair was: forward primer (GAU9) 5′CTGTCGTACCGTTGGTTGAC 3′and reverse primer (GAU10) 5′CGCACGGACGGAGACCGA 3′. PCR was performed in a final reaction volume of 25 μL. Each reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 μM (each) deoxynucleotide triphosphate (dNTPs), 2.5 U of Taq DNA polymerase (Merck, USA), 20 pmol of primers and 5 μL of extracted DNA sample. *B. bovis* DNA (previously isolated from the blood of naturally infected cattle and kindly provided by Dr. Munir Aktas, University of Firat, Turkey) and distilled water (without DNA) were run during every PCR amplification as positive and negative controls, respectively. DNA amplification was carried out in a thermal cycler (Gene Amp® PCR system 2700 Applied Biosystems Inc., UK). The thermo-profile used by Oliveira *et al*[32] and Shahnawaz *et al*[33] was modified for the present study. An initial denaturing step of 5 min at 94 ℃ was followed by 5 cycles: denaturing step of 1 min at 94 ℃, an annealing step of 1 min at 56 ℃ and an extension step of 1 min at 72 ℃. These 5 cycles were followed by 30 cycles. Each cycle consisted of denaturing step of 1 min at 94 ℃, an annealing step of 1 min at 54 ℃ and an extension step of 1 min at 72 ℃. The PCR program ended with a final extension step of 7 min at 72 ℃. Amplified products were separated by electrophoresis on a 1.5% agarose gel and visualized under a UV transilluminator (Biostep, Germany).

2.5. Hematological and serological analysis

Blood glucose concentration was measured by using ACCU-CHEK® Active blood glucose meter (Roche, Germany) while hemoglobin was determined by using Metertek SP-8SO spectrophotometer (Korea) and Randox LTD Laboratories diagnostic kit (UK). For the determination of serum biochemical activity, the blood samples were centrifuged at 13 000 rpm for 10 min to separate the serum and serum was stored at -20 ℃ until further use. Serological biochemical parameters including cholesterol, aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) were determined by using APEL PD-303S spectrophotometer (Japan) and diagnostic kits manufactured by Spinreact, Spain following their user′s manuals.

2.6. Statistical analysis

Animals were grouped into two age categories: less or equal to 1 year (calf) and 1 year or more than 1 year old (adult). Herds were divided into two size categories: herds having 1-15 animals and herds with more than 15 animals. Also, herds were divided according to their composition

into three categories: herds with cattle only, herds with buffalo only and herds with cattle and buffalo together. The absence or presence of ticks on cattle, buffalo, calf and dogs associated with the herds was also recorded. Association between the presence (positive and negative blood samples) of *B. bovis* and the various parameters, *i.e.* herd location, herd size, herd composition, gender and age of animal, and absence or presence of ticks on cattle, buffalo, calf and dogs in the herd was assessed by contingency table analysis using the Fisher's exact test (for 2×2 tables). Association of parasite prevalence with herd composition and herd location was determined by one way analysis of variance (ANOVA). Similarly one way ANOVA was also calculated for parasite prevalence and various blood and serological parameters (glucose, hemoglobin, and cholesterol, ALT, AST and LDH) in calf and cattle. All the values were expressed as mean ± standard deviations. Mini Tab (Version 16) was used for statistical analysis.

3. Results

PCR results revealed that 27 (18%) out of 144 examined ruminant blood samples, collected from 6 districts of southern Punjab, produced the 541-bp fragment specific for *B. bovis*. The 27 parasite positive blood samples included 18 cattle and 9 buffalo samples. On the other hand, only 4 (3%) of 144 blood samples were found parasite positive during microscopic examination of Giemsa stained blood smears. Out of the 6 districts, 5 (83%) had ruminant samples positive for *B. bovis* and the parasitic prevalence varied between 10% to 50% (Table 1).

