Saudi Journal of Biological Sciences 29 (2022) 2014–2025

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Review

Tick-borne haemoparasitic diseases in small ruminants in Pakistan: Current knowledge and future perspectives

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ARTICLE INFO

Article history:
Received 7 September 2021
Revised 8 November 2021
Accepted 20 December 2021
Available online 29 December 2021

Keywords:
Theileria
Anaplasma
Babesia
Goats
Sheep
Pakistan

ABSTRACT

Livestock industry is an essential part of Pakistan's economy, and a variety of ruminants (including sheep and goats) are reared for the increasing demand of milk, meat and hide products. Haemoparasitic illnesses such as *theileriosis, anaplasmosis*, and *babesiosis* are a significant health risk for small ruminants in our country. Information regarding distribution patterns, the tick species involved and effective strategies to control tick-borne diseases (TBD) in goats and sheep of Pakistan is limited. To this end, it is required to assess the present rank of TBDs in small ruminants of Pakistan with a note on their vector ticks in order to control and identify the gaps in the knowledge of TBDs. This will recommend areas for future research and will add to the understanding of these diseases and will draw attention to the need for better-quality tools for the diagnosis and control of TBDs in small ruminants of Pakistan.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.12.046
1319-562X/

1. Livestock sector of Pakistan

In Pakistan like country with a predominantly rain-fed agricultural production system and livestock production provides security against crop-failure. For low-income populations living in rural villages, livestock is a form of social security that can be cashed at the time of need. Livestock are also a resource for sports and entertainment, and are also considered as symbols of pride and prestige in rural areas of Pakistan (Zulfiqar et al., 2012). The Pakistan livestock sector is represented chiefly by small farm holders; 30–35 million people in the rural area rely on the livestock industry for their incomes, as 80% of the country's milk is produced by rural stock holders and commercial producers (de Castro, 1997). According to the 2013/14 Pakistan Livestock Survey, the livestock industry within the agricultural economy doubled from 25.3% in 1996 to 55%. The gross value of the livestock increased from Rs. 756.3 billion in 2012/ 13 to Rs. 776.5 billion in 2013/14, as compared to the previous year an increase of 2.7% (Government of Pakistan, 2017). According to Pakistan economic survey of 2020–21, conducted by ministry of finance, Government of Pakistan, livestock have a share of 60.07 % in agriculture and 11.53 % in GDP and have achieved a growth of 3.06 percent (Government of Pakistan, 2021).

2. Small ruminants of Pakistan

Small ruminants like goat and sheep are the livestock of choice of many Pakistani small farm holders due to their fast growth, economic and less hectic farming. Preference for goat and sheep meat over the flesh of large ruminants is another major reason for increased demand and hence increased production of small ruminants in Pakistan (Abubakar et al., 2015). In Pakistan, goats are mainly found in the plain regions, while sheep are reared mostly in mountainous ranges (Khan, 2004). Pakistan have the world's third largest populations of small ruminants and the estimated small ruminant population in Pakistan is almost 99 million, consisting of goats (68.4 million: 25 breeds) and sheep (29.4 million: 24 breeds) (Rehman et al., 2017). In domesticated animals, Punjab Province of the Pakistan is richer in animal genetic resources as it is the home tract of world famous Kajli sheep and Beetal goat that are increasingly raised here to meet the growing demand for meat and milk (Khan, 2004). The livestock sector is economically more important in Khyber Pukhtoonkhwa (Zulfiqar et al., 2012) (KPK) province as compared to Sindh and Punjab as these provinces have well-established industrial sectors and suitable land for crops. According to an approximation in KPK, livestock sector contributed 62.8 billion rupees to the national exchequer (Agricultural Census Organization (Pakistan), 1997). Raising livestock is also one of the major economic sources of livelihood for people living in the rural population of Baluchistan. A significant portion of national livestock population, particularly sheep and goats is reared in this part of the country (sheep 48% and goats 22% of the national flock). About 75% population of the province are reliant on livestock and agriculture (Raziq et al., 2010). Despite of the vital importance of livestock and dependency of farmers the productivity is far below than the actual potential due to several factors including inadequate feed resources, finance shortage, limited health facilities and unawareness of artificial insemination. Majority of farmers have low adoption of recommended practices and remain unaware of new practices along with poor dissemination of information through information sources leads to reduced output (Zahur et al., 2006).

3. Ticks infesting small ruminants in Pakistan

Disease is a major threat to the livestock sector of developing countries like Pakistan because of the associated animal mortality, constraints to livestock improvement and reduction in animal productivity (Oura et al., 2004). As production of small ruminants in Pakistan usually involves the animals being reared outdoors, they are exposed to a variety of vector-borne diseases that are transmitted by arthropods like ticks, fleas and mosquitoes (Maske et al., 1990). Hundreds of bacteria, viruses, protozoa and helminths have been found to require a hematophagous (**blood sucking**) arthropod for the transmission between vertebrate host (Muraleedharan, 2005). As compared to any other group of blood-sucking arthropods ticks transmit more pathogen species around the world, affecting both humans and livestock (McCall et al., 2007). Ticks belong to the class Arachnida and morphologically and biologically, they are divided into two groups: the soft ticks (*Argasidae*) and the hard ticks (*Ixodidae*) (Rahlenbeck et al., 2016). The three families of ticks contain 900 species. The family of soft ticks (*Argasidae)* contain 191 species while the family of hard ticks (*Ixodidae)* contain 701 species and the 3rd family is *Nuttalliellidae* which consist only one species, *Nuttalliella namaqua* (Guglielmone et al., 2010). Tick's life cycle in all stages depend on feeding on blood, despite the fact that the feeding target frequently changes with each developmental stage (Ewing et al., 1997). Ticks normally feed on ruminants, during the nymph and adult stages, especially when transmission of disease occurs between species (Palmer et al., 2001). During feeding from the infected host ticks acquire blood and transfer the ***rickettsia*** in the next stage onto its host after molting between their salivary glands (Munderloh et al., 2005). Tick infestation results in clinical and sub clinical infections in host animals resulting in anaemia, dermatitis, paralysis, otoacariasis and other clinical signs that can lead to reduced milk and meat production resulting in economic loss to the livestock owners **(**Sajid et al., 2017**)(**Zeb et al., 2019**)**. The distribution of ticks in any region is closely associated with certain biotic and abiotic features. For example relative humidity range of 60–80% and temperature of 27–39 °C are ideal for tick growth and survival (Guglielmone et al., 2010). Within south Asia Pakistan being located in a subtropical zone (30° N, 70° E) having favourable environmental conditions for the development of ticks and for the transmission of TBD such as babesiosis, anaplasmosis, and theileriosis (Naz et al., 2012). Although there has been some documentation of the ticks prevalence in various agrosystems of Pakistan, and these are reported to be associated with economic losses, about the diversity of ticks information is still missing that infest the small ruminant population in Pakistan. Keeping this is mind; we summarize here all the published literature regarding tick diversity as reported in small ruminants of Pakistan along with their geographical distribution (Table 1). Ramzan et al. (Ramzan et al., 2020) identified *Rhipicephalus sanguineus, Hyalomma anatolicum and Hyalomma marginatuma* from small ruminants from Multan District and they observed higher tick infestation in sheep than in goats (Fig. 1) (Table 1). Ali et al. (Ali et al., 2019) had col-

