INTERACTIONS BETWEEN BOVINE AND ROE DEER (*CAPREOLUS CAPREOLUS*) FOR *BABESIA SP.* INFECTIONS

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Introduction

Babesia divergens is the major agent of bovine babesiosis in France. This parasite expresses a lower specificity than other *Babesia* species; *B. divergens* can be cultured in human [1], sheep [2] or rat [3] red blood cells (RBC) and this protozoa can experimentally infect gerbil [4], rat [5] or sheep [2]; furthermore, *B. divergens* babesiosis is a zoonotic disease, notably in splenectomized human [6]. Field data about the potential hosts of *B. divergens* are very poor; but *Ixodes ricinus*, the vector of *B. divergens*, can bite different hosts, notably bovine or *Cervidae*.

In France, an increase of roe deer populations is observed and roe deers are more frequent in the fields where cattle are also present. Small forms of *Babesia* have been described in *Cervidae*, notably *B. capreoli* in roe deer in Europe [7]. They showed major serogical cross-reactions with *B. divergens* [8, 9].

The aims of this study were to confirm the seroprevalence of *Babesia* in roe deer in France using *B. divergens* antigens and to isolate and characterize roe deer *Babesia* for further investigations on interactions between bovine and roe deer for *Babesia* infections.

Material and Methods

A total of 355 samples were collected in roe deers killed by hunters in the 6 départments of Loire Atlantique, Mayenne, Maine et Loire, Sarthe, Vendée and Ille et Vilaine. Sera were extracted from the cardiac thrombus. Serological investigations were done using IFAT with *B. divergens* parasitized gerbil blood as antigen. The roe deer sera were diluted in phosphate buffered saline (PBS) pH 7.2 from 1:80 to 1:1280. Fluorescein isothiocyanate-labelled rabbit anti-goat immunoglobulins (SIGMA) diluted in PBS (1:100) were used to detect specific antibodies.

For blood parasite isolation, a total of 53 roe deer were investigated: 5 from the previous protocol, the others were trapped in the three wild fauna reserves of "Chizé" (Deux sèvres) (30 animals), "Trois Fontaines" (Meuse) (15 animals) and "La Haute Touche" (Indre et Loire) (3 animals). Blood samples were collected by jugular puncture with Citrate Dextrose Phosphate supplemented Venoject tubes.

In vitro cultures were performed as previously described [2, 10] with some modifications. Briefly, blood samples were centrifuged; plasma and buffy coat were discarded and the erythrocytes were washed in RPMI 1640 (Biowhitaker); cultures were initiated in 24 wells culture plates at 7.5 % haematocrit in RPMI 1640 supplemented with 20 % of fetal bovine serum; 50 µg/ml gentamicin and 2,5 µg/ml Amphoterincin B were added to avoid fungal, bacterial and *Trypanosoma* development. Cultures were performed in humidified 6 % CO_2 atmosphere at 37°C. The media were changed every two or three days during 3 weeks. For positive cultures, subcultures were done in autologous roe deer erythrocytes and in sheep erythrocytes with 10 % of previous culture. After 5 subcultures, cultures were performed in 25 cm^2 culture flasks, in autologous erythrocytes suspended at 2.5 % haematocrit in the same culture medium which were changed every two days. When parasitaemia reached at least 10 %, cultures were frozen in liquid nitrogen for further investigations.

For 7 *Babesia* isolates, gerbils (*Meriones unguiculatus*) were inoculated intraperitoneously with 2.10^6 parasitized roe deer erythrocytes.

Results

A total of 268 roe deers (268 / 355; 75 %) were positive in *B. divergens* IFAT. A total of 43 cultures (81 %) were positive for *Babesia sp.*; only 33 (62 %) subcultures could be performed in autologous RBC. For 32 of these isolates, a typical small form of *Babesia* was observed; morphological characteristics were very similar to those described for *B. capreoli* or *B. divergens*, with numerous parasites occupying the periphery or the margin of the erythrocyte and some tetrads (fig 1).

For these small form *Babesia*, subcultures in sheep red blood cells could not be performed; furthermore, inoculations to gerbils were unsuccessfull.

