



## Quantitative evaluation of the infection dynamics of bovine brucellosis in Tanzania

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### ABSTRACT

Brucellosis is endemic in Tanzania. A cross-sectional study was conducted at 17 cattle farms in agro-pastoral areas in Tanzania to identify risk factors associated with the within-farm prevalence of bovine brucellosis and to quantitatively assess the infection dynamics through disease modelling. Cattle blood sampling and interviews with farmers using a structured questionnaire were conducted. A total of 673 serum samples were screened using the Rose-Bengal plate test (RBPT), and sero-positivity of RBPT-positive samples was confirmed using a competitive enzyme-linked immunosorbent assay. Zero-inflated binomial regression was performed for univariable and multivariable risk factor analyses of within-farm prevalence. Several susceptible-infectious (SI) models were compared based on deviance information criteria, and age-dependent force of infection (FOI) was measured using age-specific prevalence data for the 10 infection-positive farms. Using the diagnoses of cows on the 17 farms, the basic reproduction number,  $R_0$ , was also calculated. The farm-level prevalence and animal-level adjusted prevalence were 58.8 % (10/17, 95 % confidence interval: 33.5–80.6 %) and 7.0 % (28/673, 95 % credible interval: 5.7–8.4 %), respectively. The risk factor for high within-farm prevalence was introduction of cattle from other herds. A mathematical model with constant FOI showed the annual probability of infection as 1.4 % (95 % credible interval: 1.0 %–2.0 %). The  $R_0$  was 1.07. The constant FOI could have been due to the predominant mode of infection being transmission of *Brucella* from contaminated aborted materials during grazing. Direct purchase of infected cattle could facilitate efficient transmission between susceptible animals through abortion.

### 1. Introduction

Brucellosis is one of the most prevalent bacterial zoonoses in the world, and it has been designated by the World Health Organization as a neglected zoonotic disease (WHO, 2006). The genus *Brucella* consists of 10 distinct species (Banai and Corbel, 2010), four of which are zoonotic: *Brucella abortus* maintained primarily in cattle; *B. melitensis* maintained in sheep and goats; *B. suis* maintained in pigs; and *B. canis* maintained in dogs (Corbel et al., 2006).

Bovine brucellosis causes significant economic losses for farmers due to decreased production resulting from loss of animals through abortion and due to infertility, weak offspring, weight loss, and reduced milk yield (McDermott and Arimi, 2002). *Brucella* is usually transmitted

between animals via contact with contaminated aborted tissues and vaginal discharges. Vertical transmission via the uterus or through ingestion of maternal milk and sexual transmission may also play a role in the spread of *Brucella* (Corbel et al., 2006).

In humans, the clinical symptoms of brucellosis include undulant fever, joint pain, night sweats, headache, and extreme weakness (Dean et al., 2012). More severe symptoms, such as endocarditis, meningitis, arthritis, and osteomyelitis, have also been reported (Xavier et al., 2010). Humans can become infected with *Brucella* through the consumption of uncooked meat or unpasteurized dairy products obtained from infected animals or by direct contact with infected animals (Sung and Yoo, 2014). Individuals in certain occupations, such as veterinarians, butchers, abattoir workers, meat inspectors, and livestock farmers,

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are at a greater risk of contracting brucellosis (Kunda et al., 2007)

In the pastoral and agro-pastoral farming systems of Tanzania, contact between wildlife, livestock and humans is common (Assenga et al., 2015). Outbreaks of brucellosis have been documented in various regions and zones of Tanzania since 1927, when an outbreak of abortion was reported in the Arusha region (Shirima, 2005). The prevalence of bovine brucellosis at the animal level is reportedly 9.3 % in Mbeya region (Sagamiko et al., 2018), 5.3 % in Tanga region (Swai and Schoonman, 2010), 6.2 % in Arusha and Manyara regions (Kunda et al., 2007), and 6.8 % in the Katavi-Rukwa ecosystem (Assenga et al., 2015). In Mvomero district, the farm-level prevalence is 52.9 % (9/17, 95 % confidence interval [CI] 28.5–76.1 %) and animal-level adjusted prevalence is 7.0 % (28/673, 95 % credible interval: 5.7–8.4 %), respectively, and the animal-level risk factors for brucellosis include history of abortion and older age (Asakura et al., 2018a). In this previous study (Asakura et al., 2018a) which used the same dataset with the present study, the number of sero-positive farms was reported to be nine by an error (farm level prevalence was 58.8 %, 95 %CI 33.5–80.6 %, correctly), but the present study confirms that 10 farms housed sero-positive animals. While risk factors for brucellosis in animals are generally known well, reports of factors associated with higher within-herd prevalence are limited. The present study adds knowledge on this.

Although brucellosis is endemic in pastoral and agro-pastoral areas in Tanzania, the information on *Brucella* species is limited. In the area of the present study - Morogoro region, only a study reported isolation of *B. abortus* from goat sera (Kassuku, 2017), and no study reported *B. melitensis* as far as we know. There is a knowledge gap in the prevalent *Brucella* species in Morogoro region, but the present study does not attempt the characterization. In northern Tanzania, *B. melitensis* and *B. abortus* were found from human patients (Bodenham et al., 2020) and sequence analysis and blasting confirmed the presence of *Brucella* spp. from aborted woman, cattle and goat (Ntirandekura et al., 2020).

Brucellosis has been successfully controlled or eliminated in livestock populations in many high-income countries (McDermott et al., 2013). However, in developing countries, the control of brucellosis in cattle in an endemic situation is not straightforward, as mass vaccination and ‘stamping-out’ programs depend solely on public resources and are typically impracticable or not sustainable due to cost (Makita et al., 2010). Considering the limited resources available and endemic nature of brucellosis in Tanzania, the willingness of farmers to pay for *Brucella* vaccinations as a community-based intervention was investigated in a previous study, which reported that 89.5 % of farmers expressed a willingness to pay for vaccinations targeting calves (Asakura et al., 2018b).

