ITMA – MSCTAH thesis, N° 16, 2003-2004

EXPERIMENTAL INFECTION OF *TRYPANOSOMA CONGOLENSE* IN PIGS AND A CONTRIBUTION TO THE IMPROVED DIAGNOSIS OF TRYPANOSOMOSIS IN PIGS

Dr. Nynke Deckers

"Thesis presented in fulfilment of the requirements for
the degree of Master of Science in Tropical Animal Health"

Prince Leopold Institute of Tropical Medicine,
Department of Animal Health
Antwerpen (Antwerp), Belgium

Thesis of Master of Science in Tropical Animal Health

Presented and defended on the 13th of July 2004
at the Prince Leopold Institute of Tropical Medicine (ITMA)
Antwerpen (Antwerp), Belgium

Members of the jury:

Dr G. Hendrickx, Avia-GIS, Chairman
Prof. Dr S.Geerts, Dept Animal Health, ITM, Member
Dr J. Van den Abbeele, Dept Parasitology, ITM, Member
Dr J. de Borchgrave, Dept Animal Health, ITM, Member
Dr V. Delespaux, Dept Animal Health, ITM, Member
Dr P. Van den Bossche, Dept Animal Health, ITM, Member
Dr R. De Deken, Dept Animal Health, ITM, Promoter, Secretary

Dedication

I would like to dedicate this work to my parents without whom I would not have been able to follow this course. I would like to thank them not only for the financial support I received once more this year, but most of all for their ever present support and trust in my skills. They have never let me down, I will try to do the same for them by pursuing my dreams and becoming the professional veterinarian I always wanted to be.

Mama en papa, bedankt voor alles!

I would also like to thank Jes for her listening ear and for saying a lot with little words.

Jes, op de rest van ons leven!

Acknowledgements

I would like to thank my coaches Dr. De Deken and Dr. Van den Bossche for their support and advise in writing this thesis. I would also like to thank Dr. Brandt for helping me to adjust the ELISA protocol. Furthermore, a big thank you to Dr. Kanobana and the laboratory technicians Marc, Björn, Co and Frank for their daily assistance in the lab. I am also very grateful for the help I received from Martin and Christopher and the other MSc students.

Table of contents

Tables

Figures

List of abbreviations and acronyms

CNS: central nervous system
CSF: cerebrospinal fluid
DG: darkground buffy coat technique
DRC: Democratic Republic of Congo
DTPA: diethylenetriamine penta-acetic acid
EDTA: ethylenediamine tetra-acetic acid
ELISA: enzyme-linked immunosorbent assay
FAO: Food and Agriculture Organisation
HCT: hematocrite centrifuge technique
IAEA: International Atomic Energy Agency
IFAT: indirect fluorescent antibody test
NBCS: new born calf serum
OD: optical density
OPD: ortho-phenylene diamine
PBS: phosphate buffered saline
PCR: polymerase chain reaction
PCV: packed cell volume
PP: percentage positivity
RFLP: restricted length polymorphism
SD: standard deviation
SDS: sodium dodecyl sulphate

Keywords

Trypanosomosis, *Trypanosoma congolense*, pigs, antibody ELISA

Summary

Pig trypanosomosis is becoming increasingly important with growing commercial pig production. Pigs are also potential reservoir hosts for trypanosome species that cause human sleeping sickness. Since parasitological methods are not very sensitive in detecting low parasitaemias in pigs, serological methods that detect anti-trypanosomal antibodies, such as the antibody-detecting ELISA, are often preferred for epidemiological surveys.

In this work, 6 pigs of the Belgian landrace were infected with *Trypanosoma congolense* and followed up during 4 weeks. The rectal temperature, packed cell volumes (PCV), parasitaemia and antibody response were monitored. For this last purpose, an indirect ELISA test using an "in-vitro" cultured antigen of *Trypanosoma congolense* was elaborated to detect anti-trypanosomal antibodies starting from both serum samples as well as full blood samples collected on filter paper.

The pigs did not show overt clinical symptoms notwithstanding trypanosomes could be demonstrated in 5 of the 6 pigs from 2 weeks after the infection onwards. However, the parasitaemia was low and scanty. Antibody levels started to rise 2-3 weeks after the infection, but the individual response was highly variable.

In a second part of the study the ELISA-test was used to examine 493 porcine blood samples from 171 farms in the vicinity of Kinshasa (Democratic Republic of Congo). Positive farms were found in distinct locations: most of these farms were found in Mont Ngafula, Kisenso and Selembao districts. All the samples from N'djili and most of the samples from Masina were negative.

Further research using species specific testing (PCR) of infected pigs will examine whether pigs are reservoir host for humans sleeping sickness in that area.

1. Introduction

Trypanosomosis is a major limiting factor to large-scale and profitable livestock production in the tsetse-infested areas of Africa. This is not only due to apparent clinical disease and mortality but also to unapparent production losses, especially in commercial farming systems. This is also the case for trypanosomosis in pigs. Indeed, the importance of pig trypanosomosis is likely to increase with increasing commercial pig production. Semi-industrial production systems, mainly pigs and poultry, are appearing around all major African cities. In the peri-urban areas of Kinshasa (Democratic Republic of Congo), pigs and poultry are already the most important livestock present (Pers. com., De Deken R., 2004).
Porcine trypanosomosis is not only of economic importance for pig rearing but also has important public health repercussions. Pigs can be carriers of *Trypanosoma brucei gambiense* and *T. brucei rhodesiense* and thus be the reservoir of trypanosome species that can infect man (Gibson *et al*. 1978; Waiswa *et al.*, 2003). In the Republic of Congo, pigs are considered to play a role as reservoir of sleeping sickness (Truc *et al*., 1991). Further studies are required to clarify the role of pigs in the epidemiology of human trypanosomosis. If there is a correlation between the probability pigs get infected with trypanosomosis and the probability man gets infected in a certain area, the study of porcine trypanosomosis can generate important information on the epidemiology of human sleeping sickness. Unfortunately, up to now, limited research has been done on this subject.
A major constraint to studies on the epidemiology of porcine trypanosomosis is the lack of suitable diagnostic tools. For example, current parasitological techniques have low sensitivity and thus underestimate the prevalence of trypanosome infections in pigs. Epidemiological studies would clearly benefit from a test that would provide a good estimate of the level of challenge. An antibody detection test, for example, would be excellent for this purpose. Various ELISA systems have been developed for detection of bovine trypanosomal antibodies (Rebeski *et al*., 2000). Indirect ELISAs for detection of anti-trypanosomal antibodies in bovine serum have been adapted for serodiagnosis in goats (Lejon *et al*., 2003) but up to now, such tests do not exist for the detection of anti-trypanosomal antibodies in pigs.

The purpose of this study was to develop and evaluate an anti-trypanosomal antibody-detection ELISA for use in pigs. For this purpose, pigs were infected experimentally with *Trypanosoma congolense* and an indirect ELISA for detection of antibodies against *Trichinella spiralis* in pigs was adapted to detect anti-trypanosomal antibodies in pigs serum and blood spots collected on filter paper. Furthermore, in order to detect zones around Kinshasa, where the risk of trypanosomosis transmission is relatively high, 493 field samples, collected from pigs located in the vicinity of the city, were tested for the presence of antibodies. The possible role of pigs as reservoir for human sleeping sickness in this area is discussed.

2. Literature review

2.1. Trypanosomosis in pigs

2.1.1. Trypanosoma (Nannomonas) simiae

2.1.1.1. Introduction

At the beginning of the last century various outbreaks of acute porcine trypanosomosis were attributed to different trypanosome species (*T. suis, T. brucei, T. vivax, T. uniforme, T. rhodaini, T. porci* and *T. simiae*). Hoare (1936) demonstrated that all these parasites exhibited a similarity of structure and polymorphism, which according to this author left no doubt that they all represented the same species, *Trypanosoma simiae*.
At present, the prevalence of *T. simiae* throughout tropical Africa is a well-established fact (Hoare, 1972a). In his work, Stephen (1986) gives an overview of outbreaks of pig trypanosomosis caused by *T. simiae* throughout the whole of Africa: Mali, Ivory Coast, Ghana, Nigeria, Democratic Republic of Congo, Zambia, Zimbabwe, South Africa, Mozambique, Malawi, Tanzania, Kenya, etc.

2.1.1.2. Clinical aspects of *T. simiae* infection in pigs

Hoare (1936) describes the characteristics of acute porcine trypanosomosis. The onset of the disease is always sudden. An apparently healthy pig suddenly falls ill, becomes progressively worse and dies within the next few days. Infection spreads rapidly through the herd, killing the pigs in large numbers within a very short period of time and usually before any precaution can be taken or treatment can be carried out. Other authors mention sudden death, pyrexia up to 41°C, dullness, no appetite, sometimes anorexia, decreased activity (stiffness, paraplegia, incoordination), respiratory distress (thumping breathing), coldness of the extremities and hyperaemia of the skin (Stephen, 1986; Otaru & Nsengwa, 1987). Infected pigs show a marked drop in packed cell volume and a significantly poor performance (Ilemobade & Balogun, 1981). There are no pathognomonic signs of *T. simiae* infections in domestic pigs. Intercurrent helminth infections or babesiosis can complicate the clinical picture (Ocholi *et al.*, 1988).
The prepatent period (i.e. the time between the injection of metacyclic trypanosomes and the appearance of trypanosomes in the peripheral blood) is remarkably constant varying between 4-6 days (Stephen, 1986). The parasitaemia is very high and the trypanosomes are usually swarming in the blood (Hoare, 1936).
Bruce *et al.* (1913) described *T. simiae* infections as "the lightning destroyer of pigs". They added: "in the whole range of trypanosome diseases in animals there is nothing so striking as the rapidly fatal action of *T. simiae* on the domestic pig". Isoun (1968) saw that infected animals died within 3-4 days following clinical signs. During another outbreak comprising 22 pigs, 5 pigs died within 12 hours after showing clinical signs and 6 pigs died after a short illness (Otaru & Nsengwa, 1987). All these observations confirm the rapidly fatal course of the disease.
However, sometimes *T. simiae* appears to cause a less acute disease with infected pigs surviving for 3-4 weeks or even longer (Janssen & Wijers, 1974). According to these authors the virulence of *T. simiae* is determined by the species of tsetse that transmits the trypanosomes. For example, *T. simiae* transmitted by *Glossina brevipalpis* is said to be more virulent than those developing in *G. austeni* (moderately virulent) and *G. pallidipes* (chronic strain). However, this so called chronic strain was then first identified as *T. suis* (Stephen,

1986). Finally Gibson *et al.* (2001) showed beyond any doubt that this isolate was in fact *T. congolense* Tsavo type. Moloo *et al.* (1992) saw that the patent period in days to death was somewhat longer when a *T. simiae* stock was transmitted by *G. pallidipes* than when transmitted by *G. brevipalpis* although the effect was much less marked than that seen by Janssen & Wijers (1974). Hoare (1972a) mentioned an atypical strain of *T. simiae* described by Chardome & Peel (1967) in Rwanda-Burundi. It was characterised by a longer incubation period with the animals surviving for more than a month, and – instead of a progressive rising parasitaemia – periods when trypanosomes were numerous in the peripheral blood were followed by aparasitaemic periods.
The question rises whether these more chronic infections were caused by *T. simiae* or in fact by *T. godfreyi* or *T. congolense* Tsavo type. McNamara *et al.* (1994) stated that chronic or subacute experimental infections in pigs previously attributed to *T. simiae* are in fact similar to those produced by *T. godfreyi*.

2.1.1.3. Pathological aspects

Histopathological examination is characterised by generalized thrombosis, hypertrophy of endothelial cells in severely thrombosed capillaries and depletion of lymphocytes in lymph nodes and spleen (Stephen, 1986). According to Isoun (1968) vascular changes are the most predominant and constant lesions.
Stephen (1986) says there is no evidence of trypanosomes in the sediment of a centrifuged sample of cerebro-spinal fluid (CSF) or in the smears of the spinal and sternal bone marrow. However, Zweygarth & Röttcher (1987a) proved this wrong when they were able to detect trypanosomes in CSF.
The actual cause of death in *T. simiae* trypanosomosis is still not clear. Considering the fact that pigs show laboured respirations and their blood is often dark, purple-coloured, Stephen (1986) concluded that at least one of the major causes of death is anoxaemia. The blood is frequently swarming with trypanosomes, which probably utilize large quantities of oxygen in their metabolic processes. Generalised thrombosis of medium sized blood vessels and the plugging of capillaries with remnants of trypanosomes in various vital organs may also be the cause of, or a factor contributing to death from acute *T. simiae* trypanosomosis (Isoun, 1968).

