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Study on prevalence of bovine trypanosomosis and density of its vectors in three selected districts of Wolaita Zone, Southern Ethiopia

Zemedkun Gona[1], Ayichew Teshale[2]* and Alebachew Tilahun[2]

[1]Agricultural Research Center Wolaita Zone District, Wolaita Sodo, Ethiopia.
[2]Wolaita Sodo University School of Veterinary Medicine, Wolaita Sodo, Ethiopia.

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The study was conducted from November, 2013 to March, 2014 in three selected districts of Wolaita zone, Southern Ethiopia with objective of determining the prevalence of bovine trypanosomosis and density of its vectors. Blood samples from 480 randomly selected cattle of both sexes different age, coat color and body condition score groups were collected and examined with conventional hematological and entomological techniques. Out of total 480 cattle examined, 32(6.67%) were found to be positive for *Trypanosoma congolense* and *Trypanosoma vivax* with the individual prevalence of 5% (24/480) and 1.67 (8/480), respectively. This indicated 75% of the infection was caused by *T. congolense* while only 25% was by *T. vivax*. The areal distribution of the trypanosomosis infection was found to be 5.6, 7.3 and 7.1% in Humbo, Duguna fango and Damot woyde districts respectively. The risk factors analysis revealed that the likelihood of the occurrence of the trypanosomosis in male (p=0.046, OR=2.3, 95%CI=1.0, 5.3), age category of 3 to 7 years old (p=0.019, OR=3.2, 95% CI=1.2, 8.3), poor body conditioned animals (p=0.001, OR=12.5, 95%CI=2.8, 50.0), black coat colored animal (p = 0.02, OR=12.5, 95%CI=1.5, 117.7) was higher when compared to female, other age categories, medium and good body condition and with other coat colored animals respectively. One way ANOVA used to compare the PCV values of parasitemic and aparasitemic animals revealed mean PCV values in animal infected by *T . congolense* (21.38%) and *T . vivax* (23.75%). Accordingly, one way ANOVA employed to compare the mean PCV value among the three categories (Negative, Positive for *T. congolense* and positive for *T. Vivax*) revealed significant (p=0.000, F=25.8) difference in PCV values were observed. Likewise, the *Bonferroni* multiple comparisons test indicated the existence of significant difference (p=0.000) in the PCV value between negative group and positive group for *T. congolense* (p=0.000). From 90 traps deployed for three consecutive days at 6 kebeles in three districts, a total of 328 flies were caught. Of these, 37 (11.28%) belong to *Glossina pallidipes*, the remaining were 193 (58.84%) *Tabanus* and 98 (29.87%) *Stomoxys*. The overall apparent tsetse fly density was 0.14 flies/trap/days (F/T/D).

Key words: Cattle, *T. congolense, T. vivax, Trypanosomosis, Glossina pallidipes,* Southern, Ethiopia.

INTRODUCTION

Ethiopia has high livestock resource potential with estimated number of 40.9 million head of cattle, 22.5 million heads of sheep, 23.4 million heads of goats and more than 7.5 million equines and 2.3 heads of camels (CSA, 2007). However, much of the livestock resources are not fully utilized to maximum potential due to various constraints. Trypanosomosis is one of the major animal diseases affecting sub Saharan African countries in

general, and Western and South western part of Ethiopia in particular (NTTICC, 2004; Enwezor et al., 2006).

Trypanosomosis is a serious haemoprotozoan disease caused by different species of uni-cellular eurykaryotic parasite of the genus trypanosome found in the blood and other tissues of vertebrates including livestock, wild life and people and transmitted cyclically by tsetse flies of *Glossina* species and many other insects mechanically (Tesfaheywet and Abraham, 2012; Kumar et al., 2012). Animal trypanosomosis is an important livestock disease in Africa which is considered as a threat to the ongoing effort on poverty alleviation in the continent (Wint et al., 2010). It is a serious disease in domestic livestock that causes a significant negative impact in food production and economic growth in many parts of the world (Kumar et al., 2012) particularly in sub-Saharan Africa (Taylor et al., 2007; Cecchi et al., 2008).

