Heliyon 9 (2023) e17172

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Review article

Babesiosis: Current status and future perspectives in Pakistan and chemotherapy used in livestock and pet animals

Muhammad Azhar [a], Javaid Ali Gadahi [a,*], Bachal Bhutto [a], Sambreena Tunio [b], Waseem Ali Vistro [c], Haleema Tunio [d], Sahar Bhutto [e], Teerath Ram [f]

[a] *Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam, Pakistan*
[b] *Department of Animal Product Technology, Sindh Agriculture University, Tandojam, Pakistan*
[c] *Department of Veterinary Anatomy, Sindh Agriculture University, Tandojam, Pakistan*
[d] *Department of Poultry Husbandry, Sindh Agriculture University, Tandojam, Pakistan*
[e] *Department of Veterinary Pathology, Sindh Agriculture University, Tandojam, Pakistan*
[f] *Department of Animal Nutrition, Sindh Agriculture University, Tandojam, Pakistan*

A R T I C L E I N F O

Keywords:
Babesiosis
Chemotherapy
Pakistan
Veterinary medicine

A B S T R A C T

Babesiosis is a protozoal disease affect livestock and pet animals such as cattle, buffaloes, sheep, goats, horses, donkeys, mules, dogs, and cats. It causes severe economic losses in livestock as well as in pet animals. A large number of dairy animals are imported in order to fulfill the demands of milk, milk, meat and its products. In addition, different pet animals are transported from Pakistan to various parts of the world, therefore, it is important to identify the current status and distribution of babesiosis throughout Pakistan in order to control the disease and draw attention for future research, diagnosis, treatment and control of this diseases. No work has been done on a complete review on up-to-date on blood protozoal disease burden in Pakistan. This article will provide about the complete background of babesiosis in ruminants, equines and pet animals, its current status, distribution, vectors in Pakistan and allopathic and ethnoveterinary treatments used against babesiosis. Babesiosis may be subclinical (apparently normal) and may be clinical with acute to chronic disease and sometimes fatal. Babesia is found and develops inside the erythrocytes (red blood cells). Clinically, it causes fever, fatigue, lethargy, pallor mucus membranes, malaise, cachexia, respiratory distress, jaundice, icterus, hemolytic anemia, hemoglobinuria, lymphadenopathy, chollangocytitis, hepatomegaly, and splenomegaly. Chemotherapy for babesiosis includes Imidocarb dipropionate, Diaminazine aceturate Atovaquone and Bupravaquone, Azithromycin, Quinuronium sulfate and Amicarbalidesio-thionate are most widely used. Supportive therapy includes multivitamins, fluid therapy, antipyretics intravenous fluids, and blood transfusions are used if necessary. In addition, there are certain ethnoveterinary (homeopathic) ingredients which having anti-babesial activity. As the resistance against these drugs is developing every day. New more specific long-lasting drugs should be developed for the treatment of Babesiosis. Further studies should be done on disease genome of different species of *Babesia* for vaccine development like malarial parasites.

* Corresponding author.
E-mail address: jagadahi@sau.edu.pk (J.A. Gadahi).

https://doi.org/10.1016/j.heliyon.2023.e17172
Received 26 July 2022; Received in revised form 6 June 2023; Accepted 9 June 2023
Available online 18 June 2023

1. Introduction

Babesia protozoan parasites are responsible for serious diseases in livestock, pets and humans all over the world. Globally, the most significant haemoprotozoan diseases which affect livestock health and animal productivity are Babesiosis, Theileriosis, Anaplasmosis, and Trypanosomiasis [1] which are caused by various species of *Bebsia* spp*, Theileria* spp*, Anaplasma, spp and Trypanosoma* spp in different species of livestock and human [2–4]. Babesiosis and theileriosis are transmitted by the *Boophilus* spp (Ixodid) and *Hyalloma anatolicum, H. marginalatum* and *H. excav*um ticks respectively where trypanosomiasis are transmitted by hematophagous biting flies such as *Glossina* (Tsetse) fly and blood transfusion. *Babesiaesia* spp (*B. bigemina*) is round, amoeboid found in the erythrocytes (RBCs) their growth and multiplication are taken places by the process of binary division (asexual reproduction). *Theileria* spp (*T. parva, T. mutants, and T. annulata*) are the irregular small rod-like ovoid-round organism found in the lymphocytes, erythrocytes, and histiocytes [5,6]. *Anaplasma* spp (*A. marginale, A. centrale*) is the small spherical bodys, dark red in color found inside the RBCs after the giemsa staining. A hot and humid area is favorable climate for the development and survival of ticks (vectors) [7]. Globally, haemoprotozoans infection causes significant effect on the health and productivity of in livestock animals and human which lead to numerous economic losses and considerable constraints in the dairy and meat industry around the world [8]. Clinic findings vary between species and animals, however it cause fever, weakness, loss of body condition, cachexia, anemia, decrease in milk production, poor draught power, abortion and death in severe cases [8].

Piroplasmosis, also known as Babesiosis, is a disease caused by a haemoprotozoan belonging to the phylum Apicomplexa, order Piroplasmidia, family Bebesiidae, and genus *Babesia*. The erythrocytes of wild and domestic animals are parasitized by this malaria-like protozoan. It's a well-known veterinary illness that affects cattle, horses, and dogs, and it's becoming more well-known as a zoonotic disease. This bacterium can produce symptoms similar to malaria, such as fever, hemolysis, and hemoglobinuria. Infections with Babesia have most likely been disrupting human lives since antiquity, especially through infections in domestic cattle.

1.1. History

The oldest documented of babesiosis is most likely in Exodus which describes a plague of the Egyptians Pharaoh Ramses II's livestock, which might have been red water fever (caused by *Babesia bovis*) and includes hemoglobinuria as a common symptom. However, it was not until Babes' study in 1888 that the genus was properly named, when he discovered an intra-erythrocyte pathogen (suspected to be a bacterium) producing fever hemoglobinuria in Rumanian cattle [9]. Shortly thereafter, the agent of Texas Cattle Fever, initially called *Pyrosoma,* was discovered [10] and later identified as *Babesia bigemina*. Wilson and Chowning [11] observed piriform, intra-erythrocytic inclusions in the blood of patients with Rocky Mountain spotted fever in the western US, provided the first evidence that humans might be infected with *Babesia* parasites. *Pyroplasma hominis* was the name given to this parasite. Babesiosis in cattle cause by *B. bovis*: In 1934, it is the most virulent species of Babesia protozoa and it was first identified in Argentina [12].

Due to inadequate practices, management and preventive techniques, haemoprotzoan lead to great economic losses in the form high infection rate, death rate, reduction in the productive performance of animals, survival of cross breeds, draught power, increase in the cost of control and preventive measures [13,14] which lead to reduction in the development of socio-economic and agriculture [15, 16]. Economically, only it cause annual loss of US$800 million [17]. Due to high economic losses in livestock as well as in pet animals. Large number of diary animals are imported in order to fulfil the demands of milk, milk, meat and its products. Large number of pet animals are transported from Pakistan to various parts of the world; therefore, it is important in order to identify the current status and distribution of babesiosis throughout the Pakistan in order to control the diseases and draw an attention for future research, diagnosis, treatment and control of this diseases. No work has been done on complete review on up-to-date blood protozoal diseases burden in Pakistan. This article will provide about complete background of babesiosis in ruminants, equines and pet animals, current status, distribution, vectors in Pakistan and different allopathic babesiacidal drugs and ethnoveterinary treatment can be used against babesiosis.

In addition, drugs and combination of the drugs which are used for the treatment in different animals are sometime are not registered or approved for specific indication by the national agencies and their use is extra then the required dose.

2. Etiology

(Table 1).

3. Tramission and vector

Babesiosis is tick borne disease and it is transmitted by various species of tick genus including *Rhipicephalus, Ixodes, Boophilus, Hyalomma, Dermacenter* and *Haemophysalis.* it showed that, *Rhipicephalus* spp*, Boophilus* spp*, Haemaphysalis* spp*, Ixodes* spp and *Hyalomma* spp are involved in the transmission of *Babasia* spp in Cattle and Buffaloes. *Dermacenter* spp*, Hyalomma* spp and *Rhipicephalus* spp in equines, *Rhipicephalus* in sheep, goat, cat, and pigs. However, *Rhipicephalus sanguineus, Dermacenter* spp, *Hyalomma* spp and *Haemaphysalis bispinosa* tick species are involved in the transmission of babesiosis in canines.

4. Diagnosis

Babesiosis can be diagnosed by microscopic detection technique including thin, thick, brain and hemolymph smear, serological

Table 1
Etiology.

Diseases	Species	Transmitted	Animal	References
		Ticks:	Cattle	[18–24]
	1. Babesia bovis	*1. Rhipicephalus* spp		
	2. B. bigemina	*2. Boophilus* spp		
	3. B. major	*3. Haemaphysalis* spp	Buffaloes	
	4. B. divergen	*4. Ixodes* spp		
	5. B. beliceri	*5. Hyalomma* spp		
		Ticks:	Horse	[19]
		Dermacenter spp		
	1. B. equi	*Hyalomma* spp		
	2. B. caballi	*Rhipicephalus* spp		
	1. B. motasia	**Ticks:**	Sheep	[13,19]
Babesiosis	*2. B. ovis*	*Rhipicephalus* spp	Goat	
Red Water Diseases		**Ticks:**	Dog	[16,20,25–30],
Piroplasmosis	*1. B. canis* (Large)	*1. Rhipicephalus sanguineus*		
Tick Fever	*2. B. gabsoni* (small)	*Dermacenter* spp		
	B. rossi (Large)	*Hyalomma* spp		
	3. B. vogeli	*Haemaphysalis bispinosa*		
	4. B. valpes	*2. Haemophysalis eliptica*		
	5. B. microti	*H. leachi*		
	6. B. conradae	*3. Rhipicephalus sanguineus*		
	B. felis	**Ticks:**	Cat	[13,31]
		Rhipicephalus sp		
	B. trautmanni	**Ticks:**	Pig	[13,31]
	B. perroncitoi	*Rhipicephalus* spp		

technique including indirect flouroscent antibody test (IFAT), Competitive Enzyme linked immunosorbent assay (cELISA), Immunochromatography (ICT), complement fixation test (CFT), and molecular techniques including DNA probes, Polymerase Chain Reaction (PCR), nested PCR, Real-time PCR, Restriction fragment length polymorphism (RFLP) PCR, Reverse line blot hybridization (RLB), loop-mediated isothermal amplification (LAMP) The most common methods used for the diagnosis is thin blood smear technique and microscopic examination of the stage of *Babesia* parasite in the erythrocytes or blood and tissue fluid. Chronic stages of these diseases are challenging for the examination under microscope of invasively acquired specimen and treatment for these cases [32,33].

