BABESIA CRASSA N.SP. (SPOROZOA, BABESIIDAE) OF DOMESTIC SHEEP IN IRAN (*)

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SUMMARY

A large Babesia sp., isolated from a sheep in Iran, proved to be serologically and morphologically different from B. motasi and B. ovis.

The parasite, designated B. crassa n. sp., is characterized by the frequent occurrence of four organisms in one erythtocyte, which is the result of quadruple division and, in other cases, of two successive binary divisions. Parasites resulting from the first of two successive binary divisions are exceptionally broad. B. crassa appears to be of low pathogenicity for sheep and goats, to which it is also infective.

The vector is unknown.

INTRODUCTION

Two species of *Babesia* are generally recognized in domestic sheep, *Babesia motasi* Wenyon, 1926, large in size, and *B. ovis* Starcovici, 1893, small and frequently in a marginal position in the red cell. In a recent review of the *Babesiae* of small ruminants Uilenberg *et al.* (1980) mention the occurrence of a large *Babesia* species of sheep in Iran, discovered by one of us (R.H.-F.), which differs from *B. motasi* by the fact that quadruple division is common. In this paper we report on further studies on this parasite, at first designated *Babesia* sp. (Iran).

MATERIAL AND METHODS

ORIGIN OF BABESIA SPP

Babesia sp. (Iran). Blood smears of one lamb out of sixty, which were splenectomized during studies on haemoparasites of sheep in Iran, revealed a *Babesia* species differing morphologically from known species in sheep.

Eperythrozoon ovis Neitz, Alexander, and Du Toit, 1934, was also found in the blood of this animal. Its blood was subinoculated into other splenectomized

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lambs and cryopreserved at – 70°C, for further study.
B. motasi (Turkey) originates from Turkey and was received from Prof. E.Schein in Berlin. The strain has been described by Uilenberg et al. (6).
B. motasi (Netherlands) was isolated on Dutch islands in the North Sea and described by Uilenberg et al. (6).
B. ovis (Iran) was isolated in Iran and maintained at the Razi Institute.
B. ovis (Turkey) was received from Prof. K. T. Friedhoff in Hanover. It was originally isolated in Turkey (2).

EXPERIMENTAL ANIMALS

All lambs used in Iran were of local breed, aged from three to five months. Five intact and 18 splenectomized animals were used to study the parasite. They were housed in a tick-free stable and regularly sprayed with acaricide. In addition two adult splenectomized sheep and one adult splenectomized goat, born and bred in the Netherlands, were used at Utrecht.
No haemoprotozoa were found in the animals following splenectomy. Monitoring consisted of taking the rectal temperature daily, making blood smears daily, and preparing serum once a week. Blood smears were fixed in methanol and stained according to Giemsa, while sera were examined by the indirect fluorescent antibody test (IFAT).

SEROLOGICAL TEST

The IFAT was performed as described by Brocklesby *al.* (1), with minor modifications. Antigens were prepared of *Babesia* sp. (Iran), *B, motasi* (Turkey), *B, motasi* (Netherlands) and *B. ovis* (Turkey). They were tested against sera of animals infected with one of these four strains. Serum dilutions were twofold.

MORPHOLOGICAL STUDY

Pear or oval forms of *Babesia* sp. (Iran) which had just finished either quadruple or binary division were measured with a calibrated ocular micrometer.

CRYOPRESERVATION

Parasites in Iran were frozen and kept at – 70°C, according to the method described by Hashemi-Fesharki and Shad-Del (3). Stabilates with glycerol as cryoprotectant were also prepares from infected blood at Utrecht and kept in liquid nitrogen.

TRANSMISSION

Subcutaneous or intravenous injection of fresh or cryopreserved blood was carried out in Iran. 25 To 30 ml of blood with a parasitaemia of 3 to 4% was used per animal. In Utrecht inoculation was subcutaneous; one sheep received 17 ml of cryopreserved blood from an Iranian lamb with a parasitaemia of over 10%, the second sheep and the goat were inoculated with 2 ml of cryopreserved blood from the first sheep, with a parasitaemia of approximately 5%.

