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Review

Tick-, flea- and mite-borne pathogens and associated diseases of public health importance in Bangladesh: a review

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ABSTRACT

Background: This scoping review provides a baseline summary of the current records of the ticks, fleas, and mites of public health importance that are present in Bangladesh. It summarizes their geographic distributions and reports the levels of their infestation of livestock, pets, wildlife, and humans, and the clinical and epidemiological studies pertinent to these vectors and their pathogens.

Methods: Sixty-one articles were identified in a literature search, including 43 published since 2011.

Results: Twelve articles contained reliable information on ticks and their associated hosts. However, information on fleas and mites in Bangladesh is very limited. Seventeen species of ixodid ticks that commonly parasitize peridomestic animals and can bite humans are described: *Rhipicephalus microplus*, *R. appendiculatus*, *R. sanguineus*, *Haemaphysalis bispinosa*, *Hyalomma anatolicum*, and *Amblyomma testudinarium*. Thirty-eight veterinary articles describe livestock pathogens, including *Babesia, Anaplasma*, and *Theileria*, and the diseases they cause. Few of those studies used modern molecular techniques to identify these pathogens. Eleven articles reported human diseases or surveillance studies, 10 from the last 10 years. Two country-wide serosurveys of 1,209 and 720 patients, using Enzyme Linked Immunosorbent Assay (ELISA) and Indirect Immunofluorescence Assay (IFA), respectively, reported human exposure to *Orientia tsutsugamushi* (8.8%–23.7%), typhus and spotted-fever group rickettsiae (19.7%–66.6%), and *Coxiella burnetii* (3%). The seropositivity rates varied regionally. PCR-based studies confirmed that febrile patients in Bangladesh may be infected with *O. tsutsugamushi, Rickettsia typhi, Rickettsia felis*, or *Bartonella elizabethae*. Only limited molecular research has been done with dogs and cats. These studies have reported PCR-confirmed canine infections with *Babesia gibsoni* (30%), *Anaplasma bovis* (58%), or *Rickettsia monacensis* (14%, *n*=50), and feline infections with *Rickettsia felis* (21%, *n*=100). Similarly, fleas from cats tested positive for *Rickettsia felis* (20.6%).

Conclusions: These findings indicate that diseases borne by non-mosquito vectors in Bangladesh urgently require more attention from public health, medical, and veterinary specialists to establish their true occurrence.

1. Introduction

Bangladesh is the eighth most populous country in the world (168 million people). It is situated between latitudes 20°34′ and 26°38′ N and longitudes 88°01′ and 92°41′ E in the subtropical monsoon region of southeast Asia. Bangladesh (formally known as East Pakistan) became independent of Pakistan in 1971 [1]. Bangladesh shares most of its western, northern, and eastern terrestrial borders with eastern India and shares a short border in the south-east with Myanmar. Bangladesh has three distinct seasons: a hot humid summer from March to June, a cool rainy season from June to October, and a cool dry winter from October to March. April is the warmest month (maximum temperature of 40°C), whereas January is the coldest month (average temperature of 10°C). The average annual rainfall is 2000 mm, with most precipitation occurring during the rainy season. The landscape of Bangladesh consists of three geomorphological divisions. Most of the country is floodplain (80%), with smaller areas of terrace (8%) and hills (12%) [2]. These terrains are unevenly distributed among the eight admin-

Abbreviations: SFGR, spotted-fever-group rickettsiae; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescence assay.

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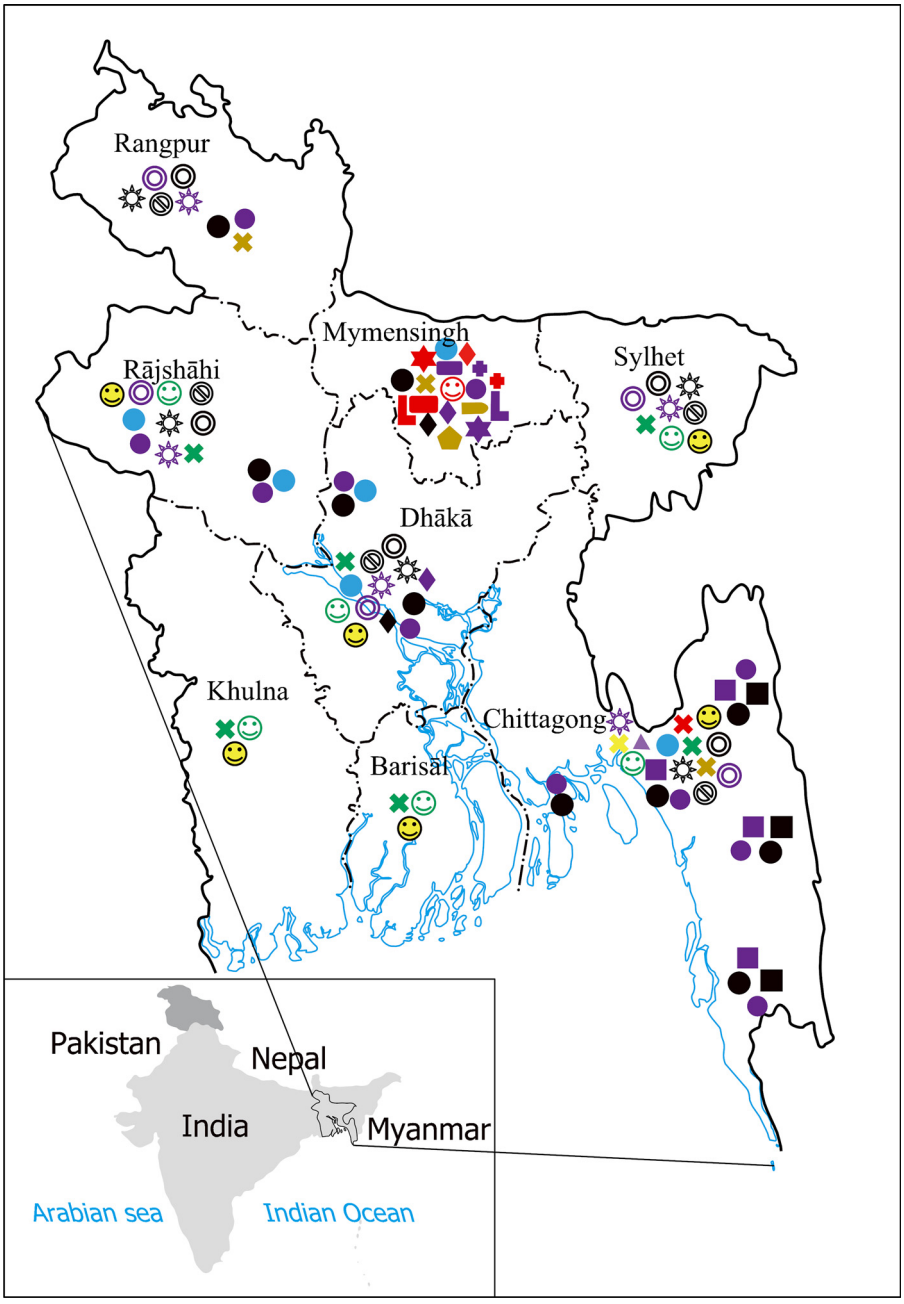

Figure 1. Geographic location of Bangladesh and identification of major areas where non-mosquito vector-borne diseases were studied. Administrative districts are identified by their current names. The following symbols were used to label research subjects including humans, animas hosts, and ectoparasites ◯ — cattle, □ — gayal, △ — goat, ◇ — dog, ⬠ — cat, ✲ — rat, ◍ — mouse, ◎ — shrew, ☺ — human serology studies, ⛒ — human PCR studies, ✡ — *Rhipicephalus microplus*, ▭ — *Rhipicephalus sanguineus*, ✚ — *H. bispinosa*, ⌊ — *H. anatolicum*, ⊃ — *Ctenocephalides felis*. Colored symbols correspond to samples tested positive for the following organisms: *Anaplasma* sp. — violet, *Babesia* sp. — black, *Theileria* sp. — teal, *Rickettsia* sp. — red, *R. felis* — brown, *R. typhi* — yellow, *Orientia tsutsugamushi* — green.

istrative divisions, which are named after the major city in each jurisdiction (Fig. 1). The floodplain occurs in the Ganges delta (Barisāl and Khulna divisions) and along the main rivers (Padma, Jamuna, Meghna) that traverse the country, which drain into the Bay of Bengal. The major terrace area is in the Rājshāhi division, whereas hilly landscapes are mostly located in the Chittagong and Sylhet divisions. Approximately 16% of Bangladesh consists of tropical moist deciduous forest, tropical moist evergreen forest, tropical moist semi-evergreen forest, or mangrove forest. The forest zones are located in the Rangpur, Khulna, Dhākā, Sylhet, Mymensingh, and Chittagong divisions [3].

The lifestyle of the Bangladeshi people is fundamentally defined by their specific physical environment and their local cultures and traditions. The population den-

sity of the country is uneven. Industrial production is limited to the urban areas in all administrative divisions, but the highest density of industries is in the Dhākā and Chittagong divisions. The rest of Bangladesh, especially Rangpur, Rājshāhi, Khulna, Barisāl, and Sylhet, is agricultural, with the various administrative divisions specializing in growing vegetables, tobacco, jute, tea, banana, mango, lychee, or other produce. Most villagers keep livestock as sources of income, as power for various agricultural operations, and for family nutrition (cited in [4,5]). According to a recent Bangladesh livestock assessment, the densities of cattle, goat, sheep, and buffalo are estimated to be 362, 359, 43 and 15 animals per square kilometer, respectively [6]. These large numbers suggest that the well-being of local populations is highly dependent upon the health of their livestock animals and animal products [7].