2.1.1.4. Chemotherapy and chemoprophylaxis

Because of the rapid fatal course of the disease, the best solution to pig trypanosomosis is prevention (Stephen, 1986). *Trypanosoma simiae* is more refractory to treatment than any of the other trypanosome species. In an outbreak in Nigeria both antimosan and antrycide methyl sulphate were ineffective in controlling infections with *T. simiae* (Unsworth, 1952). Mahaga & Röttcher (1981) saw that commercial drugs (diminazene aceturate, suramin, isometamidium chloride, antrycide prosalt, homidium chloride, mel-B) and experimental drugs (6 different test diamidines) equally failed to cure pigs once the disease was established. Parasitaemias disappeared for a period but invariably recurred. Pigs treated 6 hours after the inoculum, however, remained consistently negative. Zweygarth & Röttcher (1987a) demonstrated that *T. simiae* can invade the central nervous system (CNS), where it becomes inaccessible to trypanocidal drugs. This explains both the observations of Mahaga & Röttcher and the discrepancy they saw in their own experiments: diminazene aceturate and quinapyramine prosalt were ineffective in the treatment of patent *T. simiae* infections but the same drugs did have a protective effect when administered prophylactically before infection. Only trypanocides that penetrate the blood-brain barrier could cure *T. simiae* infections with CNS involvement. According to these authors, the only commercially available drug that

enters the CNS is Mel B (Arsobal ®). However, it is ineffective against trypanosomes of the *Nannomonas* subgroup to which *T. simiae* belongs (Zweygarth & Röttcher, 1987b). They conclude that treatment would therefore be most successful if performed before *T. simiae* invades the CNS and they stress the importance of chemoprophylaxis to prevent the invasion of the CNS by the parasite. Onah & Uzoukwu (1991) state that suramin (Naganol ®) also crosses the blood-brain barrier and could thus treat parasites sequestered to the CSF. In work performed by other authors however, suramin failed to cure *T. simiae* infection (Mahaga & Röttcher, 1981).

2.1.1.5. Epidemiological aspects

Outbreaks of pig trypanosomosis sometimes occur when tsetse flies are apparently absent in the immediate neighbourhood of the infected farms. This could suggest that infection is introduced in a piggery by tsetse flies but then spread to other pigs by other blood-sucking flies such as *Stomoxys* and the Tabanidae (Unsworth, 1952; Stephen, 1986).
In pigs infected by tsetse flies the incubation period is said to be longer, the parasitaemia lower and the disease more protracted than in infection introduced by syringe or mechanical vectors (Hoare, 1972a).
The warthog (*Phacocoerus aethiopicus*) is the principal reservoir, but the trypanosome is also found in the bush-pig (*Potamochoerus* spp.) that can transmit the disease to domestic pigs (Otaru & Nsengwa, 1987). Another mode of transmission is given by Mols & Lenaerts (1950). They claimed that the disease can be propagated by monkeys that approach the fields of maize and groundnuts in search of food. The tsetse flies, which accompany the monkeys in their foraging, leave their hosts to feed on the pigs, thus introducing an infection in the herd. Most pigs in Africa, however, roam around and approach the rivers where tsetse flies (*Palpalis* group) can be found.

2.1.2. Trypanosoma (Nannomonas) congolense

Parkin (1935) performed experimental infections with *T. congolense* in pigs. These infections resulted in a low parasitaemia and had a subacute course. Hoare (1936) described *T. congolense* as the most common trypanosome of pigs, causing a chronic infection without impairing the health of the host.
Several reports have been made on *T. congolense* infections in pigs (Stephen, 1986; Killick-Kendrick & Godfrey, 1963; Ilemobade & Balogun, 1981). They all noted that *T. congolense* causes mild chronic infections with low parasitaemias. Pigs show hardly any clinical symptoms. Other authors conclude that although the course of infection is mild, the disease affects pig productivity and could cause serious losses in a herd since pigs are raised almost exclusively for meat production (Stephen, 1970; Omeke, 1994).
Kageruka (1987) saw that in fattening pigs *T. congolense* causes chronic and asymptomatic infections contrary to pregnant sows where it is responsible for abortion and even death. Contradictory to the findings of others, Katunguka-Rwakishaya (1996) noted high parasitaemias in pigs. The animals were apparently healthy, but had lower PCV's than non-infected animals.
Trypanosoma congolense is, together with *T. brucei*, the major cause of subclinical and chronic trypanosomosis in some areas (Omeke, 1989).

Trypanosoma congolense has recently been subdivided in several "types" which can be distinguished by isoenzymatic differences and molecular techniques. One type has received a separate species name, *T. godfreyi* (see below). The others are designated as *T. congolense*

savannah type, *T. congolense* Tsavo type, *T. congolense* forest type and *T. congolense* Kilifi type.
Already in 1982 Godfrey described a kind of *T. congolense* that only infects pigs. Gibson *et al.* (2001) identified a trypanosome isolate by means of PCR analysis and DNA sequencing as *T. congolense* Tsavo type. The host range of this *congolense* type is indeed restricted to pigs.
McNamara *et al.* (1994) experimentally infected animals with savannah, forest and Kilifi type of *T. congolense*. The Kilifi type did not produce a patent infection, the other two infections were both chronic and caused mostly subpatent parasitaemias.

2.1.3. Trypanosoma (Nannomonas) godfreyi

Trypanosoma godfreyi has been distinguished recently from *T. congolense* on the basis of isoenzymatic and DNA differences (McNamara *et al.*, 1994). The course of the disease is also different from *T. congolense*. The host range is restricted to pigs and it causes a subacute infection. Warthogs appear to be its normal host and constitute a reservoir of infection for domestic pigs.
The observed prepatent period was 7-10 days and parasitaemias remained patent during the whole period of infection. The more chronic, occasionally lethal, infection with *T. godfreyi* is clearly different from the acute rapidly fatal disease after infection with *T. simiae* and the mild infection characteristic of *T. congolense*. Previously, chronic or subacute experimental infections in pigs, similar to that produced by *T. godfreyi*, have been attributed to *T. simiae*.

2.1.4. Trypanosoma (Trypanozoon) brucei

For decades, researchers believed that *T. brucei* infections ran a chronic course in pigs, sometimes causing no apparent symptoms at all (Stewart, 1947).
Killick-Kendrick & Godfrey (1963) noted that *T. brucei* (and *T. congolense*) can persist in the blood of experimentally infected pigs for many months and the pig may therefore be an important reservoir of infection. The outcome of a survey they conducted in Nigeria showed a high prevalence of *T. congolense* and *T. brucei* in domestic pigs. Ilemobade & Balogun (1981) experimentally infected pigs with *T. brucei* which resulted in mild or asymptomatic infections. These infections had no significant effect on PCV, voluntary feed intake, mean liveweight gain and feed conversion efficiency. The course of the disease was similar whether infection is by needle or by tsetse fly challenge.
In Nigeria, *T. brucei* is a major cause of subclinical and chronic trypanosomosis (Omeke, 1989) causing following clinical signs: pyrexia, ataxia, anaemia, anoestrus, etc. Exotic breeds are more susceptible than their crossbreds with indigenous breeds.

Recently, however, there have been several reports of severe acute *T. brucei* infections in pigs. One case report (Agu & Bajeh, 1986) mentioned anorexia, emaciation, pale mucous membranes and death within 1-3 months due to *T. brucei*. The same authors also conducted an experimental infection with the same strain and observed an undulating parasitaemia for several weeks and intermittent pyrexia (Agu & Bajeh, 1987). The pigs became progressively emaciated, some showed nervous signs and were recumbent in the terminal stages. Large subcutaneous haemorrhages were visible. Five out of six piglets died. The packed cell volume dropped from the fourth week and remained low. These experiments showed clearly that *T. brucei* could be more pathogenic in pigs than hitherto believed.
Similarly, during an outbreak of *T. b. brucei* in pigs in Nigeria (Onah & Uzoukwu, 1991) the pigs showed pyrexia, anorexia, severe emaciation, weight loss, anaemia and low PCV values. All the animals were treated with diminazene aceturate (Berenil ®). However, two pigs

relapsed and succumbed as a result of brain involvement. At the post-mortem examination there were numerous trypanosomes present in the brain impression smears.
Otesile *et al.* (1991) also confirmed that *T. brucei* might cause severe disease in pigs. In an experimental infection one pig developed nervous symptoms. At necropsy it showed a very severe meningo-encephalitis with trypanosomes in the brain.

According to these reports *T. brucei* can cause mild chronic as well as severe acute disease in pigs, depending on the trypanosome strain. Büngener & Mehlitz (1984) infected mini-pigs with different strains of *T. brucei* and subsequently performed histopathological examination. Infections with a mild strain disappeared spontaneously, leaving no histological lesions. The parasitaemia was low and parasites were undetectable from 6 months post infection onwards. Infections with the other strain led to moderate to severe lesions of the central nervous system without marked clinical symptoms. Other pigs infected with the same strain died and post mortem examination revealed an intense myocarditis. These pigs developed a higher parasitaemia detectable up to 1 year post-infection. A very virulent strain caused death of all the pigs. Necropsy showed severe myocarditis and nephritis and only relatively mild cerebral lesions.

Different factors influence the severity of infections and impact of the disease on the pigs.
First of all, nutrition modulates the host response to infection with trypanosomes. This is probably true for all trypanosome species. Adequate dietary energy levels seem to alleviate the disease produced by *T. brucei* in growing pigs (Fagbemi *et al.*, 1990). Otesile *et al.* (1991) also saw that adequate feeding may assist in reducing the deleterious effects of trypanosomosis on production in endemic areas.
Secondly, Omeke (1994) noted that trypanosomosis was more severe in pigs with single rather than mixed infections. Multiple pathogen infections tend to lower their individual impact on their hosts.

A very important aspect of *T. brucei* infections in pigs is the possible role of these animals as reservoir host of human sleeping sickness. Gibson *et al.* (1978) screened 29 *Trypanozoon* stocks from Liberian pigs and found two of them to be resistant to human plasma. These stocks also had an isoenzyme marker previously only found in *T. b. gambiense* from man. These findings constitute evidence that the pig is a reservoir of human trypanosomosis in West Africa.
A survey conducted in Uganda (Okuna *et al.*, 1986) showed that *T. brucei* infection is quite prevalent in domestic pigs from tsetse-infested areas around Lake Victoria where sleeping sickness is epidemic. Infected animals appeared clinically normal. Two out of six isolates were resistant to human plasma so it is likely that domestic pigs constitute a reservoir host for *T. b. rhodesiense* in this area. Other surveys conducted in Uganda (Katunguka-Rwakishaya, 1996; Waiswa *et al.,* 2003) have led to the same conclusion. The latter identified the pig-tsetse-human cycle as a major transmission cycle. The proportion of trypanosome-positive animals carrying parasites of the *T. brucei* subgroup was much higher for pigs than for cattle. The authors therefore conclude that, in some areas, the mass treatment of pigs with trypanocides may be justified.

2.1.5. Trypanosoma (Trypanozoon) evansi

In pigs infected with *T. evansi* the disease is mild and as a rule asymptomatic (Hoare, 1970). However there are a few reports from Asia where pigs suffered from clinical symptoms due to *T. evansi* infections. Gill *et al.* (1987) describe an outbreak in a government farm in India

where 13 pigs were lost. The pigs showed several symptoms such as fever, shivering, laboured breathing, necrosis of the tips of ears and tail, oedema of the eyelids and emaciation just before death. The animals, however, had mange lesions and therefore could have been more susceptible to *T. evansi* because of the immunodepressive effect of the mange. Another outbreak occurred in South-East Asia in a breeding stock characterized by fever, anorexia, emaciation, abortion and death (Arunasalam *et al.*, 1995).
Although *T. evansi* is not considered as an important pathogen in pigs, it may interfere with other pathogens or vaccinations through its immunosuppressive nature. Holland *et al.* (2003) compared pig performance and induction of immune responses by vaccination against classical swine fever between *T. evansi*-infected and non-infected animals. *T. evansi* did not have a significant influence on growth performance, feed conversion or PCV. They saw however, that the presence of a *T. evansi* infection lowered the immunoresponsiveness of fattening pigs to concurrent immunisations.
The most important aspect of *T. evansi* infection in pigs is perhaps the role of the pig as reservoir host. Baldrey (1910) inoculated a pig with a large dose of infected blood resulting only in a transient infection with low fluctuating parasitaemia. There were no clinical symptoms. He concluded that the pig is a possible carrier of *T. evansi* and hence source of infection for other animals. Other experimental studies in pigs with *T. evansi* (Reed *et al.*, 1999) also showed that pigs have a high tolerance for infection with *T. evansi* and are suitable reservoir hosts.

