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

2  
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13  
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### 15 16    **Abstract**

17    Brucellosis is endemic in Tanzania. A cross-sectional study was conducted at 17 cattle farms in  
18    agro-pastoral areas in Tanzania to identify risk factors associated with the within-farm prevalence of  
19    bovine brucellosis and to quantitatively assess the infection dynamics through disease modelling.  
20    Cattle blood sampling and interviews with farmers using a structured questionnaire were conducted.  
21    A total of 673 serum samples were screened using the Rose-Bengal plate test (RBPT), and sero-  
22    positivity of RBPT-positive samples was confirmed using a competitive enzyme-linked  
23    immunosorbent assay. Zero-inflated binomial regression was performed for univariable and  
24    multivariable risk factor analyses of within-farm prevalence. Several susceptible-infectious (SI)  
25    models were compared based on deviance information criteria, and age-dependent force of infection  
26    (FOI) was measured using age-specific prevalence data for the 10 infection-positive farms. Using the  
27    diagnoses of cows on the 17 farms, the basic reproduction number,  $R_0$ , was also calculated. The  
28    farm-level prevalence and animal-level adjusted prevalence were 58.8% (10/17, 95% confidence

29 interval: 33.5-80.6%) and 7.0% (28/673, 95% credible interval: 5.7-8.4%), respectively. The risk  
30 factor for high within-farm prevalence was introduction of cattle from other herds. A mathematical  
31 model with constant FOI showed the annual probability of infection as 1.4% (95% credible interval:  
32 1.0%-2.0%). The  $R_0$  was 1.07. The constant FOI could have been due to the predominant mode of  
33 infection being transmission of *Brucella* from contaminated aborted materials during grazing. Direct  
34 purchase of infected cattle could facilitate efficient transmission between susceptible animals  
35 through abortion.

36

## 37 **Keywords**

38 Brucellosis; infectious disease modelling; force of infection; basic reproductive number

39

## 40 **1. Introduction**

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

46 Bovine brucellosis causes significant economic losses for farmers due to decreased production  
47 resulting from loss of animals through abortion and due to infertility, weak offspring, weight loss,  
48 and reduced milk yield (McDermott and Arimi, 2002). *Brucella* is usually transmitted between  
49 animals via contact with contaminated aborted tissues and vaginal discharges. Vertical transmission  
50 via the uterus or through ingestion of maternal milk and sexual transmission may also play a role in  
51 the spread of *Brucella* (Corbel et al., 2006).

52 In humans, the clinical symptoms of brucellosis include undulant fever, joint pain, night sweats,  
53 headache, and extreme weakness (Dean et al., 2012). More severe symptoms, such as endocarditis,  
54 meningitis, arthritis, and osteomyelitis, have also been reported (Xavier et al., 2010). Humans can  
55 become infected with *Brucella* through the consumption of uncooked meat or unpasteurized dairy

56 products obtained from infected animals or by direct contact with infected animals (Sung and Yoo,  
57 2014). Individuals in certain occupations, such as veterinarians, butchers, abattoir workers, meat  
58 inspectors, and livestock farmers, are at a greater risk of contracting brucellosis (Kunda et al., 2007)

59 In the pastoral and agro-pastoral farming systems of Tanzania, contact between wildlife,  
60 livestock and humans is common (Assenga et al., 2015). Outbreaks of brucellosis have been  
61 documented in various regions and zones of Tanzania since 1927, when an outbreak of abortion was  
62 reported in the Arusha region (Shirima, 2005). The prevalence of bovine brucellosis at the animal  
63 level is reportedly 9.3% in Mbeya region (Sagamiko et al., 2018), 5.3% in Tanga region (Swai and  
64 Schoonman, 2010), 6.2% in Arusha and Manyara regions (Kunda et al., 2007), and 6.8% in the  
65 Katavi-Rukwa ecosystem (Assenga et al., 2015). In Mvomero district, the farm-level prevalence is  
66 52.9% (9/17, 95% confidence interval [CI] 28.5-76.1%) and animal-level adjusted prevalence is  
67 7.0% (28/673, 95% credible interval: 5.7-8.4%), respectively, and the animal-level risk factors for  
68 brucellosis include history of abortion and older age (Asakura et al., 2018a). In this previous study  
69 (Asakura et al., 2018a) which used the same dataset with the present study, the number of sero-  
70 positive farms was reported to be nine by an error (farm level prevalence was 58.8%, 95%CI 33.5-  
71 80.6%, correctly), but the present study confirms that 10 farms housed sero-positive animals. While  
72 risk factors for brucellosis in animals are generally known well, reports of factors associated with  
73 higher within-herd prevalence are limited. The present study adds knowledge on this.

