Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/July-2014/13.pdf

RESEARCH ARTICLE
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Prevalence and risk factor's analysis of bovine brucellosis in peri-urban areas under intensive system of production in Gujarat, India

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Received: 31-03-2014, **Revised:** 14-06-2014, **Accepted:** 18-06-2014, **Published online:** 23-07-2014

doi: 10.14202/vetworld.2014.509-516 **How to cite this article:** Patel MD, Patel PR, Prajapati MG, Kanani AN, Tyagi KK and Fulsoundar AB (2014) Prevalence and risk factor's analysis of bovine brucellosis in peri-urban areas under intensive system of production in Gujarat, India, *Veterinary World* 7(7): 509-516.

Abstract

Aim: A study on surveillance of bovine brucellosis in dairy herds of peri-urban areas under intensive system of production was carried out by milk-ELISA. Various risk factors were identified having significant association with occurrence of bovine brucellosis in dairy herds of peri-urban areas.

Materials and Methods: Five randomly selected peri-uban areas of six cities of Gujarat were included in the present study. Five randomly selected dairy herds under intensive system of production from each selected peri-urban area were included for further investigation. In total, 199 bulk and 582 individual milk samples were screened by milk-ELISA. Forty three different risk factors were identified and grouped into four major categories as general characteristics of farms, introduction of infection to farms, management systems of farms and exposure of disease. Further, their distribution and association with prevalence of bovine brucellosis was studied.

Results: The overall herd and animal prevalence in peri-urban areas was 33.70 and 11.90%, respectively. Out of 11 risk factors on general characteristics of dairy farms, only five (herd size, type of animals, type of breed, age of owner and knowledge gained by owners) showed significant ($p<0.05$) association with occurrence of bovine brucellosis. None of risk factors on introduction of infection to farms (n=6) and management systems of farms (n=11) was found significantly associated with occurrence of brucellosis. Among risk factors on exposure of disease (n=15), history of abortion, retention of placenta, still birth and metritis/endometritis showed significant ($p<0.05$) association with prevalence of bovine brucellosis.

Conclusion: It was concluded that prevalence of bovine brucellosis in dairy herds under intensive system of production in peri-urban areas of Gujarat was comparatively higher than reported overall prevalence of brucellosis. Risk factors like larger herd in close confinement without adequate sheds, type of animal, type of breed and knowledge/awareness of dairyman, unrestricted animal market, replacement without prior testing, reproductive disorders with absence of their testing are the important risk factors under the intensive production system of peri-urban areas of Gujarat, India.

Keywords: bovine brucellosis, milk-ELISA, peri-urban area, risk factors.

Introduction

The economic wellbeing of dairy farmers depends upon healthy, productive and sound reproductive livestock. Among the various prevalent diseases which considerably affect production and reproduction performance of dairy animals, bovine brucellosis is perhaps the most economically important reproductive disease of the rapidly growing Indian dairy industry. In India, brucellosis was first recognized in 1942 and is now endemic throughout the country. The disease has been reported in cattle, buffaloes, sheep, goats, pigs, dogs and humans. Brucellosis in India is a very common but often neglected disease [1]. The most significant feature of bovine brucellosis epidemiology is the shedding of large numbers of organisms during 10 days after abortion or calving of infected cows and the consequent contamination of the environment [2].

The prevalence of infection in animal reservoirs provides a key to its occurrence in humans also. Therefore, the correct and prompt diagnosis is important in controlling and eradicating the disease in animals. The diagnosis of the disease can be challenging and is frequently delayed or missed because the clinical picture may mimic other infectious and non-infectious conditions [3]. Recently, ELISA has taken over as an important serological tool in the diagnosis of brucellosis because of its economy, sensitivity, specificity, rapidity, reproducibility, and easy interpretation through colorimetric end product [4]. Further, the advent of milk based I-ELISA (Milk-ELISA) brings revolution in screening of large population. Milk-ELISA performed on bulk milk samples are now routinely and effectively used for screening and monitoring dairy cattle for brucellosis [5]. Overall in country as a whole and

Gujarat in particular, the village milk production system is being transforming into a milk production industry which is more concentrated in and around the city areas (peri-urban areas). Ahir, Bharvad, Rabari communities as well as other farmer communities are focusing on this profitable business on a large scale and taking dual benefits of already established milk co-operatives infrastructure and direct market of city areas. A parallel milk marketing system is growing rapidly in and around each city due to readily available raw milk market. Earlier research work carried out on bovine brucellosis has mostly focused on organized government farms or farmers under milk co-operatives. These studies did not cover peri-urban milk producers maintaining good quality animals under intensive system of production and which were reluctant to provide necessary support and information.

