Equine babesiosis: A disease linked to the extensive horse raising in the pasture land of Extremadura ("dehesa")

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in

Etienne M. (ed.).
Dynamics and sustainability of Mediterranean pastoral systems

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 39

1999
pages 273-276

Equine babesiosis: A disease linked to the extensive horse raising in the pasture land of Extremadura ("dehesa")

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SUMMARY - Serologic testing was carried out to show antibodies to *babesia equi* and *babesia caballi* in mares raised in extensive regime in the "dehesa" of Extremadura, their transmission to the mares' offspring and their permanence in the foals. We have studied 33 Andalusian mare and foal serums. The technique used has been that of indirect immunofluorescence (IFI). The result was 63.6% of the mares and 54.5% of the foals seropositive to *B. equi*. All serums were negative to *B. caballi*.

Key words: "Dehesa" of Extremadura, equine babesiosis, indirect immunofluorescence.

RESUME *- "La babésiose équine : Une maladie liée à l'élevage extensif des chevaux dans les pâturages d'Estrémadure". On a effectué des tests sérologiques pour mettre en évidence des anticorps de* Babesia equi *et* Babesi caballi *chez des juments élevées dans des systèmes extensifs dans la "dehesa" d'Estrémadure, leur transmission aux descendants des juments et leur permanence chez les poulains. Nous avons étudié 33 sérums de juments et poulains Andalous. La technique utilisée a été l'immunofluorescence indirecte (IFI). Le résultat montre que 63.6% des juments et 54.5% des poulains sont séropositifs pour B. equi. Tous les sérums étaient négatifs pour* B. caballi.

Mots-clés *: Dehesa d'Estrémadure, Babésiose équine, Immunofluorescence indirecte.*

Introduction

Equine Babesiosis is a parasitic disease caused by the presence and multiplication of protozoa of the babesiidae family (*B. equi* and *B. caballi*) in the erythrocytes.

Equine Babesiosis is usually transmitted by carriers, ticks of the genera *Hyalomma, Dermacentor* and *Rhipicephalus*.

Nevertheless, in enzootic areas, like the "dehesa" of Extremadura, the *in utero* infection, though uncommon, should be taken into account since it may be the cause of abortion (Neitz, 1956).

The infection of babesias through placenta in cattle, sheep, horses and pigs has been pointed out by different authors, such as Hall (1960); Bruner and Gillespie (1966); and Correa (1974).

The aim of this paper is to evidence antibodies to babesias in foals of seropositive mares, raised in extensive regime in the "dehesa" of Extremadura.

The technique used is that of indirect immunofluorescence (IFI).

We would also like to show the importance of the serologic diagnosis through the IFI to make evident asymptomatic equine babesiosis, very common in the mares grazing in the "dehesa" of Extremadura.

Clinically babesiosis can be acute, sub-acute and chronic. When it is acute or sub-acute the animal usually presents symptoms which allow a clinical diagnosis: hyperthermy, anorexia, sadness and also signs of a haemolytic syndrome: anaemia, haemoglobinuria, icterus, etc.

When chronic, which it can come from the acute or primary form of the disease, due to either a low infection or the resistance of the animal to the disease, the clinical signs are slight or even non-existent.

Therefore, the search for parasites in blood smear tests is unsuccessful most of the times. This situation can last for years and meanwhile the animal will be a reservoir of infection for the vector ticks. Hence it is important to evidence the antibodies to discover the sick asymptomatic animals.

Some countries like USA and Canada do not allow seropositive horses to cross their borders. Before horses are allowed into their countries they must undergo a complement fixation test. Thus, the importance of diagnosis before travelling is clear.

Material and methods

Eleven Andalusian mares and their offspring have been studied. Thirty-three sera have been tested. These 33 sera correspond to 11 samples from the mares and 22 samples from the foals (2 samples per foal) taken at approximately one month interval.

The samples were taken in a farm in the province of Badajoz in the municipality of Merida. The blood samples were vacuum-kept in test tubes with EDTA as an anticoagulant, and they were frozen immediately after the extraction. The serum was extracted by centrifugation at 3,000 rpm during 15 minutes.

The 33 problem sera were tested at dilutions ranging from 1/40 to 1/640 in PBS.

Antigen of *B. equi* obtained in this faculty from a chronic *b.equi* seropositive mare whose spleen had been removed, and also antigen of *B. caballi* USDA type from the Faculty of Veterinary Science in Utrecht (Holland), were used.

We obtained the positive and negative control sera to both antigens from the Department of Parasite Diseases of the Faculty of Veterinary Science in Caceres.

The control sera were tested, together with the problem sera, at dilutions ranging from 1/40 to 1/20,480 in P.B.S. Moreover, the positive and negative control sera of *B. equi* were confronted with the *B. caballi* antigen and *vice versa* to detect possible crossreaction.