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

81 Brucellosis has been successfully controlled or eliminated in livestock populations in many  
82 high-income countries (McDermott et al., 2013). However, in developing countries, the control of  
83 brucellosis in cattle in an endemic situation is not straightforward, as mass vaccination and

84 ‘stamping-out’ programs depend solely on public resources and are typically impracticable or not  
85 sustainable due to cost (Makita et al., 2010). Considering the limited resources available and  
86 endemic nature of brucellosis in Tanzania, the willingness of farmers to pay for *Brucella*  
87 vaccinations as a community-based intervention was investigated in a previous study, which  
88 reported that 89.5% of farmers expressed a willingness to pay for vaccinations targeting calves  
89 (Asakura et al., 2018b).

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

97

## 98 **2. Materials and methods**

### 99 **2.1. Field survey**

100 A cross-sectional study involving cattle blood sampling and a structured interview using a  
101 questionnaire was conducted between August and December 2015 in Morogoro region of Tanzania.  
102 The questionnaire was administrated via in-person interviews to collect information regarding the  
103 farm owner, farm characteristics, animals kept on the farm, purchase of animals, contact with  
104 wildlife, use of veterinary services, and animals sampled, including any history of abortion. More  
105 detailed information can be found in our previously published article (Asakura et al., 2018a). In this  
106 previous study, the formula for a comparison of two proportions was used for the calculation of the  
107 animal-level sample size, in order to compare brucellosis prevalence between urban areas of  
108 Morogoro municipality and agro-pastoral areas of Mvomero district. A total of 667 and 673 cattle  
109 from 106 and 17 farms were studied in the respective areas. At farms with  $\leq 50$  eligible cattle, all  
110 cattle older than 3 months were selected to avoid any effect of maternal antibodies on new-born

111 calves. For larger farms, a maximum of 50 cattle were sampled to reflect the age structure of the  
112 herd. In this previous study (Asakura et al., 2018a), only one positive animal was identified in  
113 Morogoro municipality; thus, the present study targeted the animals in Mvomero district.

114

## 115 **2.2. Serological tests**

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

124

## 125 **2.3. Descriptive epidemiology**

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

130

$$131 \quad P = (P^T + Sp - 1)/(Se + Sp - 1), \quad \text{(Equation 1)}$$

132

133 where

134

$$135 \quad Se = Se_1 \times Se_2, \text{ and} \quad \text{(Equation 2)}$$

$$136 \quad Sp = 1 - \{(1 - Sp_1) \times (1 - Sp_2)\}, \quad \text{(Equation 3)}$$

137

138 where  $P$  represents the true prevalence, and  $P^T$  represents the test prevalence. As the maximum

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

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

150

#### 151 **2.4. Relationship between sero-positivity and history of abortion**

152 Odds ratios (ORs) were calculated for 3- to 14-year-old females at infected farms to determine  
153 whether there is an association between history of abortion as a potential exposure and the  
154 occurrence of brucellosis. Data for animals younger than 3 years were not used, as cows infected  
155 with brucellosis before abortion occurs often do not have antibodies (Nielsen and Duncan, 1990);  
156 indeed, no sero-positive cows between 0 and 2 years of age were identified (see Results). Brucellosis  
157 can cause abortion in cows at the first pregnancy after infection (Nielsen and Duncan, 1990).  
158 Farmers were asked about any history of abortion for animals of any age. In animals between 3 and  
159 4 years of age, when the parity number is low, examinations of any causal relationship between  
160 infection with *Brucella* and abortion can proceed without caution. However, in animals  $\geq 5$  years of  
161 age, abortion might be occurred several years ago, and in such cases, OR will not show recent  
162 relationship between brucellosis infection and abortion. Therefore, the data were stratified into two  
163 groups, one for animals between 3 and 4 years of age, and the other for animals over 5 years of age,  
164 and these data were analyzed using the Mantel-Haenszel (MH) OR. In this analysis, crude and strata-  
165 specific ORs were calculated, and a test for homogeneity was performed using Woolf's statistics  
166 (Woolf, 1955) to assess differences between strata regarding the strength of the association between

167 infection status and abortion. Finally, the crude OR, MH OR, or strata-specific OR was selected  
168 based on the results of tests for homogeneity and rate of change in MH OR compared with the crude  
169 OR  $([\text{crude OR} - \text{MH OR}]/\text{crude OR})$ .

170

## 171 **2.5. Comparison of reproduction performances of sero-positive and sero-negative cows**

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

177

## 178 **2.6. Identification of risk factors for highly efficient transmission: within-farm prevalence**

### 179 **2.6.1. Univariable analysis**

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

186

### 187 **2.6.2. Multivariable analysis**

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

191 Zero-inflated binomial multivariable regression was conducted using data for the 17 farms,  
192 selecting within-herd prevalence as the outcome variable and the variables identified in the causal  
193 web, including potential confounders, as explanatory variables. Stepwise backward and forward  
194 model simplification was performed, retaining potential confounders identified in the causal web, by

195 comparing the deviance of models using Akaike's Information Criterion. In addition, the within-herd  
196 prevalence and 90% credible/confidence intervals predicted were visually compared between the  
197 selected model and the observed data. The within-herd prevalence and the credible interval of bovine  
198 brucellosis in each of 17 farms were predicted using the estimates and standard normal distributions  
199 adjusted by the standard errors of explanatory variables. Means of 30 predicted values were  
200 simulated for 1000 iterations, and transformed into proportions. The median within-herd prevalence  
201 and confidence interval were also calculated using  $qbeta(s+1, n-s+1)$  function, where the number of  
202 sero-positive animals in a herd is  $s$ , and the number of animals tested is  $n$ .

