*Pak. J. Agri. Sci., Vol. 59(3), 511-517;2022*
*ISSN (Print) 0552-9034, ISSN (Online) 2076-0906*
*DOI:10.21162/PAKJAS/22.879*
http://www.pakjas.com.pk

# Detection of *Babesia ovis* in small ruminants by using microscopic and molecular techniques

Asif Masih, Azhar Rafique*, Farhat Jabeen and Shabana Naz

Department of Zoology, Government College University, Faisalabad-38040, Pakistan.
*Corresponding author's e-mail: azharrafique96@gmail.com

The present study was planned to detect *Babesia ovis* (*B. ovis*) in naturally infected sheep and goat herds of Faisalabad, Toba Tek Singh and Jhang districts of Punjab, Pakistan through microscopy and molecular diagnostic techniques *i.e*., PCR and nPCR. For this purpose, a total of 892 blood samples were collected *i.e*., sheep (n=544), goats (n=348) in both seasons (summer + winter). Initially, the samples were screened via the blood film and the blood smear examinations revealed parasite merozoites as tear drop in pair identified as babesia. The overall prevalence in summer based upon microscopic analysis was 22.05% (60/272) in sheep and 16.05% (28/174) in goat, respectively. In winter the prevalence was 7.34% (20/272) and 4.59% (8/174) in sheep and goat, respectively. In summer the results based upon PCR were 29.7% (81/272) and 23.56% (41/174) and n-PCR were 31.98% (87/272) and 27.01% (47/174) in sheep and goats respectively. On the other hand, in winter results showed that 12.13% (33/272), 9.77% (17/174) and 13.60% (37/272), 13.21% (23/174) samples positive for *B. ovis* through PCR and nPCR respectively. The results have shown that sensitivity of NPCR is more as compared to conventional PCR. The product size of *B. ovis* through PCR and NPCR was 186 base pair (bp) and 549 bp. Furthermore, the prevalence based upon different risk factors *i.e*., age, sex, housing, floor system, presence or absence of ticks was determined. Statistical analysis of different risk factors indicated that, increased age, non-cemented floor, closed housing, weak body condition and heavy infestation of ticks increase the incidence of disease by many folds.

**Keywords:** *Babesia ovis, PCR, NPCR, prevalence, small ruminants.*

## INTRODUCTION

Livestock sector plays an important role in Pakistan's economy and share of livestock in national GDP is 11.6% along with it contributes about 55.1% of agriculture value added during 2011-2012. Most of the rural economy of developing countries is dependent directly or indirectly on livestock (Abdel-Rahman *et al*., 2020; Bukhari *et al*., 2020; Rahat *et al*., 2020; Štrbac et al., 2021). However, livestock production systems have always been threatened with huge burden of infectious diseases (Anees et al., 2020; Muhammad *et al*., 2020). Among livestock, rearing of sheep and goats has much importance in rural areas especially for non-agricultural poor families (Shahzad *et al.,* 2013). Total number of heads of sheep and goats in Pakistan assessed as 28.24 M and 49.14 Million, respectively (Shahzad *et al.,* 2013) and they yielded 0.040 and 0.915 billion liters of milk for human consumption, 0.629 Million tons of meat and 0.043 Million tons of wool during 2011-2012. Despite of huge number of animals in Pakistan production is very low, reasons behind the low production are many few of them are poor breeding, poor management, improper housing and increased infestation of ticks, which lead to increased prevalence of hemoparasitic diseases. Pakistan being in the sub-tropical region in the world has environment, which favors the growth of ticks, which play an important role in the transmission of such diseases in small ruminants such as babesiosis. Ovine babesiosis develops in RBCs of peripheral blood of small ruminants (Abed *et al.*, 2017).

Three species of genus *Babesia* namely *B. motasi, B. crassa* and *B. ovis* are responsible for disease in small ruminants. *Babesia motasi* and *B. crassa* are considered to be less pathogenic and infection is mostly subclinical, while *B. ovis* is extremely pathogenic and instigates severity of infection along with 30-50% mortality rate in sheep and goats particularly in aged animals (Mathieu *et al.,* 2018; Hurtado *et al.,* 2018). The main clinical signs of babesiosis in sheep and goats include high fever, anemia, hemoglobinuria, depression and loss of appetite, as a consequence death may occurred (Rodastits, 2007; AbouLaila *et al*., 2021). Because of

Masih, A., A. Rafique, F. Jabeen and S. Naz. 2022. Detection of Babesia ovis in small ruminants by using microscopic and molecular techniques. Pakistan Journal of Agricultural Science. 59:511-517.

