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Prevalence of Bovine Trypanosomosis and Its Apparent Vector Densities in Dabo Hana District, Western Ethiopia

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Abstract

A cross-sectional study was conducted from December 2014 to April 2015 in Dabo Hana district, Illubabor Zone, Western Ethiopia with main objectives to determine the prevalence of bovine Trypanosomosis and to assess the apparent densities of vectors of the Trypanosomosis. A parasitological study using Buffy coat technique was employed for the determination of prevalence of Trypanosomosis while mono pyramidal traps were used for the vector studies. A total of 505 cattle randomly selected from the study population and examined for the parasitological study. The result of parasitological study revealed that from the total cattle examined during the study period 73 (14.45%) animals were infected with trypanosomes. From the positive samples, the prevalence for trypanosome species were *Trypanosoma congolense* 66 (90.4%), mixed (*Trypanosome Congolese* and *Trypanosome vivax*) 4 (5.5%) and *Trypanosoma vivax* 3(4.1%), respectively.

The prevalence in all purposively selected peasant associations (PA) were 23.45%, 16.29%, 14.38% and 6.97% for Kerkeha, Lilo, Didessa and Loko respectively. In this study, there were statistically significant differences ($P < 0.05$) between PA and body condition, the prevalence was significantly higher ($P < 0.05$) in cattle which were in poor body condition. There were no statistically significant difference between age and sex of the animals ($P > 0.05$). The mean PCV value of infected animals (22.71 ± 3.95 s.d.) was significantly lower ($P < 0.05$) than that of non-infected animals (25.53 ± 4.926 s.d.). About 47 mono pyramidal traps deployed and a total of 377 flies were caught in study area. Of these; 288 (76.4%) belong Glossina species, 73 (19.4%) were Stomoxys and 16 (4.24%) were Tabanus. The overall apparent fly density was 4.01 f/t/d. The apparent density of *Glossina, Stomoxys* and *Tabanus* were 3.06 f/t/d, 0.77 f/t/d and 0.17 f/t/d, respectively. According to this study, the prevalence of bovine Trypanosomosis and the presence of its vector are evident that can pose impact to the performance of livestock in the area. Therefore, possible control options should be implemented to reduce the disease associated economic loss.

Keywords: *Bovine; Dabo Hana; Glossina; Trypanosomosis*

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Introduction

In developing African countries, livestock production remains crucial and represents a major asset among resource-poor smallholder farmers by providing milk, meat, skin, manure and traction. However, the economic benefits of livestock populations remain marginal due to prevailing livestock diseases which are among the principal hindrance to livestock performance and cause of high economic losses of the resource of poor farmers [1]. About 30% of the total cattle population in the African continent and about 60 million pastoralists in 37 Sub- Sahara African (SSA) countries are exposed to African animal Trypanosomosis (AAT) and Human African Trypanosomosis (HAT) or human sleeping sickness, respectively [2].

Trypanosomosis is the main haemoparasitic disease in domestic animals that causes a significant negative impact in food production and economic growth in many parts of the world, particularly in Sub-Saharan Africa and is caused by the protozoan parasite *Trypanosoma* [3]. The most important trypanosoma species affecting livestock are *Trypanosoma Congolese, Trypanosoma vivax*, and *Trypanosoma brucei*, in cattle, sheep and goat, *Trypanosoma evansi* in camel and *Trypanosoma equiperdum* in horse [4].

In Ethiopia, Trypanosomosis is one of the major impediments to livestock development and agricultural production due to its high prevalence in the most arable and fertile land of South West, West and North West part of the country following the greater river basins of Abay, Omo, Ghibe, Baro, Akobo and Didessa [4,5]. Currently, about14 million heads of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at risk of contracting Trypanosomosis at any one time [6].

Trypanosomosis transmission in livestock is either cyclically (*T. congolense, T. brucei*) by tsetse flies or non-cyclically (*T. evansi, T. vivax*) by haematophagus flies (like tabanids and stomoxys) [7]. The exception is infection with *T. equiperdum*, which is transmitted by sexual contact [8]. Most tsetse (*Glossina*) transmissions are cyclical, which is the primary vector of African *Trypanosomosis* and infest the physical landscape covering approximately 11.6 million km^2 of Africa representing 37% of the land area of the continent and affecting 37 countries in Africa [9]. At present, 23 different species and eight sub species of the genus Glossina are recognized belonging to three groups on the basis of their preference for habitat: the riverine (*palpalis*) group, the forest (*fusca*) group and the savannah (*morsitans*) group [10].

