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Virulence profiles in mice of *Trypanosoma congolense* forest isolated from domestic animals in the democratic republic of Congo (DRC)

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Abstract
During four years, an investigation was conducted to examine the virulence profiles of eight strains of *Trypanosoma congolense* Forest isolated from domestic animals in four sites of African Animal Trypanosomosis (TAA) in the west of the Democratic Republic of Congo. Virulence tests conducted in NMRI mice revealed that, five of those strains (62.5%) had an average virulence profile and three of them had low virulence profile (37.5%). Both strains of Kimwenza and one of N'Djili Brasseries in the city of Kinshasa Province, showed a moderate virulence profile. At Mbanza-Ngungu in Kongo Central Province, two strains revealed low virulence profile and another strain an intermediate virulence profile while at Mushie in the Province of Mayi Ndombe, a strain had a low virulence profile and another a moderate virulence profile. The low profile virulence strains showed a prepatent period between 10 ± 4.5 and 20 ± 4.9 days while those of intermediate virulence profile had a prepatent period between 2 ± 0.0 to 6 ± 3.7 days. The results of this study revealed that the disease should normally have a low impact on the health of animals and livestock.

Keywords: virulence, *Trypanosoma congolense* forest, domestics animals, West of DRC

Introduction

In sub-Saharan Africa, tsetse trypanosomiasis is a major problem in both animal and human health and agricultural development (Pangui, 2001) [18]. This disease causeseconomic losses through abortions, premature births, prenatal losses, infertility in men through testicular lesions, a reduction in milk production in dairy animals and an increase in expenditure in the purchase of drugs (trypanocides). According to FAO, about 60 million cattle and 100 million small ruminants are at risk of the disease (Gardiner and al., 1989) [5]. Direct losses and the cost of controlling animal trypanosomiasis are estimated to be between US $ 600 million and US $ 1,200 million per year for sub-Saharan Africa (Osorio and al., 2008) [17]. This disease accounts for only one quarter of the economic losses due to animal diseases (Gibson, 2003) [7].

However, the distribution of the aforementioned losses remains variable depending on the environment. Indeed, the studies conducted so far have been able to reveal different epidemiological situations in the endemic areas of African trypanosomiasis (Van den Bossche and al., 2000) [22]. In some areas, the disease is rather endemic with little impact on productivity, especially in farmed animals. On the other hand, outbreaks are also observed in some areas, suggesting a variety of factors that affect these different areas. The most studied factor remains the variability of the host. Whether in humans or animals, it has been clearly demonstrated that genetic and physiological factors related to the host play a very important role in the expression of the interaction between the host and the parasite. It is also becoming increasingly clear that the parasite itself can influence the expression of this interaction with very wide variations within the same subspecies or subgroup (Bengaly and al. (2002a, 2002b) [1].

Human African Trypanosomiasis (HAT), for example, is caused by two different types of parasites, including *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. Field data show that *T. b. gambiense* generally give rise to a chronic form of sleeping sickness in West and Central Africa which may persist for several years, whereas *T. b. rhodesiense* generally causes acute infection in East Africa (Garcia and al., 2006) [4]. However, the diversity of asymptomatic clinical changes to acute forms has also been described in *T. b. gambiense*. Similarly, human trypanosomiasis caused by *T. b. rhodesiense* is rather chronic in southern countries, such as Malawi and Zambia

(Garcia and al., 2006) [4], while it has a rather acute profile with a rapid progression towards the late stage in other countries such as Uganda (MacLean and al., 2004).

In animals, is transmitted cyclically by tsetse flies in Africa and mechanically by biting flies in South America. Here again, differences in virulence have been reported between strains of *T. vivax* circulating in East and West Africa with strains from West Africa being more pathogenic to livestock compared to strain from East Africa (Stephen, 1986) [19] although some studies have demonstrated the presence of severe haemorrhagic disease caused by *T. vivax* in East Africa (Gardiner and al., 1989) [5]. In South America, however, most *T. vivax* infections are chronic and asymptomatic, with rare signs of clinical disease (Osorio and al., 2008) [17].

These different profiles of the virulence as observed in trypanosomes infecting humans as well as animals sufficiently demonstrate the existence of variations not only between the different species of the trypanosomes but also within the same species. Until now, variations in the virulence profiles within a single species have only been demonstrated in *T. congolense* type Savannah. Using mice model, experimental works carried out some authors have been able to show the occurrence of different virulence profiles of *T. congolense* savannah strains occurring in the same host (Masumu *et al.*, 2006) [12] or in different hosts (Van den Bossche and al., 2011; Motloang and al., 2014) [22].

