

Review

Diversity and Distribution of *Theileria* Species and Their Vectors in Ruminants from India, Pakistan and Bangladesh

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Abstract: Tropical theileriosis, caused by the apicomplexan hemoparasite of the genus *Theileria*, is a major constraint to livestock production in various parts of world, including South Asia. Several studies have been carried out over the last five decades; however, comprehensive information in this region regarding the diversity and distribution of *Theileria* is lacking. Therefore, keeping in mind the economic importance of theileriosis, we have systematically reviewed the current knowledge about *Theileria* spp. diversity and distribution affecting cattle, water buffaloes, goats and sheep in three countries included India, Pakistan and Bangladesh of the Indian sub-continent. The data collected indicated that the microscopic method is the widely used method for evaluating *Theileria* species in the three countries from 1970 to 2021. This is the first study in this region to compile a comprehensive knowledge about the diversity and distribution of *Theileria*. Our study revealed the existence of 11 different species of *Theileria*, including *Theileria* spp. *Theileria annulata*, *T. orientalis*, *T. mutans*, *T. velifera* circulating in cattle and buffalo while *T. annulata*, *T. lestoquardi*, *T. luwenshuni* *T. ovis*, *Theileria* spp. and *T. lestoquardi-like* spp., were infecting goats and sheep from various regions of India, Pakistan and Bangladesh. We find that *T. annulata* can be found in both small and large ruminants and is widely distributed in the different regions of India, Pakistan and Bangladesh. In addition, our analysis revealed that the existence of possible tick vectors of the genera *Hyalomma*, *Haemophysalis*, *Rhipicephalus* and *Amblyomma* may be responsible for the diverse and wide distribution of different *Theileria* species. However, the competence of these tick vectors for different *Theileria* species still need to be explored. Therefore, further studies are needed to bridge this gap and to improve the health and production of livestock and reduce economic losses due to theileriosis in India, Pakistan and Bangladesh. Furthermore, we selected representative 18S rRNA sequences for *T. annulata* from the different regions to infer phylogenetic relationship. Phylogenetic analysis of the selected isolates clustered in different clades which might be due to the variation in a hypervariable region of 18S rRNA. The outcome of this analysis is expected to provide a coherent and integrated framework about the different *Theileria* species prevailing in these countries and contribute to improving the surveillance and control plans of various *Theileria* species in the region.

Keywords: diversity; distribution; *Theileria*; tick vectors; South Asia



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1. Introduction

Tick-borne diseases (TBDs) are considered to be one of the main threats to the ruminants' health in both tropical and sub-tropical regions of the world, representing a serious obstacle to livestock farming. Several TBDs cause significant economic losses associated

with high mortality rate and decreased production output in domestic livestock worldwide [1,2]. Among these, theileriosis is considered one of the significant tick-borne diseases and a major constraint in the growth of the livestock business in many areas of the world [3,4] (Ica et al., 2007; Jenkins 2018). It causes severe morbidity and mortality in livestock, reducing meat and milk production, leading to significant economic losses each year worldwide. The estimated range of economic losses due to theileriosis is 5–25% of the total farm losses worldwide [5]. Resource-poor farming communities are at a greater risk due to the lack of systematic acaricide use and limited access to veterinary health care centers leading to a high mortality rate in untreated animals [6,7].

The lifecycle of *Theileria* parasites in the ruminant host and tick vector has been reviewed [8,9]. Shortly, the lifecycle of *Theileria* is completed in two stages i.e., the vertebrate host stage (asexual reproduction) and tick vector stage (sexual reproduction). The lifecycle begins when an infected tick bites and transmits sporozoites into vertebrate hosts during blood-feeding, where it may transform into schizonts [10]. Subsequently, upon releasing from the infected leukocytes, the merozoites may infect host erythrocytes (RBCs) and then develop into piroplasms. Further multiplication of the piroplasms (merogony) takes place in the RBCs [11]. In non-transforming *Theileria*, merogony has been observed in RBCs [12]. Finally, the tick acquires blood-stage *Theileria* parasites including the gametes, when they feed on an infected host. Sexual reproduction of the gametes occurs in the midgut of the competent vector tick species, where, during meiosis, genetic recombination occurs [13,14]. In this way, *Theileria* parasites' transmission occurs trans-stadially by the tick vectors, and therefore, the known transmission vectors may be 2- or 3-host tick species [9]. The modified form of life cycle of *Theileria* species is shown in Figure 1.

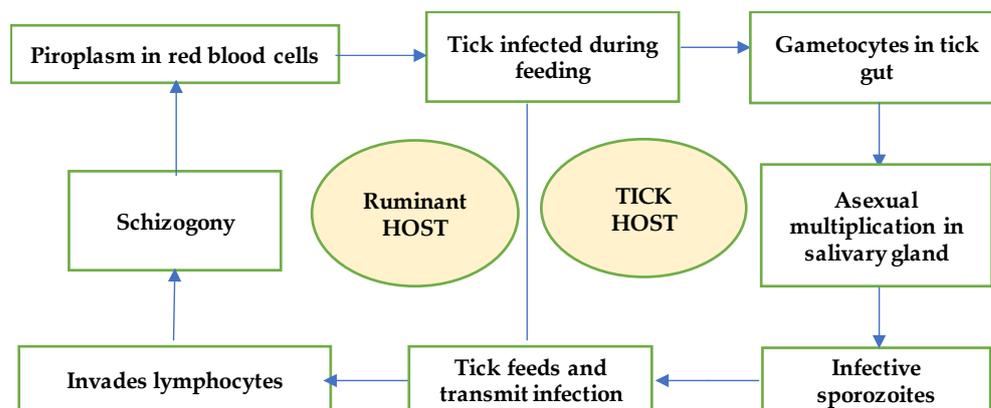


Figure 1. Life cycle of *Theileria* showing different stages in ruminants and tick hosts.

Theileria is distributed worldwide and is a significant cause of disease in livestock in tropical and subtropical regions of the world, including Asia. The most pathogenic and economically important *Theileria* species infecting large ruminants (Cattle; *Bos Taurus* and *Bos indicus*, and water buffalo; *Bubalus bubalis*) are *T. annulata*, which causes Tropical theileriosis (TT) or Mediterranean theileriosis, *T. parva*, which causes East Coast fever (ECF), and *T. mutans*, which causes benign theileriosis and *T. orientalis*, (*T. orientalis/buffeli* group), which causes Oriental theileriosis (OT) or *Theileria*-associated bovine anemia (TABANA). However, on the other hand, *T. lestoquardi*, which causes malignant ovine theileriosis (MOT) *T. uilenbergi* and *T. luwenshuni* are the most pathogenic species of economic significance infecting small ruminants (goats; *Capra aegagrus hircus* and sheep; *Ovis aries*), *T. taurotragi*, and *T. ovis*, mostly cause asymptomatic infections in livestock [2,15]. Depending upon the *Theileria* species, different tick vector species of the genera *Hyalomma*, *Rhipicephalus*, *Haemaphysalis*, and *Amblyomma* are involved in disease transmission [16].

Different diagnostic techniques are used for the detection of *Theileria*. Most widely used and standard techniques are Giemsa-stained blood smears and lymph node needle biopsy smears. These methods are more useful in acutely infected animals than the chroni-

cally infected carriers due to the low level of parasitaemia. Furthermore, species-specific identification based on Giemsa staining is difficult as most *Theileria* piroplasms share morphological identity except for *T. parva*, *T. annulata* and *T. velifera*. Similarly, schizonts cannot always be detected in the superficial lymph nodes during the disease time. In addition, these methods need more field investigation [15,17]. As an alternative, a serological method for detecting parasites antibodies has been developed. However, these methods may only detect previous exposure to the infection as compared to the current one due to its poor sensitivity and specificity [15,18]. New advanced techniques such as next-generation genomic resources have been adapted to overcome these limitations of traditional gross parasitological diagnostic techniques. Different piroplasm species can be differentiated based on variations in the hyper-variable region of 18S rDNA sequence [19–25]. For the amplification of the 18S region, various PCR methods like reverse line blot (RLB)-PCR, quantitative PCR (qPCR) and multiplex PCR have been described to [19,26–28].