Table 1
Diversity and geographical distribution of ticks hosting small ruminants in Pakistan.

Sampling locations	Host Animals	*Hyalomma* spp.	*Haemaphysalis* spp.	*Rhipicephalus* spp.	*Dermacentor* spp.	Other tick species	Parasites detected in ticks	References
Multan District in Punjab	Sheep and goat	*Hyalomma anatolicum, Hyalomma marginatum*	–	*Rhipicephalus sanguineus*	–	–	–	(Ramzan et al., 2019)
Khyber Pakhtunkhwa (various parts)	Cattle, buffalo, sheep, goat, domestic fowl, dogs, horse	*Hyalomma impeltatum, Hyalomma anatolicum, Hyalomma marginatum*	*Haemaphysalis montgomeryi, Haemaphysalis indica, Haemaphysalis punctata*	*Rhipicephalus microplus, Rhipicephalus turanicus, Rhipicephalus haemaphysaloides, Rhipicephalus annulatus, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides*	*Dermacentor marginatus*	*Argas persicus* *Amblyomma gervaisi, Amblyomma exornatum, Amblyomma latum*	Not applicable	(Ali et al., 2019)
Mansehra, Haripur, Shangala and Kohistan Districts in KPK and Gilgit Baltistan	Sheep,goats, cattle and buffaloes	*Hyalomma anatolicum*	–	*Rhipicephalus microplus*		–	Not applicable	(Sajid et al., 2018)
Punjab, Sindh, KPK, Baluchistan Provinces and Gilgit-Baltistan	Cattle, buffalo, sheep, goat, camel, poultry, cat, dog	*Hyalomma anatolicum*	*Haemaphysalis dromedarii*	*Rhipicephalus microplus*		*Ornithodorus Argas.*	Candidatus Rickettsia amblyommii, *Theilleria annulata*	(Karim et al., 2017)
Muzaffar Garh and Layyah Districts in Punjab	Goat	–	–	*Rhipicephalus sanguineus*	–	–	Not applicable	(Sajid et al., 2017)
Lahore District in Punjab	Sheep	*Hyalomma* spp.	–	*Rhipicephalus* spp.	–	–	*Theileria ovis*, *Theileria lestoquardi*	(Durrani et al., 2011)
southern Punjab	goats	*Hyalomma* spp	–	*Rhipicephalus sanguineus*	–	–	–	(Sajid et al., 2020)
KP	cows and buffaloes	*Hyalomma* spp	–	*Rhipicephalus* spp	–			(N. Nasreen et al., 2020)
Lower Dir, Upper Dir, Chitral, Bajaur and Malakand	Cattle	*Hyalomma anatolicum, Hyalomma marginatum*	–	*Rhipicephalus microplus*	–	–	–	(Zeb et al., 2020)
District Pishin, Baluchistan	Cows	*Hylomma anatolicum, Hylomma dromedarii, Hyalomma truncatum, Hylomma scupense*			*Dermacentor andaroni, Dermacentor Variabilis*	*Annu -latus boo -philus Amblyomma americanum*		(Iqbal et al., 2021)
FATA	Goats Sheep	*Hyalomma anatolicum, Hyalomma scupense, Hyalomma detritum*	*Haemaphysalis punctata, Haemaphysalis sulcata,*	*Rhipicephalus sanguineus Rhipicephalus microplus,*				(Adil Khan et al., 2019)
Multan District in Punjab	Goats sheep	*Hyalomma anatolicum, Hyalomma marginatuma*		*Rhipicephalus sanguineus*				(Ramzan et al., 2020)
Bannu, Khyber Pakhtunkhwa	Sheep Goats	*Hyalomma anatolicum, Hyalomma marginatum*	*Haemaphysalis aciculifer*	*Rhipicephalus microplus, Rhipicephalus annulatus, Rhipicephalus turanicus*			NA	(Rooman et al., 2021)

Table 1 (*continued*)

Sampling locations	Host Animals	*Hyalomma* spp.	*Haemaphysalis* spp.	*Rhipicephalus* spp.	*Dermacentor* spp.	Other tick species	Parasites detected in ticks	References
ex-FATA region (Federally Administered Tribal Area), namely Bajaur, Mohmand, Khyber, Orakzai, and North Waziristan	Sheep Goats	*Hyalomma anatolicum*	*Haemaphysalis punctata, Haemaphysalis sulcata*	*Rhipicephalus haemaphysaloides, Rhipicephalus microplus, Rhipicephalus turanicus,*			*Rickettsia massiliae, Theileria* spp., *Anaplasma ovis, Rickettsia slovaca, Anaplasma marginale, Anaplasma centrale,* and Ehrlichia	(Ghafar et al., 2020)
Different zones of Punjab	Sheep Goats	*Hyalomma anatolicum, Hyalomma marginatum, Hyalomma dromedari,*		*Rhipicephalus sanguineus, Rhipicephalus microplus, Rhipicephalus appendiculatus, Rhipicephalys decolaratus*			*NA*	(Batool et al., 2019)
Harnai, Baluchistan	Sheep Goats	*Hyalomma anatolicum, Hyalomma dromedarii,*		*Rhipicepahalus annulatus, Rhipicephalus microplus*		*Amblyomma hebraeum*		(Bibi et al., 2020)
Different zones of Punjab province	Goat Sheep	*Hyalomma anatolicum, Hyalomma dromedarii,*		*Rhipicephalus microplus*			*Theileria ovis, Anaplasma ovis*	(Rehman et al., 2019)
Multan District in Punjab	Goat	*Haemaphysalis* spp.		*Rhipicephalus* spp.	–	–	–	(Riaz et al., 2019)

