

ECOLOGY AND PARASITOLOGICAL ANALYSIS OF HORSE FLIES (DIPTERA: TABANIDAE) IN ANTIOQUIA, COLOMBIA

Ecología y análisis parasitológico de tábanos (Diptera: Tabanidae) en Antioquia, Colombia

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ABSTRACT

During the months of June to September 2006, collections of tabanids (Diptera: Tabanidae) and ticks were conducted in the Caucasia municipality, Antioquia, Colombia. Tabanids were caught on horses during daylight using hand nets and pots at the ecotone zone between secondary forests and paddock habitats. Ticks were collected directly from cattle by hand. The purpose of the study was to identify possible vectors of bovine trypanosomosis, and register the diversity and abundance of tabanids in the zone. The arthropods were brought to the laboratory for taxonomic determination and protozoans searching in proboscis, midgut, and salivary glands of flies. In the case of ticks, protozoans were searched in hemolymph. One hundred and forty tabanids belonging to four genera and nine species were caught. Among the species, *Lepiselaga crassipes* was the most abundant (43.6%), with the highest abundance in July and a biting peak at 14:00 h. The highest diversity of tabanids was observed during September. Three tabanids were found infected with flagellates morphologically compatible with *Trypanosoma vivax*. 315 ticks belonging to *Boophilus microplus* species were collected, all of them negative to flagellates. These results suggest *T. vivax* transmission by tabanids in the study area. However, the specific status of the parasites should be determined by molecular techniques and the transmission mechanism should be established too by controlled studies.

Key words. *Trypanosoma vivax*, Tabanids, Ticks, Bovine trypanosomosis, Caucasia, Colombia.

RESUMEN

Durante los meses de junio a septiembre de 2006 se colectaron tábanos (Diptera: Tabanidae) y garrapatas en el municipio de Caucasia, Antioquia, Colombia. Los tábanos se capturaron sobre equinos durante el día, usando frascos en una zona de ecotono entre bosque secundario y potreros. Las garrapatas se colectaron de forma

manual, sobre bovinos. El propósito del estudio fue identificar los posibles vectores de la tripanosomosis bovina y registrar la densidad y la diversidad de tábanos en la zona. Los artrópodos fueron llevados al laboratorio para su determinación taxonómica y búsqueda de protozoos en probóscide, intestino medio y glándulas salivales de las moscas. En las garrapatas los protozoos fueron buscados en hemolinfa. Se capturaron 140 tábanos correspondientes a cuatro géneros y nueve especies. *Lepiselaga crassipes* fue la especie más abundante (43.6%), con mayor densidad en julio y mayor actividad de picadura a las 14:00 h. La mayor diversidad de tábanos se registró en septiembre. Tres tábanos se encontraron infectados con flagelados compatibles con *Trypanosoma vivax*. Se colectaron 315 garrapatas de la especie *Boophilus microplus*, todas negativas a flagelados. Estos resultados sugieren transmisión de *T. vivax* por tábanos en la zona de estudio. Sin embargo, se debe determinar el estatus específico de los parásitos hallados por medio de técnicas moleculares y esclarecer el mecanismo de transmisión mediante estudios controlados.

Palabras clave. *Trypanosoma vivax*, Tabánidos, Garrapatas, Tripanosomosis bovina, Caucasia, Colombia.

INTRODUCTION

Bovine trypanosomosis is a hemoparasitic disease caused by a flagellate protozoan, *T. vivax vienni*, which affects bovines, ovines and buffalos (Hoare 1972, Wells *et al.* 1982, Gardiner 1989, Sandoval *et al.* 1996, Bolívar *et al.* 2006). This disease causes economic losses due to abortions, decreased milk production and high costs of treatment and veterinary services (Betancourt & Wells 1979, Guillen *et al.* 2001). In South America, *T. vivax* was introduced by animals carried from Africa, where *Glossina* flies are the biological vectors (Wells *et al.* 1982).