EXPERIMENTS AND RESULTS

BEHAVIOUR IN SHEEP

No parasites could be detected in the blood of 3 intact lambs in Iran, inoculated subcutaneously, and their temperatures remained normal. Only one of 2 intact lambs inoculated intravenously showed very rare parasites, but no hyperthermia, on days 8 and 9 after infection (p.i.).

All splenectomized animals showed a thermal reaction and became parasitaemic. The incubation periods, both to hyperthermia and parasitaemia, varied in the 18 animals injected subcutaneously from 3 to 6 days, while they were only 1 to 2 days in the two lambs inoculated intravenously. Peak temperatures ranged from 40.2 to 41.5°C, peak parasitaemias from 5 to 14%. All animals recovered, and even in both adult Dutch sheep there were no marked symptoms of disease other than an increased rate of respiration.
The first Dutch sheep injected with blood sent from Iran also showed *Eperythrozoon ovis,* after the *Babesia* sp. had disappeared. *E. ovis* has so far not been reported to occur in the Netherlands, so that it is likely to be of Iranian origin.

BEHAVIOUR IN A GOAT

The only (splenectomized) goat used had no hyperthermia after injection, but showed a low parasitaemia, with a prepatent period of 15 days and a peak of less than 0.01% on day 18 p.i.

MORPHOLOGY

Babesia sp. (Iran) is a large species, which multiplies by binary and quadruple division. Many erythrocytes contain four parasites (exceptionally more, Fig. 8); this number is attained in two different ways:
(a) by quadruple division (Figs. 1, 2, 3, 6, 7),

(b) by two successive binary divisions. After the first, the parasites are generally not pear-shaped but ovoid or even round (Figs. 2, 3, 4), probably preparing for the next division. Disintegration of the host cell occasionally occurs at this stage (Fig. 6), but there is usually a second binary division (Figs, 5, 6), resulting in four pear-shaped parasites in the celle (Fig. 6).

The length of mature parasites just after completed division is not significantly different from that of *B. motasi* (Table 1). The principal differences from this species are that quadruple division is common, that two successive binary divisions frequently occur in one erythrocyte, and that the parasites resulting from the first binary division are exceptionally broad (1.97 + 0.30 μm wide, the length being 2.52 + 0.43 μm). *B. ovis* is markedly smaller, frequently situated near the periphery of the red cell, and like *B. motasi.* normally does not give rise to more than two parasites in one host-cell.

SEROLOGICAL COMPARISON

Table 2 shows the results of the IFAT with antigens of four different parasites and positive sera against each of these antigens. Figure 9 shows the results of the IFAT with the four antigens on sequential sera of a sheep infected with *Babesia* sp. (Iran) is clearly different in the IFAT from *B. motasi* (Netherlands), *B. motasi* (Turkey), and *B. ovis,* although there is some unilateral cross-reaction between *Babesia* sp. (Iran) and *B. motasi* (Turkey). Serological differences between the Dutch and the Turkish strains of *B. motasi,* reported by Uilenberg *et al.* (6), were once more confirmed.

VARIOUS OBSERVATIONS

Experiments in splenectomized and intact lambs have shown that there is no cross-protection between *Babesia* sp. (Iran) and *B. ovis* (Iran).
Cryopreservation, both in Iran and in Utrecht, was succesful. Stabilates prepared and maintained in Iran proved to be infective after four years.