The seasonal fluctuations have been found as one of the important risk factors that affect the distribution of this parasite. There are several species of this parasite, and they have substantial differences in their ecoepidemiology in significant parts of the Asian countries including India, Pakistan and Bangladesh. In addition, lack of proper management practices in these areas may lead to heavy economic losses [29–31]. Keeping in view the importance of the above discussion, the present review data related to the *Theileria* species diversity infecting ruminant species and distribution concerning its tick vectors in specific region of the Asia including India, Pakistan and Bangladesh. The outcome of this analysis is expected to provide an integrated scientific baseline for future vaccination programs and other control measures either at the pathogen or vector level.

2. Materials and Methods

2.1. Study Protocol

In this review study, we systemically reviewed the relevant articles published on *Theileria* in small and large ruminants in the three important countries from the livestock perspectives of South Asia, including India, Pakistan and Bangladesh as shown in Figure 2. The different studies were divided into various zones based on administrative and geomorphic features such as India divided into five zones viz Central, North, East, North-east, West, South, while Pakistan divided into four provinces such as Punjab, Khyber Pakhtunkhwa, Sindh and Balochistan and FATA and Bangladesh was divided into six zones like Central, South Eastern, North Central, Northern, and Western.

2.2. Literature Search Strategy

Our procedure was based on searching different databases such as PubMed, Science Direct, Springer, Scopus, Google Scholar and Web of Science for retrieving relevant articles published in these three countries, which mainly focused on the time period from 1970 to 2021. Furthermore, to back trace the past years published articles on *Theileria*, all collected peer-reviewed articles, and references cited from the retrieved studies were searched again. Different terms such as *Theileria*, tick borne pathogens, *Theileria* vectors, tick vectors, large ruminants, small ruminants, and region of the concerned country were used for retrieving data.

2.3. Data Extraction and Qualitative Assessment

The diversity and distribution studies were analyzed carefully and reviewed systematically before initiating the data entry process onto predesigned Microsoft Excel 2010 sheets. To further maximize the accuracy, the extracted information compiled in Microsoft Excel 2010 by the author (JZ) was screened to remove repeated studies. After the data were entered, another author (OS) checked the dataset thoroughly to avoid errors, duplications and to further enhance the quality of extracted data.

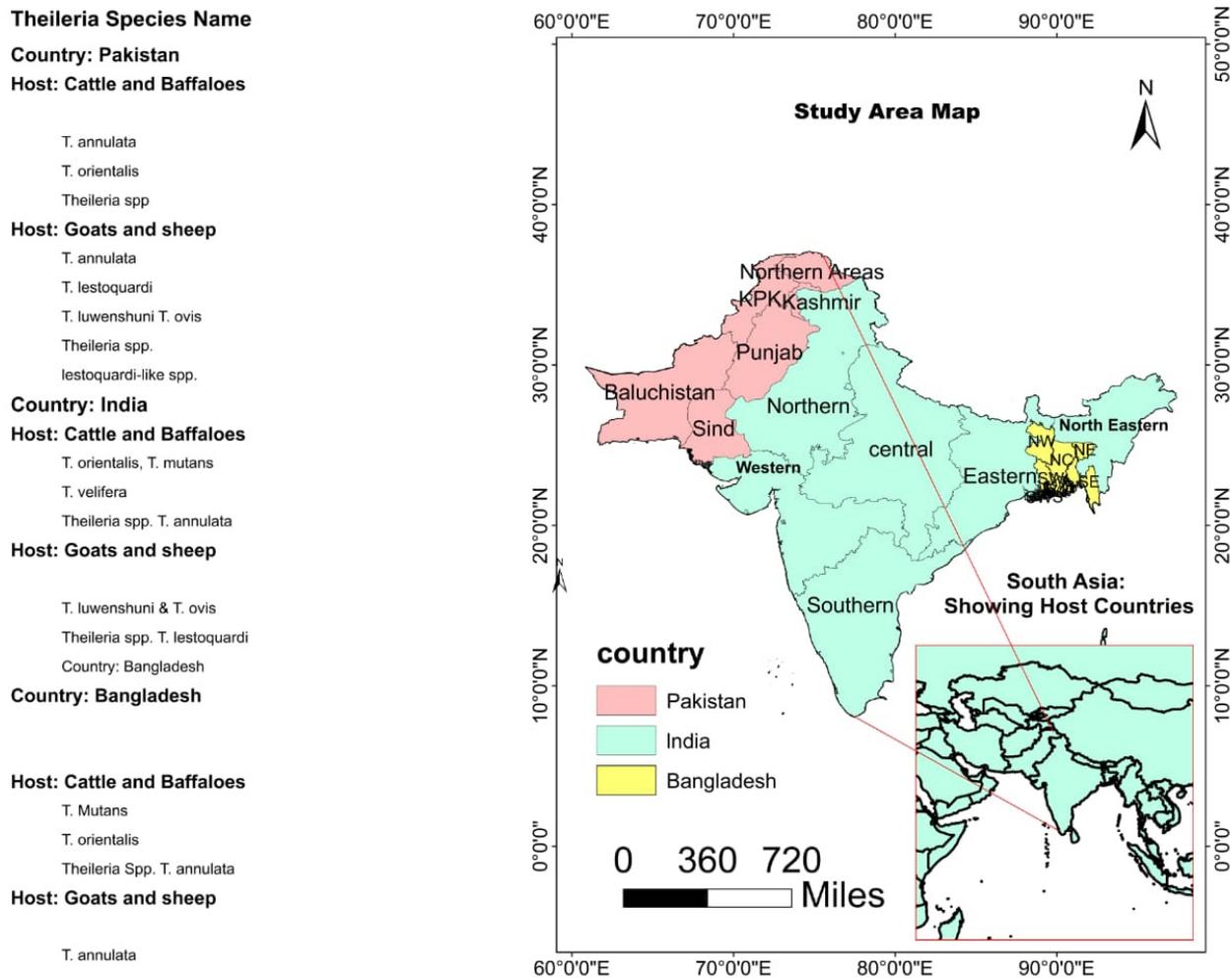


Figure 2. Map showing the location of the study area (India, Pakistan, and Bangladesh) and a list of *Theileria* Species present in each country.

Furthermore, all studies regarding *Theileria*/theileriosis conducted on large (cattle and buffaloes) and small ruminants (goats and sheep) in the region were selected for analysis. Five key pieces of information was extracted from the literature: (1) *Theileria* species and possible tick vectors detection (2) region, state or location of the study, (3) time of the study conducted, (4) study type and (5) studies that have used the standard methodology of confirmatory tests including blood smear examination with different staining methods, molecular methods by different PCRs, and serological diagnosis.

During the first step of screening, 410 articles, including $n = 250$ from India, $n = 130$ from Pakistan and $n = 30$ from Bangladesh were retrieved. Following the data retrieval step, the data were compiled in Microsoft Excel 2010 and all the duplicated studies $n = 60$ were removed from further screening while $n = 130$ theses and full length papers which were not available online were also excluded. In addition, all the irrelevant data and papers ($n = 75$) published in a language other than English were also removed. To further enhance the quality assessment, we removed the conference proceedings, which were not available with full text. Finally, a total of 136 were identified to be eligible for data analysis which included $n = 67$, $n = 56$ and $n = 13$ from India, Pakistan and Bangladesh, respectively (Figure 3). The studies were carried out between 1975 and 2021, and were grouped in three different periods: (1) 1975–2010, (2) 2011–2015, (3) 2016–2021, and were differentiated between molecular and direct diagnostic tests, as shown in Figures 4 and 5.