Infectious disease models can be used to enhance our understanding of infection dynamics. Such models make it possible to formulate policies theoretically and evaluate the adequacy of such policies *a priori*. For instance, models can be used to calculate the potential impact of a vaccination program and provide evidence useful for designing efficient, low-cost vaccination strategies. The objectives of this study were to identify the major risk factors responsible for the higher within-herd prevalence of bovine brucellosis in agro-pastoral areas of Tanzania and characterize the infection dynamics through disease modelling.

## 2. Materials and methods

### 2.1. Field survey

A cross-sectional study involving cattle blood sampling and a structured interview using a questionnaire was conducted between August and December 2015 in Morogoro region of Tanzania. The questionnaire was administrated via in-person interviews to collect information regarding the farm owner, farm characteristics, animals kept on the farm, purchase of animals, contact with wildlife, use of veterinary services, and animals sampled, including any history of abortion. More

detailed information can be found in our previously published article (Asakura et al., 2018a). In this previous study, the formula for a comparison of two proportions was used for the calculation of the animal-level sample size, in order to compare brucellosis prevalence between urban areas of Morogoro municipality and agro-pastoral areas of Mvomero district. A total of 667 and 673 cattle from 106 and 17 farms were studied in the respective areas. At farms with  $\leq 50$  eligible cattle, all cattle older than 3 months were selected to avoid any effect of maternal antibodies on new-born calves. For larger farms, a maximum of 50 cattle were sampled to reflect the age structure of the herd. In this previous study (Asakura et al., 2018a), only one positive animal was identified in Morogoro municipality; thus, the present study targeted the animals in Mvomero district.

### 2.2. Serological tests

Serum samples were collected from 673 animals in Mvomero district and screened using the Rose-Bengal plate test (RBPT) (IDvet, Grabels, France). RBPT-positive samples were then analyzed using a competitive enzyme-linked immunosorbent assay (CELISA) (Boehringer Ingelheim Svanova, Uppsala, Sweden). Both the RBPT and CELISA were performed following the manufacturers’ instructions at Sokoine University of Agriculture. For CELISAs, the optical density was measured at 450 nm using a Multiskan RC version 6.0 ELISA reader (Thermo Labsystems, Helsinki, Finland). Samples exhibiting positive results in both the RBPT and CELISA were regarded as sero-positive, and each farm with at least one sero-positive cow was classified as infected.

### 2.3. Descriptive epidemiology

Animal-level true prevalence was calculated using Eq. 1, with RBPT sensitivity ( $Se_1$ ) and specificity ( $Sp_1$ ) values of 81.2 % and 86.3 %, respectively. The corresponding sensitivity and specificity values for the CELISA ( $Se_2$  and  $Sp_2$ , respectively) are reportedly 100 % and 99.9 %, respectively (Gall and Nielsen, 2004; Portanti et al., 2006):

$$P = (P^T + Sp - 1) / (Se + Sp - 1) \quad (1)$$

where

$$Se = Se_1 \times Se_2 \quad (2)$$

and

$$Sp = 1 - \{(1 - Sp_1) \times (1 - Sp_2)\} \quad (3)$$

where  $P$  represents the true prevalence, and  $P^T$  represents the test prevalence. As the maximum number of animals sampled was 50, the number of sero-positive cattle if all cattle would have been tested within each sampled herd was stochastically estimated for herds larger than 50 animals. This particular result and the details of the method were previously published (Asakura et al., 2018a). The overall farm-level prevalence and 95 % confidence interval (CI) were calculated using the one-sample chi-square test with the statistical software R. The mean ages of male and female animals across the farm and the mean age of infected males and females were also calculated.

Farm characteristics such as tribe, location, animal species, cattle marketing activities, contact with wildlife, history of abortion, and access to veterinary services were described. Age-specific seroprevalence values were calculated using 449 samples collected from 0- to 14-year-old cattle after exclusion of cows over 15 years of age at infected farms; 95 % CIs were calculated using a generalized linear model with binomial errors.

### 2.4. Relationship between sero-positivity and history of abortion

Odds ratios (ORs) were calculated for 3- to 14-year-old females at

infected farms to determine whether there is an association between history of abortion as a potential exposure and the occurrence of brucellosis. Data for animals younger than 3 years were not used, as cows infected with brucellosis before abortion occurs often do not have antibodies (Nielsen and Duncan, 1990); indeed, no sero-positive cows between 0 and 2 years of age were identified (see Results). Brucellosis can cause abortion in cows at the first pregnancy after infection (Nielsen and Duncan, 1990). Farmers were asked about any history of abortion for animals of any age. In animals between 3 and 4 years of age, when the parity number is low, examinations of any causal relationship between infection with *Brucella* and abortion can proceed without caution. However, in animals  $\geq 5$  years of age, abortion might be occurred several years ago, and in such cases, OR will not show recent relationship between brucellosis infection and abortion. Therefore, the data were stratified into two groups, one for animals between 3 and 4 years of age, and the other for animals over 5 years of age, and these data were analyzed using the Mantel-Haenszel (MH) OR. In this analysis, crude and strata-specific ORs were calculated, and a test for homogeneity was performed using Woolf's statistics (Woolf, 1955) to assess differences between strata regarding the strength of the association between infection status and abortion. Finally, the crude OR, MH OR, or strata-specific OR was selected based on the results of tests for homogeneity and rate of change in MH OR compared with the crude OR [(crude OR – MH OR)/crude OR].

## 2.5. Comparison of reproduction performances of sero-positive and sero-negative cows

A previous study reported 15 % decrease of fertility in *Brucella* sero-positive cows (Bernués et al., 1997). An analysis of covariance was conducted to compare the relationships between age and the number of parity between sero-positive and sero-negative cows to investigate the impact of infection with brucellosis on the reproduction performance. This analysis was performed using 393 females over 3 years at 17 farms in the agro-pastoral area.

## 2.6. Identification of risk factors for high within-farm prevalence

### 2.6.1. Univariable analysis

Zero-inflated binomial regression analysis was performed due to the absence of sero-positive animals at 7 farms, selecting within-farm prevalence as the response variable and items in the questionnaire as explanatory variables. The statistical power was calculated at an effect size of 0.35 and a significance level of 0.1 using the pwr package (Champely, 2020) in R. The effect size was calculated to detect a difference of within-farm prevalence of 10 % between exposed and non-exposed group.