The impact of mosquito-borne diseases (MBD) on public health resources is larger than that of diseases transmitted by other blood-sucking ectoparasites. Therefore, we provide some perspective on MBD in Bangladesh. The climate and geographic position of Bangladesh favor the multiplication and survival of many blood-feeding ectoparasites [8,9]. Historically, malaria has been the most important mosquito-borne disease in Bangladesh, but public health services have achieved significant progress toward the elimination of malaria in most areas, except in the Chittagong Hill Tracts [10]. Japanese encephalitis, dengue disease, and chikungunya are also endemic in Bangladesh. Since 2000, the country has experienced an increasing frequency of dengue outbreaks of greater magnitude, reaching 70,188 cases in 2019, with another upsurge during the COVID-19 pandemic [11,12]. The first outbreak of chikungunya occurred in two northern villages in 2008 [13,14], and since then, chikungunya has become endemic [15]. In 2017, >13,000 clinically confirmed cases were diagnosed, and an estimated 2 million people were at risk of chikungunya infection, although no deaths were reported. Bangladesh is second only to India in its estimated burden of Japanese encephalitis [16]. Recent hospital-based surveillance in Rājshāhi, Rangpur, Chittagong and Khulna identified 548 (8%) laboratory-confirmed cases of Japanese encephalitis among 6,525 patients with acute meningitis–encephalitis syndrome [17]. Serological evidence of exposure to West Nile virus has been detected in a variety of migratory wild birds (15.9%) and residential wild birds (10.7%) in four districts of Bangladesh [18], but no case of autochthonous West Nile virus infection has yet been diagnosed in humans.

As well as mosquitoes, other vectors and vector-borne pathogens circulate in the same geographic locations in Bangladesh, with similar seasonality and causing diseases with similar clinical manifestations. Rickettsial pathogens transmitted by diverse blood-sucking ectoparasites are frequently recognized as an under-reported cause of fever of unknown origin in many tropical regions [19–24]. Similarly, numerous international travelers returning from Asia have been diagnosed with various rickettsioses [25,26].

The purpose of this review is to provide a comprehensive summary and analysis of Bangladesh studies of ticks, fleas, mites, and the pathogens they transmit, and to review the available information on rickettsial diseases important to public and veterinary health that are recognized in Bangladesh.

2. Methods and data analysis

Full-length articles were identified with searches of PubMed and Google Scholar using combination of the following keywords: tick, flea, lice, mite, non-mosquito vectors, vector-borne diseases, animals, humans, and Bangladesh. Additional publications were found by reviewing the references cited in every article acquired in the initial search. Sixty-one articles were identified and included in this review. No relevant articles on lice or louse-borne diseases were identified. Specific information on the study locations, vectors, animal hosts, infection rates, pathogens, methods of identification, and testing methods was extracted and tabulated (Tables 1–5). Both human and animal data were collected to estimate the zoonotic importance of these vectors and the pathogens they carry.

3. Ticks and tick-borne diseases in Bangladesh

3.1. Ticks of Bangladesh

The earliest published record found of ticks infesting domestic cattle in Bangladesh (then East Pakistan) was published in 1969 and described *Rhipicephalus* (previous name '*Boophilus*') *microplus, Haemaphysalis bispinosa*, and *Hyalomma anatolicum anatolicum* collected in Chittagong, Dhākā, Mymensingh, and Rājshāhi [27]. A 1985 publication summarized the identification of 5,760 of 12,778 ticks collected during 1982–1984 from various domesticated animals at eight distinct locations, including almost all the administrative districts of Bangladesh (Table 1 and [28]). Ten species of ixodid tick were described, predominantly represented by two species, *R.* (*B.*) *microplus* (48% of the collection) and *H. bispinosa* (49%), with all stages of each tick found on cattle, goats, dogs, and pigs. *Rhipicephalus microplus* was also found in the inner ear of a 9-month-old child and attached to the head of a cobra (*Naja naja*) from Mymensingh [28]. *Haemaphysalis bispinosa* was also collected from cats, foxes (*Vulpes bengalensis*), a civet (*Viverra zibathi*), and a captive monkey (*Macaca* sp.). Large numbers of *H. kinneari* from foxes, *Hyalomma anatolicum anatolicum* from cattle, and *R. san-*

Table 1
Records of ticks in Bangladesh.

Species	Location: Division (District)	Host	Source
Amblyomma (Aponoma) gervaisi (Lucas)	Dhākā, Sylhet	Lizard (*Baranus bengalensis*)	Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29]
Amblyomma testudinarium	Chittagong, Sylhet	Cattle, Goat, Gayal, Pig	Mohanta et al., 2011 [9] Mondal et al., 1996 [31] Islam et al., 2006 [34]
Amblyomma variegatum (Fabricius)	Dhākā, Sylhet	Dog, Cattle, Goat	Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29] Islam et al., 2022 [32]
Amblyomma sp.	Chittagong, Sylhet	Cattle (vegetation[a])	Islam et al., 2022 [32] Kamal et al., 1996 [35]
Argas persicus	Dhākā	Poultry	Ghosh et al., 2007 [29]
Haemaphysalis bispinosa (Neumann)[b]	Chittagong (Comilla), Dhākā (Gazipur), Dhākā metropolitan area, Rājshāhi, Mymensingh, Sylhet, Rangpur (Gaibandha)	Cattle, Buffalo, Goat, Cat, Dog, Pig, Monkey (*Macaca* sp.), Civet (*Viverra zibathi*), Fox (*Vulpes bengalensis*)	Roy et al., 2018 [5] Razzak & Shaikh, 1969 [27] Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29] Fuehrer et al., 2012 [30] Islam et al., 2022 [32] Islam et al., 2006 [34] Kamal et al., 19961 [35] Sarkar et al., 2010 [36] Rony et al., 2010 [37] Qiu et al., 2016 [38] Mohanta et al. [111]
Haemaphysalis canestrini (Supino)	Dhākā	Fox	Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29]
Haemaphysalis intermedia	Sylhet	Cattle, Goat	Islam et al., 2022 [32]
Haemaphysalis kinneari (Warburton)	Dhākā	Fox, Civet	Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29]
Haemaphysalis leachi	Sylhet	Cattle, Goat	Islam et al., 2022 [32]
Haemaphysalis sp.	Sylhet	Cattle, Goat	Islam et al., 2022 [32]
Hyalomma anatolicum anatolicum (Koch)	Khulna, Rājshāhi, Mymensingh	Cattle	Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29] Islam et al., 2006 [32] Qiu et al., 2016 [38] Mondal et al., 1995 [45]
Hyalomma truncatum (Koch)	Dhākā	Cattle	Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29]
Hyalomma sp.	Chittagong (Comilla), Dhākā, Rājshāhi, Mymensingh	Cattle	Razzak & Shaikh, 1969 [27]
Rhipicephalus annulatus	Sylhet	Cattle, Goat	Islam et al., 2022 [32]
Rhipicephalus appendiculatus	Chittagong	Cattle, Goat	Kamal et al., 1996 [35]
Rhipicephalus (Boophilus) microplus (Canestrini)[2]	Chittagong (Comilla), Dhākā (Gazipur), Dhākā metropolitan area, Khulna, Mymensignh, Rājshāhi, Sylhet, Rangpur (Gaibandha)	Cattle, Buffalo, Goat, Gayal, Dog, Pig, Cobra (*Naja naja*)	Roy et al., 2018 [5] Razzak & Shaikh, 1969 [27] Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29] Fuehrer et al., 2012 [30] Islam et al., 2022 [32] Islam et al., 2006 [34] Kamal et al., 1996 [35] Sarkar et al., 2010[36] Rony et al., 2010[37] Qiu et al., 2016 [38] Ahmed 1976 [49] Mohanta et al. [111]
Rhipicephalus decoloratus	Sylhet	Cattle, Goat	Islam et al., 2022 [32]
Rhipicephalus evertsi evertsi (Neumann)	Dhākā	Dog	Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29]
Rhipicephalus sanguineus	Barisāl, Chittagong, Dhākā (Gazipur), Dhākā metropolitan area, Khulna, Sylhet, Mymensingh, Rangpur (Gaibandha)	Dog, Cat, Cattle, Goat	Rahman & Mondal, 1985 [28] Ghosh et al., 2007 [29] Fuehrer et al., 2012 [30] Islam et al., 2022 [32] Islam et al., 2006 [34] Sarkar et al., 2010 [36] Rony et al., 2010 [37] Qiu et al., 2016 [38] Mohanta et al. [111]
Rhipicephalus sp.	Sylhet	Cattle, Goat	Islam et al., 2022 [32]

[a] Ticks were collected off vegetation in Sylhet [32].
[b] There are also records of this tick species attached to humans [28,30].

Table 2
Tick infestations in livestock and peridomestic animals in Bangladesh.