DISCUSSION AND CONCLUSIONS

Babesia sp. (Iran) is a different species from *B. motasi* and *B. ovis* on serological as well as morphological grounds. *B. foliata* Ray and Raghavachari, 1941, in sheep in India appears from its description to be rather similar to *B. motasi,* so that its validity remains in doubt (6). Sarwar (5) described a parasite of a goat in India as a new species, *Piroplasma taylori.* Its indentity is difficult to determine. Smears were made after death and the morphology of the organisms, as depicted, clearly shows post-mortem alterations. There are features in the descriptions that recall certain features of *Babesia* sp. (Iran), such as the occurrence of more than two parasites in a cell

not being uncommon. However, other features differ, such as its being reported much smaller than *B. motasi,* the abundance of extracellular parasites, the frequent occurrence of 8 or 16 organisms in one red cell, and the fact that pear-shaped forms are reported to be rare. While the possibility that Sarwar was dealing with a species similar to *Babesia* sp. (Iran), possibly in a mixed infection with a *Theileria* sp., cannot be completely ruled out, confirmation will be impossible to obtain as the description was based only on postmortem smears of a single animal. Uilenberg *et al.* (6) propose to consider *P. taylori* as a *nomen nudum,* an opinion with which we agree.

Because of its swollen shape after first binary fission, we propose the name of *Babesia crassa* n.sp., the thick *Babesia, for Babesia* sp. (Iran). *B. crassa* may be defined as follows: A large *Babesia* isolated from a domestic sheep in Iran, infective to sheep and goats, characterized by the frequent occurence of four organisms, with an average length of approximately 2.2 μm, in one red cell, the number of four being arrived at either by quadruple division or by two successive binary divisions. In the latter case, the parasites resulting from the first binary division have an average length of about 2.5 μm and are exceptionally broad. *B. crassa* appears to be non-pathogenic to intact sheep and not fatal to splenectomized sheep; it was also non-pathogenic to the single splenectomized goat used. Its virulence did not change during six mechanical passages in sheep. However, its true pathogenicity can only be assessed when it is transmitted by ticks, as it is known that mechanically passaged *Babesia* spp. often lose virulence. Transmission experiments are under way to try and identify the tick vector. Dr. H. Hoogstraal has suggested (correspondence) that the natureal hosts of *B. crassa* may well be wild sheep or goats, which frequently graze in the proximity of livestock in Iran.

Among tick species found on wild sheep and goats in Iran the genera *Haemaphysalis, Hyalomma,* and *Rhipicephalus* are especially common, while ticks of the genera *Ornithodoros, Ixodes,* and *Dermacentor* also occur (4).

Table 1. Comparative measurements of the length of mature parasites following division (average with standard deviation).

Parasite	Two parasites in cell	Four parasites in cell
Babesia sp. (Iran)	2.52 ± 0.43/ um	2.20 + 0.21/ um
B. motasi (Netherlands)	2.41 ± 0.20/ um (+)	-

(+) Results of earlier measurements of both Dutch and Turkish *B. motasi* varies from 2.26 + 0.18 μm (Uilenberg *et al.*, 1980).

Table 2. Reciprocal IFA titers to four antigens of sera of sheep infected with different parasites.

Serum of sheep no.	Infected with:	Antigen *Babesia* (Iran)	*B. motasi* (T.)	*B.motasi* (N.)	*B.ovis*
8022	*Babesia* (Iran)	*1280*	320	< 40	< 40
7819	*B.motasi* (T.)	40	*1280*	< 40	< 40
7808	*B.motasi* (N.)	40	40	*320*	< 40
7910	*B. ovis*	< 40	n.d.	n.d.	*640*

n.d. = not determined
T. = Turkey
N. = Netherlands
Underlined titers refer to homologous antigen and serum.

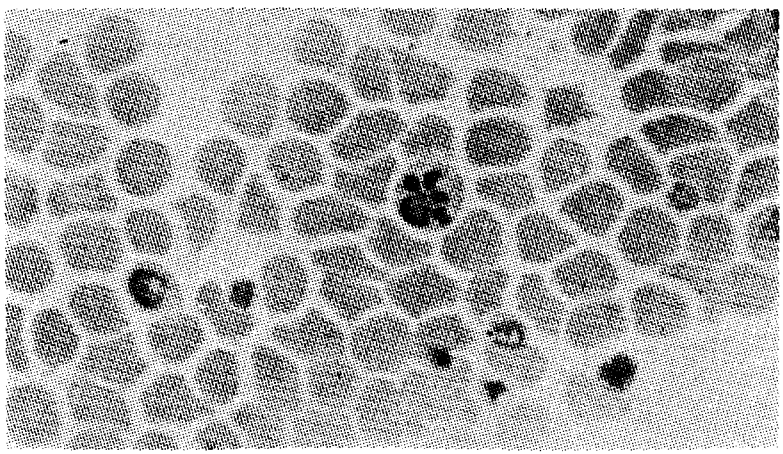


Fig. 1.