203

## 204 **2.7. Infectious disease modelling**

205 The analysis of sero-prevalence stratified by age can provide insight on the history and mode of  
206 circulation of a pathogen. Age-specific seroprevalence was calculated using 449 samples from 0- to  
207 14-year-old cows at 10 *Brucella*-infected farms, as all the seven cows with 15 years old and over  
208 were sero-negative. The FOI, defined as the rate of infection among susceptible animals by age, was  
209 then fitted to this age-specific sero-prevalence using a serocatalytic model: a simplified version of an  
210 age-structured SI (Susceptible-Infectious) model. The basic form of the mathematical infectious  
211 disease model is the Kermack and McKendrick SIR (Susceptible-Infectious-Recovered) model  
212 (Kermack and Mckendrick, 1927); however, in our case, infectious and recovered states were not  
213 distinguishable based on the sero-diagnosis results. According to the literature, infected animals can  
214 harbor *Brucella* in the body for a long period (Poester et al., 2013). In the present study, a simplified  
215 age-structured SI model was used, regarding sero-negative animals as susceptible and sero-positive  
216 as infectious. In the model, the new infection rate is defined as the product of FOI and the number of  
217 susceptible animals.

218 In total, 12 different FOI scenarios were prepared, such as age-independent (constant) FOI, and  
219 increased or decreased risk in certain age (see Table 5 for more details), and compared using  
220 deviance information criteria (DIC). Intervention models assume that risk of infection was  
221 suppressed during the period of intervention, while outbreak models assume that one or two  
222 outbreaks occurred in the region at some point. Lower DIC values are indicative of a better adequacy

223 to the data, and DIC difference of more than 5 are considered substantial. The FOI and DIC were  
 224 determined using the Rsero package (Hoze, 2020), where Markov Chain Monte Carlo (MCMC)  
 225 simulation is performed based on rstan software (Stan Development Team, 2016). To select the best  
 226 model, 5000 iterations with 4 chains for each model were carried out. For the selected model, further  
 227 iterations are added up to 40,000.

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

231

$$232 \quad 1/\mu = \sum_{x=0}^{\infty} l_x, \quad (\text{Equation 4})$$

233

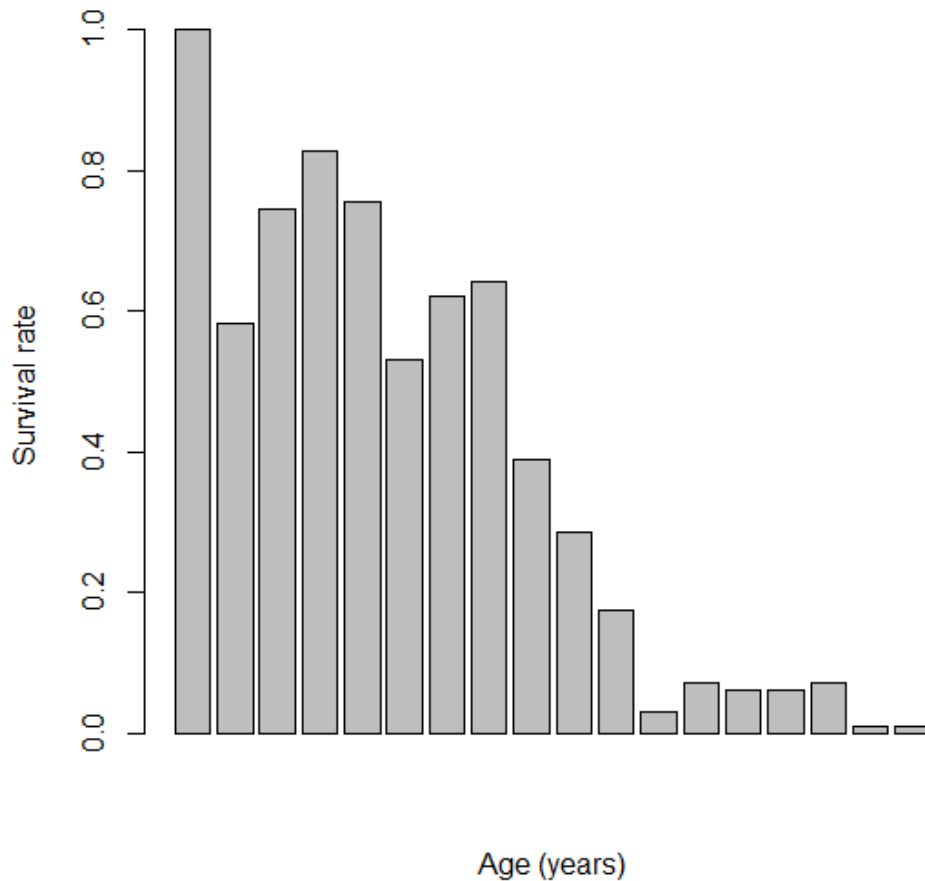
234 where  $l_x$  represents the survival rate by age ( $x$ ), calculated as the number of animals at each age  
 235 divided by the number of animals at age 0. For this calculation, the data from 673 cattle at all studied  
 236 farms were used (Figure 1). Although the average life expectancy is generally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

