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Original article

Transplacental transmission of bovine tick-borne pathogens: Frequency, co-infections and fatal neonatal anaplasmosis in a region of enzootic stability in the northeast of Brazil

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ABSTRACT

Bovine tick-borne disease (TBD) constitutes a worldwide group of diseases that result in great losses for dairy and beef cattle. With regard to the epidemiological profile of the diseases, the importance of transplacental transmission is still not very well understood. The aim of this study was to determine the transplacental transmission of TBD agents (*Anaplasma marginale*, *Babesia bovis* and *B. bigemina*) in a herd of dairy cattle that had been naturally infected in an area of enzootic stability in northeastern Brazil. Blood for serology of the three agents was collected from cows within 120 days of gestation and serology, haemogram and nPCR assays were performed after birth. Blood was collected from the calves within 3 h of birth, and haemogram and nPCR assays were performed in all animals. Pre-colostrum serology was achieved in 34 animals. The Student's *t*-test was used to compare the haemogram results between animals that were positive and negative for the haemoparasites. The cows were seropositive for all agents in at least one of the examinations. We detected 15 cases of vertical transmission of *A. marginale*, 4 of *B. bovis* and 2 of *B. bigemina* in the 60 cows. In infected animals, co-infection was detected for *A. marginale* and *B. bovis* in 1 of 60 calves, and a triple infection was detected in one other calf. Fatal neonatal anaplasmosis was observed in 1 of 15 calves, in which death occurred within 24 h of birth. From the results, we concluded that transplacental transmission of TBD agents occurs, including in cases of co- and triple-infection. Such transplacental transmission can cause neonatal death, increasing the importance of this form of epidemiological transmission and suggesting its role as a cause of undiagnosed neonatal death.

1. Introduction

Bovine tick-borne diseases (TBDs) are caused in Brazil mainly by *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*, which occur throughout the tropical, subtropical and temperate areas of the world (Guglielmone, 1995; Echaide et al., 1998; Tembue et al., 2011; Ait-Hamou et al., 2012). As TBD agents are simultaneously present in most regions of enzootic stability or instability (Barros et al., 2005; Atif et al., 2012; Shebish et al., 2012; Brito et al., 2013; Mtshali et al., 2013), they are a limiting factor in animal husbandry development in these locations (De Vos et al., 1976; Kessler, 2001; Kocan et al., 2003, 2010; Aubry and Geale, 2011).

The main means of transmission in Brazil is the tick *Rhipicephalus microplus*, direct transmission by fomites (needles, surgical instruments and piercing objects), haematophagous Diptera (Tabanidae, Culicidae and Muscidae) (Brito et al., 2010; Reinbold et al., 2010; Aubry and Geale, 2011), and transplacental pathways (Neitz, 1956; Potgieter and Van Rensburg, 1987; Ribeiro et al., 1995; Grau et al., 2013; Santarosa et al., 2013); however, little is known about the real contribution of the transplacental pathways in the epidemiology of TBDs (Kessler, 2001; Kocan et al., 2003; Aubry and Geale, 2011).

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The results of natural transplacental transmissions in several studies differ, and report absent (Piercy, 1956; Kuttler et al., 1962), low (Passos and Lima, 1984; Ribeiro et al., 1995) or moderate transmission (Maldonado et al., 2012; Silva et al., 2014) rates, which lead to the hypothesis that the importance of this transmission pathway may vary by region, depending on the climate characteristics, the cattle, and the presence of vectors, or it may be based on the genetic variability of the agent.

The transplacental transmission of *A. marginale* is the most common, being described extensively in experimental studies, including longitudinal studies (Norton et al., 1983; Potgieter and Van Rensburg, 1987; Ribeiro et al., 1995; Pypers et al., 2011; Maldonado et al., 2012; Grau et al., 2013; Silva and Fonseca, 2014; Silva et al., 2015). It mainly occurs between the second and third trimesters of pregnancy (Fowler and Swift, 1975; Swift and Paumer, 1976, 1978; Zaugg and Kuttler, 1984; Zaugg, 1985; Potgieter and Van Rensburg, 1987; Ribeiro et al., 1995). The vertical transmission of *B. bovis* has been reported, in most cases, as isolated cases (Neitz, 1956; De Vos et al., 1976; Barbosa et al., 1994; Bracarense et al., 2001; Osaki et al., 2002; Yeruham et al., 2003; Santarosa et al., 2013). Reports of transplacental transmission of *B. bigemina* were only found in older studies (Zolotareff, 1936, Roux, 1939 cited by Neitz, 1956; Atwell, 1975).

The death of calves infected transplacentally by naturally infected cows has been previously reported (De Vos et al., 1976; Paine and Miller, 1977; Norton et al., 1983; Barbosa et al., 1994; Bracarense et al., 2001; Pypers et al., 2011; Santarosa et al., 2013), and these reports characterize this epidemiologically important transmission pathway, whilst also improving estimates of economic losses due to infection.