lected large number of ticks infesting diverse hosts including goat and sheep from various regions of Khyber Pakhtunkhwa (KPK) Pakistan and morpho-taxonomically categorized ticks into six genera comprising of 17 species (Table 1). Sajid et al. (Sajid et al., 2017) collected ticks from free range livestock population of the plain and hilly areas of Kohistan, Mansehra, Gilgit Baltistan, Haripur and Shangla. 75.03% of enrolled animals (with majority of sheep) were tick infested. The two tick species that were identified were *Hyalomma anatolicum* and *Rhipicephalus microplus*. Highest tick prevalence was observed in young animals and in females than adults and males respectively (Table 1). Karim et al. (Karim et al., 2017) had morphologically identified 3866 tick specimens from all four provinces and Gilgit-Baltistan of Pakistan and identified 19 different tick species representing three hard tick genera, *Rhipicephalus, Haemaphysalis* and *Hyalomma*, and two soft tick genera, *Ornithodorus* and *Argas* (Table 1). Sajid et al. (Sajid et al., 2017) had collected 800 tick samples from goats in Muzaffar Garh and Layyah districts in Punjab and reported an overall 60.1% goats from both districts were tick infested. The tick prevalence was higher on goats of district Muzaffar Garh than in district Layyah. In both districts *Rhipicephalus sanguineus* was the predominant species. Lowest tick infestation in goat was observed during November and December and highest was documented during July. Regarding host determinants, younger animals were more burdened than older ones and female animals were more heavily infested than males. Teddy goats were the most susceptible breeds for ticks followed in order by Nachi, Beetal and crossbred (Table 1). Durrani et al. (Durrani et al., 2011) had collected 100 ticks from sheep in District Lahore during spring and summer seasons The most commonly identified tick were belonging to *Hyalomma* followed by *Rhipicephalus* and *Boophilus*. They had also reported that ticks were infected with *Theileria ovis* and *Theileria lestoquardi* (Table 1). Khan et al., 2019a,b conducted a comprehensive study targeted more than 300 small ruminants in federally administered tribal areas (exFATA) of Pakistan and concluded that *Rhipicephalus sanguineus* was observed on 135 (46.6%) sampled of small ruminants, followed by *Hyalomma excavatum* (0.3%), *Rhipicephalus microplus* (10.7%), *Hyalomma scupense* (3.4%), *Haemaphysalis punctata* (1.7%), *Hyalomma detritum* (0.7%), *Hyalomma anatolicum* (10.7%) and *Haemaphysalis sulcata* (25.9%). Iqbal et al., 2021 reported, *Dermacentor andaroni, Hylomma dromedarii, Hyalomma truncatum, Hylomma scupense, Dermacentor variabilis, Boophilus annulatus and Amblyomma americanum* and *Hylomma anatolicum* from Balochistan (Table 1). Zeb et al., 2020 reported *Hyalomma anatolicum, Hyalomma marginatum* and *Rhipicephalus microplus* from lower and upper Dir, Chitral, Bajaur and Malakand (Table 1). Nasreen et al. (2020)a,b reported some *Hyalomma* and *Rhipicephalus* spp. From different district of KP (Table 1). Sajid et al., 2020 reported the presence of *Rhipicephalus sanguineus and some Hyalomma* spp. **from** the goats of southern Punjab. Sajid et al. Sajid et al., 2018 conducted a study in Mansehra, Haripur, Shangala and Kohistan and identified *Rhipicephalus microplus and Hyalomma anatolicum* species (Table 1). In a study from Punjab province, Batool et al., 2019 had collected ticks from sheep and goats and reported that the most common tick species in goatswere *Hyalomma anatolicum, Hyalomma marginatum, Hyalomma dromedari, Rhipicephalus sanguineus, Rhipicephalus microplus, Rhipicephalus appendiculatus, Rhipicephalys decolaratus,* whereas the prevalent tick species in sheep were *Hyalomma anatolicum, Rhipicephalus sanguineus, Rhipicephalus microplus, Rhipicephalus appendiculatus* and *Rhipicephalys decolaratus.* Rehman et al., 2019 has also identified *Hyalomma anatolicum, Hyalomma dromedarii* and *Rhipicephalus microplus* ticks infesting small ruminants. Ghafar et al., 2020 conducted a study in five districts of Pakistan's ex-FATA region (Federally Administered Tribal Area), namely Bajaur, Mohmand, Khyber, Orakzai, and North Waziristan,