The course of Trypanosomosis in cattle is variable depending on the factors associated with the host and the parasite. Generally, Nangana in cattle and other domestic animals is characterized by the intermittent presence of parasites in the blood, intermittent fever, wasting, lymphadenopathy, lacrimation and abortion in pregnant animals. Post-mortem examination of an animal that died of AAT reveals generalized carcass emaciation, enlarged lymph nodes, and enlarged liver and petechial hemorrhages of the serosal membranes, especially in the peritoneal cavity [11].

There are a number of drugs available for the treatment of AAT [12]. Early diagnosis of the disease is important for effective treatment. The choice of drug, dosage and route of administration vary depending on the animal species affected, local preference and presence or absence of trypanosome drug resistance. The appearance of resistant strains of trypanosomes has been associated with the extensive and prolonged usage of the few anti-Trypanosoma drugs like Diminazene aceturate, Homidium, Isometamidium, Quinapyramine and Suramin that available on the market [13].

The control of Trypanosomosis is facilitated through understanding of the disease epidemiology as well as knowing of its vector (tsetse flies) distribution in the infected area [14]. Tsetse flies in Ethiopia, distributed in south western, western and North western regions between longitude 33° and 38°E and latitude 5° and 12°N. These tsetse infested areas lie in the low lands and also in river valleys of Baro, Akobo, Didesa, Abay, Ghibe and Omo. The areas were dominated by 5 species of tsetse fly (*Glossina*) namely G*lossina morsitans sub morsitans, Glossina pallidipes, Glossina fuscipes fuscipes, Glossina tachinoides, Glossina longipennis* among these species the first four are wide spread and more economic importance while *G. longipennis* is of minor economic importance [4].

Hence, Tsetse transmitted animal Trypanosomosis still remain as one of the largest causes of livestock production losses in Ethiopia. Although the epidemiology and impact on livestock production of Trypanosomosis is determined largely by the prevalence of the disease and its vector density in the affected areas; there was no previously done related study in Dabo Hana district. Therefore, the objectives of this study were; to estimate the prevalence of bovine Trypanosomosis, to identify and determine the dominant trypanosome species and to estimate the apparent density of vectors in Dabo Hana district.

Materials and Methods

Study Area

The study was carried out from December 2014 to April 2015 on the prevalence of bovine Trypanosomosis and apparent tsetse density in Dabo Hana district; Illubabor zone, Oromia Regional state which has twelve settlement areas of peasant associations. This area is located about 480 km west of Addis Ababa at an altitude between 1600-2200 meters above sea level. The major town is Kone which lies between 08° 41' N latitude and 36° 17' E longitude. Bordered on the south by Gechi, on the south west by Chora, on the west by Dega, on the north by the southern exclave of of the Benishangul- Gumuz Region, on the north east by the Didessa river which separates it from the east Wollega Zone. The area has temperature range of 18-24°C. It receives rainfall ranging from 1200-1800 mm. Based on altitude; it is divided into 3 agro-ecological zones as high land 10%, midland 70% and lowland 20%, respectively. The land is covered by different vegetation types namely savanna grass lands, forest and bush land, riverine and cultivated land [15]. The main farming system in the area is mixed farming and cattle are the most abundant animal species kept.

Study Design and Study Population

Cross-sectional study was conducted to determine the prevalence of bovine Trypanosomosis and the density of tsetse and biting flies' population. The livestock population of bovine is 62,264 (oxen: 16,948, cows: 18,298, heifers: 11,325, bulls: 10,678, calves: 5,015), shoats are 26, 4509 (ovine: 9200 and caprine: 17, 250), equine, 5083 (horse: 250, mule: 1,578, donkey's: 3,255) [15]. Human population is estimated to be 41,285 of whom 20,471 were men and 20,814 were women [16]. The study populations were local zebu cattle (Bos indicus) on different category of age, sex and body condition. The animals are usually kept under an extensive husbandry system and allowed to graze freely during the day and housed in poorly constructed barns at night. The age of animals was determined by dentition according to DeLahunta & Hable [17] and categorized into three age groups (< 2years, 3-5 years and >5 years). The body conditions of the animals were also grouped as 'good, medium or poor based on based on the appearance of ribs and dorsal spines applied for zebu cattle [18].