Animal trypanosomosis is among one of the most important diseases limiting livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of South West and North West part of the country following the greater river basins of Abay, Omo, Ghibe and Baro, which has a high potential for agricultural development (Shimels et al., 2005). Over 6 million heads of cattle and equivalent number of other livestock species are at risk of contracting the diseases. More than 20,000 heads die per annual, and annual loss attributed to the disease is estimated to be over US$236 million, whereas loss due to reduced meat, milk and draft power is not applicable to this figure (OAU, 2002). The most important *Trypanosoma* species affecting cattle in Ethiopia are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* (Abebe, 2005).

The tsetse flies are widely distributed in the Western, Southern and South western low lands and river valleys and 15% of the land believed to be suitable for livestock production is affected by one or more of the following species of tsetse flies; *Glossina morsitans sub morsitans, Glossina pallidipes, G. tachinoides, Glossina fuscipes fuscipes* and *Glossina longipennis* (Abebe, 2005). Apart from cyclical transmission of trypanosomosis by *Glossina* species, mechanical transmission is a potential threat to livestock productivity in some parts of Ethiopia (Abebe and Jobre, 1996). *T. vivax* infection can be transmitted mechanically by several *Tabanide* and large number of biting flies (Chernet et al., 2006). Among domestic animals, cattle are the most susceptible to *T. congolense*, *T. vivax* and *T. brucei* infections (Radostitis et al., 2007).

Currently, the livestock production and productivity of southern region is highly affected by the high incidence of trypanosomosis. The communities in the region in general and in low lands lying along Ghibe and Omo river basins in particular expand a lot of money to purchase trypanocidal drugs.

Therefore, taking this into an account this study was designed with the following specific objectives.

To determine the prevalence of bovine trypanosomes on the basis of age, sex, body condition score and color of the animals and on area basis.

To determine the dominant species of trypanosome in study areas.

To investigate the epidemiological distribution of bovine trypanosomosis and to determine the abundance of tsetse fly in selected districts.

MATERIALS AND METHODS

Study area

The study was conducted from October 2013 to April 2014 in three selected districts of Wolaita zone, Southern Ethiopia which is located about 390 km south of Addis Ababa at an altitude of 700 to 2950 meters above sea level. It has an average annual rain fall ranging from 450 to 1144 mm. The rain over much of the area is typically bimodal with the major rainy season extending from June to September and the short rainy season occurs from February to April. The mean annual maximum and minimum temperature of the area is 34.120°C and 11.4°C, respectively. The predominant farming system is mixed crop-livestock production. The livestock population of Wolaita zone is estimated to be 886, 242 bovine; 117,274 ovine; 99,817 Caprine; 41160 equines and 442,428 poultry. The zone consists of 12 districts of which Humbo, Damot woyde and Duguna Fango were selected for the study based on available information that they are tsetse infested. The information was obtained from the zones office of agriculture (CSA, 2007).

Study design

Study population and study type

The study constituted zebu cattle of various sexes, age groups, body condition scores and different coat color managed under smallholder mixed crop livestock farming system a type of study employed was cross-sectional study type.

Sample size and sampling method

Simple random sampling technique was followed to select the study animals. During sampling age, sex, color and body condition score of animals was recorded. The age was categorized into three groups: less than 3years, 3 to 7years and greater than eight years old and the body condition score was grouped into good, medium, and poor based on the appearance of ribs and dorsal spines applied for zebu cattle (Nicholson and Butterworth, 1986). The desired sample was also calculated according to the formulae given by Thrusfield (2005) as follows:

*Corresponding author. E-mail: tayichew25@gmail.com.

$n=1.96^2 x Pexp\ (1-Pexp)/d^2$

Where n=required sample
P=expected prevalence
d=desired absolute precision

Hence, with 14.2% expected prevalence rate which is done by Feyissa et al. *(*2011), desired absolute precision of 5 and 95% level of confidence, the sample size was calculated to be 187. But to increase the precision, 480 animals were sampled during the study period.

Study methodology and procedures

Parasitological Study

Buffy coat technique (BCT*):* Heparinized micro-hematocrit capillary tubes, containing blood samples were centrifuged for 5 min at 12,000 rpm. Buffy coat was drained on to microscope slide by cutting the capillary tube with sharp pointed diamond pencil 1mm below the Buffy coat to include the plasma after which it was covered with a 22x22 mm cover slip on microscope slide and examined under phase contrast or dark field microscope (40x power objective) to see motile parasite. Trypanosoma species were identified according to their morphological descriptions on Giemsa stained blood film as well as their motility in wet blood film preparations provided.