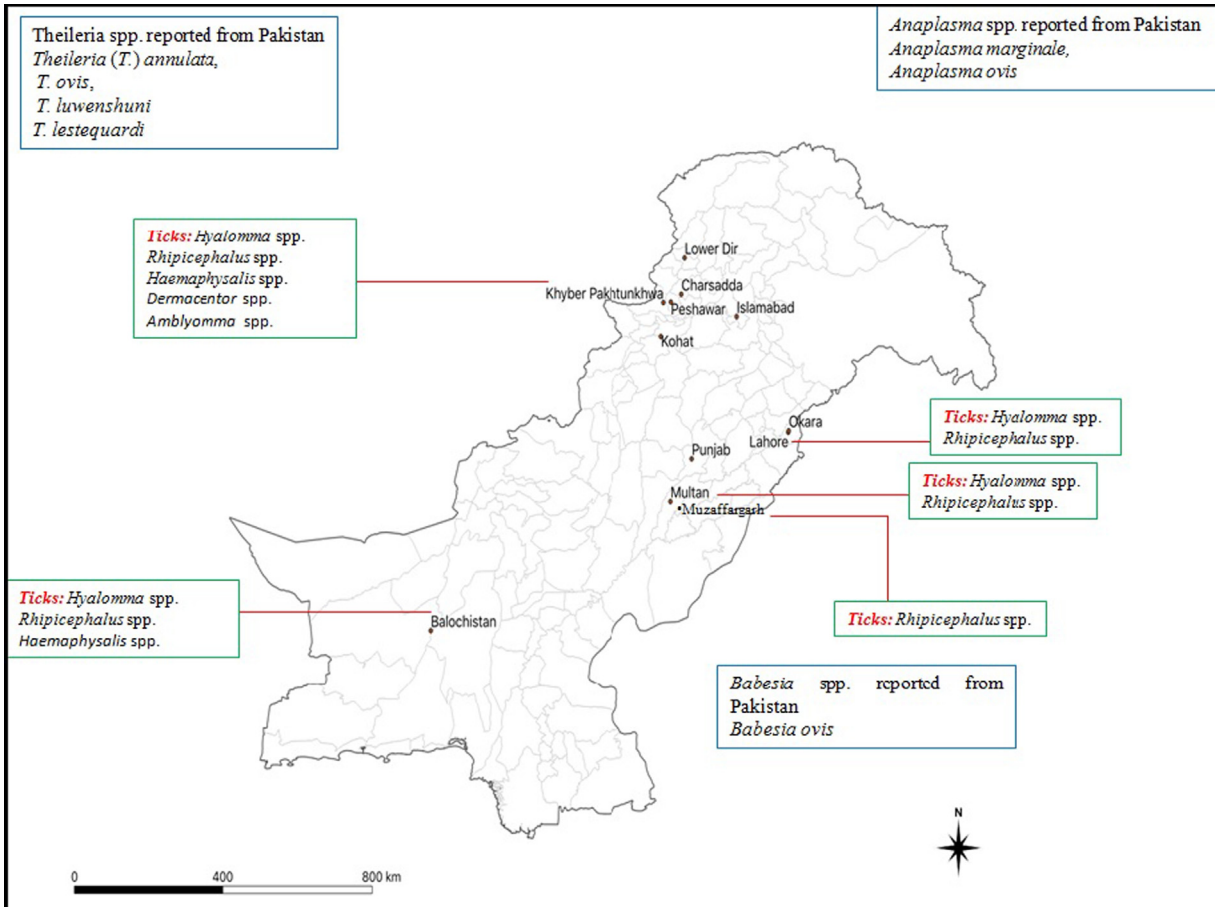

Fig. 1. Map of Pakistan showing the sampling sites from where small ruminants were enrolled to report the tick, Theileria, Babesia, and Anaplasma spp. diversity as reported from various studies from this country.

to assess the diversity of ticks and tick-borne diseases in small ruminants. The collected ticks were morphologically and molecularly characterized, revealing the presence of six Ixodid tick species i.e., *Rhipicephalus haemaphysaloides*, *Rhipicephalus microplus*, *Rhipicephalus turanicus*, *Haemaphysalis punctata*, *Haemaphysalis sulcata* and *Hyalomma anatolicum*. In a study conducted in Multan district in Punjab, Riaz et al., 2019 had reported that enrolled goats were infested with *Rhipicephalus* spp. and *Haemaphysalis* spp. and considered them as vector for ovine theileriosis.

Bibi et al., 2020 had also conducted a study at Harnai district, Balochistan and reported that *Amblyomma hebraeum, Hyalomma anatolicum, Hyalomma dromedarii, Rhipicephalus boophilus* were infesting the enrolled goats whereas *Hyalomma anatolicum, Hyalomma dromedarii, Rhipicepahalus annulatus* and *Rhipicephalus microplus* were collected and identified from sheep.

In a recent study from district Banu in KPK, Rooman et al., 2021 had collected hard ticks infesting from sheep and goats and reported the presence of *Rhipicephalus microplus, Rhipicephalus annulatus, Hyalomma anatolicum, Hyalomma marginatum, Rhipicephalus turanicus* on the enrolled small ruminants. Rooman et al., 2021 had also reported the genetic diversity of collected *Rhipicephalus microplus* by amplifying their two genetic markers: 16S ribosomal RNA (16S rRNA) and internal transcribed spacer 2 (ITS2) genes. The amplified gene fragments of *Rhipicephalus microplus* clustered in clade with *R. microplus* gene sequences from China, India and Pakistan. In another recent study, Hussain et al., 2021 has reported the tick burden at livestock farms in various districts of Punjab They did not report the tick taxonomy but has provided important information about perception and practices of livestock farmers regarding tick infestation. Hussain et al., 2021 has reported that 37.5% of investigated farms were infested with ticks. Only 28.6% of the dairy farmers were consulting veterinarians for ticks control, while 86.7% of the respondents did not consider bio security measures in the control of tick transmission. Most of the farmers were unaware that manual tick removal from their animals can lead to spreading of zoonotic diseases. Hussain et al., 2021 observed the highest tick infestation in Sheikhpura and Vehari (50%) districts followed by Kasur and Muzaffargarh (43.7%), Gujranwala and Bahawalnagar (31.3%) districts whereas tick burden was lower in district Khushab (12.5%).

This quick review of existing literature is providing us the initial patterns of distribution of some of the most commonly detected and identified tick species but large scale and extensive tick studies are recommended throughout the country. These surveys must focus to the areas that were never explored before for the tick diversity on small ruminants. This will not only provide us a complete list of ticks that are infesting our livestock but these surveys will confirm the distribution pattern of already reported ticks in unexplored areas and will also provide an opportunity to report the unreported ticks from Pakistan.