### 2.6.2. Multivariable analysis

Based on the results of the univariable analysis and findings in the previous study (Asakura et al., 2018a), a causal diagram was constructed for brucellosis within-herd prevalence using DAGitty v3.0 (Textor, 2020).

Zero-inflated binomial multivariable regression was conducted using data for the 17 farms, selecting within-herd prevalence as the outcome variable and the variables identified in the causal web, including potential confounders, as explanatory variables. Stepwise backward and forward model simplification was performed, retaining potential confounders identified in the causal web, by comparing the deviance of models using Akaike's Information Criterion. In addition, the within-herd prevalence and 90 % credible/confidence intervals predicted were visually compared between the selected model and the observed data. The within-herd prevalence and the credible interval of bovine brucellosis in each of 17 farms were predicted using the estimates and standard normal distributions adjusted by the standard errors of explanatory variables. Means of 30 predicted values were simulated for

1000 iterations, and transformed into proportions. The median within-herd prevalence and confidence interval were also calculated using  $qbeta(s+1, n-s+1)$  function, where the number of sero-positive animals in a herd is  $s$ , and the number of animals tested is  $n$ .

## 2.7. Infectious disease modelling

The analysis of sero-prevalence stratified by age can provide insight on the history and mode of circulation of a pathogen. Age-specific seroprevalence was calculated using 449 samples from 0- to 14-year-old cows at 10 *Brucella*-infected farms, as all the seven cows with 15 years old and over were sero-negative. The FOI, defined as the rate of infection among susceptible animals by age, was then fitted to this age-specific sero-prevalence using a serocatalytic model: a simplified version of an age-structured SI (Susceptible-Infectious) model. The basic form of the mathematical infectious disease model is the Kermack and McKendrick SIR (Susceptible-Infectious-Recovered) model (Kermack and Mckendrick, 1927); however, in our case, infectious and recovered states were not distinguishable based on the sero-diagnosis results. According to the literature, infected animals can harbor *Brucella* in the body for a long period (Poester et al., 2013). In the present study, a simplified age-structured SI model was used, regarding sero-negative animals as susceptible and sero-positive as infectious. In the model, the new infection rate is defined as the product of FOI and the number of susceptible animals.

In total, 12 different FOI scenarios were prepared, such as age-independent (constant) FOI, and increased or decreased risk in certain age (see Table 5 for more details), and compared using deviance information criteria (DIC). Intervention models assume that risk of infection was suppressed during the period of intervention, while outbreak models assume that one or two outbreaks occurred in the region at some point. Lower DIC values are indicative of a better adequacy to the data, and DIC difference of more than 5 are considered substantial. The FOI and DIC were determined using the Rsero package (Hoze, 2020), where Markov Chain Monte Carlo (MCMC) simulation is performed based on rstan software (Stan Development Team, 2016). To select the best model, 5000 iterations with 4 chains for each model were carried out. For the selected model, further iterations are added up to 40,000.

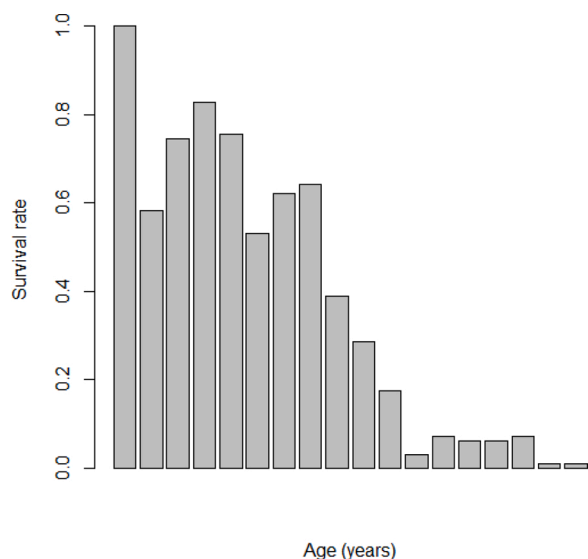
The basic reproduction number ( $R_0$ )—the average number of secondary cases caused by a single infected animal in a totally susceptible population—was calculated as follows. The average life expectancy of a cow ( $1/\mu$ ) was calculated using Eq. 4:

$$1/\mu = \sum_{x=0}^{\infty} l_x \quad (4)$$

where  $l_x$  represents the survival rate by age ( $x$ ), calculated as the number of animals at each age divided by the number of animals at age 0. For this calculation, the data from 673 cattle at all studied farms were used (Fig. 1). Although the average life expectancy is generally calculated using survival and mortality data, it was estimated using the limited data. For an individual of age  $a$ , the standard SIR model predicts that the probability that an individual is still susceptible is given by  $S(a) = \exp(-a\mu(R_0 - 1))$  under some simplifying assumptions (Capai et al., 2020). Since the numbers of the susceptible and infectious are binomially distributed, the likelihood function of these numbers was obtained as a function of  $R_0$ .  $R_0$  was then inferred as the value that maximized the logarithm of this likelihood function (Eq. 5):

$$\log L(R_0) = \sum_{i=0}^n \log(\exp(-a_i\mu(R_0 - 1))) + \sum_{i=0}^m \log(1 - \exp(-b_i\mu(R_0 - 1))) \quad (5)$$

where  $n$  ( $= 645$ ) is the number of susceptible animals (sero-negative) of age  $a_1, \dots, a_n$ , and  $m$  ( $= 28$ ) is the number of sero-positive animals (ages  $b_1, \dots, b_m$ ) for all farms (Keeling and Rohani, 2008). This estimation was



**Fig. 1.** Probability of survival at age 0 to 20 years, with age 0 having a probability of survival of 1.0 among cattle populations in agro-pastoral areas of Mvomero, Tanzania (n = 673).

carried out for the cattle population at the infected farms as well. For all statistical analyses, R version 4.0.2 (R Core Team, 2020) was used.

