Review

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

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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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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 Pakhtoonkhwa (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, Nuttalliellina 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 thiereliosis (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 Halyomma marginatum 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 microplus | – | – | – | (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 gervaesi, Amblyomma exornatum, Amblyomma latum | Not applicable | (Ali et al., 2019) |
| Manshehra, Haripur, Shangala and Kohistan Districts in KPK and Gilgit Baltistan Punjab, Sindh, KPK, Baluchistan Provinces and Gilgit-Baltistan | Sheep, goats, cattle and buffaloes | Hyalomma anatolicum | – | Rhipicephalus microplus | – | – | Not applicable | (Sajid et al., 2018) |
| Lahore District in Punjab | Sheep | Hyalomma spp. | – | Rhipicephalus spp. | – | – | Theileria ovis, Theileria lestoquardi | (Karim et al., 2017) |
| southern Punjab | goats | Hyalomma spp | – | Rhipicephalus sanguineus | – | – | – | (N. Nasreen et al., 2020) |
| KP | cows and buffaloes | Hyalomma spp | – | Rhipicephalus spp | – | – | – | (Zeb et al., 2020) |
| Lower Dir, Upper Dir, Chitral, Bajaur and Malakand | Cattle | Hyalomma anatolicum, Hyalomma marginatum | – | Rhipicephalus microplus | – | – | – | (Iqbal et al., 2021) |
| District Pishin, Baluchistan | Cows | Hyalomma anatolicum, Hyalomma dromedarii, Hyalomma truncatum, Hyalomma scupense | – | Rhipicephalus sanguineus | – | – | – | (Nasreen et al., 2020) |
| FATA | Goats Sheep | Hyalomma anatolicum, Hyalomma scupense, Hyalomma dromedarii | Haemaphysalis punctata, Haemaphysalis sulcata, | Rhipicephalus variabilis, Rhipicephalus microplus, Rhipicephalus var. scupense | – | – | – | (Adil Khan et al., 2019) |
| Multan District in Punjab | Goats sheep | Hyalomma anatolicum, Hyalomma marginatum | – | Rhipicephalus sanguineus | – | – | – | (Ramzan et al., 2020) |
| Bannu, Khyber Pakhtunkhwa | Sheep Goats | Hyalomma anatolicum, Hyalomma marginatum | Haemaphysalis acicularis | Rhipicephalus microplus, Rhipicephalus annulatus, Rhipicephalus turanicus | – | – | NA | (Rooman et al., 2021) |
Table 1 continued

| Sampling locations | Hosts detected in ticks | Other tick species | Parasites detected in ticks | References |
|--------------------|-------------------------|-------------------|----------------------------|------------|
| South Punjab, Pakistan | Sheep | Hyalomma anatolicum, Haemaphysalis sleowi, Rhipicephalus microplus, H. detritum, Hyalomma marginatum | Theleria ovir, Anaplasma ovis | (Rehman et al., 2019) |
| Pakhtunkhwa | Goats | Rhipicephalus sanguineus, H. apperencid, H. microplus | Theileria ovir | (Rehman et al., 2019) |
| Gilgit Baltistan | Goats | Rhipicephalus sanguineus, H. apperencid, H. microplus | Theileria ovir | (Rehman et al., 2019) |
| North Waziristan | Goats | Rhipicephalus sanguineus, H. apperencid, H. microplus | Theileria ovir | (Rehman et al., 2019) |
| South Waziristan | Goats | Rhipicephalus sanguineus, H. apperencid, H. microplus | Theileria ovir | (Rehman et al., 2019) |
| Federally Administered Tribal Area, Pakistan | Goats | Rhipicephalus sanguineus, H. apperencid, H. microplus | Theileria ovir | (Rehman et al., 2019) |
| Other tribal areas | Goats | Rhipicephalus sanguineus, H. apperencid, H. microplus | Theileria ovir | (Rehman et al., 2019) |