5. Material and method

The purpose of this review article is to gather the information regarding babesiosis, babesiosis burde, distribution in Pakistan in different livestock and pet animals and its distribution. In this review article, we go through the literature by using a terms Babesiosis", "Pakistan", "Sindh", "Punjab", "Balouchistan" and "Khyberpukhtunkhwa" in Google Scholar. Fif**ty research article**s were systematically reviewed from 1984 to 2019 by focusing the number of animals including cattle, buffaloes, sheep, goat, horse, dog and cat affected with Babesiosis in Pakistan, geographical area, causative agent and to identify the risk factors associated with these diseases like, gender, age, type and seasonal influence on the occurrence of babesiosis in Pakistan by using the published data by the authors. The data is presented in tabulated form.

6. Babesiosis in ruminants

It is one of the most important and economically important disease of cattle. There are about six species of *Babesia* which cause disease in cattle. *Babesia* is intra-erythrocytic protozoan parasites which cause anemia, icterus, hemoglobinuria, and death. *B. bovis* and *B. gemina* are the tick protozoan which cause great economic burden where cattle are used for the food security in the form of milk and meat in tropical and subtropical areas of the world [34].

Babesiosis in cattle cause by *B. bigemina*: In Asia, Africa, Australia, Europe, North and South America, *B. bigemina* is widespread in cattle and it is transmitted by *Boophilus* spp tick. However, calves are naturally resistant to *B. bigemina* does not produce any pathology. In old or immunocompromised animals, the disease may be acute. In addition, pathogenicity of *B. bigemina* vary in different isolated from different geographical area. In this disease, more than 75% of erythrocytes are destroyed in a few days which lead to rapid anemia and once hemoglobinuria (blood in urine) is developed then chances of the recovery are too less. Mortality varies according to the environment conditions, in susceptible animal's mortality may up to 50% without treatment, however, if animal survive from the severe phase of the disease, animal become weak with loss of conditions. Animal may survive, if animal is in good environment with proper management, in the absence of undue stress [23,35].

The epidemiology is similar to *B. bigemina.* However, it is also reported in those part of Europe where *Boophilus* tick doesn't occurs which shows that, there is another vector which is associate with the transmission of *B. bovis* in cattle. Incubation period of Babesiosis is 10–14 days but it may shorten it the inoculum is large. Infection can persist from years to throughout the life. Clinically, in both cases, there is not any resemblance but, in some cases, there is neurological and respiratory signs which lead to death of an animals. In *B. bovis* infection, there is massive intravascular erythrocytes sequestration which carry mature stage of *Babesia* parasite [23,36].

It is also reported that, in case of *B. bigemina*, 10% of hematocrit may cause death whereas in case of *B. bovis*, death may occur in 12% or higher hematocrit. In USA, in 1942 tick eradication program was completed so there was a reduction in the case of Babesiosis exception in quarantine near to the Mexican border [37].

Babesiosis cause by *B. divergens* and *B. major* in cattle: Babesia major is larger specie among all the Babesia species and slightly smaller than *B. bigemina.* Epidemiologically, they are found in England and northern part of Europe transmitted by *Haemophysalis puncata* (*H. puncata*). Clinically, all the types of Babesiosis cause similar clinical manifestations however, neurological signs are rarely seen in case of *B. divergens. B. divergen* cause serious disease, however *B. major* is non-pathogenic but can cause clinical signs and death if induce to a splenectomized calves [38].

In case of sheep and goat, Babesiosis is more severe in sheep as compare to goat and highly pathogenic species of Babesia is *B. ovis* as compare to *B. motasi*. However, mixed infection of both species is highly severe and cause highly pathogenic disease syndrome (HPDS) in sheep [39].

6.1. Prevalence of babesiosis in ruminants in Pakistan

(Table 2).

7. Babesiosis in equines

Babesiosis in equine cause by *B. equi and B. caballi*. It is characterized hemolysis of red blood cells (RBCs), Anemia, icterus, lachrymation, dullness, mucous membrane pale, hemoglobinuria, fever (40–41 °C), jaundice, respiratory distress, and death [70,71]. Clinically, the severity is variable, there is spontaneous recovery follow to the febrile with no anemia and hemoglobinuria [20]. There are other species of Babesia which affect different vertebrate species [23]. The most susceptible to babesiosis is horse whereas donkey, mule and zebras are rarely affected with babesiosis. Equine babesiosis is endemic in temperate, tropical and subtropical regions and it is also reported in Middle east, Asia, Southern Europe and Latin America. The animals which are within the age of first year are most prone to this infection with mortality rate is 5–10% in native horse may exceed to 50% with respect to the dose of transmission, horse health, treatment, population of horses and vector [71].

7.1. Prevalence of babesiosis in equines in Pakistan

(Table 3).

8. Babesiosis in dog

Canine babesiosis is cause by *Babesia canis* (large) and *Babesia gibsoni* (small) are two organisms commonly known to infect dogs. However, epidemiologically, several other species are also found in dogs worldwide [27,75]. Both organisms have *Ixodid* tick vectors and are found throughout Asia, Africa, Europe, the Middle East, and North America, with *B. canis* being more prevalent. In North America, canine *Babesia* infection cause by *B. gibsoni* is increasing, however, no any tick species was found in the transmission of the *B. gibosoni* infection [18]. In addition, three more subspecies of *Babesia canis* were also by amplification of ribosomal subunit RNA through Restriction Fragment Length Polymorphism (RFLP) – Polymerase Chain Reaction (PCR) technique named *as B. canis, B. vogeli* and *B. rossi* [76] and taxonomic classification of these subspecies was done by Uilenberg, Franssen [28]. They are differentiate on the basis of their pathogenicity [77], geographic distribution and tick vectors [16]. The clinical manifestation of Babesiosis is vary according to the types of species, subspecies, strains, their virulence and factors which are depent on their host condition = such as individual, age, status of immune system, and the presence of infection or other infections [75,78,79]**.** Clinically, the disease is due to *B. canis* and *B. gibsoni* is depend upon the severity, clinical signs are ranging from the hyperacute shock associate hemolytic (acute) to chronic and inapparent infection which was described by Refs. [75,79–82]. The disease with the onset with high fever, lethargy, pallor mucous membrane (m.m), depress, malaise, cachexic lymphadenopathy (inflammation of lymph nodes and lymphatic vessels), splenomegaly (enlargement of spleen) and abnormalities in the blood coagulation and electrolyte imbalance. Blood profile of infected animals is normochromic to hypochromic [18,83]. Anemia due to systemic inflammatory response and erythrocytes destruction (Hemolysis) lead to dysfunction of different organs such liver, lungs, kidney and central nervous system which results in the various clinical manifestation in dogs. Formerly, Babesia was diagnose and identified by microscopic appearance of the parasite in the erythrocytes. Large and small form of *Babesia* were designated *B. canis* and *B. gibsoni* respectively.

There is large diversity of Babesia parasites which cause disease in dogs based on the difference in the antigenic properties, pathogenicity and tick vector and it was proved by molecular (genetically) identification by molecular technology [26,27,75,77]. Recently, different species of Babesia has been identified like *B. gibsoni, Babesia rossi, Babesia vulpes, Babesia vogeli* (also termed *Theileria annae* and *Babesia microti*-like) *and Babesia conradae*.

8.1. Prevalence of Bebesia in dog and cat in Pakistan

(Table 4).

Table 2
Prevalence of babesiosis in ruminants in Pakistan.