In Colombia *T. vivax* has been adapted to new environmental and ecological conditions causing endemic infections in cattle of warm zones although epidemiological aspects are still unknown (Benavides *et al.* 2004).

Some authors have reported mechanical transmission of *T. vivax* by tabanids and by stable flies of the genus *Stomoxys* (Gardiner & Wilson 1987, Otte & Abuabara 1991, Jones & Davila 2001). However, the roles of particular tabanid species and other biting flies are unknown.

In Colombia, the bovine trypanosomosis has been studied by Clarkson (1976), Betancourt & Wells (1979); Betancourt (1982), Otte & Abuabara (1991), and Otte *et al.* (1994). From those studies, the regions of Atlantic Coast, Middle Magdalena, Oriental plains, low Cauca and Cauca Valley have been recognized as transmission areas; in general, the disease is more common in regions until 1500m asl. These studies have incriminated tabanids as mechanical vectors of *T. vivax*. Otte & Abuabara (1991) confirmed experimentally the occurrence of mechanical transmission of *T. vivax* by *Tabanus nebulosus*.

The purpose of this study was to identify possible vectors of bovine trypanosomosis and register the diversity, abundance and seasonality of tabanids, as well as the tick species that infest cattle in Caucasia, Antioquia.

MATERIALS AND METHODS

The study was conducted from June to September 2006. The selected sample points were located in four farms: El Asombro (7° 57,593 North; 75° 10,808 West; 62 m a.s.l.),

Villa Cristina (7° 56,095 North; 75° 13,125' West; 79 m a.s.l.), Casa Blanca (8° 1,302 North; 75° 7,163 West; 70 m a.s.l) and Severa (8° 00,249 North; 75° 11,867 West; 58 m a.s.l); located in Cauca, north of Antioquia. Cauca is about 50 m a.s.l. and areas near Cauca River are seasonally flooded every year. Annual rainfall is about 1 200-1 300 mm and mean temperature and relative humidity are 28° C and 56.6%, respectively.

Once a month, tabanid collection on a horse was conducted continually from 9:00 a.m to sunset in the ecotone habitat of each farm, by two observers with the aid of hand nets (20 cm diameter and 30 cm deep, 75 cm handle length). Tabanids that landed on different parts of the body (head, stomach and legs) of the animal were carefully captured with plastic pots. During each collection, two observers remained near (1 to 3 m) the horse at all times and captured all flies landing on the horse. The flies were immediately put in an icebox. The horse was kept stationary [tied up], except for a 5-min walk every 30-min in a pre-defined transect (about 100m) along the ecotone zone. The purpose of the walk was to increase host attractivity and improve capturing, particularly of tabanids. The sampling was conducted for 2 days each month for 4 months.

At the end of each month, flies were transported to the entomology laboratory (Instituto Colombiano de Medicina Tropical – CES) in Medellín for identification and enumeration. Identification of collected tabanids was made with the aid of taxonomic keys (Wilkerson 1979, Fairchild 1984, Coscarón & Papavero 1993, Barros & Gorayeb 1996). Representative specimens of each identified species were incorporated into the entomological collection of the laboratory.

The collected tabanids were dissected to search for protozoans compatible with *T. vivax* in their midgut, salivary glands and proboscis.

In each farm a sample of cattle was studied for the presence of ticks. The collected ticks were kept for four days, after which each was analyzed for the presence of trypanosomatids in its hemolymph, following the technique described by Burgdorfer *et al.* (1973). The collected ticks were identified with the aid of taxonomic keys (Jones *et al.* 1972, Keirans & Clifford 1978).

Samples positive for flagellates were stained with Giemsa and intraperitoneally inoculated in mice of 20 days old.