2.1.6. Trypanosoma (Pycnomonas) suis

Trypanosoma suis is by far the least known and most infrequently reported pathogenic trypanosome affecting livestock. The history of discovery of this trypanosome is very confusing. The first account of this parasite was given by Ochmann in 1905 who proposed the name *Trypanosoma suis* (Stephen, 1986). Later on, various authors identified it as *T. congolense*, *T. simiae* or *T. brucei* (Hoare, 1970). The identity of *T. suis* as a valid and independent species was established by Peel & Chardome (1954) who gave a description of its morphology, life cycle, transmission and host-parasite relationship.
The only animal Peel & Chardome (1954) were able to infect with *T. suis*, both by cyclical as mechanical transmission, was the pig. Other mammals appear to be refractory to infection with this parasite. Hence, *T. suis* is said to be a specific parasite of the Suidae.
The only isolate presumed to be *T. suis* is maintained at the Kenya Trypanosomiasis research Institute in Nairobi. In 2001, Gibson *et al*. characterised this isolate by morphology, tsetse transmission , the use of species-specific DNA probes and DNA sequence analysis. These authors identified this presumed *T. suis* isolate as *T. congolense* Tsavo type. From these results it can be concluded that *T. suis* as a separate species does not exist but is in fact a type of *T. congolense*.

2.1.7. Trypanosoma (Dutonella) vivax

Pigs are reported to be refractory to infections with *T. vivax* (Hoare, 1970).

2.1.8. Trypanosoma cruzi

It is important to know whether pigs are good reservoir hosts of *Trypanosoma cruzi* in view of transmission of Chagas' disease to humans. Pigs are certainly susceptible to *T. cruzi* infections but only one natural infection has been reported (Minter, 1976). Marsden *et al*. (1970) observed that, during experimental infection, none of the infected pigs became ill.

Furthermore, direct examinations of fresh blood films did not reveal any parasites but after subinoculation of blood into mice 3 pigs were shown to be infected.

2.2. Diagnosis of trypanosomosis in pigs

A description of the laboratory methods most commonly used for diagnosis of trypanosomosis can be found in the FAO field guide (Uilenberg, 1998). The choice of a particular diagnostic test depends on the purpose of the diagnosis. For example, the diagnostic requirements for the detection of infected animals are completely different from the diagnostic requirements for epidemiological surveys. Especially the latter situation is of special interest in our case.

Trypanosome infections in pigs do not always produce pathognomonic clinical signs or patent parasitaemias. Therefore, clinical and parasitological diagnosis is often very difficult. Although parasitological diagnosis by microscopical examination is relatively easy and fast to perform and does not require highly specialised equipment, sensitivity of these techniques is far too low for use in epidemiological studies of pig trypanosomosis. Even concentration techniques such as the hematocrit centrifugation technique (HCT, described by Woo, 1970) are not sensitive enough. The sensitivity of the most commonly used parasitological techniques decreases as follows (Luckins, 1993): DG > HCT > thick film > thin film > wet film (DG = darkground buffy coat technique, described by Murray *et al*., 1977).
Several authors advocate the use of laboratory animal inoculation to detect subpatent infections in pigs, especially for *brucei* type infections (Killick-Kendrick & Godfrey, 1963; Omeke, 1994). This method is first of all too cumbersome for large scale surveys and the results are not immediately available. Secondly, some *Trypanosoma* spp. will not infect rodents (Godfrey, 1982; Luckins, 1993), consequently this technique will also not reveal these subpatent conditions.

The low sensitivity of the standard parasitological techniques has necessitated the introduction of other diagnostic methods with improved diagnostic sensitivity and acceptable specificity. In this respect, primary binding assays that measure the antigen-antibody reaction directly, are more reliable than secondary binding assays such as complement fixation or indirect haemagglutination tests (Luckins, 1993; Rebeski *et al.*, 2000). Among the primary binding assays ELISA and IFAT are the most frequently used. Both tests have comparable sensitivity and specificity. The major drawback of the latter is its subjectivity which can make comparison of results difficult (Luckins, 1993). The ELISA method is considered superior because it is relatively simple, fast, objective and a technique that can easily be standardised. Furthermore, it is a cost-effective screening tool for large number of samples.
Several ELISAs have been developed for diagnosis of trypanosomosis in cattle and some have already been adapted for the use in small ruminants (Lejon *et al.*, 2003). The current antigen-detecting ELISAs have been proven unreliable because of their unsatisfactory diagnostic sensitivity and specificity (Thammasart et al., 2001).
Indirect ELISAs for detection of antibodies of *T. congolense* and *T. vivax* in cattle have high diagnostic sensitivity and specificity (Eisler *et al.*, 2000; Rebeski *et al.*, 2000) and were until recently commercially available from FAO/IAEA. The trypanosome antibody-detection ELISA also has a great potential for the use in epidemiological studies on porcine trypanosomosis.
However, there are some downsides on the use of the antibody ELISA. Firstly, there are cross-reactions between the different trypanosome species. Hence, the test cannot be used to

determine the trypanosome species or combination of species that causes the infection. Secondly, antibodies persist in cattle for up to 13 months after treatment (Van den Bossche *et al.*, 2000). Hence, a positive test result means that an animal is infected or has been in contact with trypanosomes. Nevertheless, ELISAs remain useful in sero-epidemiological studies aiming at assessing the presence or absence of trypanosomosis in transversal studies.

Several molecular techniques have been developed for trypanosomosis diagnosis. The polymerase chain reaction (PCR) has a very high sensitivity and specificity, but only well equipped specialised laboratories are capable to perform this test. Recently, Geysen *et al.* (2003) developed a PCR-RFLP (restricted fragment length polymorphism) that allows the differentiation of different trypanosome species of cattle in one test. Furthermore, it has a higher sensitivity than multiplex PCR tests.

2.3. Objectives of the study

The objective of this study was to develop an antibody-detecting ELISA for the diagnosis of trypanosomosis in pigs. For this purpose, 6 pigs were experimentally infected to obtain pre- and post-infection serum samples. This ELISA-test was used to test porcine blood samples from Kinshasa (DRC) for presence of anti-trypanosomal antibodies. This is part of a study conducted by the Veterinary Laboratory of Kinshasa to determine the role of reservoir animals in the transmission of human sleeping sickness. The aim is to determine the distribution of animal trypanosomosis and the zones with increased risk of trypanosomosis transmission in Kinshasa. In these zones, the role of pigs as reservoir host for human sleeping sickness will be studied by testing the blood samples with PCR for presence of *T. b. gambiense*.

3. Material and methods

3.1. Experimental infection

3.1.1. Experimental animals

Six boars of the Belgian landrace weighing app. 26 kg were used for the study. They were kept in a stable on straw bedding for a period of approximately 8 weeks. The pigs were fed on a commercially available diet; water was provided ad libitum.

3.1.2. Parasite material and infection procedure

Two rats were infected intraperitonially with the *Trypanosoma congolense* IL 1180 strain. The rats were sedated with ether and their blood was collected by heart puncture. The parasitaemia was app.10 trypanosomes per field (objective 40x) which is 7.5 on the scale of Herbert and Lumsden (1976). Each pig was infected with approximately 4 ml of blood through subcutaneous and intra-peritoneal inoculation.

3.1.3. Sample collection

Two blood samples were taken before the start of the study at an interval of one week. After infection, blood samples (10-15 ml) were collected on a weekly basis from the vena cava cranialis into tubes with EDTA (ethylenediamine tetra-acetic acid) as anticoagulant. At the same time the body temperature was measured with a rectal thermometer.

Blood samples in capillary tubes were kept at room temperature and examined microscopically a few hours after collection. The capillary tubes were sealed with wax (Hirschmann Labogeräte, Germany) and centrifuged in a microhaematocrit centrifuge for 5 min at 9000 rpm. Full blood samples were collected on filter paper (Whatman n°4) and kept at 4°C in sealed plastic bags with silica gel. For serological analysis, aliquots of serum samples were stored in a freezer at -18°C until use.

3.1.4. Parasitological and serological techniques

The parasitaemia was assessed using the haematocrit centrifugation technique (HCT) described by Woo (1970). The same microcapillary tube preparations were used to determine the packed cell volume (PCV). The presence of antibodies to *T. congolense* was evaluated using an indirect ELISA method as described below.

3.1.5. Statistical analysis

Rectal temperature and PCV values were analysed using a t-test. Stata 7.0 ™ (Stata Corporation, USA) was used as statistical software. Data from the ELISA tests were processed using Microsoft Office Excel 2003 ®.

3.2. ELISA reagents

3.2.1. Antigen

In-vitro adapted *Trypanosoma congolense* blood stream forms of stock CP-81 (a derivative of the primary isolate Trans Mara I strain) were used to produce the antigen. In vitro propagation

of *T. congolense* was carried out according to the methods described by Hirumi & Hirumi (1991) and modified by Rebeski et al. (1998). Ultrasonic cell disruption treatment (Rebeski et al., 1998) was performed to obtain a mixture of crude extracellular and intracytoplasmatic antigens from the trypanosome lysate. The protein concentration (500 µg/ml) was determined according to the method of Warburg & Christian which essentially involves measuring the absorbance at 280 nm and 260 nm of the antigen mixture. The antigen was then denaturated by heat-mediated dissociation with SDS (sodium dodecyl sulphate) and DTPA (diethylenetriamine penta-acetic acid) (Steindl et al., 1998). Aliquots were made and stored at -80°C until use.

3.2.2. Carbonate buffer

A carbonate buffer (pK 9.6) with 0.1% NaN_3 was used as coating buffer for the antigen.
Preparation:

1. 0.265 g Na_2CO_3 in 50 ml H_2O
2. 0.630 g $NaHCO_3$ in 150 ml H_2O
3. add 20 ml of (1) and 50 ml of (2) to 300 ml H_20
4. adjust pH by adding (1) or (2)
5. adjust the volume to 500 ml by adding H_20
6. add 0.1 g NaN_3 per 100 ml buffer

3.2.3. PBS-Tween 20

PBS-Tween 20 was used as washing buffer and as base for the sample and conjugate diluting buffer.
Preparation:
Dissolve 1 tablet PBS (Oxoid Ltd., England) in 100 ml de-ionized water and add 0.555 g Tween-20 (Sigma, USA) per litre PBS.

3.2.4. PBS-Tween 20 + 2% NBCS

This buffer was used to dilute the serum samples, the blood on filter paper samples and the conjugate.
Preparation:
Add 2 ml new borne calf serum (NBCS) per 100 ml PBS-Tween 20.

3.2.5. Conjugate

Rabbit anti-pig Ig G peroxidase conjugate (Sigma, Germany) was diluted in PBS-Tween 20 + 2% NBCS.

3.2.6. Substrate

OPD (DakoCytomation, Denmark) was used as substrate.
Preparation:
Dissolve 2 tablets OPD in 12 ml de-ionized water and add 5 µl H_20_2 just before use.

3.3. ELISA protocol using serum samples

An indirect ELISA for detection of antibodies against *Trichinella spiralis* in pigs (Diouf, 2000) was adapted for detection of antibodies against *T. congolense*. The optimum antigen, serum and conjugate dilutions were determined by checkerboard titration. *T. congolense* stock CP 81 was diluted in carbonate buffer with 0.1% NaN_3 at a dilution of 1/100 and used as coating antigen. The 96 wells immuno-assay plates (Nalge Nunc International, Denmark) were coated with 100 µl of antigen and incubated overnight at 37°C with shaking. Plates were then washed 2 times with PBS-Tween 20. Blocking was done with 150 µl of PBS-Tween 20 + 2% NBCS and the plates were incubated for 15 min at 37°C. No washing was done after this step. Serum samples were diluted in sample diluent buffer (PBS-Tween 20 + 2% NBCS) at a dilution of 1/400. To each well 100 µl of serum was added and incubated for 1 hour at 37°C with shaking. Thereafter, the wells were washed 4 times. The conjugate was diluted at 1/10.000 in PBS-Tween 20 + 2% NBCS and incubated for 30 min at 37°C (100 µl/well). After 4 washing cycles each well was incubated in the dark for 15 min at 30°C with 100 µl substrate-chromogen solution. The reaction was stopped by adding 50 µl of H_2SO_4 (4N) to each well. The optical density (OD) values were measured at 492/655 nm using a multichannel spectrophotometer (Thermo Labsystems Multiskan EX) and Ascent software version 2.6.

Serum samples were applied in duplicate and the control sera in quadruplicate. Results were expressed as percentage positivity (PP) values. Percentage positivity was determined by dividing the mean OD of duplicates by the OD of the strong-positive control serum (Wright *et al*., 1993). The serum obtained from pig n° 4 at 3 weeks p.i. served as strong-positive control serum (C++). Negative reference serum consisted of the serum sample of the same pig prior to infection. Moderate-positive control serum (C+) was produced by diluting strong-positive control serum 1/2 in negative control serum. The conjugate control (CC) comprised PBS-Tween 20 + 2% NBCS only.