Vertebrate host	Number of animals examined	Location: District (division)	Tick species collected (number collected)	Infestation (%)	Source
Cattle	264	Chittagong (Comilla), Dhākā, Mymensingh, Rājshāhi	*Rhipicephalus (Boophilus) microplus* (2636) *Haemaphysalis bispinosa* (77) *Hyalomma* sp. (46)[a]	83.7 10.6 5.7	Razzak & Shaikh, 1969 [27]
Cattle	240	Rājshāhi	*Hyalomma anatolicum* (1438)	64.6	Mondal et al., 1995 [45]
Cattle	165	Chittagong	*Rhipicephalus (Boophilus) microplus* (1963) *Rhipicephalus appendiculatus* (989) *Haemaphysalis bispinosa* (227) *Amblyomma* sp. (24)	18.2 9.2 2.1 0.2	Kamal et al., 1996 [35]
Cattle	250	Mymensingh (Jamalpur, Sherpur), Dhākā (Mankgonj, Faridpur), Chittagong (Khagrachari, Banderban, Rangamati), Rājshāhi (Chapainawabgonj, Bogra)	*Rhipicephalus microplus* (3790) *Haemaphysalis bispinosa* (7580) *Rhipicephalus sanguineus* (989) *Hyalomma anatolicum* (1007) *Amblyomma testudinarium* (187)	42.4 12.0 10.8 19.2 4.4	Islam et al., 2006 [34]
Cattle	206	Dhākā (Gazipur)	*Rhipicephalus* (*Boophilus*) *microplus* (1–7) *Haemaphysalis bispinosa* (1–2) *Rhipicephalus sanguineus* (1–4)	45.6 36.9 16.5	Rony et al., 2010 [37][b]
Cattle	380	Chittagong	*Rhipicephalus (Boophilus) microplus* *Rhipicephalus sanguineus* *Haemaphysalis bispinosa*	25 13.7 12.6	Kabir et al., 2011 [46]
Cattle	1000[c]	Chittagong (Bandarban, Rangamati, Khagrachari)	*Rhipicephalus (Boophilus) microplus* (5555) *Amblyomma testudinarium* (300)	86.8 5.9	Mohanta et al., 2011 [9]
Cattle	384	Mymensingh	*Rhipicephalus microplus* (1432) *Haemaphysalis bispinosa* (855)	60.4[d]	Roy et al., 2018 [5]
Gayal	15	Chittagong (Bandardan)	*Amblyomma testudinarium* (282)	100	Mondal et al., 1996 [31]
Gayal	1000[c]	Chittagong (Bandarban, Rangamati, Khagrachari)	*Rhipicephalus (Boophilus) microplus* (5555) *Amblyomma testudinarium* (300)	96.3 70	Mohanta et al., 2011 [9]
Buffalo	120	Mymensingh (Jamalpur, Sherpur) Dhākā (Mankgonj, Faridpur) Rangpur Chittagong (Khagrachari, Banderban, Rangamati) Rājshāhi (Bogra, Chapainawabgonj)	*Rhipicephalus microplus* (3790) *Haemaphysalis bispinosa* (7580)	12.5 10.8	Islam et al. 2006 [34]
Goat	322	Chittagong	*Rhipicephalus (Boophilus) microplus* (2186) *Rhipicephalus appendiculatus* (1002) *Haemaphysalis bispinosa* (263)	15.3 7.0 1.8	Kamal et al., 1996 [35]
Goat	235	Mymensingh (Jamalpur, Sherpur), Dhākā (Mankgonj, Faridpur), Rangpur, Faridpur, Chittagong (Khagrachari, Banderban, Rangamati) Rājshāhi (Bogra, Chapainawabgonj)	*Rhipicephalus microplus* (3790) *Haemaphysalis bispinosa* (7580) *Rhipicephalus sanguineus* (989)	25.5 31.5 6.8	Islam et al., 2006 [34]
Goat	1000[c]	Chittagong (Bandarban, Rangamati, Khagrachari)	*Rhipicephalus (Boophilus) microplus* (5555) *Amblyomma testudinarium* (300)	100 13.3	Mohanta et al., 2011 [9]
Black Bengal goat	125	Mymensingh, Rangpur (Gaibandha)	*Rhipicephalus* (*Boophilus*) *microplus*[e] *Haemaphysalis bispinosa* *Rhipicephalus sanguineus*	27.2 34.4 7.2	Sarkar et al., 2010 [36]
Pig	85	Mymensingh (Jamalpur, Sherpur), Dhākā (Mankgonj, Faridpur), Rangpur, Chittagong (Khagrachari, Banderban, Rangamati), Rājshāhi (Bogra, Chapainawabgonj)	*Rhipicephalus microplus* (3790) *Amblyomma testudinarium* (187)	8.2 2.3	Islam et al. 2006 [34]
Dog	62	Mymensingh (Jamalpur, Sherpur), Dhākā (Mankgonj, Faridpur), Rangpur, Chittagong (Khagrachari, Banderban, Rangamati), Rājshāhi (Bogra, Chapainawabgonj)	*Rhipicephalus sanguineus* (989)	27.4	Islam et al. 2006 [34]
Dog	85	Dhākā metropolitan area	*Rhipicephalus sanguineus* (50) *Rhipicephalus microplus* (1) *Haemaphysalis bispinosa* (2)	30.6[d]	Mohanta et al., [111]

[a] Rahman and Mondal (1985) refer to these ticks as *Hyalomma anatolicum anatolicum* [28].
[b] This study reports total tick burden but not tick numbers [37].
[c] 1,000 animals were examined for tick infestations; however, exact number was not reported for each species [9].
[d] Infestation level was not calculated for each tick species collected [5,111].
[e] Total number of ticks is not reported beyond the parasitic burden estimated for each species [36].

guineus from cattle, dogs and cats were reported. Gosh et al. (2007) repeated the 1985 work done by Rahman and Mondal (1985), and listed additional collections of the soft tick, *Argas persicus*, in two areas of the Dhākā district [28,29]. Fuehrer et al. (2012) reported human infestations with *H. bispinosa* [30].

Amblyomma testudinarium was found before 1994 in the northeastern and southeastern hills of Bangladesh, where

Table 3
Detection of tick-borne pathogens and evidence of tick-borne diseases in Bangladesh.

Tick species collected (number if reported)	Vertebrate host (number if reported)	Location: District (division)	Pathogen detected		Detection method	Source
			Host blood[1]	Tick		
Rhipicephalus (Boophilus) microplus, *Haemaphysalis bispinosa*, *Rhipicephalus sanguineus*	Cattle (19) Dog (172) Cat (6) Buffalo, cattle (1,730) Sheep, goat (62)	Not reported	*Babesia bigemina* (100%) *Babesia gibsoni* (50.6%) *Babesia gibsoni* (50.6%) *Babesia felis* (100%) *Theileria mutans* (64.9%) *Theileria ovis* (24.2%)	NT	Giemsa stain	Ahmed, 1976 [49]
Rhipicephalus (Boophilus) microplus (5,555), *Amblyomma testudinarium* (300)	Cattle (296)[a] Goat (150) Gayal (179)	Chittagong (Bandarban, Rangamati, Khagrachari)	*Babesia bigemina* (16.6%) *Anaplasma marginale* (14.9%) None (0%, 0/100) *B. bigemina* (3.7%) *A. marginale* (17.9%)	NT	Giemsa stain	Mohanta et al., 2011 [9]
Rhipicephalus sanguineus (15)	Dog (50)	Mymensingh	SFG *Rickettsia* sp. (14%) *Wolbachia* endosymbiont (26%) *Anaplasma bovis*-like (2%) *Anaplasmataceae* sp. (2%)	SFG *Rickettsia* sp. (6.7%) *Anaplasma bovis* (66.7%)	PCR	Qui et al., 2016 [38]
Rhipicephalus microplus (70) *Haemaphysalis bispinosa* (82) *Hyalomma anatolicum* (2)	Cattle[b]	Mymensingh	NT	SFG *Rickettsia* sp. (14.3%) SFG *Rickettsia* sp. (31.7%) ∘*R. monacensis* ∘*Rickettsia* sp. *Anaplasma bovis* (3.7%) SFG *Rickettsia* sp. (50%)	PCR	Qui et al., 2016 [38]
Rhipicephalus microplus (1,432) *Haemaphysalis bispinosa* (855)	Cattle (384)	Mymensingh	•*Anaplasma* sp. (4.7%)[c] •*Babesia* sp. (0%) •*Theileria* sp. (0%)	•NT	Giemsa stain	Roy et al., 2018 [5]
			•*Anaplasma* sp.-*Babesia* sp. ∘*Theleria orientalis* (62.2%)[d] ∘*Theileria orientalis* (55.2%) ∘*Anaplasma bovis* (35.7%) ∘*Anaplasma marginale* (4.2%) ∘*Anaplasma* sp. Mymensingh (13%) ∘*Babesia bigemina* (1%) ∘*Babesia bovis* (0.5%) ∘*Babesia* sp. Mymensingh (0.3%)		PCR and sequencing followed by Reverse line blot	
NR	Cattle (100)	Sylhet	*Anaplasma sp.* (42%) *Babesia sp.* (16%)	N/A	Giemsa stain	Nath et al., 2013 [50]

(*continued on next page*)

Table 3 (*continued*)