Finally an antispecies conjugated labelled with fluorescein isotiocianate (marketed by Nordic Inmunology, Tilbug, Holland), was utilized at a 1/80 dilution in PBS.

A slide cover with adhesive solution (GUR) is placed on the sera, and then they are observed through a fluorescence microscope, using a magnifying lens of 40.

Results

Seven mares out of the 11 tested were seropositive to *babesia equi*, whereas all of them were seronegative to *babesia caballi.*

As for their foals the results from the first taking, when they were between 2 days and one month old, were: six seropositive to *babesia equi* and all of them seronegative to *B. caballi*. We should state that the dams of those 6 seropositive foals were also seropositive.

The results from the second blood extraction a month later showed that only one foal was seropositive.The rest had lost their antibodies to the *babesia equi* (Table 1).

The titres found in the mares show the whole range of dilutions tested, i.e. from 1/40 to 1/640. On the other hand the titres found in the foals were never above 1/80 dilution (Table 2).

Table 1. Results from the second blood extraction

	Tested samples	Seropositivity			
		B. equi		*B. caballi*	
Mares	11	7	63,60%	0	0,0%
Foals (1)	11	6	54,50%	0	0,0%
Foals (2)	11	1	9,09%	0	0,0%

Table 2. Titres

	Samples	Negative	1/40	1/80	1/160	1/320	1/640
Mares	11	4	1	2	2	1	1
Foals (1)	11	5	3	3	0	0	0
Foals (2)	11	10	1	0	0	0	0

(1) Average age: 24 days
(2) Average age: 45 days

Discussion

If we present graphically the percentages of seropositivity in foals in relation to their ages, we find out that there is a decrease in the number of seropositive foals 15 days after their birth. Then the number is constant till they are 75 days old. Then there is another decrease, and at 90 days the percentage is very low. Though we did not continue the study we assume that at 120 days they would all become seronegative.

These results are similar to the ones found by J. Donnelly, L.P. Phipps and K.L. Watkins published in 1982 by Equine Veterinary Journal. Using the complement fixation test they observed that the antibodies in the foals studied decreased, and almost disappeared when they were 4 months old.

With regard to the diagnosis of the asymptomatic equine babesiosis, we can state that the IFI technique is very helpful to evidence those antibody carriers, and consequently we can have a better knowledge of the horse population health in Extremadura.

This control and prevention of the babesiosis can be approached from 2 viewpoints or management systems: (i) high genetic value horses, meant for exportation; and (ii) horses meant for reproduction and sale within the country.

A correct diagnosis before exporting a horse will mean a saving on money and troubles if it is seropositive and, in the case of seronegative horses it will speed up the customs formalities.

For valuable seronegative horses to be exported, contact with the transmission vectors should be avoided, since they may become infected. (As we already know even if their dams were seropositive, the foals lose their antibodies in less than four months).

As for the horses which will not be exported it would be advisable to reduce, but not eliminate, the number of ticks they can withstand. Being in contact with the infection vectors, but in a low number, we may obtain a chronic state which is good to make the animals resistant to the babesias and thus avoid a massive spread.

Conclusion

In Table 3 we have a summary of the results. From them we can state that:

(i) From the foals born seropositive, 90.9% lose their antibodies in 90/100 days. This suggests there is a passive transmission of maternal antibodies rather than an infection *in utero*.

(ii) The IFI technique for the diagnosis of the asymptomatic equine babesiosis seems to be easy, sensitive and with a good specificity. The fluorescence interpretation, though it should be done by somebody with experience, is not complicated.

(iii) The diagnosis of the asymptomatic equine babesiosis means a great advantage not only because it is a way to fight the disease, but also from the economic point of view, as it saves problems when exporting stallions to countries where the disease does not exist.

(iv) In enzootic areas it is convenient to keep the enzootic stability and this depends on:

- Continuous presence of horses resistant to ticks and babesias.
- These horses must have a low level of parasites.
- Presence of ticks with a low infection power.
- Constant and moderate inoculation.

These steps should be followed when dealing with horses meant for reproduction and sale within the country.

Table 3. Summary of results

Mare's name	Serum number	Titre *B. equi*	Foal's name	Serum number	Titre *B. equi*
Soraya	1	1/80	Halcon	12	1/40
				23	negative
Bondadosa	2	1/320	Haragan	13	1/40
				24	1/40
Niña	4	1/160	Huracan	15	1/80
				26	negative
Llamativa	6	1/640	Hugo	17	1/80
				28	negative
Baviera	7	1/80	Hormiga	18	1/80
				29	negative
Oficiala	8	1/160	Huno	19	1/40
				30	negative
Bizarra	9	1/40	Huron	20	negative
				31	negative

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