Hematological study

***PCV determination*:** Blood samples were taken by puncturing marginal ear vein with a lancet and added directly into a pair of heparinized capillary tubes to their three-fourth of length. The tubes were then sealed at one end with crystal seal, placed in micro-hematocrit centrifuge and centrifuged at 12,000 revolutions per minute (rpm) for 5 minutes. Then capillary tubes were placed in a hematocrit reader and PCV estimated as a percentage of the total volume of blood to demonstrate the general health status of the animal.

Entomological study

Entomological data collection and recording commenced with collection of base line data on the description and density of tsetse before the start of trial. Tsetse flies were sampled by deploying traps along suspected habitat baited with three week old bovine urine and acetone into two different dispending bottles. Traps were set at approximate intervals of 200 to 250 meters and deployed preferably in shade in a visible manner. The different flies going to be caught in each trap were counted and identified. The species of tsetse fly, characteristic morphology and similar other mechanical vectors were also identified.

Data analyses

Raw data generated from this study were entered into MS-Excel database and the prevalence of bovine trypanosomosis in different age, color, sex and body condition groups and different localities or sites were analyzed using logistic regression analysis test. Mean PCV values between parasitaemic and aparasitemic cattle were compared by using one way ANOVA analysis test. Also the *Bonferroni* multiple comparisons test used to indicate the existence of significant difference in the PCV value between negative group and positive group for *T. congolense* and *T. vivax.* Flies per trap per day *(*F/T/D) analysis were used to calculate an apparent tsetse and biting flies densities.

RESULT

Prevalence of trypanosome infection

Out of the total 480 cattle examined for trypanosomosis, 32 (6.67%) were found to be positive for *T. congolense* and *T. Vivax* with the relative proportion of 5 (24/480) and 1.67% (8/480), respectively. This indicated that 75% of infection was caused by *T. congolense* while only 25% was by *T. vivax*. The area distribution of the trypanosomosis infection was found to be 5.6, 7.3 and 7.1% in Humbo, Duguna Fango and Damot woyde districts, respectively (Table 1).

Analysis of the risk factors

The analysis of risk factors revealed significant difference in the occurrence of trypanosomosis among different sex, age, body condition score and color. That is, the likelihood of the occurrence of the trypanosomosis in male (p = 0.046, OR = 2.3, 95%CI = 1.0, 5.3), age category of 3 to 7 years old (p = 0.019, OR = 3.2, 95% CI = 1.2, 8.3), animal with poor body condition score (p = 0.001, OR = 12.5, 95%CI = 2.8, 50.0), animal with black color (p = 0.02, OR = 12.5, 95%CI = 1.5, 117.7) was higher when compared to female animals, other age categories, animal with medium and good body condition scores and animals with other coat color. No significant difference was observed in the occurrence of the disease among different districts (Table 2).

Hematological findings

The recorded PCV value of animals was analyzed using one way ANOVA analysis to compare the PCV value of parasitemic and aparasitemic animals. There was a significant difference (p = 0.000, F = 49.2) in mean PCV value between infected and none infected animals in which infected animals have low mean PCV value (21.97) than non-infected ones (26.93) (Table 3).

Additionally, the mean PCV value in the animal infected by *T. congolense* (21.38) and *T. vivax* (23.75) was analyzed (Table 4). Accordingly, the one way ANOVA analysis was employed to compare the mean PCV value among the three categories (Negative, Positive for *T. congolense* and positive for *T. vivax*) and significant (p = 0.000, F = 25.8) difference in PCV values were observed. Likewise, the *Bonferroni* multiple comparisons test indicated the existence of significant difference (p = 0.000) in the PCV value between negative group and positive group for *T. congolense* (p=0.000). No significant difference in PCV was observed between the negative

Table 1. Prevalence of trypanosome infection and species identified in different study areas, sex, age BCS and coat color.