RESULTS

A total of 140 tabanids were found, belonging to two subfamilies, Tabaninae and Chrysopsinae, and distributed in four genera: *Tabanus*, *Lepiselaga*, *Chrysops* and *Cryptotylus*, and nine species. The genus *Tabanus* was found in all sample points (table 1), followed by *Lepiselaga*, which was found in three sample points.

The most abundant species was *L. crassipes* (43.6%), which was found in three of the studied farms. *Chrysops variegata* (19.3%) was the second most abundant species, and was found in two farms. *Tabanus occidentalis* var. *dorsovitatus* (12.1%) was found in just one farm and *Tabanus claripennis* (10.0%) was found in two farms.

Other less conspicuous species found in this study were *Tabanus importunus*, *T. nebulosus*, *Tabanus albocirculus* and *Cryptotylus unicolor*. Some specimens belonging to genus *Tabanus* could not be identified to the species level. *L. crassipes* was found in three of the four samplings, with population peak abundance in July. *C. variegata* abundance peaked in June; *T. occidentalis* was only collected in September, which was also the month of highest species diversity (Figure 1). *L. crassipes* showed a biting peak at 14:00 h, as did *T. occidentalis*. *C. variegata* peaked at 9:00 h (figures 2-5).

Table 1. Relative abundante (RA) and Distribution of tabanids in four collecting sites at Antioquia.

| Specie | Villa Cristina | Severa | Casa Blanca | El Asombro | Total | RA (%) |
|--|----------------|-----------|-------------|------------|------------|--------------|
| <i>L. crassipes</i> Fabricius, 1805 | 14 | 41 | 0 | 6 | 61 | 43,6 |
| <i>C. variegata</i> DeGeer, 1776 | 24 | 0 | 3 | 0 | 27 | 19,3 |
| <i>T. occidentalis</i> Macquart, 1855 | 17 | 0 | 0 | 0 | 17 | 12,1 |
| <i>T. claripennis</i> Bigot, 1892 | 0 | 13 | 0 | 1 | 14 | 10,0 |
| <i>T. importunus</i> DeGeer, 1776 | 0 | 3 | 0 | 0 | 3 | 2,1 |
| <i>T. albocirculus</i> Hine, 1907 | 1 | 0 | 1 | 0 | 2 | 1,4 |
| <i>T. nebulosus</i> DeGeer, 1776 | 2 | 0 | 0 | 0 | 2 | 1,4 |
| <i>C. unicolor</i> Wiedemann,1828 | 0 | 0 | 1 | 0 | 1 | 0,7 |
| <i>C. chiriquensis</i> Fairchild, 1939 | 0 | 0 | 1 | 0 | 1 | 0,7 |
| <i>Tabanus</i> spp. | 3 | 7 | 1 | 1 | 12 | 8,6 |
| Total of captured tabanids | 61 | 64 | 7 | 8 | 140 | 100,0 |

RA: Relative abundance

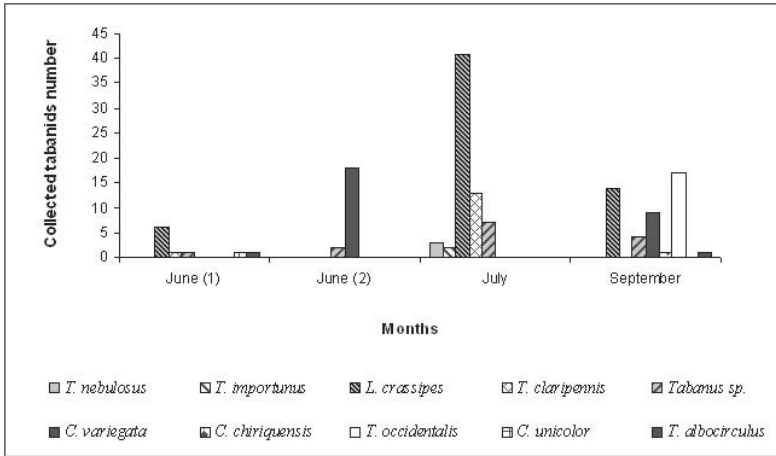


Figure 1. Monthly distribution of tabanid species collect on horses at Caucasia, Antioquia.