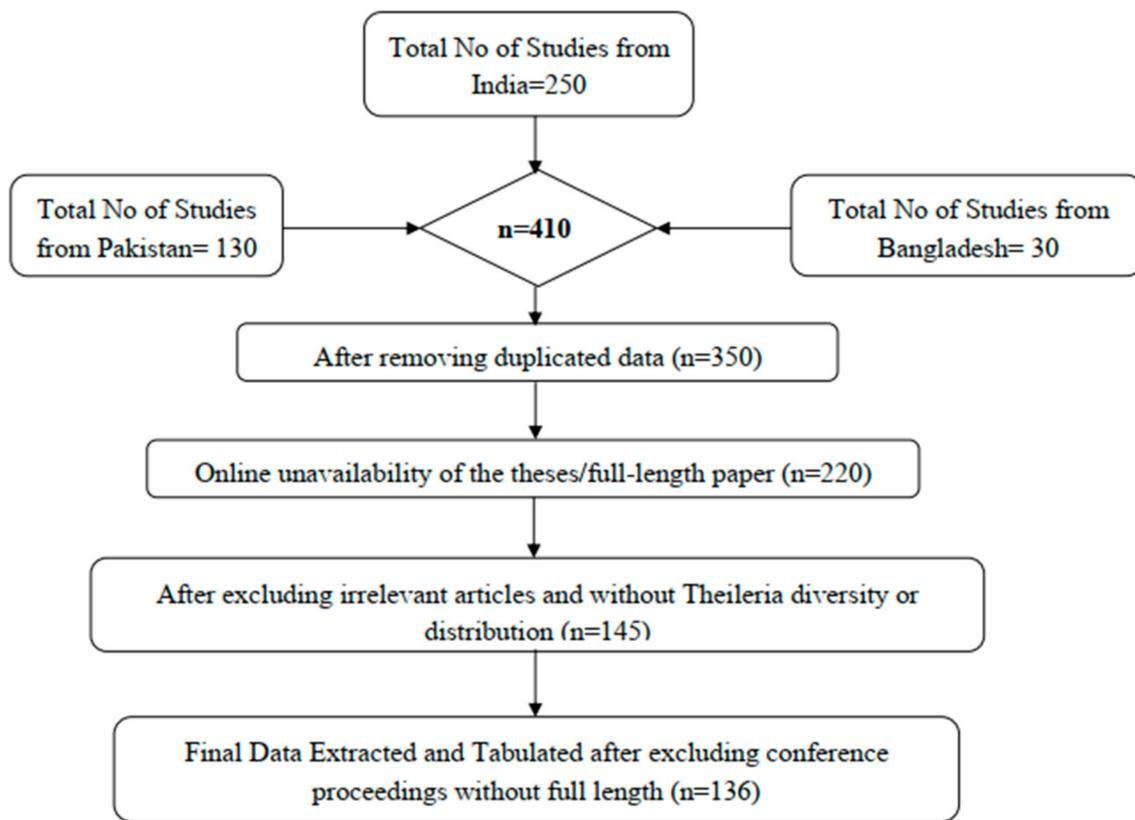


Figure 3. Flow chart diagram showing the data retrieving and extraction procedure.

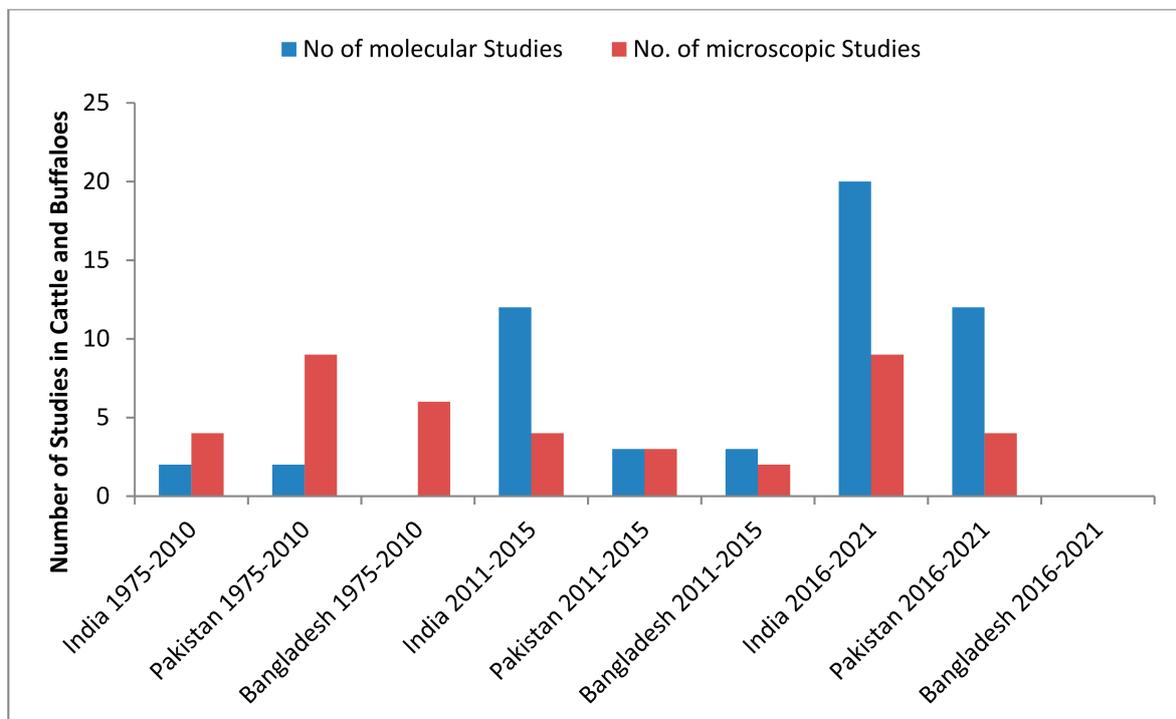


Figure 4. Comparison of the number of epidemiological studies detecting *Theileria* spp. in water buffalo and cattle using molecular and microscopic diagnostic methods in India, Pakistan and Bangladesh, according to three different periods between 1975 and 2021.

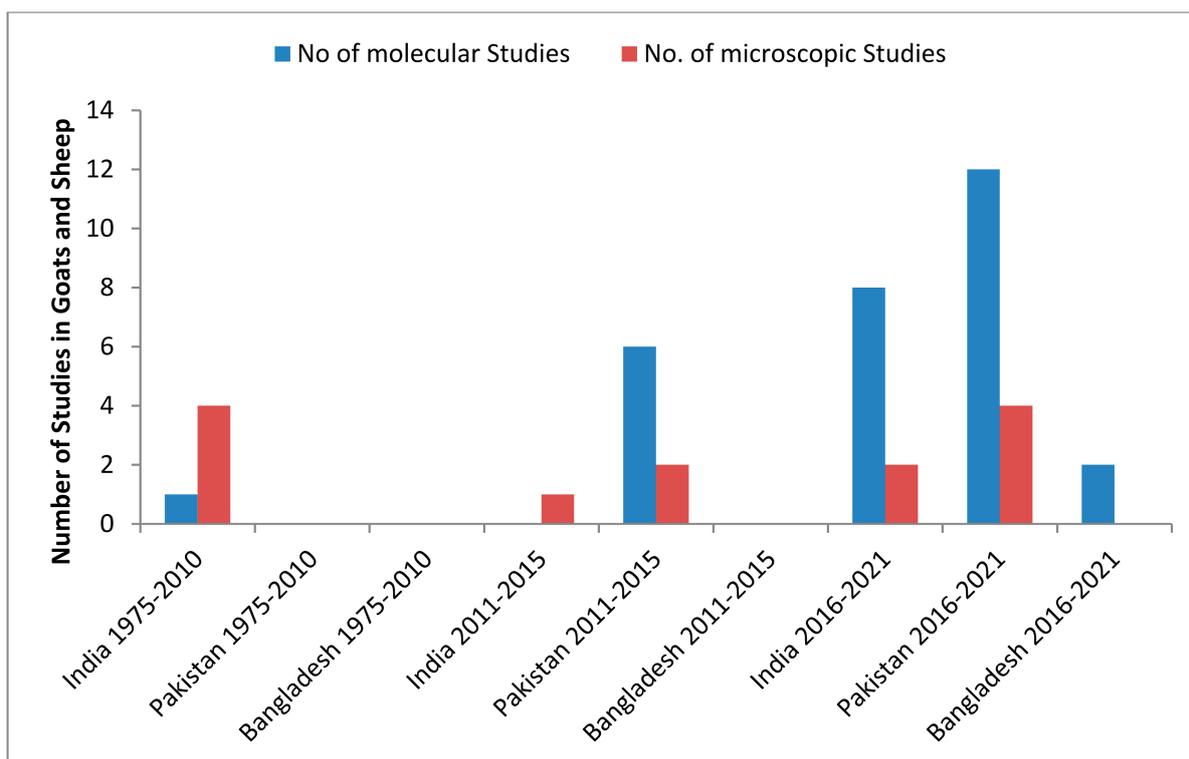


Figure 5. Comparison of the number of epidemiological studies detecting *Theileria* spp. in goats and sheep using molecular and microscopic diagnostic methods in India, Pakistan and Bangladesh, according to three different periods between 1975 and 2021.