Risk factors	No. examined	No. positive	Prevalence (%)	*T. cong* (%)	*T. vivax* (%)
District					
Humbo	144	8	5.6	6 (4.2)	2 (1.4)
Duguna Fango	96	7	7.3	5 (5.2)	2 (2.1)
Damot Woyde	240	17	7.1	13 (5.4)	4 (1.7)
Sex					
Female	223	10	4.5	10 (4.5)	0
Male	257	22	8.6	14 (5.4)	8 (3.1)
Age					
<3 year	165	6	3.6	3 (1.8)	3 (1.8)
(3, 7) year	232	22	9.5	19 (8.2)	3 (1.3)
>8 year	83	4	4.8	2 (2.4)	2 (2.4)
BCS					
Poor	103	14	13.6	12 (11.7)	2 (1.9)
Medium	245	16	6.5	11 (4.5)	5 (2.0)
Good	132	2	1.5	1 (0.8)	1 (0.8)
Color	49	7	14.3	5 (10.2)	2 (4.1)
Black White/bulla	80	2	2.5	1 (1.3)	1 (1.3)
Gray/mixed	75	1	1.3	1 (1.3)	0
Red	276	22	8.0	17 (6.2)	5 (1.8)

Table 2. Logistic regression analysis of the prevalence of trypanosomosis with assumed risk factors.

Risk factors	No. examined	No. +ve (%)	COR(95% CI)	AOR(95% CI)	P-value
District					
Humbo	144	8(5.6)	1	1	
Duguna Fango	96	7(7.3)	1.3(0.5,3.8)	2.3(0.7,7.4)	0.15
Damot Woyde	240	17(7.1)	1.3(0.6,3.1)	1.5(0.58,3.9)	0.391
Sex	2				
Female	23	10(4.5)	1	1	
Male	257	22(8.6)	1.9(0.9,4.3)	2.3(1.01,5.3)	0.046
Age					
<3 year	165	6(3.6)	1	1	
>8 year	83	4(4.5)	1.3(0.4,4.5)	1.1(0.3,4.3)	0.916
(3,7)year	235	23(9.5)	2.8(1.1,7.1)	3.2(1.2,8.3)	0.019
BCS					
good	132	2(1.5)	1	1	
Medium	245	16(6.5)	23(1.1,4.8)	2.86(12.5,6.3)	0.012
Poor	103	14(13.6)	1.0(2.3,4.6)	12.5(2.8,50)	0.001
Color					
Gray/mixed	75	1(1.3)	1	1	
Red	276	22(7.8)	1.9(0.8,48)	1.8(0.7,4.8)	0.241
White/bulla	80	2(2.5)	1.9(1.3,33.3)	1.7(1.2,33.33)	0.030
Black	49	7(14.3)	12.5(15,100)	12.5(1.5,117.7)	0.02

Table 3. Mean PCV value for parasitemic and aparasitemic animals.

Status	Mean PCV	Std. Dev.	Freq.	F	P-value
Aparasitaemic	26.93	3.85	448		
Parasitaemic	21.97	4.04	32	49.2	0.000
Total	26.60	4.05	480		

Table 4. Mean PCV value for trypanosome species.

Status	Mean PCV	Std. Dev.	Freq.	F	P-value	Bonferroni multiple comparison test	
						Categories	P-value
Negative	26.93	3.85	448	25.8	0.000	Negative and *T. congolense*	0.000
Positive for *T.congolense*	21.38	4.13	24			Negative and *T. vivax*	0.064
Positive for *T. vivax*	23.75	3.37	8			Positive for *T.congolense* and Positive for *T. vivax*	0.397

Table 5. Results of entomological survey.

District	No. PA	Altitude (mtrs)	No. traps	No. days	*Glossina Pallidipes*					Tabanus	Stomoxys
					No. of male	No. of female	U sexed	Total	F/T/D		
Humbo	2	1215-1275	30	3	2	6	2	10	0.11	113	38
Duguna Fango	1	1242-1259	12	3	8	8	2	18	0.5	35	15
Damot Woyde	3	1285-1457	48	3	3	6	0	9	0.063	45	55
Total	6	-	90	-	13	20	4	37	0.14	193	98

F/T/D= apparent fly density per trap per day, No.M =number of males, No.F =number of female, U-sexed=unknown sex.

group and the infected group with *T. vivax*. Similarly, there was no significant difference in PCV between the group infected with *T. congolense* and *T. vivax*.