Statistical analysis of the characteristics of animals showed that age (P=0.02) and presence of ticks on animals (P=0.04) might play an important role in the spread of babesiosis as animal less than 1 year old and those having ticks present on them were more infected with the parasite. Regarding the characteristics of herds, results indicated that presence of ticks on dogs associated with herds had non-significant correlation with increasing parasitic prevalence (P>0.05) (data not shown).

Blood and serum biochemical parameters (cholesterol, ALT, AST, LDH and glucose) varied non-significantly between parasite positive and negative blood samples except ALT. ALT level in cattle was the only parameter that varied significantly between parasite positive and negative large ruminants indicating that the presence of *B. bovis*, being a blood parasite, affects the blood biochemistry of host (Table 2).

4. Discussion

Babesia is one of the most important blood parasites affecting cattle and buffaloes and in its acute forms, it lowers the productive performance of the affected animals[8,34]. It is estimated that 1.2 billion cattle are exposed to babesiosis in many countries of the world including Asia, Australia, Africa, South and Central America and the United States[35].

It is the demand of time to develop sensitive tools for the effective detection and drugs for treatment of *Babesia* sp. in order to decrease the economic losses by the parasite. Several conventional and modern methods are used for the detection of *Babesia* sp. in host animals. The most commonly

Table 1

Prevalence of *B. bovis* detected according to the sampling districts and species [n (%)].

Parameters		*B. bovis* (positive)	*B. bovis* (negative)	Total
Sampling site	Multan	17 (30)	39 (70)	56
	Layyah	3 (10)	27 (90)	30
	Muzaffar Garh	3 (50)	3 (50)	6
	Bhakar	3 (14)	19 (86)	22
	Bahawalnagar	1 (10)	9 (90)	10
	Vehari	0 (0)	20 (100)	20
Species	Cattle	18 (17)	87 (83)	105
	Buffalo	9 (23)	30 (77)	39

ANOVA showed a highly significant association between the sampling sites and prevalence of parasite (P=0.006).

Table 2

A comparison of serum biochemical parameters of parasite positive and negative blood samples from calves (less than 1 year old) and cattle (more than 1 year old) (mean±SD).

Parameters (mg/dL)	Parasite (positive)				Parasite (negative)			
	Calves (n=12)		Cattle (n=15)		Calves (n=31)		Cattle (n=65)	
ALT	2.91-15.75	9.90±3.70	8.75-19.20	15.10±3.50**	7.00-22.10	12.20±3.70	7.58-23.30	13.60±4.00**
AST	16.30-52.50	37.90±14.80	17.50-48.40	32.70±11.00	16.30-52.50	34.00±12.90	12.83-48.40	28.90±9.30
Cholesterol	59.40-96.00	82.80±9.50	59.40-273.20	142.30±64.60	59.40-187.10	92.60±34.80	59.40-749.50	166.40±108.90
LDH	361.50-596.30	445.40±74.30	35.08-472.20	299.00±191.00	51.20-547.70	345.70±112.60	35.08-995.60	345.10±216.90
Hb	3.30-10.00	5.90±2.40	3.90-12.10	8.10±2.50	3.30-10.00	7.20±2.60	3.20-12.10	9.50±1.80
Glucose	11.00-75.00	39.20±19.10	19.00-57.00	35.50±15.40	11.00-75.00	49.10±14.50	11.00-70.00	39.80±17.80

Hb: hemoglobin; **: P<0.01.

used one is by microscopic examination of blood smears stained with Giemsa, but this technique is usually adequate for detection of acute infections and due to its low sensitivity this method is not used for the detection of carrier animals, where parasitemia may be low[10]. Many studies verified that PCR is a more specific and sensitive tool than conventional techniques for the detection of carrier ruminants having *Babesia* sp. present in blood without any apparent signs of babesiosis[8,33,34]. We had similar experience as the prevalence of *B. bovis* detected through PCR was 18% as compared with 3% (*n*=4) parasitic detection by microscopic examination of Giemsa-stained blood smears. Furthermore, these 4 blood samples were also found to be parasite positive by PCR. Only microscopic examination of PCR positive samples would have declared many of the positive samples as parasite free. A similar comparison was made by Durrani and Kamal[6] in Kasure, Pakistan. They found 33.3% prevalence for *B. bovis* and *Babesia bigemina* in cattle from Kasure by PCR as compared with 3% prevalence of *B. bovis* detected by blood smear examination.