243

$$244 \quad \log L(R_0) = \sum_{i=0}^n \log \left( \exp(-a_i\mu(R_0 - 1)) \right) + \sum_{i=0}^m \log(1 - \exp(-b_i\mu(R_0 - 1))), \quad (\text{Equation 5})$$

245

246 where  $n$  (= 645) is the number of susceptible animals (sero-negative) of age  $a_1, \dots, a_n$ , and  $m$  (= 28)  
 247 is the number of sero-positive animals (ages  $b_1, \dots, b_m$ ) for all farms (Keeling and Rohani, 2008).  
 248 This estimation was carried out for the cattle population at the infected farms as well. For all  
 249 statistical analyses, R version 4.0.2 (R Core Team, 2020) was used.



251

252 **Figure 1.** Probability of survival at age 0 to 20 years, with age 0 having a probability of survival of  
 253 1.0 among cattle populations in agro-pastoral areas of Mvomero, Tanzania (n = 673).

254

### 255 3. Results

#### 256 3.1. Descriptive epidemiology

257 Of 28 sero-positive animals in 10 of 17 farms, 27 were female. The mean ages of male and  
 258 female animals were 1.7 years (n = 163) and 5.3 years (n = 510), respectively. The age of the  
 259 infected bull and mean age of infected female cows were 3 years and 7.0 years, respectively. Table 1  
 260 shows the age-specific sero-prevalence of cows at the 10 infected farms. There were no sero-positive  
 261 or aborted cattle in the youngest age group (0 to 2 years old), and 7 of 117 (6.0%) had an experience

262 of parity. The age-specific prevalence increased with age (slope of logit = 1.38, se = 0.38,  $p < 0.01$ ).

263 Infected farms were widely distributed across the study areas, and the herd size ranged between 16

264 and 460 animals (Figure 2). The 5 northern-most herds were located in mountainous areas, and the

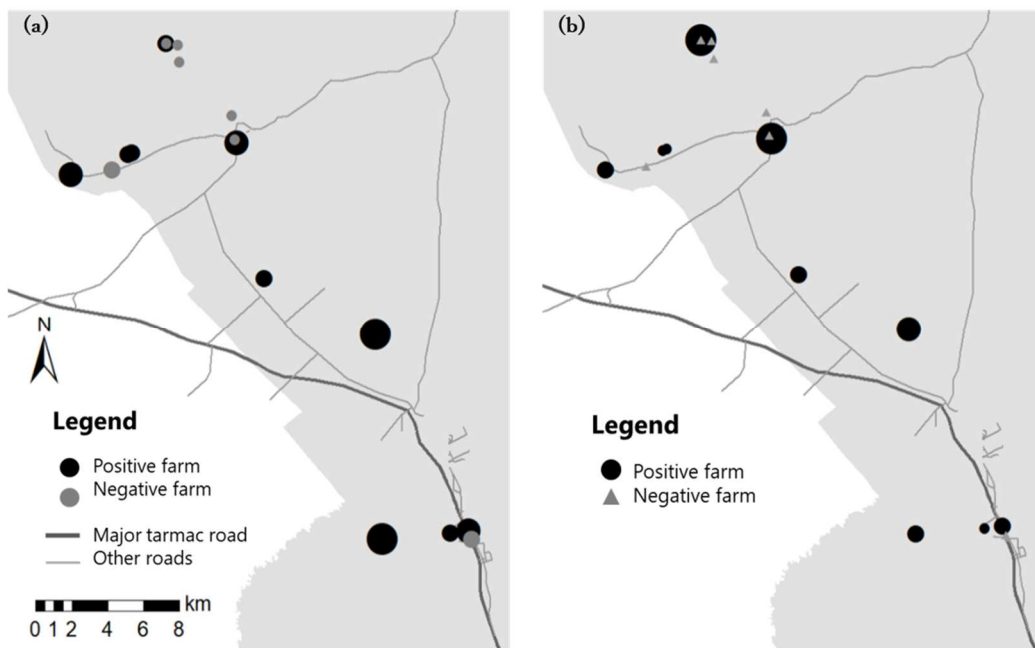
265 herd sizes were relatively small (Figure 2a). Although there were no communal grazing areas in the

266 mountainous areas, one farm had a high within-herd prevalence (Figure 2b. 16.7%). The other herds

267 were located in plain and hilly areas, and the large herds were located far from main roads (Figure

268 2a). No association between prevalence and geographical locations was observed (Figure 2b).