Sample Size Determination and Sampling Method

The sample size was determined based on the formula given by [19] and calculated at 50% prevalence with the expected precision at 5% and at 95% confidence interval. As there was no previously done published paper in the district, the required sample size was 384 animals; however a total of 505 animals were sampled to increase the precision, which were randomly selected from four peasant associations (PAs) namely; kerkeha, Didessa, Lilo and Loko. These PAs were selected purposively based on their accessibility to the main road and livestock populations. The formula used to calculate the sample size is shown below.

$$n = \frac{(1.96)^2 \, P_{exp} \, (1-P_{exp})}{d^2}$$

Where

n = the required sample size

Pexp = the expected prevalence (p = 0.5)

d = desired absolute precision (d = 0.05)

Study Methodology and Procedures

In this study, the methodology conducted were; Hematological, Parasitological and Entomological.

Hematological Survey

Packed Cell Volume (PCV) determination

First, cattle to be sampled were restrained properly and areas around ear vein prepared aseptically for sampling. The tip of lancet was used to prick ear vein of cattle. Blood samples were collected directly into heparinized micro hematocrit capillary tube up to ¾ of the capillary tube. The capillary tubes holding the sample were sealed and placed in the rack. The rack holding the tube was then placed in the ice box and taken to the laboratory for examination. After reaching the laboratory, the capillary tubes were taken out from the box and placed in a micro hematocrit centrifuge with sealed end outer most. Load the tube symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12, 000 rpm for five minutes. Tubes were then taken out and placed in hematocrit reader and expressed the reading as a percentage of packed red cells to the total volume of whole blood. Animals with PCV < 24% were considered to be anemic [20].

Parasitological survey

Buffy Coat Technique

After determination of the PCV, the previously centrifuged capillary tube containing the blood was then cut using a diamond tipped pen 1mm below the Buffy coat to include the upper most layers of the red blood cells and 3mm above to include the plasma, then the contents of capillary tube poured onto a clean microscopic slide, covered with a 22x22 mm cover slip and examined under X 40 objective and X 10 eye piece for the presence of motile Trypanosomes. A sample was considered positive for Trypanosomosis when Trypanosome was detected with the Buffy Coat Technique (BCT) [21, 22].

Thin Blood Smear

Trypanosomes positive Buffy coat samples were analyzed and Trypanosome species were identified based on their morphological structure from Giemsa-stained thin films. A small drop of blood from a micro hematocrit capillary tube was applied to a clean slide and spread by using another clean slide at angle of 45 degree, air dried and fixed for 2 minutes in methyl alcohol then, immersed in Giemsa-stain (1:10 solution) for 50 minutes. Drain and wash of excess stain using distilled water and allowed to dry by standing up right on the slide rack and examined under the microscope with oil immersion X100 objective lens [20].

Entomological survey

The apparent densities of tsetse and biting flies were determined based on the mean fly catches in mono pyramidal traps baited with acetone, octenol and cow urine which were deployed at an interval of about 100-200 meters along livestock grazing areas, watering points, savanna grass land and dense river side forests in the District [23]. The coordinates of each trap position were recorded with a Global Positioning System (GPS). In this study, a total of 47 mono pyramidal traps were deployed early in the morning and maintained in position for two consecutive days at four different peasant associations (PAs) within eight days. The cages from these traps were emptied. Caught tsetse flies and biting flies were counted, identified and sexed for the tsetse fly, other biting flies according to their morphological characteristics such as size, color, wing venation structure and proboscis at the genus level [24, 25].

Data Management and Analysis

All the collected raw data, results of parasitological and hematological examination and vector fly survey were entered into a Microsoft Excel spread sheet to create data base and descriptive statistics were used to summarize the data. For the analysis of data, statistical software program (SPSS 16.0) was used. In all cases, differences between parameters were tested for significance and the test result was considered significant when the calculated P-value was less than 0.05. Data collected on PCV values were analyzed by using student t-test to compare the mean PCV values of parastaemic and parastaemic animals. The point prevalence of trypanosome infection was

calculated by dividing the number of infected animals by the number of animals examined during the study time multiplied by 100. The total densities of vector flies were calculated by dividing the total number of flies per trap per day (f/t/d).