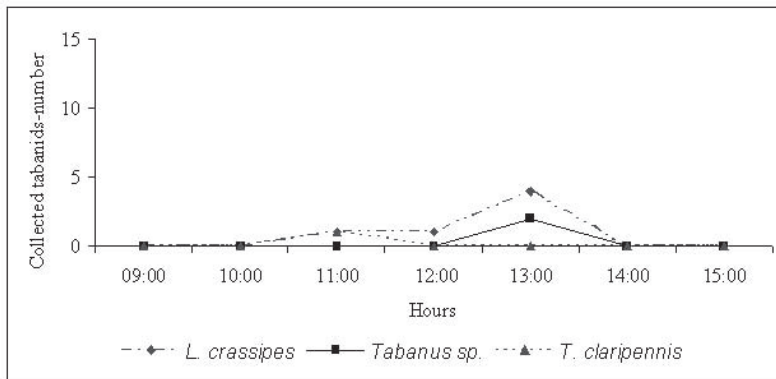


Figure 2. Diurnal biting activity of tabanids collected on horses at El Asombro farm.

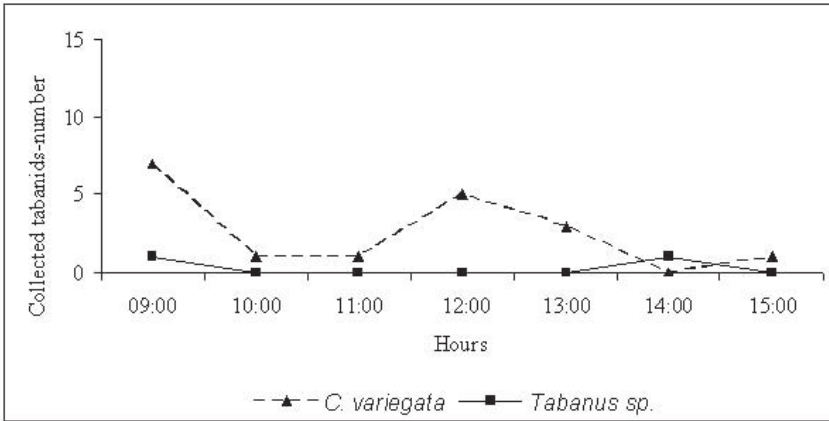


Figure 3. Diurnal biting activity of tabanids collected on horses at Villa Cristina farm. Sampling 1.

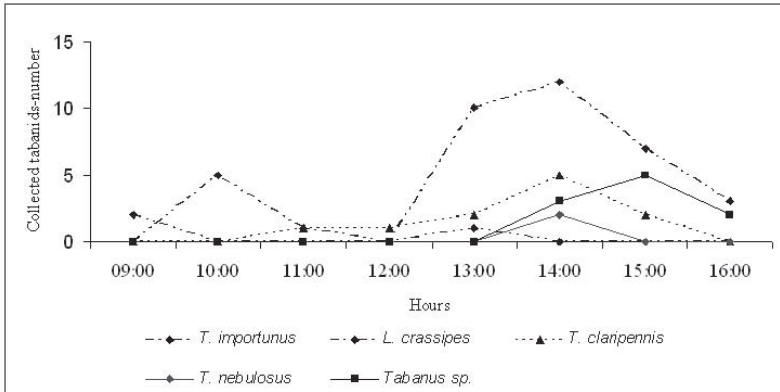


Figure 4. Diurnal biting activity of tabanids collected on horses at Severa farm.