The present investigation had been planned to study bovine brucellosis in dairy herds of peri-urban areas using milk-ELISA.

Materials and Methods

Ethical approval: The present study was carried out after the necessary permission of institutional ethical committee.

Area, sample and test detail: Six selected cities *viz.* Ahmedabad, Anand, Surat, Navsari, Valsad and Vapi covering middle and south Gujarat were included in the present study. Five peri-urban areas of each city were randomly selected for the present work. From each peri-urban area, five farmers following intensive system production with herd size ≥10 milking animals were included in the study. The species (cattle and Buffalo) and breed-wise bulk and individual milk samples at approximately 10 per cent of total milking animals, were collected for Milk-ELISA. A total of 199 bulk and 582 individual milk samples were collected aseptically in 5 ml sterile screw capped plastic sample collection vials and kept in insulated ice-box with pre-freezed ice-packs during transportation up to laboratory. The actual numbers of samples collected were differed from above mentioned selection criteria due to mixed farming (cattle and buffaloes or different breeds of cattle or buffaloes) practice on dairy farms. The available facilities of Department of Veterinary Medicine and Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University (AAU), Anand, Livestock Research Station, Navsari Agricultural University (NAU), Navsari and Regional Animal Disease Investigation Offices (ADIO) of Department of Animal Husbandry (Ahmedabad and Navsari) were used for present study. After removal of creamy part by centrifugation, milk samples were transferred to another vials and stored at -20ºC, till further use. *Brucella* indirect ELISA test kits were procured from VMRD, Inc., U.S.A and the tests were performed as per the protocol outlined in the user manual at LRS, NAU, Navsari or ADIO, Ahmedabad. The epidemiological information and necessary history regarding various risk factors were collected in surveillance performed during the present study. A total of 45 risk factors were taken into consideration. They were grouped into four major categories i.e. 1) Risk factors on general characteristics of farms, 2) Risk factors on introduction of infection to farms, 3) Risk factors on management systems of farms and 4) Risk factors on exposure of disease.

Statistical analysis: Data pertaining to prevalence based on milk testing and risk factors were analyzed on IBM SPSS statistical software version 20.0 using chi square test (probability at 5% and confidence interval at 95% level) as per method described by Snedecor and Cochran [6].

Results and Discussion

Herd and animal prevalence: In the present study, an overall 67 (33.70%) out of 199 bulk milk samples were found positive for *Brucell*a antibodies on milk-ELISA. The present result is in accordance to the previous reports on herd prevalence of bovine brucellosis based on milk-ELISA [7-10]. Similarly, an earlier report of Asfaw *et al.* [11] also found 100% herd prevalence in peri-urban, 30% in intra-urban and 12.5% in inter-urban areas with overall herd prevalence of 33.30%. In a single report from Gujarat, Varasada [12] reported only 3.53% herd prevalences on tank bulk milk testing by milk-ELISA. During the present study, the overall animal prevalence based on milk-ELISA was 11.90%. Present finding is in accordance with previous reports based on milk tests which reported 10-15% animal prevalence [13-15]. Whereas, Patel [16] and Aulakh *et al*. [17] recorded still higher animal prevalences on milk tests with corresponding figures of 28.30 and 18.26%, respectively. Whereas, few reports from India and neighboring country reported animal prevalence below 10% [18-2], the animal prevalence (11.90%) observed in present study was found higher than a wide area based bovine brucellosis prevalence (6-7%) by ELISA conducted by Renukaradhya *et al*. [1]. Further, the reports from India on sero-prevalence of bovine brucellosis also showed more or less similar trend of animal prevalence of bovine brucellosis [22-24]. The species and peri-urban areas-wise herd and animal prevalence are presented in Table-1. The prevalence was found non-significantly higher in cattle than buffaloes. Further, it was varied non-significantly between the peri-urban areas. The higher prevalence in present study may be attributed to highly intensive production system and frequent replacement of animals without prior testing.

