Bovine Trypanosomiasis in the peri-urban zone of Bamako (Mali): status report in the context of increasing livestock numbers

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Overall the manuscript is very poorly written and needs major revision

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ABSTRACT

African Animal Trypanosomiasis (AAT) is a haemoparasitic diseases ~~Haemoparatosis~~ that considerably hampers socioeconomic development in sub-Saharan Africa. One way to counter this scourge is through vector control and chemoprevalence. **Objectivefs**: This study aims to help breeders in the study zone to control AAT~~TAA~~. **Location and duration of the study:** About three-year longitudinal follow-up was conducted in seven municipalities (Mandé, Kati, Kalaban coro, Sanankoroba, Baguinééda, Tienfala) ~~and~~ in the district of Bamako from M~~m~~arch 2007 to December 2009. **Methodology**: Thirty to fifty heads of cattle were randomly selected in each municipality, and depending on herd size, 5 to 10 heads of cattle were selected. For each run, all animals diagnosed with *Trypanosoma* s~~S~~pp. positivity were systematically treated. **Results:** A total of 7,622 blood samples from 312 cattle of different breeds (…) were collected and examined. ~~monitored. A total of 7,622 blood samples were collected during the six visits and examined using the Woo technique, i.e., an average of 312 samples per visit~~. A total of 106 animals were found infected with two *Trypanosoma* species (*T. vivax*, 99% and *T. congolense*, 1%). ~~A total of 1691 doses of trypanocidal products were administered. A total of 106 infections were recorded due to two *Trypanosoma Sp* species (*T. vivax*, 99% and *T. congolense*, 1%) (P<0.05%).~~ Prevalence was significantly (P<0.05) variable not only by breed (78.25% for zebu and nil for N'Dama) but also by year (4.47%, 0.53% and 0.1%~~)~~ in 2007, 2008 and 2009, respectively). ~~(P<0.05%).~~ Variability was also observed according to locality. However, this did not vary according to the sex of animal (P= 0.07~~%~~). A total of 1691 doses of trypanocidal products were administered to infected animals. **Conclusion:** As a result of treatment, the incidence and prevalence of the disease has decreased~~, and its prevalence has decreased~~ considerably. However, the risk of disease transmission remains, as biological and mechanical vectors are omnipresent in the area. Seasonal animal movements are also a risk factor.

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Keywords: Trypanosomiasis, Cattle, Vectors, Prevalence, Mali.

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1. INTRODUCTION

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Are there reports of mechanical vectors transmitting this diosease in the study area

Trypanosomiasis is a parasitic disease of humans and animals transmitted by tsetse flies. Endemic primarily in domestic animals, this condition remains a major obstacle to livestock development in infested regions (Farougou *et al.,* 2012). According to Affognon *et al.* (2012), nearly 80% of the human population lives in tsetse-infested areas and depends mostly on agriculture. In these countries, livestock farming contributes between 10% and 20% of the country's GDP. Trypanosomiasis, a debilitating and fatal livestock disease, is one of the major constraints on the socio-economic development of populations, causing a drop in productivity and considerable economic losses (Bouyer *et al.,* 2006). The Koulikoro region and the Bamako district are 80% infested with tsetse fly (Traoré *et al.,* 2024). Every year, around 2.7 million cattle and 2.5 million people are exposed to the risk of the disease (Djitèye *et al.,* 1992). Faced with the constraints imposed by African Animal Trypanosomiasis (AAT) and its vectors on the promotion of agriculture and livestock farming in countries affected by the scourge, several national tsetse fly and trypanosome control projects have been developed in Africa. While some of these projects have been successful (reducing tsetse fly density and trypanosome infection rates in livestock), unfortunately, they have not led to the eradication of tsetse fly and trypanosomiasis. They continue to represent a major health and economic challenge in sub-Saharan Africa. Animal trypanosomiasis can lead to a progressive deterioration in the affected individual's general condition. Domestic animals infected with the disease can act as reservoirs.