Analysis of data revealed that calves (28%) were more infected by *B. bovis* as compared with adult animals (15%) (P=0.02). Further analysis showed that the calves of buffaloes (50%) were more prone to babesiosis than their adults (0%) (P=0.000 2). This result was in accordance with the findings of Niazi *et al*[15] who found a high incidence of babesiosis in calves in their study conducted at livestock experimental station Qadirabad and adjascent areas, in Sahiwal (Pakistan).

The presence of ticks on animals proved to be an important risk factor for the spread of babesiosis during the present study as ticks are the potential vectors for the transmission of parasite under study. Ticks were found on 37.5% of the infected animals (P=0.04). The incidence of babesiosis, possibly due to tick infestation, was more significant in buffaloes than in cattle during the present study as in cattle ticks were found on only 17.7% of the infected animals while 60% of the *B. bovis* positive buffaloes had ticks detected on their bodies (P=0.000 2). These results indicated that ticks may act as a vector for the transmission of *Babesia* sp. in large ruminants but parasite detection was not carried out in ticks found on the animals under study. A similar trend of tick infestation and babesiosis was previously reported by Iqbal *et al*[5] and Ghosh *et al*[26].

As *B. bovis* is a blood parasite, various hematological and serum biochemical parameters were measured in all large ruminant samples. Blood hemoglobin and glucose values were reduced in infected calves and cattle as compared with the parasite free ones. These observations were similar to those of Yuksek *et al*[13], Durrani *et al*[18], and Col and Uslu[19]. The decrease in blood glucose concentration could be due to the utilization of glucose by parasites and damage to the liver in large ruminants infected with *B. bovis*.

Serum AST and ALT concentrations are the indicators of hepatic function. The rise in serum ALT concentration may be due to alteration of liver function as a result of bovine babesiosis[13,16]. The serum ALT concentration of infected calf samples was lower than parasite negative calves while the serum ALT concentration of infected cattle was higher as compared with non infected cattle. The level of serum AST in infected calves and cattle was higher as compared with the healthy ones. Similar findings were observed by Talkhan *et al*[8]. In present study the increase in ALT and AST levels in infected animals compared with healthy animals might indicate hepatic dysfunction in parasite positive animals.

The serum LDH concentrations of infected calves were found to be higher than parasite negative calves. LDH is a cytosolic enzyme, which is essentially present in all tissues involved in glycolysis. Any destructive process of these tissues leads to the enzyme leaking into extra cellular fluids and then into body fluids. Hence detection of elevated concentration of this enzyme released into the blood stream from the damaged tissues has become a definitive diagnostic and prognostic criterion for various diseases and disorders. The higher level of LDH in calves may be related to liver dysfunction reported by Talkhan *et al*[8].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This project was financed by the Directorate of Research and External Linkages, Bahauddin Zakariya University, Multan (Pakistan) through grant No. DR & EL/D-40 dated 05-04-2010. Authors would like to thank all the veterinarians for their kind help during sample collection.

References

[1] *Livestock census: data on Punjab livestock*. Livestock Census Organization, Goverment of the Punjab; 1996.

[2] Das G, Ghosh S, Ray DD. Reduction of *Theileria annulata* infection in ticks fed on calves immunized with purified larval antigens of *Hyalomma anatolicum anatolicum*. *Trop Anim Health Prod* 2005; **37**: 345-361.

[3] Adham FK, Abd-el-Samie EM, Gabre RM, el-Hussein H. Detection of tick blood parasites in Egypt using PCR assay I-*Babesia bovis* and *Babesia bigemina*. *Parasitol Res* 2009; **105**: 721-730.

