

Original papers

A four year epidemiological and chemotherapy survey of babesiosis and theileriosis, and tick vectors in sheep, cattle and goats in Dehgolan, Iran

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ABSTRACT. *Babesia* and *Theileria* are two protozoa belonging to the phylum Apicomplexa, which result in babesiosis and theileriosis in different hosts, cause considerable problems in domestic animals and as a consequence economic losses. These two diseases are transmitted by ticks. This survey was carried out due to the lack of studies and information regarding tick vectors and *Theileria* and *Babesia* species in the Dehgolan area of Iran, which is a center of agriculture and animal holding industry. This study was conducted during a four-year period, between 2012 and 2016. Infection with *Theileria* and *Babesia* was observed throughout the year while in contrast to cattle and sheep, infection with *Babesia* in goats was not observed between December and February. Infection with *Babesia* and *Theileria* reached the highest peak in July and this was more considerable for *Theileria* in cattle and sheep with a prevalence of 37–47% and 46–79%, respectively. The infection rate in goats for both diseases was 20%. The results in this study showed that Imidocarb Dipropionate and Buparvaquone are effective treatments for *Babesia* and *Theileria*, respectively. In this survey, the ticks of *Rhipicephalus* spp. and *Hyalomma anatolicum* had the highest infection rate with *Babesia* and *Theileria*, respectively. Considering the significant relationship between the prevalence of *Theileria* and *Babesia* with tick infection in this study and other studies, more studies on climate changes and tick vector prevalence are necessary.

Key words: Apicomplexa, Ixodidae, *Babesia*, *Theileria*, epidemiology

Introduction

Theileria and *Babesia* species are important tick-borne haemoprotozoan parasites in domestic animals, especially in tropical and sub-tropical regions [1]. Theileriosis and babesiosis are a cause of clinical disease in Iranian domestic ruminants with resultant economic losses through decreasing weight, milk production and increased mortality [2]. Ixodidae ticks are vectors in the transmission of these diseases and are distributed across Iran. *Babesia ovis*, *B. motasi* and *B. crassa* in sheep and goats, and *B. bigemina* and *B. bovis* in cattle have all been reported in Iran. *B. ovis*, *B. motasi* in sheep, and *B. bigemina* and *B. bovis* in cattle are known to be pathogenic agents, with *Babesia ovis* being the most common species that causes sheep babesiosis in Iran [3,4]. Studies previously conducted show a

high prevalence of babesiosis in Iran, with high mortality rates occurring in the warm season, particularly late spring [5]. Iran is also an endemic region for *Theileria ovis*, *T. lestoquardi* and *T. annulata* infection in sheep and cattle. *Theileria annulata* which is a causal agent of malignant theileriosis in cattle, can also infect sheep, and has also been reported in sheep from Iran [6,7]. *T. lestoquardi* and *T. ovis* are the main cause of theileriosis in Iran [5] with *T. lestoquardi* causing high mortality in sheep and goats in the East and South East of the country [8]. Knowledge of prevalence of parasitic diseases in the country is very important to establish the risk of potential future economic losses and the need for effective treatment and control programs. A limited number of epidemiological studies have been done in relation to these diseases in Iran.

The aim of the present study was to gather information on the infection rate and epidemiology of babesiosis and theileriosis in Iran, as well as showing efficacy of Buparvaquone and Imidocarb Dipropionate in the treatment of theileriosis and babesiosis over a four year period.

Materials and Methods

Field study area. The study was done in the Dehgolan area, situated in the west of Iran including more than 100 villages. The climate is temperate with moderate summers and cold winters. The warmest and driest month is July with 1 ml average precipitation and 23.9°C average temperature. April has the highest precipitation with 89 ml. The coldest month is January with an average temperature of -1.5°C. It has conditions suitable for agriculture and husbandry. Flock raising in the province is very important economically. Herds are generally composed of approximately 12000 cattle and more than 130000 sheep and goats in Dehgolan.

Collection of blood samples. In total, 1625 sheep, 510 goats and 921 cattle were randomly selected from different farms in villages of the Dehgolan area by the local veterinary service of Dehgolan and the Damyaran Lab. The study was conducted between August 2012 and September 2016. First, the flocks were clinically examined for relevant clinical signs such as anemia and icterus, and blood smears were prepared from capillary veins of the ear to maximise sensitivity. The blood samples were drawn by syringe from jugular veins and collected in EDTA tubes. Finally, the bodies of the animals were inspected and attached ticks were collected into labelled specimen tubes for further identification and examination. The blood and tick specimens were kept cool and transferred to the Damyaran Laboratory and Veterinary Network Office in Dehgolan.