The peri-urban area is the dairy belt and source of meat production for a population of approximately 4.228 million (INSTAT, 2024). Livestock farming is the second most important primary sector in the Niger River basin. Livestock numbers have risen steadily in recent decades. Livestock comprised mainly of poultry (17,253,244), sheep and goats (132,206), cattle (43,285), asians (872), and only 790 horses (DNPIA, 2022). Livestock production systems are traditionally extensive and characterized by exclusive or semi-intensive herding, especially in the peri-urban area of Bamako. Most of the herds were sedentary. Cattle breeds include~~d~~ Peulh zebu, Maure zebu, N'Dama, stabilized crossbreeds (Méré), and local/exotic crossbreeds (Montbéliard, Rouge des Steppes and Holstein). Among small ruminants, the Djalonké and related breeds are predominant (Traoré *et al.,* 2019). Intensive livestock farming is booming in this area. The introduction of improved breeds during this period significantly increased milk production and led to the establishment of small milk processing units. However, transhuman herds are present during the dry season. Endoparasites such as transboundary trypanosomes can considerably reduce herd productivity, exposing almost 50% of the human population to food insecurity (Matteroni *et al.,* 2004). The asymptomatic nature of ~~TAA~~ AAT can often result in reduced productivity and weakened animals; hence, this study aimed~~, which aimed~~ to determine the prevalence of ~~TAA~~ AAT in different breeds of cattle in the peri-urban zone of Bamako to~~,~~ help breeders control ~~TAA~~ the disease and improve animal productivity.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Bamako and its suburbs from 2007 to 2009. The District of Bamako stretches 22 km from W~~w~~est to E~~e~~ast and 12 km from N~~n~~orth to S~~s~~outh, covering an area of 277 km^2 (Figure 1). In 2009, the human population was estimated at 1,810,366, with a density of 4,563.17 inhabitants/km^2 (ANMM, 2021). The city of Bamako is watered by the Niger River over a length of approximately 26 km and therefore enjoys a fairly humid tropical climate with a marked dry season and rainy season. The vegetation is savannah-Sudanese in the northeast (Province of Kati, Koulikoro, Dioïla and the District of Bamako), and sub-Sudanese or pre-Guinean in the northwest and southwest (municipalities of Bancoumana, Mandé, Siby). The Koulikoro Region is characterized by various plant formations that, vary from north to south depending on the soil and rainfall. They range from open forests (Sudano-Guinean zone) to gallery forests and shrubs (Saharan zone). The most common plant species are: *Barassus aethipum*, *V. pardoxa*, *E. camadulensis*, *A. indica*, *Bombax costatum*, *M. indica*, and *P. biglbosa*. Forage potential is characterized by a grass carpet dominated by *Loudetia togoensis* on cuirassed soils, *Andropogon pseudopricus* on hydromorphic plains, and *Schoenefelddia gracilis* on rocky soils (ANMM, 2021). The annual rainfall in the region can reach 990 mm (Traoré, 2024). In general, the relative humidity varies between 40% and 60%, with an average humidity of 81%, monthly (ANMM, 2021). The livestock are mainly cattle, sheep, and goats. These animals are a source of income for the local population~~ and are used to pull cattle~~. There are very few equidaes (François *et al.,* 2002). Poultry farming is practiced in almost every village. The invertebrate fauna is rich in insects of medical, veterinary, and agricultural interests (Muscidaes,

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Culicidaes and Simuliidae~~.......~~) (François *et al.,* 2002). These environmental conditions provide a biotope conducive to the proliferation of tsetse flies, vectors of trypanosomiasis. Hence, this site is, also an agrosilvo-pastoral zone. Dairy products, poultry, truck farm produce, and other products come from these sites, and many local livestock farmers are tempted by the idea of fattening small ruminants. Regular summer rainfall also allows the development of wooded savannahs, cereal, and industrial crops such as sorghum, corn, and cotton.