So far, *T. congolense* Forest type is considered less pathogenic to cattle. These results were obtained from the work of Bengaly and al. (2002a, b) [2] who compared the virulence profile of a single strain of *T.* congolense Forest type in both mice and cattle. These studies yielded concordant results in which the strain analyzed exhibited the same profile of low pathogenicity in these two host species. The hypothesis of this study was that, based on *T. congolense* Savannah, there is variation in the strains of *T. congolense* forest in terms of their virulence profiles. Therefore, the present study was conducted to evaluate the variation in virulence of *T. congolense* type Forest strains isolated from the endemic foci of TAA in the west of the Democratic Republic of Congo.

Material and methods

Study area

The study duration was four years from 2013 to 2016. Samples were collected at the homestead of the Mushie Ranch (Latitude: 02° 09' to 2° 95' S and Longitude: 17° 45' E) in Mayi Ndombe Province, in the Province of Kinshasa (Latitude: 04°48'08"S and Longitude: 15° 30' 43" E) and in Mbanza Ngungu in the Province of Central Kongo (Latitude: 05° 27' 127" S and Longitude: 014° 97' 173" E). All these sites are located in the west of the DRC. Laboratory analyses were carried out at the National Institute of Biomedical Research (INRB) in Kinshasa.

Isolation of trypanosome strains

Trypanosomes were isolated from cattle and pigs which were positive through parasitological diagnosis by buffy coat. After isolation, 250 µl of each positive blood was inoculated intraperitoneally in two adult mice of the NMRI breed with weight between 25 and 30 g. These mice were followed up for the development of parasitemia using the matching method (Herbert and Lumsden, 1976). Once the parasitaemic peak of 10^8 trypanosomes/ml was attained, the mice were euthanized and heart blood was collected for the preparation of a stabilate. These strains were characterized by PCR developed by Geysen and al. (2003).

Virulence test of *T. congolense* Forest

The virulence testing of *T. congolense* strains was determined using following the protocol described in Masumu *et al.* (2006a) [12]. All the strains used were in their first passage in mice. Prior to infection, each of the stabilate was subinoculated in two mice and followed up as described above. When parasitaemia reached between $10^{7.5}$ and 10^8 trypanosomes/ml, caudal blood was collected and diluted with phosphate buffered saline glucose (PSG) to obtain a concentration of 10^5 parasites in a total volume of 0.2 ml. This volume was injected intraperitoneally into six mice for each strain. A group of six mice, injected intraperitoneally with 0.2 ml of PSG was used as a control.

For each strain, parasitaemia, hematocrit, prepatent period (number of days between inoculation and first detection of parasites in the blood) and survival time were recorded up to 60 days post-infection. Mortality in infected and control mice was recorded every other day for the first two weeks and twice a week for the rest of the experimental time. One animal was considered parasitologically negative when no trypanosome was detected in at least 50 microscopic fields.

Statistical analysis

The median survival time of infected mice was estimated in parametric survival models using Excel. The strains were divided into three classes of virulence based on their median survival time (MST) periods (<10 days, 10-50 days and > 50 days). Strains where none of the infected mice died during an observation period of more than 60 days were allocated to the last class. Using this classification, strains were considered as high virulent when their MST was <10 days, those of moderate virulence had a MST between 10-50 days while the MST of strains of low virulence was above 50 days.

Results

In total eight trypanosome strains were isolated from cattle and pigs. Molecular characterization revealed that they all belonged to *Trypanosoma congolense* Forest type. Three of these strains were isolated in Kinshasa Province, three in Mbanza Ngungu in Kongo Central Province and two in Mushie in Mayi Ndombe Province. Following the virulence testing in mice, five of these strains (62.5%) had an average virulence profile while the remaining three had a low virulence profile (37.5%). The three strains of Kinshasa had a Moderate virulence profile. In Mbanza Ngungu, two strains had a low virulence profile while one had a moderate virulence profile. In Mushie, one strain had a low virulence profile and another had a moderate virulence profile. Base on the hosts these strains were isolated from, three strains were isolated from cattle and six from pigs. Among the two strains that were isolated from cattle, one had a low and another one a moderate virulence profile. Strains isolated from pigs showed a moderate profile for four of them while the remaining two had a low virulence profile. All the results are shown in Table I, III, IV and Figure 1).

Table 1: Virulence profile of *T. congolense* Forest strains isolated from western DRC.