Results

Parasitological Findings

The overall prevalence of Trypanosome infection was 14.45% (73/505) Dabo Hana District. Based on origin, the prevalence of Trypanosomosis was 23.45% (19/81) in Kerkeha, 16.29% (22/135) in Lilo, 14.38% (23/160) in Didessa and 6.97% (9/129) in Loko, respectively, with statistical significant difference ($p < 0.05$) between these four PA (Table 1). When the prevalence was assessed in terms of species; 13.069% (66/505) were *T. congolense*, 0.594% (3/505) were T. vivax and 0.792% (4/505) were mixed infection of *T. Congolense* and *T.vivax* with statistically significant difference between species (Table 2).

PA No of animals examined	No of animals Prevalence *T. congolense* *T. vivax* Mixed X^2 P- value Positive (%)						
Loko	129	9	6.97	8	1	0	11.513 0.009
Lilo	135	22	16.29	20	0	2	
Didessa	160	23	14.38	20	2	1	
Kerkeha	81	19	23.45	18	0	1	
Total	505	73	14.45	66	3	4	

Table 1: *Origin based prevalence of bovine Trypanosomosis.*

Species	No of animal positive	Prevalence (%)	X^2	P- value
T. congolense	66	13.069	5.05	0.000
T. vivax	3	0.594		
T. congolense & T. vivax	4	0.792		
Total	73	14.45		

Table 2: *Species based prevalence of bovine Trypanosomosis.*

Risk factors such as; sex, age and body condition score were assessed to know their relationship to the prevalence of the disease. The prevalence in male and female animals was 16.61% (51/307) and 11.11% (22/198), respectively; this difference was statistically not significant ($P > 0.05$) (Table 3). Based on ages; the prevalence was 17.7% (26/147) in cattle > 5 years of age, 13.7% (40/292) in cattle between 3-5 years of age and 10.6% (7/66) in animal < 2 year of age. However there was no statistically significant difference ($P > 0.05$) between these age categories (Table 4). The prevalence based on body condition score was 22.46% (31/138) in a poor body condition animals, 13.62% (32/235) in medium body condition and 7.57% (10/132) in good body condition animals respectively; with statistically significant ($P < 0.05$) difference between body conditions (Table 5).

Sex	No of animal examined	No of animal Positive	Prevalence (%)	X^2	P –value
Male	307	51	16.61	2.946	0.086
Female	198	22	11.11		

Table 3: *The prevalence of Trypanosomosis based on sex of animal.*

Age	No of animal Examined	No of animal Positive	Prevalence (%)	X^2	P –value
< 2 years	66	7	10.6	2.168	0.338
3-5 years	292	40	13.7		
> 5 years	147	26	17.7		

Table 4: *The prevalence of Trypanosomosis based on age of animal.*

Body condition	No of animal Examined	No of animal Positive	Prevalence (%)	X^2	P –value
Poor	138	31	22.46	12.343	0.002
Medium	235	32	13.62		
Good	132	10	7.57		

Table 5: *The prevalence of Trypanosomosis based on body condition.*

Hematological Findings

In the current study, the prevalence of bovine Trypanosomosis in anemic (< 24 PCV value) animals were higher (24.42%) than in non-anemic (> 24 PCV value) animals (6.94%). This difference was statistically significant (P < 0.05) (Table 6). The mean PCV values of infected animals (22.71 ± 4.395 s.d.) were significantly lower (P < 0.05) than that of non-infected animals (25.53 ± 4.926 s.d.) (Table 7).

