

E. Kyewalabye Kaggwa^{1*} | ***Babesia equi* and *Trypanosoma vivax***
 I. A. Lawal¹ | **infections in donkeys**

KYEWALABYE KAGGWA (E.), LAWAL (I. A.). Infections à *Babesia equi* et *Trypanosoma vivax* chez les ânes. *Revue Élev. Méd. vét. Pays trop.*, 1989, 42 (2) : 205-210.

Six ânes (*Equus asinus*) ont été achetés sur des marchés locaux. Avant et pendant les infections par *Trypanosoma vivax*, pour détecter *B. equi*, les techniques suivantes ont été employées : étalement de sang et gouttes épaisses, prise de température, centrifugation hématocrite, volume globulaire total, numération globulaire et immunofluorescence indirecte. Pour cette dernière épreuve, un conjugué anti-cheval a été utilisé. En dépit des parasitémies à *T. equi* ou à *T. vivax*, la température des ânes est restée inférieure à 38,5 °C. Le volume globulaire total s'est abaissé davantage lors de l'infection à *B. equi* que lors de celle à *T. vivax*. Quatre ânes sur 6 avaient des anticorps contre *B. equi* alors que 2 seulement présentaient une parasitémie décelable. Le traitement, soit avec le BérénilND, soit avec l'ImizolND, a éliminé la parasitémie décelable à *B. equi*, et le test d'immunofluorescence indirecte était négatif 35 à 45 jours après le traitement. Cependant des rechutes se sont produites 60 à 70 jours après traitement. Dans deux cas, les titres sérologiques étaient inférieurs à 1:40 (c'est-à-dire négatifs) alors que la parasitémie était manifeste. *Mots clés* : Ane - *Babesia equi* - *Trypanosoma vivax* - Babésiose - Trypanosomose - Technique immunologique - Infection expérimentale - Nigeria.

INTRODUCTION

Babesia equi is a parasite of equine species while *Trypanosoma vivax* is of particular importance in domestic ruminants. After an infection with either of the parasites, some of the haematological changes indicative of infection are parasitaemia, anaemia and presence of serum antibodies. Therefore, these and other parameters have been used in diagnosis and in determination of pathogenicity of the various parasite species in a given animal host, e.g. in cattle, sheep and goats infected with *Trypanosoma vivax* (1, 4). However, before such parameters can be meaningful in an experiment, it is important to start with animals that are as much infection-free as possible. The inadequacy of breeding centres of experimental large animals in Africa does not allow all researchers to have animals with known history. Thus in an experiment to determine the pathogenicity of *T. vivax* in donkeys (7), the animals used were bought in a

market. Most donkeys in Nigeria have been reported to be carriers of *B. equi* (5, 8), a conclusion made when experimental splenectomy of several donkeys, or infection with *T. evansi*, resulted in patent *B. equi* parasitaemia. Splenectomy, however, cannot be performed invariably prior to all experiments for screening purposes, since the operation might result in altered hosts' response to various infections.

Thus, methods which are more sensitive than the blood smear examination, in intact animals, are most useful for screening purposes. The indirect fluorescent antibody test (IFAT) has been found more reliable than the complement fixation test (CFT) in detection of *Babesia* infections in horses (6, 11, 14). Its use in donkeys has not been reported, but an anti-donkey conjugate is not as easily available as the anti-horse one.

The aim of this paper is to report on the occurrence of *B. equi* parasites, the IFAT antibodies using a rabbit anti-horse IgG conjugate, and the haematological changes in donkeys experimentally infected with *T. vivax*.

MATERIALS AND METHODS

Six donkeys (*Equus asinus*) 1.5 - 2 years old, were bought locally. They were kept without grazing in fly-proof but not tick-proof pens. They were examined for blood parasites using thin and thick blood smears, haematocrit centrifuge technique (HCT) and mice inoculation. In an effort to eliminate other infections before an experimental infection with 4 x 10⁷ *Trypanosoma vivax* parasites (7), all donkeys were treated with diminazene aceturate (BerenilTM, Hoechst), 3.5 mg/kg, and perbendazole 20 mg/100 kg. After one month, 3 of the donkeys were infected intravenously with the trypanosomes while 3 were left uninfected.

Some of the tests performed and parameters monitored at various intervals during the 150 days period of observation were HCT, parasitaemia using thin and thick blood smears, packed cell volume (PCV), total white blood cell counts, and *B. equi* antibody levels determined with the indirect fluorescent antibody test (IFAT).