Tick species collected (number if reported)	Vertebrate host (number if reported)	Location: District (division)	Pathogen detected		Detection method	Source
			Host blood[1]	Tick		
NR	Crossbred cattle (150)	Chittagong (Jointika, Nasirabad, Bayezid, Patia)	*Anaplasma* sp. (6%); *Babesia* sp. (2.7%) ∘*Anaplasma* sp. (2%) ∘*Babesia* sp. (1.3%)	N/A	Giemsa stain ∘PCR	Bary et al., 2018 [4]
	Indigenous cattle (150)		*Anaplasma* sp. (3.3%); *Babesia* sp. (1.3%) ∘*Anaplasma* sp. (1.3%) *Babesia* sp. (1.3%);			
NR	Cattle (400)	Rangpur (Gangachara, Pirgacha)	*Anaplasma* sp. (3.5%); *Babesia* sp. (1.5%) ∘*A. marginale* ∘*Babesia* sp.	N/A	Giemsa stain ∘PCR	Rahman et al., 2015 [48]
NR	Cattle (59)	Rājshāhi (Natore)	*Theileria orientalis* (66.1%) *T. annulata* (1.7%) *Babesia bovis* (3.4%)	N/A	PCR (*cox*1 gene)	Moni et al., 2019 [51]
NR	Goats (2,013)	Chittagong	Anaplasmosis (2.48%) Babesiosis (0.4%)	N/A	Clinical diagnosis only	Nath et al., 2014 [52]
NR	Goats (400)	Chattogram City	*Anaplasma* sp. (5.8%) ∘*Anaplasma ovis* (14.8%) ∘*A. marginale* (1%)	N/A	Giemsa stain ∘PCR	Rahman et al., 2022 [53]
NR (24)	Cattle (81) Black Bengal goats (91)	Rangpur (Kurigram), Rājshāhi (Shahjadpur, Pabna), Mymensingh	Q fever ELISA (6.1%) Q fever ELISA (7.6%)	0/24	ELISA PCR	Chakrabartty et al., 2016 [47]
NR (127)	Cattle (NR), Goat (NR)	Rājshāhi (Sirajgonj, Shahjadpur)	Q fever ELISA (6.97%, 12/172)	*Coxiella burnetii* (0.79%)	ELISA PCR	Rahman et al., 2018 [54]
NR	Crossbred cattle (216)	Chittagong (Noakhali sadar)	Anaplasmosis 2.78-5.56% Babesiosis 6.94-12.5% Theileriosis 1.39-2.78%	N/A	Giemsa stain	Alim et al., 2012 [55][e]
	Indigenous cattle (432)	Chittagong (Noakhali, Boakhali, Rangunia, Khagrachori)	Anaplasmosis 2.78-3.7% Babesiosis 4.62-9.25% Theileriosis 0-2.78%			
NR	Cattle (14)	Dhākā (Tangail)	*Babesia* sp. (28.6%) *Anaplasm*a sp. (14.3%) *Theileria* sp. (0%) *B. bovis* (64.3%) *A. marginale* (14.3%) *T. annulata* (35.7%)	N/A	Giemsa stain Uniplex and Multiplex PCR	Karim et al, 2012 [56]
NR	Cattle (395)	Rājshāhi (Sirajganj)	*Theileria* sp. (5.8%) *Babesia* sp. (2.3%)	N/A	Giemsa stain	Mahmud et al., 2015 [57]
NR	Cattle (192)	Rājshāhi (Rājshāhi) Rājshāhi (Natore)	*Theileria annulata* (20.4%, 30/147) *Theileria annulata* (80.0%, 36/45)	N/A	ELISA	Ali et al., 2016 [58]

(*continued on next page*)

Table 3 (*continued*)

Tick species collected (number if reported)	Vertebrate host (number if reported)	Location: District (division)	Pathogen detected		Detection method	Source
			Host blood[1]	Tick		
NR	Cattle (60)	Rājshāhi (Sirajgong)	*Anaplasma* sp. (70%) *Babesia* sp. (3.3%)	N/A	Giemsa stain	Chowdhury et al., 2006 [59]
NR	Dog (68)	Dhākā	*Babesia gibsoni* (38.2%) *Anaplasma* AnHl446 sp. (2.9%)	N/A	PCR & sequencing	Talukder et al, 2012 [60]
NR	Dog, stray (50)	Dhākā (Mymensingh)	*Babesia gibsoni* (30%)	N/A	PCR & sequencing	Terao et al., 2015 [61]
Rhipicephalus sanguineus (50) *Rhipicephalus microplus* (1) *Haemaphysalis bispinosa* (2)	Dog, stray (85)	Dhākā metropolitan area	*Babesia gibsoni* (44.7%) *Anaplasma platys* (8.2%)	*Babesia gibsoni* (1.9%)[f] *Babesia vogeli* (1.9%) *Anaplasma platys* (1.9%)	PCR & sequencing	Mohanta et al., 2024 [111]
NR	Cattle (117)	Dhākā (Mymensingh)	*Theileria annulata*: Microscopy (8.5%) CF-test (22.0%)	N/A	Giemsa stain, Complement fixation test	Samad et al., 1983 [62]
NR	Cattle (14,350)	Dhākā (Tangail), Mymensingh (Jamalpur), Sylhet, Rājshāhi (Pabna, Bogra)	*Babesia bigemina* (4.5%) *Theileria sp.* (4.3%) *Anaplasma sp.* (5.9%)	N/A	Giemsa stain	Samad et al., 1989 [63]
NR	Cattle (166)	Chittagong	*Anaplasma sp.* (3%) *Babesia* sp. (1.2%) *Theileria* sp. (4.2%)	N/A	Giemsa stain	Siddiki et al., 2010 [64]
NR	Cattle (179)	Dhākā, Mymensingh	*Babesia bigemina* (14.5%)	N/A	Capillary tube agglutination test	Banerjee et al., 1983 [65]
NR	Cattle (100)	Rājshāhi (Serajgonj)	*Anaplasma sp.* (33.3%)	N/A	Giemsa stain	Taludker & Karim, 2001 [66]
NR	Cattle (385)	Rājshāhi (Sirajganj) Rangpur (Rangpur)	*Theileria* (8.3%) *Babesia* (5.9%) *Theileria* (12.2%) *B. bigemina* (4.7%) *Theileria/Babesia* (1.8%)	N/A	Giemsa stain PCR	Hossain et al., 2023 [67]
NR	Cattle (1,070) Sheep (80)	Dhākā (Savar, Sirajganj Sadar, Shaiadpur Upazila, and Nikhansori, Chottrogram)	*Anaplasma* sp. (21.8%, 251/1150) *Babesia* sp. (9.8%, 113/1150) *Theileria* sp. (2%, 23/1150) *Anaplasma/Babesia* (16.8%, 193/1150) *Anaplasma/Babesia/Theileria* (0.5%, 6/1150)	N/A	Multiplex PCR[g]	Hassan et al., 2019 [8]

(*continued on next page*)

Table 3 (*continued*)

Tick species collected (number if reported)	Vertebrate host (number if reported)	Location: District (division)	Pathogen detected		Detection method	Source
			Host blood[1]	Tick		
NR	Dromedary camel (55)	Dhākā	*Anaplasma* sp. (9.1%) *Babesia* sp. (1.8%)	N/A	Giemsa stain	Islam et al., 2019 [68]
NR	*Suncus murinus* (299) *Rattus rattus* (125) *Mus musculus* (27)	Rangpur (Lalmonirhat, Dinajpur), Rayshani (Joypurhat), Dhākā (Rajbari, Faridpur), Sylhet (Moulovibazar), Chittagong (Khagracholri, Rangamati, Bandorban, Cox's Bazar)	*Anaplasma* sp. (10%) *Babesia* sp. (4.3%) *Anaplasma* sp. (3.2%) *Babesia* sp. (5.6%) *Babesia* sp. (3.7%)	N/A	Giemsa stain	Islam et al., 2020 [69]
NR	Human (40 febrile patients)	Mymensingh	Spotted fever (40%)	N/A	Weil-Felix test	Miah et al., 2007 [70]

Abbreviations: NT, not tested; NR, not reported; N/A, not applicable.

[1] Percentage prevalence is indicated; proportions are also included when/if only a subset of animals or patients was tested.

[a] 1,000 animals were examined for tick infestations; however, exact number was not reported for each species. Numbers of animals examined for *Babesia* and *Anaplasma* were calculated based on the prevalence reported [9].

[b] Number of animals examined was not reported [38].

[c] Total prevalence based on blood smear analysis.

[d] Total prevalence based on the Reverse Line Blot (RLB) analysis and estimated for each pathogen, including coinfections: *Theileria orientalis* infections were most common (212/384, 55.2%) followed by infections with *Anaplasma bovis* (137/384, 35.7%), *Anaplasma marginale* (16/384, 4.2%), *Babesia bigemina* (4/ 384, 1%), and *Babesia bovis* (2/384, 0.5%).

[e] This study reported prevalence based on the parameters evaluated, but estimated the overall prevalence only for babesiosis and not for other diseases [55]. The ranges of all variable estimates are included based on geographic locations.

[f] Tick species were not reported in association with positive PCR results [111].

[g] Multiplex PCR was used; however, the results are reported as gross numbers and do not differentiate positivity rates for each pathogen in the different host animals tested. Microscopic evaluation of Giemsa-stained blood smears detected blood ectoparasites in 100% of Australian sheep and 16% of native sheep, in 30% and 80% of high yielding cattle, 22% and 31% of local cows; however, the breakdown for each pathogen is not reported.