4. Tick borne diseases of small ruminants from Pakistan

The arid regions of Pakistan are notorious for the presence of the vector: tick (*Acari: Ixodidae*) and tick-borne diseases of domesticated animals as the humid and hot climate is highly encouraging and favors the survivability and multiplication of ticks (Khan et al., 2004). Three main tick borne diseases, theileriosis, anaplasmosis

and babesiosis are reported to affect small ruminants and cause huge economic losses in Pakistan (Jabbar et al., 2015). Several conventional and molecular techniques are in use for the detection of tick-borne parasites in wild and domestic animals. Blood smear screening is a conventional for morphological identification of parasites. This method is less expensive and in this do not require costly apparatus and reagents but it cannot be applied to all situations and for this technique a well-experienced microscopist is required. A great deal of work is being focused on the standardization and development of molecular techniques, which would be suitable in diagnosing parasitic infections as well as monitoring chronic phases of disease, treatment, and reactivation responses. These modern molecular technique for the detection of tick born parasites in small ruminants include polymerase chain reaction (PCR), enzyme linked immuno sorbent assay (ELISA), restriction fragment length polymorphism (RFLP), reverse line bolt (RLB), microsatellite marker method. A great deal of work is being focused on the development and standardization of molecular methods, which would be useful in diagnosing parasitic infections as well as monitoring chronic phases of disease, reactivation, and treatment responses (Figueroa et al., 1993)(Garibyan and Avashia, 2013)(Kim et al., 2007)(Iqbal et al., 2013)(Ullah et al., 2018)(Riaz and Tasawar, 2017)(Khan et al., 2017)(d'Oliveira et al., 1995)(Fatima et al., 2015)(Gomes and Inácio, 2015). In the next part of this chapter, we are going to briefly introduce theileriosis, babesiosis and anaplasmosis and their documented status in small ruminants from Pakistan.

4.1. Theileriosis

Theileriosis is a tick-borne haemoparasitic infection which leads to heavy economic losses in the livestock industry due to its association with high morbidity and mortality (Schnittger et al., 2000). The pathogenic *Theileria* species that infect small ruminants include *Theileria* (*T.*) *lestoguardi, T. leuwenshuni, T. ovis, T. separate* and the newly identified *Theileria* sp. *China 1*. (Chae et al., 1999). In small ruminants, theileriosis is mostly transmitted by ticks of the *Rhipicephalus*, *Haemaphysalis, Amblyomna* and *Hyalomma* genera (Bhispo et al., 2009). The disease presents with fever, weakness, anorexia, conjunctival petechiae, swollen lymph nodes, anemia and coughing. Symptoms such as nasal and ocular discharges, pyrexia, leukopenia and pale mucous membranes have also been observed (Naz et al., 2012). The signs and symptoms in later stages of the disease include diarrhea, dysentery, recumbency and increased risk of viral, bacterial, and fungal infections due to immunosuppression (Schnittger et al., 2000). If left untreated, this disease can cause mortality within 3–4 weeks due to lymphocytosis (Li et al., 2014). Strategies to control tropical theileriosis are immunization using live vaccines, controlling tick infestation by acaricides and treatment of infected cattle (Akat et al., 2014). Specific anti-Theileria drugs (Buparvaquone and Parvaquone) can be used for treatment (Bishop et al., 2009)(Muraguri et al., 1999). Theileriosis is mostly diagnosed based on clinical signs and symptoms, tick history, and/or microscopic examination of thin blood smears (Durrani et al., 2011)(Kirvar et al., 1998). However, major limitations in the microscopic identification of this piroplasmid lie in the low sensitivity of the method itself, and in the difficulty of differentiation of *Theileria* species, that may have morphological similarities, and varied shapes. Microscopy is not a reliable method for the detection of asymptomatic carrier animals (Aktas et al., 2006). To overcome such limitations in detecting specific *Theileria* species, the development and use of serological and molecular based assays is regarded as highly sensitive and specific (Aktas et al., 2006)(Durrani et al., 2011).

4.2. Babesiosis

Babesiosis is one of the most common animal infections that has been reported worldwide and it is caused by *Babesia* genus which have several species that are tick-borne, intra-erythrocytic protozoan parasites (Persing and Conrad, 1995). *Babesia* species infect a wide variety of animals and some of them are of zoonotic importance as they cause diseases in humans (Kim et al., 2007) (Savić et al., 2014). *Babesia* (*B.*) *motasi, B. crassa,* and *B. ovis* are the most commonly diagnosed and reported *Babesia* species in small ruminants (Longstaffe, 1984)(Morel, 1989). Among them, the infections caused by *B. motasi* varies from mild to severe (Morel, 1989), while *B. crassa* seems to have little or no pathogenicity (Friedhoff, 1997). *B. ovis* causes severe infections in sheep and leads to fever, anemia, anorexia and can cause mortality in up to 50% of the infected animals (Hashemi-Fesharki, 1997)(Bai et al., 2002). Ticks of the *Hyalomma* and *Haemaphysalis* genera can transmit piroplasmosis in cattle, buffalo, sheep, and goats in Pakistan. Major economic losses in tropical and subtropical areas are significantly associated with babesiosis, which directly affects sheep and goat production (Bai et al., 2002). Two drugs, Diminazine, and Imidocarb are available for treatment and prophylaxis of babesiosis (Mosqueda et al., 2012). Mixture of Atovaquone with Azithromycin is proved to be more beneficial for the treatment of babesiosis than mixture of Quinine and Clindamycin (Krause et al., 2000).

4.3. Anaplasmosis

Anaplasmosis is disease reported in human as well as in wild and domestic ruminants and it is caused by obligate intraerythrocytic Rickettsiae of the genus *Anaplasma* (Rickettsiales: Anaplasmatacea) and is distributed in tropical and subtropical region of the world (Torina et al., 2008). *Anaplasma ovis* is the main pathogen causing anaplasmosis in small ruminants in Pakistan (Dumler et al., 2001). Common clinical sign of anaplasmosis are dyspnoea, icterus, fever, lethargy, hyper excitability, abortion due to hypoxia (Camus and Uilenberg, 2010). Techniques that are used for the control of anaplasmosis include use of antibiotics, vaccination and arthropod control. *Anaplasma* carrier animals treatment with Oxytetracycline is normally successful for the eradication of infections (Atif, 2015).