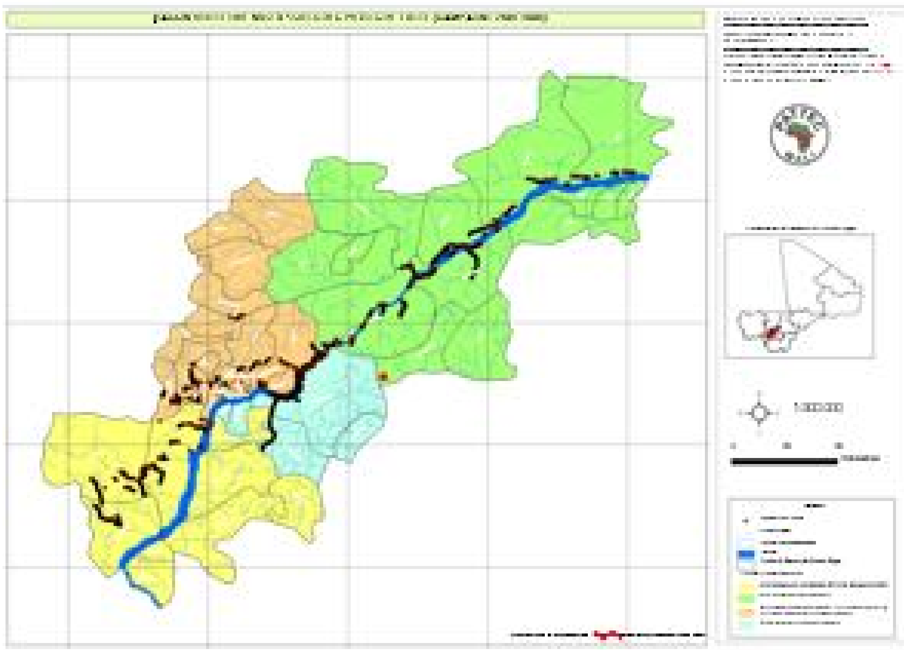

Figure 1: Location of the study area (source, PATTEC-Mali, 2007)

2.2. Methodology

This descriptive cross-sectional study with repeated passages included ~~. Blood samples were collected from~~ seven municipalities (Mande, Kalaban coro, Sanankoroba, Kati, Tienfala, Baguinéda, Koulikoro) ~~and~~ in the Bamako district. Five villages in each of these communes were randomly selected. For each run, blood samples were collected from around 12 animals in different herds, depending on the availability of the owners, for 312 cattle. The sample size was determined by using online questionnaire (Winepi.net) with a confidence level of 95% and margina of error of 5%. The selected animals were entered by noting the identification code, sex, age, breed, and date of the last trypanocidal treatment. Blood was drawn from the jugular vein and collected in v~~V~~acutainer tubes, each containing an anticoagulant. ~~The selected animals were treated with trypanocide (Diminazene acetunate) at a dose of 3.5 mg/kg. During the investigations, all animals diagnosed as positive and those with hematocrit levels below 25% were also treated.~~ ~~Blood was drawn from the jugular vein and collected in Vacutainer tubes, each containing an anticoagulant.~~

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~~The selected animals were entered by noting the identification code, sex, age, breed, and date of the last trypanocidal treatment.~~ The method of Murray (1977) was used to examine buffy coats after differential centrifugation in the capillary tubes of trypanosomes. The hematocrit level was determined using the Buffy Coat test. This procedure consisted of filling microtubes 2/3 full with blood and sealing one end with plasticine (modeling paste). The tubes were then centrifuged for 5 min at 12,000 rpm in a hematocrit centrifuge. The hematocrit level was estimated using a hematocrit reader after examination of the interface (Buffy Coat). After reading, the tube was cut 1 mm below the interface using a diamond cutter to, include the layer of erythrocytes. This fluid was then observed under a darkfield or phase-contrast microscope at 400X magnification to determine the animal's parasitemia. During the investigations, all animals diagnosed as positive for and those with hematocrit levels below 25% were treated with a trypanocide (Diminazene aceturate) at a dose of 3.5 mg/kg.

2.3. Cattle selection criteria

The sample size was determined using an online questionnaire (Winepi.net) with a confidence level of 95% and a margina of error of 5%. Cattle were selected at random, regardless of sex, age ~~class~~, or breed, according to the availability and the number of herds in the area. Verbal consent was obtained from the owner of each herd.

2.4. Data analysis

Data were entered into Excel version 2007. Statistical analyses were performed using R software (R version 4.4.2, http://www.r-project.org); Bartlett's Chi^2 test was used to compare variances at the 5% ~~threshold~~level of significance.

2.5. Ethical issues

The study protocol was approved by the institutional ethics committee of the Faculty of Science and Technology (FST). Informed, voluntary consent was obtained from all ~~breeders~~owners. All measures were taken to minimize the risks associated with participation in the study.