### 3. Results

#### 3.1. Descriptive epidemiology

Of 28 sero-positive animals in 10 of 17 farms, 27 were female. The mean ages of male and female animals were 1.7 years (n = 163) and 5.3 years (n = 510), respectively. The age of the infected bull and mean age of infected female cows were 3 years and 7.0 years, respectively. Table 1 shows the age-specific sero-prevalence of cows at the 10 infected farms. There were no sero-positive or aborted cattle in the youngest age group (0–2 years old), and 7 of 117 (6.0 %) had an experience of parity. The age-specific prevalence increased with age (slope of logit = 1.38, se = 0.38,  $p < 0.01$ ). Infected farms were widely distributed across the study areas, and the herd size ranged between 16 and 460 animals (Fig. 2). The 5 northern-most herds were located in mountainous areas, and the herd sizes were relatively small (Fig. 2a). Although there were no communal grazing areas in the mountainous areas, one farm had a high within-herd prevalence (Fig. 2b, 16.7 %). The other herds were located in plain and hilly areas, and the large herds were located far from main roads (Fig. 2a). No association between prevalence and geographical locations was observed (Fig. 2b).

Table 2 shows the characteristics of the 17 farms studied. The sale and purchase of animals at livestock markets is common in these areas, according to the farm owners (personal communications); however, 52.9 % of farms purchased animals directly from other farms. A history of abortion was also common (82.4 %, 14/17 farms).

There was no significant difference in the relationship between brucellosis sero-positivity and history of abortion in younger age group

**Table 1**  
Age-specific brucellosis prevalence and number of animals studied.

Age (years)	Positive (n)	Total no. of animals	Prevalence (%) (95 % confidence interval)
0–2	0	122	0.0 (0.0–3.0)
3–5	12	159	7.5 (4.0–12.8)
6–8	9	116	7.8 (3.6–14.2)
9–11	4	37	10.8 (3.0–25.4)
12–14	3	15	20.0 (4.3–30.0)

(OR = 11.50 [95 % CI 0.31–184.34]) or older age group (OR = 2.26 [95 % CI: 0.68–6.46]), and the ORs were not significantly different between two groups, according to the test of homogeneity ( $\chi^2 = 1.25$ ,  $df = 1$ ,  $p = 0.26$ ). The crude and adjusted ORs were 3.02 (95 % CI 1.01–7.79) and 2.64 (95 % CI 0.92–7.55), respectively. The rate of change in the ORs was 12.6 %, and the crude OR was adopted; there was a significant relationship between *Brucella* sero-positivity and history of abortion, regardless of age group. Moreover, analysis of covariance comparing the relationships between age and the number of parities, between *Brucella* sero-positive (slope = 0.37) and sero-negative cows (slope = 0.64), showed a degraded reproduction performance by infection with brucellosis (difference of slopes = -0.27, se = 0.1,  $p = 0.01$ , and 42.2 % reduction, Fig. 3).

#### 3.2. Identification of risk factors for high within-herd prevalence

##### 3.2.1. Univariable analysis

Table 3 shows the results of the univariable analysis. Grazing is known to be a major risk factor for brucellosis (Makita et al., 2011), and cross-species transmission of brucellosis between cattle and goats has been reported (Muendo et al., 2012). However, these factors were not examined, because all of the studied farms allowed cattle to graze, and only one of the farms did not raise goats. The effect of keeping infected bulls was also not examined, as there was only one infected bull among all of the farms studied; the within-herd prevalence was 2.0 % at this farm. One significant factor was found to be associated with higher within-herd prevalence of bovine brucellosis: direct purchase of cattle from other farms ( $p = 0.01$ ). Variables with  $p$  values  $< 0.2$  included cattle herded with sheep, visiting animal markets, and farms located in a mountainous area. Although the number of farms was small (n = 17), the study's power was sufficient (0.80).