Examination of blood smears. The blood smears were air-dried, fixed in methanol and stained in 10% Giemsa solution diluted in PBS pH 7.2. The stained slides were examined with an oil immersion lens at a total magnification of 1000×. To identify *Babesia* species, the full length of intraerythrocytic mature *Babesia* organisms were measured using a graded ocular lens at a magnification of 1000×. 100 different microscopic fields were carefully examined per animal for estimating parasitemia, and the percentage of infected erythrocytes determined. A total, 3107 ticks were collected and

speciation was conducted using the identification-keys of Hoogstraal [9,10] and Mazlum [11]. Haemolymph and egg smears were prepared to search for kinetes of *Babesia* spp. and *Theileria* spp. The engorged female ticks were kept for oviposition by incubation at 28°C and 80% humidity. Egg masses were collected and respective smears prepared, dried, fixed with methanol and stained by the Giemsa method. Haemolymph was collected from each surviving tick by removing the distal portion of the first leg and smears were stained by the Giemsa method. All smears were microscopically examined under oil immersion at 1000× magnification. The length and width of 120 kinetes were measured, and shape and position of the kinete nucleus were recorded. The collected ticks were dissected and the salivary gland removed. Each salivary gland was stained by the Feulgen technique [12] and examined for the presence of infected acini.

Chemotherapy study. A total of 439 cattle, 657 sheep and 194 goats positive for babesiosis and theileriosis were selected for treatment. After two weeks, blood smears were prepared, as described above, to show the effect of drug treatment. In this study, the hosts were treated using available drugs Butalex (Buparvaquone) 1ml/20 kg of body weight for theileriosis and Imizol (Imidocarb Dipropionate) 1ml/100 kg of body weight for babesiosis.

Results and Discussion

The prevalence of *Babesia* spp. and *Theileria* spp. infections in sheep, cattle and goats is summarized in Table 1. *Babesia* spp. were identified in sheep and cattle throughout the year. *Babesia* spp. were absent from goats in this area from December through February but infection was present throughout the rest of the year. *Theileria* spp. were present in cattle, sheep and goats throughout the year. In sheep, cattle and goats, the prevalence of infection with *Babesia* spp. and *Theileria* spp. increased from January, peaking in July, before decreasing again through the rest of the year. The prevalence peaked for *Theileria* spp. at 79% in sheep, 47% in cattle and 20% in goats. *Babesia* spp. prevalence was equal to or lower than *Theileria* spp. throughout the year peaking at 46% in sheep, 37% in cattle and 20% in goats. *B. ovis* was the most prevalent *Babesia* spp. in sheep and goats, while *B. bigemina* was the most prevalent *Babesia* spp. in cattle.

Table 1. Seasonal prevalence of *Babesia* spp. and *Theileria* spp. infection, sorted by month and host in the Dehgolan area over four years

Months in four year	No. Positive (%) sheep		No. Positive (%) cattle		No. Positive (%) goat		Total
	<i>Babesia</i> spp.	<i>T. lestoquardi</i>	<i>Babesia</i> spp.	<i>T. annulata</i>	<i>Babesia</i> spp.	<i>T. lestoquardi</i>	
January	4	8	3	4	0	2	2
February	5	11	3	1	0	1	1
March	7	19	9	5	4	2	6
April	16	42	13	15	11	9	20
May	25	50	18	30	18	19	37
June	38	58	31	39	17	18	35
July	46	79	37	47	28	29	40
August	34	52	26	37	17	17	34
September	22	40	17	28	3	3	6
October	14	29	13	23	6	3	9
November	12	23	8	16	1	2	3
December	7	18	6	10	0	1	1
Total	230/1625 (14.15%)	427/1625 (26.27%)	184/921 (19.97%)	255/921 (27.68%)	97/510 (19.01%)	97/510 (19.01%)	194/510 (38.03%)
Treatment with Butalex		418/427 (97.89%)		237/255 (92.94%)		91/97 (93.81%)	
Treatment with Imizol	214/230 (93.04%)		167/184 (90.76%)		93/97 (95.87%)		

The prevalence of *Babesia* spp. and *Theileria* spp. in sheep cattle and goats treated for infection is also summarized in Table 1. *Theileria* spp. infection was absent from sheep, cattle and goats treated with Buparvaquone. There was no significant difference between *Babesia* spp. prevalence in sheep cattle and goats treated with Buparvaquone compared with untreated animals. *Babesia* spp. were absent from sheep cattle and goats treated with Imidocarb Dipropionate. There was no significant difference between cattle, sheep and goats treated with Imidocarb Dipropionate compared with those that were not treated.

The prevalence of *Babesia* spp. and *Theileria* spp. present in tick species in Dehgolan province is summarized in Table 2. *Babesia* spp. were found in all species of tick examined with the exception of *Dermacentor marginatus*. *Theileria* spp. were present in all species of tick examined with the exception of *D. marginatus* and *Haemaphysalis punctata*. *Rhipicephalus* spp. had the highest prevalence of *Babesia* spp. infection (*R. bursa* 63.95%, *R. sanguineus* 59.89%, *R. annulatus* 66.43%), significantly higher than other genera of ticks examined ($p < 0.01$). *Hyalomma anatolicum* had the highest prevalence of *Theileria* spp. infection (55.54%), significantly higher than the other tick species examined ($p < 0.05$).