269



271 **Figure 2.** Maps showing the distribution of *Brucella* sero-positive and -negative herds studied in the

272 Mvomero district (shaded gray): (a) herd size is indicated by four categories based on the number of

273 cattle (16-30, 31-80, 91-130, and 130-460); and (b) sero-prevalence indicated by four categories

274 (<2.0%, 2.0-6.0%, 6.0-10.0%, and >10.0%).

275

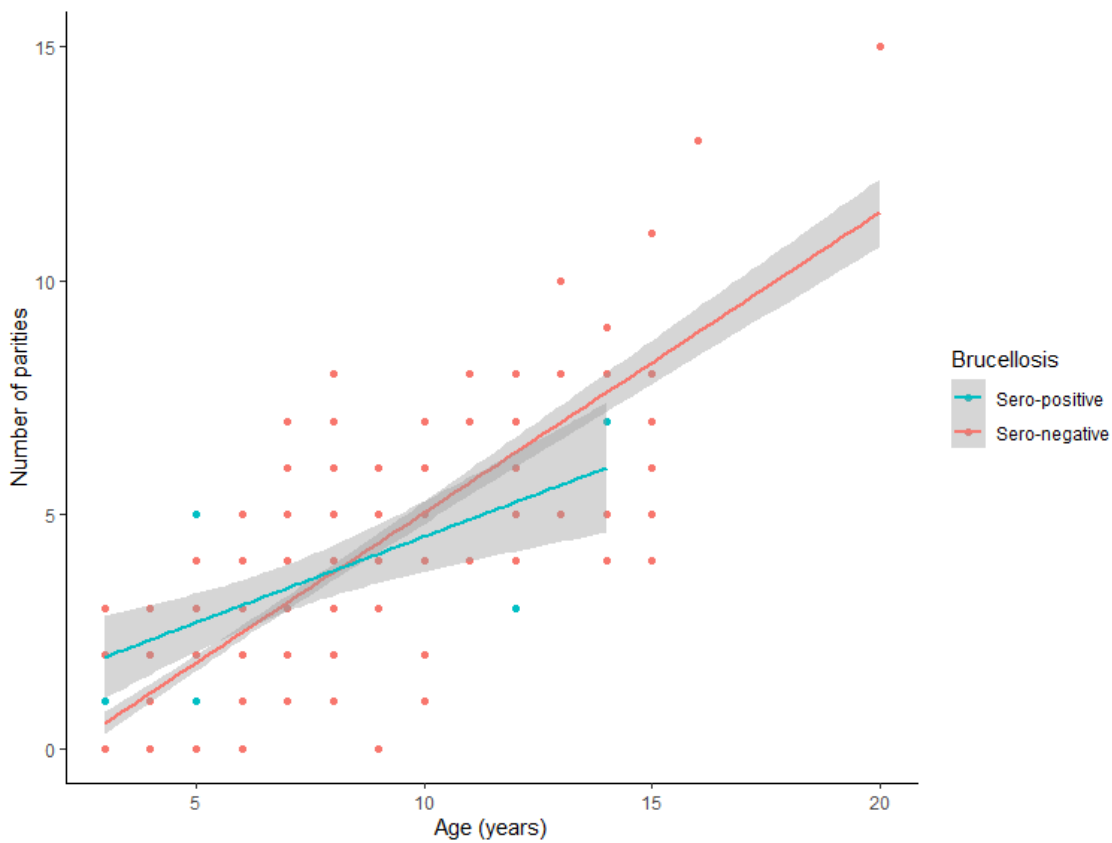
276 Table 2 shows the characteristics of the 17 farms studied. The sale and purchase of animals at

277 livestock markets is common in these areas, according to the farm owners (personal

278 communications); however, 52.9% of farms purchased animals directly from other farms. A history

279 of abortion was also common (82.4%, 14/17 farms).

280 There was no significant difference in the relationship between brucellosis sero-positivity and  
 281 history of abortion in younger age group (OR = 11.50 [95% CI 0.31-184.34]) or older age group  
 282 (OR = 2.26 [95% CI: 0.68-6.46]), and the ORs were not significantly different between two groups,  
 283 according to the test of homogeneity ( $\chi^2 = 1.25$ ,  $df = 1$ ,  $p = 0.26$ ). The crude and adjusted ORs were  
 284 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  
 285 was 12.6%, and the crude OR was adopted; there was a significant relationship between *Brucella*  
 286 sero-positivity and history of abortion, regardless of age group. Moreover, analysis of covariance  
 287 comparing the relationships between age and the number of parities, between *Brucella* sero-positive  
 288 (slope = 0.37) and sero-negative cows (slope= 0.64), showed a degraded reproduction performance  
 289 by infection with brucellosis (difference of slopes = -0.27,  $se = 0.1$ ,  $p = 0.01$ , and 42.2% reduction,  
 290 Fig. 3).  
 291



292  
 293 **Figure 3.** Analysis of covariance comparing the relationships between age and the number of  
 294 parities: between *Brucella* sero-positive and sero-negative cows showing degraded reproduction