[4] Ghirbi YM, Hurtado A, Brandika J, Khlif K, Ketata Z, Bouattour A. A molecular survey of *Theileria* and *Babesia* parasites in cattle, with a note on the distribution of ticks in Tunisia. *Parasitol Res* 2008; **103**: 435-442.

[5] Ghosh S, Bansal GC, Gupta SC, Ray D, Khan MQ, Irshad H, et

al. Status of tick distribution in Bangladesh, India and Pakistan. *Parasitol Res* 2007; **101**: 207-216.

[6] Durrani AZ, Kamal N. Identification of ticks and detection of blood protozoa in friesian cattle by polymerase chain reaction test and estimation of blood parameters in district Kasur, Pakistan. *Trop Anim Health Prod* 2008; **40**: 441-447.

[7] Rehman WU, Khan, IA, Qureshi AH, Hussain S. Prevalence of different species of Ixodidae (hard ticks) in Rawalpindi and Islamabad. *Pak J Med Sci* 2004; **43**: 42-46.

[8] Talkhan OFA, Radwan MEI, Ali MA. Cattle babesiosis and associated biochemical alteration in Kalubyia Governorate. *Nat Sci* 2010; **12**: 24-27.

[9] Bastos RG, Ueti MW, Knowles DP, Scoles GA. The *Rhipicephalus* (*Boophilus*) *microplus Bm86* gene plays a critical role in the fitness of ticks fed on cattle during acute *Babesia bovis* infection. *Parasit Vectors* 2010; **3**: 111.

[10] Mesplet M, Palmer GH, Pedronic MJ, Echaided I, Christensen MF, Schnittger L, et al. Genome-wide analysis of peptidase content and expression in a virulent and attenuated *Babesia bovis* strain pair. *Mol Biochem Parasitol* 2011.

[11] Brown C. *Foreign animal diseases*. Boca Raton, Florida: United States Animal Health Association; 2008.

[12] Ferreri L, Benitez D, Dominguez M, Rodriguez A, Asenzo G, Mesplet M, et al. Water buffalos as carriers of *Babesia bovis* in Argentina. *Ann N Y Acad Sci* 2008; **1149**: 149-151.

[13] Yuksek N, Altug N, Gul AR. Therapeutic effect of the combination of trichlobendazole and levamisole in sheep with endoparasite infection. *J Vet Med* 2006; **11**: 34-37.

[14] Perez-Llaneza A, Caballero M, Baravalle F, Mesplet M, Mosqueda J, Suarez CE, et al. Development of a tandem repeat-based multilocus typing system distinguishing *Babesia bovis* geographic isolates. *Vet Parasitol* 2010; **167**: 196-204.

[15] Niazi N, Khan MS, Avais M, Khan JA, Pervez K, Ijaz M. A study on babesiosis in calves at livestock experimental station Qadirabad and adjascent areas, Sahiwal, Pakistan. *Pak J Agri Sci* 2008; **45**: 13-16.

[16] Schneider DA, Yan IH, Bastos RG, Johnson- WC, Gavin PR, Allen AJ, et al. Dynamics of bovine spleen cell populations during the acute response to *Babesia bovis* infection: an immunohistological study. *Parasite Immunol* 2011; **33**: 34-44.

[17] Mahmoud MS, Abou-Zeina HAA. Current state in the serological diagnosis of babesiosis and haematological changes in splenectomised buffaloes. *Glob Vet* 2008; **2**: 271-281.

[18] Durrani AZ, Kamal N, Khan MS. Incidence of theileriosis and estimation of packed cell volume, total erythrocyte count and hemoglobin in buffaloes. *J Anim Plant Sci* 2006; **16**: 85-88.

[19] Col R, Uslu U. Changes in selected serum components in cattle naturally infected with *Theileria annulata*. *Bull Vet Inst Pulawy* 2007; **51**: 15-18.

[20] Terkawi MA, Huyen NX, Shinuo C, Inpankaew T, Maklon K, Aboulaila M, et al. Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in water buffaloes in the northeast region of Thailand. *Vet Parasitol* 2011; **178**: 201-207.