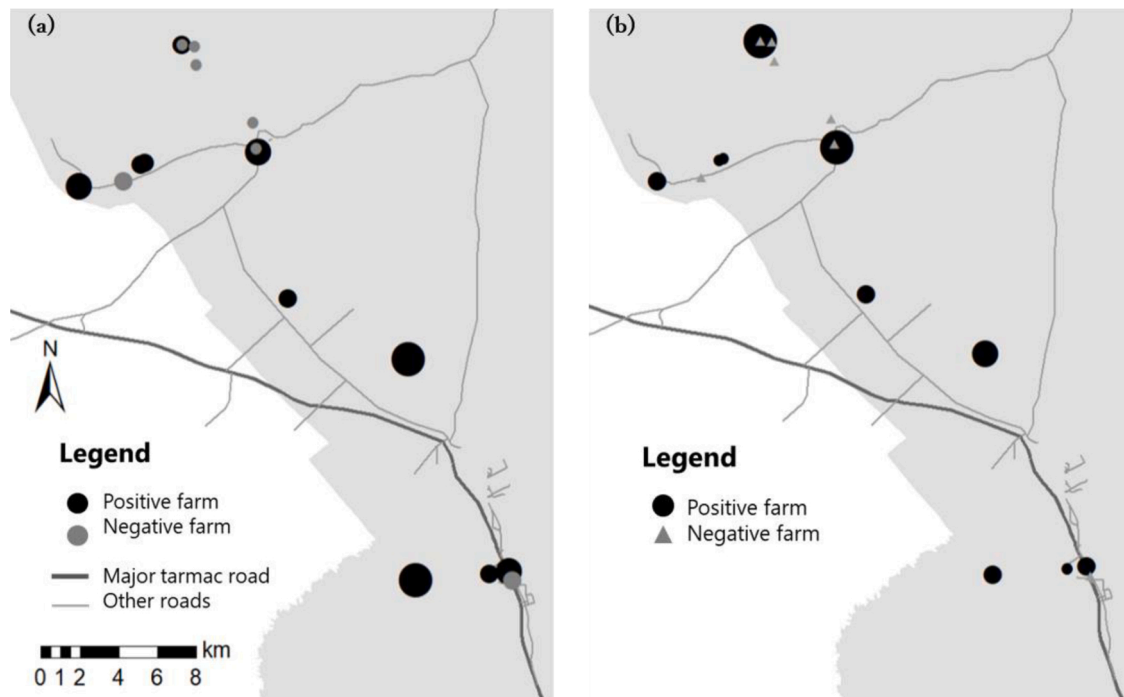
##### 3.2.2. Multivariable analysis

Fig. 4 shows the causal web constructed based on the results of the univariable analysis, our previous report (Makita et al., 2011), and potential confounders. Maasai tribe and herd size were chosen as potential confounders, as traditional cattle herding involving a large number of animals, including sheep and goats, has been associated with higher brucellosis prevalence (Asakura et al., 2018a). In the multivariable analysis, grazing, keeping goats, and keeping infected bulls were excluded from the analysis for the same reason explained for the univariable analysis. In addition, cattle herded with sheep was eliminated as an exposure factor, as it was considered to be a characteristic of the Maasai tribe rather than a factor influencing within-herd brucellosis prevalence in cattle.

The final multivariable model included direct purchase of cattle from other farms (direct purchase not at livestock markets,  $p = 0.01$ ) and farm owners visiting animal markets ( $p = 0.01$ ) (Table 4). The median within-herd prevalence and 90 % credible intervals of 17 studied farms predicted by the selected multivariable model and beta distribution using observed data generally overlapped (Fig. S1), and the model was validated.

#### 3.3. Infectious disease model

Table 5 shows descriptions and DIC of the 12 models prepared. Besides the model of independently estimated annual FOI, all chosen models had similar low DIC values and were adequate to explain the data. Fig. 5 shows the age-specific FOI with the 95 % credible interval for five of the selected different epidemiological models. The simplest model, the constant model, was adopted because there had been no previous interventions or notable outbreaks such as abortion storm in the study area, and there was no significant difference in DIC value between the models. The annual probability of infection was estimated at 1.4 % (95 % credible interval 1.0–2.0%) in the constant model. Fig. 6 shows observed and estimated age-specific sero-prevalence values



**Fig. 2.** Maps showing the distribution of *Brucella* sero-positive and -negative herds studied in the Mvomero district (shaded gray): (a) herd size is indicated by four categories based on the number of cattle (16-30, 31-80, 91-130, and 130-460); and (b) sero-prevalence indicated by four categories (<2.0 %, 2.0-6.0 %, 6.0-10.0 %, and >10.0 %).

**Table 2**  
Characteristics of farms studied (n = 17).

Category	Number of farms	Percentage
Cattle herded with sheep	11	64.7
Cattle herded with goats	16	94.1
History of bought-in cattle	10	58.8
Direct purchase of cattle from other farms	9	52.9
Owner visits animal markets	12	70.6
Contact with wild animals	4	23.5
History of abortion of cattle	14	82.4
Receiving veterinary services	11	64.7
Maasai tribe	5	29.4
Located in mountainous area	5	29.4

according to the constant FOI model.

The average life expectancy and  $R_0$  were estimated at 6.9 years and 1.07, respectively. The  $R_0$  calculated for the 10 infected farms was 1.10.

#### 4. Discussion

The present study was conducted to quantitatively assess the risk factors of within-herd highly efficient spread and infection dynamics of bovine brucellosis in agro-pastoral areas of Tanzania. Three approaches were used to achieve the objective, namely risk factor analysis for within-farm prevalence, age-specific FOI, and estimation of  $R_0$ ; a combination of these approaches provided valuable information.

In the risk factor analysis, direct purchase of cattle from other farms and visiting animal markets by farm owners were identified as risk factors associated with higher within-farm prevalence. A previous study conducted in the same areas but using a separate survey found that farmers unhesitatingly sold cattle with a history of abortion (Asakura et al., 2018b). Farmers had little knowledge about brucellosis, and thus, they may not consider abortion as being related to any infectious disease, probably because of the endemic nature of brucellosis (brucellosis-induced abortion can happen in any animal herd). However, the significance of these risk factors (i.e., introduction of animals and

visiting markets by farm owners) suggests that introduction of brucellosis-infected cows into a naïve herd could result in a higher brucellosis prevalence or that frequent buying-in of infected cattle could contribute to the maintenance of a high prevalence at such farms. The location of farms in a mountainous area was assumed to be a preventative factor for high within-herd prevalence of brucellosis because herds in such areas should have less contact with other herds, as compared with herds grazing in plain areas, but this was not the case. Contact with wild animals was also not a significant risk factor in the present study; infected cows thus appear to play a more significant role than infected wildlife in the infection dynamics. In this regard, keeping goats (both *B. melitensis* and *B. abortus* can infect goats (Muendo et al., 2012)) and infected bulls in a herd are matters of interests; however, these animals were not investigated in the present study, as all of the farms kept goats, and only one bull was sero-positive. Further research is needed to elucidate the infection dynamics between livestock animals (including small ruminants) and humans with the strains isolated in these areas (Godfroid et al., 2013). One reason for the lower prevalence of brucellosis among male animals might be the lower mean age of males compared with females; aged bulls may be sold for beef. This difference between sexes is consistent with a previous report (Junaidu et al., 2011). In contrast, another study reported either a significantly higher prevalence in males than females or no difference between sexes (Mai et al., 2012).

In the FOI analysis, a model with a constant FOI was selected, suggesting that the most common source of infection with *Brucella* in cattle is ingestion of contaminated materials, such as an aborted fetus or placenta during pasture grazing. Grazing was conducted at all of the studied farms but not included in the analyses; however, previous studies have examined this obvious risk factor for brucellosis transmission (Makita et al., 2011).