Table 4
Fleas, flea borne-pathogens and flea-borne diseases in Bangladesh.[a]

Flea species collected (number)	Vertebrate host (number)	Location	Infestation (%)	Pathogen[b]		Detection method	Source
				Host blood	Fleas		
Ctenocephalides felis	Cat (100)	Mymensingh	68	*Rickettsia felis* (28%)	*Rickettsia felis* (20.6%, 14/68)	PCR & sequencing (17kDA protein gene)	Ahmed et al., 2016 [88]
NR	Human (50 febrile patients)	Mymensingh (North central part)	N/A	*Rickettsia felis* (42%)	N/A		Ahmed et al., 2016 [88]
NR	Human (414 febrile patients)	Dhākā, Rangpur, Rājshāhi, Mymensingh, Sylhet, Khulna, Barisāl Chittagong	NR	Weil-Felix cross-reactive antibodies (74%) ∘OX2-antigen antibodies (50%)	N/A	Weil-Felix test	Choudhury et al., 2017 [89]
				PCR *Rickettsia felis* (19.6%, 81): ∘Dhākā (24%, 18/75) ∘Rangpur (36%, 4/11) ∘Rājshāhi (30%, 3/10) ∘Mymensingh (20%, 43/216) ∘Sylhet (5%, 1/20) ∘Khulna (3%, 1/30) ∘Barisāl (24%, 10/42) ∘Chittagong (10%, 1/10)		PCR & sequencing	
				PCR *Rickettsia typhi* (0.2%) ∘Barisāl (2.4%, 1/42)			
NR	Human (150 febrile patients)	Mymensingh	NR	*Rickettsia* sp. (46%) including *R. felis*[c]	N/A	PCR	Ferdouse et al., 2015 [90]
NR	Human (416 febrile patients)	Chittagong	NR	Antibodies reacting to *Rickettsia* sp. antigen (3.6%, 15/415)	N/A	IFA	Kingston et al., 2019 [91]
				PCR *Rickettsia* (7%) ∘*Rickettsia typhi* (5.8%) ∘*Rickettsia felis* (0.5%) ∘*Rickettsia* sp. (0.7%)		PCR	
NR	Human (720 febrile patients)	Dhākā, Khulan, Barisāl, Chittagong, Rājshāhi, Sylhet	NR	Antibodies reacting to ∘Typhus group *Rickettsia* (1%) ∘SFGR (18%)	N/A	IFA, ELISA	Faruque et al., 2017 [85]
				PCR: ∘Typhus group *Rickettsia* (0.1%)		PCR	
				Culture: *Bartonella elizabethae* (0.1%)		Blood culture	
NR	Human (1,244 patients)[d]	Dhākā, Sylhet, Rājshāhi (Bogra), Chittagong (Comilla)	NR	Antibodies reacting to *R. typhi* (66.6%, 805/1,209)	N/A	ELISA (IgM)	Maude et al., 2014 [92]
NR	Human (300 malaria smear negative patients)	Chittagong	NR	*Rickettsia typhi* (0.7%)	N/A	PCR (TaqMan)	Maude et al., 2016 [93]
NR	Human (402 febrile patients)	Mymensingh	NR	*Rickettsia* sp. (11.5%, 13/113)	N/A	PCR[e]	Nila et al., 2022 [94]

[a] Additional entomological reports of *Xenopsylla cheopis* in Bangladesh can be found in Fuehrer et al., 2012 [30]; testing was not performed as a part of that study.
[b] Percentage prevalence is indicated; proportions are also included when/if only a subset of animals or patients was tested.
[c] Only 20 of 69 17-kDa protein gene amplicons were sequenced, so other *Rickettsia* etiology cannot be excluded [90].
[d] This serological study recruited patients seeking hospital care in their corresponding catchment areas with no exclusion criteria; IgM ELISA testing was performed using *R. typhi* antigen for all samples [92].
[e] *Rickettsia* PCR was performed only for patients who tested positive on *Orientia* Immunochomatographic Test and/or PCR [94].

Table 5
Scrub typhus and *Orientia tsutsugamushi* reports in Bangladesh.

Host	Location	Serological methods (antibody type and antigens)	Serological findings	Molecular methods (target)	Molecular findings	Source
Human (416 febrile patients)	Chittagong	IFA (IgM/IgG) Karp, Kato, Gilliam	Scrub typhus (13.7%, 57/415)	PCR (56 kDa and 47 kDa antigen genes)	*Orientia tsutsugamushi* (10.9%, 45/414)	Kingston et al., 2019 [91]
Human (720 febrile patients)	Dhākā, Khulna, Barisāl, Chittagong, Rājshāhi, Sylhet	ELISA (NR)[a]	Scrub typhus (30%, 107/360)	PCR (NR)[a]	*Orientia tsutsugamushi* (0.6%, 2/360)	Faruque et al., 2017 [85]
		IFA (NR)[a]	Scrub typhus (18%, 63/360)			
Human (1,244 patients)[b]	Dhākā, Sylhet, Rājshāhi (Bogra), Chittagong (Comilla)	ELISA(IgM)	Scrub typhus (23.7%, 287/1,209)	NT	NT	Maude et al., 2014 [92]
Human (300, malaria smear negative patients)	Chittagong	N/T	Scrub typhus (0.3%, 1/300)	TaqMan PCR (47 kDa antigen gene)	*Orientia tsutsugamushi* (0.3%, 1/300)	Maude et al., 2016 [93]
Human (402 febrile patients)	Mymensingh	ICT[c], (IgG/IgM)	Scrub typhus (22%, 89/402)	PCR (47 kDa antigen gene)	*Orientia tsutsugamushi* (16.2%, 65/402)	Nila et al., 2022 [94]
Human (40 febrile patients)	Mymensingh	Weil-Felix test	Scrub typhus (60%, 24/40)	NT	NT	Miah et al., 2007 [70]

Abbreviations: NT, not tested; NR, not reported; N/A, not applicable.

[a] This article does not contain information about antibody classes tested, and/or antigen or primers used [85].

[b] This serological study recruited patients seeking hospital care in their corresponding catchment areas with no exclusion criteria; IgM ELISA testing was performed for *Orientia tsutsugamushi* for all samples [92].

[c] ICT, Immunochromatographic test, commercial test for IgG and IgM against *Orientia* (Mytest Scrub Ab test card, India) [94].

adult ticks predominantly infested pigs and cattle. An *A. testudinarium* female can suck 11 times the volume of blood as can *R. microplus,* with a detrimental effect on livestock [31]. A conference report described *A. testudinarium* from domesticated gayals in the hills of Naikhonchari, Banbardan, where this tick infestation peaked during the dry months [31]. Similar veterinary surveillance of livestock in other hilly areas of Bangladesh also identified *A. testudinarium*, predominantly infesting semidomesticated gayals and *Bos frontalis* (70% of findings), but it was also present on goats (13.33%) and cattle (5.88%) [9].

During 2018–2019 diverse tick species were collected from cattle and goats in Sylhet and from vegetation in Lawachara National Forest[32]. Not only were historical records of *R. microplus, R. sanguineus, A. variegatum*, and *H. bispinosa* infesting farm animals corroborated, but previously undescribed ticks were also identified: *R. decoloratus, R. annulatus, H. intermedia*, and *H. leachi.* Other species of *Rhipicephalus, Amblyomma*, and *Haemaphysalis* ticks may occur in Bangladesh (Table 1), but have not been identified due to a lack of reliable taxonomic keys, especially for the immature stages of ticks [28,32,33].

The population of dogs in Bangladesh, including pets and service and stray dogs, is estimated to be 14–85 dogs/km^2 in different parts of the country, based on the rabies control program [39,40]. The dogs of Bangladesh are susceptible to the many tick-borne pathogens circulating in the country. Dogs can act as reservoirs for tick-borne pathogens and as the hosts of different ticks. Two veterinary articles summarized ectoparasitic infestations of dogs and cats [41,42], but they did not specify the types and species of ectoparasites found. Nevertheless, these papers indicated that 8.3% of companion and working dogs of non-local breeds in Dhākā were infested with ectoparasites [42]. In Chattogram (Chittagong division), 48% of 488 dogs and 32% of 361 cats were infested with ectoparasites, with the highest prevalence in winter [41]. A survey of domestic dogs from the Bandarban district (Chittagong division) yielded 342 *H. bispinosa* but only 12 *R. sanguineus* ticks [41]. Stray dogs in the Mymensingh division were infested with adults and nymphs of *R. sanguineus*, but the degree of infestation and the prevalence of adult and immature ticks were not reported [38]. The most recent study conducted in the Dhākā metropolitan area corroborated this observation and reported that ticks were found on 30.6% of 85 stray dogs, 94% of which were *R. sanguineus* [111]. According to our analysis, dogs in Bangladesh are infested with at least five different species of ticks (Table 1).

Livestock animals have shown significant levels of tick infestation (Table 2), with up to 100% affected in Chittagong and Dhākā [9,27], and this situation has persisted for decades. With few exceptions, *R. microplus* is the most frequently collected tick in Bangladesh, and it is the most economically important tick due to its wide distribution and the occurrence of acaricide resistance [43]. Phylogenetic and morphological analyses have shown that *R. microplus* from Bangladesh belongs to *R. microplus* clade C, together with ticks from Pakistan and Myanmar [44], although clade C ticks display large morphological diversity, and many features overlap the descriptions of *R. mi-*

croplus clade A and *R. australis*. The taxonomic and biological properties of the circulating endemic populations of *R. microplus, R. sanguineus, Amblyomma, Hyalomma*, and *Haemaphysalis* ticks in Bangladesh require more study.