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The IFA test was performed following the method of BROCKLESBY *et al.* (2) with minor modifications. Two-fold serum dilutions were used, starting at 1:40 up to 1:2560. However, the conjugate used was a commercial rabbit anti-horse fluoresceine-labelled immunoglobulin. The negative and positive control sera were from a horse. Antibody titres equal to or below 1:40 were considered negative in the test.

RESULTS

The temperatures, total white blood cell counts, packed cell volume, reciprocal antibody titre to *B. equi* and parasitaemia for *B. equi* and *T. vivax*, in each of the six donkeys, are given in figures 1, 2, 3, 4, 5, 6.

At the time of purchase, two donkeys (Nos. 3287 and 3289) were parasitologically positive for *B. equi* (Fig. 1, 2). The infection was accompanied by a drop in PCV in No. 3289 though not in 3287. Treatment with

Berenil™ 3.5 mg/kg cleared detectable parasitaemia, and antibodies were not detectable at 37 days post-treatment in the two donkeys.

Figures 1, 3 and 5 show donkeys (Nos. 3287, 3286, 3288 respectively) that were infected with *T. vivax*. During the days 15-80 post-infection none of these donkeys showed *B. equi* parasitaemia, and only No. 3286 (Fig. 3) developed a low antibody titre. The PCV values were slightly depressed at 50-70 days, coinciding with trypanosome parasitaemia. Other clinical parameters during this period have been reported (6). Also during this time the total white blood cell counts were increased though not to abnormal ranges. There was a comparable increase in No. 3289 (Fig. 2) which animal had no *T. vivax* but was treated with babesicidal drugs against *B. equi* infection.

Beyond one month of infection, trypanosomes continued to be detected in blood, the highest parasitaemia, seen at only two occasions, was 10-15 trypanosomes per capillary haematocrit tube in the HCT method during the 2nd and 3rd month of infection. Otherwise

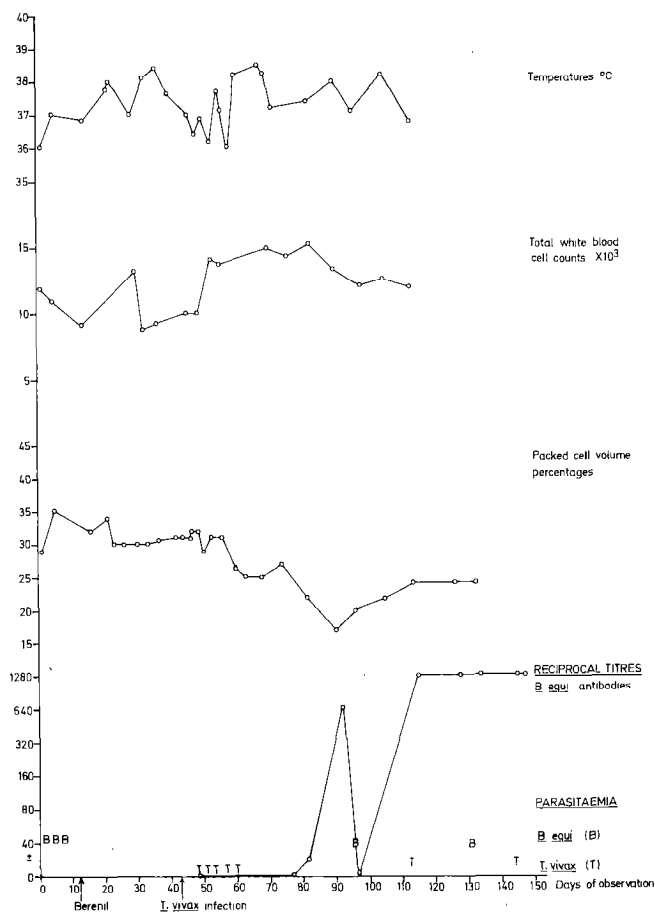


Fig. 1 : *Babesia equi* serological and other haematological observations of donkey No. 3287.