3.4. Adaptation of ELISA using blood samples on filter paper

The optimal dilution equivalent to the serum dilution (1/400) was determined by titration. Five-mm disks were punched out of the dried blood spots on filter paper and eluted overnight at 4°C in 700 µl of PBS-Tween 20 + 2% NBCS. The same protocol as above was followed for the ELISA test, only now 100 µl of eluens was applied instead of 100 µl of the diluted serum samples.

3.5. Calculation of cut-off value

A total of 152 negative serum samples were tested to calculate the cut-off value. Details can be found in table I. These samples were tested in duplicate and their overall average percent positivity (PP) value and standard deviation (SD) was calculated. The cut-off value was then calculated from the mean PP value plus 2 or 3 SD.

Table I: Pig serum samples used for calculating the cut-off value for *T. congolense* antibody detecting ELISA

Number of samples	Origin	Remarks
79	Belgium	Commercial piggery
23	Peru	Roaming pigs infected with *Cysticercus tenuicollis*, *Fasciola hepatica*, *Echinococcus* spp.
8	Vietnam	Roaming pigs infected with *Cysticercus tenuicollis*
4	Belgium	Experimental infection with *Trichinella spiralis* and *Ascaris suum*
8	Belgium	Experimental infection with *Taenia asiatica*
18	Peru	Closed piggery, pigs aged 1, 3 and 5 months
12	Belgium	Experimental animals used in this study, samples prior to infection

3.6. Field samples Kinshasa

3.6.1. Context of the study

The administrative region of the City of Kinshasa ("Ville de Kinshasa"), province harbouring the capital of the Democratic Republic of Congo, has a surface of 9 965 km² and consists of 24 districts. The population in the region is estimated at 6.7 million inhabitants, 10% of the total population of the country. The actual city of Kinshasa covers a surface of 20 km by 12 km and consists of approximately 20 villages. The region can be divided in an urban environment (i.e. the city centre), surrounded by a peri-urban area with open space building alternating with small plots of irrigated arable land. This peri-urban zone turns farther from town in a sparcely populated rural zone with farmers living in small villages surrounded by farmland, fallowland and groves. Since 1996, there has been a vast rise in the number of cases of human sleeping sickness in this region (Ebeja *et al.*, 2003).

As part of a study « Etude du role du réservoir animal dans la transmission de la trypanosomose humaine dans la ville de Kinshasa et son hinterland » the blood of around 1000 pigs in and around Kinshasa city was sampled on filter paper and checked for trypanosomes by the personnel of the Veterinary Laboratory of Kinshasa. This work was carried out in order to determine the distribution of animal trypanosomosis around Kinshasa. One of the objectives of this study is to identify sites of increased risk of trypanosomosis transmission and in these zones to determine the possible role of domestic animals as reservoir hosts for human sleeping sickness. These studies would increase our knowledge of the epidemiology of this disease in a urban/peri-urban environment.

3.6.2. Samples

493 of the blood samples, collected by the Veterinary Laboratory of Kinshasa and originating from 171 different farms (table II), were tested for anti-trypanosomal antibodies with the ELISA test described earlier. This time, the cut-off value of 32% (PP + 3 SD) was used. These samples came from one of the following predilection sites in Kinshasa:

- peri-urban and urban farms around tsetse habitat: *Glossina fuscipes quanzensis* which is found in Kinshasa belongs to the *Palpalis* group ("riverine" group) with its habitat along the rivers,
- farms in peri-urban areas where cases of human sleeping sickness were reported,
- farms with parasitological positive cases of animal trypanosomosis.

Table II: Overview of pig samples from the vicinity of Kinshasa tested for anti-trypanosomal antibodies

District	Nr. farms	Nr. samples
Kimbanseke	44	104
Kisenso	17	40
Masina	15	29
Mont Ngafula	48	211
N'djili	13	25
N'sele	16	35
Selembao	18	49
Total	171	493

3.6.3. Statistical analysis

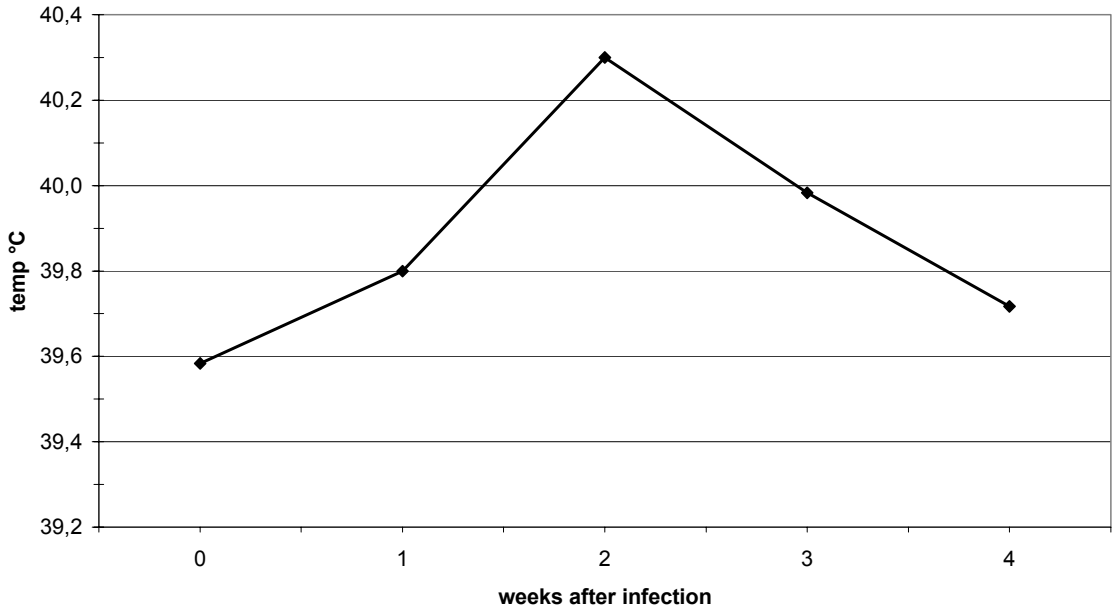
Data were processed with Microsoft Office Excel 2003 ®. Results per district were compared using logistic regression. Stata 7.0 ™ (Stata Corporation, USA) was used as statistical software.

4. Results

4.1. Experimental infection

Throughout the whole observation period the pigs appeared to be in good health and showed no overt clinical symptoms. However, all pigs developed fever starting from 1-2 weeks after the infection. The average temperature pre-infection was 39.58°C (SD=0.06). This was significantly lower ($p<0.05$) than the average maximum temperature (40.50°C, SD=0.05). The average temperature reached a maximum (40.3 °C, SD=0.36) at 2 weeks after the infection, thereafter it dropped again to normal values (figure I).

Figure I: Average rectal temperature of 6 pigs experimentally infected with *Trypanosoma congolense*



The packed cell volumes showed no significant decline after the infection. However, three PCV values clearly differed from the others (table III). In week 2, pigs n° 1, 4 and 6 had PCV values of 41, 22 and 42% respectively.

Table III: Packed cell volumes (PCV) of 6 pigs experimentally infected with *Trypanosoma congolense* between one and four weeks after infection

	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6	Average PCV (SD)
Week 1	33	38	35	36	35	32	34.8 (2.14)
Week 2	41	38	39	22	37	42	36.5 (7.34)
Week 3	36	35	35	34	32	36	34.7 (1.51)
Week 4	35	36	35	39	35	37	36.2 (1.60)

Examination of the buffy coat was negative the first week after infection (table IV). Two weeks after infection trypanosomes could be detected in the blood of all the pigs except pig n° 6. This animal stayed aparasitaemic for the rest of the observation period. Pigs n° 1 and 4 no longer had a patent parasitaemia after 4 weeks.

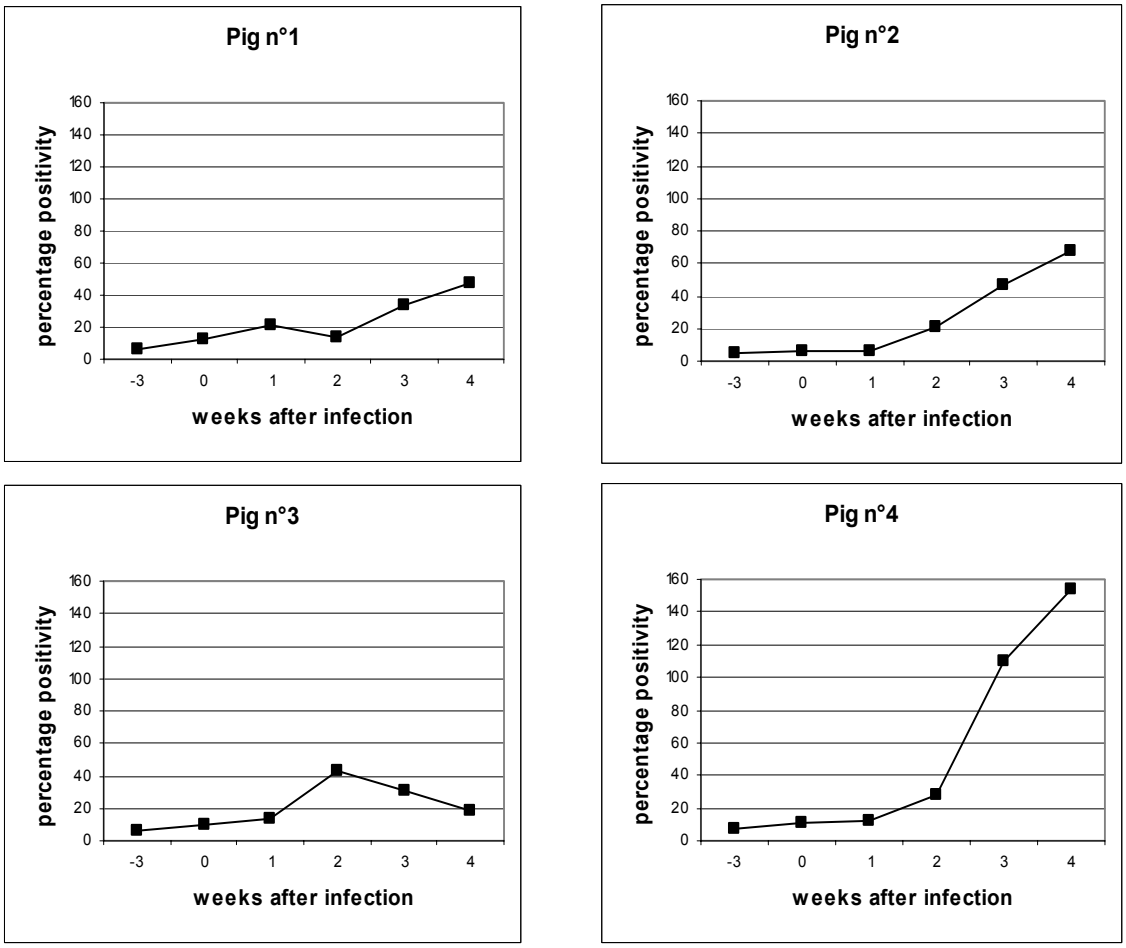
Table IV: Parasitaemia of 6 pigs experimentally infected with *Trypanosoma congolense* using haematocrit centrifugation technique between one and four weeks after infection

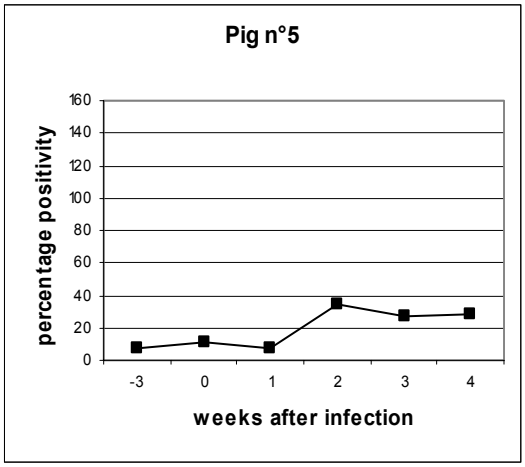
	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6
Week 1	-	-	-	-	-	-
Week 2	+-	+	+	+	++	-
Week 3	+-	+-	+-	+-	+	-
Week 4	-	+-	+-	-	+-	-

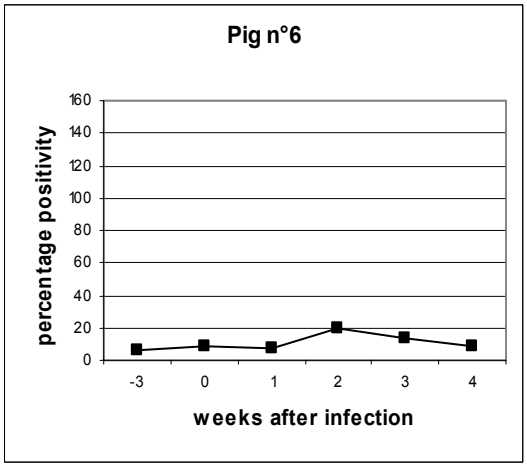
- negative; +- weak positive; + positive; ++ strong positive

Figure II shows the antibody response of the pigs after infection. In general, the pigs became positive from 2-3 weeks after infection onwards but the individual response was highly variable. Pig n° 4 clearly showed a high and vast rise in antibody level. On the contrary, the antibody levels of pig n° 6 remained below the cut off level of 26% (see below) throughout the whole period of observation. Pigs n° 3 and 5 only developed a small increase in antibody titre.