[Received 27 Jul 2021; Accepted 2 Jun 2022; Published 27 Jun 2022]

**Table 1. Primer sets used for *Babesia ovis* DNA detection.**

| Parasite | Assay | Primer name | Primer sequence | Size | Reference |
|---|---|---|---|---|---|
| *B. ovis* | PCR | Bov-F | TGGGCAGGACCTTGGTTGTTCT | 549bp | (Esmaeilnejad *et al.*, 2014) |
| | | Bov-R | CCGCGTAGCGCCGGCTAAATA | | |
| | nPCR | iBov-F | AAGAATTTCACCTATGACAG | 186bp | (Haghi *et al*., 2013) |
| | | iBov-R | GTCTGCGCGCGGCCTTTGCG | | |

zoonotic nature some of *Babesia* species cause diseases in human population so increased emergence of such tick-borne parasites are considered to be a major threat to public health (Fakhar *et al.,* 2012; Zheng *et al.,* 2018; Young *et al.,* 2019). Different investigators have reported the occurrence of babesiosis in different parts of country, which were geographically different from the study like PCR based prevalence of babesiosis in sheep was 50% (Iqbal *et al.,* 2011) and Durrani *et al.,* (2012) documented other hemoparasite incidence that was 35%. However, before the present study few data is available on molecular prevalence of babesiosis in small ruminants through conventional and nested PCR in Faisalabad, Pakistan.

Traditionally, parasites are detected by clinical signs and microscopy (Hussain *et al.,* 2017; Karatepe *et al.,* 2019). These methods are insufficient to diagnose the carrier animals with low parasitemia. The disadvantages of conventional diagnostic methods can be improved by using modern molecular diagnostic approaches (Wang *et al.,* 2018).

Keeping in view the benefits of molecular techniques like PCR and nested PCR (nPCR) in terms of high specificity and sensitivity, the current study was planned to detect *Babesia ovis* in naturally infected sheep and goat herds of Faisalabad, Toba Tek Singh and Jhang districts of Punjab, Pakistan. Before that no such kind of work has been reported in purposed areas.

## MATERIALS AND METHODS

***Sample collection and experimental ethics*:** A total of 892 blood samples in both seasons (summer + winter) from ovine were collected *i.e.*, sheep (n=544), goats (n=348) with a history of high fever or presence of ticks on the body residing in Faisalabad, Toba Tek Singh and Jhang districts in Punjab, Pakistan. Samples were taken in tube free from any kind of anticoagulant. Thin blood smears were formed and fixed with absolute methanol and stained with Giemsa, all the laboratory precautions were followed. The presence of piroplasm was determined by examining the blood smears under light microscope with 100X magnification. Piroplasm were identified by morphological characteristics.

***DNA Isolation*:** Babesia genomic DNA was extracted from suspected sheep and goat blood as depicted beforehand (Almeria, 2001). Phenol chloroform isoamyl liquor technique was utilized for separating DNA. Erythrocytes were slicked in lysis cradle, at that point 20 ml proteinase K (10 mg/ml) was added, and the incubation for 2 hours at 56ºC was done to process the proteins. At that point, phenol chloroform isoamyl liquor was added and centrifuged at 13400 rpm for 15 min. Upper fluid layer was transferred to another smaller scale eppendorf containing ethanol 96% (2.5 ml volumes of the sample) and centrifuged at 13400 rpm for 15 min. Subsequent to washing with ethanol 70%, at long last removed DNA air-dried, disintegrated in TE cushion (10mM Tris-HCl pH 8, 0.1mM EDTA) at 55º C, and kept up at - 4º C for 24 hours at that point kept at - 20º C until use.

***PCR Analysis*:** So as to amplification of DNA parts of *B. ovis*, the PCR and nPCR methods were utilized with explicit ground works previously revealed for Babesia spp. (Table 1) got from flanking part of hyper variable locale of 18SrRNA. The PCR was acted in an all-out volume of 25 µl including 5µl of DNA master mix, 1 x PCR cradle, 0.1 U Taq polymerase, 0.5 µl of each primer (P1/P2, 20 mM), 125 µM of each of dNTPS (Fermentas) and 1.5 mM $MgCl_2$ in a programmed DNA Thermocycler (Bio-Rad); with the accompanying conditions: 5 min at 95°C to denature twofold strand DNA, 38 patterns of 45s at 94°C, 45s at 56°, 45s at 72°C and a last expansion step for 10 min (Esmaeilnejad *et al.,* 2014). The intensified items will be settled by 1.5% agarose gel electrophoresis and recolored with ethidium bromide for visual location by bright transillumination. Tests introducing noticeable groups of around 549 base sets (PCR) thought about positive for *B. ovis,* PCR positive groups were of 549 bp for *B. ovis* (Fig. 5).