Province	Region	Species	Host	Period of Study	Diagnostic Method	Cattle/Sheep			Buffaloes/Goat			Total			Ref:
						%	NOP	TN	%	NOP	TN	%	NOP	TN	
Sindh	Karachi	*Babesia* spp	Cattle and Buffalo	Sept 1984 to Feb 1985	Thin Smear	4.2	4	95	1.4	3	219	2.2	7	314	[40]
	Karachi	*Babesia bovis*	Buffaloes	April to Oct 2009	Thin smear	–	–	–	3.0	3	100	3.0	3	100	[41]
	Hyderabad	*Babesia* spp	Cattle and Buffaloes	1990–1991	Thin Sm ear	1	1	100	1	1	100	1.0	2	200	[42]
Punjab	Lahore	*Babesia* spp	Sheep and Goat	Aug–Nov 2010	Thin Smear	23.5	57	243	13.5	51	377	17.4	108	620	[43]
	Lahore	*Babesia* spp	Cattle	N/A	Thin smear and ELISA	24.7	22	89	–	–	–	24.7	22	89	[44]
	Lahore	*B. bovis*	Cattle	N/A	Thin Smear and PCR	8.3	5	60	–	–	–	8.3	5	60	[21]
	Islamabad	*Babesia* spp	Cattle Buffalo	1999 to 2000	Thin Smear	0.6	2	307	0	0	155	0.5	1	220	[45]
	Attock	*Babesia* spp	Cattle Buffalo	1999 to 2000	Thin Smear	–	–	–	–	–	–	0.4	1	242	
	Rawalpindi	*Babesia bigemina*	Cattle and Buffalo	N/A	Thin Smear and PCR	–	–	–	–	–	–	58.9	1278	2170	Siddique, Sajid [46]
	Toba tek Singh	*Babesia bigemina*	Cattle and Buffalo	N/A	Thin Smear and PCR	–	–	–	–	–	–	59.5	1291	2170	[46]
	Toba tek Singh	*B. bigemina* & *B. bovis*	Cow and Buffaloes	N/A	PCR	9.4	9	96	7.1	3	42	8.3	9	108	[47]
	Multan	*Babesia bigemina*	Cattle and Buffalo	N/A	Thin Smear and PCR				–	–	–	54.7	1187	2170	[46]
	Multan	*B. bovis*	Sheep and Goat	N/A	Thin Smear and PCR	–			–	–	–	22.2	4	18	[48]
	Sahiwal	*Babesia* spp	Cattle	2010	Thin Smear and PCR	23.5	47	200	–	–	–	23.5	47	200	[49]
	Sahiwal	*Babesia* spp	Cattle (Sheep)	May–July 2005	Thin smear	7.2	30	415	–	–	–	7.2	30	415	[50]
	Kasur	*Babesia* spp	Cattle	2003 to 2004	Thin smear	5	10	200	–	–	–	5.0	10	200	[51]
	Kasur	*B. bovis* & *B. bigemina*	Cattle	N/A	Thin smear and PCR	29	29	100	–	–	–	29.0	29	100	[5]
	Faisalabad	*Babesia* spp	Cattle	N/A	Thin Smear and PCR	10.4166	40	384	–	–	–	10.4	40	384	[52]
	Faisalabad	*B. bigemina*	Cattle & Buffaloes	N/A	Thin smear and PCR							18.5	402	2176	[46]
	Faisalabad	*B. bigemina*	Cattle & Buffaloes	N/A	PCR	14.16667	17	120	7.0	4	57	11.9	21	177	[47]
	Jhang	*B. bigemina* & *B. bovis*	Cattle and buffaloes	N/A	PCR	18.75	27	144	13.3	8	60	17.2	35	204	[47]
	Dera Ghazi Khan	*B. bovis*	Sheep and Goat	N/A	Thin Smear and PCR	–	–	–	–	–	–	18.4	7	38	[48]
	Bhawalnagar	*B. bovis*	Sheep and Goat	N/A	Thin Smear and PCR	–	–	–	–	–	–	20.0	1	5	
	Khanewal	*B. ovis*	Sheep and Goat	N/A	Thin Smear and PCR	–	–	–	–	–	–	33.3	1	3	
	Khanewal	*B. bigemina*	Cattle and Buffaloes	N/A	Thin Smear and PCR	–	–	–	–	–	–	22.7	495	2176	[46]
	Chakwal	*B. bigemina*	Cattle	N/A	Thin Smear and PCR	–	–	–	–	–	–	10.5	228	2176	
	Muzaffargarh	*B. ovis*	Sheep and Goat	N/A	Thin Smear and PCR	–	–	–	–	–	–	38.5	5	13	[48]
	Vehari	*B. ovis*	Sheep and Goat	N/A	Thin Smear	–	–	–	–	–	–	40.0	2	5	

(*continued on next page*)

Table 2 (*continued*)

Province	Region	Species	Host	Period of Study	Diagnostic Method	Cattle/Sheep			Buffaloes/Goat			Total			Ref:
						%	NOP	TN	%	NOP	TN	%	NOP	TN	
	Layyah	*B. ovis*	Sheep and Goat	N/A	Thin Smear	–	–	–	–	–	–	60.0	15	25	
	Okara	*B. ovis*	Sheep (Lohi)	N/A	Thin smear and PCR	55.0	110	200				55.0	110	200	[53]
	Mandi Bhauddin-Qadirabad	*Babesia* spp	Sheep (Lohi)	N/A	Thin Smear and PCR	9.7	30	310	–	–	–	9.7	30	310	[54]
	Mandi Bhauddin-Qadirabad	*B. bigemina* & *B. bovis*	Cattle	N/A	Thin Smear and PCR	29.0	29	100	–	–	–	29.0	29	100	[49]
KPK	Peshawar	*B. bigemina* & *B. bovis*	Cow and Buffaloes	2001	Thin smear	4.6	13	285	–	–	–	4.6	13	285	[55]
	Peshawar	*B. bigemina* & *B. bovis*	Cattle and Buffaloes	N/A	Thin smear & PCR	23.3	17	73	74.0	20	27	37.0	37	100	
	Peshawar	*Babesia* spp	Cattle and Buffalo	2013–2016	Thin smear	15.9	109	690	51.4	125	243	25.0	234	933	[56]
	Peshawar	*Babesia* spp	Sheep & Goat	2013–2016	Thin smear	14.8	16	108	41.6	25	60	37.9	41	108	
	Peshawar	*Babesia* spp	Sheep and Cattle	N/A	Thin smear	–	–	–	–	–	–	7.0	21	300	[57]
	Malakhand Agency	*B. bigemina*	Cattle	N/A	Thin Smear	6.1	115	1892	–	–	–	6.1	115	1892	[58]
	Charsada	*B. bovis* & *B. bigemina*	Cattle	2010 to 2011	Thin Smear	20.0	20	100	–	–	–	16.0	16	100	[59]
	Swabi	*B. bovis* & *B. bigemina*	Cattle	2010 to 2011	Thin Smear	20.0	20	100	–	–	–	14.0	14	100	
	Bannu	*B. bovis* & *B. bigemina*	Cattle	2012	Thin Smear	61.3	92	150	–	–	–	61.3	92	150	[60]
	Bannu	*B. bovis* & *B. bigemina*	Cattle	2018	Thin Smear	58.7	176	300	–	–	–	58.7	176	300	[61]
	Karak	*B. bovis* & *B. bigemina*	Cattle	2018	Thin Smear	49.3	148	300	–	–	–	49.3	148	300	
	Karak	*B. bovis* & *B. bigemina*	Cattle	2012	Thin Smear	9.3	111	1200	–	–	–	9.3	111	1200	[62]
	Chatt	*B. bovis* & *B. bigemina*	Cattle	2018	Thin Smear	56.0	168	300	–	–	–	56.0	168	300	[61]
	Lakki Marwat	*B. bovis* & *B. bigemina*	Cattle	2018	Thin Smear	45.3	136	300	–	–	–	45.3	136	300	
	Lakki Marwat	*B. bovis* & *B. bigemina*	Cattle	2012	Thin Smear	48.0	72	150	–	–	–	48.0	72	150	[60]
	Dera Ismail Khan	*B. bovis* & *B. bigemina*	Cattle	2018	Thin Smear and PCR	64.7	194	300	–	–	–	64.7	194	300	[63]
	Koghat	*Babesia* spp	Cattle	2012	Thin Smear	10.5	126	1200	–	–	–	10.5	126	1200	[62]
	Kohat	*Babesia* spp	Cattle	2015 to 2016	Thin Smear	9.0	28	311	–	–	–	9.0	28	311	[64]
	Muhammad Agency	*B. bovis* & *B. bigemina*	Cattle	July to Oct 2007	Thin Smear	12.1	23	190	7.9	15	190	10.0	38	380	[65]
	Northern Central and Southern	*B. bovis*	Cattle and Buffaloe	April–September 2015	Microscopy & PCR	11.9	57	479	8.1	34	421	10.0	90	900	[66]
Balouchistan	Quetta	*B. ovis* & *B. motasi*	Sheep and Goat	1998 to 2000	Thin Smear	23.2	232	1000	10.9	76	700	18.1	308	1700	[67]
	Quetta	*Babesia* spp	Cattle	2013 to 2014	Thin Smear	51.8	57	110	–	–	–	51.8	57	110	[68]
	Quetta	*B. bovis*	Cattle	2011 to 2012	Thin Smear and PCR	20.5	123	600	–	–	–	20.5	123	600	[69]
	Sibi	*B. bovs*	Cattle	2011 to 2012	Thin Smear and PCR	15.2	91	600	–	–	–	15.2	91	600	

Note: %: Prevalence Percentage, NOP: Number of positive and TN: Total Number.

Table 3
Prevalence of babesiosis in equines in Pakistan.

Province	Region	Species	Host	Period of Study	Diagnostic Method	Horse			Donkey			Mule			Total			Ref:
						%	NOP	TN	%	NOP	TN	%	NOP	TN	%	NOP	TN	
Sindh	Larkana, Ratodero, Bakrani, Dokri (Northern Sindh)	*Babesia* spp	Horse and Donkey	N/A	Thin Smear	0	0	150	–	–	–	–	–	–	–	90	300	[72]
Punjab	Remount Veterinary Farm and Corps (RVFC), Jhelum	*Babesia* spp	Horse	Aug-2007	Thin Smear	–	21	–	–	–	–	–	–	–	–	21	–	[70]
	Lahore, Faisalabad, Mulatan, Bhawalpur & Gujranwala	*B. cabali*	Horse, Donkey and Mules	July 2007 to March 2008	Competitive enzyme-linked immunosorbent assay (cELISA)	24.6	16	65	20.2	67	332	30.3	10	33	21.6	93	430	
	Lahore	*Babesia* spp	Horse, Mule and Donkey	March 2012 to February 2013	cELISA	19.3	32	166	26.0	30	115	23.6	27	114	22.5	89	395	[73]
	Lahore	*B. cabali*	Horse, Donkey and Mules	July 2007 to March 2008	cELISA	–	–	–	–	–	–	–	–	–	29.0	20	69	[74]
	bhawalpur	*B. cabali*	Horse, Donkey and Mules	July 2007 to March 2008	cELISA	–	–	–	–	–	–	–	–	–	16.2	11	68	
	Faisalanad	*B. cabali*	Horse, Donkey and Mules	July 2007 to March 2008	cELISA	–	–	–	–	–	–	–	–	–	22.0	33	150	
	Gujranwala	*B. cabali*	Horse, Donkey and Mules	July 2007 to March 2008	cELISA	–	–	–	–	–	–	–	–	–	22.7	17	75	
	Multan	*B. cabali*	Horse, Donkey and Mules	July 2007 to March 2008	cELISA	–	–	–	–	–	–	–	–	–	164.7	112	68	

Note: %: Prevalence Percentage, NOP: Number of positive and TN: Total Number.

Table 4
Prevalence of babesiosis in dogs and CAT in Pakistan.

