TRANSMISSION AND DIAGNOSIS OF EQUINE BABESIOSIS IN SOUTH AFRICA

F.T. POTGIETER; D.T. DE WAAL & ELSA S. POSNETT**

Protozoology- and *Molecular Biology Divisions, Veterinary Research Institute, Onderstepoort, 0110, Republic of South Africa

The transmission and prevalence of Babesia equi *and* B. caballi *are being studied.* Rhipicephalus evertsi mimeticus *an ixodid tick from Namibia was identified as a new vector of* B. equi, *however,* R. turanicus, *previously reported to be a vector, failed to transmit both* B. equi *and* B. caballi *in the laboratory.*

The accurate diagnosis of B. caballi *is being investigated because the nature of its low level parasitaemia does not allow easy detection in thin blood smears, routinely used for diagnosis, by clinicians. Consequently its role as a pathogen remains obscure. The importance of identifying infected horses, destined for export to* Babesia-*free countries, is also stressed.*

Thick and thin blood smears, serology (IFAT) and DNA probes are currently employed to study disease prevalence. To date 293 healthy, adult, thoroughbred horses have been screened by all three methods. The percentage positives are as follows: B. equi *4.4%, 70.6%, 13% and* B. caballi *0.7%, 37%, 18.4% respectively.*

The DNA probes were more sensitive than blood smear examination for diagnosing carrier infections but are probably not sensitive enough to identify all carrier infections. A poor correlation was found between detection of the parasites' DNA and seropositivity. However, polymerase chain reaction could be used to amplify parasite DNA in a particular sample and this could result in more accurate diagnosis.

Key words: *Babesia equi* – *Babesia caballi* – diagnosis – transmission – epidemiology – South Africa – immunofluorescent test – DNA probes

During the past seven years studies on the epidemiology of equine babesiosis in South Africa and the relative importance of the two organisms involved namely, *Babesia equi* and *B. caballi*, have been conducted. Laboratory investigations have concentrated on the transmission of these infections and the development of accurate diagnostic techniques.

It was already known that both species occur in this country and that *B. equi* is transmitted by *Rhipicephalus evertsi evertsi* prior to 1983, but the vector of *B. caballi* remained unknown. Most earlier reports referred to equine babesiosis without differentiating between the causative organisms. The relative importance of the disease in South Africa was demonstrated in a survey which indicated that the number of cases treated exceeded those of every other infectious disease of horses, including respiratory diseases and African horsesickness (Littlejohn et al., 1977).

Most clinical cases of equine babesiosis in South Africa are diagnosed as *B. equi* infections (De Waal, 1991 unpublished data). *B. caballi* is seldom associated with clinical disease probably because it is not always possible to demonstrate the parasites microscopically because of the low parasitaemia. There are no grounds to differentiate between *B. equi* and *B. caballi* on clinical signs alone.

The well recognized phenomenon of *in utero* infection of the fetus by *B. equi*, resulting in abortions or neonatal deaths, is not uncommon in South Africa and probably plays an important role in equine reproductive failure (De Waal & Coetzer, 1990 unpublished data).

Recently it was found that, as in the case of *B. equi*, *R. e. evertsi* will also transmit *B. caballi* transstadially (De Waal & Potgieter, 1987). *Hyaloma truncatum*, a two host tick, is capable of transovarial (adult to adult) transmission of

B. caballi (De Waal, 1990). Both these ticks have a widespread, overlapping distributions (Howell et al., 1978), leaving *B. caballi* with a potentially more extensive occurrence.

The thick blood smear technique (Mahoney & Saal, 1961) is more sensitive for detecting parasites, specially in the case of *B. caballi* infections, than thin blood smears (De Waal, 1990).

The indirect fluorescent antibody test (IFAT) is currently the preferred test and has successfully been used to detect pure infections of *B. equi* and *B. caballi* from field horses. Subsequent blood inoculations to susceptible splenectomized horses have confirmed its accuracy (De Waal et al., 1988).