3. RESULTS AND DISCUSSION

3.1. RESULTS

Parasitological analysis was carried out on a total of 7,622 blood samples from 312 cattle in eight communes over three survey years, i.e., 2015, 2,646 and 2,961 ~~cattle~~ samples in 2007, 2008, and 2009, respectively. ~~(Figure 2).~~

Comment [DNS10]: Give the names of breeds, how many animals of particular breed sampled. Merge this sub heading with methodology in line 4

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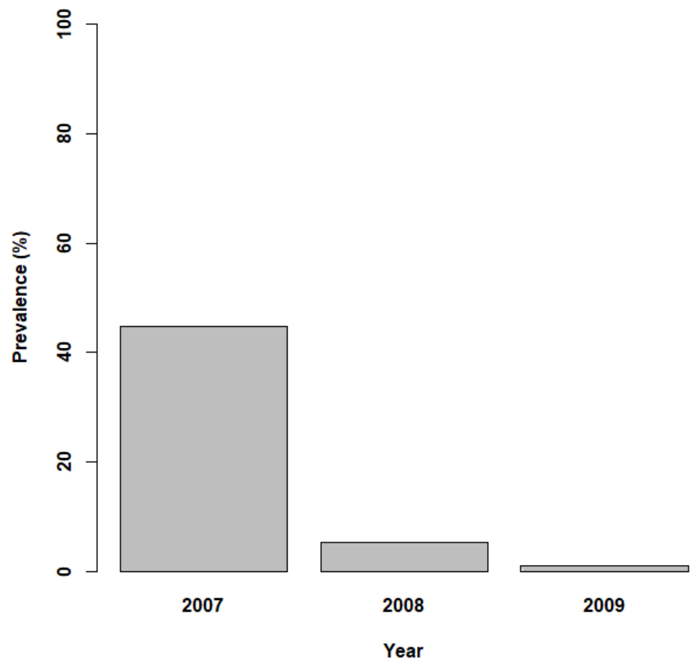

Figure 2: Change in prevalence during the study period.

The highest infection rate was ~~achieved~~ observed in 2007, ~~and~~ while the lowest was ~~achieved~~ observed in 2009. In 2008, the prevalence rate was less than 1% (Figure 2).
A statistically significant variation in prevalence was observed in the study population according to breed (Figure 3).

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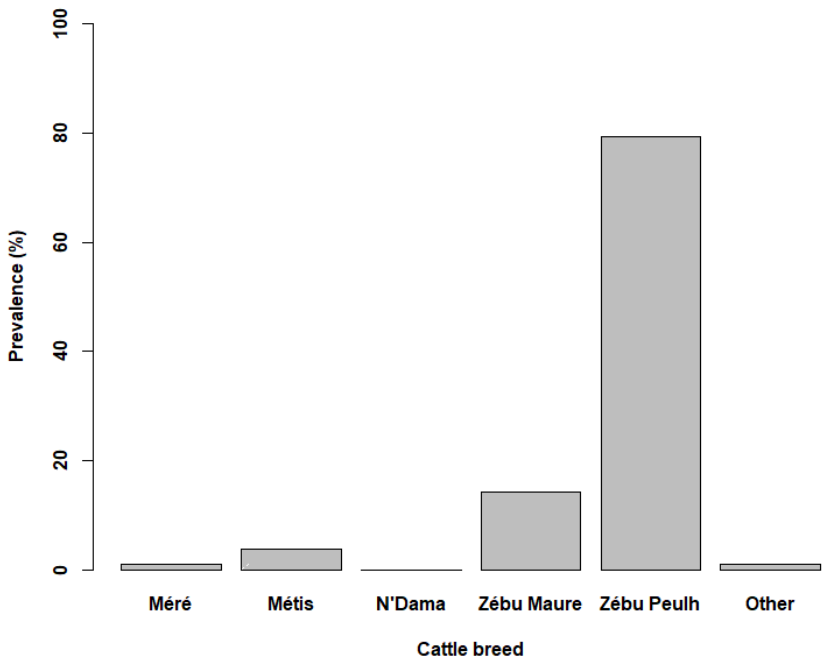

Figure 3: Prevalence varies by breed

More than 12 breeds were recorded, with a predominance of the Peulh zebu breed (33.5%), mixed breeds (32.3%), and Mérés (30.6%). The N'Dama breed is the only species (0.5%). A few isolated breeds, such as Azawak, Goudale, Bodouro, Holsten, and Batorodji, accounted for only 0.5%. The breeds most affected were Peulh Zebu (79.25%), Moorish Zebu (14.15%), and mixed breeds (3.14%). The values for mixed and improved breeds were, 3.14% and 1.89%, respectively. The prevalence rate of N'Dama is zero.