Province	Region	Species	Host	Period of Study	Diagnostic Method	Dog			Cat			Total			
						%	NOP	TN	%	NOP	TN	%	NOP	TN	References
SINDH	Hyderabad	*Babesia canis*	Dogs	N/A	Thin Smear	5.0	15	300				5.0	15	300	[84]
PUNJAB	Lahore	*Babesia canis*	Dog	Jan 2004 to Dec 2005	Thin Smear	13.2	1566	11,840				13.2	1566	11,840	[85]
	Lahore	*Babesia* spp	Dog	Jan 2005 to Dec 2007	Thin Smear	1.7	337	19,546				1.7	337	19,546	[86]
	Lahore	*B. canis, B. gibsoni*	Dog	Dec 2018 to May 2019	Thin Smear and PCR	34.0	17	50				34.0	17	50	[33]
	Lahore	*B. microti*	Dog and Cat	N/A	Thin smear and PCR							7.4	23	309	
	Lahore	*B. felis*	Cat	January 2007 to December 2009	Thin Smear				3.1	163	5183	3.1	163	5183	[86]
	Multan	*B. microti*	Dog and Cat	N/A	Thin smear and PCR	0.0	0	49	0	0	49	0.0	0	49	[87]
	Rawalpindi and Islamabad	*B. microti*	Dog and Cat	N/A	Thin smear and PCR	2.0	1	49	0	0	49	2.0	1	49	
	Rawalpindi	*B. gibsoni* & *B. vogeli*	Dog	May to Oct 2016	Thin Smear and PCR	17.3	78	450				17.3	78	450	[88]
	Narowal	*B. canis, B. gibsoni*	Dog	Dec 2018 to May 2019	Thin Smear and PCR	44.7	67	150				44.7	67	150	[33]
	Kasur	*B. gibsoni* & *B. vogeli*	Dog	May to Oct 2016	Thin Smear and PCR	23.3	105	450				23.3	105	450	[89]
	Muzaffargarh	*B. gibsoni* & *B. vogeli*	Dog	May to Oct 2016	Thin Smear and PCR	13.8	62	450				13.8	62	450	[88]

Note: %: Prevalence Percentage, NOP: Number of positive and TN: Total Number.

9. Effect of babesiosis in hematology

9.1. Ruminants

In sheep, it is reported that there is sharp reduction in the hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), packed cells volume (PCV) and thrombocytes (platelets) in infected sheep as compared to healthy sheep. However, in case of goat, there is reduction in the Hb, RBC, PCV and thrombocytes significantly but there was no any effects of WBCs in infected goat as compare to health goats [43,50,90]. Similar results were also reported by Shah, Khan [57]. It is reported that, there is significant increase in glucose and triacyl glyceride and decrease in thyroxine (T4), Cholesterol, and High density lipoprotein (HDL) in infected animal as compare to healthy cattle [44].

9.2. Canines

In dogs, it is reported that there is significant reduction in the Hb, RBC, WBC, PCV and thrombocytes in infected dogs as compared to healthy dogs [91,92]. However, biochemical parameters show that, there is reduction in the level of total protein, albumin, globulin, Albumin/globulin ratio, glucose, serum glutamic pyruvic transaminase (SGPT), and creatinine in infected dogs as compared to health dogs. It is also reported that, there is increase in the level of blood urea nitrogen (BUN) in Babesiosis in dogs [92]. Moreover, it is reported that, along with the reduction in RBC, HB, WBC and PCV (HCT) there is reduction in other hematological parameters such as MCH, MCV, MCHC & Platelets [91].

9.3. Equines

Similar results are also reported in equines, there was a sharp reduction in total erythrocytes count (TEC), total leucocytes count (TLC) and hemoglobin (Hb) as compared to recovered animals [70].

10. Specie wise prevalence of babesiosis

According to the species of *Babesia*, there are various species which cause disease in various animals. It is reported that, the prevalence of *B. bovis* was higher than *B. bigemina* with 34% and 20.6% in case of cattle [60,61,65]. However, by Faryal, Muhammad [93] reported 21% of *B. bigemina* and 11% *B. bovis*, Masih, Rafique [47] who reported 14.6% *B. bigemina* and 9.2% *B. bovis* respectively, Chaudhry, Suleman [49] reported 19% *B. bigemina* and 11% *B. bovis* infection rate in cattle, Ahmad, Khawja [59] reported 19% *B. bigemina* and 11% *B. bovis* in cattle. In case of dogs, it is reported that prevalence of *B. gibsoni* and *B. vogeli* was 46.8 and 7.3% respectively. Shahzad, Haider [53] reported 55% of in lohi sheep breed in Okara, Punjab, Pakistan.

11. Animal wise prevalence of babesiosis in Pakistan

11.1. Ruminants

The infection rate of babesiosis is higher in cattle as compared to buffaloes and goat as compared to sheep. Studies shows that, the infection rate of Babesiosis is higher in the cattle as compared to buffalo [43,45,66]. However, by Rashid [56], Faryal, Muhammad [93] reported higher in buffaloes than cattle. Ijaz, Rehman [43] reported that, the infection rate was higher in sheep as compare goat with 23.4 and 13.5% respectively. In case of goat and sheep, the infection rate was higher in goat as compare to sheep (Rashid, 2018).

11.2. Equines

The infection rate of babesiosis is higher in mule as compared to horse and donkey. In equines, the infection rate of babesiosis was higher in mule followed by horse and donkey with 30.3, 24.2 and 20.2% respectively [74]. The results are not significant according to the animals. Moreover, it is reported by Rashid, Mubarak [70], Javed, Ijaz [73], Hussain, Saqib [74], the infection rate was higher in horses followed by mules and lowest in donkey. Whereas, Kumbhar, Shah [72] reported high prevalence in donkey as compare to mule and horse.

12. Age wise prevalence of babesiosis in Pakistan

According to the age, literature shows that, the infection is higher in young animals as compared to old animals. It is reported that, the infection is higher in adult animals in cattle and buffaloes as compared to young stocks [47,60,61]. Similar finding were also reported by Ahmad, Rashid [89] in canine. However, by Siddique, Sajid [46], Khan, Rehman [60], Hussain, Ashraf [94] who reported higher in young stock as compared to adult animals. It is reported that, the prevalence of babesiosis in case of cattle was higher in those animals who are <2 years followed by <4, <6, <9 and lowest in >9 years old age animal. It shows there is decrease in the infection rate as age advances, which may be due to advancement in the immunity of the body according to the age of the animals. However, in case of dogs, Shah, Khan [91] reported higher disease incidence in adults as compare to young animals. Ahmad, Khawja [59], Khan, Rehman [60], Zaman, Atif [95] reported higher infection rate in <1 followed by 1–3, 3–6 and lowest in 6–9 years of age in cattle and buffaloes with 66.0, 55.2, 51.9 and 50.49 respectively. In case of cats, the prevalence was higher in 25 months of age group followed by 13–24 months, <6 months, and lowest in 7–12 months of age [96]. In equines, it is reported that, infection rate of babesiosis is higher in between 5 and 10years followed >10 and lowest in <5 years [74].

13. Breed wise prevalence of babesiosis in Pakistan

In Pakistan, Lohani breed is more prone blood parasitic infection followed by cross breeds, Dhanni and Red Sindhi Cattle breed with 36.8, 19.4, 19.23, and 16.94% respectively [45]. Siddique, Sajid [46] reported that, exotic breeds of cattle are more severely affected with the babesiosis followed by cross breed and indigenous breeds (Sahiwal). In case of buffaloes, Kundhi (Sindh) breed is more affected with babesiosis as compared to Nilli Ravi (Punjab). Furthermore, infection was higher in Holstein Frisian followed by Jersey (Exotic), cross breeds, Sahiwal and lowest in Achai breed of cattle. Morevoer, infection was higher in Kundhi followed by Nilli Ravi and lowest in non-descriptive buffalo. In case of dogs, the disease is higher in Gull terrier (80.9) followed by German shepherd (62.2), Tazi (38), Spaniel (30), Labrador (22.2) and lowest in Pointer (14.2%) [91]. In case of cat, the prevalence of babesiosis was 3.22, 2.72, 3.27 and 0% in cross breed, Persian, Siemese cat and Himalyan breeds respectively [96]. Hussain, Ashraf [94] reported that, there is high prevalence in cross breed cattle's as compared to indigenous cattle with 14.3 and 3.3% respectively.

14. Gender-wise prevalence

Gender wise prevalence showed that, the infection rate was higher in female as compared to male which may be due to physiological stress during lactation, dry period, estrus, and pregnancy. Bhutto, Gadahi [41], Siddique, Sajid [46], Rafique, Kakar [68], Zaman, Atif [95], Farooqi, Ijaz [97] reported, there is high infection rate of babesiosis in female as compare to male. Masih, Rafique [47], Khan, Rehman [60]reported that, the infection of babesiosis cause by *B. bovis* and *B. bigemina* was higher in female as compare to male. The high prevalence of babesiosis in female may be due to its physiological status such as dry periods, lactation periods, estrus period etc. Which are the predisposing factors of the stress on female which lead them more prone to infectious disease. It is also reported that, it may be due to use of contaminated needles, syringes, and injections of drugs, hormone for milk production and milk let down (Oxytocin) [60]. Similar results were also reported by Ahmad, Khan [96], who reported high babesiosis prevalence in female as compare to male with 3.6 and 2.69% respectively. However, the results are opposites in buffalo and cows [66,93], in equine Kumbhar, Shah [72]. In the results are opposite in case of dogs [91] and in cattle [59]. In case of equines there is no any significant difference in the gender wise infection of babesiosis. It is reported that, the infection rate was in male and female was 21.7 and 21.5% respectively [74].

15. Seasonal aspect

There are four seasons in Pakistan, winter (December–February), Spring (March to May), Summer (June to August) and Autumn (September to November. According to the season, The rate of infection of babesiosis is higher in summer season followed by autumn, spring and lower in winter season which may be due to favorable environment for the growth and development, survival and tick burden in these seasons [46,59–61,95,98–100]. Similar results were also reported by Ahmad, Khan [96] in cats. It is also reported that, the infection is higher in summer season which may be due to association of tick activity with humidity and temperature of an environment. Moreover, in case of dogs, the disease incidence was higher in summer season as compared to winter season [91]. Ijaz, Rehman [43] reported in goat and sheep, the infection rate was higher in August, followed by September, October and lower in November with 72.9, 22.0, 7.94 and 5.4% respectively. Niazi, Khan [50] reported that, there is high infection in the month of July followed by June and lowest in May. Rashid, Khan [54] reported greater prevalence percentage of babesiosis in Lohi sheep breeds in June followed by May and lowest in April with 15.4, 8.18, 3.6 respectively.