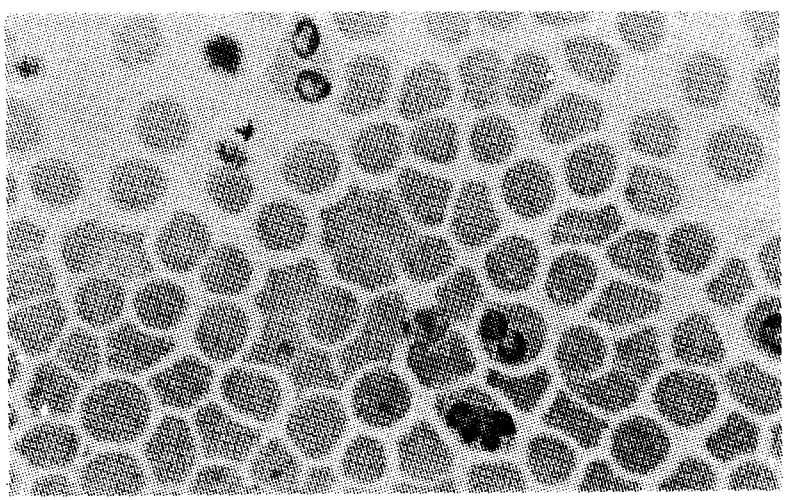


Fig. 2.

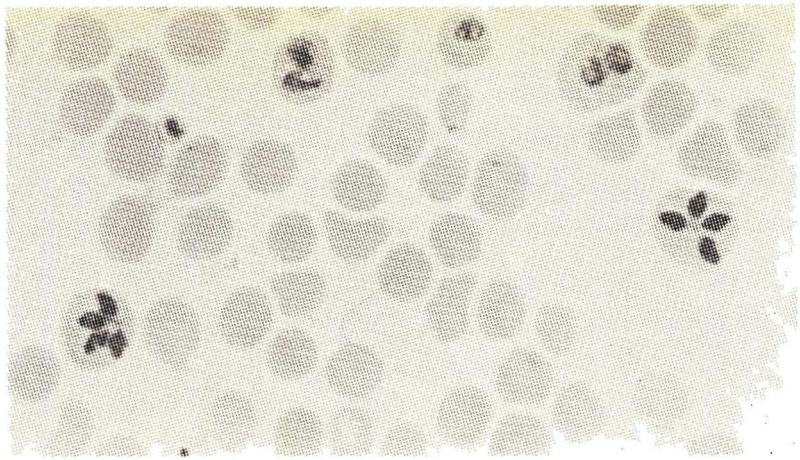


Fig. 3.

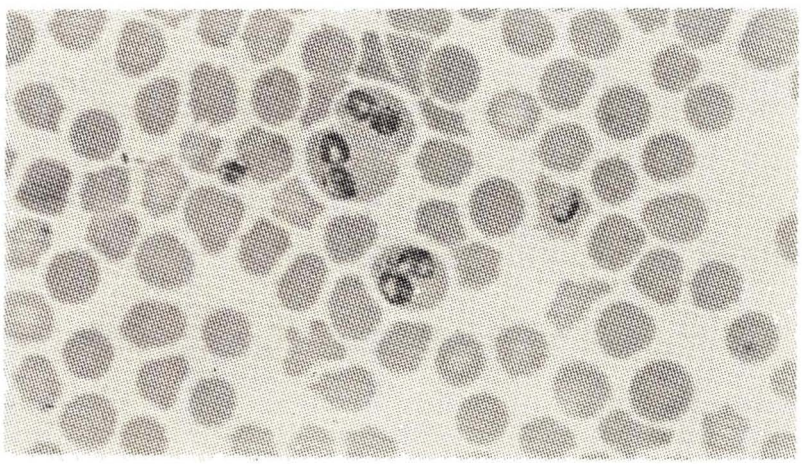


Fig. 4.

