Major reproductive disorders and seroprevalence of brucellosis in dairy cows of Kembata-Tembaro zone, Southern Ethiopia.

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Abstract

A cross-sectional study was conducted from October 2016 to May 2017 in Kembata-Tembaro Zone, Ethiopia to estimate the prevalence of major reproductive disorders and brucellosis in dairy cattle. A total of 733 dairy cows were selected from Kedida-Gambella and Damboya districts by a systematic random sampling technique. Clinical examination for reproductive disorders (n=733 cows) was carried out to investigate reproductive problems. From each cow, about 10 ml blood sample was collected and serum separated. Serum samples were screened for *Brucella* antibody by Rose Bengal plate test (RBPT) and positive samples were further tested by Complement Fixation Test (CFT). Results showed that the overall seroprevalence of brucellosis using RBPT and CFT were 2.0% (95% confidence interval [CI]: $1.15 - 3.35\%$) and 0.8% (95% CL 0.2-1.5%)., respectively. About 61% of the study cows had exhibited one or more of the reproductive disorders. The major reproductive disorders identified during this study were anoestrus (34.2%), repeat breeding (19.8%), dystocia (12.4%), retained placenta (8.5%), metritis (4.8%), abortion (3.8%), vaginal prolapse (1.8%) and stillbirth (0.9%). Reproductive disorders were significantly associated with the management system, body condition, watering point, and age of the animals (p< 0.05). Accordingly, the disorders were more frequent in the extensive management system, in cows with poor body conditions, and among those using communal watering points. The problems of reproductive disorders were found to increase with age, especially in cows older than eight years of age. The overall seroprevalence of brucellosis reflects a low level of *Brucella* infection in the study area. In general, reproductive disorders are widely prevalent in dairy cows of the study area and undoubtedly hinder the

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production performances of dairy farms. Thus, there is a need for further study on the causes of clinical reproductive disorders in the study areas.

Keywords: Brucellosis; Dairy cows; Reproductive disorders; Seroprevalence; Ethiopia.

Introduction

Ethiopia is one of the developing countries endowed with a huge livestock population. Dairy farming is becoming an important component of livestock farming in Ethiopia owing to increasing demand for dairy products in urban and peri-urban areas along with a favorable policy are indicators of the importance and potential of dairying in the country (Tegegne *et al.*, 2013).

Even though the aforementioned opportunities exist for livestock production and most of the rural poor farmers are dependent on this sector to sustain their livelihood (MoARD, 2007), the country has not been benefited as such from the livestock sector in terms of economic gain compared to its huge potential. Per capita annual milk consumption is extremely low (19 liters), which is far below the world average of 105 liters and the African average of 40 liters (AGP, 2013). Various constraints hinder the country from full utilization of the potential of the dairy sector. These constraints include feed shortage, reproductive disorders, spread and occurrence of tropical animal diseases, poor productive and reproductive traits of local animals, inadequate health facilities, and weak veterinary extension systems (Tadesse and Mengistie, 2016). Reproductive disorders and animal diseases are the major problems that reduce the productivity and productivity of dairy cows (Duguma and Zewdie, 2014; Lawson *et al.*, 2004). The most common reproductive disorders reported in cattle include anoestrous, repeat breeding, endometritis, retained placenta, and abortions (Zemenu *et al.*, 2018; Duguma and Zewdie, 2016; Khan *et al.*, 2016; Haile *et al.*, 2010). Of these, anoestrous and repeat breeding are very serious reproductive problems affecting nearly 30-40% of the total breeding cow population (Srijit, 2015).

According to Ball and Peters (2005), the underlining causal factors for reproductive disorders are classified as infectious and noninfectious. Among infectious causes, brucellosis is one of the most important causes of reproductive disorders that result in higher economic loss in the dairy industry (Nicoletti,

1998). In Ethiopia, the reported prevalence of bovine brucellosis is ranging from 0% to 32.9% (Weldegebriel, 2015; Megersa *et al.*, 2011; Hunduma *et al.*, 2009; Asmare *et al.*, 2007). In Ethiopia, despite the problem being multifactorial; there is a tendency of associating these disorders with brucellosis by most animal health professionals. Hence, assessing the magnitude of reproductive disorders and their determinants in the study area is instrumental to develop an appropriate intervention strategy. Therefore, the present study was designed to estimate the prevalence of reproductive disorders and brucellosis seroprevalence in dairy cows in the Kembata Tembaro Zone.