Figure II: Antibody response in percentage positivity of six pigs after experimental infection with *Trypanosoma congolense*







4.2. Cut-off values ELISA test

Four different cut-off values were calculated (table V). This resulted in specificities of 96.0% (+ 2 SD) and 98.7% (+ 3 SD) when all 152 samples are included. When excluding the 23 samples from Peru and 8 from Vietnam, this resulted in specificities of 97.5% (+ 2 SD) and 99.2% (+ 3 SD).

Table V: Cut-off values for antibody-detecting ELISA in pigs

Negative samples included	Avg PP	SD	Cut-off 1	Cut-off 2
All samples (n=152)	17.57	9.54	36	46
All samples except 23 from Peru and 8 from Vietnam (n=121)	14.93	5.71	26	32

Cut-off 1 = PP + 2 SD; cut-off 2 = PP + 3 SD

4.3. Field samples Kinshasa

The full overview of results for each individual farm can be found in the annex. Table VI shows the results per district. A positive farm is defined as a farm where at least one positive sample is found.

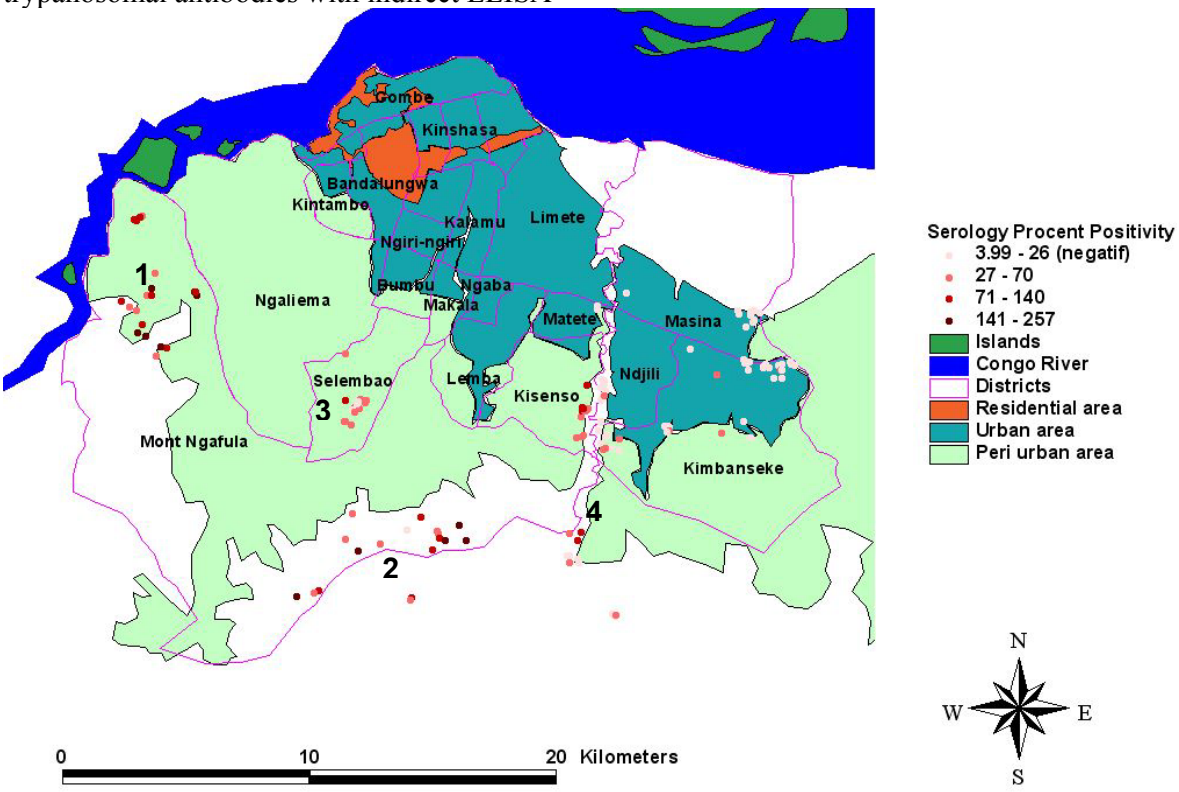
Table VI: Results of anti-trypanosomal antibody-detecting ELISA conducted on 493 pig blood samples collected in the vicinity of Kinshasa. Overview per district.

District	Nr. farms	Nr. samples	Positive farms	Positive samples	Maximum PP
Kimbanseke	44	104	12 (27%)	20 (19%)	100,6
Kisenso	17	40	16 (94%)	35 (88%)	179,57
Masina	15	29	2 (13%)	3 (10%)	32,87
Mont Ngafula	48	211	46 (96%)	187 (89%)	575,75
N'djili	13	25	0 (0%)	0 (0%)	31,32
N'sele	16	35	5 (31%)	7 (20%)	205,43
Selembao	18	49	15 (83%)	25 (51%)	84,03
Total	171	493	96 (56%)	277 (56%)	

PP = percentage positivity; cut-off value = 32 PP

The highest number of seropositive farms was found in the districts of Kisenso, Mont Ngafula and Selembao. There was no significant difference ($p<0.05$) between these 3 districts in number of seropositive premises. However, looking at the individual samples, there were less positive pigs in Selembao than in Kisenso or Mont Ngafula. The maximum PP values found in Selembao, Kisenso and Mont Ngafula districts were 84%, 180% and 575% respectively. Farms in Kimbanseke, Masina and N'sele were less seropositive. There was no difference between these 3 districts, neither in number of positive farms as number of positive samples. One sample from N'sele district had a relatively high PP value (PP = 205.43) compared with the other positive samples from that district. All the samples from N'djili were negative. From the 493 samples tested, 277 were positive, i.e. 56.2%.

Figure III: Location of pig farms in the vicinity of Kinshasa tested for presence of anti-trypanosomal antibodies with indirect ELISA



1: Lutendele quarter 2: Lukaya river 3: Funa river 4: Ndjili river

Figure III shows the location of the sampled farms. The infected farms are located in three foci: Lutendele quarter in the west, along the Lukaya and Ndjili rivers in Kisenso and Mont Ngafula and in Selembao along the Funa river. Figure IV shows the location of the sampled farms in comparison with the presence of tsetse flies in the area. Tsetse flies are present along Lukaya and Ndjili rivers and in Lutendele quarter where most of the infected farms are situated. The flies are rare in Selembao. Nevertheless there are infected farms present in this district. Figure V also shows the farms where parasitologically positive cases of pig trypanosomosis were found. From 1008 pig samples tested, only 33 were positive, i.e. 3.27% (Sumbu, 2003). All positive samples, except one, originated from Mont Ngafula district with a distinct concentration of positive cases in Lutendele quarter (16 out of 32, i.e. 50%).

Figure IV: Location of pig farms in the vicinity of Kinshasa tested for presence of anti-trypanosomal antibodies with indirect ELISA and presence of tsetse flies

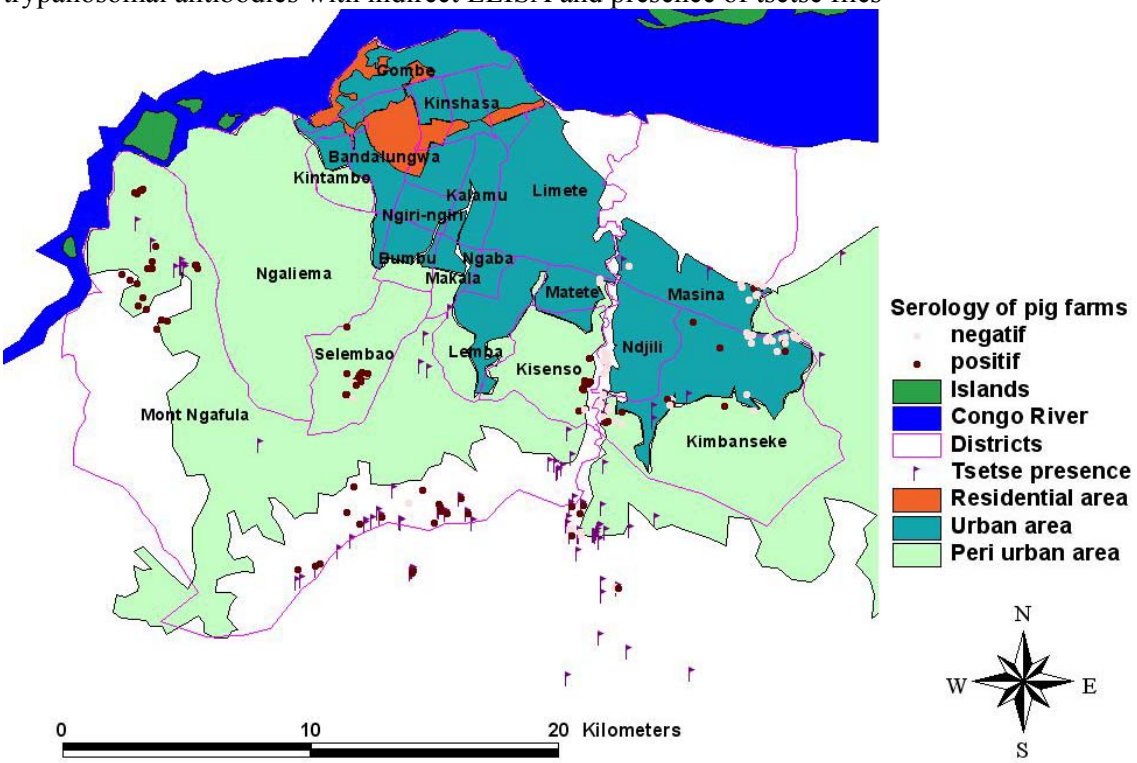
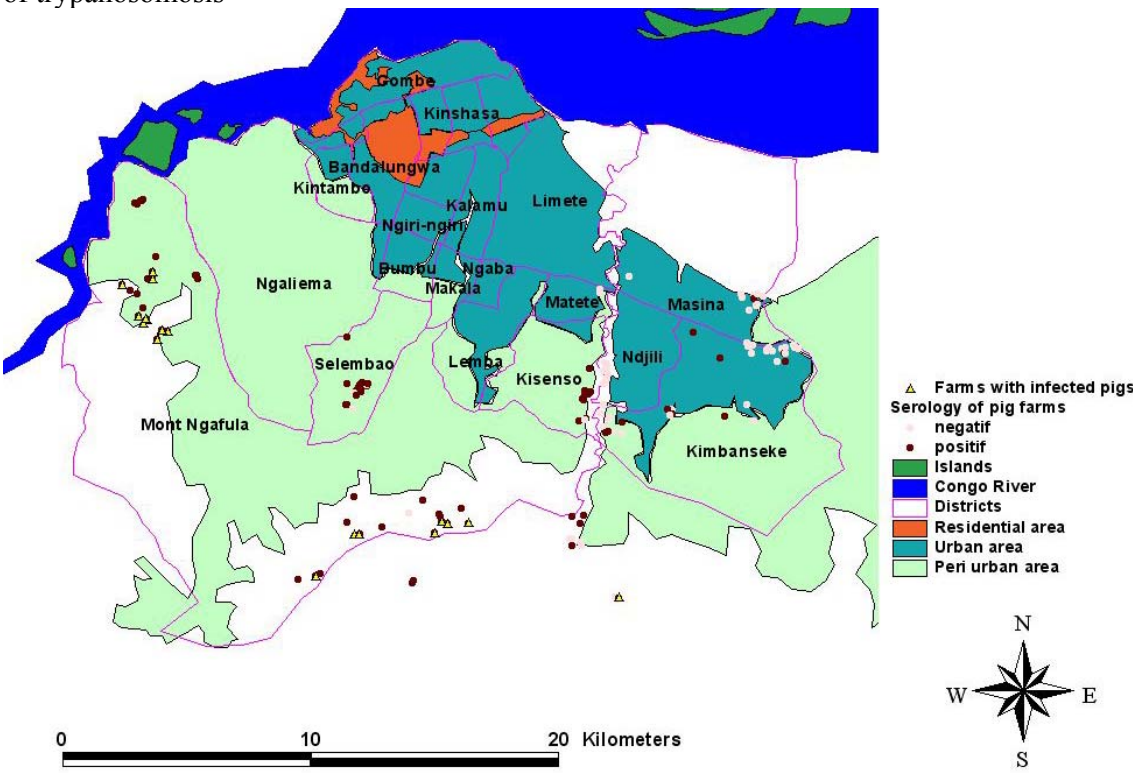