16. Housing system and animal keeping

It is revealed that, the rate of babesiosis is higher in intensive type of housing as compared to semi-intensive and free housing system. It may be due to high risk of tick infestation and environmental stress in the intensive type of housing as compared to semi-and free housing system. Siddique, Sajid [46], Masih, Rafique [47], Farooqi, Ijaz [66], Zaman, Atif [95] reported that, animal which are tie in the barns (tethered) are more prone to parasitic diseases as compared to the open type of keeping animal system. It is also reported that, the infection rate was higher in those animals who are fed in ground as compared to trough feeding [47]. Similarly, the rate of infection is higher in closed types and uncemented type of housing system followed by semi-closed and lowest in open types and cemented types of housing system which may be due to breeding and sheltering of ticks population in crevices, heaps and cracks in bricks lead to higher disease burden [101,102]. Siddique, Sajid [46], Masih, Rafique [47] reported higher incidence of disease in uncemented followed by partially cemented and uncemented. It may be due to hiding of the vector in the cracks, spaces of uncemented and partially cemented floor and houses. It is reported that, the herd having more than 20 animals have greater rate of infection as compare to herd having less than 20 animals Masih, Rafique [47]. In case of equines, the infection rate higher in those barn where the tick infestation persist for a long time as compare too tick free house [74].

17. Chemotherapy to babesiosis

There are various drugs and combination of drugs which are used against Babesiosis [25,27,75,103]. The mechanism of action of these drugs and combination of drugs against Babesia were not known and not studied in detail. However, explanatory and clinical trial has been done but they were not well understood [25,78,104,105]. It is reported that, infected dogs are susceptible to different drugs and produce its action differently therefore, it is more important to treat with the most effective antiprotozoal drugs and combination with other drugs.

Treatment for the Babesiosis is dependent upon amicarbalides, diminazene and quinuronium from last 10 years. It is reported that, the size of protozoal parasites is important for their susceptibility against antiprotozoal drugs. Large form of haemoprotozoans is highly susceptibility to the antiprotozoal drugs but no treatment can be relied upon to give a radical cure. This is rarely required because elimination of the parasites may break down premunition and render the subject susceptible to reinfection.

18. Babesiacidal drugs

(Table 5).

18.1. Imidocarb dipropionate (ID)

Imidocarb is Babesia drugs which is approved by Food and Drug administration (FDA) for the treatment of Babesiosis in cattle, buffaloes, horse, dog, cat, and camel. However, it is reported that, it is highly effective against large Babesia species as compared to small Babesia species [78,104].

18.1.1. Mechanism of action

ID act on the protozoa by various mechanism. It is reported that, imidocarb act on the Babesia by disrupting the formation and use of polyamines in different parasites like these polyamines are also found in *trypanosome brucei* [118], interrupting in the entrance of

Table 5
Drugs used against babesiosis.

Drug	Spectrum	Purpose	Animal	Dose and route	Ref
Imidocarb dipropionate	*Babesia bovis* *B. bigemina* *B. divergens* *B. canis B. gabsoni* *B. rossi B. vogeli* *B. valpes* *B. microti* *B. conradae* *B. caballi* *B. equi*	Treatment and Protection	Bovines Horse Sheep	Bovine: Treatment: 1.2 mg/kG IM Prevention: 3 mg/kg IM Sheep: 2 mg/kg Horse: 2–4 mg/kg IM	[21,22,32,54,82, 106–108]
Diaminazine aeturate	*Babesia bovis* *B. bigemina* *B. rossi* *Trypnosoma* spp *Anaplasma* spp	Treatment and Curative	Camel Horse Ruminants Dogs	Treatment: 3.5mg/kgbw IM Prevention: 3.5mg/KgBW for 2–4 weeks 10 mg/kg IM	[10,32,109–113].
Atovaquone	*Babesia* spp, *Theileria* spp	Treatment and Curative	Bovines	2.5 mg/kg	[114]
Bupravaquone	*Babesia* spp, *Theileria* spp	Treatment and Curative	Bovines	2.5 mg/kg	[114]
Azithromycin	*B. gibsoni, B. conradae* *B. vulpes* *B. microti* *Cytauxzoon feli*	Treatment			[115,116]
Atovaquon combination with Azithromycin	*B. gibsoni, B. conradae* *B. vulpes* *B. microti* *Cytauxzoon feli*	Treatment	Cattle Dogs Cat	Aqvaquone: 13.3 mg/kg PO Azithromycin: 10 mg/kg PO	[104,117]
Quinuronium sulfate	*Babesia* spp	Treatment			[25]
Amicarbalidesio-thionate	*Babesia* spp	Treatment			
Oxytetracyline and Doxycycline	*B. canis* (Large) *B. gabsoni* *B. felis*	Treatment and Curative	Sheep, Goat, Dogs and cat		
Imidocarb dipropionate + Oxytetracyline	*B. ovis*	Treatment	Sheep and Goat	ID: 2 mg/kg BW IM Oxytetracyline: 10 mg/kg BW i.m	[108]

inositol in the infected RBCs which is important for the survival of Babesia parasites, this lead to starvation of Babesia in the RBCs result in the death of the parasites [119], combination with damaging and inhibition of DNA replication and cell repairing [109]. ID is excreted via hepato-enteral and renal route in urine and feces.

18.1.2. Adverse effect

ID are available in parental form, so it causes pain during administration. It also have a cholinergic effect which lead to drooling of saliva, nasal discharge and emesis. Anticholinergic such as atropine sulfate at dose rate of 0.05 mg/kg are used to counter effect the cholinergic effect. It also causing, panting, lethargy, diarrhea, nephrotoxicity, hepatotoxicity, inflammation and ulceration (rarely) at the site of injection which will be healed in few days.

18.2. Diaminazine

Diaminazine is Babesiacidal drugs which is available with aceturate salt in the form of diaminazene aceturate (brenil). It is most used in the Asian countries such as Pakistan, India, and Bangladesh whereas in Europe, quinornium sulfate and amicarbalideisothionate are used for the treated of Babesiosis in different livestock animals. However, it is not approved for the treatment of dogs in USA but is widely used in South Africa where DA is main drugs for the treatment of *B. rossi* infections in dogs Babesiosis in dogs [120].

18.2.1. Mechanism of action

The mechanism of action of diaminazine is not clear yet, however, it is suggested that it disrupt glycolysis, DNA synthesis of trypanosome species and leishmania species (Plumb, 2015). It is also reported that, it also inhibition the replication of DNA and affect the respiratory activity of mitochondria [121,122]. Chemotherapy of Babesia parasites varies according to the size and stage of the parasites. This shows that, the efficacy is variable and this drug is most effective against large form of Babesia spp. DA is excreted through hepatoenteric and renal system by urine and feces.

18.2.2. Adverse effect

At normal and high doses, DA affects the central nervous system, causing neurological symptoms. With dosages exceeding 10 mg/kg IM or repeated lesser doses over a short period of time, the safety index is regarded poor, resulting in a cumulative impact and severe to lethal toxicity [109]. In dogs given therapeutic dosages of diaminazene aceturate, side effects include gastrointestinal problems such as vomiting and diarrhea, discomfort and inflammation at the injection site, a transitory reduction in blood pressure, and, less commonly, neurological symptoms such as ataxia, seizures, and death. Additionally, in severe cases of babesiosis, supportive therapy such as blood transfusions, anti-inflammatory medicines, tick removal, iron repairs, dextrose, vitamins (B complex), purgatives, and fluid replacements may be required [109].

18.3. Atovaquone and Bupravaquone

Antiprotozoal agent atovaquone is a synthetic hydroxynaphthoquinone effective against Babesia, plasmodium, and theileria. In cats, infection with piroplasmosis such as *Cytauxzoon feli* is treated with atovaquone [104,117].

18.3.1. Mechanism of action

Atovaquone and other hydroxy1,4 naphthoquinones are thought to preferentially impede protozoan mitochondrial electron transport, inhibiting pyrimidine and ATP production [109,123].

18.3.2. Resistance against atovaquone

Resistance to atovaquone has been found in *B. gibsoni*-infected dogs from Japan and Taiwan, and is thought to be caused by changes in the parasite's cytochrome *b* gene, which alter atovaquone site of action [124–128]. Mutations in the cytochrome *b* gene that cause the amino acid methionine to be replaced with isoleucine, known as the M121I mutation, were shown to be the source of resistance to atovaquone in resistant *B. gibsoni* [105,124]. In vitro testing of 15 different medications for *B. gibsoni* growth suppression revealed atovaquone to be the most effective against the parasite's Aomori strain [124]. Theileria parva (bovine theileriosis) and horse theileriosis are treated with buparvaquone (*Thieleria equi*).

18.3.3. Adverse effect

Atovaquone (Mepron) and pill containing atovaquone and proguanil HCl (Malarone®) is also available for human malaria therapy and prophylaxis; however, due to the proguanil component, this combination has been documented to produce gastrointestinal side effects in dogs and should be avoided. Atovaquone has no recognized side effects in dogs at this time. In cats Infection with the piroplasma such as *Cytauxzoon feli* is also treated with a combination of atovaquone and azithromycin [129].

18.4. Azithromycin

Azithromycin is a macrolide antibiotic that works by inhibiting protein synthesis. Antimicrobial and antiprotozoal medicines are commonly used to treat bacterial infections in the lungs.

18.4.1. Mechanism of action

Azithromycin inhibits mRNA translation and bacterial protein synthesis by binding to the 50S component of the prokaryote ribosome. It also has antiprotozoal properties by acting on apicoplasts, which are relict non-photosynthetic plastid organelles with a restricted genome seen in apicomplexan parasites, including Babesia spp [115,116].

Azithromycin has been demonstrated to have efficacy against *Toxoplasma gondii* [130] and to create a delayed mortality effect in *P. falciparum* by acting on the parasite's apicoplast [131].