Materials and methods

Study area description

The study was conducted in Southern Nations, Nationalities, and People Region (SNNPR) Kembata Tembaro zone (Figure 1). The altitude of the study area ranges from 1500 to 3500 meters above sea level. The study area is located between 7º12' to 7º42' latitude and 37º44' to 38º longitude. The area is characterized by bimodal rainfall, a short rainy season that extends from March to April, and a long rainy season from June to Mid-September. The area receives an average annual rainfall of 900-1400mm. The mean minimum and maximum temperatures were 7ºC and 25ºC, respectively (ZDA, 2014).
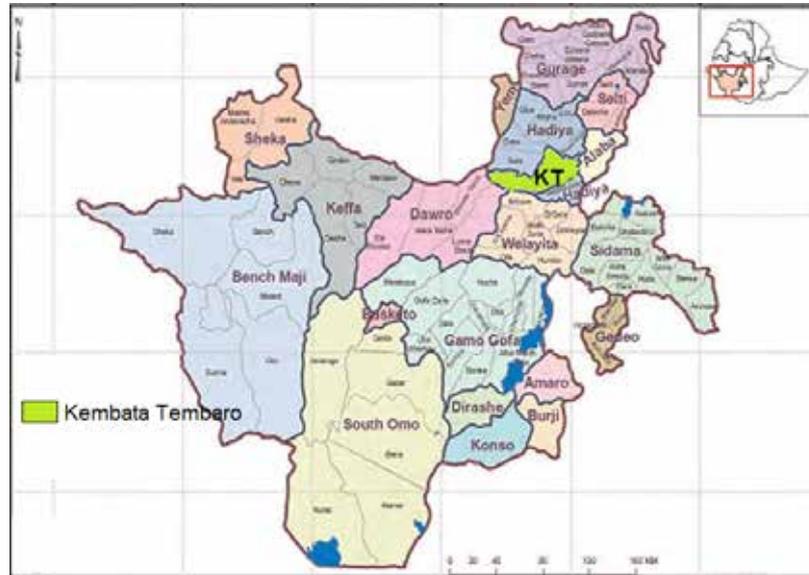
Figure 1. Zonal Map of Southern Region showing the study area

Study animals

The study animals were dairy cows selected from the Kedida Gambella and Damboya districts of the Kembata Tambaro Zone. The districts were purposively selected based on the presence of a large number of dairy cows. A total of 733 cows were selected from the two districts and 17 villages that managed under semi-intensive (n = 367 cows) and extensive (n = 366 cows) management systems. All cows that calved in the last year were purposively included in the study. Risk factors like management system, age of animals, watering point, body condition score, breed, and breeding systems were considered for this study.

Study design and sample size

A cross-sectional study design was undertaken, from October 2016 to April 2017, to estimate the prevalence of major reproductive disorders in addition to assessing the seroprevalence of brucellosis. The study cows were selected

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from 17 villages, which were selected using a systematic random sampling technique. The minimum number of samples required for the study (i.e. 246) was computed by using prevalence (43.1%) reported by Haile *et al.* (2014) from Hossana and considered 95% level of confidence and 5% absolute precision (Thrusfield, 2018). So the computed sample size was increased to 733 (i.e. ≈3 folds) to improve the precision and validity of the results.

Blood sample collection and laboratory tests

Blood samples (≈10 ml) were collected from the jugular vein of the 733 cows separately, using sterile needles and plain vacutainer tubes. The samples were transported to Sodo Regional Veterinary Laboratory and allowed to stand overnight at room temperature, and the serum was decanted into cryovials. After extraction, the serum samples were labeled with all necessary information, and then in the laboratory kept at -20°C in deep freeze until examined for antibodies against natural Brucella exposure using Rose Bengal Plate Test (RBPT). Thirty microliters of RBPT antigen (VLA Weybridge, UK) was added to equal volumes of test sera dropped on a test plate. The antigen and test serum were thoroughly mixed with an applicator stick, and after 4 minutes checked for the occurrence of agglutination. Any degree of agglutination was taken as evidence for the presence of antibodies. Those positive sera were sent to National Veterinary Institute for Complement Fixation Test (CFT). CFT was performed using *Brucella* antigen (Central Veterinary Laboratory, Weybridge, UK), following the standard protocol described by OIE (2018). An animal was considered positive if it was seropositive both in RBPT and CFT.