2.4. Phylogenetic Analysis and Evolutionary Divergence

Representative sequences with accession numbers were selected from the previously published studies and were used for phylogenetic and evolutionary (genetic) divergence analysis. The sequences selected from the different regions of India were included, MF287947.1 (Central India), MF287920.1, (West India), MF287949.1 (Eastern India), MF287937.1 *T. annulata* South India and MF287934.1 (North India) while sequences with accession numbers from Pakistan are included JQ743631.1 (Pakistan), JQ743636.1, (Pakistan), MW046053.1 (Pakistan) and MW046054.1 (Pakistan). The phylogenetic tree of the selected *T. annulata* isolates was inferred using the 18S rRNA genetic marker. Phylogenetic analyses were conducted with a Tamura 3-parameter (T92 + G) Model using MEGA version 7.0 [32]. CLUSTAL W alignment was performed to align the selected sequences retrieved from the GenBank. The neighbor-joining algorithm was used to establish the phylogenetic analysis. Bootstrap values were obtained with 1000 replicates.

3. Results and Discussion

3.1. Diversity and Distribution of *Theileria* Species Infecting Livestock

A general overview of the *Theileria* species identified in the three countries is depicted in the Table 1. We have collected the data regarding different species of *Theileria* infecting livestock i.e., large (cattle & buffalo) and small ruminants (goats & sheep) conducted in the different geographical regions of the three selected countries of South Asia. Our analysis revealed that different species of *Theileria* in these countries circulating in large ruminants include *T. annulata*, *T. orientalis*, *T. mutans*, *T. Ovis* and *T. velifera* and *Theileria* spp., while in small ruminants, the different species of *Theileria* reported were *T. annulata*, *T. lestoquardi*, *T. luwenshuni*, *T. ovis*, *Theileria* spp. and *T. lestoquardi*-like spp. from various geographical locations of India, Pakistan and Bangladesh. Among these, the most common and widespread species circulating in all ruminants is *T. annulata*.

Table 1. Common *Theileria* species found in India, Pakistan and Bangladesh.

Country	Host	<i>Theileria</i> Species Name
India	Cattle & Buffaloes	<i>Theileria</i> spp. <i>T. annulata</i> , <i>T. orientalis</i> , <i>T. mutans</i> & <i>T. velifera</i>
	Goats & Sheep	<i>Theileria</i> spp. <i>T. lestoquardi</i> <i>T. luwenshuni</i> & <i>T. ovis</i>
Pakistan	Cattle & Buffaloes	<i>T. annulata</i> , <i>T. orientalis</i> & <i>Theileria</i> spp.
	Goats & Sheep	<i>T. annulata</i> , <i>T. lestoquardi</i> , <i>T. luwenshuni</i> <i>T. ovis</i> & <i>Theileria</i> spp. <i>T. lestoquardi</i> -like spp.
Bangladesh	Cattle & Buffaloes	<i>Theileria</i> spp. <i>T. annulata</i> , <i>T. orientalis</i> , <i>T. Mutans</i> ,
	Goats & Sheep	<i>T. annulata</i>

3.2. Diversity and Distribution of *Theileria* Species Infecting Livestock in the Different Regions of India

Theileria species reported from different regions of India are listed in Table 2. According to the data collected, only one species of *Theileria* reported in cattle and buffalo from the central region of India is *T. annulata*, [33,34] while, on the other hand, in Eastern India, two species of *Theileria*. (*T. mutans* & *T. velifera*) were identified; however, no *Theileria* species were reported in small ruminants from these two regions [35,36]. Furthermore, *Theileria* species identified in the large ruminants from South India included *Theileria* spp., *T. annulata* [37–39] and new *T. orientalis* genotype [40–43], whereas, in East and North East India, trans placental transmission of *T. annulata* in young borne calves and its detection in tick *Hy. anatolicum* has been identified [44,45]. In the case of small ruminants, *T. lestoquardi* was identified molecularly. *T. luwenshuni* & *T. ovis* [46–48] were detected in South India while only *T. luwenshuni* has been recorded in the East and Northeast region of India [49,50]. Furthermore, *Theileria* spp., [51] *T. annulata*, [52–57] and *T. orientalis* [58] were detected to be circulating in cattle and buffalo while *T. annulata*, [59], *Theileria* spp. [60] and *T. luwenshuni* [61–63] were found infecting goats and sheep (small ruminants) from West Bangal and North India. The difference in the distribution of diversity of various *Theileria* species in different regions may be attributed to various factors such as competent vector tick species, geo-climatic conditions favoring different vector tick species growth, lack of education of farmers, and improper use of acaricidal use.

Table 2. Regional wise distribution of *Theileria* Species in different regions of India.

India					
Province/State	<i>Theileria</i> spp.	Identification Method	Host	Year of Study	References
Central India					
		Cattle & Buffaloes			
Central India	<i>T. annulata</i>	Microscopy	Ticks & Cattle	1975	[64]
Uttar Pradesh	<i>T. annulata</i>	Molecular	Cattle	1977	[65]
Uttar Pradesh	<i>T. annulata</i>	Microscopy	Cattle	2012	[66]
Uttar Pradesh	<i>T. annulata</i>	Molecular	Cattle	2015	[67]
Chhattisgarh	<i>T. annulata</i>	Microscopy	Cattle	2016	[34]
Central India	<i>T. annulata</i>	Molecular	Cattle	2017	[68]
Uttar Pradesh	<i>T. annulata</i>	Molecular	Cattle	2018	[69]
Hisar	<i>T. annulata</i>	Microscopy	Cattle	1989	[33]
Eastern India					
West Bangal	<i>T. annulata</i>	Molecular	Cattle	2003	[35]
Guinea	<i>T. mutans</i> & <i>T. velifera</i>	Molecular	Cattle	2021	[36]
South India					
North Banglore	<i>T. annulata</i>	Microscopy	Cattle	2009	[37]
Kerala	<i>Theileria</i> spp. & <i>T. annulata</i>	Microscopy & Molecular	Cattle	2011	[38]
South India	<i>T. orientalis</i>	Molecular	Cattle	2011	[70]
South India	<i>T. annulata</i> & <i>Theileria</i> spp.	Molecular	Cattle	2013	[40]
Tamil Nadu	<i>Theileria</i> spp.	Microscopy	Cattle	2014	[71]
Telangana and Andhra Pradesh	<i>T. orientalis</i>	Molecular	Cattle	2015	[41]
Karnataka	<i>T. annulata</i>	Microscopy	Cattle	2016	[42]

Table 2. Cont.