The livestock agribusiness world is particularly affected by TBDs because more than 1.2 billion heads are at risk for infection and developing disease (Bock et al., 2004). It is estimated that in the next decade, TBDs will cause losses in the order of $282 million in Australia as a direct consequence of the infections when considering miscarriages, reductions in milk production and weight gain, deaths and the costs of prevention and treatment (Gohil et al., 2013). These estimates may increase due to the continuing increase in the distribution of the infections, as a function of the intense transportation of asymptomatic animals and the subsequent transmission to susceptible individuals, as well as the effects of global warming, which significantly alters the distribution of vectors and parasites throughout the world (Kocan et al., 2010).

On the basis of the above and in view of the great damage caused by anaplasmosis and babesiosis in global livestock, this study aims to determine the frequency of transplacental transmission of TBD agents and reports one case of fatal neonatal anaplasmosis in a naturally infected, crossbred herd occurring in an endemic region of zoonotic stability in northeastern Brazil.

2. Materials and methods

2.1. Area of study

The study was conducted from October 2010 to June 2011 on a property with a history of clinical babesiosis and anaplasmosis cases in young animals. The property is in the municipality of Ibicaraí in a micro-region of Itabuna-Ilhéus in the state of Bahia in Brazil's northeast region (14°51′54″ South and 39°35′16″ West at an altitude of 270 M). The study area is in the Atlantic forest area. The annual average rainfall is 1445 mm, with a relative humidity of 80% and a temperature of 24 °C (MAPA, 2010).

The selected property has 135 acres and 350 animals. The herd was composed of crossbred cattle (1/4 *Bos taurus indicus* combined with 3/4 *Bos t. taurus* to 1/4 *Bos t. taurus* combined with 3/4 *Bos t. indicus*) that are maintained in a semi-intensive system. The daily production of milk from 110 lactating cows is 1200 l, obtained from two daily milkings using a closed system, mechanical milking machine. Nutrition is based on a rotational grazing system, with mineral supplementation offered ad libitum. The adult animals received concentrated supplements composed of corn, soybeans and urea once a day, while the calves were fed milk and a concentrate composed of corn and soybeans. Ectoparasite control was carried out strategically by spraying the whole herd at intervals of 21 days with ectoparasiticides indicated by a tick-susceptibility bioassay.

2.2. Sample collection and processing

Sixty pregnant cows with an average age of 7.4 years (±1.7 years) were included in the study sample and had their initial blood collection within the first 120 days of gestation for TBD serological diagnoses. These cows and their calves had their blood collected at the time of the calf's delivery, or in some cases, a maximum of 3 h after birth. The blood of these animals was conditioned in tubes with an anticoagulant (EDTA) and without an anticoagulant for performing the haemogram, molecular parasitic identification, the determination of the calves' parasitemia by blood smear and the serology of the cows and 34 calves that had not ingested colostrum, confirmed by the low concentration of the gamma-glutamyl transferase serum, as described by Perino et al. (1993).

The haemogram was carried out in an automatic ABX Vet counter (Horiba, São Paulo, Brazil). The total plasma protein values were obtained using manual refractometry. The parasitemia was estimated by counting the number of parasitized erythrocytes in a sample size of 1000, the result was then expressed as a percentage. After the haemogram, whole blood and serum aliquots were placed in 2.0 mL sterile plastic cryotubes free of DNase and RNase, in duplicate, and stored at −20 °C.

2.3. Genomic DNA extraction, PCR and nested-PCR (nPCR) for the diagnosis of Anaplasma marginale, Babesia bigemina and Babesia bovis

The extraction of DNA from blood samples was performed using the QIAamp DNA Blood mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's recommendations. The extracted DNA was kept at −20 °C. A Nested-Polymerase Chain Reaction (nPCR) was performed independently for each agent using the primers described by Lew et al. (2002), Suarez et al. (1991) and Terkawi et al. (2011) as presented in Table 1.

Babesia bovis was detected using primers described by Suarez et al. (1991). The reactions were performed using a final volume of 25 μL, with 5 μL of DNA template, reaction buffer (5×),

Table 1
The primers used in PCR (1st reaction) and nPCR (2nd reaction) for the identification of the transplacental transmission of tick-borne disease agents in a naturally infected crossbred herd in Ibicaraí in the northeast of Brazil.

Agent	Sequence of oligonucleotides (5′–3′)	Reaction	Reference
Babesia bovis	CACGAGGAAGGAACTACCGATGTTGA	1[a]	Suarez et al. (1991)
	CCAAGGAGCTTCAACGTACGAGGTCA		
	TCAACAAGGTACTCTATATGGCTACC	2[a]	
	CTACCGAGCAGAACCTTCTTCACCAT		
Babesia bigemina	GAGTCTGCCAAATCCTTAC	1[a]	Terkawi et al. (2011)
	TCCTCTACAGCTGCTTCG		
	AGCTTGCTTTCACAACTCGCC	2[a]	
	TTGGTGCTTTGACCGACGACAT		
Anaplasma marginale	TGTGCTTATGGCAGACATTTCC	1[a]	Lew et al. (2002)
	AAACCTTGTAGCCCCAACTTATCC		
	TGTGCTTATGGCAGACATTTCC	2[a]	
	TCACGGTCAAAACCTTTGCTTACC		