[21] Iseki H, Zhou Z, Kim C, Inpankaew T, Sununta C, Yokoyama N, et al. Seroprevalence of *Babesia* infections of dairy cows in northern Thailand. *Vet Parasitol* 2010; **170**: 193-196.

[22] Aktas M, Altay K, Dumanli N. Development of a polymerase chain reaction method for diagnosis of *Babesia ovis* infection in sheep and goats. *Vet Parasitol* 2005; **133**: 277-281.

[23] Nagore D, García-sanmartín J, García-pérez AI, Juste RA, Hurtado A. Identification, genetic diversity and prevalence of *Theileria* and *Babesia* species in sheep population from northern Spain. *Int J Parasitol* 2004; **34**: 1059-1067.

[24] Bhoora R, Franssen L, Oosthuizen MC, Guthrie AJ, Zweygarth E, Penzhorn BL, et al. Sequence heterogeneity in the 18S rRNA gene within *Theileria equi* and *Babesia caballi* from horses in south Africa. *Vet Parasitol* 2009; **159**: 112-120.

[25] Martins TM, Pedro OC, Caldeira RA, Do Rosário VE, Neves L, Domingos A. Detection of bovine babesiosis in Mozambique by a novel seminested hot-start PCR method. *Vet Parasitol* 2008; **153:** 225-230.

[26] Iqbal F, Fatima M, Shahnawaz S, Naeem M, Shaikh RS, Shaikh AS, et al. A study on the determination of risk factors associated with babesiosis and prevalence of *Babesia* sp., by PCR amplification, in small ruminants from southern Punjab (Pakistan). *Parasite* 2011; **18**.

[27] Cariado-Fornelio A, Martinez-Marcos A, Bulinga-Sarana A, Barba-Carretero JC. Molecular studies on *Babesia*, *Theileria* and *Hepatozoon* in Southern Europe Part 1. Epizootological aspects. *Vet Parasitol* 2003; **113**: 189-201.

[28] Altay K, Dumanli N, Holman PJ, Aktas M. Molecular identification, genetic diversity and distribution of *Theileria* and *Babesia* spp infecting small ruminants. *Vet Parasitol* 2007; **147**: 121-127.

[29] Ravindran R, Saravanan BC, Rao JR, Mishra AK, Bansal GC, Ray D. A PCR-RFLP method for the simultaneous detection of *Babesia bigemina* and *Theileria annulata* infections in cattle. *Curr Sci* 2007; **93**: 1840-1843.

[30] Altay K, Aydin MF, Dumanli N, Aktas M. Molecular detection of *Theileria* and *Babesia* infections in cattle. *Vet Parasitol* 2008; **158**: 295-301.

[31] Shaikh R, Ramzan K, Nazil S, Sattar S, Khan SN, Raizuddin S, et al. A new locus for non syndromic deafness DFNB51 maps to chromosomes 11p 13-p12. *Am J Med Genet A* 2005; **138**: 295-392.

[32] Oliveira DC, Van Der WM, Habela MA, Jacquiet P, Jongejan F. Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. *J Clin Microbiol* 1995; **33**: 2665-2669.

[33] Shahnawaz S, Ali M, Aslam MA, Fatima R, Chaudhry ZI, Hassan MU, et al. A study on the prevalence of a tick transmitted pathogen, *Theileria annulata*, and hematological profile in cattle from Southern Punjab (Pakistan). *Parasitol Res* 2011.

[34] Ziapour SP, Esfandiari B, Youssefi MR. Study of the prevalence of babesiosis in domesticated animals with suspected signs in Mazandaran province, north of Iran, during 2008. *J Anim Vet Adv* 2011; **10**: 712-714.

[35] Terkawi MA, Thekisoe OMM, Katsande C, Latif AA, Mans BJ, Matthee O, et al. Serological survey of *Babesia bovis* and Babesia *bigemina* in cattle in South Africa. *Vet Parasitol* 2011.