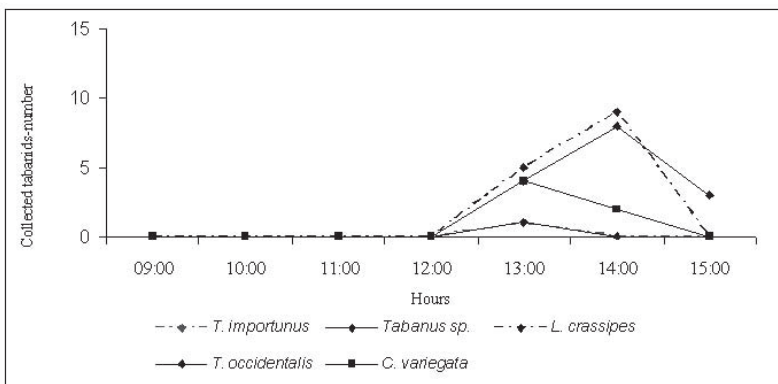


Figure 5. Diurnal biting activity of tabanids collected on horses at Villa Cristina farm. Sampling 2.

During the study period, the month of July has the highest temperature (33.6°C) and the lowest relative humidity (58.7%), and this coincided with the highest abundance of tabanids (figure 6). In general, relative

humidity and temperature values have a strong variation during the study months. Some of the most representative species of this study are presented in figure 7.

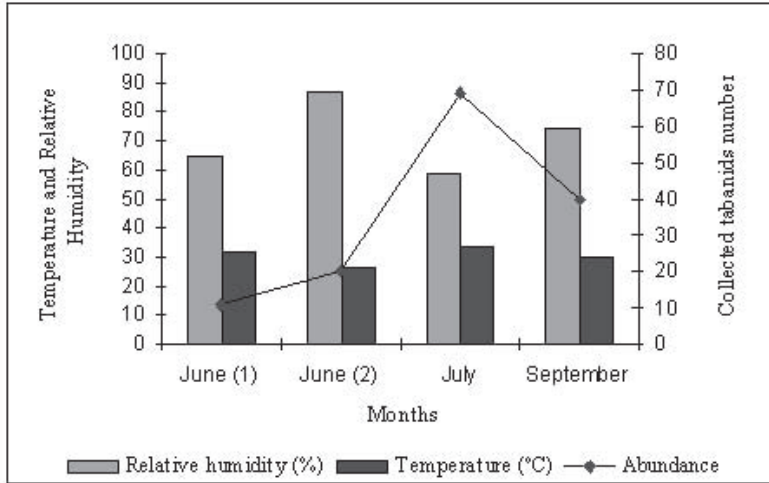


Figure 6. Climatic data and tabanid abundance, from June to September 2006, at Antioquia.