This study demonstrated a high prevalence of *Theileria* spp. and *Babesia* spp. in the West of Iran, consistent with studies carried out in the East of the country [5,8]. Seasonal variation in infection with both *Theileria* and *Babesia* spp. was demonstrated in sheep, goats and cattle. Although this has been demonstrated in other parts of Iran, the peak of

infection occurring in July is slightly later than those recorded in the East of the country [5]. This is likely to be an effect of climate on the feeding activity of the tick vectors. A seasonal variation in the prevalence of clinical babesiosis has been observed in other countries, with the greatest incidence occurring soon after the peak of the tick population [13]. Air temperature and humidity are important factors influencing feeding activity [14]. Similarly, seasonal outbreaks of theileriosis have been demonstrated to be due to rainfall allowing feeding of tick vectors [15]. The absence of *Babesia* spp. between December and February may also be due to vector activity and climate but may not be a consistent finding over subsequent years and cannot be assumed to absent at this time in any given year. Knowledge of when peak transmission is occurring however, in this area of Iran is vital in helping farmers to assess when to maximise benefits from tick control and chemoprophylaxis in their animals.

The examination of tick salivary gland and uterine infections revealed *Hyalomma* spp. to have the highest prevalence of *Theileria* spp. and *Rhipicephalus* spp. to have the highest prevalence of *Babesia* spp. but both *Babesia* spp. and *Theileria* spp. were present in significant numbers in both genera of tick. While *Dermacentor marginatus* and *Haemaphysalis punctata* do not seem to be significant reservoirs of *Theileria* spp. infection, *H. punctata* was demonstrated to be a significant reservoir of *Babesia* spp. infection. Control of these ticks is therefore desirable to limit exposure of livestock to parasitic infection at times of peak tick activity. This can be achieved through application of acaricides through dipping or pour on application.

Table 2. Prevalence of tick infection with *Theileria* spp. and *Babesia* spp. in Dehgolan area, Iran

Species of tick	Number of males	Number of females	Total	No. infected with <i>Babesia</i> spp. (%)	No. infected with <i>Theileria</i> spp. (%)
<i>Hyalomma anatolicum</i>	203	573	776	221 (28.47%)	431 (55.54%)
<i>Hyalomma asiaticum</i>	102	178	280	18 (6.42%)	101 (36.07%)
<i>Hyalomma excavatum</i>	93	151	244	31 (12.70%)	78 (31.96%)
<i>Hyalomma detritum</i>	75	101	176	25 (14.20%)	41 (23.29%)
<i>Rhipicephalus bursa</i>	211	391	602	385 (63.95%)	113 (18.77%)
<i>Rhipicephalus sanguineus</i>	189	357	546	327 (59.89%)	98 (17.94%)
<i>Rhipicephalus annulatus</i>	125	301	426	283 (66.43%)	77 (18.07%)
<i>Dermacentor marginatus</i>	10	11	21	0 (0%)	0 (0%)
<i>Haemaphysalis punctata</i>	15	21	36	13 (36.11%)	0 (0%)

These techniques while effective can be expensive, labour intensive and lead to resistance. Chemotherapy therefore, remains an important component of tick-borne disease control.

Imidocarb has been the primary treatment used for babesiosis in cattle for the past 20 years and has been demonstrated to be effective in clearing the parasite from carrier animals [16]. It has also been demonstrated to be effective in the treatment of babesiosis in sheep and goats when combined with a tetracycline [17]. This study demonstrated that it is effective when used alone in clearing the parasite from cattle, sheep and goats and should remain the first line choice in treating this parasite in ruminants. It has been suggested that treatment with buparvaquone does not eliminate *T. parva* infection and can lead to a carrier state in the host. While previous studies would suggest this is a possibility, this study demonstrated high efficacy in clearing *Theileria* spp. from infected animals.

Tick-borne diseases in livestock remain a major economic burden and welfare concern. *Theileria* and *Babesia* spp. are highly prevalent in Iran and capable of causing significant disease and economic loss. Understanding their distribution in the country and seasonal occurrence is vital in advising farmers when chemoprophylaxis and tick preventative measures are to be employed. Imidocarb and Buparvaquone are highly efficacious treatments for *Babesia* spp. and *Theileria* spp. infections respectively, but their use may be cost prohibitive and therefore, timing prophylaxis around times of peak transmission will help to maximize impact of chemoprophylaxis while minimizing costs. Climate change and shifting distributions of tick-borne diseases and their vectors means that continuing studies of seasonal patterns and distributions of infection are vital so that control programs can be adjusted appropriately, therefore limiting adverse economic and welfare impacts.

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