Strain	Area	Host	Median survival time	Virulence profile
K01	Kinshasa	Pig	33	Moderate
K02	Kinshasa	Pig	30	Moderate
N01	Kinshasa	Pig	33	Moderate
Mb01	Mbanza Ngungu	Pig	51	Low
Mb02	Mbanza Ngungu	Pig	59	Low
Mb03	Mbanza Ngungu	Pig	25	Moderate
Mu01	Mushie	Cattle	55	Low
Mu02	Mushie	Cattle	41	Moderate

Strains of low virulence profile had a prepatent period varying between 10 ± 4.5 and 20 ± 4.9 days whereas the prepatent period of strains of moderate virulence profile varied between 2 ± 0.0-6 ± 3.7 days. The evolution of parasitemia of most strains including those of low and moderate profiles showed two peaks except two strains of moderate profile that had a single peak. (Table II)

Table 2: Pathogenicity of strains of *T. congolense* Forest isolated in western DRC

Strain	Virulence profile	Prepatent period in days	1st Peak in days	Number of peaks
K01	Moderate	5 ± 2,9	25 ± 9,1	2
K02	Moderate	4 ± 0,8	19 ± 5,6	1
Mb01	Low	20 ± 4,9	33 ± 2,3	2
Mb02	Low	17 ± 5,9	33 ± 10,0	2
Mb03	Moderate	6 ± 2,9	11 ± 4,1	2
Mu01	Low	10 ± 4,5	21 ± 6,0	2
Mu02	Moderate	2 ± 0,0	9 ± 4,3	2
N01	Moderate	6 ± 3,7	30 ± 10,1	1

Table 3: Proportion of virulence profiles of *T. congolense* Forest strains in western DRC according to sites

Site	Number of strains of *T. congolense* Forest	Number of *T. congolense* Forest strains with average virulence	Number of *T. congolense* Forest strains with low virulence
Mushie	2	1	1
Mbanza Ngungu	3	1	2
Kinshasa	3	3	0
Total	8	5	3

Table 4: Proportion of virulence profiles of *T. congolense* Forest strains in western DRC according to hosts

Host	Number of strains of *T. congolense* Forest	Number of *T. congolense* Forest strains with moderate virulence	Number of *T. congolense* Forest strains with low virulence
Cattle	2	1	1
Pigs	6	4	2
Total	8	5	3

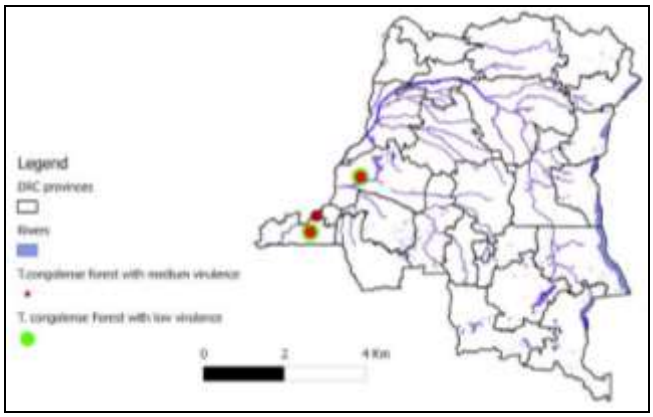

Fig 1: Distribution of the virulence profile of *T. congolense* Forest in the Western DRC.

Discussion

Studies on the virulence of trypanosomes infecting livestock in Africa which have been carried out so far in different laboratories in the continent and elsewhere confirm considerable variations between the different species on the one hand and, on the other hand, between different strains belonging to the same species. Concerning *T. congolense*, which is considered the most pathogenic species in cattle, the Savannah type is the most studied of the three. Indeed, previous studies on the virulence profiles of *T. congolense* indicated that the Savannah type is highly virulent compared to the Forest and Kilifi types that are considered to be low or not pathogenic for cattle, respectively (Bengaly and al., 2002a, b) [2]. However, most of these studies were conducted using very limited number of isolates for each of the *T. congolense* types.

The variation in the virulence profiles among *T. congolense* strains belonging to the Savannah type was first demonstrated by Masumu and al. (2006) [12]. In this study, these authors used thirty-five isolates of *T. congolense* Savannah type that were collected from cattle in Eastern Zambia. Results obtained from their experiment were able to explain why strains of *T. congolense* Savannah type circulating in trypanosomiasis-susceptible bovine breeds (Nguni) in Eastern Zambia, induced a less severe infection in these animals.

Further studies on AAT in South Africa (Van den Bossche and al., 2011; Motloang and al., 2014) [22] confirmed the assertions made previous authors (Masumu and al., 2006) [12] concerning the variation between the *T. congolense* Savannah whereby different strain circulating in the same area exhibited different virulence profiles. But results from these later studies (Van den Bossche and al., 2011; Motloang and al., 2014) [22] revealed higher virulence profile of *T. congolense* Savannah strains circulating in wild animals (buffaloes) compared to those circulating in cattle. Although all these studies have been conducted in mice, a concordance between the virulence profile of *T. congolense* in mice and in cattle has previously been demonstrated (Bengaly and al., 2002a, b) [2] suggesting that the results obtained using mouse model can be extrapolated in cattle.

