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Transmission of *Babesia* sp to sheep with field-collected *Haemaphysalis qinghaiensis*

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Abstract *Haemaphysalis qinghaiensis* ticks collected in the Gannan Tibet Autonomous Region were infested onto a sheep from a *Babesia*-free area. A strain of small *Babesia* (1.8–2.1 μm in length) was isolated from the sheep. Most of the *Babesia* in erythrocytes were round, oval, single pyriform, double pyriform, budding or elongated in form. Measurements were made of 100 single sides of the double-pyriform *Babesia* and compared with those for *B. motasi* and *B. ovis* from Holland, using Student's *t*-test. The Gannan small *Babesia* was similar to the *B. ovis* from Holland, but differed significantly from the Dutch *B. motasi*.

Introduction

Since *Babesia ovis* was first reported by Babes as the causative agent of ovine babesiosis in 1892, other *Babesia* species have been found to occur in sheep, like *B. crassa*, *B. foliata*, *B. motasi*, *B. ovis* and *B. taylori*, etc. (Levine 1985). Ovine babesiosis occurs over a wide geographic area, ranging from China in the east to Algeria in the west (Lewis et al. 1981). In China, ovine babesiosis has been reported in Sichuan and Heilongjiang Provinces (Chen 1982; Zhao et al. 1986) and in the eastern Gansu Province (Lian et al. 1997; Yin et al. 1996, 1997). The latter authors isolated and identified two strains as *B. motasi* and *B. ovis*. In the present study, we analysed the morphology, pathogenicity and transmission of *Babesia* sp by *Haemophysalis qinghaiensis*.

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Materials and methods

Animals

Sheep (5–7 months old) and goats (1.0–1.5 years old) were purchased from a *Babesia*-free area. They were splenectomized 6 months before experiments. Microscopic examination of blood films for *Babesia* showed them to be negative.

Ticks

Haemaphysalis qinghaiensis adults were collected in the field in Lintan County, Gannan Tibet Autonomous Region, in April 2000.

Transmission test

A total of 45 male and 15 female *H. qinghaiensis* collected in the field in the Gannan Tibet Autonomous Region were fed on an intact sheep (no. 2002). Every day after tick infestation, the rectal temperature was taken and blood smears were examined microscopically for the presence of haemoprotozoa.

Infection of animals

Aliquots of 15 ml of blood containing *Babesia* sp. (preserved in liquid nitrogen) were inoculated into splenectomized goats (nos. 9945, 9946). After inoculation, splenectomized goat 9946 was injected with dexamethasone at a dosage of 2 mg/day, for 3 days.

Morphological observation and morphometric analysis

When parasitaemia began to increase, blood smears from the ear vein were prepared, fixed in methanol, stained with Giemsa and examined microscopically. Measurements were made of 100 single sides of double-pyriform specimens of the Gannan *Babesia* sp., as illustrated by Uilenberg et al. (1980) and compared with *B. motasi* and *B. ovis* originating from Holland. The mean length of each strain was calculated and an analysis of variance was performed on the data, using Student's *t*-test.

Observation of pathogenicity

Every day after inoculation, the rectal temperature of each animal was taken. The erythrocyte level and haemoglobin level were also measured daily, using blood collected from the ear vein. Clinical symptoms were also observed daily.

Results

Transmission test

At 22 days after tick infestation, double-pyriform parasites were seen in the blood smears. When *Babesia* parasitaemia reached 10 parasites/100 viewing fields, blood was collected from sheep 2002 with anti-clotting agents and inoculated intravenously into an intact sheep (no. 2013). Two days after inoculation, parasites of *Babesia* sp. were seen in the blood smears. When parasitaemia reached 12 parasites/100 viewing fields, blood was collected with anti- clotting agents and preserved in liquid nitrogen.

Infection of animals

After blood containing *Babesia* sp. was inoculated into splenectomized goats 9945 and 9946, blood smears showed *Babesia* sp. on day 10 post-infestation and on day 8 post-inoculation, respectively. The parasitaemia of goat 9945 rose slowly, reached 1 parasite/3 viewing fields on day 14 post-inoculation and later reduced slowly. On day 19 post-inoculation, the parasitaemia was 0 parasites/100 viewing fields. The parasitaemia of goat 9946 increased rapidly and reached 1.5% on day 11 post-inoculation, when the goat was injected with Dexamethasone. On day 15 post-inoculation, parasitaemia reached 85% in goat 9946.

Morphological observation

The parasite described here was a small *Babesia* sp. During the early days of infestation, the most common forms were round, oval and single pyriform. Double pyriform, budding and elongated forms increased as parasitaemia increased. Various forms were described, as follows.