PCV value (%)	Test result		Total	Prevalence (%)	X^2	P- value
	Negative	Positive				
< 24	164	53	217	24.42	30.577	0.000
>24	268	20	288	6.94		

Table 6: *The prevalence of Trypanosomosis on the basis of PCV value.*

Status of infection	No of animals	Mean PCV (%)	95% CI (%)	X^2 P- value
Infected	73	22.71	21.69-23.74	30.577 0.000
Non-infected	432	25.53	25.07-26.00	

Table 7: *The Mean PCV of infected and non – infected animals in the study area.*

Entomological Findings

A total of 377 flies were caught. Of these; 288 (76.4%) belong *Glossina* species, 73 (19.4%) were Stomoxys and 16 (4.24%) were Tabanus (Table 8). From the Glossina species 275 (95.5%) were *Glossina tachinoides* were 13 (4.5%) were *Glossina morsitans sub morsitans* (Table 9). The overall apparent fly density was 4.01 f/t/d. The apparent density of Glossina, Stomoxys and Tabanus were 3.06 f/t/d, 0.77 f/t/d and 0.17 f/t/d, respectively (Table 8).

Area	No of traps Deployed	Flies identified in number and fly/trap/day (F/T/D)			
		Tsetse	Tabanus	Stomoxys	Total
Didessa	9	265 (14.72)	8 (0.44)	46 (2.55)	319 (17.72)
Kerkeha	9	12 (0.66)	2 (0.11)	4 (0.22)	18 (1)
Loko	19	9 (0.24)	3 (0.079)	18 (0.47)	30 (0.79)
Lilo	10	2 (0.1)	3 (0.15)	5 (0.25)	10 (0.5)
Total	47	288 (3.06)	16 (0.17)	73 (0.77)	377 (4.01)

Table 8: *The distribution and apparent densities of vectors of Trypanosomosis in the study area.*

Area	Tsetse fly species	Sex		Total	Percentage (%)
		Male	Female		
Didessa	*G.m.submorsitans*	-	2	2	0.69%
	G. tachinoides	114	149	263	91.32%
Kerkeha	*G. m.submorsitans*	-	-	-	-
	G. tachinoides	4	8	12	4.2%
Loko	*G. m. submorsitans*	3	6	9	3.1%
	G. tachinoides	-	-	-	-
Lilo	*G. m. submorsitans*	1	1	2	0.69%
	G. tachinoides	-	-	-	-
Total		122	166	288	100

Table 9: *The total catch and percentage of tsetse fly species, in respect of the areas.*

Discussion

The parasitological findings indicated that the overall prevalence of bovine Trypanosomosis in the study area was 14.45%, which is virtually similar with the result of Waktole [26] 13.44% in Gawo Dale District. The result is higher than the reports in different areas; 4.2% in Bedele District of North-West Ethiopia [27] and 6.86% in Lalo Kile District of Western Ethiopia [28]. The relatively high prevalence of Trypanosomosis in this area might be attributed to tsetse distribution and high fly–animal contact. However, the prevalence was relatively lower than that of 29% along the escarpement of the upper Didessa Valley [29]. This might be possibly due to the more abundance of tsetse density along the area.

The prevalence of bovine Trypanosomosis between the peasant associations are statistically significant ($P < 0.05$). Higher prevalence rate was recorded in Kerkeha 23.45% followed by Lilo 16.29% and Didessa 14.38% and least prevalence was recorded in Loko 6.97%, this might be possibly due to the grazing of animals nearby river banks and dense forests of vectors infested areas. The least prevalence in Loko peasant association could be attributed to the seasonal clearing of vegetation and bush for ploughing purpose. When compared to others, Didessa Pa is the highest in its vector density; in contrast the prevalence in this area is lower than that of Kerkeha Pa and Lilo Pa respectively, this might be possibly due to the grazing areas of the animals.

The dominant trypanosome species in the Dabo Hana District was *T. congolense* 90.4% (66/73) followed by mixed infection of T. congolense and *T. vivax* 5.5% (4/73) and *T. vivax* 4.1% (3/73) were detected. This may be due to a highly presence of a biological vector (Glossina), whereas T. vivax is more readily transmitted mechanically by biting flies than tsetse flies [30]. According to Abebe [4], *T. congolense and T. vivax* are the most prevalent trypanosomes that infected cattle in the tsetse-infested and tsetse-free areas of Ethiopia, respectively.