Risk factor's analysis: The risk factors showed significant associations with occurrence of bovine brucellosis were discussed whereas the information of distribution of those risk factors had statistically non-significant association with occurrence of brucellosis are given in respective table.

Table-1: Species-wise and peri-urban area-wise prevalence of bovine brucellosis under intensive system of production.

Type of samples	Particular		No. of samples	No. of positive (%)	p value
Bulk milk samples	Species	Cattle	68	28 (41.20)	0.291
		Buffalo	131	39 (29.80)	
	Peri-urban areas	Anand	40	19 (47.50)	0.116
		Ahmedabad	34	12 (35.30)	
		Navsari	33	07 (21.20)	
		Surat	33	07 (21.20)	
		Valsad	30	10 (33.30)	
		Vapi	29	12 (41.40)	
		Overall	199	67 (33.70)	
Individual milk samples	Species	Cattle	199	27 (13.60)	0.357
		Buffalo	383	42 (11.00)	
	Peri-urban areas	Anand	147	23 (15.60)	0.481
		Ahmedabad	66	05 (07.60)	
		Navsari	87	07 (08.00)	
		Surat	97	11 (11.30)	
		Valsad	107	13 (12.10)	
		Vapi	78	10 (12.80)	
	Overall	**582**	**69 (11.90)**		

Table-2: Distribution of risk factors on general characteristics of farms and their association with herd prevalence of bovine brucellosis.

Sr. no.	Type of risk factor		Bulk milk testing		p value
			No. of samples (%)	No. of positive (%)	
1	Herd Type	Single	118 (59.30)	41 (34.70)	0.695
		Mixed	81(40.70)	26 (32.10)	
2	Herd Size	<25	129 (64.80)	28 (21.70)	0.000*
		26-50	57 (28.60)	27 (47.40)	
		51-75	08 (04.10)	07 (87.50)	
		76-100	01 (00.50)	01 (100.00)	
		101-125	00 (00.00)	00 (00.00)	
		126-150	02 (01.00)	02 (100.00)	
		>150	02 (01.00)	02 (100.00)	
4	Type of species	Cattle	68 (34.20)	28 (41.20)	0.291
		Buffalo	131 (65.80)	39 (29.80)	
5	Type of animal	Indigenous	155 (77.90)	44 (28.40)	0.012*
		Crossbred	44 (22.10)	23 (52.30)	
6	Type breed	Mahesani	101 (50.80)	35 (34.70)	0.013*
		Jafarabadi	18 (09.00)	02 (11.10)	
		Bunni	10 (05.00)	01 (10.00)	
		HF crossbred	40 (20.10)	23 (57.50)	
		Jersey crossbred	04 (02.00)	01 (25.00)	
		Gir	23 (11.60)	04 (17.40)	
		Other	03 (01.50)	01 (33.30)	
7	Animal SexAll	Female	191 (95.90)	62 (32.50)	0.114
		Mixed	08 (04.10)	05 (62.50)	
8	Owner sex	Male	178 (89.40)	58 (32.60)	0.501
		Female	021 (10.60	09 (42.90)	
9	Owner's education	Primary	063 (31.70)	17 (27.00)	0.540
		Secondary	066 (33.20)	26 (39.40)	
		High secondary	039 (19.60)	15 (38.50)	
		Graduate	024 (12.00)	08 (33.30)	
		Professional	007 (03.50)	01 (14.30)	
10	Owner's age	<30	001 (00.50)	01 (100.00)	0.002*
		31-40	052 (26.10)	09 (17.30)	
		41-50	088 (44.20)	30 (34.10)	
		>50	058 (29.20)	27 (46.60)	
11	Knowledge gained	Inherited	175 (88.00)	63 (36.00)	0.034*
		Self	024 (12.00)	04 (16.70)	