Assessment of reproductive disorders and general husbandry practices

Examination for reproductive disorders (n = 733 cows) was carried out to investigate reproductive problems like repeated breeder, retention of fetal membrane, anoestrus, abortion, uterine and vaginal prolapse, dystocia, stillbirth, and metritis. During this time, information on the risk factors considered in this study like age, body condition, breed, breeding methods, herd size, watering points, setting, and management system were recorded. The body condition score of cattle was described as 1 to 5 using the method of Richard (1993). For ease of the study it was categorized into three as follows: poor (BCS 1and 2), medium (BCS 3), and good (BCS 4 and 5).

Definitions of some terms:

Repeated breeder: Cows that are cycling with no clinical abnormalities, which have failed to conceive after at least three successive inseminations.

Retention of fetal membrane: It is defined as failure to expel fetal membranes within 24 hours after parturition (Sheldon, 2019).

Anoestrus: It is a prolonged period of sexual rest during which the genital system is quiescent (Parkinson, 2019).

Abortion is the expulsion of a fetus before the time of expected viability. Stillbirth is the delivery of a dead fetus within the period of expected viability (Schafer and Miller (2016).

Dystocia: A difficulty encountered in the expulsion of the fetus

Questionnaire survey and clinical examination of reproductive disorders

The management system and reproductive disorders of dairy cattle in the study areas were assessed by using a semi-structured and pretested questionnaire. During the study, the objectives of the study were briefed for the participants, and then the questionnaire was given for 200 dairy farm owners to collect information for the occurrence of reproductive disorders like dystocia, abortion, stillbirth, retained fetal membrane, uterine/vaginal prolapse, anoestrous, metritis and repeat breeding in the past one year. Moreover, information on the management system, breeding method, watering, age of the animals, sex and education level of respondents, awareness about the causes of reproductive disorders, keeping of animals in the dwelling house, heat detection, knowledge of owners about the correct time of insemination and constraints of livestock production were assessed by the investigators. Following the questionnaire, observation and rectal examination (i.e. palpation) were done. Any observed reproductive disorders were recorded in the format prepared for this purpose.

Data analysis

Collected data were entered into Microsoft Excel and then coded. Descriptive statistics was used to summarize the data into mean, frequency distribution,

and percentage. Associations of the risk factors with the occurrence of reproductive disorders were analyzed using the Chi-square test. All statistical analyses were carried out using STATA software version 14 for statistical analysis (Stata Corp, College Station, Texas). The 95% confidence interval and p-value ≤ 0.05 were considered statistically significant.

Ethical clearance

Before beginning the research, dairy cow owners were provided with clear and detail information. Then, informed consent, for their willingness and participation in responding the questionnaire as well as blood sampling was obtained from participants. As a result, we attest that this research activity was carried out in accordance with professional ethical standards and regulations.

Results

Seroprevalence of brucellosis

The seroprevalence of brucellosis with the screening test (RBPT) and confirmatory test (CFT) and in the two study districts was shown in Table 1. From a total of 733 examined dairy cows, 15 (2.0%, 95% CI: $1.15 - 3.35\%$) and 6 (0.82%, 95% CI: 0.2-1.5%) were found to be seropositive for brucellosis with RBPT and CF, respectively. Since the seroprevalence of brucellosis was low the statistical analysis was not performed for the risk factors considered for this study.

Table 1. Seroprevalence of brucellosis in dairy cows in Kembata Tambaro zone

Tests	No examined	Districts		Management		Total	95% CI
		Kadida-Gamella (n = 509)	Damboya (n = 224)	Semi-intensive (n = 367)	Extensive (n = 366)		
RBPT	733	8 (1.6%)	7 (3.1%)	-	15 (4.1%)	15 (2.0%)	1.0-3.1
CFT	733	3 (0.6%)	3 (1.3%)	0	6 (1.6%)	6 (0.8%)	0.2-1.5

CI = Confidence interval

Prevalence of reproductive disorder in dairy cows

Of the total 733 examined dairy cows, 450 (61.4%) were affected at least by one or more types of reproductive disorders. A total of eight different types of cases of reproductive disorders were recorded in the study areas. A higher

proportion of reproductive disorders was observed in animals with poor body conditions (69%) than those animals with medium (57%) and good (56%) body conditions. Anoestrus, repeat breeding syndromes, dystocia, and retained fetal membrane were among the major reproductive problems encountered in the area (Table 2).

