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THEILERIA ORIENTALIS IN IRAN

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Abstvact

A bening species of Theileria *of cattle in northern Iran proved to be indistinguishable from* T. orientalis *in the indirect fluorescent antibody test as well as in the morphology of its piroplasms. It was transmissible transstadially by* Haemaphysalis punctata.

INTRODUCTION

Delpy (3) reported two species of *Theileria* in cattle in Iran, T. annulata (Dschunkowsky and Luhs, 1904), pathogenic to animals belonging to European breeds, and the benign *T. mutans* (Theiler, 1906). Rafyi and Maghami (9) confirmed these findings and added that *T. mutans* was less frequently found than *T. annulata and occurred only in certain parts of the country, according to Hooshmand-Rad (5) only in the Caspian Sea area. The latter author believed that the parasite was more likly to be T. sergenti* Yakimoff and Dekhtereff, 1930, than *T. mutans,* because its distribution coincided with that of one of the *Haemaphyaslis* vectors of *T. sergenti.* Recently, it has been argued that the name *T. sergenti* is invalid for a *Theileria* species of cattle and that the correct name for the benign species associated with *Haemaphysalis* ticks in Eurasia and Australia should be *T. orientalis* (Yakimoff and Soudatschenkoff, 1931), although the name *T. buffeli* Neveu-Lemaire, 1912 is also a candidate (7, 10).

In order to identify the benign species in northern Iran, a strain was isolated and studied.

MATERIAL AND METHODS

A strain of' *T. mutans*' was isolated in northern Iran, near the Caspian Sea, where blood examination had shown over 80% of local Mazandaranian cattle to be carriers of the parasite, by inoculating blood from a local animal into experimental Holstein and Sarabian calves. The latter breed, originating from

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northern Iran, is more susceptible to theilerial infections than other indigenous cattle.

infected blood, after having been maintained at – 70° C for 8 years (4), was inoculated into Holstein calves for cross-immunity studies with *T. annulata*. Deep frozen blood was also sent to Utrecht and injected subcutaneously and intravenously into a splenectomized Friesian calf, no. 409, in which piroplasms were first seen after 4 weeks. The strain was again cryopreserved at Utrecht, with 10 per cent dimethyl sulfoxide used as a protectant, and further studied in two more splenectomized Friesian calves, no. 421 and 433.

The morphology of the piroplasms in calves 409, 421, and 433 was studied in blood smears fixed in methanol and stained whit Giemsa. Parotid lymph node biopsy smears, made from one calf following an attempted transmission by ticks, were processed in the same way. Serum was collected once a week. Antigen smears were prepared from the blood of calf 409 according to the method of Burridge (2), as soon as suitable parasitaemia had developed. The packed cell volume (PVC) was measured in two calves during part of the observation period, for which a microhaematocrit centrifuge was used.

One attempt was made to transmit the *Theilera* transstadially with the tick *Haemaphysalis longicornis* (a bisexual strain originating in Jeju island, South Korea), from the larval to the nymphal stage. Another transmission experiment was conducted with *Haemaphyaslis punctata* (a Dutch strain), from the nymphal to the adult stage. The ticks were confined in clothbags on the ears. The non-parasitic stages of the ticks were maintained at approx. 90 per cent relative humidity and 20° C, apart from the ovipositing, hatching or moulting stages of *H. longicornis*, which were kept at 27° C.

The indirect fluorescent antibody (IFA) test was used for serological comparisons of *Theileria* sp. (Iran) with strains of *T. orientalis* ('Britain', a strain isolated in Essex, U.K. (8), 'Korea', a strain isolated on the Korean island of Jeju in 1981 by Dr. T. W. Han, 'Japan', the Fukushima strain isolated in Japan (6), and 'Australia', isolated at Brisbane, Australia by Dr. L.L.Callow In 1976 and also with African strains of *T. velifera* (Uilenberg, 1964) and *T. mutans*. Serum from an Ethiopian bovine, infected with *T. orientalis* (1), was also included. The IFA test was carried out according to Burridge (2) with minor modifications. Antigen of the Iranian *Theileria* was tested on sera known to be positive to these species and strains, and sera of calf 409 and various control sera were tested against a range of known antigens.

RESULTS

Influence on the host

No clinical symptoms were shown by the Sarabian and Holstein calves inoculated in Iran. Although the parasitaemia in some of the animals reached

levels of 12 per cent of red cells infected, there was at most a slight, transient anaemia and hyperthermia.

None of the three splenectomized calves at Utrecht showed any hyperthermia or specific signs associated with the infection, not even during the tick-borne infection of calf 433 (see below). A relatively long prepatent period in all calves (25 days or more) was followed first by a wave of relatively low parasitaemia (1 to 5 per cent). Observations were then discontinued on calf 421. Calf 409 had a parasitic relapse, starting 2 months after infection, during which the parasitaemia attained approximately 10 per cent, associated with a minimum packed cell volume of 14 per cent. Calf 433, infected by ticks, showed a parasitaemia oscillating between 0.1 and 1 per cent from 25 to 85 days after infection, followed by a higher wave, not quite attaining 10 per cent, 3 months after infection; the packed cell volume during this wave fell temporarily to 17 per cent.