Double pyriform

The piroplasms measured 1.8–2.1 μm × 0.9–1.7 μm. The narrow end of each piroplasm linked them together. The angle between them was generally obtuse. The piroplasms was almost round, because the commonest form was wide. Either the nucleus was located in the narrow end of the piroplasm or there were nuclei at each end. The ratio was about 24%.

Single pyriform

The size of the piroplasms was 1.9–3.0 μm × 0.9–2.1 μm; and they were narrow and long. Most piroplasms had a two-lobed nucleus. The ratio was about 25%.

Round and oval

This form was thick and stained blue at the margin; and the central part was transparent. The piroplasms were 2.0–3.2 μm × 1.0–1.8 μm. The ratio was 27%.

Budding form

The central part is occupied by the nucleus. Two major leaves and a branching leaf extended in three orientations. This form was characteristic of *Babesia* sp. The ratio was 7%.

Elongated

When the parasitaemia was high, many elongated parasites appeared, shaped like an amoeba. The ratio was 17%.

Morphometric analysis

Morphometric analysis revealed significant differences in the long axis and short axis between double merozoites of *B. motasi* from Holland and *Babesia* sp. from Gannan, but *B. ovis* from Holland and *Babesia* sp. were similar.

Pathogenicity

The results concerning the pathogenicity of this parasite are presented in Table 1. It is evident that this strain was almost non-pathogenic for small ruminants. No tested animals showed the typical symptoms of babesiosis, except for one goat which was injected with Dexamethasone.

Discussion

According to data in the literature, the known vectors of *Babesia ovis* are *Dermacentor marginatus*, *Haemaphysalis punctata*, *Hyalomma anatolicum excavatum*, *Rhipicephalus bursa*, *R. turanicus*, etc. (Friedhoff 1997). However, none of these vector ticks of *B. ovis* has yet been identified in Gannan, China. In contrast, there are several reports indicating that the main ticks species are *D. silvarum, Haemaphysalis longicornis* and *H. qinghaiensis*, etc. in regions where ovine babesiosis occurs. It is clear from the results of the present study that *H. qinghaiensis* is the vector species of *Babesoa* sp in the Gannan Tibet Autonomous Region, China.

The morphological observations on *Babesia* sp from Gannan were in agreement with *B. ovis* described by Lian et al. (1997) and Yin et al. (1997) for strains isolated in eastern Gansu, China. The size agreed with the

Table 1 Reactions of intact sheep and splenectomized goats to infection with *Babesia* sp. (Gannan strain). Animals 2002 and 2013 were intact sheep, animals 9945 and 9946 were splenectomized goats. *Hb* Haemoglobin, *RBC* red blood cells

Animal no.	Maximum parasitaemia (%)	Prepatent period (Days)	Body temperature (°C)	Haematological counts			
				RBC		Hb	
				($\times 10^{-4}$/l)		(mg/l)	
				Low	High	Low	High
2002	10	1	40.0	1032	1082	800	1020
2013	12	1	40.2	950	1115	820	920
9945	33	2	40.2	–	–	–	–
9946	85	5	41.2	382	701	410	720

ranges of *B. ovis* given by Ristic and Kreier (1981; 1.0–2.5 μm) and Papadopoulos et al. (1996; 1.4–2.2 μm), but differed a little from the size given by Habela et al. (1990; 1.12 μm × 0.23 μm). Budding-form parasites similar to *B. motasi* (Lewis et al. 1981; Alani and Herbert 1988) and *B. ovis* (Lian et al. 1997; Yin et al. 1997) were observed. No cross forms were found, as described by Yeruham et al. (1998). Although four pyriform parasites were seen in blood smears, those were more similar to two pairs of splitting double pyriforms. Using Student's *t*-test, morphometric analysis of double pyriforms (length 1.8–2.1 μm, width 0.9–1.7 μm) in smears showed they were similar to *B. ovis* from Holland, but significantly different from *B. motasi* from Holland. However, the identity of the *Babesia* sp described here needs to be further studied as very little information has been known about it.

B. ovis is considered to be highly pathogenic (Ristic and Kreier 1981; Ristic 1988; Friedhoff 1997). The mortality rates in susceptible sheep ranged from 30% to 50% after experimental infection or field infection (Friedhoff 1997). The results of the laboratory experiment demonstrated that *B. ovis* from Ganan, like that isolated from Somalia (Edelstein 1975), is not pathogenic for intact sheep and goats. Nevertheless, it is possible that strains from different regions differ in their virulence. In the Gannan Tibet Autonomous Region, ovine babesiosis never occurs naturally in sheep and goats; and *Babesia* spp have not been detected by local veterinarians in small ruminants yet. This confirms that the strain of *B. ovis* described here is non-pathogenic for sheep and goats.

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