Entomological survey

From 90 traps deployed for three consecutive days at 6 PAs (kebeles) in three districts, a total of 328 flies were caught. Of these, 37 (11.28%) belong to *Glossina* specie (Tsetse flies), the remaining were 193 (58.84%) *Tabanus* and 98 (29.87%) *Stomoxy*s, which belongs to biting flies. Furthermore, all *Glossina* species caught were identified to be *G. pallidipes.* The overall apparent tsetse fly density was 0.14 flies/trap/days (F/T/D). The overall fly density at district level was 0.11, 0.5 and 0.063 F/T/D in Humbo, Duguna Fango and Damot Woyde, respectively. In Damot Woyde district, which is located at altitude range of 1285 to 1457 m.a.s.l., the fly density is relatively lower (0.0625 F/T/D) when compared to Duguna fango (0.5 F/T/D) and Humbo (0.11 F/T/D) districts, which are located at altitude range of 1215 to 1275 m.a.s.l and 1242 to 1259 m.a.s.l, respectively. The sex category indicated 13 (35.14%) male, 20 (54.05%) females and 4 (10.81%) unknown sexed (U-sexed) tsetse flies. The summary of entomological survey is indicated in Table 5.

DISCUSSION

The overall prevalence of bovine trypanosomosis in this study was 6.7% which was in agreement with the previous findings by Habtwolde (1995), Feyisa et al. (2011) and Bitew et al. (2011) who reported 9.3% at Humbo Larena of Wolaita zone, 6.3% at Humbo district , and 11.7% at Jabi Teheran district, West Gojam of Amhara regional state, respectively. However, this finding was relatively lower than the reports of Terzu (2004), Mesfin and Getachew (2001), and Amare (1995) who reported 15.8, 35.5 and 21.0% prevalence of bovine trypanosomosis respectively at Omo river basin of South Western Ethiopia.

The possible explanation for the lower report in the current study could be attributed to the fact that action of Southern Valley Tsetse and Trypanosomosis Eradication (STEP) project, expansion of cultivation in the area which directly affects fly distribution, expansion of veterinary clinic, and awareness towards the control and treatment

of the disease. There was no significance difference in the selected districts, Humbo (5.6%), Duguna Fango (7.3%) and Damot Woyde (7.1%), since they are located relatively at similar agro ecology and tsetse belt of Ethiopia.

T. *congolense* was the most prevalent trypanosome species in the study area that accounts for the overall percentage of about 75% (24/32). This result was in line with Abebe and Jobre (1996) for tsetse infested areas of Ethiopia (58.5%); Muturi (1999) at Southern rift valley of Ethiopia (66.1%); Afework et al. (2001) at Pawe, North West Ethiopia (60.9%); Terzu (2004) in selected site of southern region (63.4%) and Bitew et al. (2011) in West Gojam (54.3%). An increased proportion of infection with T. congolense in the study area may be due to the major cyclical vectors of Savannah tsetse flies, (Glossina morsitans and Glossina pallidipes) which are effective transmitters of T. congolense and T. vivax (Langride, 1976) since the study area is located at tsetse belt of Ethiopia. Another reason also may be due to high number seroderms of *T. congolense* as compared to *T*. *vivax* and the development of better immune response to *T*. *vivax* infected animals (Leak et al., 1999; MacLennan, 1970).

Higher infection rate was observed in male animals than in females and the significant difference was also observed between two sex groups. Similar results have been reported by different works (Afework, 1998; Muturi, 1999; Tewolde, 2001; Mulugeta et al., 2013). The possible explanation from the present finding would be that the male animals are more exposed to traction power and also cross different vegetation for grazing and watering where tsetse challenge is higher than females.

In the present study, there was a statistically significant difference among age groups. The higher infection rate was observed in adults (3 to 7) years) than young's (<3years) and older (≥8years) animals. This result was in agreement with the previous research result reported by Sinshaw (2004). This could be due to the fact that adult animals travel long distance for grazing and draft as well as harvesting of crops to the tsetse challenged areas. Similar to the case in report by Rowlands et al. (1999) in Ghibe valley, suckling calves do not go out with their dams but graze at homesteads until they are weaned off. Young animals are also protected to some extent by maternal antibodies (Fimmen et al., 1999). This could result in low prevalence of trypanosome of that was observed in calves. *T*. *congolense* is usually higher in adult animals than the young ones (McDermott et al., 2003).