India					
Province/State	<i>Theileria</i> spp.	Identification Method	Host	Year of Study	References
Southwest India	<i>T. annulata</i>	Microscopy	Buffalo & Cattle	2016	[39]
Karnataka	<i>T. annulata</i>	Microscopy	Cattle	2017	[72]
Kerala	<i>T. orientalis</i>	Molecular	<i>R. annulatus</i> Ticks	2019	[43]
South India	<i>Theileria</i> spp.	Molecular	Ticks & Cattle	2021	[73]
West Bengal	<i>T. hirci</i> (<i>T. lestoquardi</i>)	Microscopy	Goats & Sheep	1990	[46]
Karnataka	<i>Theileria</i> spp.	Microscopy	Goat	1985	[74]
South India	<i>Theileria</i> spp.	Microscopy	Sheep	2017	[47]
Kerala	<i>Theileria</i> spp.	Microscopy & Molecular	Goat & Ticks	2017	[75]
Karnataka	<i>T. luwenshuni</i>	Molecular	Goats	2017	[48]
Karnataka	<i>T. luwenshuni</i> & <i>T. ovis</i>	Molecular	Goats & Sheep	2017	[48]
Karnataka	<i>Theileria</i> spp.	Microscopy	Sheep	2019	[76]
Assam	<i>T. annulata</i> & <i>T. orientalis</i>	Molecular	Sheep	2020	[77]
Odisha	<i>T. annulata</i> & <i>T. orientalis</i>	Molecular	Cattle	2015	[78]
Odisha	<i>T. annulata</i>	Microscopy & Molecular	Cattle	2017	[79]
Odisha	<i>T. orientalis</i>	Microscopy and Molecular	Cattle	2017	[80]
Odisha	<i>T. orientalis</i>	Molecular	Cattle	2020	[81]
Odisha	<i>T. annulata</i>	Molecular	Cattle	2021	[44]
Odisha	<i>T. annulata</i>	Molecular	Cattle	2021	[45]
Assam	<i>T. luwenshuni</i>	Goats & Sheep	Goats	2018	[50]
Guwahati of Assam	<i>T. luwenshuni</i>	Microscopy & Molecular	Goat	2019	[49]
Anand	<i>T. annulata</i>	West India	Buffalo & Cattle	2014	[52]
Gujrat	<i>T. annulata</i>	Molecular	Cattle	2015	[54]
Gujrat	<i>T. annulata</i>	Microscopy and Molecular	Cattle & Buffalo	2015	[53]
Maharashtra	<i>T. annulata</i> & <i>T. orientalis</i>	Microscopy & Molecular	Cattle	2017	[58]
Bihar	<i>T. annulata</i>	Molecular	Cattle	2017	[58]
Anand	<i>T. annulata</i> & <i>T. orientalis</i>	Microscopy	Buffalo	2018	[82]
Maharashtra & tamil Nadu	<i>T. annulata</i>	Molecular	Cattle	2019	[55]
Telangana, Gujarat, Haryana, and Bihar	<i>T. annulata</i>	Molecular	Buffalo & Cattle	2019	[58]
Maharashtra	<i>Theileria</i> spp.	Microscopy	Vaccine Isolate	2019	[83]
Bihar	<i>Theileria</i> spp.	Microscopy	Buffalo	2020	[51]
Tamil Nadu	<i>T. annulata</i>	Microscopy	Cattle	2021	[84]
Bihar	<i>T. annulata</i>	Molecular	Cattle	2021	[57]
Haryana	<i>T. annulata</i>	Microscopy & Molecular	Cattle	2021	[85]
Tamil Nadu	<i>Theileria</i> Spp.	Microscopy	Goat, cattle, sheep	1998	[86]
Haryana	<i>T. annulata</i>	Microscopy	Sera	2005	[87]
Tamil Nadu	<i>Theileria</i> spp.	Molecular	Sheep	2006	[59]
Tamil Nadu	<i>T. luwenshuni</i>	Microscopy	Tick	2015	[60]
Maharashtra	<i>T. luwenshuni</i>	Molecular	Goats & Sheep	2019	[61]
Punjab	<i>T. annulata</i>	Microscopy & Molecular	Goats & Sheep	2021	[62]
Gujrat	<i>Theileria</i> spp.	Microscopy	Sheep	2010	[88]
Ludhiana Punjab	<i>T. annulata</i>	Microscopy	Tick Hy. anatolicum	2021	[89]
Punjab	<i>T. annulata</i>	Molecular	Buffalo & Cattle	2012	[90]
Ludhiana Punjab	<i>T. annulata</i>	Molecular	Cattle	2015	[91]
Rajasthan	<i>Theileria</i>	Molecular	Cattle	2015	[92]
Uttara hand	<i>Theileria</i> genus	Microscopy	Tick & Cattle	2015	[93]
Haryana	<i>T. annulata</i>	Microscopy & Molecular	Cattle	2014	[94]
Haryana	<i>T. annulata</i>	Molecular	Cattle	2017	[95]
Telangana, Gujarat, Haryana, and Bihar	<i>T. annulata</i>	Molecular	Cattle	2020	[96]
Gujrat	<i>T. annulata</i>	Molecular	Vaccine	2021	[83]
Himachal Pradesh	<i>T. orientalis</i>	Molecular	Cattle	2021	[97]
Himachal Pradesh	<i>T. luwenshuni</i>	Molecular	Cattle	2021	[98]
Himachal Pradesh	<i>T. luwenshuni</i>	Goats & Sheep	Goats & Sheep	2021	[63]

3.3. Possible Tick Vectors for *Theileria* Species in India

The diverse array of *Theileria* species in the region may be due to different tick vectors infesting livestock, which is shown in Table 3. Several studies have reported different vector tick species from different regions of India included *R. microplus* Hae. *Bispinosa*

Hy. truncatum *Hy. dromedarii* *Hy. anatolicum* *R. Sanguineus* [70,78,99]. It may be assumed that the presence of different species of *Theileria* circulating in large and small ruminants may be linked to the presence of a wide variety of these tick vectors in the particular area as these ticks have the potential to transmit various *Theileria* species reported from other parts of the world [100,101]. For example, it has been found in Uttar Pradesh that *H. anatolicum* and *R. appenticulatus* could play a vector role in the *T. annulata* and *T. lestoquardi* transmission in large and small ruminants, respectively [64]. Similarly, *Hy. anatolicum* and *R. microplus* *Hy. m. isaaci* have been identified to transmit *T. annulata* in cattle, while, in buffalo, *Hy. anatolicum* may be the only vector transmitting *T. buffeli* (*T. orientalis*). On the other hand, *Hy. anatolicum* and *Haemaphysalis* are widely distributed in different regions of India [64,102], which may transmit various species of *Theileria* such as *T. lestoquardi* (*hirci*) and other *Theileria* species in goats and sheep. Recently, in India, some studies have also confirmed the role of various tick species such as *R. microplus*, *Hy. anatolicum* and *Hae. bispinosa* in the transmission of *T. orientalis* [70,78]. Similar reports have been observed in Africa [103]. Besides the above-mentioned tick species, *Hy. detritum*, *Hy. dromedarii*, and *Hy. lusitanicum* can also be the potential vectors for the transmission of this pathogen in different hosts [9].

Table 3. Distribution of the possible vector tick species of *Theileria* in different regions of India.

Tick Species	Host	States/Region	References
<i>R. microplus</i> , <i>R. haemaphysaloides</i>	Cattle & Buffalo	It is found in all places except Manipur, Kerala, Nagaland, Tripura & Maharashtra	[78,102]
<i>Hy. anatolicum</i>	Ruminants	It may be present in all parts except Andhra Pradesh, Jharkhand, Manipur, Meghalaya, Sikkim, Tripura	[33,46,103]
<i>Hae. Bispinosa</i>	Goats & Sheep	Widely distributed except Delhi, Haryana, Kerala, Nagaland, Uttar Pradesh and Chhattisgarh	[47,64,70,99]
<i>Hy. truncatum</i>	Goats & Sheep	It is restricted to only Gujrat, Maharashtra & Uttar Pradesh	[102]
<i>Hy. dromedarii</i>	Goats & Sheep	It can be found only in Andhra Pradesh, Delhi, Gujrat, Haryana, Himachal Pradesh, Jammu & Kashmir, Odisha, Punjab, Rajasthan, Uttar Pradesh	[102]
<i>R. Sanguineus</i>	Goats & Sheep	It is reported from all places except Gora, Delhi, Manipur, Meghalaya, Nagaland, Tripura and Uttar Pradesh	[47,64,102]

3.4. Diversity and Distribution of *Theileria* Species in Different Regions of Pakistan

Theileria species that have been reported in different provinces of Pakistan are listed in Table 4. Approximately three different species of *Theileria*, including *Theileria* spp., *T. annulata* and *T. orientalis* have been reported in cattle and buffalo from different places of the Punjab province. Among these, the predominant and widely distributed pathogen is *T. annulata*, which many authors have reported across the province either microscopically or molecularly [104–107]. In addition to *T. annulata*, other *Theileria* species such as *T. orientalis* [108–110] and *Theileria* spp. have also been identified recently [111–113]. On the other hand, different species of *Theileria* identified in small ruminants (goats and sheep) from various geographic areas of Punjab included *Theileria* spp. and *T. annulata*, [114–116] *T. ovis* and *T. ovis*, *T. lestoquardi*, [115,117–119]. In Khyber Pakhtunkhwa province, several studies from different areas investigated that *T. annulata* is the only species circulating in cattle and buffalo [101,120–122]; however, diverse species of *Theileria* such as *T. annulata*, *T. lestoquardi*, *T. luwenshuni* *T. ovis* and *Theileria* spp. have been identified in goats and sheep [123–126]. *Theileria* spp. and *T. annulata* were identified in cattle and buffalo from Sindh and Balochistan Province [116,127]. No single study has reported *Theileria* species infecting ruminants from Sindh province; however, in Balochistan and FATA, different species *Theileria* identified from small ruminants include *T. annulata*, *T. ovis* and *T. lestoquardi* [7,107,116]. Some studies were carried out in the adjoining areas of two provinces and reported different species of *Theileria*; for example, in a study Ghafar et al. [128] interestingly identified *T. lestoquardi*-like spp., *T. orientalis* and *T. annulata*

from the ticks collected from the cattle and buffalo in different agro-ecological zones of Punjab and Sindh Province, while, on the other hand, Durrani et al. [129] and Karim et al. [130] identified *T. ovis* and *T. annulata* in small and large ruminants from the different regions of Punjab and Khyber Pakhtunkhwa, respectively.