***NPCR Analysis*:** Recently portrayed conventions were utilized for the nPCR measures for location by explicit enhancement of DNA pieces of *B. ovis*. Experiment was performed in a volume of 25 µl: 12.5 µl of Master Mix, 5.5 µl of ultrapure water, 10 pM of each primer (Table 1) and 5 µl of DNA fragment. The nPCR thermocycling conditions for *B. ovis* were: 1 min at 94 ˚C, 1 min at 62 ˚C and 1 min at 72 ˚C. The nPCR conditions were indistinguishable with the exception of the tempering advance, which was at 69˚C for 30 seconds (Haghi *et al*., 2013). Decontaminated tests of *B. ovis* was submitted to DNA extraction and utilized as positive control. DNA tests were viewed as positive if in nPCR explicit groups will be imagined on 1.5% agarose gel *B. ovis* 186 bp (Fig. 4).

## RESULTS

In this study a total of 892 blood samples in both seasons (summer + winter) from ovine were collected *i.e.*, sheep (n=544), goats (n=348) residing in Faisalabad, Toba Tek Singh and Jhang districts in Punjab, Pakistan. First of all, the samples were screened via the blood film in both seasons (summer & winter) and the blood smears examination revealed parasite as tear drop in pair identified as Babesia and the overall microscopic prevalence in summer was 22.05% (60/272) and 16.05% (28/174) in sheep and goat, respectively and in winter was 7.34% (20/272) and 4.59% (8/174) in sheep and goat, respectively.

Furthermore, the prevalence based upon different risk factors *i.e.,* age, sex, housing, floor system, presence or absence of ticks was determined. Statistical analysis of different risk factors (Table 2) (Fig. 2) indicated that, age (*P= 0.026*), sex (*P = 0.048*), floor (*P= 0.033*), housing (*P= 0.047*), body condition (*P = 0.033*), tick burden (*P = 0.036*) increased age, female, non-cemented floor, closed housing, weak body condition and heavy infestation of ticks increase the incidence of disease by many folds.

The district wise prevalence of babesiosis in goats and sheep was also vary in both seasons i.e in summer in goats the prevalence was 15.38% (12/78) in Faisalabad, 12.50% (6/48) in Toba Tek Singh and 20.83% (10/48) in Jhang, respectively, while in sheep prevalence was 10.90% (12/110), 20.00% (16/80) and 39.02% (32/82) in Faisalabad, Toba Tek Singh and Jhang respectively. While in winter the prevalence of *Babesia ovis* in goats was 5.12% (4/78) in Faisalabad, 2.08% (1/48) in Toba Tek Singh and 6.26% (3/48) in Jhang respectively, while in sheep *B. ovis* prevalence in Faisalabad was 5.66% (03/53), 3.44% (03/87) in Toba Tek Singh and 10.60% (14/142) in Jhang, respectively (Table 3) (Fig. 3).

The samples were further analyzed through the conventional PCR and nPCR. In summer the results based upon PCR were 29.7% (81/272) and 23.56% (41/174) and nested PCR were 31.98% (87/272) and 27.01% (47/174) in sheep and goats

**Table 2. Prevalence of *Babesia ovis* in ovine in relation to different risk factors in both seasons.**

| Risk factors | Variables | Summer | | | Winter | | |
|---|---|---|---|---|---|---|---|
| | | Prevalence % | Chi-square | P-Value | Prevalence % | Chi-square | P-Value |
| Age | Young | 22/214(10.28) | 34.22 | 0.038 | 07/220(3.18) | 38.35 | 0.026 |
| | Adult | 66/232(28.44) | | | 21/216(9.72) | | |
| Sex | Female | 76/338(22.48) | 16.43 | 0.026 | 23/306(7.51) | 17.22 | 0.048 |
| | Male | 12/108(11.11) | | | 05/140(3.57) | | |
| Feeding System | Ground | 53/210(25.23) | 18.33 | 0.053 | 20/229(8.73) | 21.42 | 0.053 |
| | Trough | 35/236(14.83) | | | 08/217(3.68) | | |
| Floor | Cemented | 20/203(09.85) | 17.43 | 0.045 | 07/232(3.01) | 14.32 | 0.033 |
| | Non-cemented | 68/243(27.98) | | | 21/214(9.81) | | |
| Housing | Open | 23/213(10.79) | 28.33 | 0.028 | 04/226(1.76) | 28.21 | 0.047 |
| | Closed | 63/233(27.03) | | | 24/220(10.9) | | |
| Herd size | >20 Animals | 58/231(25.10) | 19.33 | 0.046 | 21/209(10.0) | 21.44 | 0.056 |
| | <20 Animals | 28/215(13.02) | | | 07/237(2.95) | | |
| Body conditions | Good | 14/209(06.69) | 11.31 | 0.062 | 04/206(1.94) | 13.43 | 0.033 |
| | Poor | 64/237(27.00) | | | 24/240(10.0) | | |
| Ticks | Present | 55/203(27.09) | 22.53 | 0.057 | 22/224(9.82) | 27.43 | 0.036 |
| | Absent | 33/243(13.58) | | | 06/222(2.70) | | |
| Dog Association | Present | 56/213(26.29) | 20.43 | 0.035 | 19/226(8.40) | 32.45 | 0.065 |
| | Absent | 32/233(13.73) | | | 09/220(4.09) | | |