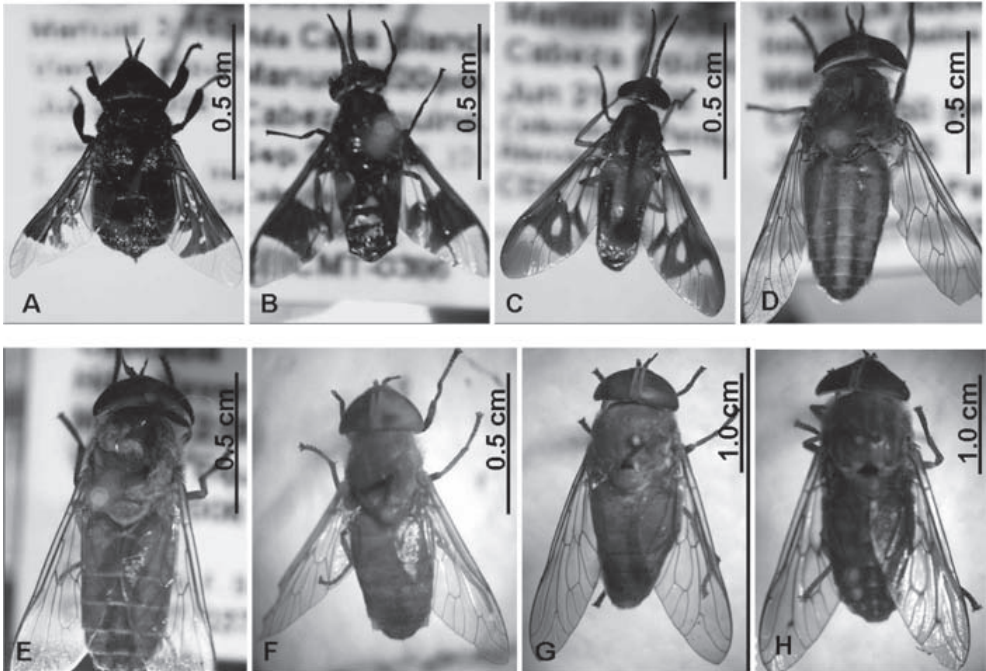


Figure 7. Some species of tabanids that occurs at Cauca, Antioquia. A: *L. crassipes* B: *C. chiriquensis* C: *C. variegata* D: *T. occidentalis* E: *T. claripennis* F: *C. unicolor* G: *T. nebulosus* H: *T. importunus*. Pictures by Gabriel Parra and Erika Alarcón.

In the parasitological analysis of the collected tabanids we found that three of them have flagellates in their midgut. Two of them were *T. occidentalis* and the other was *T. nebulosus*. In addition, one of the positive *T. occidentalis* also was positive-for flagellates in the salivary glands.

The flagellates found in the tabanids were morphologically compatible with *T. vivax*. However, after mouse inoculation with the flagellates, no parasitemia was observed after two months. All positive tabanids were collected in the same farm (Villa Cristina).

A total of 315 ticks was collected, all *B. microplus*. The distribution between farms was: Villa Cristina (52.5%), Casa Blanca (22.5%), Severa (19.0%), and El Asombro (6.0). No tick was found to be positive for *Trypanosoma* in the hemolymph tests.

DISCUSSION

The present study extends the knowledge about the diversity of tabanids species present in the Caucasia municipality. The highest abundance of tabanids was found in July, that was the month with the higher temperature and the lowest relative humidity during the sampling period. This is in accordance with the work of Koller *et al.* (2002), Barros (2001) y Barros & Foil (1999), who investigated the seasonal abundance of tabanids in the Pantanal region of Brazil and found an increase of the horse fly population during the first part of the rainy season and an increase of the populations by the end of that period.

As indicated by Kettle (1995), the activity and dispersion of adult tabanids is influenced by meteorological factors, principally luminosity and temperature.

The highest diversity of tabanids was in September, corresponding to the dry climatic period, this finding was in accordance with

Velásquez de Ríos *et al.* (2004) in Guarico State, Venezuela; Gorayeb (1985) in east Amazonian region of Brazil and Barros & Foil (1999) in Pantanal, Brazil.

Of the nine collected species, *L. crassipes* had the highest relative abundance (43.6%) and general tabanid populations peak was mainly due to this species, and similarly findings were reported by Barros (2001). Remaining species showed low relative abundances, fluctuating between 0.7 and 19.3% (table 1, figure 1).

L. crassipes has a wide distribution in America, with records from Mexico to north of Argentina including the Caribbean islands of Cuba, Jamaica, and Puerto Rico. In Colombia *L. crassipes* have been found in the departments of Chocó, Valle, Cauca, Antioquia, Córdoba and Amazonas. The biting peak of *L. crassipes* (14:00 h), coincided with the reports of Wilkerson (1979) who found that the biting peak for that species was at 13:00 h.

C. variegata was the second most abundant species and was most conspicuous on June, its geographical range goes from Mexico to Argentina, including the Antilles. In Colombia, *C. variegata* was registered at the departments of Chocó, Valle, Cauca, Antioquia, Santander, Magdalena, Meta, Putumayo and Vaupés. Bequaert & Rengifo (1946) and Porter (1976), both cited by Wilkerson (1979), reported the presence of *C. variegata* in the municipality of Caucasia (Antioquia department).