Figures in parenthesis indicate percentage * indicates significant at p<0.05

Risk factors on general characteristics of farms: The distribution of 11 risk factors on general characteristics of farms and their association with herd prevalence of bovine brucellosis is given in Table-2. The highest prevalence (87.50%) was observed in a group of herds with herd size between 51-75 animals whereas it was the lowest (21.70%) for a group of herds with herd size below 25 animals. Increase in prevalence with increased herd size was observed with highly significant association with occurrence of bovine brucellosis (p= 0.000). Such finding is in accordance with the earlier report of Tun *et al.* [10] who reported significantly higher risk when the herd size is greater than 50 animals as it was 28.6% for herd size above 50 and only 3.1% for herd size less than 50 animals. Likewise, scientists had also reported significant association of herd size with prevalence of brucellosis [11, 19, 25-32]. On contrary, non-significant association between herd

Table-3: Distribution of risk factors on introduction of infection to farms and management systems of farms and their association with herd prevalence of bovine brucellosis.

Sr. No.	Type of risk factor	Bulk milk testing		p value
		No. of samples (%)	No. of positive (%)	
A.	**Risk factors on introduction of infection to farms**			
1	Vaccination			
	Routine vaccines	193 (97.00)	66 (34.20)	0.292
	Both (*Brucella* and above)	006 (03.00)	00 (33.30)	
2	Breeding methods			
	AI	126 (63.20)	44 (34.90)	0.205
	Natural services	010 (05.00)	01 (10.00)	
	Mixed	063 (31.80)	22 (34.90)	
3	Milking methods			
	Hand milking	171 (85.90)	51 (29.80)	0.055
	Machine milking	021 (10.60)	12 (57.10)	
	Mixed	007 (03.50)	04 (57.10)	
4	Farm replacement			
	From own farm	006 (03.00)	04 (66.70)	0.350
	From known source	008 (04.00)	02 (25.00)	
	From market	150 (75.40)	50 (33.30)	
	Mixed	035 (17.60)	11 (31.40)	
	No	199 (100.00)	67 (33.70)	
B.	**Risk factors on management systems of farms**			
1	Type of housing system			
	Loose	001 (00.50)	00 (00.00)	0.257
	Tying	186 (93.50)	65 (34.90)	
	Mixed	012 (06.00)	02 (16.70)	
2	Type of floor			
	Concreted	173 (86.90)	62 (35.80)	0.054
	Kaccha	000 (00.00)	00 (00.00)	
	Other	026 (13.10)	05 (19.20)	
3	Level of hygiene at farm			
	Good	087 (43.70)	31 (35.60)	0.968
	Fair	107 (53.80)	34 (31.80)	
	Poor	005 (02.50)	02 (40.00)	
4	Level of waste management			
	Good	091 (45.70)	35 (38.50)	0.580
	Fair	100 (50.30)	30 (30.00)	
	Poor	008 (04.00)	02 (25.00)	
5	Grazing practice			
	Yes	006 (03.00)	02 (33.30)	0.883
	No	193 (97.00)	65 (33.70)	
7	Disinfection practice			
	Yes	006 (03.00)	00 (00.00)	0.061
	No	193 (97.00)	67 (33.70)	
9	Water system			
	Tap	034 (17.10)	10 (29.40)	0.906
	Under ground	165 (82.90)	57 (34.50)	
	Surface	000 (00.00)	00 (00.00)	
10	Milk and other records			
	Yes	014 (07.00)	05 (35.70)	0.970
	No	185 (93.00)	62 (33.50)	

Figures in parenthesis indicate percentage.

size and prevalence of brucellosis was also reported by Kebede *et al*. [33], Tolosa *et al*. [34] and Chand and Chhabra [35].

Out of 199 herds, 155 (77.90%) were of indigenous animals whereas only 44 (22.10%) herds had crossbred animals. But, the prevalence of brucellosis was significantly (p=0.012) higher in herds of crossbred animals (52.30%) than indigenous animals (28.40%). The highest prevalence (57.50%) was observed in herds of Holstein-Frisian crossbreds followed by 34.70% for Mahesani buffaloes, 33.30% for other breed of buffalo, 25.00% for Jersey crossbred, 17.40% for Gir cattle, 11.10% for Jafarabadi buffaloes and the lowest (10.00%) in Bunni buffaloes. The difference in prevalences due to breed was also statistically significant (p=0.013). The results are in accordance to earlier reports indicated significantly higher prevalence in crossbred than indigenous animals [23, 36-38]. In this regard, Swai *et al*. [39-40] reported significant association of exotic blood with prevalence of brucellosis. Tesfaye *et al*. [41] also observed higher prevalence in crossbreds than local breed but it was statistically non-significant. The findings of Kebede *et al*. [33] and Chand and Chhabra [35] reported seropositivity, independent to breed and species, respectively. Only a single report of Karimuribo *et al*. [42] reported higher prevalence in indigenous cattle than crossbreds.