**Table 3. District wise prevalence of *Babesia ovis* in small ruminants in summer and winter season.**

| Districts | Specie | Summer | | | Winter | | |
|---|---|---|---|---|---|---|---|
| | | Prevalence % | Chi-square | P-Value | Prevalence % | Chi-square | P-Value |
| Faisalabad | Goat | 12/78(15.38) | 12.53 | 0.039 | 04/78(5.12) | 15.64 | 0.042 |
| | Sheep | 12/110(10.90) | | | 03/53(5.66) | | |
| Toba Tek Singh | Goat | 06/48(12.50) | 16.36 | 0.042 | 01/48(2.08) | 20.53 | 0.049 |
| | Sheep | 16/80(20.00) | | | 03/87(3.44) | | |
| Jhang | Goat | 10/48(20.83) | 21.53 | 0.050 | 03/48(6.25) | 19.43 | 0.027 |
| | Sheep | 32/82(39.02) | | | 14/132(10.60) | | |
| Total | Goat | 28/174(16.09) | 18.55 | 0.030 | 08/174(4.59) | 14.74 | 0.064 |
| | Sheep | 60/272(22.05) | | | 20/272(7.35) | | |

respectively. On the other hand, in winter results showed that 12.13% (33/272), 9.77% (17/174) and 13.60% (37/272), 13.21% (23/174) samples positive for *B. ovis* through PCR and nPCR respectively. The results have shown that sensitivity of nPCR is more as compared to conventional PCR. The product size of *B. ovis* through PCR and nPCR was 186 bp and 549 bp (Fig. 4 and Fig. 5)

**DISCUSSION**

Tick borne diseases (TBDs) are major threat to animals particularly in small ruminants common in temperate zone in tropical and sub-tropical countries (Misgana *et al.,* 2017; Zeb *et al.,* 2020). Most of them are zoonotic in nature and leads to severe economic losses to a country in terms of decreased production and trade allegations (Shah *et al.,* 2017; Jelicic *et al.,* 2020). Babesiosis is one of them, babesia was first discovered by Babes (1888) in the cattle populations of Romania that caused high mortalities; previously it was considered as bacteria. The prevalence of babesiosis in different parts of the world ranges from 0% to 100% (Jorgensen *et al*., 1992; Noaman, 2013).

Babesiosis is characterized by fever, decreased production, poor quality wool, anemia, hemoglobinuria and birth of weak offspring. Sheep and goats are prone to babesiosis and the disease endemic in different areas of Pakistan (Shahabuddin *et al.,* 2006; Rashid *et al.,* 2010; Iqbal *et al.,* 2011; Durrani *et al.,* 2011; Durrani *et al.,*2012; Naz *et al.,* 2012). Babesiosis is commonly diagnosed by microscopic examination (Reddy *et al.,* 2016; Haq *et al.,* 2017; Vannier *et al.,* 2020) but this practice is useful in acute cases where the parasitemia is high. Drawback of this technique is that subclinical infections and carrier animals can not be detected by this method. Another assay which is indirect fluorescent antibody assay (IFAT) also used previously but this method has also some limitations like lack of skilled persons in reading the microscopic slides of specimen (Persichetti *et al*., 2017). It is need of time to develop a more sensitive tool and accurate treatment of disease to decrease the economic losses because of the babesiosis. Different researchers were agreed with the fact that PCR and nPCR are more sensitive and specific tool for detection of hemoparasites without showing any apparent signs which mostly occurs in carrier animals (Talkhan et al., 2010; Shahnawaz *et al.,* 2011; Ziapour *et al.,* 2011). In the past sheep and goats were investigated separately either by PCR or nPCR, but the current study firstly, encompasses the collectively investigation of sheep and goat by two molecular techniques.