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Prevalence data does not match with that shown in Figure 3

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The cattle in our study sample were predominantly female (70.6%), with a ~~sex~~male: female ratio of 1: 2.62 (Figure 4).

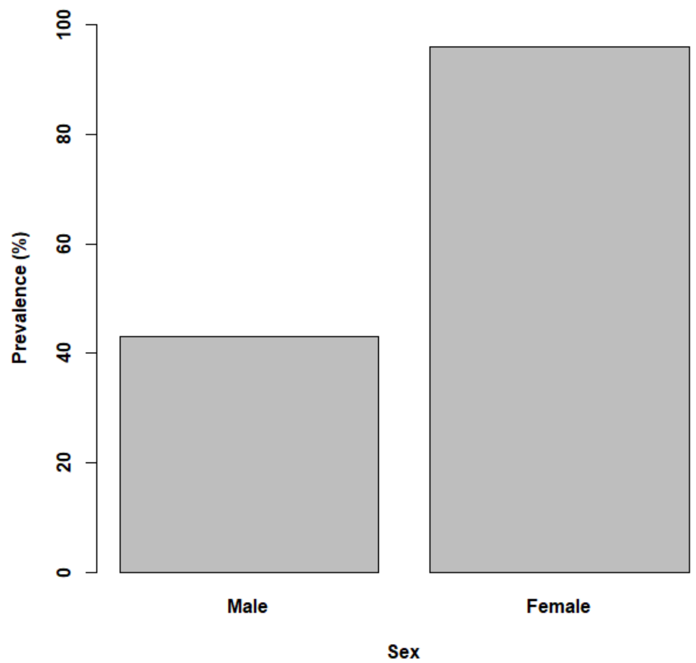

Figure 4: Variation in prevalence according to sex.

The prevalence rate was 43% in male and 96% in female. The prevalence rate varies greatly from one commune to another and according to year (Figure 5).

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Better to predict the data in tables rather than in figures.

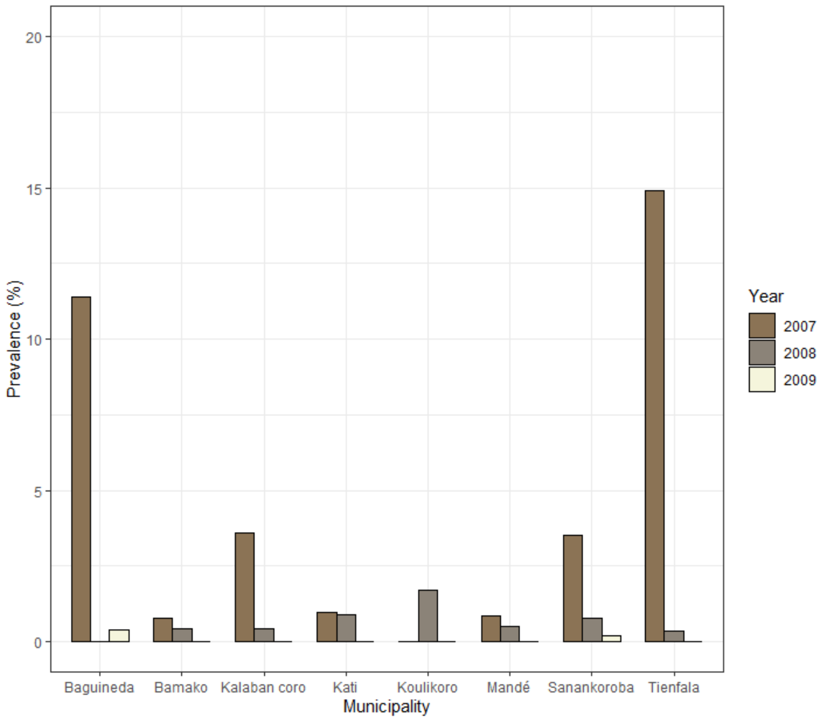

Figure 5: Variation in prevalence by municipality

It is highest in the villages of Tienfala and Baguineda. The herds in these two municipalities were the most affected by the disease, accounting for almost 15% and 11.49% in 2007, while Koulikoro (1.72%) and Kati (0.9%) recorded the highest prevalence rates in 2008. The prevalence of *T. congolense* was almost zero in all the municipalities in 2009, with the exception of Baguinéda (0.38%) and Sanankoroba (0.19%). 99% of the cases detected were due to *Trypanosoma vivax* (102/106) compared with only 1% (04/106) for *T. congolense*.