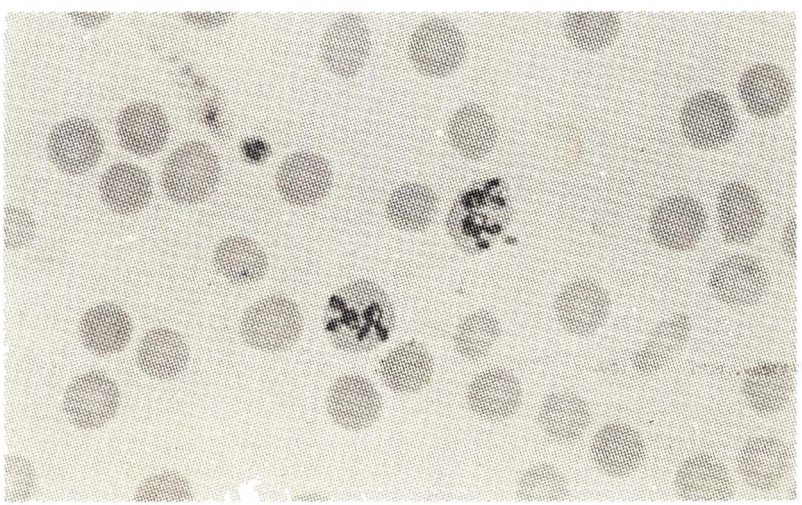


Fig. 5.

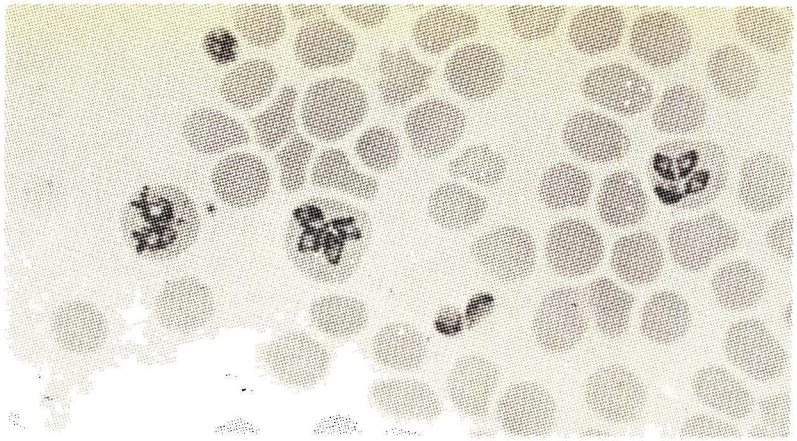


Fig. 6.

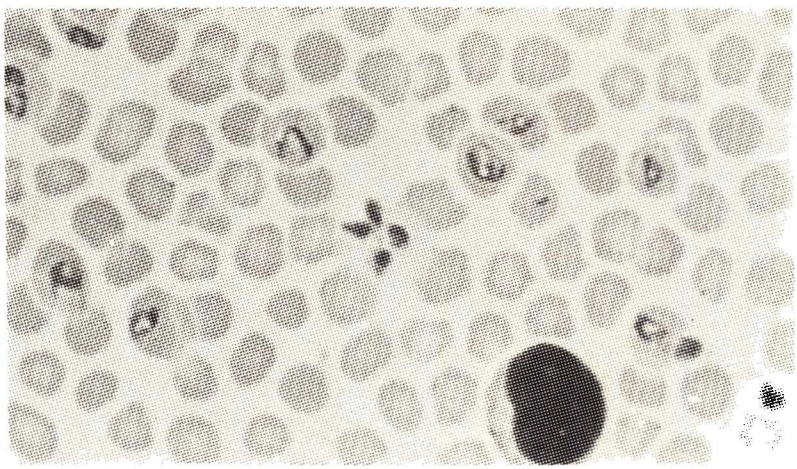


Fig. 7.