During a present study, risk factors related to owner's age, sex, education level, experience were studied first time in Gujarat. The prevalence of brucellosis showed increasing trend with increase in age of owner with an exception of a single herd owned by owners aged below 30 years. The highest prevalence was in

Table-4: Distribution of risk factors on exposure of disease and their association with herd prevalence of bovine brucellosis.

Sr. No.	Type of risk factor	Bulk Milk Testing		p value
		No. of samples (%)	No. of positive (%)	
1	Control of visitors			
	Yes	035 (17.60)	12 (34.30)	0.797
	No	164 (82.40)	55 (33.50)	
2	Control of stray animals			
	Yes	007 (03.50)	03 (42.90)	0.600
	No	192 (96.50)	64 (33.30)	
3	Washing facilities			
	Yes	195 (98.00)	66 (33.80)	0.561
	No	004 (02.00)	01 (25.00)	
4	Protective clothing			
	Yes	006 (03.00)	03 (50.00)	0.390
	No	193 (97.00)	64 (33.00)	
5	Awareness of brucellosis			
	Yes	030 (15.00)	13 (43.30)	0.376
	No	169 (85.00)	54 (32.00)	
6	Veterinary help in case of abortion			
	Yes	029 (14.60)	07 (24.10)	0.144
	No	170 (85.40)	60 (35.30)	
7	Fate of aborted animal			
	Retain	190 (95.50)	62 (32.60)	0.155
	Sale	009 (04.50)	05 (55.60)	
	Panjarapole	000 (00.00)	00 (00.00)	
8	History of abortion			
	Yes	111 (55.80)	52 (46.80)	0.000*
	No	088 (44.20)	15 (17.00)	
9	History of retention of placenta			
	Yes	130 (65.30)	52 (40.00)	0.031*
	No	069 (34.70)	15 (21.70)	
10	History of still birth			
	Yes	080 (40.20)	35 (43.80)	0.015*
	No	119 (59.80)	32 (26.90)	
11	History of repeat breeding			
	Yes	128 (64.30)	49 (38.30)	0.080
	No	071 (35.70)	18 (25.40)	
12	History of metritis/endometritis			
	Yes	086 (43.20)	40 (46.50)	0.003*
	No	113 (56.80)	27 (23.90)	

Figures in parenthesis indicate percentage.

herds owned by owner's age above 50 years (46.60%) followed by prevalence in herds owned by owner's age group of 41-50 years (34.10%) and 31-40 years (17.30 %). The overall effect of age groups of owner was significant (p=0.002). Further, the prevalence in herds owned by owners having inherited knowledge of dairying was significantly (p=0.034) higher (36.00%) than herds owned by owners who gained knowledge of dairying by themselves (16.70%). In this regard, Tebug *et al*. [43] reported that higher awareness in farmers about the existence of zoonotic infections and practices with above primary education and more than six years of dairy farming experience.

Risk factors on introduction of infection to farms: A total of six different risk factors on introduction of infection to farms were studied for the first time in Gujarat. Of these, distribution of four risk factors and their association with herd prevalence of bovine brucellosis is given in Table-3. None of risk factors showed significant association with occurrence of bovine brucellosis. Risk factors such as quarantine practice and testing before introduction were not followed in the herds covered under the study and therefore, statistical significance could not be drawn out. The results observed in present study are in accordance to the findings of Tun *et al.* [10] who reported statistically non-significant effects of the risk factor variables (vaccination, breeding, milking methods and production system) concerned with the introduction of the infection into the herd. Vaccination against disease is considered to be a protective factor as reported by Azevedo *et al.* [44]. This was also supported by observation of Muma *et al*. [45] who found negative correlation of *Brucella* vaccine history with prevalence of brucellosis. Further, unrestricted movement of animals [46] and purchase of animals for farm replacement or breeding [36, 47-48] and removal of sero-positive reactors after testing [49] are considered to be important risk factors for introduction of infection.