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, 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 cross-bred (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 Theleria ovir and Theliera westogardi (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 scapatus (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, Hyalomma dromedarii, Hyalomma truncatum, Hyalomma scapata, Dermacentor variabilis, Boophilus annulatus and Amblyomma americanum and Hyalomma 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 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., 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 goats were Hyalomma anatolicum, Hyalomma marginatum, Hyalomma dromedarii, Rhipicephalus sanguineus, Rhipicephalus microplus, Rhipicephalus appendiculatus, Rhipicephalus decolaratus, whereas the prevalent tick species in sheep were Hyalomma anatolicum, Rhipicephalus sanguineus, Rhipicephalus microplus, Rhipicephalus appendiculatus and Rhipicephalus 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,
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*, *Rhipicephalus 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 Sheikhupura 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 (*Acaric: 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, anaplasmiosis

![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.](image-url)
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 Avasha, 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.) lestoquardi, T. lewenshuni, 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, Amblyomma and Hyalomma genera (Bispo 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, leukenopoenia 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 (Buparvaquine and Parvaquine) 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 (Aktaş 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 (Aktaş 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-erythrocitic protozoan parasites (Persing and Conrad, 1995). Babesia species infect a wide variety of animals and some of them are of zoonotic importance as it cause diseases in humans (Kim et al., 2007)(Savič 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 vary 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: Anaplasmatace) 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. Piroplasmosis 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 to 10.5% 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 |
|-----------------------|---------------|-----------------|-----------------|------------------|-------------------------------------|--------------------------------------|-----------|
| **Sheep Goats**       | Lahore District | Healthy and infected animals | November 2005-October 2006 | Microscopy | 21/256 (8.20%) | N/A 59/529 (11.20%) | (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 | N/A Sheep 44/200 (22%) | (Durrani et al., 2011) |
| **Sheep Goats**       | Lahore District | Healthy and infected animals | November 2005-October 2006 | Microscopy | 38/273 (13.92%) | N/A Sheep 44/200 (22%) | (Durrani et al., 2011) |
| **Sheep Goats**       | Sheep ticks Lahore District in Punjab | Animals with history of relapse of fever and tick infestation | Spring and summer of 2007 | Microscopy | 70/200 (35%) | N/A Sheep 27/41 (65.85%) | (Durrani et al., 2011) |
| **Sheep Goats**       | Multan, Muzaffargarh, 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%) | N/A Sheep 27/41 (65.85%) | (Durrani et al., 2011) |
| **Lohi sheep**        | Livestock Experiment station Bahadar Nagar Okara in Punjab | Healthy animals | May-July 2011 | Microscopy | 54/200 (27%) | 48/200 (24%) | (Shahazad et al., 2013) |
| **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%) | N/A 523/2870 (18.22%) | (Khan et al., 2017) |
| **Sheep Goats**       | Multan District in Punjab and Khyber Pakhtunkhwa | Healthy sheep | 2013 | PCR (no sequencing) | 6/114 (5.26%) | 32/196 (16.32%) | (Iqbal et al., 2013) |
| **Sheep Goats**       | Shujabad Tehsil of Multan District in Punjab | Healthy animals | 2013 | PCR (no sequencing) | 14/150 (9.33%) | 11/300 (3.7%) | (Riaz and Taxawar, 2017) |
| **Sheep Goats**       | Kohat and Peshawar Districts in Khyber Pakhtunkhwa | Healthy sheep and goats | 2015 | PCR (no sequencing) | 3/121 (2.47%) | 5/165 (3.03%) | (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%) | 18/300 (6%) | (Fatima et al., 2015) |
| **Sheep Goats**       | Peshawar and Khyber Agency in Khyber Pakhtunkhwa | Random blood sample collection | 2015 | Microscopy | N/A | 18/300 (6%) | (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 | (Ullah et al., 2018) |
| **Lohi sheep**        | Livestock Production Research Institute, Bahadur Nagar, Okara in Punjab | Healthy animal | 2010 | Microscopy | 62/400 (15.5%) | N/A | (Zia-ur-Rehman et al., 2010) |
| Investigated Animals | Sampling Site | Sampling design | Sampling period | Detection Method | Detection Test positive animals (%) | Prevalence of detected piroplasm (%) | Reference |
|----------------------|---------------|-----------------|-----------------|------------------|-------------------------------------|-----------------------------------|-----------|
| Goats and Sheep      | Malakand Division, Khyber Pakhtunkhwa | Animals symptomatic for Theileria infection | October 2017 to September 2018 | Microscopy | 1/74 (1.3%) | 3.02% | NA |
|                      | Malakand, Swat, Bajaur and Shangla | Random blood sample collection | January and December 2019 | PCR | 31/74 (41.9%) | 0.42% | NA |