In the current study, the overall prevalence in summer based upon microscopic analysis was 22.05% (60/272) in sheep and 16.05% (28/174) in goats. In winter the prevalence was 7.34% (20/272) in sheep and 4.59% (8/174) in goat respectively. The prevalence reported on PCR and nPCR based upon molecular techniques was 29.7% (81/272) and 23.56% (41/174), 31.98% (87/272) & 27.01% (47/174) in sheep and goats respectively in summer. On the other hand, in winter results showed that 12.13% (33/272), 9.77% (17/174) and 13.60% (37/272), 13.21% (23/174) samples positive for *B. ovis* through PCR and n-PCR respectively. Similar findings have been reported by Iqbal *et al.,* (2012) in southern Punjab, Pakistan. They reported 50% samples positive through molecular techniques i.e., PCR and 3% detected positive via microscopic analysis. Similarly, 18% blood samples were reported positive through PCR as compared to microscopic 3% (Zulifqar *et al.,* 2012).

Further risk factors analysis of data revealed that, adult animals were more prone to babesiosis (28.44%) (*P = 0.026*) as compared to young ones. This is because of adult animals are active grazers and when they go out for grazing, they are more at risk to ticks secondly immunity decreases with increase in age, these findings were in accordance with previous literature (Niazi *et al.,* 2008). Females were more infected with babesiosis (22.48%) (*P = 0.048*) as compared to males. High prevalence in females might be due to the fact that females were reared for longer period of time, and female are more prone to physiological stress like milk production, breeding, parturition and post parturition as compared to male (Alim *et al.,* 2011; Kabir *et al*., 2011). High occurrence of disease was associated with non-cemented floor system (27.98%) (*P = 0.033*) as compared to cemented floor system, because in non-cemented there is no proper drainage system, cleanliness of shed along with some cracks in the floor which harbor the parasites that why the eggs of ticks were not properly removed that leads to the incidence of babesiosis in animals. Similar results have been reported by Roy *et al.,* (2018). Disease was found to be more prevalent in closed housing system (27.03%) (*P= 0.047*) as compared to open housing system. This is because of zoonotic nature of disease was more frequent in intensive farming (Tolkacz *et al.,* 2017). Weak animals were more infected with babesiosis (27%) (*P = 0.033*), as compared to healthy ones (Ward *et al*., 2018). It was also noted that animals with high tick infestations were more suffered with *Babesia* infection (27.09%) (*P = 0.036*) as compared to less tick burden. This finding was similar to Surez *et al.,* (2017). The transmission of disease is due to ticks because they act as an intermediate host **(**Ybanez *et al.,* 2019). According to current study presence of dogs (*P = 0.065*) and herd size (*P = 0.056*) has no effect on the prevalence of disease (Theodoropoulos *et al.,* (2006).

***Conclusion*:** *Babesia ovis* is much prevalent in sheep and goat herds that leads to huge economic losses in terms of poor growth rate, poor quality wool and increased treatment cost after disease outbreak. Commonly smear method is used for detection of blood parasite i.e., babesia, this is not useful for detection of carrier animals or very low parasitemia. Therefore, current study was conducted to investigate the babesiosis in ovine and caprine species in Faisalabad Division. PCR for the detection of *B. ovis* is specific and sensitive. The test is suitable for tracing carrier animal and provide a qualitative and validated measure that is useful in epidemiological surveys, follow ups for drug treatment and

ticks control programs in endemic areas to enhance the livestock productivity.

***Authors Contributions statement*:** AM and AR designed and executed the work; FJ and SN help in research work.

***Conflict of interest***: There is no conflict of interest

***Acknowledgement*:** Not applicable

## REFERENCES

Abed, M.J. and S.A. Hasso. 2017. Molecular detection of *Babesia bigemina* in ticks infesting water buffaloes (Bubalus bubalis) in Iraq. Al-Qadisiyah Journal of Veterinary Medical Science. 18:15-22.

AbouLaila, M., A.R. AbdEl-Aziz, S. Menshawy, N. Yokoyama, I. Igarashi, M. Al-Wabel and M. Omar. 2021. Evaluation of the inhibitory effects of coumermycin A1 on the growth of Theileria and Babesia parasites in vitro and in vivo. Pakistan Veterinary Journal. 41:469-474.

Alim, M.A., S. Das., K. Roy, M. Masuduzzaman, S. Sikder, M. Mahmudul and M.A. Hossain. 2011. Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. Veterinary Journal. 32:221-24.