Table 4. Provincial wise distribution of the different *Theileria* species in Pakistan.

Pakistan					
Province/State	<i>Theileria</i> Species	Identification Method	Host	Year	Reference
Punjab					
Cattle & Buffaloes					
Punjab	<i>Theileria</i> spp.	Microscopy	Cattle	1983	[131]
Faisalabad	<i>Theileria</i> spp.	Microscopy	Cattle	1999	[132]
Faisalabad	<i>T. annulata</i>	Microscopy	Buffaloes & Cattle	2004	[133]
Kasur	<i>Theileria</i> spp.	Microscopy	Cattle	2005	[134]
Punjab	<i>T. annulata</i>	Microscopy	Buffaloes	2006	[135]
Kasur	<i>T. annulata</i>	Molecular	Cattle	2008	[136]
Punjab	<i>T. annulata</i>	Microscopy & Molecular	Cattle	2008	[137]
Sahiwal	<i>Theileria</i> spp.	Microscopy	Cattle	2010	[111]
Southern Punjab	<i>T. annulata</i>	Microscopy	Cattle	2011	[104]
Sargodha	<i>T. annulata</i>	Microscopy	Cattle	2012	[138]
Faisalabad, Jhang, Khanewal	<i>T. annulata</i>	Molecular	Ticks of Cattle & Buffaloes	2013	[105]
Faisalabad	<i>T. annulata</i>	Molecular	Cattle & Buffaloes	2013	[106]
Punjab	<i>T. orientalis</i>	Molecular	Cattle	2021	[108]
Punjab	<i>T. annulata</i>	Molecular	Cattle	2018	[109]
Punjab	<i>T. annulata</i> & <i>T. orientalis</i>	Molecular	Cattle	2018	[139]
Lahore	<i>T. annulata</i>	Microscopy	Cattle	2018	[5]
Agro-ecological Zones Punjab	<i>T. orientalis</i> & <i>T. annulata</i>	Molecular	Ruminants	2019	[110]
Agro-ecological Zones Punjab	<i>T. annulata</i>	Molecular	Cattle	2020	[107]
Layyah	<i>T. annulata</i> & <i>T. orientalis</i>	Molecular	Cattle	2021	[113]
Dera Ghazi Khan & Lodhran	<i>T. annulata</i>	Molecular	Cattle	2021	[112]
Attock	<i>Theileria</i> spp.	Microscopy	Goats & Sheep	2010	[140]
Okara	<i>Theileria</i> spp.	Microscopy	Sheep	2010	[141]
Lahore	<i>T. lestoquardi</i> & <i>T. ovis</i>	Microscopy & Molecular	Sheep	2011	[142]
Lahore	<i>Theileria</i> spp.	Microscopy	Goats & Sheep	2011	[143]
Okara	<i>T. ovis</i>	Molecular	Sheep	2013	[114]
Southern Punjab	<i>T. lestoquardi</i>	Molecular	Goats & Sheep	2015	[144]
Multan	<i>T. ovis</i> & <i>T. lestoquardi</i>	Microscopy & Molecular	Goats & Sheep	2017	[115]
Multan	<i>T. lestoquardi</i> & <i>T. ovis</i>	Microscopy & Molecular	Goats & Sheep	2017	[145]
Punjab	<i>T. annulata</i> <i>T. ovis</i> & <i>T. lestoquardi</i>	Molecular	Ruminants	2019	[117]
Multan	<i>T. lestoquardi</i> & <i>T. ovis</i>	Molecular & Microscopy	Goats	2019	[118]
Layyah	<i>T. annulata</i>	Molecular	Sheep	2021	[119]
Lahore	<i>Theileria</i> Spp.	Microscopy	Goats & Sheep	2021	[116]
Peshawar	<i>T. annulata</i>	Microscopy	Buffalo & Cattle	2005	[146]
KPK (Southern KP)	<i>T. annulata</i>	Molecular	Cattle	2012	[147]
KPK	<i>T. annulata</i>	Molecular	Cattle	2017	[120]
Northern Pakistan	<i>T. annulata</i>	Molecular	Cattle (Ticks)	2019	[101]
North-Western Pakistan	<i>T. annulata</i>	Molecular	Cattle	2021	[148]
DI Khan	<i>Theileria</i> spp.	Microscopy	Cattle	2021	[149]
Central KPK	<i>T. annulata</i>	Microscopy & Molecular	Cattle	2021	[122]
KPK	<i>T. lestoquardi</i> & <i>T. ovis</i>	Molecular	Goats & Sheep	2013	[147]
KPK	<i>T. lestoquardi</i>	Molecular	Goats & Sheep	2015	[150]
Peshawar & Periphery	<i>Theileria</i> spp.	Microscopy	Ruminants	2017	[151]
Peshawar & Khyber Agency	<i>Theileria</i>	Microscopy	Goats & Sheep	2017	[152]
Southern KPK	<i>T. ovis</i> <i>T. lestoquardi</i>	Molecular	Goats & Sheep	2018	[123]

Table 4. Cont.

Pakistan					
Province/State	Theileria Species	Identification Method	Host	Year	Reference
Southern KPK	<i>Theileria</i> spp.	Microscopy	Sheep	2018	[153]
KPK	<i>T. annulata</i> , <i>T. lestoquardi</i> , <i>T. luwenshuni</i> <i>T. ovis</i> & <i>Theileria</i> spp.	Molecular	Goats & Sheep	2020	[124]
Malakand Division	<i>Theileria</i> spp.	Microscopy	Buffalo, Cattle, Goat & Sheep	2021	[125]
KPK	<i>Theileria</i> Spp. <i>T. annulata</i> , <i>T. lestoquardi</i> , <i>T. ovis</i>	Molecular	Goats & Sheep	2021	[126]
Hyderabad	<i>Theileria</i> spp.	Sindh Microscopy	Cattle	1994	[154]
Karachi	<i>Theileria</i>	Microscopy	Buffalo	2012	[127]
Quetta	<i>T. annulata</i>	Microscopy	Cattle	2021	[116]
Baluchistan	<i>Theileria ovis</i> & <i>T. lestoquardi</i>	Molecular	Goats & Sheep	2017	[7]
FATA	<i>T. ovis</i>	Molecular	Goats & Sheep	2020	[107]
Punjab & KPK	<i>T. ovis</i>	Microscopy & Molecular	Goats & Sheep	2012	[129]
Pakistan/Punjab-KPK	<i>T. annulata</i>	Molecular	Ruminants	2017	[130]
Sindh & Punjab	<i>T. lestoquardi</i> -like spp., <i>T. orientalis</i> & <i>T. annulata</i>	Molecular	Cattle	2021	[128]

3.5. Tick Vectors for Transmission of *Theileria* in Pakistan

In Pakistan, different studies have reported a wide variety of tick species from various geographical areas as shown in Table 5. In these studies, different tick species have been identified, which may be due to different prevailing conditions in the country such as seasonal fluctuation, relative humidity, temperature, association and lifestyle of different species of animals, lack of education in farmers, and farm management practices which may favor tick growth and survival. In the current study, existence of a wide variety of *Theileria* species may be due to the presence of different tick vectors, which may possibly play a role in the transmission of these pathogens. Common genera which may be possibly involved in the transmission of these pathogens include *Hyalomma*, *Rhipicephalus* and *Haemaphysalis*. Several studies have reported different tick species across the country such as *Hy. hussaini*, *Hy. scupense*, *Hy. anatolicum*, *Hy. scupense*, *Hy. excavatum*, *R. microplus*, *R. sanguineus* [105,123,138,155–158]. These ticks may have the capacity to transmit different *Theileria* species in different hosts [103,107]. However, further molecular studies are needed to find out their vector competencies.

Table 5. Distribution of various tick vectors for the transmission of *Theileria* species in Pakistan.