DNA probes have recently been developed for *B. equi* (Posnett & Ambrosio, 1989; Posnett et al., 1991) and *B. caballi* (Posnett & Ambrosio, 1991). The sensitivity of these probes in diagnosing these infections is superior to that of blood smear examination.

In the present study tick transmission trials were undertaken to identify vectors and a total of 293 horses were screened for *Babesia* parasites and species specific antibodies using blood smear, DNA probes and serology.

MATERIALS AND METHODS

Tick transmission experiments – The Claratal strain of the two-host tick *R. e. mimeticus*, isolated in Namibia, was obtained from the Entomology Division, Veterinary Research Institute, Onderstepoort. It has been maintained in the laboratory for several generations by initially feeding the immature stages on rabbits and the adults on sheep and subsequently all parasitic stages on rabbits.

The Kaalplaas strain of the three-host tick *R. turanicus* was bred from two engorged females collected from horses on the farm Kaalplaas (De Waal et al., 1988). The parasitic stages have been maintained in the laboratory on rabbits, using the method of Neitz et al. (1971).

The free-living tick stages of both ticks were maintained in an acaridarium at 25 °C and 85% relative humidity. In all experiments the ticks were fed on the shoulder region of horses kept

in tick-free stables as described by De Waal & Potgieter (1987). Six, one to three-year old, intact horses, born and reared under strict tick-free conditions, were used to infect ticks and demonstrate transmission. All horses were subjected to serological screening for *B. equi* and *B. caballi* specific antibodies before being used in this study. Failure to transmit the respective infections was serologically confirmed up to 60 days post tick infestation.

Experimental procedure

R. e. mimeticus – One horse was infected intravaneously on Day 0 with frozen *B. equi* blood (OP-isolate, used throughout this study), isolated and prepared as described by De Waal et al. (1988). On Day – 3 and Day 0, 1000 non-infected larvae of *R. e. mimeticus* were placed on the horse. A total of 250 engorged nymphae were collected from the 12th to the 22nd day post infestation (p.i.) and allowed to moult in the acaridarium. Twenty days post moulting, 20 male and 20 female ticks were placed on another horse. Only five engorged females had been recovered by Day 11 p.i., the rest were damaged.

R. turanicus – Two attempts were made to infect *R. turanicus* nymphae with *B. equi* and the SWA/Namibia-isolate of *B. caballi* (De Waal, 1990) respectively, in order to demonstrate transstadial transmission by the adult stage. In both instances the numphal ticks engorged during patent parasitaemias.

In the case of the *B. equi/R. turanicus* transmission attempt, the experiment was designed to expose engorging nymphae of *R. e. evertsi*, *R. e. mimeticus* and *R. turanicus* to a patent *B. equi* parasitaemia on a single horse, in order to ensure equal opportunities for acquiring infection.

Clinical reactions – Early morning rectal temperatures and haematocrit readings were taken daily. Thick and thin blood smears were prepared daily and the parasitaemia expressed as described by De Waal & Potgieter (1987).

Diagnostic procedures

DNA probes and blood smears – The collection of blood samples from 293 horses, preparation for microscopy examination of

blood smears and the use of species specific DNA probes in a spot hybridization assay to detect *B. equi* and *B. caballi* DNA were done as described by Posnett et al. (1991); Posnett & Ambrosio (1991).

Serology – A standard IFAT protocol was followed to detect species specific antibodies (Madden & Holbrook, 1968).

RESULTS

Tick transmission

R. e. mimeticus – *R. e. mimeticus* successfully transmitted *B. equi* transstadially. The infection was picked up during the larval-nymphal feeding phase and transmitted by the ensuing adult ticks. The important aspects of this experiment include: a prepatent period of eight days following the artificial infection with *B. equi*; the larvae and nymphae engorged during a patent parasitaemia score of one to two; the horse that received the 40 adults ticks exhibited a *B. equi* infection showing a prepatent period of 13 days p.i.; an intermittent febrile reaction followed and the second peak coincided with maximum parasitaemia; the lowest haematocrit recorded was 15% on Days 19 and 20 p.i., followed by a slow increase to pre-infection levels; the horse recovered without treatment.