Almeria S., J. Castella., D. Ferrer., O.A. Ortun., A.E. Pen and J.F. Gutierrez. 2001. Bovine piroplasms in minorca (Balearic Islands Spain): a comparison of PCR-based and light microscopy detection. Veterinary Parasitology. 99:249-259.

Anees, M., I. Ali, M.O. Sadiq N. Khan, I.Q. Afridi and N. Ali. 2020. Evaluation of Antibiotics Pattern of Extended Spectrum Beta-Lactamase Producing Multi-Drug Resistant *Pseudomonas aeruginosa*. Advancements in Life Sciences. 7:146-150.

Bukhari, A., N. Ahmed, F. Khan, M. Shafique and A.S. Sahi. 2020. Assessment of genetic diversity and genetic characterization of Nili Ravi buffalo breed utilizing microsatellite markers. Advancements in Life Sciences. 7:277-280.

Durrani, A.Z., M. Younus., N. Kamal., N. Mehmood and A.R. Shakoori. 2011. Prevalence of ovine Theileria species in district Lahore, Pakistan. Pakistan Journal of Zoology. 43:234-47.

Durrani, S., Z. Khan., R.M. Khattak., M. Andleeb., M. Ali., H. Hameed and M. Riaz. 2012. A comparison of the presence of *Theileria ovis* by PCR amplification of their SSU rRNA gene in small ruminants from two provinces of Pakistan. Asian Pacific Journal of Tropical Diseases. 2:43-47.

Esmaeilnejad, B., M. Tavassoli., S. Asri-Rezaei., B.D. Naghadeh., K. Mardani., G.J. Amin and J. Arjmand. 2014. PCR-based detection of *Babesia ovis* in *Rhipicephalus* bursa and small ruminants. Journal of Parasitology Research. 12:77-102.

Fakhar, M., A. Hajihasani, S. Maroufi, H. Alizadeh, H. Shirzad, F. Piri and A.S. Pagheh. 2012. An epidemiological survey on bovine and ovine babesiosis in Kurdistan Province, western Iran. Tropical Animal Health and Production. 44:319-322.

Haghi, M.M., F. Etemadifa., M. Fakhar, S.H. Teshnizi, M. Soosaraei, A. Shokri and H. Mashhadi. 2013. Status of babesiosis among domestic herbivores in Iran: A systematic review and meta-analysis. Parasitology Research. 116:1101-09.

Haq, A.U., N.A. Tufani, M.B. Gugjoo, S.U. Nabi and H.U. Malik. 2017. Therapeutic amelioration of severely anemic local Kashmiri goats affected with babesiosis. Advances in Animal and Veterinary Science. 5:463-67.

Hurtado, O.J.B and C. Giraldo-Ríos. 2018. Economic and health impact of the ticks in production animals. In Ticks and Tick-Borne Pathogens. Intech Open. 4:342-49.

Hussain, S., K. Ashraf., N. Anwar., M.A. Jamal., H. Naeem., N. Ahmad and A.U. Rahman. 2017. Diagnosis of *Babesia bovis* infection in indigenous and crossbred cattle with comparison between conventional and molecular diagnostic techniques. Journal of Infections and Molecular Biology. 5:1-6.

Iqbal F, M. Ali., M. Fatima., S. Shahnawaz., S. Zulifqar, R. Fatima., R.S. Shaikh., A.S. Shaikh., M. Aktas and M. Ali. 2011. A Study on prevalence and determination of the risk factors of infection with *Babesia ovis* in small ruminants from southern Punjab (Pakistan) by PCR amplification. Parasite. 18:229-234.

Iqbal, F., R.M. Khattak., S. Ozubek., M.N.K. Khattak., A. Rasul and M. Aktas. 2012. Application of the reverse line blot assay for the molecular detection of Theileria and Babesia spp. in sheep and goat blood samples from Pakistan. Iranian Journal of Parasitology. 8:289-295.

Jelicic, M.S. 2020. Ticks and Tick-borne diseases surveillance with special reference to Babesia infections in small ruminants in northern Greece (Master's thesis). pp. 1-110.

Jorgensen, W.K., D.J. Weilgama, M. Navaratne and R.J. Dalgliesh. 1992. Prevalence of *Babesia bovis* and *Anaplasma marginale* at selected localities in Sri Lanka. Tropical Animal Health and Production. 24: 9-14.

Kabir, M.H.B., M.M.H. Mondal, M. Eliyas, M.A. Mannan, M.A. Hashem, N.C. Debnath, O.F. Miazi, C. Mohiuddin, M.A. Kashem, M.R. Islam and M.F. Elahi 2011. An epidemiological survey on investigation of tick infestation in cattle at Chittagong District, Bangladesh. African Journal Microbiological Research. 5:346-352.