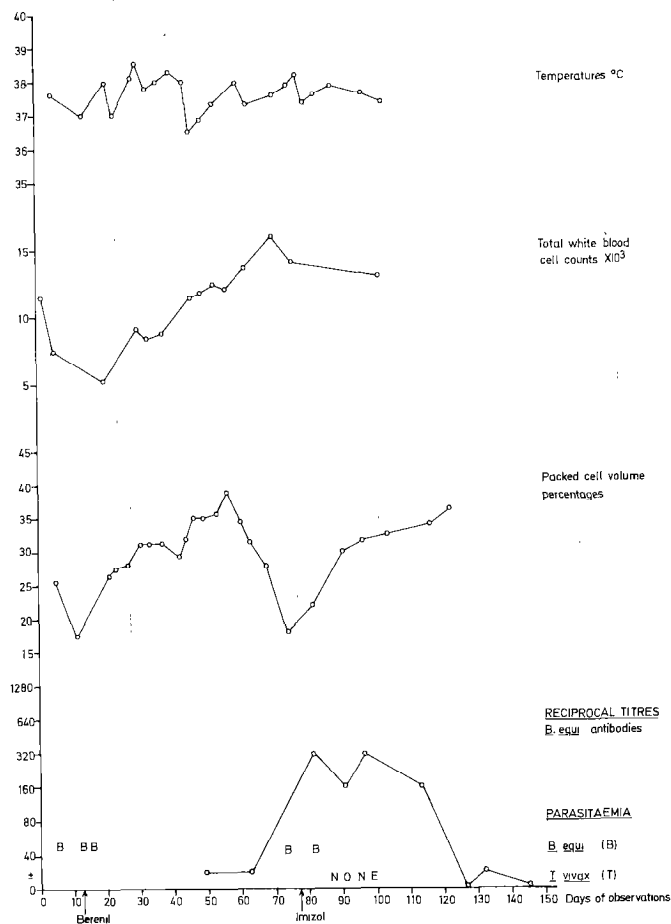


Fig. 2 : *Babesia equi* serological and other haematological observations of donkey No. 3289.

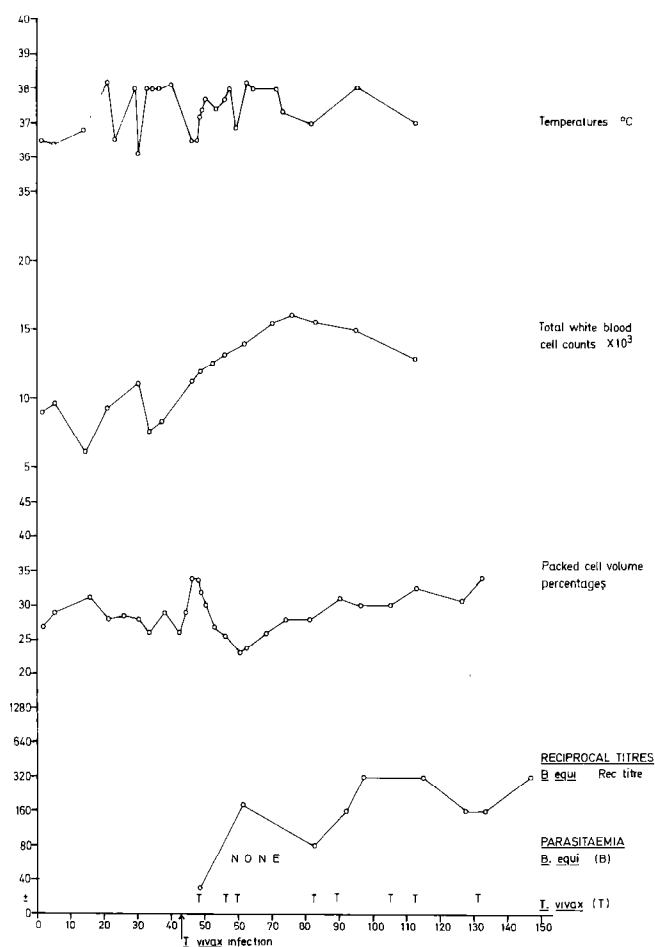


Fig. 3 : *Babesia equi* serological and other haematological observations of donkey No. 3286.

parasitaemia was always very low and not detectable on every occasion; no detectable haematological abnormalities in donkey 3286 (Fig. 3), and only slightly lowered PCV values in donkey 3288 (Fig. 5), while the depression in PCV values in No. 3287 (Fig. 1) is due to both *T. vivax* and *B. equi*.