19. Ethnoveterinary medicinal plant (homeopathic) agents which are used for babesiosis

Pakistan is a developing country rich with medicinal plants which are regularly used traditionally for the treatment of various parasitic diseases. In developing countries, due to a lack of resources for health facilities, most of the rural communities rely on traditional medicine in humans as well as in animals to alleviate a variety of ailments. Sometimes commercial pharmaceutical products are available, but the instruction printed on them are difficult to read and understand by uneducated and unaware farmers, this led to the misuse of drugs [132]. Therefore, it is worth important for rural communities to utilize all the available resources including ethnoveterinary medicinal plants (EVMP) to improve their animal health care services. However, the evaluation of traditional animal treatment is important due to the safety of animals and its effectiveness in the treatment of ailments. EVM's profile would benefit from the confirmation of specific procedures in the view of possibly skeptical, traditionally educated vets who may have only seen the outcomes of harmful handling by rural farmers seeking to treat infectious and parasitic infections. EVMPs are important due to the increase in the resistance to drugs for the development of new extracted and lead compound for the pharmaceutical industry even though ethnoveterinary practices can serve as a starting point for drugs development, a more beneficial and cost-effective exercise would be to improve a selected preparation through pharmacological research and development, and the resulting treatment can be returned to the community with added value. Possible adverse effects, as well as toxicity that may be taken by the animals and subsequently transfer to people via milk or meat, are other concerns that must be addressed. There are certain ethnoveterinary medicinal plants that are reported against babesia (Table 6).

Table 6
Homeopathic agents WHICH are used for babesiosis.

S. No	Plant	Part	Preparation	Animal	Dose Rate	References
1	*Peganum harmala* (Harmal)	Seeds	Extract	Babesia spp	12.5 mg/kg	[52]
2	*Zantedeschia aethiopic*	Rhizome	Boil in water	Babesia spp		[133]
3	*Boophone disticha*	Inner bulb	Boil in water after crushing	Babesia spp		
4	*Cussonia spicata*	Bark and leaves	Boil in water after crushing	Babesia spp		
5	Chrysocoma tenuifolia	Roots	Boil in water	Babesia spp		
6	*Combretum caffrum*	Leaves and barks	Boil in water after crushing	Babesia spp		
7	*Heteromorpha arborescen*	Root	Cold after crushing	Babesia spp Anaplasma spp		
8	*Salix capensis*	Leaves & Barks	Boil in water after crushing	Anaplasma spp Babesia spp		
9	*Plectranthus laxiflorus*	Root and Barks	Boil in salt water after crushing	Anaplasma spp Babesia spp		
10	*Hypoxis colchicifolia*	Seeds & Leaves	Boil with *Salix capense* barks	Anaplasma spp Babesia spp		
11	*Schotia latifolia*	Bark Leaves	Boil in water after crushing	Babesia spp		
12	*Lippia javanica*	Leaves	Boil in water	Boil in water after Crushing		
13	*Tithonia diversifolia*	Leaves	Ethanol Extract	In-vitro againt *Plasmodium falciparum, L. divergen & B. divergen*	3 μg/ml	[134]
14	*Cyclea barbata*	Leaves and Stem	Ethanol Extract	In-vitro againt *Plasmodium falciparum, L. divergen & B. divergen*	3 μg/ml	
15	*Tinospora crispa*	Stem	Ethanol Extract	In-vitro againt *Plasmodium falciparum, L. divergen & B. divergen*	3 μg/ml	
16	*Arcangelisia flava*	Stem	Ethanol Extract	In-vitro againt *Plasmodium falciparum & B. divergen*	3 μg/ml	
17	*Pycnarrhena cauliflora*	Powdered + Dichloromethane	n-hexane lipophilic compound extract	In-vitro againt *Plasmodium falciparum, L. divergen & B. divergen*	3 μg/ml	
18	*Elephantorrhiza elephantina*	N/A	Acetone Extract	In-vitro- *B. caballli*	100 μg/ml	[135]
19	*Urginea sanguinea*	N/A	Acetone Extract	In-vitro- *B. caballli*	Non-effective	
20	*Aloe marlothii*	N/A	Acetone Extract	In-vitro- *B. caballli*	Non-effective	
21	*Rhoicissus tridentata*		Acetone Extract	In-vitro- *B. caballli*	Non-effective	

20. Conclusions

It is concluded that, babesiosis is endemic and the most common diseases in cattle, buffaloes, sheep, goat, horse, mule, donkey, dogs and cats among the blood protozoal diseases in Pakistan. There are limited number of drugs which are effective against Babesia parasites. However, there is increasing in the resistance of these drugs against certain species of *Babesia.* Therefore, further studies should be conducted to isolate new chemical and herbal compounds which having anti-babesiacidal properties. Further studies should be done on disease genome of different species of *Babesia* for vaccine development like malarial parasites.

Credit author statement

Conceived And Designed The Experiments: JA. Gadahi & Bachal Bhutto. **Performed The Experiments:** MA, Memon & JA Gadahi. **Analyzed And Interpreted The Data:** S. Tunio, T. Ram & H. Tunio. **Contributed Reagents, Materials:** Analysis Tools Or Data; WA Vistro, S Bhutto. **Wrote The Paper:** MA Memon, JA Gadahi.

Ethics approval

Review article is not an experimental based study. Therefore, there was no need of approval for the research from the Animal Ethical Committee.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

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

References

[1] Z. Rajput, Comparative study of Anaplasma parasites in tick carrying buffaloes and cattle, J. Zhejiang Univ. - Sci. B 6 (11) (2005) 1057.
[2] B.R. Maharana, et al., Important hemoprotozoan diseases of livestock: challenges in current diagnostics and therapeutics: an update, Vet. World 9 (5) (2016) 487.
[3] H. Singh, et al., A PCR assay for detection of Babesia bigemina infection using clotted blood in bovines, J. Appl. Anim. Res. 32 (2) (2007) 201.
[4] TEWARI, A., et al., Identification of immunodominant polypeptides common to Babesia bigemina and Theileria annulata. The Indian Journal of Animal Sciences. 71(7).
[5] A. Durrani, et al., Prevalence of theileriosis in buffaloes and detection through blood smear examination and polymerase chain reaction test in district Lahore, J Anim Plant Sci 18 (2008) 59–62.
[6] E. Soulsby, Helminths, Arthropods Protozoa of Domesticated Animals (1982) 291.
[7] N. Afifi, et al., Prevalence of theileriosis in cross-bred cattle: its detection through blood smear examination and polymerase chain reaction in Dehradun district, Uttarakhand, India, Vet. World 7 (3) (2014) 168.
[8] P. Juyal, et al., Management of Surra Due to Trypanosoma Evansi in India: an Overview vol. 75, 2005, pp. 109–120.
[9] V. Babes, Sur l'hemoglobinurie bacterienne du boeuf, C. R. Acad. Sci. 107 (1888) 692–694.
[10] T. Smith, F.L. Kilborne, Investigations into the Nature, Causation, and Prevention of Texas or Southern Cattle Fever, US Department of Agriculture, Bureau of Animal Industry, 1893.
[11] L.B. Wilson, W.M. Chowning, Studies in pyroplasmosis hominis ("spotted fever" or "tick fever" of the Rocky mountains), Rev. Infect. Dis. (1979) 540–558.
[12] K.L. Kuttler, World-wide impact of babesiosis, in: Babesiosis of Domestic Animals and Man, CRC Press, 2018, pp. 1–22.
[13] O. Radostits, et al., Veterinary Medicine: A Textbook of Disease of Cattle, Sheep, Pigs, Goats and Horses, *Balliere Tindall.* English Language Book Society, London, 2000, pp. 1805–1810.
[14] L.H. Makala, et al., The current status of major tick borne diseases in Zambia, Vet. Res. 34 (1) (2003) 27–45.
[15] K. Ananda, J. Adeppa, Prevalence of Haemoprotozoan infections in bovines of Shimoga region of Karnataka state, J. Parasit. Dis. 40 (3) (2016) 890–892.
[16] G. Uilenberg, International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health, Vet. Parasitol. 57 (1-3) (1995) 19–41.
[17] K. Ananda, P.E. D'Souza, G. Puttalakshmamma, Prevalence of Haemoprotozoan diseases in crossbred cattle in Banglore north, Vet. World 2 (1) (2009) 15.
[18] J. Taboada, S.R. Merchant, Babesiosis of companion animals and man, Vet. Clin. Small Anim. Pract. 21 (1) (1991) 103–123.
[19] M. Ristic, Babesiosis, in: Diseases of Cattle in the Tropics, Springer, 1981, pp. 443–468.
[20] R. Purnell, in: M. Ristic, J.P. Kreier (Eds.), Babesiosis in Various Hosts, Babesiosis, vol. 25, Academic Press, New York, 1981, p. 63.
[21] A. Durrani, N. Kamal, Identification of ticks and detection of blood protozoa in friesian cattle by polmerase chain reacton test and estimation of blood parameters in district Kasur, Pakistan, Trop. Anim. Health Prod. 40 (6) (2008) 441–447.
[22] A. Jabbar, et al., Tick-borne diseases of bovines in Pakistan: major scope for future research and improved control, Parasites Vectors 8 (1) (2015) 1–13.
[23] R. Aryeetey, V. Jimenez-Lucho, Babesiosis, Curr. Treat. Options Infect. Dis. 4 (2002) 319–326.
[24] G. Uilenberg, Babesia—a historical overview, Vet. Parasitol. 138 (1-2) (2006) 3–10.
[25] H.J. Vial, A. Gorenflot, Chemotherapy against babesiosis, Vet. Parasitol. 138 (1-2) (2006) 147–160.
[26] M. Zahler, et al., Detection of a new pathogenic Babesia microti-like species in dogs, Vet. Parasitol. 89 (3) (2000) 241–248.
[27] L. Solano-Gallego, G. Baneth, Babesiosis in dogs and cats—expanding parasitological and clinical spectra, Vet. Parasitol. 181 (1) (2011) 48–60.
[28] G. Uilenberg, et al., Three groups of Babesia canis distinguished and a proposal for nomenclature, Vet. Q. 11 (1) (1989) 33–40.
[29] S.E. Shaw, et al., Tick-borne infectious diseases of dogs, Trends Parasitol. 17 (2) (2001) 74–80.
[30] F. Jongejan, G. Uilenberg, The global importance of ticks, Parasitology 129 (S1) (2004) S3–S14.
[31] W.J. Foreyt, Diagnostic parasitology, Vet. Clin. Small Anim. Pract. 19 (5) (1989) 979–1000.
[32] J. Mosqueda, et al., Current advances in detection and treatment of babesiosis, Curr. Med. Chem. 19 (10) (2012) 1504–1518.