Table 2. Prevalence of major reproductive disorder of dairy cattle in Kemba-ta-Tambaro zone (n=733)

| Reproductive disorders | Total number of cases | Cases by Study districts | | Total Prevalence (%) | 95% CI |
		Kadida-Gamella (n = 509) Cases (%)	Damboya (n = 224) Cases (%)		
Dystocia	91	46 (9.0)	45 (20.1)	12.4	10.02- 14.80
Abortion	28	16 (3.1)	12 (5.4)	3.8	2.43 - 5.21
Stillbirth	7	5 (1.0)	2 (0.9)	0.9	0.25 - 1.66
Retained placenta	62	43 (8.5)	19 (8.5)	8.5	6.44 - 10.48
Prolapsed	13	10 (2.0)	3 (1.3)	1.8	0.82 - 2.73
Metritis complex	35	19 (3.7)	16 (7.1)	4.8	3.23 - 6.32
Anoestrus	251	181 (35.6)	70 (31.3)	34.2	30.80 - 37.69
Repeat breeder	145	98 (19.3)	47 (21.0)	19.8	16.89 - 22.67
Over all	450	314 (61.7)	136 (60.7)	61.4	57.85 -64.92

Questionnaire survey results

Among the respondent of dairy cows owners, 76% didn't know the causes of the reproductive disorders. About 96% of the respondents were consuming raw milk and 42% of owners kept animals in their houses or shared with people (Table 3).

Table 3. Summary of questionnaire results

Description of variables	Number. of respondents	Percent (%)
Sex of respondents		
Male	186	93
Female	14	7
Total	200	100
Education level		
Primary	152	76
Secondary	46	23
Degree	2	1
Awareness about the cause of RDs		
Know some causes	48	24
Do not know any causes of RDs	152	76
Keep animals in the dwelling house		
Yes	84	42
No	116	58
Heat detection		
Detect based on oestrus signs	184	92
Unable to detect	16	8
Knowledge of owners correct time of insemination		
Know the correct time of insemination	30	15
Do not know the correct time of insemination	170	85
Constraints of livestock		
Feed shortage	178	89
Mastitis	104	52
RDs	124	62
Low productivity	64	32

RDs = Reproductive disorders

A summary of the potential risk factors for reproductive disorders is shown in table 4.

Table 4. Chi-square test of reproductive disorders with different risk factors

Risk factors	No. examined	RD No (%)	χ^2	P-Value
Management				
Semi-intensive	367	201 (55)	13.60	≤0.001
Extensive	366	249 (68)		
Breeding method				
Bull	510	311 (61)	0.12	0.730
AI	223	139 (62)		
Breed				
Jersey	178	106 (59)	0.35	0.841
Holstein Friesian	275	171 (62)		
Local	280	173 (62)		
Setting				
Urban	123	74 (60.1)	0.12	0.94
Per urban	417	258 (62)		
Rural	193	118 (61)		
BCS				
Poor	247	171 (69)	9.68	0.008
Medium	382	220 (57)		
Good	104	59 (56)		
Watering				
Communal	430	309 (71.86)	48.10	≤0.001
Individual	303	141 (46.53)		
Cow age(years)				
≤8 Years	368	201 (54)	14.30	≤0.001
>8 years	365	249 (68)		

AI = artificial insemination, BCS= body condition score, RD= Reproductive disorder