Ticks	Host	References
Punjab <i>R. microplus</i> , <i>Hy. anatolicum</i> , <i>Hy. aegyptium</i> , <i>Hy. dromedarii</i> , <i>R. appendiculatus</i> , <i>R. sanguineus</i> Khyber Pakhtunkhwa	Cattle and buffaloes	[102,105,138,147,153,157–162]
<i>R. microplus</i> , <i>R. appendiculatus</i> , <i>Hy. anatolicum</i>	Cattle & buffaloes	[101,163]
Sindh <i>Hy. hussaini</i> , <i>Hy. scupense</i> , <i>R. annulatus</i> , <i>R. microplus</i> , <i>Hy. anatolicum</i> , <i>Hy. scupense</i> , <i>Hy. excavatum</i>	Buffaloes	[155,156,164]
Balochistan <i>R. microplus</i> , <i>Hy. anatolicum</i> , <i>Hy. scupense</i> , <i>Hy. aegyptium</i> , <i>Haemaphysalis</i>	Cattle & buffaloes	[107,165–169]
Punjab <i>Hy. anatolicum</i> , <i>Hy. excavatum</i> <i>R. appendiculatus</i> , <i>Hy. dromedarii</i> , <i>R. microplus</i> , <i>R. sanguineus</i> , <i>R. Turanicus</i>	Goats & Sheep	[158,160,170–174]
Khyber Pakhtunkhwa <i>Hy. anatolicum</i> , <i>Hy. detritum</i> , <i>Hy. excavatum</i> , <i>Hy. scupense</i> , <i>Haemaphysalis longicornis</i> , <i>Hyalomma</i> <i>impeltatum</i> , <i>R. appendiculatus</i> , <i>R. microplus</i>	Goats & Sheep	[107,175–180]
Sindh <i>Hae. bispinosa</i> , <i>Hy. anatolicum</i> , <i>Hy. detritum</i> , <i>Hy. dromedarii</i> , <i>Hy. hussaini</i> , <i>Hy. impeltatum</i> , <i>Hy. marginatum isaaci</i> , <i>R. microplus</i> , <i>R. Sanguineus</i>	Goats & Sheep	[181,182]
Balochistan <i>Hy. anatolicum</i> , <i>Hy. dromedarii</i> , <i>Hy. excavatum</i> , <i>Hy. scupense</i> , <i>R. microplus</i>	Goats & Sheep	[168,183–185]

3.6. Regional Wise Distribution of Theileria Species and Its Possible Tick Vectors in Bangladesh

Different species of *Theileria* from large and small ruminants from various geographical regions are listed in Table 6. Among these, most of the species such as *Theileria* spp., *T. annulata*, *T. mutans* and *T. orientalis* have been identified from the large ruminants of Central and North Central regions of Bangladesh [186–189] while, from the South-western, Northern and Western region, only *Theileria* spp. and *T. annulata* have been reported [190–193]. Furthermore, only one species, i.e., *T. annulata*, was reported in goats and sheep from the central part of Bangladesh [189,194].

Table 6. Regional wise distribution and diversity of *Theileria* species in various regions of Bangladesh.

Bangladesh					
City	<i>Theileria</i> spp.	Identification Method	Host	Year of Study	References
Central Region					
Dhaka Targil	<i>T. annulata</i>	Microscopy	Cattle	1983	[195]
Dhaka	<i>T. annulata</i> and <i>T. mutans</i>	Microscopy	Cattle	1989	[196]
Dhaka, Sirajganj and Nikhangsori	<i>T. annulata</i>	Microscopy & Molecular	Cattle goats & Sheep	2019	[194]
Dhaka	<i>Theileria</i> spp.	Goats & Sheep Microscopy & Molecular	Goats	2021	[189]
Chittagong	<i>Theileria</i> spp.	South Eastern Region Microscopy	Cattle	2010	[190]
North Central Region					
Sirajganj	<i>Theileria</i> spp.	Microscopy	Cattle	2015	[187]
Sirajganj	<i>T. annulata</i> & <i>T. mutans</i>	Microscopy	Cattle	1976	[186]
Sirajganj	<i>T. annulata</i>	Molecular	Cattle	1977	[197]
Mymensingh	<i>T. orientalis</i>	Molecular	Cattle	2018	[188]
Northern Region					
Dinajpur	<i>Theileria</i> spp.	Microscopy	Cattle	2016	[191]
Western region					
Rajshahi	<i>T. annulata</i>	Molecular	Cattle	2016	[192]
Natores	<i>T. annulata</i>	Molecular	Cattle	2019	[193]

The possible vector tick species from the different geographical places of Bangladesh that could be involved in the transmission of various *Theileria* species in small and large ruminants are shown in Tables 7 and 8. The common tick species responsible for the transmission of *Theileria* in cattle and buffalo are *R. microplus*, *Hae. bispinosa*, *R. sanguineus*, and *Hy. anatolicum anatolicum* [102], while, in the case of small ruminants (goats and sheep), these may be *R. sanguineus* and *Hy. anatolicum anatolicum* [102].

Table 7. Distribution of possible tick vectors for *Theileria* species in various regions of Bangladesh.

Divisions	Possible Tick Vector	Reference
Braisel, Dhaka, Savar, Narayanganj, Tangali	<i>R. microplus</i>	[102]
Dhaka, Rajshahi, Savar	<i>Hae. bispinosa</i>	[102]
North western dry Region (Rajshahi, Rangpur, and Dinajpur districts)	<i>Hy. anatolicum anatolicum</i>	[102]
Savar	<i>Hy. Truncatum</i>	[102]
Braisal, Chitagang, Dhaka, Narayanganj, Tangail, Rangpur, Sylhet	<i>R. sanguineus</i>	[102]

Table 8. Common tick species in different hosts in Bangladesh.

Possible Tick Vectors for TT	Host	References
<i>R. microplus</i> , <i>Hae. bispinosa</i> , <i>R. sanguineus</i> , <i>Hy. anatolicum anatolicum</i>	Cattle & Buffaloes	[102]
<i>R. sanguineus</i> , <i>Hy. anatolicum anatolicum</i>	Goats & Sheep	[102]

3.7. Phylogenetic Analysis, Genetic Divergence and Multiple Sequence Alignment

We have selected *T. annulata* for phylogenetic and genetic divergence analysis as this species may infect all ruminants and could be of great economic concern. The evolutionary history was inferred using an *18S rRNA* taxonomic marker. Homology searches of the selected *18S rRNA* isolates shared 99–100% similarities with local and global isolates deposited in the NCBI GenBank. The neighbor-joining algorithm was used to establish a phylogenetic relationship among different selected isolates. The selected *18S rRNA* isolates were clustered into different clades (Figure 6). However, no single isolate was selected from Bangladesh as *18S rRNA* sequence was not available for *T. annulata* from Bangladesh. We select the *18S rRNA* genetic marker as several previously published studies used this genetic marker in Pakistan, India and other parts of the world to identify and establish the phylogenetic profile of *T. annulata* circulating in ruminants [41,101,106,109,198]. The *18S rRNA* gene play an important role in genetic variability of *Theileria* spp. due to the presence of conserved sequences and some hypervariable regions which are crucial in determining the evolutionary patterns and discriminating the various *Theileria* species [16,199]. Furthermore, targeting the amplification of the hypervariable V4 region of the *18S rRNA* gene is preferably used for the accurate identification, classification and exploring the population structures of the piroplasm parasites [117,200]. Nucleotide sequence heterogeneity analysis (evolutionary/genetic divergence) showed that *T. annulata* isolates were different from each other by 0–4.7 bp as shown in Table 9. Maximum divergence (4.7 bp) was observed with isolates from Pakistan. Additionally, the multiple alignment analysis also showed changes in the nucleotide sequences of different isolates as can be seen in Figure 7. These genetic variations may be due to genetic variability contributed by the deletions, insertions and substitutions in the nucleotide sequences of different isolates [41,55], which may result in the various degrees of pathogenicity and treatment measures in the field. Thus, on the basis of this analysis and previous findings, we concluded that, due to the presence of a hypervariable region and genetic variability, vaccine development against *Theileria* species is still challenging, and we may suggest that using a single diagnostic or immunogenic molecule may not be sufficient in achieving the required goals.