[33] M. Tayyub, et al., Genetic diversity of canine Babesia species prevalent in pet dogs of Punjab, Pakistan, Animals 9 (7) (2019) 439.
[34] P.H. Hamid, et al., First autochthonous report on cattle babesia naoakii in central java, Indonesia, and identification of Haemaphysalis bispinosa ticks in the investigated area, Pathogens 12 (2023) 59.
[35] E. Vannier, P.J.J.H.s. Krause, E.I. Diseases, Babesiosis (2020) 799–802.
[36] D.R. Allred, Babesiosis: persistence in the face of adversity, Trends Parasitol. 19 (2) (2003) 51–55.
[37] A.A. Pérez de León, et al., Integrated Strategy for Sustainable Cattle Fever Tick Eradication in USA Is Required to Mitigate the Impact of Global Change vol. 3, 2012, p. 195.
[38] J. Kamani, et al., Prevalence and Significance of Haemoparasitic Infections of Cattle in North-Central, Nigeria, 2010.
[39] T. Alessandra, C.J.S.R.R. Santo, Tick-borne Diseases in Sheep and Goats: Clinical and Diagnostic Aspects vol. 106, 2012, pp. S6–S11.
[40] M. Haider, F. Bilqees, Babesiosis in some mammals in Karachi, Proc. Parasitol. 3 (1987) 5–11.
[41] Bhutto, B., et al., A survey on haemo-protozoan parasites in buffaloes of Landhi Dairy Colony, Karachi-Pakistan. 2012. 6: p. 73-76.
[42] S. Buriro, et al., Incidence of some haemo-protozoans in Bos indicus and Bubalis bubalis in Hyderabad, Pak. Vet. J. 14 (1) (1994) 28–29.
[43] M. Ijaz, et al., Clinico-epidemiology and therapeutical trials on babesiosis in sheep and goats in Lahore, Pakistan, J. Animal Plant Sci. 23 (2) (2013) 666–669.
[44] Hussain, M., et al., Assessment of lipid and thyroid biomarkers in cattles infected with babesiosis in Lahore, Pakistan. 2020. 10(1): p. 311-317.
[45] M. Khan, et al., Prevalence of blood parasites in cattle and buffaloes, Pak. Vet. J. 24 (4) (2004) 193–194.
[46] R.M. Siddique, et al., Association of different risk factors with the prevalence of babesiosis in cattle and buffalos, Pakistan J. Agric. Sci. 57 (2020).
[47] A. Masih, et al., Molecular epidemiology of bovine babesiosis in Punjab, Pakistan, Acta Sci. Vet. 49 (2021).
[48] F. Iqbal, et al., A study on the determination of risk factors associated with babesiosis and prevalence of Babesia sp., by PCR amplification, in small ruminants from Southern Punjab (Pakistan), Parasite: journal de la Société Française de Parasitologie 18 (3) (2011) 229.
[49] Z.I. Chaudhry, et al., Molecular detection of Babesia bigemina and Babesia bovis in crossbred carrier cattle through PCR, Pakistan J. Zool. 42 (2) (2010).
[50] N. Niazi, et al., A study on babesiosis in calves at livestock experimental station Qadirabad and adjacent areas, Sahiwal (Pakistan), Pakistan J. Agric. Sci. 45 (2) (2008) 209–211.
[51] I. Zahid, M. Latif, K. Baloch, Incidence and treatment of theileriasis and babesiasis, Pak. Vet. J. 25 (3) (2005) 137.
[52] M. Saleem, et al., Evaluation of anti-protozoal activity of peganum harmala (harmal) against babesiosis in cattle: department of clinical medicine and surgery, university of agriculture, faisalabad, Pakistan, Pakistan J. Agric., Agric. Eng. Veterinary Sci. 36 (1) (2020) 55–60.
[53] Shahzad, W., et al., Prevalence and molecular diagnosis of Babesia ovis and Theileria ovis in Lohi sheep at livestock experiment station (LES), Bahadurnagar, Okara, Pakistan. 2013. 8(4): p. 570.
[54] A. Rashid, et al., Prevalence and chemotherapy of babesiosis among lohi sheep in the livestock experiment station, qadirabad, Pakistan, and environs, J. Venom. Anim. Toxins Incl. Trop. Dis. 16 (4) (2010) 587–591.
[55] Z.K. Afridi, I. Ahmad, Incidence of anaplasmosis, babesiosis and theileriosis in dairy cattlein Peshawar [Pakistan], Sarhad J. Agric. (2005).
[56] M.I.J.J.A. Rashid, Epidemiology of tick-borne infection in ruminants in Peshawar, J. Adv. Parasitol. 5 (1) (2018) 6–10.
[57] S. Shah, M. Khan, H.J.P.J.a. Rahman, Epidemiological and hematological investigations of tick-borne diseases in small ruminants in Peshawar and Khyber Agency, J. Adv. Parasitol. 4 (1) (2017) 15–22.
[58] Shoaib, M., et al., Prevalence and Molecular Characterization of Anaplasma marginale in Cattle Population of Khyber Pakhtunkhwa Province, Pakistan.
[59] I. Ahmad, et al., Detection of babesiosis and identification of associated ticks in cattle, Int. J. Bioassays 3 (2014) 3195–3199.
[60] A. Khan, et al., Burden of babesiosis among domestic cattle of southern Khyber Pakhtunkhwa, Pakistan, Pak J Entomol Zool Stud 4 (2016) 305–307.
[61] A. Khan, et al., Prevalence of tick born Babesia infection in domestic cattle of Khyber Pakhtunkhwa, Pakistan, Pakistan J. Zool. 52 (6) (2020) 2401–2403.
[62] K.P. Pakistan, Epidemiology and molecular detection of babesiosis in household dairies in districts kohat and karak, Life Sci. J. 10 (10s) (2013).
[63] Khan, A., et al., Prevalence of tick born Babesia infection in domestic cattle of Khyber Pakhtunkhwa, Pakistan. 2020. 52(6): p. 2401-2403.
[64] Khan, Z., et al., Incidence of Blood Protozoans in Dairy Cattle in Kohat Region, Khyber Pakhtunkhwa, Pakistan. Livestock Development Foundation®(LDF): p. 771.
[65] B. Khattak, et al., Study the incidence of babesiosis in cattle of afghan refugees in Mohmand agency, Pakistan, 5, 2017, pp. 1422–1424.
[66] S.H. Farooqi, et al., Molecular epidemiology of Babesia bovis in bovine of Khyber Pakhtunkhwa, Pakistan, 37, 2017, pp. 275–280.
[67] Y. Nawaz, J. Nawaz, M. Nawaz, Epidemiological Study on the Occurrence of Natural Babesiosis in Sheep and Goats of Balochistan, Pakistan, Proceedings of Parasitology, Pakistan), 2006.
[68] N. Rafique, et al., Pervasiveness of Tick Borne Disease, Babesiosis in Quetta City of Province Balochistan, Pakistan, Biological Forum, 2015 (Research Trend).
[69] M.E. Kakar, CLINICO-EPIDEMIOLOGICAL AND THERAPEUTIC STUDY ON BABESIOSIS IN DIFFERENT BREEDS OF CATTLE IN BALOCHISTAN, UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES LAHORE, 2015.
[70] A. Rashid, A. Mubarak, A. Hussain, Babesiosis in equines in Pakistan: a clinical report, Vet. Ital. 45 (3) (2009) 391–395.
[71] C.M. Rothschild, Equine piroplasmosis, J. Equine Vet. Sci. 33 (7) (2013) 497–508.
[72] M. Kumbhar, et al., Prevalence of haemoprotozoan diseases in equines of northern region of sindh, Pakistan: department of veterinary parasitology, sindh agriculture university tandojam, Pakistan, Pakistan J. Agric., Agric. Eng. Veterinary Sci. 36 (1) (2020) 61–67.
[73] K. Javed, et al., Prevalence and hematology of tick borne hemoparasitic diseases in equines in and around Lahore, Pakistan J. Zool. 46 (2) (2014).
[74] M.H. Hussain, et al., Seroprevalence of Babesia caballi and Theileria equi in five draught equine populated metropolises of Punjab, Pakistan, Vet. Parasitol. 202 (3-4) (2014) 248–256.
[75] P.J. Irwin, Canine babesiosis: from molecular taxonomy to control, Parasites Vectors 2 (1) (2009) 1–9.
[76] C. Carret, et al., Babesia canis canis, Babesia canis vogeli, Babesia canis rossi: differentiation of the three subspecies by a restriction fragment length polymorphism analysis on amplified small subunit ribosomal RNA genes, J. Eukaryot. Microbiol. 46 (3) (1999) 298–301.
[77] T.P. Schetters, et al., Different Babesia canis isolates, different diseases, Parasitology 115 (5) (1997) 485–493.
[78] A.J. Birkenheuer, et al., Detection and molecular characterization of a novel large Babesia species in a dog, Vet. Parasitol. 124 (3-4) (2004) 151–160.
[79] L.S. Jacobson, The South African form of severe and complicated canine babesiosis: clinical advances 1994–2004, Vet. Parasitol. 138 (1-2) (2006) 126–139.
[80] R. Lobetti, F. Reyers, J. Nesbit, The comparative role of haemoglobinaemia and hypoxia in the development of canine babesial nephropathy, J. S. Afr. Vet. Assoc. 67 (4) (1996) 188–198.
[81] A.L. Leisewitz, et al., The mixed acid-base disturbances of severe canine babesiosis, J. Vet. Intern. Med. 15 (5) (2001) 445–452.
[82] R.M. Eichenberger, et al., Prognostic markers in acute Babesia canis infections, J. Vet. Intern. Med. 30 (1) (2016) 174–182.
[83] T.P. Schetters, et al., Parasite localization and dissemination in the Babesia-infected host, Ann. Trop. Med. Parasitol. 92 (4) (1998) 513–519.
[84] J. Gadahi, et al., Prevalence of Blood parasites in stray and pet Dogs in Hyderabad Area: comparative sensitivity of different Diagnostic techniqes for the detection of microfilaria, Vet. World 1 (8) (2008) 229.
[85] S. Ahmad, M. Khan, M. Khan, Prevalence of canine babesiosis in Lahore, Pakistan, J. Anim. Plant Sci 17 (2007) 11–13.
[86] S. Ahmad, M. Khan, M. Khan, Epidemilogy and seasonal abundance of canine babesiosis in Lahore, Pakistan, J. Animal Plant Sci. 21 (2) (2011) 351–353.
[87] I. Akram, et al., Short Communication Molecular detection of Babesia microti in dogs and cat blood samples collected from Punjab (Pakistan), Trop. Biomed. 36 (1) (2019) 304–309.
[88] A.S. Ahmad, et al., Molecular occurrence of canine babesiosis in rural dog population in Pakistan, Trop. Biomed. 35 (3) (2018) 593–603.
[89] A.S. Ahmad, et al., Molecular occurrence of canine babesiosis in rural dog population in Pakistan, Trop. Biomed. 35 (3) (2018) 593–603.
[90] S. Rahbari, et al., Clinical, Haematologic and Pathologic Aspects of Experimental Ovine Babesiosis in Iran, 2008.
[91] S. Shah, et al., Tick-borne Diseases-Possible Threat to Humans-Dog Interspecie Bond vol. 5, 2017, pp. 115–120.
[92] M. Tayyub, et al., Efficacy of anti-babesia drugs in relation to hematological parameters in dogs, J. Animal Plant Sci. 30 (5) (2020) 1341–1346.
[93] S. Faryal, et al., Prevalence and molecular detection of babesiosis in the slaughter animals of peshawar khyber pakhunkhawa Pakistan, Int.J.Curr.Microbiol. App.Sci. 4 (8) (2015) 1030–1036.