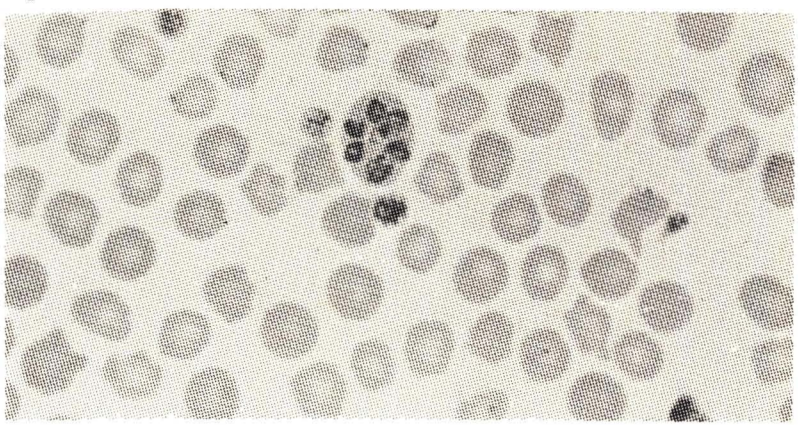
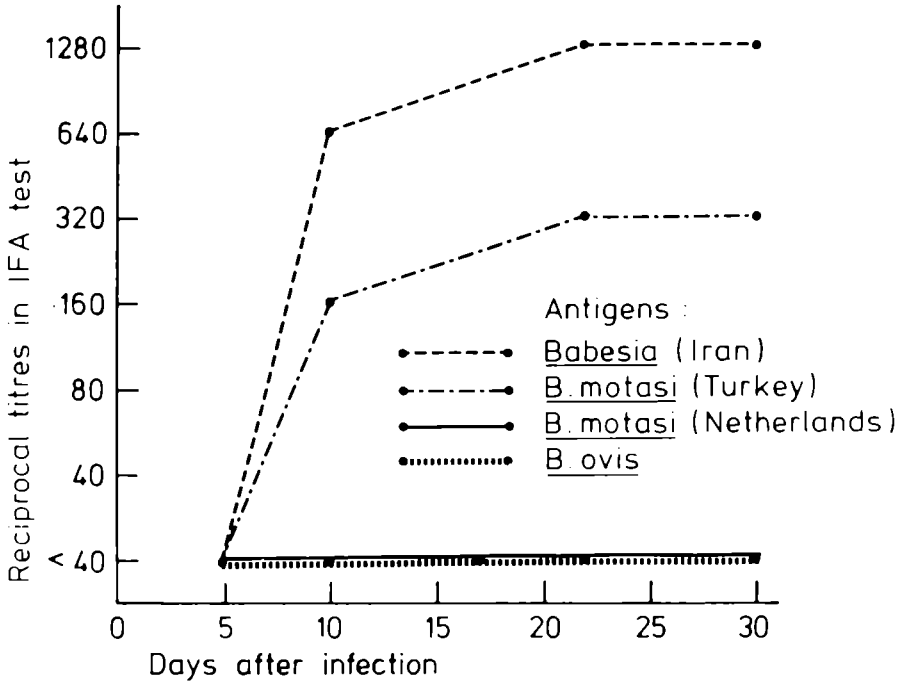


Fig. 8.

Fig. 1 to 8. Microphotographs of *Babesia* sp. (Iran) in blood of sheep. Magnification 1500 x.

Fig. 9. Results of IFAT using four antigens, on sequential sera of sheep 8022, infected with *Babesia* sp. (Iran).



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