The  $R_0$  estimated in the present study was slightly greater than 1.0, which was consistent with the endemic nature of brucellosis (Keeling and Rohani, 2008). By comparison, the  $R_0$  for the cattle populations at infected farms was 1.10, which was not markedly higher than the overall  $R_0$ , 1.07, suggesting limited heterogeneity in disease transmission

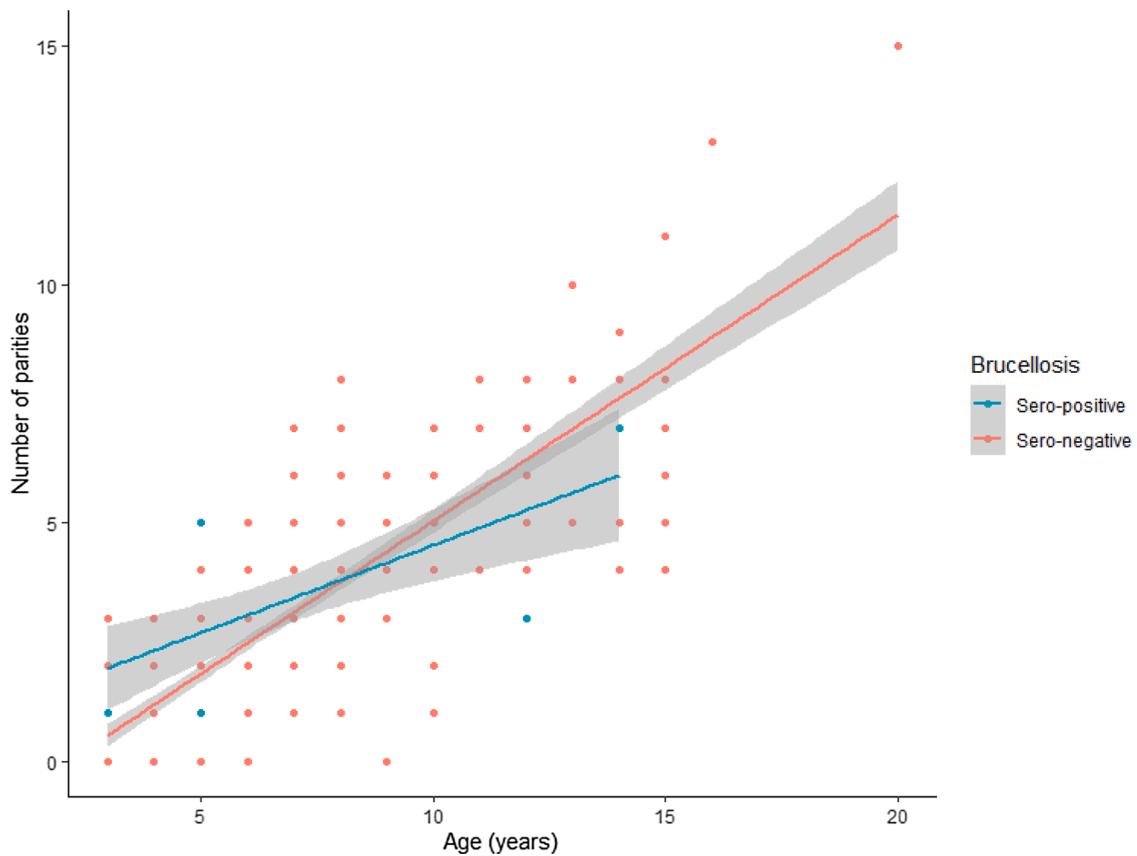


Fig. 3. Analysis of covariance comparing the relationships between age and the number of parities: between *Brucella* sero-positive and sero-negative cows showing degraded reproduction performance by infection with brucellosis (difference of slopes = -0.27, se = 0.10,  $p = 0.01$ ).

Table 3

Univariable analysis results showing the median brucellosis within-herd crude prevalence and range and the number of farms giving true and false responses for the variables assessed (n = 17).

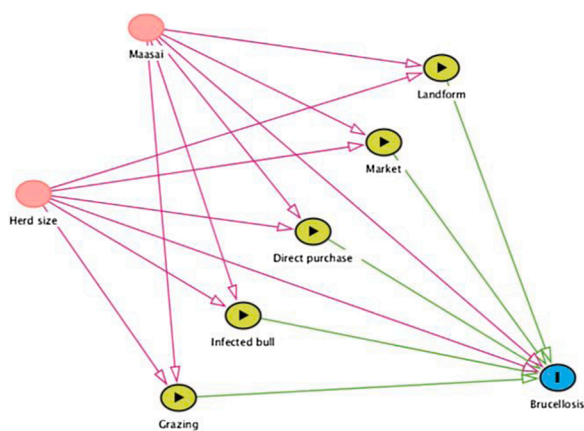
Variables	True			False			p-value
	Prevalence (%)		Number of farms	Prevalence (%)		Number of farms	
	Median	Range		Median	Range		
Cattle herded with sheep	2.0	0–18.0	6	0	0–16.7	11	0.13
History of bought-in cattle	1.0	0–16.7	10	4.0	0–18.0	7	0.29
Direct purchase of cattle from other farms	4.0	0–18.0	8	2.0	0–6.0	9	0.01
Owner visits animal markets	3.0	0–18.0	5	0	0–16.7	12	0.09
Contact with wildlife	3.0	0–10.0	4	2.0	0–18.0	13	0.82
History of abortion of cattle	2.0	0–18.0	14	0	0–6.0	3	0.66
Receiving veterinary services	0	0–18.0	6	4.0	0–10.0	11	0.69
Maasai tribe	4.0	2.0–10.0	5	0	0–18.0	12	0.80
Located in mountainous area	0	0–16.7	5	3.0	0–18.0	12	0.15

between infected and non-infected farms. These data might also suggest that there is regular contact between infected animals and animals at non-infected farms. For future intervention planning discussions, methods for the control of brucellosis over a wide area, including non-infected farms, should be considered.

As no records of the time of abortion occurrence were available, leaving only data on cumulative occurrence, MH analyses were performed in the present study. The crude OR chosen suggested a significant relationship between brucellosis and history of abortion. Under field conditions, in which access to facilities for diagnosing animal brucellosis is limited, symptom-based monitoring using a history of abortion can play a key role in identifying high-risk herds. The analysis of covariance for the reproduction performance suggested that infection with brucellosis not only causes abortion in the first pregnancy after infected, but also significant reduction of reproduction performance

(42.2%), which has a long-term effect on the herd productivity.