3.2. Tick-borne pathogens in Bangladesh

Theileriosis, babesiosis, and anaplasmosis are recognized livestock scourges in Bangladesh (Table 3). However, few studies have evaluated the prevalence of their etiological agents in ticks [38,47,48]. Most studies have only tested the host blood for these agents with Giemsa staining and more recently with PCR and antibody assays (see section 3.3, and Table 3). Seventy *R. microplus*, 82 *H. bispinosa*, and two *Hyalomma anatolicum* from cattle in the Mymensingh district of Bangladesh were tested for Anaplasmataceae with a 16S-rRNA-gene-directed seminested PCR assay [38]. Only three (3.7%) were positive for *H. bispinosa*, and only one PCR-positive sample was further shown to contain *Anaplasma bovis* DNA [38]. Testing the same tick DNA for spotted-fever-group rickettsiae (SFGR) identified 27 (17.5%, *n*=154) samples positive for the citrate synthase gene (*glt*A). However, subsequent PCR amplification and sequencing of the OmpA gene fragment yielded informative results for only three of 10 *glt*A-positive *R. microplus* and three of 16 *glt*A-positive *H. bispinosa*. The positive samples clustered with *Rickettsia monacensis,* a known human pathogen [71,72]. The genotype of the SFGR detected in *H. bispinosa* was not fully characterized, but it appeared to be closely related to an SFGR previously detected in Korea (NCBI accession DQ402485), Japan, and China (AB114807) and shared a OmpA gene sequence with *Candidatus* Rickettsia longicorni (MN026548) and *Candidatus* Rickettsia jingxinensis (MH932061), which was identical within the OmpA gene region compared [73,74]. Only one *Hyalomma anatolicum* tested positive for *Rickettsia glt*A, but the identification was incomplete [38]. Dogs from the same areas were infested with *R. sanguineus* and 10 of 15 tested positive for the Anaplasmataceae 16S rRNA gene. Two different genotypes of *Anaplasma bovis* were identified when the amplicons were sequenced [38]. One *R. sanguineus* from a stray dog in the Dhākā metropolitan area tested PCR-positive for *Anaplasma platys* [111], the causative agent of canine cyclic thrombocytopenia. These findings underscore the need for continued surveillance of brown dog ticks to better understand the various associations of *Anaplasma* spp. with this ectoparasite and its appropriate transmission control.

Unidentified ticks collected in three districts from seropositive cattle and black Bengal goats with a history of reproductive disorder were tested for the *Coxiella burnetii* IS1111 fragment with a TaqMan PCR assay, but no sample was positive [47]. Testing 127 unidentified ticks from goats and cattle in Shahjadpur Upazila in the Sirajgonj district with TaqMan PCR detected only one *Coxiella*-IS1111-positive tick [54]. The role of ticks in the eco-epidemiology of Q fever is not fully understood, although the reported prevalence of *C. burnetii* in ticks ranges from 2.5% to 14.0% in different countries [75]. The confounding issue is that most studies do not differentiate between *C. burnetii* sensu stricto and *Coxiella*-like tick endosymbionts [75].

Only a limited number of molecular surveillance projects targeting ticks have been undertaken in Bangladesh. Such work performed in neighboring countries with similar climates and faunal characteristics have reported many tick-borne pathogens that are not currently recognized in Bangladesh. Therefore, their potential impacts on human and animal health in Bangladesh are unknown [21,76–78].

3.3. Livestock burden of tick-borne protozoan and Anaplasma pathogens

The earliest record of the blood parasite *Babesia bigemina* in Bangladesh was based on microscopic findings in Giemsa-stained blood smears from cattle and buffalo in 1976 [49]. Since then, endemic babesiosis has been diagnosed with microscopy or serological or molecular methods, or their combination (Table 3). *Babesia bigemina* infections in livestock vary seasonally in the hilly areas of the country, with maximum rates of 28.3% in the rainy months (July–October) [9]. The lowest infection rate of 15% occurs in the winter months, November–February. The prevalence of *Babesia* positivity in cattle is also seasonal and age-specific [57]. Serological veterinary surveys performed with a capillary tube agglutination test showed that 14.5% (*n*=179) of cattle in Dhākā and Mymensingh had antibodies against *B. bigemina* [65]. A study at two upazilas of Rangpur district found that six of 400 cattle (1.5%) were microscopically positive for *Babesia* spp., which was confirmed with PCR targeting the multicopy *vesA-1a* gene of *B. bovis* [48]. A microscopic study of cattle (*n*=395) revealed that *Babesia* (1.6%) infections were more common in females than in male animals [57]. Molecular surveillance targeting the *Babesia* 18S rRNA gene demonstrated that 2.7% of 300 cattle were positive for *Babesia* sp., and that cross-bred animals were more vulnerable to infection than the local breed of cattle [4]. In the Mymensingh district, PCR-based molecular surveillance of cattle (*n*=385) confirmed the low occurrence of *B. bigemina* (1%) and *B. bovis* (0.5%) in blood samples collected in February–May [5].

Anaplasmosis is another serious veterinary problem in Bangladesh due to its wide prevalence and negative effects on the animal industry. The prevalence of clinically diagnosed or Giemsa-stain-positive anaplasmosis in cattle has been reported to be 25.8%–70% in Sirajganj, 8.21% in Chattogram, and 33% in Baghabari milk

shed areas [53,59,66,79]. However, caprine anaplasmosis was identified in only 2.1%–3.8% of different breeds of goats in Chattogram [52]. Molecular and serological diagnostic methods have been used to only a limited extent to confirm anaplasmosis. In the Mymensingh district, PCR identified *Anaplasma bovis* in 35.7% of 385 cattle and *Anaplasma marginale* in 4.17% [5]. Fifty-nine goats from Chattogram district (14.75%, *n*=400) were positive for *Anaplasma ovis* but only four (1%) were positive for *Anaplasma marginale* when PCR and sequencing of the *msp*4 fragment was used for testing [53]. The circulation of *A. marginale* and other *Anaplasma* spp. was also confirmed with PCR in Rangpur, Chattogram City, and Chittagong [4,48,53].

Theileria, another genus of tick-borne blood protozoan, occurs widely in Bangladesh, as expected from its endemicity in South Asia [80]. Different species of *Theileria* cocirculate in the country and affect different livestock and domestic animals to various degrees [81]. Bovine theileriosis attributable to *T. annulata, T. orientalis*, or *T. mutans* is well established in different breeds of cattle [57,82]. The highest prevalence (64.9%) in a historic report was based on the Giemsa-staining diagnostic method [49] and typically occurred in older animals. When *Theileria* infection is primarily detected and identified microscopically, its estimated prevalence ranges from 4.2% to 8.5% [57,62–64]. However, serological and molecular tools have become more readily available in recent years [5,51,58,83]. The seroprevalence of *Theileria* in cattle was measured with an enzyme-linked immunosorbent assay (ELISA) based on the recombinant major piroplasm surface protein antigen of *T. annulata* [58]. That study found significant differences in the seroprevalence of *T. annulata* between two areas of the Rājshāhi division, reporting 20.4% (*n*=147) and 80.0% (*n*=45) seropositivity in the Natore and Rājshāhi districts, respectively. This suggests that different exposure parameters may exist in these two areas. In the Mymensingh district, PCR surveillance detected *T. orientalis* infection in 55.2% of the 385 cattle tested [5]. The detection and identification of *T. luwenshuni* was reported in 34 of 400 goats in Chattogram examined at the university's teaching hospital. That study was based on the PCR amplification and subsequent sequencing of the 18S rRNA gene of *Theileria* spp. [83]. Goats from medium and small herds were found to be at higher risk of theileriosis than animals from large herds, and cross-bred goats had a higher rate of *Theileria* infection than Black Bengal or Jamuanpari goats. The availability of molecular diagnostic methods for veterinary laboratories will provide the additional information required to identify tick-borne protozoans to the species level, to confirm the animals' carrier status, to detect the introduction of new pathogens, and to identify coinfections with these pathogens [55,56].

3.4. Tick-borne pathogens and tick-borne diseases in dogs and cats (Table 3)

The blood of stray dogs infested with *R. sanguineus* in Mymensingh tested positive with TaqMan PCR for the *glt*A of SFGR (14%, *n*=50). However, none of these samples yielded larger *glt*A or *omp*A nested PCR fragments suitable for sequencing and species identification [38]. In contrast, 58% of the same canine blood samples were positive for the 16S rRNA gene of Anaplasmataceae (*n*=50). However, only one dog was infected with *Anaplasma bovis*, whereas the others carried the *Wolbachia* endosymbiont *Dirofilaria immitis*, a common canine nematode [38].