Although there was no significant difference (P > 0.05) in prevalence of *Trypanosomosis* between sexes, in this study, highest prevalence of Trypanosomosis was recorded in male animals (16.61%) than female animals (11.11%). This agrees with the previous report by Efrem., *et al.* [28] in Lalo Kile District of Western Ethiopia. These could be possibly due to the more use of male animals for draught purposes, travel long distances to an area of tsetse challenge for grazing or plowing and stressed by draught power and as a result the risk of contracting Trypanosomosis is high [31].

Although the difference in prevalence of Trypanosomosis between age groups was not significant (P > 0.05), the current study indicated high prevalence in those animals > 6 years and 3-5 year than young < 2). This might be possibly due to the reason that adult animal in the area kept in the field for long time for grazing, that might predispose them to fly infestation than young animal [32]. According to Masamu., *et al*. [33] this might also be due to the reason that tsetse flies are more attracted by the odor of adult animals and least by calves.

In this study, the prevalence of Trypanosomosis based on body condition of animals were assessed and the prevalence was high in animals with poor body condition (22.46%) followed by medium (13.62%) and good (7.57%) body conditions with statistically significant difference (P < 0.05) between these body condition categories. This is in agreement with the works of Teka., *et al*. [34] which conducted in selected villages of Arbaminch, Ethiopia, showing that the highest prevalence was in poor body conditioned (12.22%) followed by in medium (2.32%) and good body conditioned (2%) animals. This might be related to the chronic nature of the disease.

This study revealed that the prevalence of Trypanosomosis in anemic animals is higher than non-anemic (Table 6). This might indicate that trypanosome involve in reduction of PCV. The mean PCV values of infected and non-infected animals were also assessed and trypanosome infection and mean PCV obtained between them had statistically significant difference (P < 0.05) (Table 7) and it was lower in parastaemic animal than aparasitemic one. This was due to the lower PCV value that might be resulted from the debilitating nature of the disease [35]. Poor nutrition and undercurrent gastro-intestinal helminthes infection could also contribute to the general low PCV [36]. So that, in absence of these two factors anemia is a good indicator of Trypanosomosis.

In this study, the vector density and types of vectors available in the area were determined, that can possibly influence the prevalence rate of Trypanosomosis in the area. The apparent density of Glossina, Stomoxys and Tabanus were 3.06 f/t/d, 0.77 f/t/d and 0.17 f/t/d, respectively. These findings were higher than the reports of Girma., *et al*. [37], on the densities of vectors in and around Arbaminch areas; 0.194 and 0.069 f/t/d for tsetse and biting flies, respectively with a very less prevalence rate of Trypanosomosis (1.4%) than the study area. Therefore, the higher prevalence rate of Trypanosomosis is the result of more vector density. This is in agreement with Shimelis and Shibeshi [38] in control (Sibu Sire) and non-control (Guto Gida) Districts of East Wollega Zone, Western Ethiopia.

In this study, from the Glossina species very high percentage of tsetse species observed were Glossina tachinoides (95.52%). This is due to the predominant abundance of Glossina tachinoides in and around Didessa [5].

Conclusions and Recommendations

In general, this study indicated that the prevalence of bovine Trypanosomosis in Dabo Hana District was high that can pose threat to livestock owners in the area, due to loss in production and productivity of cattle. In the findings of the study, Trypanosomosis was found to be negatively affecting the PCV value and body condition score of affected animals. Also aged animals were more likely to be infected with Trypanosomosis. The most commonly encountered Trypanosoma species in cattle of the area was T.cogolense followed by mixed infections of (T. vivax and T.cogolense) and T. vivax. Entomological survey from this study indicated that, relatively there was a higher tsetse fly (Glossina) density than other biting flies which play the main crucial role in the prevalence of Trypanosomosis particularly to the area.

Based on the above conclusions, the following recommendations are forwarded;

- Tsetse burden in the area should be reduced through continual use of traps and insecticide-impregnated targets or through application of available chemicals on the animal.
- Regular screening of bovine Trypanosomosis and early treating of positive animals with trypanocides are necessary.
- Educating farmers, especially those nearest to the main tsetse challenge areas, is critical to reduce the chance of contact of animal with flies.
- Continuous community-based tsetse monitoring and more Trypanosomosis surveillance programs should be instituted in tsetse infested areas of the district.
- The areas should be provided with adequate veterinary service.

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Author's conflict of interest

The authors declare that there is no conflict of interest for this manuscript.

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