Till recently, *T. congolense* Forest has only been considered as less virulent for livestock. This statement reported from previous studies conducted in mice and in cattle respectively using a single strain isolated in Burkina Faso (Bengaly and al., 2012a, b) [2]. The current study was the first to have used a fairly large number of *T. congolense* Forest strains isolated from three AAT foci in different provinces of DRC (Figure 1). In total eight *T. congolense* Forest strains were used among which six were isolated from pigs while two were from cattle. The results obtained demonstrated, for the first time, the variation of virulence profiles among *T. congolense* Forest strains confirming the observation made by the previous authors related to significant variations in the virulence profiles of *T. congolense* Savannah type (Masumu and al., 2006a; Van den Bossche and al. 2011; Motloang and al., 2014) [12, 22].

The present results has several implications. First, at the epidemiological level, these strains were isolated from two different animal species. In pigs, four of the six strains tested (67%) showed a moderate virulence profile and the remaining two (33%) had a low profile. In cattle, however, the two profiles (moderate and low) were found in each of the strains tested respectively. These strains were isolated either in areas where only pigs (Kinshasa and Mbanza Ngungu) or only cattle (Mushie) were exploited as livestock. On the other hand, in areas where pigs were raised (Kinshasa and Mbanza Ngungu), large game are non-existent, unlike Mushie, which is located in the middle of the equatorial forest and where large game may co-habit with cattle. Based on previous data (van den Bossche and al., 2011; Motloang and al., 2014) [22], trypanosome strains isolated from wildlife exhibited a high virulence profile compared to those isolated from domestic animals kept away from wild animals. We would have expected to find strains of higher virulence profile in Mushie (Forest zone) compared to Kinshasa and Mbanza-Ngungu (Savannah zones). In contrast, in areas where large wild animals were absent (Kinshasa and Mbanza-Ngungu), two third of the strains were of moderate virulence profile compared to half in Mushie where large wild animals are present. This situation can be explained either by the consequence of the coexistence of the strains of different virulence profiles or by the existing local control strategies.

Indeed, it has been suggested that in areas where trypanosomiasis is endemic, trypanosome strains of high virulence profile are gradually eliminated (Hide and al., 1994). This hypothesis is supported by the fact that high virulent trypanosome strains would require frequent treatment or induce higher mortality in sensitive hosts and thus reduce their overall presence. In the area where *T. congolense* Forest strains were collected, none of the strains circulating in the animals had a high virulence profile. However, the majority of strains had a moderate or low virulence profile resulting in benign infections. In addition, further analysis made by Masumu and al. (2006a) [12] revealed that infection with low virulent trypanosome strains protects animals in the event of co-infection with those of high virulence profile. Under these circumstances, even when infected with trypanosome strains of high virulence profile, animals in this area will rarely need treatment as confirmed by Van den Bossche and al. (2000) [22]. Therefore, there is a low possibility to encounter high virulent trypanosome strains in the study area presumably due to death or treatment (Masumu and al., 2009a) [14].

As far as the control strategies are concerned, cattle in the Mushie ranch are regularly treated with trypanocides (unpublished data). Since this ranch is of the commercial type, this treatment is systematic given the availability of trypanocides, especially diminazene. As in many other AAT foci, treatments are applied to any animal in poor physical condition without a confirmatory examination being carried out (van den Bossche and al., 2011) [22]. It is quite evident that even if virulent trypanosome strains were present in these foci due to the presence of wild animals, such a treatment regime would eliminate most of them. However, such a blind treatment may, over time, weaken the resistance of the animals even to moderate virulence strains. The results of this study therefore deserve to be taken into consideration by local decision-makers in the implementation of more appropriate control strategies that would favor the establishment of a lasting endemicity in the animals exploited in these different foci.

Conclusion

The results of this study showed that *T. congolense* Forest strains circulating in domestic animals in western DRC have not only low virulence profiles but the moderate virulence profile has also been found in these animals especially in pigs reared in Kinshasa and Mbanza-Ngungu. Therefore, in these zones, these strains with moderate and low virulence profiles induce protection against trypanosomes which can have a high virulence profile. As a consequence, the disease found in these areas should in principle have a low impact on animal and livestock health. This leads us to have many reservations about cases of morbidity and mortality declared clinically as being caused by trypanosomiasis in the west of the DRC. This could be the case where, for example, there is mixed infection with other pathologies such as tick-borne diseases, verminosis and tuberculosis, which may lead to immunosuppression in animals. And especially since trypanosomiasis has almost the same symptoms as tick-borne diseases and certain helminthiosis. Further studies are needed to verify these assumptions.

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