PCR detected *B. gibsoni* in 30% of blood from a cohort of stray dogs from the Mymensingh district [61]. Similar results were obtained with a PCR survey of mongrel and pure-bred dogs at the animal hospital in Dhākā; 38.2% (*n*=68) of dogs tested positive for *B. gibsoni* and 2.9% for the DNA of *Anaplasma* sp. AnHI446 [60]. Both *Anaplasma*-positive dogs were coinfected with *Babesia. Anaplasma* sp. AnHI446 was first detected in *H. lagrangei* collected from a bear in Thailand [77]. It is most closely related to *Anaplasma bovis* (99.6%) and *Anaplasma phagocytophilum* (96.5%), but its pathogenicity for humans and animals remains unknown. A recent study with PCR detected *B. gibsoni* in 44.7% and *Anaplasma platys* in 8.2% of blood samples from stray dogs in the Dhākā metropolitan area, and 4.7% of these dogs were coinfected [111]. Although it is known for its cosmopolitan occurrence, *Anaplasma platys* is considered an emerging pathogen in Bangladesh [111], so specific attention should be paid to its distribution and vector associations, and veterinary awareness must ensure its proper recognition, timely diagnosis, and management.

3.5. Tick-borne pathogens in rodents and other animals (Table 3)

The occurrence of tick-borne pathogens in rodents and shrews has been based on the microscopic analysis of Giemsa-stained blood smears [69]. An evaluation of 451 animals trapped in four urban, peri-urban, rural, and hilly areas of the country, including 299 shrews (*Suncus murinus*), 125 black rats (*Rattus rattus*), and 27 house mice (*Mus musculus*), identified *Anaplasma* in 34 (7.5%) of the animals examined and *Babesia* in 21 (4.7%) of them. Shrews were most frequently infected, with 10% (30 of 299) testing positive for *Anaplasma* and 4.3% (13 of 299) testing positive for *Babesia*. Rats had a lower *Anaplasma* infection rate of 3.2% (4 of 125; p=0.0182), but their rate of *Babesia* infection (5.6%, 7 of 125) was similar to that of shrews (p=0.579 based on a χ^2 test). *Babesia* was only detected in a single mouse trapped in a peri-urban setting. This suggests that rodents and shrews harboring these agents play roles in human and veterinary health in

Bangladesh, but the molecular identification of individual pathogens is still required.

3.6. Tick-borne pathogens and tick-borne diseases in humans

A small prospective study at the Mymensingh Medical College identified 40 febrile patients presenting with rash, who were not responsive to either antimalarial or ciprofloxacin treatment, but recovered after doxycycline or tetracycline treatment [70]. The insensitive nonspecific Weil–Felix test was used to diagnose 16 patients with SFG rickettsiosis, which were presumed to have Indian tick typhus. As well as fever, headache was the other prevalent symptom, followed by splenomegaly, arthralgia, hepatosplenomegaly, and lymphadenopathy. Rashes were observed in only 37.5% of patients, and none had eschar, a useful clinical symptom of *Rickettsia conorii* infections elsewhere [84]. This suggested that other genotype(s) of SFGR may have been responsible for these conditions.

Country-wide hospital-based surveillance of 720 febrile patients identified 132 individuals (18%) with immunofluorescent antibody titers > 1/64 consistent with probable SFG rickettsiosis[85]. Unfortunately, the specific rickettsial antigen used in this study was not reported. Therefore, despite the known antigenic cross-reactivity among SFGR, its actual prevalence maybe underreported due the use of heterologous antigens or the delay in seroconversion caused by some SFGR [86]. Fever, headache, and body aches were the most common symptoms of infection, but rash was identified in one patient. As in the report of Miah et al. [70] described above, eschar was not detected in any of these patients. A similar concurrent illness in family members was identified in 11 patients (12%), suggesting cluster exposure. Five patients (5%) succumbed to infection, indicating its delayed recognition, severe comorbidity with scrub typhus or arboviral infections, and/or the lack of etiologically appropriate treatment. There is a significant gap in our knowledge of tick-borne human diseases and their burden in Bangladesh.

4. Fleas and flea-borne diseases in Bangladesh

4.1. Fleas of Bangladesh

Only a few reports have described the flea species present in Bangladesh (Table 4), a significant omission given the ubiquitous occurrence of these ectoparasites in the tropics and their medical and veterinary importance [87]. The dog flea, *Ctenocephalides canis*, frequently infests dogs and goats in the Chittagong Hill Tracts in the southeastern part of the country [30]. The rat flea, *Xenopsylla cheopis*, has been collected from peridomestic mice (*Mus musculus*) and diverse rat species, including *Rattus sikkimensis, Bandicota savilei, Bandicota indica*, and *Niviventer* sp. [30]. The cat flea, *Ctenocephalides felis*, infested 68% (*n*=100) of stray cats in Mymensingh city and neighboring rural areas in the central-north region of the country [88].

4.2. Flea-borne pathogens and flea-borne diseases in dogs and cats (Table 4)

Twenty-eight percent of stray cats in the Mymensingh area were nested-PCR positive for a gene encoding a 17-kDa protein of *Rickettsia felis*; 85.7% of these animals were also infested with *Ctenocephalides felis* (20.6% of 68 fleas were PCR positive for *Rickettsia felis*) [88]. Two different genotypes of flea-borne rickettsiae were found in both cats and cat fleas. One was identical to *Rickettsia felis* URRWXCal2, a known human pathogen, and the other genotype was Rf31, which is most similar to *Candidatus* Rickettsia senegalensis of unknown pathogenicity [88,95]. Therefore, cats may be simultaneously infected with two different genotypes of flea-borne rickettsiae. Another common flea-borne rickettsia, *Rickettsia asembonensis*, was not identified despite its frequent detection in other countries, such as Thailand [96]. No records of *Rickettsia typhi* in either rodents or their fleas in Bangladesh were found, but this issue has been inadequately studied.

4.3. Flea-borne pathogens and flea-borne diseases in humans (Table 4)

The endemic nature of flea-borne rickettsioses was first demonstrated in a 2012–2013 cross-sectional study of febrile patients unresponsive to common antimicrobial drug therapies in Mymensingh, north-central Bangladesh [90]. That study reported the high prevalence (46%, *n*=150) of *Rickettsia* DNA in blood samples tested with PCR that amplified a gene encoding a 17-kDa antigen. DNA sequencing confirmed the pathogen to be *Rickettsia felis*. PCR amplification and sequencing of multiple genes showed that several patients, including 2–17-year-old children, were infected with *Rickettsia felis*, confirming the etiological role of *Rickettsia felis* in the febrile illness of this cohort of patients. A follow-up study conducted in 2013–2014 in the same region showed that the blood of 42% (*n*=50) of febrile patients with fever of unknown origin tested positive for *Rickettsia felis* on PCR [88]. Another cross-sectional study investigated 414 febrile patients with fever of unknown origin in all eight administrative divisions of Bangladesh between July 2015 and December 2016 [89]. Although 19.6% of the blood samples were PCR positive for *Rickettsia felis*, the detection rates varied substantially from 3.3% (*n*=30) in Khulna to 36.4% (*n*=11) in Rangpur district. The largest numbers of positive samples were from three districts: Mymensingh (19.9%, 43/216), Dhākā (24%, 18/75), and

Barisāl (23.8%, 10/42). A more recent evaluation in 2019–2020 identified *Rickettsia felis* as a concurrent infection in 11.5% (13/113) of patients diagnosed with scrub typhus in the same region [94].

There is only limited information on the occurrence of murine typhus in Bangladesh, in contrast to the situation in India [97]. Among patients treated at the Chittagong Medical College Hospital between August 2014 and September 2015, *Rickettsia typhi* infection was confirmed by sequencing the PCR amplicons in 24 of the 29 patients PCR-positive for the gene encoding the *Rickettsia* 17-kDa antigen (5.8% [24/416] of all patients), whereas only two patients were PCR positive for *Rickettsia felis* [91]. In the same cohort of patients, 15 of 415 patients tested positive on an immunofluorescence assay (IFA) for a *Rickettsia typhi* antigen, including five patients who had seroconverted and showed four-fold increases in titer, to ≥ 3,200 [91]. These findings contrast with other studies that have reported the infrequent diagnosis of murine typhus infection in Bangladesh. Only one febrile patient from Barisāl tested positive for *Rickettsia typhi* on PCR in a 2015–2016 cross-sectional study conducted in all eight administrative regions of the country [89]. A 2016 laboratory survey of 300 malaria-smear-negative patients in Chittagong division identified only two (0.6%) patients positive for *Rickettsia typhi* when tested for the OmpB gene with TaqMan PCR [98]. A hospital-based serosurvey of febrile patients seeking medical care in each of the administrative divisions identified 10 patients (1.3%, *n*=720) with serological evidence of a typhus group infection, but only one of those individuals tested positive for *Rickettsia typhi* on PCR [85].

Blood cultures from the same cohort of febrile patients yielded one sample positive for *Bartonella elizabethae*, a rat-associated *Bartonella* species [85]. This is an infrequent human pathogen that can cause culture-negative endocarditis [99]. In the Kamalapur residential area of Dhākā, *Bartonella* sp. almost identical to *Bartonella elizabethae* was found to be associated with *Rattus rattus, Bandicota bengalensis*, and *Suncus murinus* [100].