4.4. Piroplosmosis in small ruminants of Pakistan

The Giemsa staining technique of blood smears is mainly used for the microscopic examination of piroplasm. However, this technique requires expertise and has low sensitivity because of similarity in the morphology of these pathogen different species may be confused. The detection of Babesia and Theileria infection in carrier animals by DNA amplification is a powerful tool for epidemiological investigations, since it is more specific and sensitive than Giemsa staining technique (Aktaş et al., 2005). Compared to other haemoparasites, *Theileria* species are extensively investigated in Pakistan (eleven studies in sheep and nine in goats). Majority of investigations were carried out in Punjab (n = 16) followed by Khyber Pakhtunkhwa (n = 6) Baluchistan (n = 1), Islamabad (n = 1) and Sindh (n = 1) (Table 2). In majority of these studies, samples were collected randomly from flocks in rural and urban areas and sometimes from the major veterinary health facilities without adopting any specific exclusion or inclusion criteria (such as health status or signs of infection or disease), which may, ultimately, result in sampling bias. By using conventional microscopy, the most common diagnostic method used to detect theileriosis in small ruminants, the prevalence of this disease in Pakistan ranges from 1.01 to 27% in sheep and 0.90 to10.5% in goats (Table 2). By using molecular techniques, various studies from Pakistan have reported the

Table 2
List of key studies on Babesia/Theileria species in goats and sheep in Pakistan.

Investigated Animals	Sampling Site	Sampling design	Sampling period	Detection Method	Detection Test positive animals (%)		Prevalence of detected piroplasm (%)			Reference
					Goats	Sheep	*Babesia*	*Theileria*	Co–infection	
Sheep Goats	Lahore District	Healthy and infected animals	November 2005-October 2006	Microscopy	21/256 (8.20%)	38/273 (13.92%)	N/A	59/529 (11.20%)	N/A	(Naz et al., 2012)
Sheep Ticks	Lahore District in Punjab	Animals with history of relapse of fever and tick infestation	Spring and summer of 2007	Microscopy	N/A	Sheep 44/200 (22%) Ticks 70/200 (35%)	N/A	Sheep 44/200 (22%) Ticks 70/200 (35%)	0	(Durrani et al., 2011)
				PCR (no sequencing)	N/A	*Rhipicephalus* 27/41 (65.85) *Hyalomma* 30/45 (66.66%)	N/A	Sheep 27/41 (65.85%) Ticks 30/45 (66.66%)		
Sheep	Islamabad and Attock City in Punjab	Healthy farmed animals.	October and November 2009	Microscopy	7/184 (3.8%)	7/95 (7.36%)	N/A	14/279 (5.01%)	0	(Irshad et al., 2010)
Sheep Goats	Multan, Muzaffarghar, Layyah, Dera Ismail Khan, Khanewal, Vehari, and Bahawalnagar Districts in Southern Punjab	Healthy Animals Randomly selected herds from seven districts	2011	PCR (No sequencing)	16/67 (23.88)	20/40 (50%)	36/107 (33.64%) ***Babesia* spp.**	N/A	0	(Iqbal et al., 2011)
Lohi sheep	Livestock Experiment station Bahadar Nagar Okara in Punjab	Healthy animals	May-July 2011	Microscopy	N/A	54/200 (27%)	32/200 (16%)	48/200 (24%)	26/200 (13%)	(Shahzad et al., 2013)
				PCR (no sequencing)	N/A	117/200 (58.5%)	86/200 (43%) ***B. ovis***	73/200 (36.5%)	42/200 (21%)	
Sheep Goats	Province of Balochistan in the northwest of Pakistan	Healthy animals Small village flocks and pastures	March 2012-February 2013	PCR (no sequencing)	65/670 (9.70%)	458/2200 (20.82%)	N/A	523/2870 (18.22%)	0	(Khan et al., 2017)
Sheep Goats	Multan District in Punjab and Khyber Pakhtunkhwa	Healthy sheep	2013	PCR RLB (No sequencing)	6/114 (5.26%)	26/82 (31.70%) ***Babesia* spp.**	0	32/196 (16.32%)	0	(Iqbal et al., 2013)
Sheep Goats	Shujabad Tehsil of Multan District in Punjab	Healthy animals	2013	Microscopy	14/150 (9.33%)	37/150 (24.66%)	N/A	11/300 (3.7%)	N/A	(Riaz and Tasawar, 2017)
				PCR (no sequencing)	(10.5%)	(18.5%)	N/A	51/300 (17%)	N/A	
Sheep Goats	Kohat and Peshawar Districts in Khyber Pakhtunkhwa	Healthy sheep and goats	2015	PCR (No sequencing)	3/121 (2.47%)	2/44 (4.5%)	N/A	5/165 (3.03%) ***T. lestoquardi***	0	(Saeed et al., 2015)
Sheep Goats	Bahawalnagar, Dera Ghazi Khan, Layyah, Multan, and Muzafargarh Districts in Punjab	Healthy animals of randomly selected flocks	2015	PCR (no sequencing)	0/66 (0%)	4/49 (8.16%)	N/A	4/115 (3.47%)	0	(Fatima et al., 2015)
Sheep Goats	Peshawar and Khyber Agency in Khyber Pakhtunkhwa	Random blood sample collection	2015	Microscopy	N/A	N/A	21/ 300 (7%)	18/300 (6%)	0	(Shah et al., 2017)
Goats	Bannu, Tank and Dera Ismail Khan Districts in Khyber Pakhtunkhwa	Healthy animals	2016	PCR (No sequencing)	86/600 (14.33%)	N/A	N/A	86/600 (14.33%)	N/A	(Ullah et al., 2018)
Lohi sheep	Livestock Production Research Institute, Bahadur Nagar, Okara in Punjab	Healthy animal	2010	Microscopy	N/A	62/400 (15.5%)	N/A	62/400 (15.5%)	N/A	(Zia-ur-Rehman et al., 2010)

Table 2 (*continued*)