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The variation in prevalence according to the animal's health status is shown in figure 6.

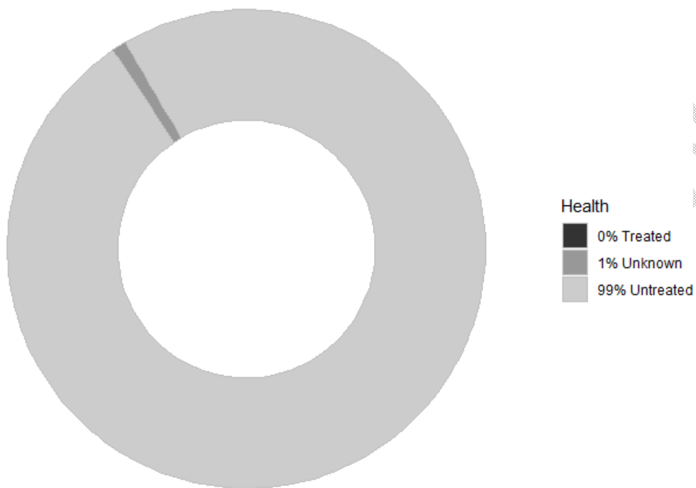

Figure 6: Variation in prevalence according to animal health status.

The prevalence of trypanocide treatment is highest among untreated heads (99%), very low among heads whose status is unknown, and zero among heads that have received a dose of trypanocide.

3.2. DISCUSSION

Careful monitoring can help reduce disease incidence. Lowering pressure in an area can reduce disease transmission by biological vectors (Traoré *et al.,* 2019). The reduction in the number of infestation cases recorded during the study period could be explained by the systematic investigation and treatment of all animals detected positive and those suspected of being infected, in order to interrupt the chain of transmission in the area between 2007 and 2009. These results corroborate those of Traoré *et al.* (2019) and Diall et al. (1987) on the Madina Diassa ranch. In 2005, a project to control tsetse flies and trypanosomosis in Mali (PLMT) was able to control the ~~TAA~~ AAT epidemic in the Tienfala-Baguinéda agropastoral zone by reducing the average prevalence from 17.6% to 1.1%, then 1.48% and 1.22% for three consecutive years (UCLT, 2005). The average prevalence rate was 1.4%. Despite the high number of female, the prevalence of male and female sex was not significant (P>0.05%). These results are contrary to those reported by Tanenbe *et al.* (2010) in Cameroon, who reported a prevalence rates of 46.1% in males and 35.6% in females.

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Comment [DNS24]: Were the same animals monitored before and after treatment for prevalence of infection in different years to check lowering in disease pressure?
Give split wise how many animals found infected and how may treated in different years and which breeds and in which areas. **The study design is not accurate.**

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Farougou *et al.* (2012) identified two *Trypansoma* s~~S~~p. species (*T. vivax* and *T. congolense*); however, they found that 33.3% of infections were due to *T. vivax* and 44.5% to *T. congolense*, in contrast to our study where 99% of cases were caused by *T. vivax* versus 1% for *T. congolense* (P<0.05%). Tanenba *et al.* (2010) found that *T. congolense* predominated in their study area and attributed this increase to the probable proximity of tsetse flies to other animals (Tanenbe *et al.,* 2010). The predominance of this species over *T. congolense* in this study is explained by the presence of *Glossina palpalis gambiensis*, the only species identified for over 10 years in the municipalities of Mandé, Tienfala, Baguinéda, and even Kati, and the main biological vector of *T. vivax* (Traoré *et al.,* 2024). No cases of coinfection were recorded. In 1986, in Madina Diassa (Diall *et al.,* 1986), identified three species (*T. vivax*, *T. congolence* and *T. brucei*), with a predominance of *T. congolence*. Boka *et al.* (2019) identified only one species (*T. vivax*) with a prevalence of approximately 6%. There was a highly significant variability in prevalence between breeds (P=0.00). The percentage was high for the zebus (79.25%) and null for the N'Dama breed. Aka *et al.* (2022) also showed the tolerance of certain breeds to trypanosomes, such as the Lagunaire breed in Côte d'Ivoire, compared with the N'Dama breed. In southern Mali, Diall *et al.* (1986) reported that the N'Dama breed is more resistant to trypanosomiasis infections. Significant variability was recorded between different localities (P=0.00). In 2007, it ranged from 0% (KouliKoro) to 14.89% (Tienfala), while in 2009, it was 0.38% in Baguinéda, 0.19% in Sanankoroba, and nil in the other municipalities (P<0.05%). This variability in prevalence has also been shown by Bengaly *et al.* (1998) in the provinces of Boulagouriba, Kénédougou, and Mouhoun in Burkina Faso (Bengaly *et al.,* 2001), as well as by Tanenbe *et al.* (2010). a few cases of *T. theileri* have been reported, but since its pathogenic role is poorly known, as shown by Amato *et al.* (2019), they have been neglected (Amato *et al.,* 20191).