5. Mites and mite-borne diseases (scrub typhus)

5.1. Mites of Bangladesh

Only a few published records of mites in Bangladesh were found. In total, five species of mites were reported on livestock, dogs, and wild rodents in Chittagong division in Bangladesh: *Laelaps echidninus, Laelaps nuttali, Laelaps* sp., *Lyponissoides* sp., and *Ornithonyssus bacoti* [30]. *Laelaps* parasitic mites represented the largest portion of the collection, and were removed from a variety of peridomestic and wild rats, including *Rattus sikkimensis, Rattus rattus, Niviventer* sp., *Cannomys badius*, and *Bandicota bengalensis*. Typically, these mites live in the bedding of animal burrows and feed during the night on shed skin and body fluids. Although they are blood-feeding arthropods, they are not known to transmit any disease agents of concern to human health, and their infestations are not associated with any clinical symptoms [101]. *Laelaps echidninus,* the spiny rat mite, is a natural vector of *Hepatozoon muris,* which can cause mild or asymptomatic infections in rats [101]. *Lyponissoides* sp. mites were collected only from *Mus musculus,* and *Ornithonyssus bacoti* only from the lesser bamboo rat, *Cannomys badius.* Whether these mites carry any human pathogens in Bangladesh is unknown, but they are known vectors of *Rickettsia akari*, the etiological agent of rickettsialpox [102] and some other *Rickettsia akari*-like genotypes of rickettsiae [103].

5.2. Scrub typhus (*Table 5*)

Miah et al. reported 19 cases of scrub typhus diagnosed with the insensitive OXK Weil–Felix test among febrile patients treated in Mymensingh Hospital in 2003–2005 [70]. These were mostly young male patients from rural areas, who responded well to doxycycline or tetracycline and were discharged from the hospital within 7 days of admission with no complications. Unfortunately, this report did not differentiate the clinical symptoms of scrub typhus and those of SFG rickettsiosis, discussed above. However, it is noteworthy that none of the patients in this cohort developed eschar.

A 2008–2009 hospital-based survey of 720 febrile patients presenting at six tertiary hospitals in each administrative division of Bangladesh was conducted with the assistance of the U.S. Centers for Disease Control and Prevention (CDC) reference laboratories [85]. One hundred and seven patients (30%, *n*=360) tested positive for scrub typhus on ELISA (December 2008 to May 2009) and 63 (17.5%, *n*=720) patients tested positive on indirect IFA (June–November 2009). However, only two patients in this cohort tested positive for *Orientia* on PCR. Seventy-three patients seropositive for scrub typhus also tested positive for *Rickettsia* (46, 6.1%) or contained antibodies reactive to *C. burnetii,* dengue virus, or chikungunya virus. Seven *Orientia*-seropositive patients died, but none of them was diagnosed clinically with scrub typhus, and only three patients were treated with doxycycline or tetracycline. Patients with scrub typhus were diagnosed in each administrative region. Skin rash was noted in only 1% of patients diagnosed with scrub typhus, but there was no report of eschar [85], a typical hallmark of many of these infections [104,105].

A TaqMan PCR assay targeting the gene encoding a 47-kDa protein of *O. tsutsugamushi* detected only one case of scrub typhus (0.3%) among 300 malaria-smear-negative febrile patients, including 154 children, admitted to the Chittagong Medical College Hospital in January–June 2012 [93]. A 2010 serological survey of 1,209 febrile

patients seen in medical college hospitals in Chittagong, Dhākā, Sir Salimullah (Dhākā), Comilla, Bogra, and Sylhet, tested with an immunoglobulin M (IgM) ELISA, detected 287 (23.7%) patients seropositive for *O. tsutsugamushi* (Karp and Gilliam), 805 (66.6%) seropositive for *Rickettsia typhi* Wilmington, and 77 (6.4%) seropositive for both organisms [92]. These rates are similar to those for scrub typhus in studies previously undertaken in the Asia–Pacific region but much higher than those for murine typhus [106,107]. The relatively high rates of exposure to *Rickettsia typhi* in Bangladesh may be related to poor sanitation and the large numbers of rodents. However, as noted earlier, these serological findings were not confirmed with PCR analyses of patient blood or ectoparasites.

Patients with a history of fever for < 3 weeks who were referred to a hospital's malaria screening program were tested to determine the proportion of patients with rickettsial diseases [91]. A robust diagnostic approach was used that included PCR testing of blood samples collected upon admission and an IFA for *O. tsutsugamushi*-reactive antibodies in convalescent plasma samples and those collected at admission using *Orientia* Karp, Kato, Gilliam antigens) and *Rickettsia typhi* Wilmington antigen. Paired plasma samples were available for only 62% of the patients. Of the patients tested, 16.8% (70/416) had scrub typhus and 5.8% (24/416) had murine typhus based either on the molecular or serological test, or both. On PCR, 45 patient blood samples and three eschar swabs tested positive for *Orientia*. Twenty-nine patient blood samples tested positive for *Rickettsia* on PCR: 24 for *Rickettsia typhi*, two for *Rickettsia felis*, and two for undifferentiated *Rickettsia*. One of the *Rickettsia felis*-positive patients was also positive for *Orientia* on PCR but negative for *Rickettsia felis* on IFA, suggesting skin contamination with this flea-borne rickettsia [91]. Eighty-five percent of patients diagnosed with scrub typhus were from rural areas. The gene sequence of a 56-kDa *Orientia* protein detected in these patients' samples clustered most frequently with Karp and Karp-like UT76 sequences from Thailand, and with Gilliam-type sequences. Only one sequence clustered with the Kato strain and one with Thai animal strain TA763. This diversity is similar to that observed in other regions endemic for scrub typhus.

Another study in Mymensingh undertaken between March 2019 and February 2020 evaluated 402 febrile patients to determine the rate of comorbidity with scrub typhus and rickettsiosis [94]. The commercial Immunochromatic (Immunochomatographic) Mytest Scrub Ab Test Card (Meditech International, India) detected scrub typhus antibodies in 89 patients (22.1%, n=402). Moreover, 65 patient samples tested positive for the gene encoding a 47-kDa protein of *Orientia* on nested PCR, so 113 cases of scrub typhus were diagnosed by either or both tests. The blood DNA of 13 of these patients (11.5%) also tested positive for the gene encoding a 17-kDa *Rickettsia* protein on nested PCR. DNA sequencing confirmed the infecting agent to be *Rickettsia felis*, indicating that many patients in this region suffer from more than one infection that respond to doxycycline or tetracycline treatment.

6. Conclusions and future directions

In this review, we have summarized the literature on non-mosquito-borne diseases and their research in Bangladesh. There is a broad awareness of tick-borne diseases that affect livestock animals, particularly anaplasmosis, babesiosis, and theileriosis. Local veterinarians have conducted numerous surveys and regional studies, although many of those reports and their associated conclusions are based on relatively inaccurate microscopic analyses of blood smears. Use of this insensitive methodology has caused the status of these infections to be underestimated, and it cannot identify the etiological agents to the species level, especially in coinfections.

Frequent contact between humans and animals in Bangladesh implies their consequent exposure of humans, livestock, and peridomestic animals to pathogens carried by various blood sucking ectoparasites. However, the frequency and specific sources of these interactions are not fully understood. Similarly, the limited cross-sectional and short-term prospective surveillance projects have provided little information on the true spectrum of these diseases or identified the etiological agents circulating in ticks, fleas, or their peridomestic and sylvatic animal hosts. For example, a recent study used unspecified generic *Rickettsia* antigens for serosurveillance and therefore reported only the cross-reactive antibody titers from different regions of Bangladesh [85]. In a similarly limited small prospective study from Mymensingh Medical College, 40 febrile patients presenting with rash were diagnosed with scrub typhus or SFG rickettsiosis with a nonspecific Weil–Felix tests [70]. Similar clinical manifestations made it nearly impossible to differentiate the two groups of diseases. Rash was present in only 37.5% of patients and none had eschar, a useful clinical symptom in individuals diagnosed with scrub typhus and *Rickettsia conorii* infections elsewhere [84,104,105]. A purpuric rash is frequently observed in patients infected with *Rickettsia conorii* subsp. *indica*, but eschar is rarely reported [84]. Although the frequency of eschar in patients with scrub typhus may show geographic and population-specific differences, eschar is commonly reported in up to 78.9% of patients in other parts of the scrub-typhus-endemic triangle [105]. The characterization of the types of *Rickettsia* and *Orientia* circulating in Bangladesh is important, and should allow the laboratory diagnosis of these infections with geographically relevant etiological

agents and kits and the detection of any antibiotic resistance present [108].

At least a dozen species of ixodid ticks are known in Bangladesh, although this inventory seems very incomplete compared with the tick species identified in neighboring countries [109,110]. Similarly, only limited information is available on the identities of the fleas present in this country, and none on louse infestations in animals was found. Although current approaches to the identification of ectoparasites are based solely on morphological keys, no Bangladesh-specific keys are available. Therefore, efforts must be made to catalogue the circulating ectoparasites borne by non-mosquito vectors, including ticks (particularly their immature stages) and fleas. Molecular identification tools for representative species are required to ensure the accuracy of surveillance and the early tracking of newly invasive species. Of course, the implementation of these measures will depend on the development and training of a local cadre of specialists who are knowledgeable about contemporary techniques and protocols for laboratory and field studies. Understanding the true diversity of tick-borne and flea-borne pathogens affecting humans and animals in Bangladesh and the associated risk of human exposure will be important for reducing the human and animal morbidity and mortality caused by these diseases and the associated health-care costs and economic losses. The broad time scale reviewed here and the insufficient and inconsistent methodologies used in these studies are barriers to establishing priorities for future research directions. Filling existing knowledge gaps is essential for choosing interventions that will most effectively remove any significant barriers to veterinary and public health in Bangladesh.

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Data available statement

All data generated as a part of this study are included in the manuscript.

Ethics statement

Not applicable.

Informed consent

Not applicable.

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