Risk factors on management systems of farms: Similar to risk factors on introduction of infection to farm, 11 different risk factors were also studied under this group. The distribution of 10 risk factors and their association with prevalence of bovine brucellosis is given in Table-3. In relation to risk factor on feeding practice in herds covered, all herds followed manual feeding system and therefore, its association with occurrence of bovine brucellosis could not be draw out. None of the risk factors had statistical significant association with occurrence of brucellosis. Though,

keeping good hygiene at dairy farm [26] and zero grazing [39, 50] are considered as a protective factor for brucellosis, unhygienic practices were identified as factors that will facilitate the spread of *Brucella* infections [51].

Risk factors on exposure of disease: A total 15 different risk factors were covered under this group. Except reproductive disorders, remaining risk factors were covered for the first time under this study. The distribution of 12 different risk factors on exposure of disease and their association with prevalence of bovine brucellosis is presented in Table-4. In none of the herds, provision of calving box and isolation of diseased animals was in practice. Proper disposal of aborted material was followed in all herds. Therefore, statistical significance of these three risk factors could not be drawn out. Majority of herds were not having control over visitors (82.40%) and stray animals (96.50%). It is a known fact that restriction over visitors and stray animals is helpful in reducing spread of infection which is further supported by an observation of Tun *et al.* [10] who found significant increased prevalences with poor bio-security measures like control of visitors and stray animals.

None of the herd was practicing isolation of diseased animal and providing calving box to down calver as they were maintaining high density of animals in lesser space due to high market price of each square feet of land area in peri-urban areas. It is a fact that the shedding of large numbers of organisms occurs during 10 days after abortion or calving of infected cows is the most significant feature of bovine brucellosis epidemiology and therefore, provision of calving box to down cowers is definitely helpful to reduce the chance of spread of infection if any [2]. The awareness of brucellosis among dairymen was also reported as significant risk factor [10, 36, 50]. In the present study, a risk factor such as veterinary help/aid in cases of abortion/still birth showed statistically non-significant association with prevalence of bovine brucellosis. However, scientists had reported the presence of adequate veterinary services as protective factors in prevalence of brucellosis [25].

As per Table-4, the risk factors such as history of abortion, retention of placenta, still birth and metritis/endometritis had statistically significant effects on prevalence of brucellosis. The results are in accordance to the findings of scientists who had reported significant association with reproductive disorders like abortion, retention of placenta and repeat breeding [17, 35, 43, 44, 50, 52-55]. Some scientists also found higher prevalence of brucellosis with reproductive disorders but their association with prevalence was non-significant [22, 26, 28-29, 33, 56-60].

Conclusion

It can be concluded that prevalence of bovine brucellosis in dairy herds maintained under intensive system of production in peri-urban areas was comparatively higher than overall prevalence of brucellosis. Risk factors like larger herd in close confinement without adequate sheds, type of animal, type of breed and knowledge/awareness of dairyman, unrestricted animal market, replacement without prior testing, reproductive disorders with absence of their testing are the important risk factors under the intensive production system of peri-urban areas.

Authors' contributions

MDP carried out the study, tabulated and analysed the data, drafted the manuscript. PRP helped to draft the manuscript and approved the final draft. MGP and ANK helped in sample collection and processing. KKT helped in data assembling and statistical analysis. ABF rendered necessary infrastructure facilities and grant for purchasing necessary kits and other materials required during the study period. All authors read and approved the final manuscript.

Acknowledgements

The authors are thankful to S. K. Raval, Professor, Department of Medicine, J. H. Purohit (Retired) and M. K. Jhala, Professor and Head, Department of Microbiology, Veterinary College, AAU, Anand and S. B. Patel, CEO, Amul dairy, Anand for their necessary help and encouragement during the study. Authors are grateful for support given by all dairy farmers and the help rendered by field veterinarians during the study. The present work was carried out as Ph. D. research work of corresponding author who is working at Livestock Research Station, Navsari Agricultural University, Navsari. Necessary funding for the present work was mainly made under the plan project of University at LRS, NAU, NAvsari and partly from Department of Medicine, Veterinary College, Anand Agricultural University, Anand.

Competing interests

The authors declare that they have no competing interests.

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