At days 80 and 73 of observation, *B. equi* antibodies and parasitaemia were detected in donkeys 3287 (Fig. 1) and 3289 (Fig. 2) respectively. The rise in antibodies coincided with big drops in PCV, the drop being greater than that in No. 3286 (Fig. 3) which showed only *T. vivax*. This time the *B. equi* infection in 3287 (Fig. 1) was not treated; the animal looked sick unlike the others with *T. vivax*, and superficial lymphnodes were swollen. Its antibody titres remained at 1:1280. No. 3289 (Fig. 2) was treated with Imizol™ (Wellcome Foundation Ltd.), and its antibodies could not be detected at day 45 post-treatment, while the PCV values picked up better than in 3287.

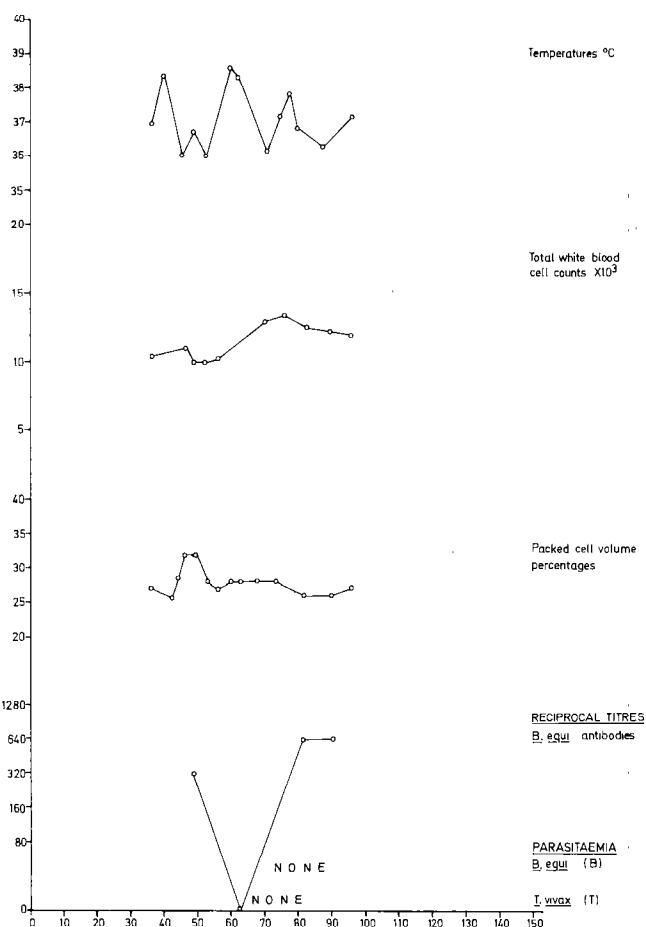


Fig. 4 : *Babesia equi* serological and other haematological observations of donkey No. 3290.

Two donkeys, 3286 (with *T. vivax*, Fig. 3) and 3290 (without *T. vivax*, Fig. 4), were serologically positive for *B. equi* but parasitaemia was never detected. Their titres ranged between 1:40 to 1:640. Their PCV dropped slightly, more in 3286 than in 3290, while the WBC counts increased slightly in 3286.

Two donkeys Nos. 3288 (with *T. vivax*, Fig. 5) and 3291 (without *T. vivax*, Fig. 6) were serologically and parasitologically negative for *B. equi* throughout the period of observation. In the *T. vivax*-infected 3288, the PCV values were slightly lower while the WBC counts tended to be higher than in the non-infected one.

DISCUSSION

There was no definite pyrexia in spite of the varying degrees of parasitaemia and anaemia due to either or