PCR = Polymerase Chain Reaction.
PCR /RLB = PCR and Reverse Line Blot Hybridization Assay.
ELISA = Enzyme-Linked ImmunoSorbent Assay.
SSI = Single species infection.
MSI = multiple species infection.
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), R. luwenshumi (Nasreen et al., 2020a,b), and T. lestoquardi (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). 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; Khan et al., 2019a,b; Hussain et al., 2017). In a cELISA based study that was conducted in Mar- dan 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, 15% 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 marginale using cELISA (Nasreen et al., 2016).

| Investigated Animals | Sampling Site | Sampling design | Sampling period | Detection Method | Detection test positive animals (%) | Anaplasma spp. positive animals (%) | Reference |
|----------------------|---------------|-----------------|-----------------|-----------------|-------------------------------------|------------------------------------|-----------|
| **Goats**            |               |                 |                 |                 |                                     |                                    |           |
| Sheep                | Peshawar in Khyber Pakhtunkhwa | Suspected animals | 2013–2014       | Microscopy      | N/A                                 | 23/40 (70%)                        | Ali et al., 2014          |
|                     | Lahore in Punjab | Infected animals | 2014            | Microscopy      | 46/150 (30.67%)                     | 129/300 (43%)                      | Ali et al., 2014          |
| Sheep                | Khyber Agency and Peshawar District in Khyber Pakhtunkhwa | Random blood sample collection | 2015           | Microscopy      | N/A                                 | 120/300 (40%)                      | Shah et al., 2017         |
| Sheep                | Mardan District in Khyber Pakhtunkhwa | Healthy animals, Random blood collections | January 2014–December | Microscopy      | 15/180 (8.33%)                     | 80/360 (22.22%)                    | Niaze et al., 2016       |
| Sheep                | Peshawar in Khyber Pakhtunkhwa | Four healthy sheep breeds | May 2012      | ELISA           | N/A                                 | 92/376 (24.47%)                    | Kashif and Ahmad, 2014 |
| Sheep                | Khyber Pakhtunkhwa | Samples collected slaughterhouses | June and July 2003 | Microscopy      | 7/73 (9.59%)                      | N/A                                 | Talat et al., 2005     |
| Sheep                | Charsadda District in Khyber Pakhtunkhwa | Healthy animals | January and December 2017 | cELISA         | N/A                                 | 75/300 (25%)                      | Khan et al., 2019a,b     |
| Sheep                | Karat District | Infected animals. | March to August 2015–2016 | Microscopy      | 17.25% (34.58%)                     | 111/800 (13.8%)                    | Hussain et al., 2017    |
| Sheep                | Layyah District | Healthy animals | September 2019 until March 2020 | –              | 32.8% (A. marginale)                | 15/218 (6.9%)                      | Abid et al., 2021       |
| **Goats**            | Mirpurkhas     | Randomly selected animals | 2019 | Microscopy      | 40/300 (13.3%) | 44/150 (29.33%) | Memon et al. (2019) |
| Sheep                | Lahore         | Diseased animals | 2018 | PCR             | 20/75 (25.3%) | 47.25% (A. marginale) | Ghafer et al., 2020 |
| Sheep                | Malakand, Swat, Bajaur and Shangla | Random blood sample collection | January and December 2019 | PCR             | 63/800 (7.8%) | 174/800 (21.7%) | Niaz et al., 2021 |
Anaplasma spp. antibodies (Kashif and Ahmad, 2014). There are three PCR-based studies reported from Pakistan regarding ovine anaplasmosis. Two studies are from KKP 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 District. 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.

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