Investigated Animals	Sampling Site	Sampling design	Sampling period	Detection Method	Detection Test positive animals (%)		Prevalence of detected piroplasm (%)			Reference
					Goats	Sheep	*Babesia*	*Theileria*	Co–infection	
Goats Sheep	Dera Ghazi Khan, Layyah, Multan, and Rahim Yar Khan Districts in Punjab and Kohat District in Khyber Pakhtunkhwa	Healthy small ruminants	2012	Microscopy	1/111 (0.90%)	1/99 (1.01%)	N/A	2/210 (0.95%)	N/A	(Durrani et al., 2012)
				PCR	1/111 (0.90%)	11/99 (11.11%)	N/A	12/210 (6%) ***T. ovis***	N/A	
Goats Sheep	Lahore in Punjab	Healthy animals of urban and preurban areas	August till November 2010	Microscopy	51/377 (13.52%)	57/243 (23.46%)	108/620 (17.41%)	N/A	N/A	(Ijaz et al., 2013)
Goats Sheep	Lower Dir District in Khyber Pakhtunkhwa	Healthy animals	December 2017 to April 2018	PCR (no sequencing)	12/120 (10%) SI 37/120 (30.8) MI	15/80 (18.7%) SSI 43/80 (53.7) MSI	N/A	107/200 53.5% ***T. luwenshuni***	27/200 13.5% SI 80/200 40% MI	(Nasreen et al., 2020)
Goats	Multan, Punjab	Healthy animals	2018	Microscopy	25/463 (5.4%)	NA	NA			
				PCR	74/463 (16%) MSI			*T. ovis* 36/74 (48.6%), *T. lestoquardi* 30/74 (40.5%)	8/74 (10%) MI	(Riaz et al., 2019)
Goats and Sheep	Malakand Division, Khyber Pakhtunkhwa	Animals symptomatic for *Theileria* infection	October 2017 to September 2018	Microscopy	3.02%	0.42%	NA	NA	NA	(Mohsin et al., 2021)
Goats and Sheep	Malakand, Swat, Bajaur and Shangla	Random blood sample collection	January and December 2019	PCR	71/800 (8.8%)	116/800 (14.5%)	NA	Overall 187/800 (23.37%) *T. ovis* 115/800 (14.3%) Other *Theileria* (T. lestoquardi and T. annulata) 72/800 (9%)	NA	(Niaz et al., 2021)

PCR = Polymerase Chain Reaction.
PCR /RLB = PCR and Reverse Line Blot Hybridization Assay.
ELISA = Enzyme-Linked InmunoSorbent Assay.
SSI = Single species infection.
MSI = multiple species infection.

Table 3
List of key studies on Anaplasma species in goats and sheep in Pakistan.

Investigated Animals	Sampling Site	Sampling design	Sampling period	Detection Method	Detection test positive animals (%)		*Anaplasma* spp. positive animals (%)	Reference
					Goats	Sheep		
Balkhi sheep	Peshawar in Khyber Pakhtunkhwa	Suspected animals	2013–2014	Microscopy	N/A	28/40 (70%)	28/40 (70%)	(Ali et al., 2014)
				PCR	NA	34/40 (85%)	34/40 (85%)	
Sheep Goats	Lahore in Punjab	Infected animals	2014	Microscopy	46/150 (30.67%)	83/15 (55.33%)	129/300 (43%)	(Ali et al., 2014)
Sheep Goats	Khyber Agency and Peshawar District in Khyber Pakhtunkhwa	Random blood sample collection	2015	Microscopy	N/A	N/A	120/300 (40%)	(Shah et al., 2017)
Sheep Goats	Mardan District in Khyber Pakhtunkhwa	Healthy animals, Random blood collections	January 2014-December	Microscopy	15/180 (8.33%)	25/180 (13.89%)	40/360 (11.11%)	(Nasreen et al., 2016)
				cELISA	37/180 (20.56%)	43/180 (23.33%)	80/360 (22.22%)	
Sheep	Peshawar in Khyber Pakhtunkhwa	Four healthy sheep breeds	May 2012	ELISA	N/A	92/376 (24.47%)	92/376 (24.47%)	(Kashif and Ahmad, 2014)
Sheep Goats	Khyber Pakhtunkhwa	Samples collected slaughterhouses	June and July 2003	Microscopy	7/73 (9.59%)	19/136 (13.2)	N/A	(Talat et al., 2005)
Sheep Goats	Charsadda District in Khyber Pakhtunkhwa	Healthy animals	January to and December 2017	cELISA	58/300 (19%)	75/300 (25%)	N/A	(Khan et al., 2019a,b)
Sheep Goats	Karat District	Infected animals.	March to Agust 2015–2016	Microscopy	17.25%	22%	33.87%	(Hussain et al., 2017)
				PCR	34.58 % ***A. marginale***	47.25% ***A. marginale***		
				ELISA	32.8	36.8%		
Sheep	Layyah District	Healthy animals	September 2019 till March 2020	PCR	–	15/218 (6.9%) *A. marginale*	15/218 (6.9%)	**(**Abid et al., 2021**)**
Goats	**Mirpurkhas**	**Randomly selected animals**	**2019**	**Microscopy**	**40/300 (13.3%)**	**NA**	**40/300 (13.3%**	**(Memon et al., 2019)**
Sheep Goats	**Lahore**	**Diseased animals**	**2018**	**PCR**	**20/75 (25.3%)**	**24/75 (32%)**	**44/150 (29.33%)**	**(**Ghafar et al., 2020**)**
Sheep Goats	Malakand, Swat, Bajaur and Shangla	Random blood sample collection	January and December 2019	**PCR**	63/800 (7.8**%**) A. ovis	111/800 (13.8**%**) A. ovis	174/800 (21.7**%**) **A.ovis**	**(**Niaz et al., 2021**)**

prevalence of theileriosis ranging between 0.90 and 23.8% in goats and from 4.5 to 58% in sheep (Table 2). *Theileria* spp. (Mohsin et al., 2021), *Theileria* (*T.*) *annulata* (Jabbar et al., 2015)(Niaz et al., 2021), *T. ovis* (Riaz et al., 2019)(Durrani et al., 2012)(Niaz et al., 2021) (Durrani et al., 2011)[69, Riaz et al., 2019], *T. luwenshuni* (Nasreen et al., 2020a,b), and *T. lestequardi* (Saeed et al., 2015) (Riaz et al., 2019)(Niaz et al., 2021) are various *Theileria* species that have been reported from Pakistan (Table 2). So far, one study has been documented from Pakistan whereby *Theileria* species were identified in ticks collected from small ruminants by using the molecular technique. The results of this study showed that 67% of collected *Hyalomma* and 66% of *Rhipicephalus* tick species were infected with *T. lestoquardi* and *T. ovis,* respectively. This study also provides evidence that the potential vectors of *T. ovis* are mainly *Rhipicephalus* spp., while those of *T. lestoquardi* are *Hyalomma* spp. (Durrani et al., 2011)(Table 2).