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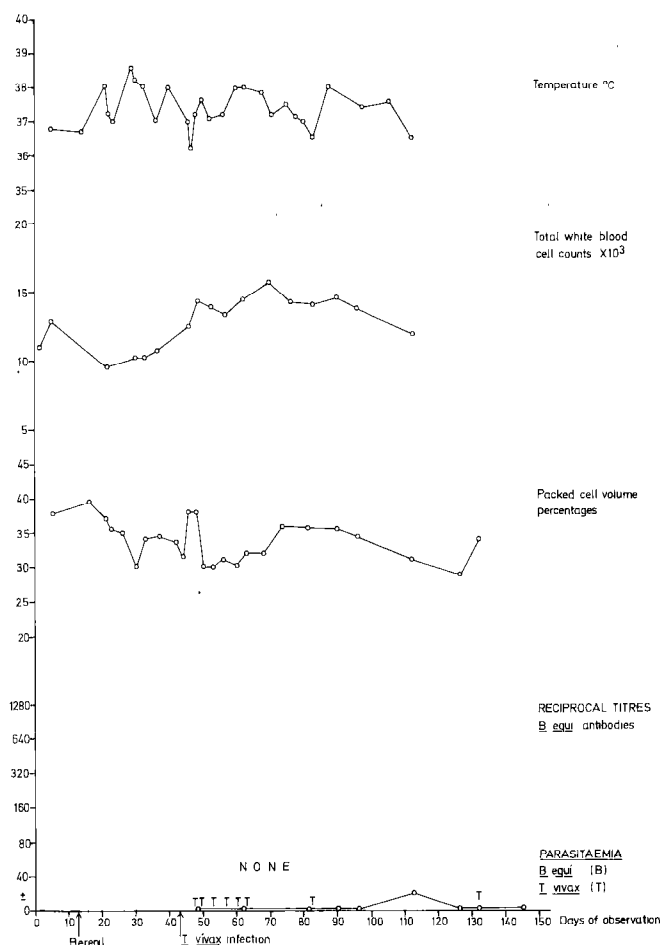


Fig. 5 : *Babesia equi* serological and other haematological observations of donkey No. 3288.

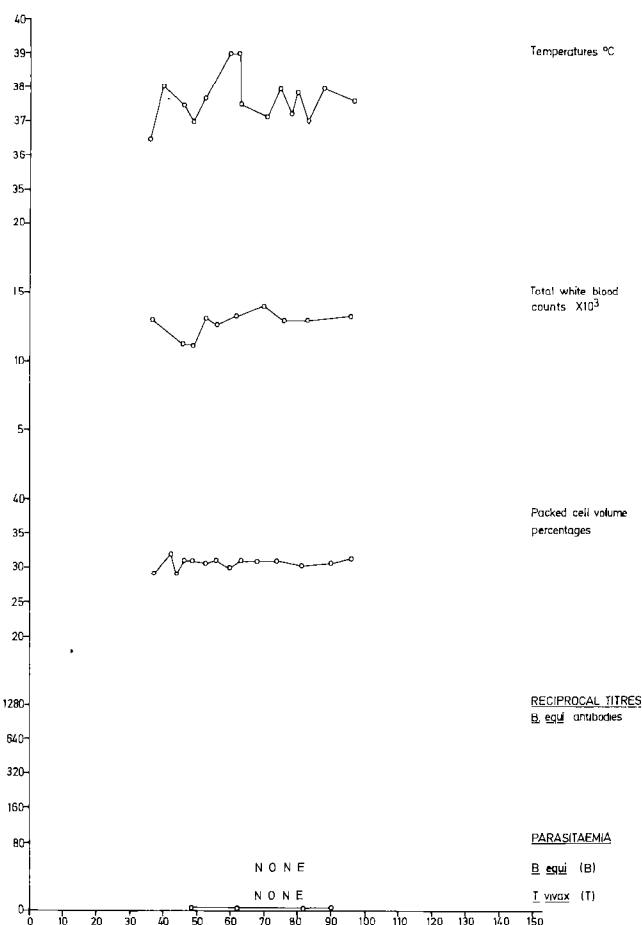


Fig. 6 : *Babesia equi* serological and other haematological observations of donkey No. 3291.

both of the infections. This is not very surprising in case of *B. equi*, since the donkeys may have been long time « healthy carriers », with, perhaps parasitaemic relapses from time to time, and such relapses, in other animal species, are not associated with pyrexia. On the other hand, the *T. vivax* infection may have been too mild to induce a temperature reaction.

Babesia equi appeared to induce greater blood loss than *T. vivax* during the period of observation. Changes in the white cell counts seem to be inconclusive in this experiment where a general increase during *T. vivax* infections has been observed ; but the transient leucopenia reported in *B. equi* infections in horses (10), would agree with the rising of counts in the « treated » *B. equi* donkeys.

The findings of 4 out of 6 donkeys being serologically positive for *B. equi* confirm earlier observations (8) that *B. equi* is common in donkeys in Nigeria.