295 performance by infection with brucellosis (difference of slopes = -0.27, se = 0.10,  $p = 0.01$ )

296

## 297 **3.2. Identification of risk factors for high transmission efficiency: within-herd prevalence**

### 298 **3.2.1. Univariable analysis**

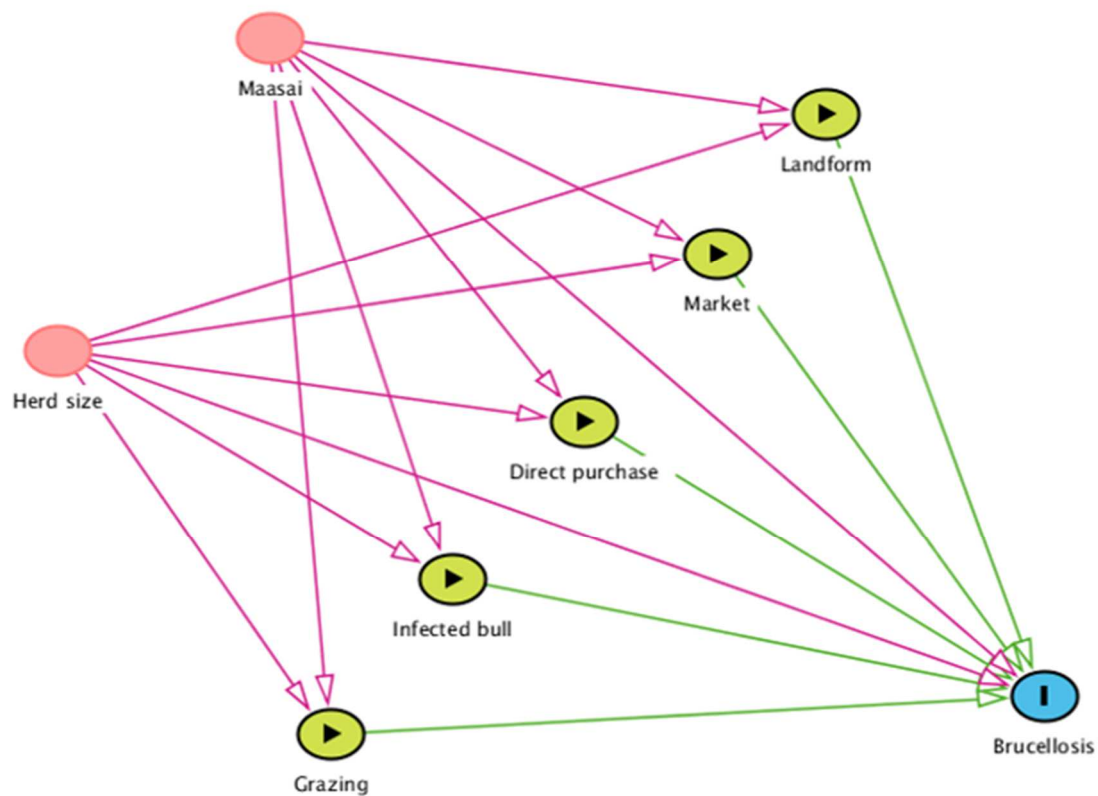
299 Table 3 shows the results of the univariable analysis. Grazing is known to be a major risk factor  
300 for brucellosis (Makita et al., 2011), and cross-species transmission of brucellosis between cattle and  
301 goats has been reported (Muendo et al., 2012). However, these factors were not examined,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

309

### 310 **3.2.2. Multivariable analysis**

311 Figure 4 shows the causal web constructed based on the results of the univariable analysis, our  
312 previous report (Makita et al., 2011), and potential confounders. Maasai tribe and herd size were  
313 chosen as potential confounders, as traditional cattle herding involving a large number of animals,  
314 including sheep and goats, has been associated with higher brucellosis prevalence (Asakura et al.,  
315 2018a). In the multivariable analysis, grazing, keeping goats, and keeping infected bulls were  
316 excluded from the analysis for the same reason explained for the univariable analysis. In addition,  
317 cattle herded with sheep was eliminated as an exposure factor, as it was considered to be a  
318 characteristic of the Maasai tribe rather than a factor influencing within-herd brucellosis prevalence  
319 in cattle.