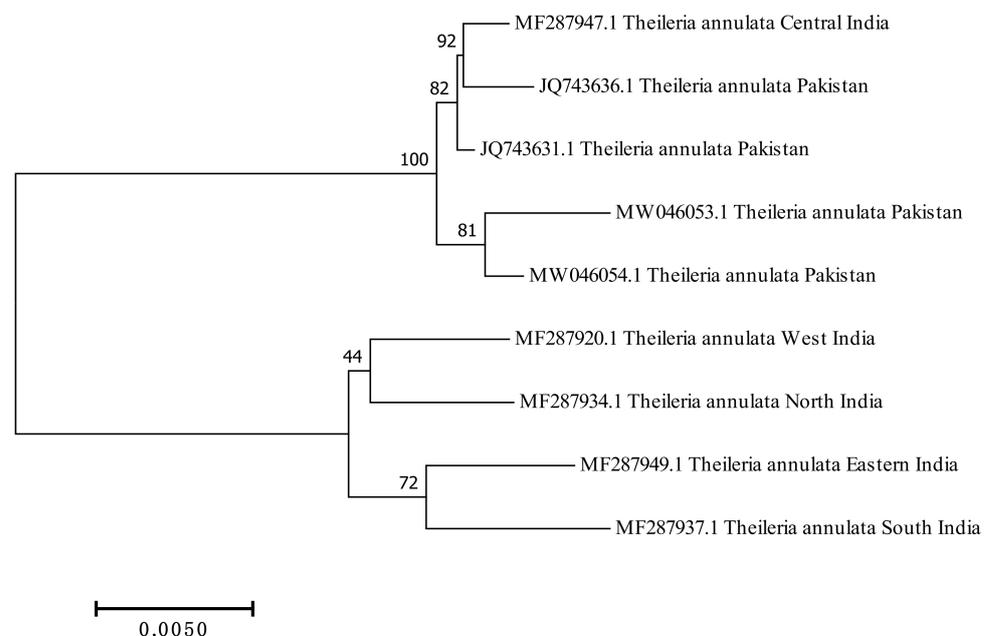


Figure 6. Phylogenetic analysis of selected *T. annulata* isolates (*18S rRNA* gene).

Table 9. Evolutionary divergence analysis of *T. annulata* 18S rRNA gene isolates.

	1	2	3	4	5	6	7	8	9
MF287947.1 <i>T. annulata</i> Central India									
MF287920.1 <i>T. annulata</i> West India	0.58								
MF287949.1 <i>T. annulata</i> Eastern India	0.62	0.22							
MF287937.1 <i>T. annulata</i> South India	0.63	0.25	0.19						
MF287934.1 <i>T. annulata</i> North India	0.58	0.16	0.19	0.28					
JQ743631.1 <i>T. annulata</i> Pakistan	0.4	0.55	0.59	0.6	0.56				
JQ743636.1 <i>T. annulata</i> Pakistan	0.7	0.6	0.63	0.065	0.6	0.5			
MW046053.1 <i>T. annulata</i> Pakistan	2.14	1.65	4.7	4.7	0.65	0.12	0.15		
MW046054.1 <i>T. annulata</i> Pakistan	0.9	0.57	2.63	3.62	0.57	3.8	0.11	0.0	
	1	2	3	4	5	6	7	8	9

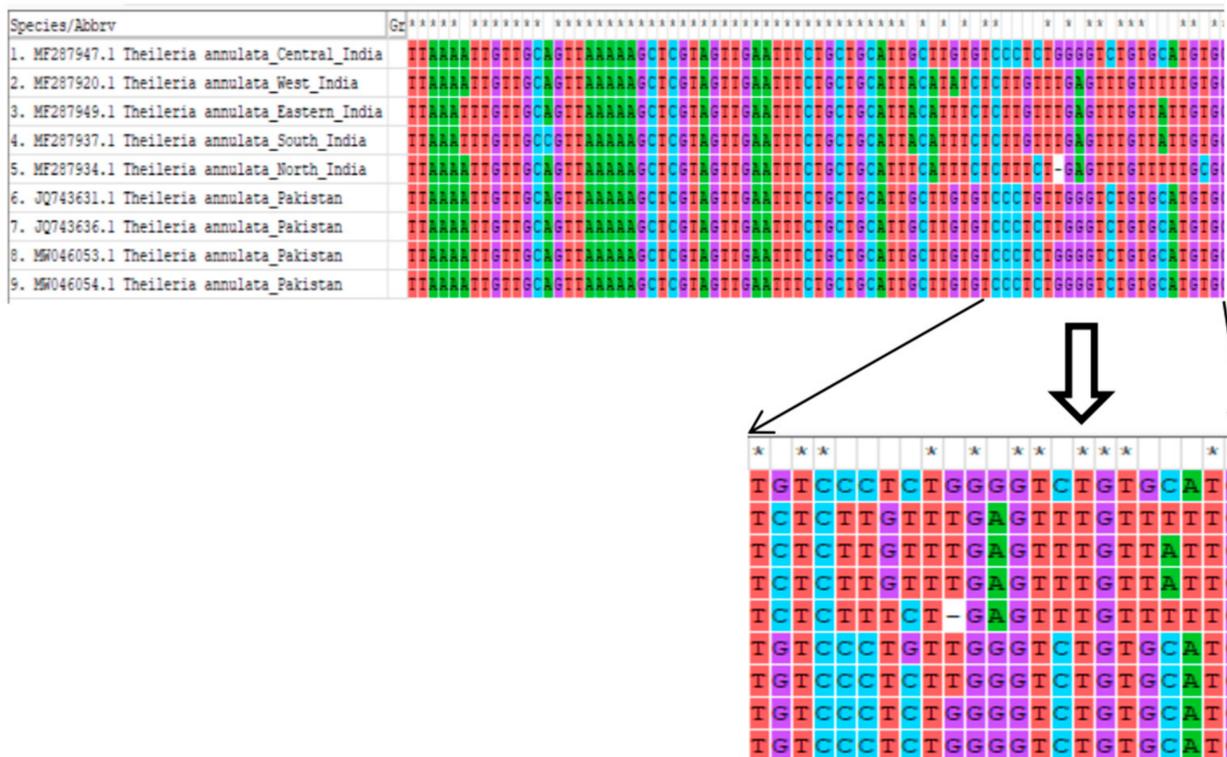


Figure 7. CLUSTAL W multiple sequence alignment analysis for the different *T. annulata* isolates from India and Pakistan. The box below showing the changes in nucleotide sequences between *T. annulata* isolates from Pakistan and India. * means nucleotide similarities.

3.8. Overall Comparison (Why Vector-Borne Diseases like Theileria Are Increasing Day by Day)

The global climate has been changing over the last century due to greenhouse gas emissions. It will continue to change over this century, accelerating without effective global efforts to reduce emissions. Ticks and tick-borne diseases (TTBDs) are inherently climate-sensitive due to the sensitivity of tick lifecycles to climate. Key direct climate and weather sensitivities include survival of individual ticks and the duration of development and questing (host-seeking) activity of ticks [201].

We have concluded that our data contribute to the knowledge of *Theileria* species diversity and distribution circulating in ruminants in different regions of India, Pakistan and Bangladesh. The diverse species and wide distribution of *Theileria* in the region may be linked with the presence of their possible vector tick species belonging to the main four genera including *Hyalomma*, *Haemaphysalis*, *Rhipicephalus* and *Amblyomma* as they have been identified as *Theileria* vectors from several parts of the world [101–103,107,117]. Paucity still exists; however, molecular studies have been updated and increased during the last decade regarding *Theileria* species identification and their possible vectors. Different

ticks have been adapted to diverse climatic conditions such as aridity in the desert, and seasonal and daily fluctuations may result in widespread distribution of these ticks and subsequently lead to the diversity in *Theileria* species. This adaptation offers the tick a significant comparative advantage over other tick species under the predicted scenarios for climate change [103], particularly in the context of South Asian countries where these tick species might gain importance in the future. Our analysis suggests the needs of further molecular studies to discover different species and strains, and their potential vectors as microscopic studies have not been so effective in identifying different species and strains. Additionally, genetic variation among different *T. annulata* isolates may be due to the mutations in the hypervariable region and genetic variability, which makes the therapeutic and single molecule based vaccine development against *Theileria* challenging and necessitates the needs for alternative control measures. This study also helps in providing a baseline for devising integrated control measures to reduce the losses due to these pathogens being either at the pathogen or vector level.

Thus, on the basis of this analysis and previous findings, we may suggest that using a single diagnostic or immunogenic molecule may not be sufficient in achieving the required goals regarding *Theileria* control strategies.

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