The treatment with Berenil™ or Imizo™ was just an attempt, since we had no effective drug available against this parasite. The low susceptibility to babesicidal drugs is one of the arguments, as reviewed by UILENBERG (12), that would support the reclassification of *Babesia equi* as *Theileria* spp. rather than *Babesia* sp. Parasitaemia, low in any case, disappeared however. The fact that antibodies were not detected at 34-45 days post-treatment is surprising, especially when antibodies and relapse parasitaemia, believed to originate from recrudescence infections, were detected in just 60-77 days post-Berenil™ treatment. Even if recovery were to have occurred, still within this short period antibodies should still be detectable. Work in horses (14) showed antibodies to be detectable up to 3-4 months after successful treatment of *B. caballi* with Berenil™. No work has been found using IFAT in donkeys for detection of either *B. caballi* or *B. equi*. In horses, however, the test is not reported to give false negatives, unlike the complement fixation test (CFT) (3, 6, 11).

The absence of detectable antibodies for that period could be due to the use of an anti-horse rather than anti-donkey conjugate, leading to lower detection level. This could be supported by the recording of only 1:1280 as the highest titre while in horses (our control serum inclusive) titres above 1:5000 have been reported. Another probability is that the IFA test (at least when performed as it was) might sometimes give false negative results for *B. equi*. This has been further exemplified by No. 3287, day 96, and No. 3290, day 62, even when parasitaemia was detectable in one case. Similar observations have been made with regard to cattle *Theileria* sp. where antibodies would sometimes disappear although the animals remained carriers of either *T. mutans* (13) or *T. parva* (9). More recently (EEC/RUU/ABU Project Report, unpublished data), field samples had a relatively high percentage of parasitologically positive but serologically negative *T. mutans* cases, and also an experimental calf carried *T. mutans* but showed no detectable IFA antibodies.

The general efficacy of IFA test in the diagnosis of individual cases of *Theileria* sp. can therefore be difficult to state, but should *B. equi* be considered to be a *Theileria* sp., then this is another factor they might have in common.

It could be concluded from our observations that *B. equi* is a slightly more pathogenic parasite than *T.*

vivax in donkeys. The latter infection, after three months, induced only gradual and slight drops in the PCV, which did not go below normal ranges. The use of an anti-horse conjugate allows detection of *B. equi* infections in the donkey, giving titres of 1:80-1:1280. A few weeks after therapy, however, it does not seem to give realistic results in spite of the assumed presence of recrudescing parasites. The IFAT test has shown some false negative reactions (< 1:40) for *B. equi*, at least when using the anti-horse conjugate in donkeys.

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KYEWALABYE KAGGWA (E.), LAWAL (I. A.). *Babesia equi* and *Trypanosoma vivax* infections in donkeys. *Revue Élev. Méd. vét. Pays trop.*, 1989, 42 (2) : 205-210.

Six donkeys (*Equus asinus*) were purchased locally. To screen them before and during *Trypanosoma vivax* infection, thin and thick blood smears, temperature, haematocrit centrifuge technique (HCT), packed cell volume (PCV), white blood cell counts, and indirect immunofluorescent antibody test (IFAT) were done for *Babesia equi*. For the IFAT, an anti-horse conjugate was used. In spite of patent *B. equi* or *T. vivax* parasitaemia, the donkeys' temperatures remained below 38.5 °C ; PCV was depressed more in *B. equi* infection than in *T. vivax* infection. Four out of the 6 donkeys had *B. equi* antibodies while 2 of them had detectable parasitaemia. Treatment with either Berenil™ or Imizol™ cleared the detectable *B. equi* parasitaemia, and IFAT was negative at 35-45 days post treatment. However, relapses occurred within 60-70 days after the treatment. In 2 circumstances serological titres were below 1:40 (negative) while there was detectable parasitaemia. *Key words* : Donkey - *Babesia equi* - *Trypanosoma vivax* - Babesiosis - Trypanosomosis - Immunological test - Experimental infection - Nigeria.

KYEWALABYE KAGGWA (E.), LAWAL (I. A.). Infecciones por *Babesia equi* y *Trypanosoma vivax* en los asnos. *Revue Élev. Méd. vét. Pays trop.*, 1989, 42 (2) : 205-210.