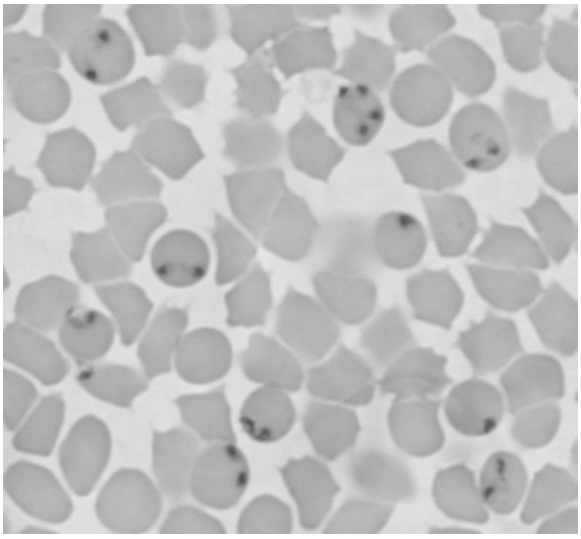

figure 1 : in vitro cultures of the small form of roe deer Babesia.

In one animal originating from "La Haute Touche", a large form of *Babesia* was observed with typical large geminated merozoites and numerous ovoid forms occupying the all diameter of the erythrocyte (fig 2). Subcultures in sheep erythrocytes were successful and this isolate could be continuously subcultured every 2 or 3 days. Inoculation to gerbil was unsuccessfull.

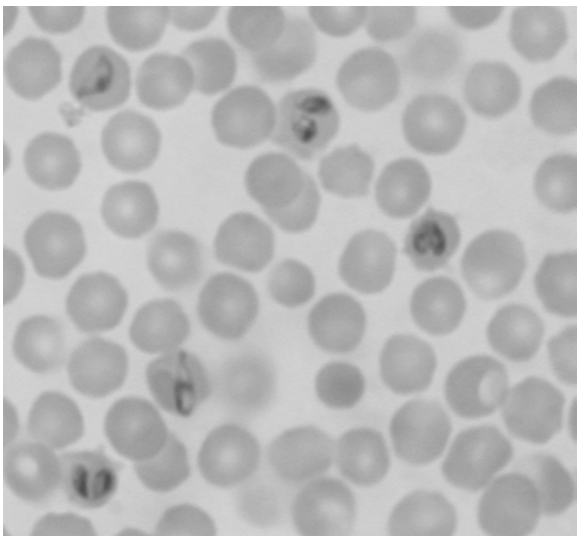

figure 2 : in vitro cultures of the large form of roe deer Babesia.

Discussion

As previously described [9], the serological prevalence against *B. divergens* was very high in investigated roe deer populations. Using in vitro isolation of the blood parasites, the prevalence of small forms of *Babesia* was similar to serological prevalence and 32 small forms isolates were produced.

B. divergens can be cultured in sheep red blood cells [2,10] and gerbils are experimental hosts [5]. In the contrary, the small *Babesia* isolates were unable to infect gerbils and were unable to grow in sheep red blood cells; furthermore, for 6 isolates, in vitro cultures were tested in human red blood cells as described for *B. divergens* [1] and were unsuccessfull. Because of these different biological properties, there is a high probability that the isolated small forms are *B. capreoli* and not *B. divergens*; the roe deers are probably not reservoir hosts for *B. divergens*. Further biological and molecular investigations should be done to finally identify the parasite.

A large form of *Babesia* was isolated in one case. Additional serological investigations were then performed using IFAT with sheep erythrocytes parasitized with this large form as antigen. About 20 % (70/355) of the roe deer investigated were positive. Further investigations are then needed about serological cross reactions between this *Babesia* isolate and *B. capreoli*. In roe deers, a arge form of *Babesia* is described in Siberia as *B. jakimovi* [11] but it has never been described in western Europe. Other large forms of Babesia are described in *Bovidae* in France, notably *B. motasi* in sheep and goat and *B. major* in bovine. The hypothesis of bovine or ovine origin of our isolate can be supported by the fact that it can be in vitro cultured in sheep red blood cells and also in bovine red blood cell (data not shown). Further studies are also needed to identify this parasite and finally evaluate the possible role of the roe deer as reservoir host for bovine *Babesia*.

The question of the interactions between roe deer and bovine for babesiosis should also be evaluated in the vector. For both *B. capreoli* and *B. divergens*, the vector is the three hosts tick, *Ixodes ricinus*. Infection with one of these two *Babesia* could modify the development in the tick and the transmission of the other one.

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