Figure V: Location of pig farms in the vicinity of Kinshasa tested for presence of anti-trypanosomal antibodies with indirect ELISA and farms with parasitologically positive cases of trypanosomosis



5. Discussion and conclusions

5.1. Experimental infection

Since no uninfected control pigs were available during the observation period, the pre-infection data were used as control values. All the pigs clearly developed pyrexia approximately two weeks after infection. This coincided with the first appearance of trypanosomes in the peripheral blood. Pyrexia is a common finding in *T. congolense* infections in pigs (Omeke & Ugwu, 1991; Omeke, 1989). In pigs infected with *T. brucei*, Agu & Bajeh (1986) saw that the pigs developed an intermittent pyrexia that accompanied the peaks of parasitaemia with peaks reaching 40.5°C at times.
The packed cell volumes did not decrease after infection. The PCV values of pigs n° 1, 4 and 6 at week 2 are probably due to errors since it is very unlikely that PCV values taken at an interval of one week differ from each other with 10% or more. Other authors also noted no significant changes in PCV of pigs after experimental infection with *T. congolense* (Ilemobade & Balogun, 1981). Omeke & Ugwu (1991), however, observed lower PCV values in pigs after challenge with *T. congolense*.
In general, the parasitaemia stayed low during the observation period. Although a concentration technique was used to assess the parasitaemia, it was not always easy to detect trypanosomes in the buffy coats. Trypanosomes could not be detected in the blood of pigs n° 1 and n° 4 in the fourth week after infection. Pig n° 6 stayed aparasitaemic during the whole observation period and its antibody titre never exceeded the cut-off value. It is very likely that the infection did not establish itself in this animal. Confirmation with PCR technique would have been useful. Other authors also mention a scanty and sporadic parasitaemia after infection with *T. congolense* (Ilemobade & Balogun, 1981; Omeke & Ugwu, 1991). Only Katunguka-Rwakishaya (1996) could detect a high parasitaemia in pigs infected with *T. congolense*.
Due to this low parasitaemia in pigs, a lot of infections will be missed when only parasitological methods are used. It is therefore better to use indirect tests that determine antibody levels. The antibody-detecting ELISA is such a technique.
The antibody response varied from one pig to another. Pigs n° 1, n° 2 and n° 4 showed a distinct rise in antibody level from two weeks after the infection onwards. Goats infected with *T. congolense* and tested for antibodies with an indirect ELISA also seroconverted at 2 weeks post infection (Lejon *et al*., 2003). These animals all consistently tested positive during the observation period of 10 weeks. In this experiment, however, pig n° 3 showed a decrease in antibody level below cut-off four weeks after infection although it was still parasitologically positive. N'Depo *et al*. (2000) applied an antibody-detection ELISA for diagnosis of bovine trypanosomosis. They also saw that some parasitologically positive animals showed low percent positivity values in the antibody-ELISA. Therefore, it will be necessary to combine serological and parasitological techniques to complete the epidemiological picture.
In conclusion, the results of this experiment are in agreement with those of other authors (Stephen, 1986): *T. congolense* causes very little harm to pigs and the animals only develop scanty and sporadic parasitaemias.

5.2. ELISA test

According to Wright *et al.* (1993), the negative samples for calculating the cut-off value should originate from animals that have no epidemiological, clinical or other serological

evidence of the disease in question. Since there were no such samples available from African countries, the cut-off value was calculated based on samples originating from Belgium, Peru and Vietnam. It is indeed unlikely that these animals have been in contact with *T. congolense*. However, the possibility that the roaming pigs from Peru and Vietnam have been in contact with *Trypanosoma evansi* cannot be ruled out. Since *T. evansi* cross reacts with *T. congolense* in antibody ELISAs (Tuntasavan *et al.*, 2003), it is better not to include these samples as negative samples for calculating the cut-off. Therefore, the cut-off levels of 26% and 32% are preferred above the cut-off levels of 36% and 46%.
Which cut-off value to use – plus 2 or 3 standard deviations – depends on the test objectives. In the case it is important to minimise the number of false positive test results, it is better to use the cut-off value calculated from PP + 3 S.D. Since this is not the case for the experimental infection, the cut-off value of 26% (PP + 2 S.D.) was used.
Cross-reactions with other pathogens such as cysticercosis do not seem to occur. It would be useful to test for cross reactions with other blood parasites of pigs. This has not been done in this study. In view of the pig as reservoir host for human trypanosomosis, it would also be useful to test for cross reactivity with *T. brucei.*
If this ELISA test is going to be used extensively in the field, local validation tests will be necessary and a statistically significant number of samples should be tested. Wright *et al*. (1993) state that a sufficient number of samples should be tested to provide a 95% confidence in the estimate of the diagnostic sensitivity and specificity. Jacobson (1998) recommends that a minimum of approximately 300 samples from infected and uninfected reference animals from the target population should be tested. In this study, only a small number of negative samples were used to determine the specificity. Furthermore, these samples did not come from animals in the target population. However, as samples from animals in the target population are tested and several thousand results are acquired, it is possible to estimate a reasonable (intrinsic) cut-off for the assay even when there are no reference animals available (Jacobson, 1998).

5.3. Field samples

For the field samples a cut-off level of 32% was used instead of 26%. This was done to avoid false positive results since local validation tests were not conducted and cross reactions with other pathogens are not fully known. The prevalence of trypanosomosis in pigs in Kinshasa was not calculated since only a few districts were tested in this study and not all samples from a district were tested. Calculating the prevalence in this situation would lead to unreliable results. A good sample survey with correct sample size and taking into account the survey design (stratification, clustering, sampling weights) is necessary to calculate the prevalence.
Another disadvantage is the lack of information of the age of the sampled pigs. Were there an equal amount of young and adult pigs tested or were there significant age differences between the different districts? If this was the case, this could lead to biased results. For example, if in one district there were more young pigs present and in another district more adult pigs, then it would be very likely that there are more seropositive animals present in the second district. This age-factor should be taken into consideration when calculating the seroprevalence.
Nevertheless, some conclusions can be drawn from the results presented in this study. Firstly, there is a conformity between the location of farms with parasitologically positive cases of trypanosomosis and the location of the seropositive farms with the highest percentage positivity values. There are two foci present: one in Lutendele and one along the Lukaya river, both in Mont Ngafula district. Where parasitology only revealed presence of trypanosomes in 3.27% of the samples, 56.2% of the samples were positive when tested with

the indirect ELISA. This once again confirms the fact that parasitology alone is not sufficient to detect trypanosome infections in pigs.
Secondly, there is also a conformity between the distribution of tsetse flies and the serology results. The flies are mainly found along the banks of the rivers, especially the Lukaya and Ndjili rivers, and these areas also harbour many seropositive farms. Recently a non governmental organisation ADC started tsetse control operations along the Funa river in Selembao, as well as along the Lukaya river in Mont Ngafula. In Selembao, in spite of the presence of seropositive farms, there seem to be much less flies present than along the Lukaya (Pers. com., Pankwa Wotang X., 2004). This could explain why seropositive pigs from Selembao in general have lower PP values than seropositive pigs from Mont Ngafula. Some rivers (e.g. the Funa river) flow into the urban areas of Kinshasa. By following these rivers tsetse flies possibly penetrate deeply into the city along the vegetation on the banks of the rivers and could be responsible for some of the cases of human sleeping sickness in the urban area. However, a recent case-control study on sleeping sickness cases detected in the centre of Kinshasa demonstrated that these infections were most likely contracted elsewhere, e.g. in the province of Bandundu or the rural area around Kinshasa (Pers. Com., Robays J., 2004). On the other hand, in 1999 an epidemic outbreak of human sleeping sickness in the district of Kisenso (Ebeja et al., 2003) indicates that sudden upsurgences of the disease and local transmission of trypanosomes is possible.
All districts have reported cases of human sleeping sickness but in 1999 a concentration of cases was among others observed in Kisenso district (classified as an urban/peri-urban area) and Kimbanseke district (rural area) (Ebeja *et al*., 2003). Considering that pigs can harbour *T. b. gambiense* and the presence of tsetse flies as well as seropositive pigs in these districts, it is possible that these pigs are reservoir hosts for human sleeping sickness. Further research is required to determine the trypanosome species present in the pigs. This can be done by conducting PCR tests on blood samples from seropositive pigs. If these tests reveal the presence of *T. brucei gambiense* in pigs, a role of an animal reservoir for human sleeping sickness in Kinshasa must be considered and its importance further examined.
Some farmers in Kinshasa already apply systematic trypanocidal drug treatment to the animals. Taking into consideration the results of this study, this could be justified. However, systematic drug treatments alone will never completely solve the problem. Further epidemiological studies are still necessary to come to an integrated control program. The combination of the use of trypanocidal drugs in animals, vector control and an active surveillance program in humans is necessary to tackle the problem of trypanosomosis in man and animal.

6. Recommendations

Provided that the ELISA test is validated properly, it can offer an excellent tool for sero-epidemiological surveys for trypanosomosis in pigs. The results obtained in this study for the field samples from Kinshasa, can already give a first insight in the local situation of porcine trypanosomosis and its role in the transmission of human sleeping sickness. Further surveys will reveal the actual role of pigs as reservoir host for human sleeping sickness in this region. Considering these results, it will be possible to adjust the local trypanosomosis control program to come to a more integrated approach. This will hopefully end the recent rise in human sleeping sickness cases.

Bibliographical references

Agu W.E. & Bajeh Z.T. 1986. An outbreak of fatal *Trypanosoma brucei brucei* infection of pigs in Benue State of Nigeria. *Tropical Veterinarian* **4:** 25-25.

Agu W.E. & Bajeh Z.T. 1987. Studies on experimental infection of pigs with *Trypanosoma brucei*. *Acta Tropica* **44:** 371-372.

Arunasalam V., Chandrawathani P. & Sivanandan S. 1995. An outbreak of *Trypanosoma evansi* infection in pigs. *Journal Veterinary Malaysia* **7:** 71-73 In: Holland W.G., Do T.T., Huong N.T., Dung N.T., Thanh N.G., Vercruysse J. & Goddeeris B.M. 2003. The effect of *Trypanosoma evansi* infection on pig performance and vaccination against classical swine fever. *Veterinary parasitology* **111:** 115-123.

Baldrey F.S.H. 1910. Transmission of Surra. *The Journal of Tropical Veterinary Science* **5:** 595-596.

Bruce D., Harvey D., Hamerton A.E. & Bruce M.E. 1913. *Proceedings of the Royal Society of London*. Series B **87:** 48. In: Stephen L.E. 1986. *Trypanosomiasis: a veterinary perspective.* Pergamon Press, Oxford.

Büngener W. & Mehlitz D. 1984. Histopathological findings in mini-pigs infected with different strains of *Trypanosoma brucei*. *Tropenmedizin und Parasitologie* **35:** 109-114.

Diouf S. 2000. Mise au point de la technique d'ELISA pour le diagnostic de la trichinellose et son utulisation par enquête épidémiologique. MSc thesis ITM.

Ebeja A.K., Lutumba P., Molisho D., Kegels G., Miaka mia Bilenge C. & Boelaert M. 2003. La maladie de sommeil dans la région Ville de Kinshasa : une analyse rétrospective des données de surveillance sur la période 1996-2000. *Tropical Medicine and International Health* **8:** 949-955.

Eisler M.C., Hopkins J.S., Machila N., Van den Bossche P., Peregrine A.S. & Luckins A.G. 2000 Evaluation of antigen and antibody ELISA's for epidemiological surveys of tsetse-transmitted trypanosomosis in cattle. In: Animal trypanosomosis: diagnosis and epidemiology. Results of a FAO/IAEA co-ordinated research program on the use of immunoassay methods for improved diagnosis of trypanosomosis and monitoring tsetse and trypanosomosis control programs, IAEA, Vienna, Austria, p. 21-36.