[94] S. Hussain, et al., Diagnosis of babesia bovis infection in indigenous and crossbred cattlewith comparison between conventional and molecular diagnostic techniques, J. Infect. Mol. Biol. 5 (1) (2017) 1–6.
[95] M.A. Zaman, et al., Climatic Regions Based Molecular Prevalence of Babesiosis and Theileriosis in Cattle and Water-Buffalo in Pakistan, 2022.
[96] S. Ahmad, et al., Prevalence of babesiosis in cats in Lahore, Pakistan, J. Animal Plant Sci. 21 (2 Suppl) (2011) 354–357.
[97] S.H. Farooqi, et al., Molecular epidemiology of babesia bovis in bovine of khyber pakhtunkhwa, Pakistan, Pak. Vet. J. 37 (2017) 275–280.
[98] Sayin, F., et al., Studies on the epidemiology of tropical theileriosis (Theileria annulata infection) in cattle in Central Anatolia, Turkey. 2003. 35(6): p. 521-539.
[99] M. Qayyum, et al., Prevalence, clinicotherapeutic and prophylactic studies on theileriosis in district Sahiwal (Pakistan), J. Animal Plant Sci. 20 (4) (2010) 266–270.
[100] S. Naz, et al., Prevalence of Theileriosis in Small Ruminants in Lahore-Pakistan vol. 2, 2012, pp. 16–20.
[101] F. Jouda, J.-L. Perret, L.J.J. Gern, Ixodes ricinus density, and distribution and prevalence of Borrelia burgdorferi sensu lato infection along an altitudinal gradient, J. Med. Entomol. 41 (2) (2004) 162–169.
[102] Muhammad, G., et al., Tick control strategies in dairy production medicine. 2008. 28(1): p. 43.
[103] F. Beugnet, et al., The ability of an oral formulation of afoxolaner to block the transmission of Babesia canis by Dermacentor reticulatus ticks to dogs, Parasites Vectors 7 (1) (2014) 1–7.
[104] R. Checa, et al., Efficacy, safety and tolerance of imidocarb dipropionate versus atovaquone or buparvaquone plus azithromycin used to treat sick dogs naturally infected with the Babesia microti-like piroplasm, Parasites Vectors 10 (1) (2017) 1–12.
[105] M.-Y. Lin, H.-P. Huang, Use of a doxycycline-enrofloxacin-metronidazole combination with/without diminazene diaceturate to treat naturally occurring canine babesiosis caused by Babesia gibsoni, Acta Vet. Scand. 52 (1) (2010) 1–4.
[106] M. Kalinowski, et al., Changes in selected subpopulations of lymphocytes in dogs infected with Babesia canis treated with imidocarb, Tierärztliche Praxis Ausgabe K: Kleintiere/Heimtiere 43 (2) (2015) 94–100.
[107] G. Baneth, Antiprotozoal treatment of canine babesiosis, Vet. Parasitol. 254 (2018) 58–63.
[108] A.-G. Ramin, The chemotherapeutic effect of'Imidocarb'against ovine babesiosis in Iran, Indian Vet. J. 77 (12) (2000) 1078–1080.
[109] D. Plumb, Plumb's Veterinary Drug Handbook, 8th ed., PharmaVet Inc Wiley, Blackwell, 2015.
[110] C. Cordoves, R. Polanco, Efficacy of Ganasegur (diminazene) in the control of trypanosomiasis and babesiosis, Veterinaria 5 (1983) 133–138.
[111] Y.O. Aliu, S. Ødegaard, Pharmacokinetics of diminazene in sheep, J. Pharmacokinet. Biopharm. 13 (2) (1985) 173–184.
[112] A. Mohamed, I. Yagoub, Outbreaks of babesiosis in domestic livestock in the eastern region of the Sudan, Trop. Anim. Health Prod. 22 (2) (1990) 123–125.
[113] I. Manget, Package of Practices in Veterinary and Animal Husbandry for Livestock and Poultry, Punjab Agriculture University, Ludhiana, 1983.
[114] N. McHardy, et al., Antitheilerial activity of BW720C (buparvaquone): a comparison with parvaquone, Res. Vet. Sci. 39 (1) (1985) 29–33.
[115] A. Chakraborty, Understanding the biology of the Plasmodium falciparum apicoplast; an excellent target for antimalarial drug development, Life Sci. 158 (2016) 104–110.
[116] T. Wang, et al., The apicoplast genomes of two taxonomic units of Babesia from sheep, Vet. Parasitol. 233 (2017) 123–128.
[117] M.F. Di Cicco, et al., Re-emergence of Babesia conradae and effective treatment of infected dogs with atovaquone and azithromycin, Vet. Parasitol. 187 (1-2) (2012) 23–27.
[118] C.J. Bacchi, et al., Prevention by polyamines of the curative effect of amicarbalide and imidocarb for Trypanosoma brucei infections in mice, Biochem. Pharmacol. 30 (8) (1981) 883–886.
[119] N. McHardy, et al., Efficacy, toxicity and metabolism of imidocarb dipropionate in the treatment of Babesia ovis infection in sheep, Res. Vet. Sci. 41 (1) (1986) 14–20.
[120] D. Miller, et al., The pharmacokinetics of diminazene aceturate after intramuscular administration in healthy dogs, J. S. Afr. Vet. Assoc. 76 (3) (2005) 146–150.
[121] W. Leon, R. Brun, S.M. Krassner, Effect of Berenil on growth, mitochondrial DNA and respiration of Leishmania tarentolae promastigotes, J. Protozool. 24 (3) (1977) 444–448.
[122] A.J. Bitonti, J.A. Dumont, P.P. McCANN, Characterization of Trypanosoma brucei brucei S-adenosyl-L-methionine decarboxylase and its inhibition by Berenil, pentamidine and methylglyoxal bis (guanylhydrazone), Biochem. J. 237 (3) (1986) 685–689.
[123] N. Sen, H.K. Majumder, Mitochondrion of protozoan parasite emerges as potent therapeutic target: exciting drugs are on the horizon, Curr. Pharmaceut. Des. 14 (9) (2008) 839–846.
[124] A. Matsuu, et al., In vitro evaluation of the growth inhibitory activities of 15 drugs against Babesia gibsoni (Aomori strain), Vet. Parasitol. 157 (1-2) (2008) 1–8.
[125] J.E. Siregar, et al., Mutation underlying resistance of Plasmodium berghei to atovaquone in the quinone binding domain 2 (Qo2) of the cytochrome b gene, Parasitol. Int. 57 (2) (2008) 229–232.
[126] G. Guan, et al., Babesia sp. BQ1 (Lintan): molecular evidence of experimental transmission to sheep by Haemaphysalis qinghaiensis and Haemaphysalis longicornis, Parasitol. Int. 59 (2) (2010) 265–267.
[127] A. Iguchi, et al., Development of in vitro atovaquone-resistant Babesia gibsoni with a single-nucleotide polymorphism in cytb, Vet. Parasitol. 185 (2-4) (2012) 145–150.
[128] M. Sakuma, A. Setoguchi, Y. Endo, Possible emergence of drug-resistant variants of Babesia gibsoni in clinical cases treated with atovaquone and azithromycin, J. Vet. Intern. Med. 23 (3) (2009) 493–498.
[129] L. Cohn, et al., Efficacy of atovaquone and azithromycin or imidocarb dipropionate in cats with acute cytauxzoonosis, J. Vet. Intern. Med. 25 (1) (2011) 55–60.
[130] K. Değerli, et al., Efficacy of azithromycin in a murine toxoplasmosis model, employing a Toxoplasma gondii strain from Turkey, Acta Trop. 88 (1) (2003) 45–50.
[131] E.L. Dahl, P.J. Rosenthal, Multiple antibiotics exert delayed effects against the Plasmodium falciparum apicoplast, Antimicrob. Agents Chemother. 51 (10) (2007) 3485–3490.
[132] E. Mathias, Ethnoveterinary Medicine in the Era of Evidence-Based Medicine: Mumbo-Jumbo, or a Valuable Resource, 2007.
[133] P. Masika, A. Afolayan, An ethnobotanical study of plants used for the treatment of livestock diseases in the Eastern Cape Province, South Africa, Pharmaceut. Biol. 41 (1) (2003) 16–21.
[134] P.R. Arba, L. Paloque, H. Belda, Antiprotozoal properties of Indonesian medicinal plant, Med. Sci. 76 (2007) 869–875.
[135] V. Naidoo, et al., Identification of anti-babesial activity for four ethnoveterinary plants in vitro, Vet. Parasitol. 130 (1-2) (2005) 9–13.