In the present study, body condition has shown to have significant effect on prevalence of trypanosome infection ($p<0.05$) with high prevalence recorded (13.59%) at poor body conditioned animals. Animals with poor body condition score were more associated with disease as compared to animals with medium (6.53%) and good (1.52%) body condition. This was in agreement with Habtwolde (1995), Dawud and Molalegne (2011) and Abiy (2002). Obviously, the disease itself results in progressive emaciation of the infected animals; nevertheless, non-infected animals under good condition have well developed immune status that can respond to any foreign protein better than those of non-infected cattle with poor body condition score which can be immune compromised due to other diseases or malnutrition and concurrent infections depress the immune responsiveness in the same cases (Collins, 1994).

Comparison conducted between the different skin color of cattle indicated that higher prevalence was observed in cattle's having black skin color (14.3%) followed by 8% in red, 2.5% in white/bulla and 1.3% in gray/mixed skin color. Tsetse flies by nature are attracted toward a black color, so in animals having black skin color there is high prevalence of trypanosomosis recorded (Teka et al., 2012).

One of the main symptoms of the disease is anemia (Murray, 1997) consequently the present study also indicated significant difference between mean PCV values of infected and non-infected cattle. Out of the observed animals, 32 of them were positive and their mean PCV value was 21.97±4.04, and 448 of them were negatives and their mean PCV was 26.93±3.85. The result of this study was in accordance with Rowlands et al. (1999) who observed an increase in PCV value, the proportions of positivity decreases and hence mean PCV was a good indicator for the health status of animals in an endemic area. The lower mean PCV value in parasitemic animals than the aparasitemic animals was reported by several authors (Leak et al., 1999; Afework, 1998; Muturi, 1999; Tewolde, 2001). Comparison of the mean PCV of infected animals within species of trypanosome out of 32, twenty four were infected with *T. congolense* and their mean PCV was 21.38±4.13 and eight were infected with *T*. *vivax* and their mean PCV was 23.75±3.37. Mostly, *T. vivax* invades other tissues in addition to blood such as lymph node, eyes and heart (Hoare, 1972; Stephen, 1986; Whitelaw et al., 1988), but *T*. *congolense* confined in the blood that might results low PCV values. Other than this, it can also be assumed that numerous concurrent diseases like helmenthiasis, tick borne diseases and nutrition imbalance cause anemia in both trypanosome positive and negative animals. There is a significance difference ($p<0.05$) in none infected and *T. congolense* positive animals.

The overall apparent density of tsetse and biting flies were 0.14 and 1.14 flies/trap/day respectively. There was no a great variability in tsetse apparent density between the study area (selected districts). This may be due to their similarity in agro ecologies and similar tsetse control measures done by Southern Tsetse Eradication Project (STEP). The lower apparent density of tsetse flies might be due to high temperature and low relative density of the dry period, which could limit the spread as described by Pollock (1982).

In the present study, the only *G. Pallidipes* were caught. The result of this study agreed with that of Leak et al. (1999), who found higher apparent density *G. Pallidipes* in Ghibe valley, which was followed by *G. Morsitans*. The present study disagreed with research by a team from NTTICC (2004), specific species of tsetse flies were recovered in Abay valley and its tributaries.

Finally, the study added knowledge to the overall prevalence of trypanosomosis in selected districts (Humbo, Duguna Fango and Damot Woyde) in Wolaita zone, Southern Ethiopia. It also indicated that the dominant trypanosome species in the study area was *T. congolense.* The host risk factors analysis of the study showed higher prevalence in males than females; in adult cattle than in younger and older and in animals with poor body condition score. The black coats colored animals were highly prevalent with disease than other color groups. The mean PCV of aparasitemic animals was higher than parasitemic animals. The overall apparent density of tsetse and biting flies was 0.14 and 1.14 flies/trap/day, respectively.

Conclusion

The study found that bovine trypanosomosis was economically an important disease that affects the health as well as the productivity of cattle in selected districts, and the findings may be used in the design of appropriate control and treatment strategies for existing problem.

Conflict of interests

The authors have not declared any conflict of interests.

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