R. turanicus – In spite of ideally synchronized patent, rising parasitaemias and nymphal engorgement, all laboratory attempts to demonstrate transstadial transmission (nymph to adult) of *B. equi* and *B. caballi* by *R. turanicus*, under controlled laboratory conditions failed, as confirmed by absence of clinical reactions, negative blood smears and serology. The highest *B. equi* parasitaemia during nymphal engorgement was 7.9%.

In the case where nymphae of all three tick species engorged on the same horse, only the ensuing adults of *R. turanicus* failed to transmit the *B. equi* infection.

Survey of horses – The results of an attempt to diagnose equine babesiosis in randomly selected blood samples from field horses are summarized in Table.

As expected, a higher percentage of positive blood smears were found in the case of *B. equi* than that of *B. caballi*. These infections could only be demonstrated in thick blood smear preparations.

All the horses with positive blood smears also tested positive with the respective DNA probes. However, there was poor correlation between the detection of the parasites' DNA and seropositivity.

DISCUSSION

Theiler (1906) reported the transmission of *B. equi* by *R. e. evertsi* and for many years it was believed to be the only vector. Another subspecies of *R. e. evertsi* namely, *R. evertsi mimeticus*, was described by Dönitz (1910). Although it was speculated that the latter tick could be a vector of equine babesiosis, it was only in this study that it was positively identified as a vector of *B. equi*, for the first time. This tick "replaces" *R. e. evertsi* in the more arid South-Western regions of southern Africa including Namibia (Theiler, 1950; 1964) from where the strain was isolated.

R. turanicus is listed as a vector of *B. equi* by Friedhoff (1988). It was also found that the nymphae of this tick could acquire the infection from parasitaemias as low as 0.05 to 0.1% and even became infected feeding on a horse that was negative, as assessed by microscopic examination and the complement fixation test (Sigrist, 1983; Friedhoff, et al., 1985) cited by Friedhoff (1988).

TABLE

Results of blood smears, IFAT and DNA probes in 293 horses screened for *Babesia equi* and *Babesia caballi* infections

Parasite	Horses tested (n)	Positive tests (%)		
		Blood smear	IFAT	DNA Probe
Babesia equi	293	4,4	70,6	13
Babesia caballi	293	0,7	37	18,4

It is believed that *R. turanicus* could have been confused with *Rhipicephalus sangiuneus* in the past (Friedhoff, 1988). Reports of the transmission of equine babesiosis by *R. sangiuneus* (Enigk, 1943, 1944) may therefore in fact refer to *R. turanicus*.

The initial failure to transmit *B. equi* with *R. turanicus* in the present study has been followed by three more unsuccessful attempts. Consequently this particular tick and parasites' interaction will probably not result in transmission of *B. equi*.

The DNA probes used in this study have been applied to detect carriers of *B. equi* (Posnett et al., 1991) and *B. caballi* (Posnett & Ambrosio, 1991). They have also found to be useful in the diagnosis of *B. caballi* infections in poor performing racehorses where specific treatment of positive cases resulted in recovery (Posnett & Ambrosio, 1991).

Although there was a 100% correlation between positive blood smears and DNA probe results in the present study, it was not practically possible to confirm the higher sensitivity of the probes by subinoculation of blood to susceptible horses. The poor correlation between serum antibody titres and DNA probes may demonstrate the inability of the probes to detect low level parasitaemias, or the persistance of detectable antibody titers after sterilization of the infection.

The polymerase chain reaction could be employed to amplify DNA in a particular sample and this could result in more accurate diagnosis of low level parasitaemias.

In conclusion in can be said that we are able to make more accurate diagnoses of carrier animals with the available DNA probes. Used in combination with other diagnostic tests a better service can be offered to facilitate the translocation of horses. Specifically the movement from countries where equine babesiosis is endemic, to disease-free countries where vector or potensial vector ticks are present.

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