Se compraron seis asnos (*Equus asinus*) en mercados locales. Para evidenciar *B. equi* antes y durante tripanosomosis por *T. vivax*, se utilizaron las técnicas siguientes : extensión de sangre y gotas espesas, toma de temperatura, centrifugación hematocrita, volumen globular total, recuento de glóbulos e inmunofluorescencia indirecta. Para la última prueba, se utilizó un conjugado anti-caballo. A pesar de las parasitemias por *T. equi* o *T. vivax*, la temperatura de los asnos quedó inferior a 38,5 °C. El volumen globular total bajó más durante la infección por *B. equi* que durante la por *T. vivax*. Cuatro asnos de 6 tenían anticuerpos contra *B. equi* mientras que 2 sólo mostraban una parasitemia patente. El tratamiento, sea con el BerenilND, sea con el ImizolND, eliminó la parasitemia a *B. equi* y la prueba de inmunofluorescencia indirecta fue negativa 35 a 45 días después del tratamiento. Sin embargo, recaídas ocurrieron 60 a 70 días después del tratamiento. En dos casos, los títulos serológicos era inferiores a 1:40 (es decir negativos) mientras que la parasitemia era evidente. *Palabras claves* : Asno - *Babesia equi* - *Trypanosoma vivax* - Babesiosis - Tripanosomosis - Técnica inmunológica - Infección experimental - Nigeria.

REFERENCES

1. ANOSA (V. O.), ISOUN (T. T.). Experimental *T. vivax* infection in sheep and goats: the relationship between the parasitaemia, the growth rate and the anaemia. *J. Nig. vet. Med. Ass.*, 1974, **3**: 102-108.
2. BROCKLESBY (D. W.), ZWART (D.), PERIÉ (N. M.). Serological evidence for the identification of *Babesia major* in Britain. *Res. vet. Sci.*, 1971, **12**: 285-287.
3. DONNELLY (J.), JOYNER (L. P.), OLIVER GRAHAM-JONES, ELLIS (C. P.). A comparison of the complement fixation and immunofluorescent antibody tests in a survey of the prevalence of *Babesia equi* and *Babesia caballi* in horses in the Sultanate of Oman. *Trop. Anim. Hlth Prod.*, 1980, **12** (1): 50-60.
4. FIENNES (R. N. T. -W.). The cattle trypanosomiases. Some considerations of pathology and immunity. *Ann. trop. Med. Parasit.*, 1950, **44**: 42-54.
5. ILEMOBADE (A. A.). In: Proceedings of 13th Meeting of the International Scientific Council for Trypanosomiasis Research, Lagos, 1971. Pp. 107-114. (CCTA Publications No. 105).
6. KUTTLER (K. L.), GOFF (W. L.), GIBSON (C. A.), BLACKBURN (B. O.). Serologic response of *Babesia equi* infected horses as measured by complement-fixation and indirect fluorescent antibody tests. *Vet. Parasit.*, 1988, **26**: 199-205.
7. KYEWALABYE KAGGWA (E.), KWARI (H. D.), AJAYI (M. O.), SHINGGU (P.). Clinical parameters of donkeys before and after *T. vivax* infection. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (3): 265-269.
8. LEEFLANG (P.), ILEMOBADE (A. A.). Tick-borne diseases of domestic animals in Northern Nigeria. II. Research summary, 1966 to 1976. *Trop. Anim. Hlth Prod.*, 1977, **9**: 211-218.
9. ROBSON (J.), PEDERSEN (V.), UILENBERG (G.), ODEKE (G. M.). Theileriosis in Uganda. Parasitological and serological responses in cattle continually exposed to natural infection. *Trop. Anim. Hlth Prod.*, 1981, **13**: 1-11.
10. TAYLOR (W. M.), BRYANT (J. E.), ANDERSON (J. B.), WILLERS (K. H.). Equine piroplasmiasis in the United States. A review. *Am. vet. Med. Ass.*, 1969, **155**: 915-919.
11. TENTER (A. M.), FRIEDHOFF (K. T.). Serodiagnosis of experimental and natural *Babesia equi* and *B. caballi* infections. *Vet. Parasit.*, 1986, **20**: 49-61.
12. UILENBERG (G.). Highlights in recent research on tick-borne diseases of domestic animals. Invited review. *J. Parasit.*, 1986, **72** (4): 485-491.
13. UILENBERG (G.), SILAYO (R. S.), MPANGALA (C.), TONDEUR (W.), TATCHELL (R. J.), SANGA (H. J. N.). Studies on *Theileriidae* (Sporozoa) in Tanzania. X. A large-scale field trial on immunisation against cattle theileriosis. *Tropenmed. Parasit.*, 1977, **28**: 499-506.
14. WEILAND (G.). Species-specific serodiagnosis of equine piroplasma infections by means of complement fixation test (CFT), Immunofluorescence (IIF), and enzyme linked immunosorbent assay (ELISA). *Vet. Parasit.*, 1986, **20**: 43-49.