320



321

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

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

332

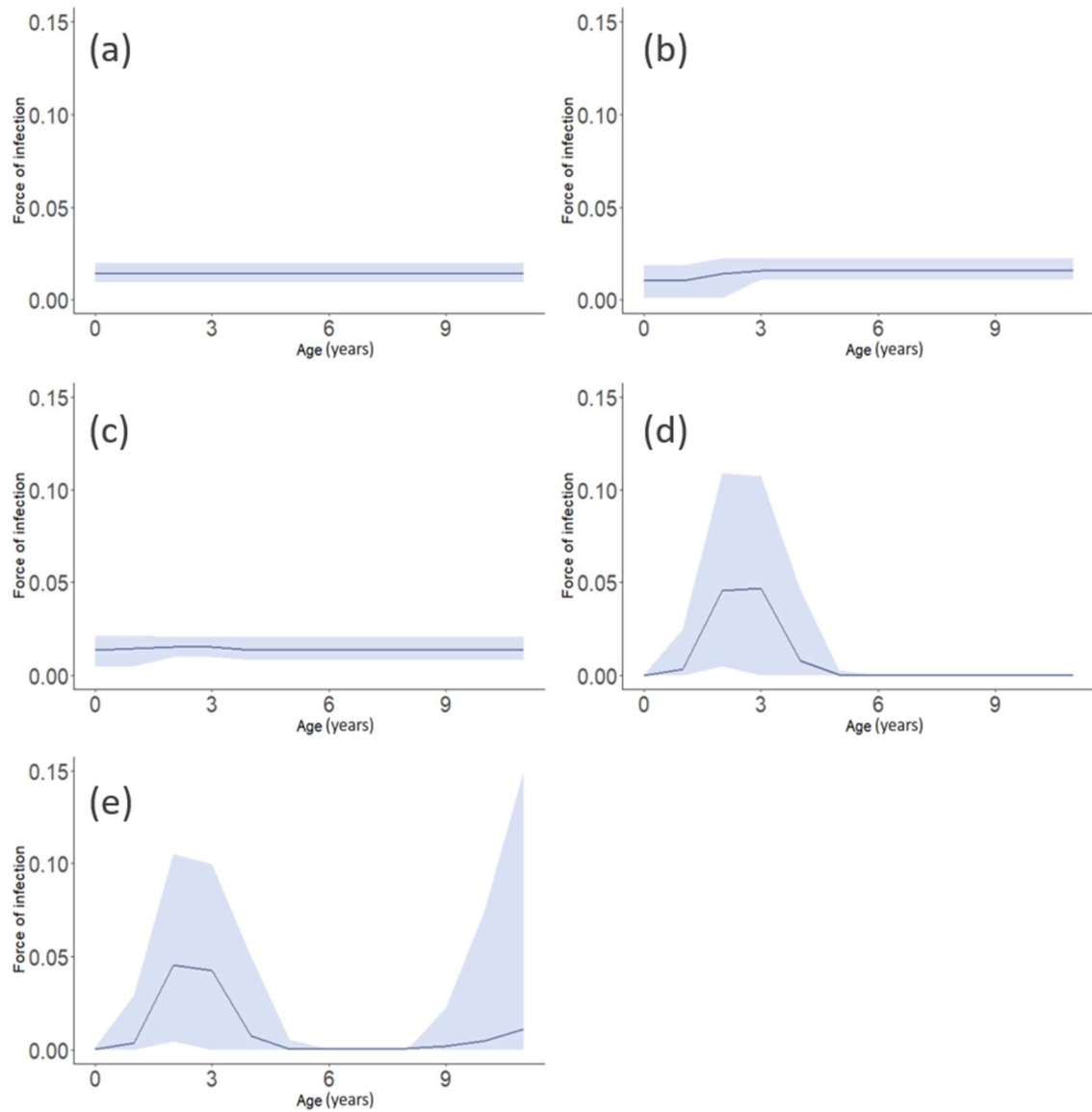
### 333 3.3. Infectious disease model

334 Table 5 shows descriptions and DIC of the 12 models prepared. Besides the model of  
 335 independently estimated annual FOI, all chosen models had similar low DIC values and were

336 adequate to explain the data. Figure 5 shows the age-specific FOI with the 95% credible interval for  
337 five of the selected different epidemiological models. The simplest model, the constant model, was  
338 adopted because there had been no previous interventions or notable outbreaks such as abortion  
339 storm in the study area, and there was no significant difference in DIC value between the models.  
340 The annual probability of infection was estimated at 1.4% (95% credible interval 1.0-2.0%) in the  
341 constant model. Figure 6 shows observed and estimated age-specific sero-prevalence values  
342 according to the constant FOI model.