Relatively few studies are available in literature regarding babesiosis in small ruminants from Pakistan. Five studies have been conducted from Pakistan in total including three from Punjab (from eight districts) (Iqbal et al., 2011)(Shahzad et al., 2013)(Ijaz et al., 2013) and two from Khyber Pakhtunkhwa (from two districts) (Shah et al., 2017)(Iqbal et al., 2013) (Table 2) The reported range of prevalence of babesiosis is from 7 to 58.5% in sheep and 7 to 23% in goats. (Iqbal et al., 2011)(Shahzad et al., 2013)(Ijaz et al., 2013)(Shah et al., 2017). Out of these limited studies from Pakistan regarding ovine babesiosis, only three have used molecular tools for the confirmation of babesiosis in Pakistan and they have reported the presence of *Babesia* spp. and *Babesia ovis* in sheep and goats of Pakistan. Keeping in view the limited number of studies from Pakistan, the overall prevalence of babesiosis in small ruminants across the country remains largely unknown. The worldwide reported vector of babesiosis is *Rhipicephalus microplus* but in Pakistan no such study has been conducted to assess the role of *R. microplus* or any other vectors in the spread of babesiosis. We recommend large scale studies in all provinces of **Pakistan** to report the prevalence of babesiosis in small ruminants along with notes on geographical distribution of vector ticks in order to design disease control strategies to uplift the livestock output.

4.5. Anaplasmosis in small ruminants of Pakistan

To date, four studies have been conducted regarding anaplasmosis in small ruminants from Pakistan (Table 3). Based on conventional methods (i.e., stained blood smear), the mean prevalence of anaplasmosis ranges between 8.33 and 30.67% in goats and 13.89 to 70% in sheep (Shah et al., 2017)(Ali et al., 2014)(Talat et al., 2005)(Memon et al., 2019) (Table 3). There are few studies from Pakistan in which serological testing was performed, by using ELISA, for the detection of antibodies against *Anaplasma* spp (Nasreen et al., 2016)(Khan et al., 2019a)(Kashif and Ahmad, 2014). In a cELISA based study that was conducted in Mardan district of KPK, anaplasmosis was reported in 22.22% of enrolled sheep and 23.33% of sheep (Nasreen et al., 2016). In a similar investigation, 19% sheep and 25% goats from Charsadda District were found infected with *Anaplasma* (Khan et al., 2019a). In a recent study from KPK, 25% sheep were found serum positive for

Anaplasma spp. antibodies (Kashif and Ahmad, 2014). There are three PCR-based studies reported from Pakistan regarding ovine anaplasmosis. Two studies are from KPK and one from Punjab province. Hussain et al., 2017 reported that 56.25% sheep and 34.85% goat blood samples that were collected from Karak District were found infected with *Anaplasma marginale*. Niaz et al., 2021 reported that 13.8% sheep and 7.8% goat blood samples were infected with *Anaplasma ovis* also collected from Karak Distrit. In a recent study from Layyah district in Punjab, Abid et al., 2021 has reported 6.9% of *A. marginale* in sheep blood samples (Table 3). From this literature review, it is evident that *A. marginale* is the only species that has been targeted in small ruminants from Pakistan and obviously all other species that can infect small ruminants are open to be explored in Pakistan.

5. Conclusion and future perspectives

In this review, we are reporting the ovine anaplasmosis, babesiosis, and theileriosis reporting from various geographical regions of Pakistan. Some of these studies have reported epidemiological data that has provided some insights but various important informations readings production system, age, season, grazing areas etc. were compromised because of limitations in study design. Often the focus was on animals with clinical symptoms instead of enrolling the whole ruminant population. None of the studies that have been reported from small ruminants in Pakistan has reported the zoonotic importance of TBDs. For example, there is no data regarding *A. phagocytophilum* that is an important zoonotic pathogen, causing tick-borne fever in small ruminants and it is also responsible for causing human granulocytic anaplasmosis and it should be explored in Pakistan as a risk for the production system and as a potential zoonotic disease. At present, mostly conventional tools are in use for the detection of parasites in small ruminants. Although, molecular tools are also in use for this purpose, but their more frequent use must be encouraged as they are more specific and sensitive than conventional blood smear screening. The use of multiplex-tandem and real-time PCR is highly recommended for future studies to detect different species in a single sample. Along with routine molecular tests, loop-mediated isothermal amplification (LAMP) can also be used to detect tick-borne pathogens in small ruminants in the future. The advantage of LAMP over other molecular tests is that it can be used even in field conditions, as a pen side test while being more specific and sensitive than PCR [79, 80, 81]. Even most of the studies that used PCR for the detection of piroplasmosis and anaplasmosis have not DNA sequenced pathogen-specific and conserved genes to confirm single or multiple species infections. We recommend the confirmation of amplified genes of detected parasites from small ruminants in Pakistan by DNA sequencing and data must be submitted to repositories like GenBank. Accurate identification of tick-borne pathogens is an important avenue to alleviate many taxonomic discrepancies, adopting the perfect therapeutic approach and proceedings of the preventive policies. Information regarding the genetic diversity of *Theileria, Babesia and Anaplasma species* in small ruminants from Pakistan is limited. Hence, the sequenced PCR products should be used for phylogenetic analysis in order to study the evolutionary history of detected parasites and to correlate this information to virulence of the parasites.

Despite of the fact that Pakistan is among the larger countries of the World, as far as the land area is concerned, and also rich in livestock as well, studies regarding ticks and TBD are limited and several regions of Pakistan are unexplored for TBDs where sheep and goats play a key role in the food security and livelihood of resource-poor farmers. So huge knowledge gap is there to be covered by conducting epidemiological and diagnostic surveys in whole country, especially on large scales in Sindh and Balochistan provinces as they are almost totally unexplored regarding the status of TBDs and livestock is major income source for majority of their population.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the support of Dr. Abdul Jabbar from University of Melbourne who help design the study.

Funding

There were no external funding sources for this study.

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