Karatepe, B., S. Ozubek., M. Karatepe and M. Aktas. 2019. Detection of Theileria and Babesia species in sheep and goats by microscopy and molecular methods in Nigde province, Turkey. Review in Medicine Vet-Toulouse. 170:136-143.

Mathieu, A., A.R. Pastor., C.N. Berkvens., C.G. Boivin., M. Hébert., A.N. Léveillé and A.D. Smith. 2018. *Babesia odocoilei* as a cause of mortality in captive cervids in Canada. Canadian Veterinary Journal. 59:52-56.

Misgana, N., 2017. Species Diversity and Seasonal Pattern of Ticks and Tick-Borne Pathogens of Cattle in Ada'a And Boset Districts, Central Oromia, Ethiopia. Addis Ababa University, Addis Ababa, Ethiopia. Parasite Epidemiology and Control. 45:23-56.

Muhammad, A., S. Nawaz, Z.A. Bhutta, M.F.A. Kulyar, M. Rashid, S. Shabir, M.K. Khan and M. Qadir. 2020. Viral outbreaks: A real threat to the world. Advancements in Life Sciences. 8:08-19.

Naz, S., A. Maqbool., S. Ahmed., K. Ashraf., N. Ahmed., K. Saeed and I.A. Nagra. 2012. Prevalence of theileriosis in small ruminants in Lahore-Pakistan. Journal of Veterinary and Animal Science. 2:16-20.

Niazi, N., M.S. Khan., M. Avais., J.A. Khan., K. Pervez and M. Ijaz. 2008. A study on babesiosis in calves at livestock experimental station Qadirabad and adjacent areas, Sahiwal (Pakistan). Pakistan Journal of Agricultural Sciences. 45:209-11.

Noaman, V. 2013. A molecular study on Theileria and Babesia in cattle from Isfahan province, Central Iran. Journal of Parasitic Diseases. 37:208-210.

Persichetti, M. F., G. L. Solano., A. Vullo., M. Masucci., P. Marty., P. Delaunay and M.G. Pennisi. 2017. Diagnostic performance of ELISA, IFAT and Western blot for the detection of anti-Leishmania infantum antibodies in cats using a Bayesian analysis without a gold standard. Parasites and Vectors. 10:1-8.

Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff. 2007. Veterinary Medicine: A textbook of the disease of cattle, sheep, pigs, goats and horses. 9th Ed, Saunders Elsevier Ltd, Pennsylvania, USA.

Rahat, M.A., M. Haris, Z. Ullah, S.G. Ayaz, M. Nouman, A. Rasool and M. Israr. 2020. Domestic animals' identification using PCR-RFLP analysis of cytochrome b gene. Advancements in Life Sciences. 7:113-116.

Rahman, A., E. Hashim, F.S. Alzahrani and A.M.E.H. Sulieman. 2020. Potential of Camel Dung as Promising Organic Manure in Saudi Arabia. Advancements in Life Sciences. 7:202-207.

Rashid, A., J.A. Khan., M.S. Khan., K. Rasheed., A. Maqbool and J. Iqbal. 2010. Prevalence and chemotherapy of babesiosis among Lohi sheep in the Livestock Experiment Station, Qadirabad, Pakistan, and environs. Journal of Venom and Animal Toxins. 16:587-91.

Reddy, B.S., S. Sivajothi., L.V. Reddy and K.S. Raju. 2016. Clinical and laboratory findings of Babesia infection in dogs. Journal of Parasitic Diseases. 40:268-72.

Roy, B.C., J. Krücken., J.S. Ahmed., S. Majumder., M.P. Baumann., P.H. Clausen and A.M. Nijhof. 2018. Molecular identification of tick-borne pathogens infecting cattle in Mymensingh district of Bangladesh reveals emerging species of Anaplasma and Babesia. Transboundary Emerging Diseases. 65:231-42.

Shah, S. S.A., M.I. Khan and H.U. Rahman. 2017. Epidemiological and hematological investigations of tick-borne diseases in small ruminants in Peshawar and Khyber agency. Pakistan. Journal of Advanced Parasitology. 4:222-34.

Shahabuddin, Y. Nawaz., J. Nawaz and M. Nawaz. 2006. Epidemiological study on the occurrence of natural babesiosis in sheep and goats of Balochistan, Pakistan. Proceedings Parasitology. 42:47-60.