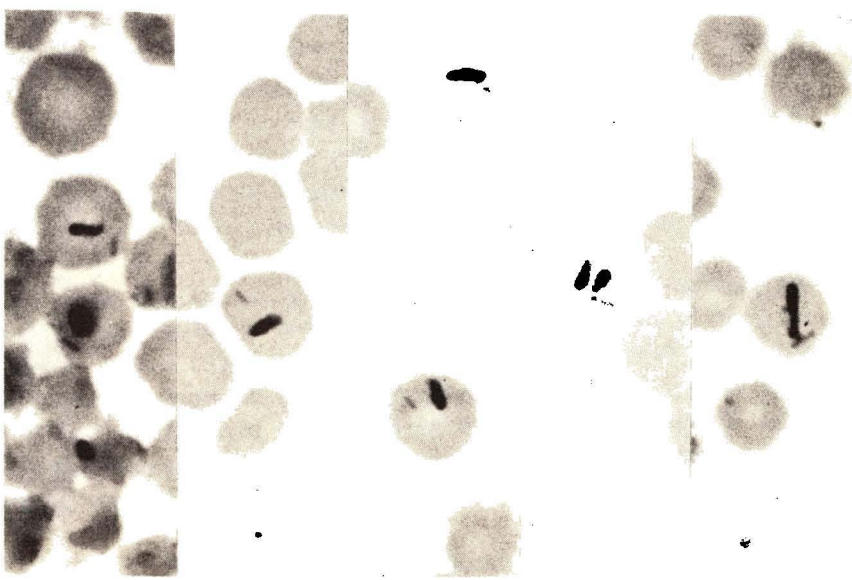

Fig. 1. Compound microphotograph showing piroplasms of *T. orientalis* (Iran), each associated with the intraerythrocytic bar structure.

Cross-immunity tests

Two calves which had recovered from acute *T. annulata* infection became parasitaemic following inoculation of blood containing *Theileria* sp. (Iran). Two calves recovered from infection with *Thelieria* sp. (Iran) showed typical clinical symptoms of *T. annulata* after inoculation with blood containing the latter species.

Morphology

The piroplasms (Fig. 1), were associated with the intraeryhrocytic 'bar' structure Known to occur in *T. orientalis* infections, while the intraerythrocytic 'veil' structure was only exceptionally seen. The parasite is in this respect similar to the Japanese Fukushima strain and a strain of *T. orientalis* from the USA, while the British as well as the Australian strains are associated whit both bar and veil (10), as is the Korean strain (unpublihed observations). Shape and size of the piroplasms were also typical of *T. orientalis,* with rod-shaped, elongate, often exceptionally long forms predominating.

Transmission experiments with ticks

1. *H. longicornis,* infected in the larval stage on calf 409 while it had a parasitaemia of nearly 5 per cent, failed to transmit the parasite to a splenectomized calf on which the ticks were fed in the nymphal stage between 6 and 7 weeks. after having dropped as larvae. The number of nymphs was less than 50.
2. *H. punctata, infected in the nymphal stage on calf 421,* while it had a parasitaemia of approx. 1 per cent, transmitted the *Theileria* in the adult stage to calf 433 on which the ticks were fed 2 months after having dropped as nymphs. Over a 100 females engorged, and an equivalent number of males also fed. The period prepatent to the appearance of piroplasms was 25 days. No schizonts were found in biopsy smears of the parotid lymph nodes, made from 7 to 13 days after application of the ticks, while the nodes were swollen.

Serological comparisons

Table 1 shows complete cross-reactivity between *Theileria* sp. (Iran) and *T. orientalis.*

Table 1. Reciprocal IFA titres of various sera to different theilerial antigens.

Sera (tag number of calf)	Antigens						
	T. orientalis					*T. mutans*	*T. velifera*
	Iran	Britain	Korea	Japan	Australia		
409. *Theileria* sp. (Iran)							
Before infection	< 40	< 40	< 40				
Day 43 p.i. (1)	1280/2560	1280	1280/2560				
Day 50 p.i.	1280/2560	2560	1280			< 40	40/80 (2)
Day 55 p.i.	1280	1280	1280				
296. *T. orientalis* (Britain) control serum	320	640	320/640				
412. *T. orientalis* (Korea) control serum	2560	2560	2560/5120				
324. *T. orientalis* (Japan) control serum	2560	1280	1280				
328. *T. orientalis* (Japan) control serum	1280/2560		2560	2560	1280/2560	< 40	
675. *T. orientalis* (Ethiopia) (3)	1280		1280	1280	1280		
282. *T. mutans* control serum	80					1280	
310. *T. mutans* control serum	< 40		40/80 (2)			2560	
277. *T. velifera* control serum	< 40					< 40	1280

(1): p.i. = post infection

(2): Pre-infection sera of calves 310 and 409 also gave titres of 40/80 with antigen of *T. orientalis* (Korea) and *T. velifera*, respectively. These titres are therefore to be regarded as negative.

(3): Animal 675 is animal A in Becerra *et al.* (1).

CONCLUSIONS

The benign *Theileria* species of cattle in northern Iran is identified as *T. orientalis,* because it is morphologically and serologically indistinguishable from *T. orientalis* and can be transmitted by the tick *H. punctata,*one of the known vectors of this species. One attempt with another vector, *H. longicornis,* failed but the number of ticks was low and the smaller larval stage presumably has less chance of becoming infected than the larger nymphs used in the successful experiment with *H. punctata.* Various species of *Haemaphysalis,* including *H. punctata,* are known to occur in the area where the strain was isolated. Although the parasite caused anaemia in splenectomized calves, these recovered spontaneously, and it is unlikely to be significant disease problem for intact cattle in endemic regions.

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