T. occidentalis has a distribution range from Mexico to Argentina, including the Trinidad Island (Wilkerson 1979, Fairchild 1986).

T. claripennis occurs in the Antilles, Colombia (Cauca, Córdoba, Magdalena, Meta and Valle departments) to Brazil, Argentina, Chile and Paraguay (Wilkerson 1979, Gorayeb 1985, Barros 2001).

T. claripennis did not show marked biting peaks but in general was more conspicuous at 14:00 h, our findings were not different of Wilkerson (1979) who reported a peak at 15:00 h.

It is known that *T. vivax* is transmitted cyclically in Africa by flies of the genus *Glossina* and is accepted that in America the transmission is mechanical (Gardiner & Mahmoud 1990). In Latin America there is no presence of *Glossina* flies and it is considered that the transmission of *T. vivax* is carried out by tabanids and flies of the genus *Stomoxys* (Gardiner & Wilson 1987, Mihok *et al.* 1995, Desquesnes 2004).

Mechanical transmission of *T. vivax* has been proved in experimental conditions (Ferenc *et al.* 1988, Raymond 1990, Otte & Abuabara 1991). In general, is accepted that the vector population abundance is an important factor to the transmission of pathogen agents, however, although *L. crassipes* was the most abundant species, it was not found the presence of flagellates in the midgut and in salivary glands of the analyzed insects. This could be explained because Trypanosomes can be found inside flies for a relatively short period of time (usually less than 24h) after feeding on an infected host. However, we found the presence of flagellates compatible with *T. vivax* in the midgut and salivary glands of *T. occidentalis*. These results suggest *T. vivax* transmission by tabanids in the study area. However, the specific status of the parasites should be determined by molecular techniques because it has been demonstrated that morphologically is hard to differentiate epimastigote stage of *Blastocrithidia* from epimastigote stage of *Trypanosoma* present in tabanids (Hoare 1972, Krinsky & Pechuman 1975, Desquesnes 2004) and the transmission mechanism should be established too by controlled studies.

Studies about transmission of *T. vivax* in the New World are scarce and are suspected

the presence of a biological vector that disseminates the disease (Betancourt 1978). In Colombia, the only study about *Trypanosoma* mechanical transmission by hematophagous dipterans was developed by Otte & Abuabara (1991), however, Wells *et al.* (1970) are doubt about the importance of mechanical transmission for the maintenance of endemic situations. Other experimental studies carried out elsewhere have confirmed this type of transmission by tabanids in South America (Ferenc *et al.* 1988, Raymond 1990) as well as in Africa (Desquesnes & Dia 2004).

López *et al.* (1979) suggested that *B. microplus* ticks could be potential vectors of *T. vivax*; in a laboratory study a splenectomized Holstein calf was inoculated with an identified *T. vivax* strain and later infested with *B. microplus* larvae which were found to be positive for the parasite in hemolymph, ovaries and salivary glands after the parasitic cycle. The current study did not find evidence of *T. vivax* in ticks. The possible absence of circulating parasites in the cattle may explain these results. Shastri & Deshpande (1981), Morzaria *et al.* (1986), and Ribeiro *et al.* (1988), cited by Rodríguez *et al.* (2003) have found *Trypanosoma* in different ticks species.

ACKNOWLEDGEMENTS

To Dirección de Investigaciones and Facultad de Medicina Veterinaria y Zootecnia, CES University for financial support. To Dr. Antonio Thadeu M Barros (Embrapa, Brazil) for taxonomic confirmation of the tabanid species. To Dr. Miguel Delgado, Asogauca Hemoparasites lab director and to Dra. Bertha Nelly Restrepo ICMT-CES.

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Recibido: 19/01/2007

Aceptado: 01/01/2008