The Bamako district is at the center of the abovementioned municipalities and is a focal point for trade. In the area, most livestock breeders fatten their animals and, in most cases, sell their calves, while others use them for dairy production. Controlling certain diseases can help improve livestock production. Animal production capacity can be influenced by several factors (Diaf, 2008), including zoonoses. As livestock farming is the main economic activity in the peri-urban area of Bamako, infection of animals with haemoparasites, such as trypanosomes, can affect the quantity and quality of animal productivity. Houndje *et al.* (2024) reported that knowledge of dominant parasitic pathologies can help estimate the costs of agropastoral products and that assessing the prevalence of these parasitic pathologies is vital for the agropastoral sector (Houndje *et al.,* 2024). This low prevalence does not negate the risk of disease transmission. Although tsetse densities have been low in recent years, the very remarkable roaming of animals, as well as transhumance, are factors that favor close contact between hosts and vectors and consequently increase the risk of transmission. Houndje *et al.* (2024) reported that even if the prevalence of trypanosomosis is declining, interbreeding and/or roaming of animals can increase the risk of transmission. Diaf (2008) showed that despite booming animal productivity in southern countries, supply is not keeping pace with demand because of demographic growth (Diaf, 2008). Controlling endoparasitic diseases could guarantee better animal production and contribute to improving food safety.

4. CONCLUSION

The aim of this study was to determine the prevalence of ~~TAA~~ AAT in cattle in the peri-urban zone of Bamako district to help breeders control ~~TAA~~ the disease and improve animal productivity. Trypanosomosis control campaigns in and around Bamako have reduced trypanosome infection rates in cattle. However, transhumance and the presence of tsetse flies (biological vector~~s~~ of ~~TAA~~AAT) and other insects (mechanical vectors) in the study area are risk factors for disease resurgence.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Affognon, H., Mutungi, C., Sanginga, P., Borgemeister. (2015). Unpacking Postharvest Losses in Sub-Saharan Africa: A Meta-Analysis. World Development. ;66:49-68.

Aka, S., Soro, B., Kanh, M., Kpandji, I., Koffi, M., Sokouri, D. (2022). Dwarf short-horned bulls in the West African cattle herd: the case of the Lagunaire and Muturu breeds. *Revue Marocaine des Sciences Agronomiques et Vétérinaires* • p-ISSN. 2028; 991X.

Amato, B., Mira, F., Di Marco Lo Presti, V., Guercio, A., Russotto, L., Gucciardi, F. & Cannella, V. (2019). A case of bovine trypanosomiasis caused by Trypanosoma theileri in Sicily, Italy. Parasitology research, 118, 2723-2727.

ANMM. (2010). Climate and year-round weather averages for the regions of Mali. https://planificateur.a-contresens.ne t ›

Bengaly, Z., Ganaba, R., Sidibé, I., & Desquesnes, M. (2001). Animal trypanosomiasis in cattle in the South Sudan zone of Burkina Faso. Results of a serological survey. *Revue d'élevage Et De médecine vétérinaire Des Pays Tropicaux* , 54(3-4), 221–224.

Boka, O.M., Boka, E.E. J., Yapi, G.Y., Traoré, S.I., & Kouamé, K.E. (2019). Epidemiology of African animal trypanosomosis in cattle in the Korhogo department (Ivory Coast). *Revue d'élevage Et De médecine vétérinaire Des Pays Tropicaux*, 72(2), 83–89.