No sero-positive cattle under 2 years of age were included in the present study. A previous study did not detect antibodies in calves despite vertical transmission, thereby maintaining disease transmission (Wilesmith, 1978). A number of studies have examined the immune response to *Brucella* infection (Golding et al., 2001; Rafiei et al., 2006), and a review has been published (Sung and Yoo, 2014). None of 0–2 years old sero-negative cows had a history of abortion in this study, although they should have included vertically or horizontally transmitted brucellosis infected cows. Such silently infected cows may exhibit sero-positivity after causing abortion. However, why young animals with brucellosis do not test sero-positive remains unclear. In the future, development of a diagnostic tool for infected calves and heifers could enhance our understanding of the contribution of vertical transmission to brucellosis infection dynamics and thus aid in planning an eradication



**Fig. 4.** Causal diagram for factors associated with within-farm bovine brucellosis prevalence. Explanatory variables include landform where cattle herd is located, owner visitation of animal markets, direct purchase of cattle from other farms, infected bulls, and grazing. Potential confounders are Maasai tribe and herd size. Infected bull was shown as a logical risk factor but was not included in the analysis because of too few animals in the dataset.

**Table 4**  
Final multivariable model for brucellosis within-herd prevalence, with potential confounders.

Variable	Estimate	Standard error	p-value
Intercept	-6.72	1.10	<0.01
Direct purchase of cattle from other farms	3.18	1.11	0.01
Owner visits animal markets	1.85	0.63	0.01
Maasai tribe	-0.66	0.62	0.31
Herd size	-0.001	0.002	0.83

**Table 5**  
Descriptions and DIC of the 12 models prepared. Models with background infection include the effect of individuals testing sero-positive in response to similar antigens.

Model	Description	DIC
1	Constant model	194.6
2	Intervention model: two constant phases	194.8
3	Intervention model: three constant phases	194.8
4	One outbreak	193.3
5	Two outbreaks	194.3
6	One outbreak and constant annual FOI	196.4
7	Independent annual FOI	203.2
8	Constant model with background infection	196.6
9	Intervention model: two constant phases with background infection	196.5
10	Intervention model: three constant phases with background infection	196.7
11	One outbreak with background infection	197.7
12	Two outbreaks with background infection	197.6

strategy.

Another concern in the analysis related to sero-positivity was that all the seven cows with 15 years of age or over were sero-negative, though they accounted for only 1.5 % of the animals sampled. In previous studies, the duration of immunity induced by the S19 vaccine in cattle vaccinated as calves was proven to be quite long (fifth pregnancy (McDiarmid, 1957)), spanning almost the entire productive life of the animals (Dorneles et al., 2015). However, these previous studies focused on commercial farms and did not include cows as old as those in our dataset (>15 years old). After infection with *Brucella*, humoral components remove microorganisms via opsonization and bactericidal mechanisms, including antigen-specific antibody production and cellular

components, including macrophages, dendritic cells, neutrophils, and innate T cells, which kill both invading microorganisms and infected cells via phagocytic or cytotoxic activity. However, IL-10 produced by T and B cells suppresses the function of IFN- $\gamma$ -activated macrophages, which increases susceptibility to infection with *Brucella* (Fernandes et al., 1995; Zhan and Cheers, 1995; Ko and Splitter, 2003). Although antigen-specific antibodies limit the number of extracellular *Brucella*, the humoral immune response is not protective during the intracellular phase of *Brucella* infection (Sung and Yoo, 2014). Although the immunologic responses that affect the removal and survival of *Brucella* are known, again, whether the absence of antibodies in very old cows suggests a true absence of intracellular *Brucella* is not known. In our analysis, data for cows >15 years old were removed. Considering the limited number of old animals in a herd, even if such old cows were truly infected, they may not be used for reproduction, and thus, their contribution to the infection dynamics should be limited.

The small sample size was a limitation to our study, as the primary purpose of the study was to compare brucellosis prevalence between urban and agro-pastoral areas (Asakura et al., 2018a). However, the power analysis demonstrated that the study was valid. The generalizability of this study may need future confirmation from studies in different agro-pastoral areas of Tanzania.