Fagbemi B.O., Otesile E.B., Makinde M.O. & Akinboade O.A. 1990. The relationship between dietary energy levels and the severity of *Trypanosoma brucei* infection in growing pigs. *Veterinary Parasitology* **35:** 29-42.

Geysen D., Delespaux V. & Geerts S. 2003. PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in cattle. *Veterinary Parasitology* **110:** 171-180.

Gibson W., Mehlitz D., Lanham S.M. & Godfrey D.G. 1978. The identification of *Trypanosoma brucei gambiense* in Liberian pigs and dogs by isoenzymes and by resistance to human plasma. *Tropenmedizin und Parasitologie* **29:** 335-345.

Gibson W.C., Stevens J.R., Mwendia C.M.T., Makumi J.N., Ngotho J.M. & Ndung'u J.M. 2001. Unravelling the phylogenetic relationships of African trypanosomes of suids. *Parasitology* **122:** 625-631.

Gill B.S., Singh J., Gill J.S. & Kwatra, M.S. 1987. *Trypanosoma evansi* infection in pigs in India. *The Veterinary Record* **120:** 92.

Godfrey D.G. 1982. Diversity within *Trypanosoma congolense*. In: *Perspectives in trypanosomiasis research.* Baker J.R., editor. Research Studies Press, Chichester, p. 37-46.

Herbert W.J. & Lumsden W.H. 1976. *Trypanosoma brucei*: a rapid "matching" method for estimating the host's parasitemia. *Experimental Parasitology* **40:** 427-31.

Hirumi H. & Hirumi K. 1991. *In vitro* cultivation of *Trypanosoma congolense* bloodstream forms in the absence of feeder cell layers. *Parasitology* **102:** 225-236.

Hoare C.A. 1936. Morphological and taxonomic studies on mammalian trypanosomes. II. *Trypanosoma simiae* and acute porcine typanosomiasis in tropical Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **29:** 619-645.

Hoare C.A. 1970. Systematic description of the mammalian trypanosomes of Africa. In: *The African Trypanosomiases.* Mulligan H.W., editor. George Allen & Unwin, London, p. 24-59.

Hoare C.A. 1972a. *Trypanosoma (Nannomonas) simiae* Bruce *et al*., 1912. In : *The trypanosomes of mammals. A zoological monograph*. Hoare C.A. Blackwell Scientific Publications, Oxford and Edinburgh, p. 449-473.

Holland W.G., Do T.T., Huong N.T., Dung N.T., Thanh N.G., Vercruysse J. & Goddeeris B.M. 2003. The effect of *Trypanosoma evansi* infection on pig performance and vaccination against classical swine fever. *Veterinary parasitology* **111:** 115-123.

Ilemobade A.A. & Balogun T.F. 1981. Pig trypanosomiasis : effects of infection on feed intake, liveweight gain and carcass traits. *Tropical Animal Health and Production* **13:** 128-136.

Isoun T.T. 1968. The pathology of *Trypanosoma simiae* infection in pigs. *Annals of Tropical Medicine and Parasitology* **62:** 188-192.

Jacobson R.H. 1998. Validation of serological assays for diagnosis of infectious diseases. *Révue Scientifique et Technique. Office International des Epizooties.* **17:** 469-486.

Janssen J.A.H.A. & Wijers D.J.B. 1974. *Trypanosoma simiae* at the Kenya coast. A correlation between virulence and the transmitting species of *Glossina*. *Annals of Tropical Medicine and Parasitology* **68:** 5-19.

Kageruka P. 1987. Trypanosomes dans les élévages porcins du bas Zaïre. *Revue d'Elévage et de Médécine vétérinaire des Pays tropicaux* **40:** 49-53.

Katunguka-Rwakishaya E. 1996. The prevalence of trypanosomosis in small ruminants and pigs in a sleeping sickness endemic area of Buikwe County, Mukono district, Uganda. *Revue d'Elévage et de Médécine vétérinaire des Pays tropicaux* **49:** 56-58.

Killick-Kendrick R. & Godfrey D.G. 1963. Observations on a close association between *Glossina tachynoides* and domestic pigs near Nsukka, Eastern Nigeria. I- *Trypanosoma congolense* and *T. brucei* infections in the pigs. *Annals of Tropical Medicine and Parasitology* **57:** 225-231.

Lejon V., Rebeski D.E., Ndao M., Baelmans R., Winger E.M., Faye D., Geerts S. & Büscher P. 2003. Performance of enzyme-linked immunosorbent assays for detection of antibodies against *T. congolense* and *T. vivax* in goats. *Veterinary Parasitology* **116:** 87-95.

Luckins A.G. 1993. Diagnostic methods in trypanosomiasis in livestock. In: *Improving the diagnosis and control of trypanosomiasis and other vector-borne diseases of African livestock using immuno-assay methods*. IAEA, Vienna, Austria, p. 27-35.

Mahaga M.T. & Röttcher D. 1981. Chemotherapy of *Trypanosoma simiae* in pigs. In: International Scientific Council for Trypanosomiasis Research and Control. Publication nr. 112, O.A.U./S.T.R.C. Seventeenth Meeting, Arusha, Tanzania, p.284-288.

Marsden P.D., Blackie E.J., Rosenberg M.E., Ridley D.S. & Hagstrom J.W.C. 1970. Experimental *Trypanosoma cruzi* infections in domestic pigs (*Sus scrofa domestica*). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **64:** 156-158.

McNamara J.J., Mohammed G. & Gibson W.C. 1994. *Trypanosoma godfreyi* sp. nov. from tsetse flies in the Gambia: biological and biochemical characterization. *Parasitology* **109:** 497-509.

Minter D.M. 1976. Effects of transmission to man of the presence of domestic animals in infested households. In: New approaches in American trypanosomiasis research. Proceedings of an international symposium, Belo Horizonte, Brazil, 18-21 March 1975. Pan American Health Organization Scientific Publication 318, p.330-337.

Moloo S.K., Zweygarth E. & Sabwa C.L. 1992. Virulence of *Trypanosoma simiae* in pigs infected by *Glossina brevipalpis*, *G. pallidipes* or *G. morsitans centralis*. *Annals of Tropical Medicine and Parasitology* **86:** 681-683.

Mols A. & Lenaerts A. 1950. Quelques observations faites à Elisabethville sur la trypanosomiase des porcs causée par le *Trypanosoma simiae*. *Bulletin Agricole du Congo belge* **41:** 427-436.

Murray M., Murray P.K. & McIntyre W.I.M. 1977. An improved parasitological technique for the diagnosis of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **71:** 325-326.

N'Depo A., Komoin-Oka C. & Kone P. 2000. Application of an antibody-detection ELISA for the diagnosis of bovine trypanosomosis in Côte-D'Ivoire. In: Improving the diagnosis and control of trypanosomiasis and other vector-borne diseases of African livestock using immunoassay methods. Results of an FAO/IAEA/DGIS co-ordinated research programme organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria, p.79-83.

Ocholi R.A., Ezeugwu R.U. & Nawathe D.R. 1988. Mixed outbreak of trypanosomiasis and babesiosis in pigs in Nigeria. *Tropical Animal Health and Production* **20:** 140.

Okuna N.M., Mayende J.S. & Guloba A. 1986. *Trypanosoma brucei* infection in domestic pigs in a sleeping sickness epidemic area of Uganda. *Acta Tropica* **43:** 183-184.

Omeke B.C.O. 1989. Field performance of exotic pigs and their crosses in tsetse infested area of Nigeria. *Bulletin of Animal Health and Production in Africa* **37:** 79-83.

Omeke B.C.O. 1994. Pig trypanosomosis: prevalence and significance in the endemic Middle Belt zone of Southern Nigeria. *Revue d'Elévage et de Médécine vétérinaire des Pays tropicaux* **47:** 381-386.

Omeke B.C.O. & Ugwu D.O. 1991. Pig trypanosomosis : comparative anaemia and histopathology of lymphoid organs. *Revue d'Elévage et de Médécine vétérinaire des Pays tropicaux* **44:** 267-272.

Onah D.N. & Uzoukwu M. 1991. Porcine cerebral *Trypanosoma brucei brucei* trypanosomiasis. *Tropical Animal Health and Production* **23:** 39-44.

Otaru M.M.M. & Nsengwa G.R.M. 1987. An outbreak of trypanosomiasis in swine in southern Tanzania. *Bulletin of Animal Health and Production in Africa* **35:** 73-74.

Otesile E.B., Akpavie S.O., Fagbemi B.O. & Ogunremi A.O. 1991. Pathogenicity of *Trypanosoma brucei brucei* in experimentally infected pigs. *Revue d'Elévage et de Médécine vétérinaire des Pays tropicaux* **44:** 279-282.

Otesile E.B., Fagbemi B.O. & Adeyemo O. 1991. The effect of *Trypanosoma brucei* infection on serum biochemical parameters in boars on different planes of dietary energy. *Veterinary Parasitology* **40:** 207-216.

Parkin B.S. 1935. Symptomatology of some trypanosomiases of domestic animals. *Onderstepoort Journal of Veterinary Science and Animal Industry* **4:** 251-267.

Peel E. & Chardome M. 1954. *Trypanosoma suis* – Ochmann 1905 – trypanosome monomorphe pathogène des mammifères, évoluant dans les glandes salivaires de *Glossina brevipalpis*. Newst., Mosso (Urundi). *Annales de la Société Belge de la Médecine Tropicale* **34:** 277-296.

Rebeski D.E., Winger E.M., Lelenta M., Colling A., Robinson M.M., Ndamkou Ndamkou C., H. Aigner H., Dwinger R.H. & Crowther J.R. 1998. Comparison of precoated and freshly coated microtitre plates using denaturated antigen for the detection of antibodies against *Trypanosoma congolense* by indirect enzyme-linked immunosorbent assay. In: Livestock production for rural development. Proceedings of the IXth international conference of Association of Institutions Of Tropical Veterinary Medicine, 14-18 September 1998, Harare, Zimbabwe. Volume I. Mukaratirwa S. & Obwolo M.J., editors, p. 376-386.

Rebeski D.E., Winger E.M., Okoro H., Kowalik S., Bürger H.J., Walters D.E., Robinson M.M., Dwinger R.H. & Crowther J.R. 2000. Detection of *Trypanosoma congolense* antibodies with indirect ELISAs using antigen-precoated microtitre plates. *Veterinary Parasitology* **89:** 187-198.

Rebeski D.E., Winger E.M., Robinson M.M., Duringer R.H. & Crowther J.R. 2000. Development, standardisation and validation of ELISA methods to improve the control of trypanosomosis. In: Animal trypanosomosis: diagnosis and epidemiology. Results of a FAO/IAEA co-ordinated research program on the use of immunoassay methods for improved diagnosis of trypanosomosis and monitoring tsetse and trypanosomosis control programs, IAEA, Vienna, Austria, p. 11-19.

Reed S.A., Husein A., Hutchinson G.W. & Copeman D.B. 1999. A possible role for Russa deer (*Cervus timorensis russa*) and wild pigs in spread of *Trypanosoma evansi* from Indonesia to Papua New Guinea. *Memorias do Instituto Oswaldo Cruz* **94:** 195-197.

Steindl F., Armbruster C., Pierer K., Purtscher M. & Katinger H.W. 1998. A simple and robust method for the complete dissociation of HIV-1 p24 and other antigens from immune complexes in serum and plasma samples. *Journal of Immunological Methods* **217:** 143-151.

Stephen L.E. 1970. Clinical manifestations of the trypanosomiasis in livestock and other domestic animals. In: *The African Trypanosomiases.* Mulligan H.W., editor. George Allen & Unwin, London, p. 774-794.

Stephen L.E. 1986. *Trypanosomiasis: a veterinary perspective.* Pergamon Press, Oxford.

Stewart J.L. 1947. Porcine trypanosomiasis. *The Veterinary Record* **59:** 648.

Sumbu J. 2003. Rapport annuel d'activité 2003: Etude du role de réservoir animal dans la transmission de la trypanosomose humaine dans la ville de Kinshasa et son hinterland, Laboratoire Vétérinaire de Kinshasa. Projet Trypanosomoses Africaines IMT/INRB/LABOVET/PNLTHA, Kinshasa, RDC.

Thammasart S., Kanitpun R., Saithasao M. & Kashiwazaki Y. 2001. Preliminary studies by ELISA on the antigen and antibody dynamics in the early stages of experimental infections with *Trypanosoma evansi* in cattle. *Tropical Animal Health and Production* **33:** 189-99.

Truc P., Mathieu-Daudé F. & Tibayrenc M. 1991. Multilocus isozyme identification of *Trypanosoma brucei* stocks isolated in Central Africa: evidence for an animal reservoir of sleeping sickness in Congo. *Acta Tropica* **49:** 127-135.