Bouyer, J., Guerrini, L., Desquesnes, M., De La Rocque, S., Cuisance, D. (2006). Mapping African Animal Trypanosomosis risk from the sky. Vet Res. t;37(5):633-45.

Diall, O., Bocoum, Z., Sanogo, Y., & Yattara, Z. (1986). Incidence of bovine trypanosomosis on the Madina-Diassa ranch (Mali). Curative treatment of sick animals. *Revue d'élevage Et De médecine vétérinaire Des Pays Tropicaux,* 39(3-4), 301–305.

Djiteye, A., Moloo, S.K., Bi, K.F., Coulibaly, E., Diarra, M., Ouattara, I., et al. (1988). Control trial against Glossina palpalis gambiensis (Vanderplank, 1949) using traps and screens impregnated with deltamethrin in the Sudanian zone of Malii. *Revue d'élevage et de médecine vétérinaire des pays tropicaux*;51(1):37-45.

Diaf Hocine, D. (2008). Effets des facteurs externes sur les performances de reproduction des bovins locaux en Afrique intertropicale. Montpellier : Agritrop, https://agritrop.cirad.fr ›UM2, 34 p.

DNPIA. (2022). Annual Report (Bamako: Direction Nationale des Productions et des Industries Animales). 2022 ; Avenue de la Liberté, Route de Koulouba. BP : 265 ; Bamako - Mali, Annual Report, 118 p

Farougou, S., Allou, S.D., Sankamaho, I., Codjia, V. (2012). Prevalence of Trypanosome Infections in Cattle and Sheep in the Benin's West Atacora Agro-ecological zone. *TROPICULTURA*, 30, 3, 141- 1146

Houndje, E., Gnammi, G., Yessinou, E., Cyrille, B., Ahounou, S., Farougou, S. (2024). Estimation of the economic impact of main cattle diseases in herds in the commune of Matéri, Benin. *Moroccan Journal of Agronomic and Veterinary Sciences*.;12(4):223 6.

INSTAT. (2011). Report by the DNPD (National Development Planning Directorate) of Mali.

Mattioli, E., Pittet, B., Palliani, R., RÖhl, H.J., Schmid-RÖhl, A., Morettini, E. (2004). Phytoplankton evidence for the timing and correlation of palaeoceanographical changes during the early Toarcian oceanic anoxic event (Early Jurassic). JGS. 161(4):685-93.

Michel, J.F., Tyc, J., Messad, S. (2002). National census of transhumant and nomadic livestock in Mali. Montpellier : CIRAD-EMVT, 2 vol. (103, 228 p.)5. Agritrop https://agritrop.cirad.fr › document_563912.

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(4):325-6.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL https://www.R-project.org/.

Tanenbe, C., Gambo, H., Musongong, A.G., Boris, O. and Achukwi, M.D. (2010). Prevalence of bovine trypanosomosis in the departments of Faro and Déo, and Vina in Cameroon: Assessment of twenty years of tsetse fly control. *Revue d'élevage et de médecine Vétérinaire des Pays Tropicaux*, 63, 53-55.

Traoré, A., Koné, O.I., Ly, B., Kéita, Y.F., Bass, B., Diawara, M.O. et al. (2024). Spatial Distribution and Ecological Determinants of Tsetse Flies in Trypanosomiasis-endemic Regions of Mali. *J Appl Life Sci Int*. 27(6):99-107.

Traoré, A., Yaro, A.S., Bass, B., Diarra, B., Koné, T. (2019). Effect of deltamethrin and diminazene acetunate treatments on tsetse flies and cattle as a prelude to a tsetse fly eradication campaign in the niger river basin, Mali . *American Journal of Innovative Research & Applied Sciences*.; 9(4): 320-327.

UCLT. (2005). Integrated control of animal trypanosomosis through the creation of a tsetse fly free zone. Mali/Burkina Fasso/IAEA tripartite project, summary of the results of tsetse fly control campaigns from 2003 to 2005.

Comment [DNS34]: Uniformity in writing references as per the journal guidelines is required.

Please match all the references with that cites in the text including the year of publication. Like the reference Diall et al 1987 is still missing.

Arrange the references alphabetically
Like Diaf 2008 should come before Diall et al 1986
Please see how to write the reference R Core Team 2021
The reference INSTAT 2021 is not complete. Also write its full name

Also write full name of references UCLT 2005, ANMM 2010, DNPIA 2022
Also write full name in text