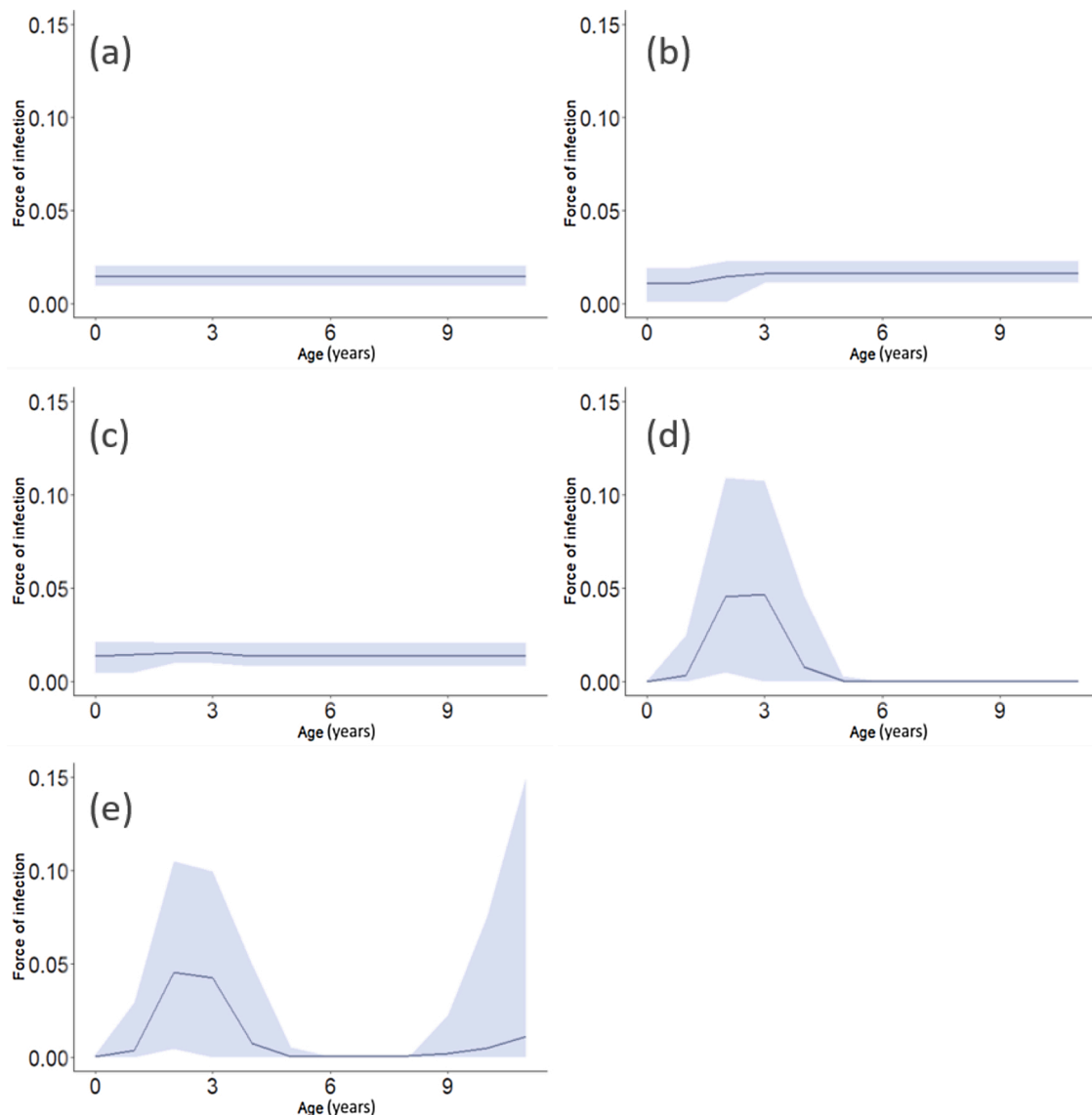
Our results suggest that extensive vaccination of animals would be an effective strategy for preventing brucellosis, considering the link between infection and environmental exposure to *Brucella*-contaminated aborted materials. Such a strategy may be feasible given the high willingness of farmers to pay for calf vaccination reported previously (Asakura et al., 2018b). However, because cattle are often kept until they are older than 15 years, long-term continuation of vaccine programs would be needed if only calves are targeted. Therefore, in parallel with calf vaccination, the number of infected cattle at infected farms must be reduced. A previous study proposed selling off cows that experienced abortion for slaughtering for food purposes, which can secure additional income for the farmer (Asakura et al., 2018b).

Brucellosis is currently a tragedy of the commons making livestock farmers and human patients suffer (Hardin, 1968) as nobody is willing to invest in its control. Tanzania could become free of brucellosis if a national brucellosis mass vaccination campaign is conducted, which requires to find a way of societal cooperation (Ostrom, 2015). Therefore, studies for designing vaccination campaign and identifying the incentives for farmers to participate in it are required.

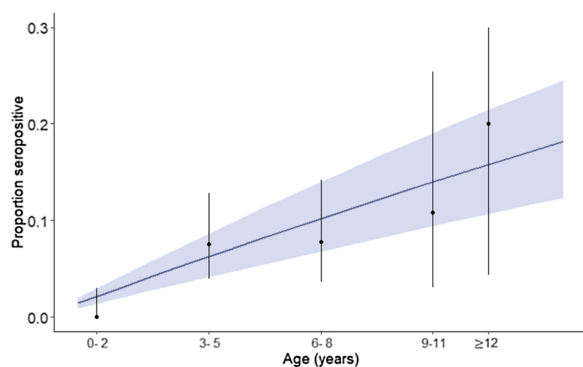
In the future, between-herd networks that take into account cattle and small ruminants in addition to livestock marketing should be investigated to obtain a better understanding of brucellosis transmission. Moreover, the multiple economic benefits of vaccination and other intervention strategies in Tanzania on livestock production and human health (Roth et al., 2003) should be studied to identify favorable brucellosis control practices. In fact, we have conducted a serological survey for humans in the study area, finding high sero-prevalence, 33.3 % (44/132), and a negative association in sero-positivity between farmers and the milk from their animals; the prevalence in cattle farming families with sero-negative milk results (18/31, 58.1 %) was significantly higher than those with sero-positive milk results (1/22, 4.5 %,  $p < 0.01$ ) (Asakura et al., 2020), suggesting the needs of studying infection source for humans, which may elucidate the magnitude of positive public health impacts expected from interventions in animals. Such studies would also be useful for many other countries in which brucellosis is endemic.

### 5. Conclusion

The results of this study suggest that the most common mode of brucellosis transmission among cattle in agro-pastoral areas of Mvomero, Tanzania, may be contact during grazing with *Brucella*-contaminated abortion tissues present on pastures. At infected farms, introducing infected cattle directly from other herds or from livestock



**Fig. 5.** Age-specific force of infection with 95 % credible intervals in different FOI model scenarios: (a) constant (age-independent) model, (b) intervention model (once), (c) intervention model (twice), (d) outbreak model (once), and (e) outbreak model (twice).



**Fig. 6.** Observed age-specific sero-prevalence (points) and 95 % confidence interval (vertical lines) and age-specific sero-prevalence (curve solid line) and 95 % credible interval (blue area) estimated using based on the constant FOI model.

markets was identified as a significant risk factor for highly efficient transmission within the farm. Bovine brucellosis exhibited limited heterogeneity between infected and non-infected herds. Future studies should examine the dynamics of between-herd transmission to plan effective targeted intervention strategies, most likely involving vaccination of animals.

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**Ethical considerations**

This study was approved by the Ethical Committee of the Graduate School of Dairy Science, Rakuno Gakuen University (approval number



16-3). Permission to conduct the study in Morogoro municipality and Mvomero district was sought and granted by municipal and district executive officers (reference numbers E.10/MMC-138/185 and MVDC/C.80/4 VOLII/126, respectively). Verbal informed consent was obtained from each participant after livestock field officers explained the aim of the study.

### Declaration of Competing Interest

The authors report no declarations of interest.

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### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prevetmed.2021.10.5425>.

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