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

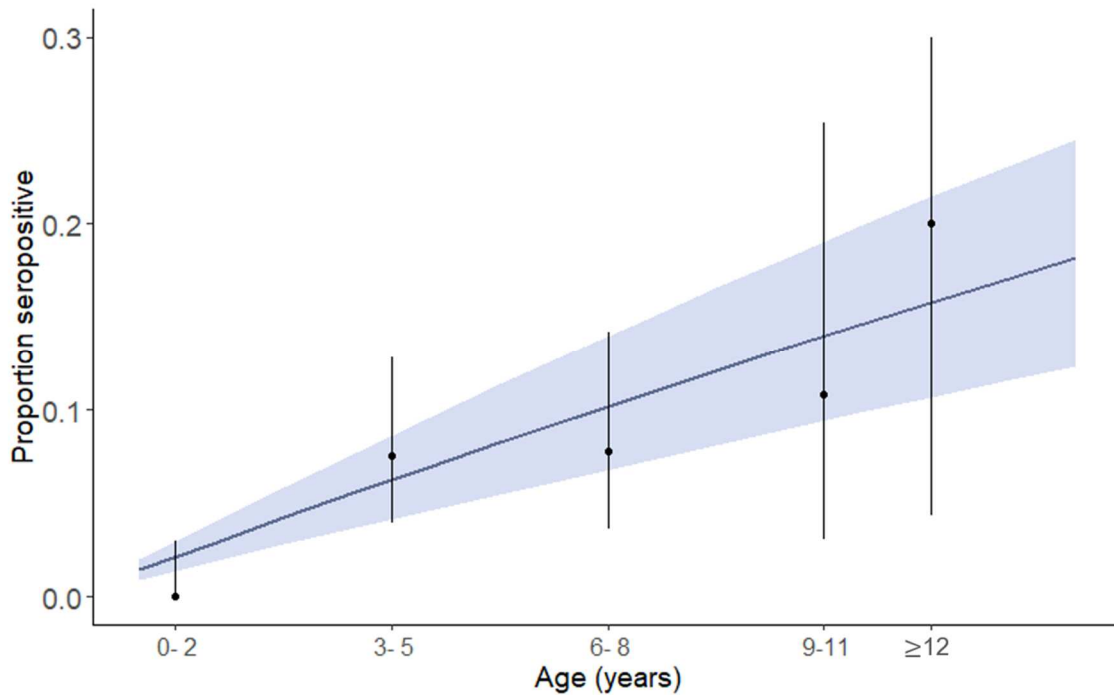
345



346

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

350



351

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

355

#### 356 **4. Discussion**

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

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

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

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

391 The  $R_0$  estimated in the present study was slightly greater than 1.0, which was consistent with  
392 the endemic nature of brucellosis (Keeling and Rohani, 2008). By comparison, the  $R_0$  for the cattle  
393 populations at infected farms was 1.10, which was not markedly higher than the overall  $R_0$ , 1.07,  
394 suggesting limited heterogeneity in disease transmission between infected and non-infected farms.  
395 These data might also suggest that there is regular contact between infected animals and animals at

396 non-infected farms. For future intervention planning discussions, methods for the control of  
397 brucellosis over a wide area, including non-infected farms, should be considered.

398 As no records of the time of abortion occurrence were available, leaving only data on  
399 cumulative occurrence, MH analyses were performed in the present study. The crude OR chosen  
400 suggested a significant relationship between brucellosis and history of abortion. Under field  
401 conditions, in which access to facilities for diagnosing animal brucellosis is limited, symptom-based  
402 monitoring using a history of abortion can play a key role in identifying high-risk herds. The  
403 analysis of covariance for the reproduction performance suggested that infection with brucellosis not  
404 only causes abortion in the first pregnancy after infected, but also significant reduction of  
405 reproduction performance (42.2%), which has a long-term effect on the herd productivity.

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

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

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

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

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

449 Brucellosis is currently a tragedy of the commons making livestock farmers and human patients  
450 suffer (Hardin, 1968) as nobody is willing to invest in its control. Tanzania could become free of  
451 brucellosis if a national brucellosis mass vaccination campaign is conducted, which requires to find a

452 way of societal cooperation (Ostrom, 2015). Therefore, studies for designing vaccination campaign  
453 and identifying the incentives for farmers to participate in it are required.

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

466

## 467 **5. Conclusion**

468 The results of this study suggest that the most common mode of brucellosis transmission among  
469 cattle in agro-pastoral areas of Mvomero, Tanzania, may be contact during grazing with *Brucella*-  
470 contaminated abortion tissues present on pastures. At infected farms, introducing infected cattle  
471 directly from other herds or from livestock markets was identified as a significant risk factor for  
472 highly efficient transmission within the farm. Bovine brucellosis exhibited limited heterogeneity  
473 between infected and non-infected herds. Future studies should examine the dynamics of between-  
474 herd transmission to plan effective targeted intervention strategies, most likely involving vaccination  
475 of animals.

476

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481

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487 of a strategic research platform for private universities”.

488

## 489 **Ethical considerations**

490 This study was approved by the Ethical Committee of the Graduate School of Dairy Science,  
491 Rakuno Gakuen University (approval number 16-3). Permission to conduct the study in Morogoro  
492 municipality and Mvomero district was sought and granted by municipal and district executive  
493 officers (reference numbers E.10/MMC-138/185 and MVDC/C.80/4 VOLII/126, respectively).  
494 Verbal informed consent was obtained from each participant after livestock field officers explained  
495 the aim of the study.

496

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629 **Tables**

630

631 **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)

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633

634 **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

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648 Table 3. Univariable analysis results showing the median brucellosis within-herd crude prevalence  
 649 and range and the number of farms giving true and false responses for the variables assessed (n =  
 650 17).

Variables	True		Number of farms	False		Number of farms	<i>p</i> -value
	Prevalence (%)			Prevalence (%)			
	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

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

Variable	Estimate	Standard error	<i>p</i> -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

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661 Table 5. Descriptions and DIC of the 12 models prepared. Models with background infection include  
662 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

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