Discussion

Seroprevalence of bovine brucellosis was reported from various parts of the country (Asmare *et al*., 2014). But, to the best of our knowledge, the present report is the first from the Kembata-Tembaro zone. Based on the CFT result of the current study, the seroprevalence of brucellosis was regarded as low, 0.8% (95% CL 0.2-1.5%). This finding is consistent with some reports from various areas of the country (Pal *et al*., 2016; Bashitu *et al*., 2015 and Degefa *et al*., 2011). Contrary to the current findings, several other authors have reported a relatively higher seroprevalence of bovine brucellosis (Yohannes *et al*., 2012; Megersa *et al*., 2011; Teklehaimanot and Gangwar, 2011; Asmare *et al*., 2010; Ibrahim *et al*., 2010; Mekonnen *et al*., 2010; Kebede *et al*., 2008; Tolosa *et al*., 2008; Berehe *et al*., 2007). All the reports were from the extensive management system. Hence, the differences in the prevalence may be due to variation in the study areas or the management systems (Matope *et al*., 2011). Moreover, variation in the practice of infected animals culling, herd size/density, and keeping mixed animal species (Mugizi *et al*., 2015; Asmare *et al*., 2013; Megersa *et al*., 2011; Richard, 2004) might have accounted for the differences. In general, our findings corroborate with the finding of Asmare *et al*. (2014) whose quantitative review revealed that the prevalence of dairy cattle brucellosis was low, and not widely distributed.

Factors such as animal management system, age of animals, water sources, body condition score, breed, and breeding systems were considered as potential predisposing factors for brucellosis seropositivity. But, since only six cows were seropositive with CFT, it was not necessary to conduct a statistical test. Analysis of *Brucella* seropositivity in dairy cows with the occurrence of specific and/or overall reproductive disorders did not show statistically significant association (p> 0.05), which could be due to the effects of very low seroprevalence. This may indicate the role of *Brucella* as the cause of reproductive disorders in dairy cattle in the study area is negligible. Hence, the higher prevalence of reproductive problems might be associated with other causative agents.

The present study showed that 61.4% of dairy cattle were affected by reproductive disorders. This finding is comparable to the report of Abreham *et al*. (2010). During this study period, eight different types of reproductive disorders were recorded. Among these, the top four disorders, relatively with higher prevalence, were anoestrus (34.2%), repeat breeder (19.8%), dystocia (12.4%), and

retention of the placenta (8.5%). This observation is in general agreement with some other studies in the country (Haile *et al.*, 2014; Gebrekidan *et al.*, 2009; Shiferaw *et al.*, 2005). Reports from various parts of the country showed that the prevalence of anoestrus ranges from 1.3 to 37.8% (Misebo *et al.*, 2018; Mekonnin *et al.*, 2015; Gashaw *et al.*, 2011), repeat breeder 1.3 to 26.8% (Misebo *et al.,* 2018; Hunduma, 2013 and Gashaw *et al.,* 2011), dystocia 0.79 to 16.7% (Misebo *et al.*, 2018; Mekonnin *et al.*, 2015; Ayele *et al.*, 2014) and retention of placenta 3.36 to 28.9% (Misebo *et al.*, 2018; Ayele *et al.*, 2014; Tekleye *et al.*, 1992). Overall, the major differences in the prevalence of reproductive disorders could be due to management factors, mainly feeding and nutrition, age and breed of cattle, and climatic condition differences (Zemenu *et al.*, 2018; Berihu and Abebaw, 2009).

The management system, watering, animal age, and body condition were significantly associated with the occurrence of reproductive disorders ($p < 0.05$). The prevalence of reproductive disorders was significantly higher in extensive management, communal watering, poor body condition score, and cows greater than eight years old. Under extensive management, less care is given to the animals, and contact between animals at a communal watering point is very high. Such factors facilitate the spread of infection among the animals (Khan *et al.*, 2016; Getachew and Nibret, 2014; Abreham *et al.*, 2010). According to Angesom *et al.* (2013) and Abebaw *et al.* (2011), poor body condition was associated with the reproductive health of dairy cows. Body condition change is associated with reproduction problems (like anestrous) and health (Roche *et al.*, 2009). The rate of occurrence of repeated breeding increased due to dystocia (Bonneville-Hébert *et al.*, 2011).

Limitation

The study's shortcoming is that the reasons of reproductive issues have not been discovered. Because there were few seropositive animals for statistical analysis, the data was summarized using frequency and average.

Conclusions

The seroprevalence of brucellosis was very low (0.8%) compared to most previous reports in the country. This entails brucellosis could not be the principal cause for the reported high occurrence of reproductive disorders (61%). There-

fore, further investigation on the causative agents of reproductive disorders is necessary. Meanwhile, awareness creation to the farm owners to improve their farm management system that enables to reduce the problem in the farm is imperative.

Acknowledgements

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