Shahnawaz, S., M. Ali., M.A. Aslam., R. Fatima., Z.I. Chaudhry., M.U. Hassan and F. Iqbal. 2011. A study on the prevalence of a tick-transmitted pathogen, *Theileria annulata*, and hematological profile of cattle from Southern Punjab (Pakistan). Parasitology Research. 109:11-55.

Shahzad, W., N. Haider., A. Mansur-ud-Din., R. Munir., M.S. Saghar., M.H. Mushtaq and F. Mehmood. 2013. Prevalence and molecular diagnosis of *Babesia ovis* and *Theileria ovis* in Lohi sheep at livestock experiment station (LES), Bahadurnagar, Okara, Pakistan. Iranian Journal of Parasitology. 8:570-87.

Štrbac, F., A. Bosco, A. Amadesi, L. Rinaldi, D. Stojanović, N. Simin, D. Orčić, I. Pušić, S. Krnjajić and R. Ratajac. 2021. Ovicidal potential of five different essential oils to control gastrointestinal nematodes of sheep. Pakistan Veterinary Journal. 41(3): 353-358.

Suarez, C.E., R.P. Bishop., H.F. Alzan., W.A. Poole and B.M. Cooke. 2017. Advances in the application of genetic manipulation methods to apicomplexan parasites. International Journal of Parasitology. 47:701-10.

Talkhan O.F.A., M.E.I. Radwan and M.A. Ali. 2010. Cattle babesiosis and associated biochemical alteration in Kalubyia Governorate. Nature Science. 12:24-27.

Theodoropoulos, G., M. Gazouli, J.A. Ikonomopoulos, V. Kantzoura and A. Kominaki. 2006. Determination of prevalence and risk factors of infection with Babesia in small ruminants from Greece by polymerase chain reaction amplification. Veterinary Parasitology. 135:99-104.

Tołkacz, K., M. Bednarska, M. Alsarraf, D. Dwużnik, M. Grzybek, R.W. Falęciak and A. Bajer. 2017. Prevalence, genetic identity and vertical transmission of Babesia microti in three naturally infected species of vole, Microtus spp. (Cricetidae). Parasites and Vectors. 10: 1-12.

Vannier, E and P.J. Krause. 2020. Babesiosis. In Hunter's Tropical Medicine and Emerging Infectious Diseases. pp. 799-802.

Wang, J., J. Zhang., P. Kelly., X. Zheng., M. Li., J. You and J. Li. 2018. First description of the pathogenicity of *Babesia vogeli* in experimentally infected dogs. Veterinary Parasitology. 253:1-7.

Ward, S.J., S.L. Stramer and Z. M. Szczepiorkowski. 2018. Assessing the risk of Babesia to the United States blood

supply using a risk-based decision-making approach: Report of AABB's Ad Hoc Babesia Policy Working Group (original report). Transfusion. 58:1916-23.

Ybanez, A.P., T. Sivakumar, R.H.D. Ybañez., M.R.B. Vincoy., J.A. Tingson., Z.O. Perez and H. Inokuma.2019. Molecular survey of bovine vector-borne pathogens in Cebu, Philippines. Veterinary Parasitology. 196:13-20.

Young, K.M., T. Corrin., B. Wilhelm., C. Uhland., J. Greig., M. Mascarenhas and L.A. Waddell. 2019. Zoonotic Babesia: A scoping review of the global evidence. Plos one 14:187-213.

Zeb. J., S. Shams., I.U. Din., S. Ayaz., A. Khan., N. Nasreen and H. Senbill. 2020. Molecular epidemiology and associated risk factors of *Anaplasma marginale* and *Theileria annulata* in cattle from North-western Pakistan. Veterinary Parasitology. 279:1090-1144.

Zheng, W. Q., X.N. Xuan., R.L. Fu., H.Y. Tao., Y.Q. Liu and X.Q. Liu. 2018. Tick-borne pathogens in ixodid ticks from Poyang Lake Region, southeastern China. Korean Journal of Parasitology. 56:589-596.

Ziapour, S.P., S. Kheiri., M. Fazeli-Dinan., F. Sahraei-Rostami., R.A. Mohammadpour., M. Aarabi and A. Enayati. 2011. Susceptibility status of field populations of Rhipicephalus bursa (Acari: Ixodidae) to pyrethroid insecticides. Tropical Biomedicine. 33:446-461.

Zulfiqar, S., S. Shahnawaz., M Ali, A.M. Bhutta., S. Iqbal., S. Hayat and F. Iqbal. 2012. Detection of *Babesia bovis* in blood samples and its effect on the hematological and serum biochemical profile in large ruminants from Southern Punjab. Asian Pacific Journal of Tropical Biomedicine. 2:104-108.