Tuntasavan D., Chompoochan T., Bunnoy W., Mohkaew K., Winger E. & Crowther J. Determination of the *Trypanosoma congolense* and the *Trypanosoma evansi* antibodies detection ELISA for the diagnosis of surra in cattle in Thailand. In: Proceedings of the Second Research Coordination meeting of the FAO/IAEA Co-ordinated Research Program (CRP) D3 20.21. Rio de Janeiro, Brazil.

Uilenberg G. 1998. Chapter 3: Diagnosis. In: *A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis*. FAO, Rome, p. 59-86.

Unsworth K. 1952. An outbreak of *T. simiae* infection in pigs in Nigeria and its attempted control using antrycide methyl sulfate. *The Veterinary Record* **64:** 353-354.

Van den Bossche P., Doran M. & Connor R.J. 2000. An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta Tropica* **75:** 247-258.

Waiswa C., Olaho-Mukani W. & Katunguka-Rwakishaya E. 2003. Domestic animals as reservoirs for sleeping sickness in three endemic foci in south-eastern Uganda. *Annals of Tropical Medicine and Parasitology* **97:** 149-155.

Woo P.T. 1970. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Tropica* **27:** 384-386.

Wright P.F., Nilsson E., Van Rooij E.M.A., Lelenta M. & Jeggo M.H. 1993. Standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibody in infectious disease diagnosis. *Révue Scientifique et Technique. Office International des Epizooties.* **12:** 435-450.

Zweygarth E. & Röttcher D. 1987a. The occurrence of *Trypanosoma (Nannomonas) simiae* in the cerebrospinal fluid of domestic pigs. *Parasitology Research* **73:** 479-480.

Zweygarth E. & Röttcher D. 1987b. The treatment of *Trypanosoma (Nannomonas) simiae* in pigs with diminazene aceturate in combination with a 5-substituted 2-nitroimidazole compound (Ro 15-0216). *Tropical Medicine and Parasitology* **38:** 175-176.

Annex: Results of pig blood samples (n=493) collected from farms in the vicinity of Kinshasa and tested for presence of anti-trypanosomal antibodies with an antibody-detecting ELISA

Farm	District	Nr. of samples	Average PP	Maximum PP	Nr. of positives
Maman Toko	Kimbanseke	2	19,12		0
Baga	Kimbanseke	3	16,08		0
Lukoji	Kimbanseke	2	25,93	36,31	1
Djoni Kiala	Kimbanseke	1	36,53	36,53	1
Joseph Dianzenza	Kimbanseke	1	16,48		0
Chine Populaire	Kimbanseke	1	8,41		0
Batakuawu	Kimbanseke	1	5,8		0
Boli	Kimbanseke	2	21,73		0
Jean Bisaka	Kimbanseke	2	22,06		0
Mabangi Fils	Kimbanseke	2	27,73	45,66	1
Mavinga JB	Kimbanseke	2	12,44		0
Salalunda et fils	Kimbanseke	4	17,81		0
Masamba	Kimbanseke	2	29,87	40,08	1
Prefel	Kimbanseke	1	26,59		0
JMO	Kimbanseke	2	9,36		0
Talamaku	Kimbanseke	2	18,94		0
Mbiyavanga	Kimbanseke	2	12,12		0
Lukau	Kimbanseke	1	11,74		0
Mungulu	Kimbanseke	11	28,25	55,06	4
Sala Lunda	Kimbanseke	1	39,32	39,32	1
Jean Bangadia	Kimbanseke	1	14,19		0
Pascal Bazebiuna	Kimbanseke	1	13		0
Jules Kibuala	Kimbanseke	1	3,99		0
Dr. Ndjoku	Kimbanseke	1	14,91		0
Kanda	Kimbanseke	3	19,32		0
Roger Mulumba	Kimbanseke	1	17,7		0
Papa Onana	Kimbanseke	3	12,54		0
Bakatufwila	Kimbanseke	1	44,77	44,77	1
Kalonji	Kimbanseke	2	16,54		0
Ceforea Dokolo	Kimbanseke	3	14,15		0
Site Ngwanza	Kimbanseke	5	21,62	32,25	1
Mambu Mbombo	Kimbanseke	2	10,14		0
Ceforea	Kimbanseke	5	19,67		0
Didier Ekuaka	Kimbanseke	3	13,72		0
Wangata	Kimbanseke	3	21,71	36,93	1
Papa Donas	Kimbanseke	2	15,3		0
Mamango	Kimbanseke	1	12,06		0
Kikuema	Kimbanseke	1	12,34		0
Katumbayi	Kimbanseke	1	17,54		0
Kasanji	Kimbanseke	1	19,08		0
Espoir	Kimbanseke	1	37,02	37,02	1

Farm	District	Nr. of samples	Average PP	Maximum PP	Nr. of positives
Mwamba	Kimbanseke	10	42,43	100,6	5
Papa Mavungu	Kimbanseke	4	16,82		0
Banga Banga	Kimbanseke	3	32,47	38,94	2
Maman Rosalie	Kisenso	4	60,08	67,87	4
Kaba	Kisenso	1	28,22		0
Basindika Lusakuenu	Kisenso	2	46,03	50,08	2
Nsana Nzita	Kisenso	5	51,91	83,94	5
ONG Mwinda	Kisenso	2	154,97	179,57	2
Maman Evala	Kisenso	2	41,97	46,58	2
Edouard Luntala	Kisenso	3	23,9	37,52	1
Nzenza Landu	Kisenso	4	84,2	121,62	4
Mavinga	Kisenso	2	47,38	50,95	2
Nzita Zau	Kisenso	2	84,78	119,16	2
Mbondo	Kisenso	2	55,84	59,7	2
Kimona Meso	Kisenso	2	58,35	71,3	2
Bavuidi Ngoma	Kisenso	3	47,48	65,42	2
Kiamwendo Mana	Kisenso	2	60,53	65,1	2
Matondo	Kisenso	2	64,83	93,48	1
Kibula	Kisenso	1	85,69	85,69	1
Ngiambidilu	Kisenso	1	75,83	75,83	1
Charles Musungayi	Masina	1	16,48		0
Mutombo Mubenga	Masina	1	16,13		0
Frère Papa	Masina	1	21,24		0
Menu	Masina	3	7,52		0
Kande Pierre	Masina	1	23,79		0
Jerome Toko	Masina	2	10,97		0
Kombi Kataliko	Masina	3	23,63	32,91	2
Pasteur Mulumba	Masina	2	19,41		0
Papa Ndambu	Masina	2	10,44		0
Jean-Marie Avion	Masina	2	24,76	32,87	1
Nzalabantu	Masina	2	6,57		0
Comat	Masina	2	5,98		0
Kabombo Zula	Masina	2	5,28		0
Muzinga Mutombo	Masina	2	14,92		0
Bambu	Masina	3	12,76		0
Domaine Nseinsi	Mt Ngafula	6	135,81	341,09	5
Nguya De Kimbala	Mt Ngafula	7	53,46	76,51	7
EDK/ONGD	Mt Ngafula	10	112,3	174,99	10
Baobab	Mt Ngafula	5	194,68	494,48	5
Bendel Saigo	Mt Ngafula	5	246,72	493,77	5
Fambando	Mt Ngafula	1	42,05	42,05	1
Hexagone	Mt Ngafula	3	48,53	85,51	2

Annex

Farm	District	Nr. of samples	Average PP	Maximum PP	Nr. of positives
Kayembe	Mt Ngafula	2	75,61	79,87	2
Mavinga	Mt Ngafula	3	38,56	53,88	2
Nlandu et fils	Mt Ngafula	1	60,17	60,17	1
Mbumba Tedika	Mt Ngafula	6	56,19	94,3	6
Papy Lumbu	Mt Ngafula	2	20,05		0
Nzola Ntima	Mt Ngafula	2	44,55	61,64	1
Mukandilwa	Mt Ngafula	5	119,82	290,87	6
Dindamba Michel	Mt Ngafula	3	40,87	47,86	3
De La Lukaya	Mt Ngafula	4	160,88	199,13	4
Feproka	Mt Ngafula	5	208,37	411,59	5
Gourmet	Mt Ngafula	8	75,09	187,09	5
Bemy	Mt Ngafula	3	52,94	72,87	2
Kaninda	Mt Ngafula	2	33,95	35,2	2
Mbiya	Mt Ngafula	10	43,2	73,45	7
Carrefour ECC	Mt Ngafula	10	44,34	85,83	9
Nlemvo	Mt Ngafula	7	59,51	91,7	7
Kitenge	Mt Ngafula	3	37	52,27	2
Nkee	Mt Ngafula	5	85,49	113,04	4
Rivo Torto	Mt Ngafula	6	95,01	254,15	4
Jacques	Mt Ngafula	10	81,38	168,79	10
Melu	Mt Ngafula	7	165,59	499,71	7
Fortuna I	Mt Ngafula	4	69,61	113,43	3
Costa	Mt Ngafula	12	114,15	575,75	12
Mansueki	Mt Ngafula	2	44,99	50,99	2
Labamba	Mt Ngafula	3	195,48	304,36	3
Nzuzi	Mt Ngafula	6	165,96	440,7	6
Makikongo	Mt Ngafula	2	214,35	265,97	2
Wivine	Mt Ngafula	2	206,31	315,93	2
Mbelemo	Mt Ngafula	10	129,83	447,04	10
BMM	Mt Ngafula	3	256,95	519,95	2
Fiston Nzolo	Mt Ngafula	2	59,9	60,75	2
Papa Jean	Mt Ngafula	3	102,03	108,83	3
Dr. Masumu	Mt Ngafula	1	42,56	42,56	1
Michel Bavon	Mt Ngafula	5	28,33	50,09	2
Joelle	Mt Ngafula	1	21,99		0
Somue	Mt Ngafula	1	41,99	41,99	1
Roland	Mt Ngafula	2	104,44	119,79	2
Nioka	Mt Ngafula	3	73,15	86,02	3
Ziama	Mt Ngafula	1	92,36	92,36	1
Fortuna II	Mt Ngafula	4	155,87	363,96	4
Matala	Mt Ngafula	3	74,57	128,51	2
Tuendi	N'djili	3	5,83		0
Sadam	N'djili	2	5,49		0

Annex

Farm	District	Nr. of samples	Average PP	Maximum PP	Nr. of positives
Doudou	N'djili	2	11,29		0
Maman Germaine	N'djili	2	8,6		0
Nkiba Michel	N'djili	2	4,32		0
Radja	N'djili	3	14,18		0
Papa Georges	N'djili	2	19,09		0
Samba	N'djili	1	11,32		0
Nguala Bertin	N'djili	1	31,32		0
Lukau Georges	N'djili	2	20,02		0
Matuanga	N'djili	2	11,95		0
Mbisi Jean	N'djili	1	14,9		0
Bazonzisa	N'djili	2	9,18		0
Major Molasso	N'sele	2	10,25		0
Mbelolo	N'sele	3	39,58	86,55	1
Pièrre	N'sele	2	6,92		0
Mukawa Robert	N'sele	3	8,43		0
Maman Nsimba	N'sele	2	4,32		0
Madiata	N'sele	2	13,6		0
Gemima	N'sele	2	6,07		0
Sikatende	N'sele	5	75,91	205,43	3
Mawawu Lawu	N'sele	3	28,5	54,8	1
Papa Luzitu	N'sele	1	85,5	85,5	1
Isa	N'sele	1	4,32		0
Patrice Kabamba	N'sele	2	5,34		0
Mabiala	N'sele	2	19,55		0
Astride Njoku	N'sele	1	12,72		0
Adamo	N'sele	1	9,47		0
Mukendi	N'sele	3	30,24	45,3	1
Simbi Mengi	Selembao	2	29,71		0
La Gracia	Selembao	3	28,52	32,29	1
Ngelesi	Selembao	3	26,28	38,36	2
Tshibwaba	Selembao	3	44,26	60,85	2
Kazadi	Selembao	2	31,79		0
Wasolue	Selembao	3	59,15	72,89	2
Biyoko	Selembao	4	39,88	52,24	2
Papa Nsimba	Selembao	2	49,33	81,5	1
Papa Anicet	Selembao	2	82,7	84,03	2
Papa Mbwabwa	Selembao	1	39,52	39,52	1
Pero	Selembao	5	59,32	83,42	5
Papa Lambert	Selembao	2	28,55	45,13	1
La Benediction	Selembao	2	32,54	39,57	1
Luzolo	Selembao	2	10,95		0
Boni	Selembao	2	39,18	44,03	2
SIRO	Selembao	3	30,94	49,04	1

Farm	District	Nr. of samples	Average PP	Maximum PP	Nr. of positives
Cibola	Selembao	4	25,59	43,9	1
Affairé	Selembao	4	22,52	38,52	1