Seroprevalence of brucellosis and its associated risk factors in cattle from smallholder dairy farms in Zimbabwe

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Please cite the paper as: Matope, G., Bhebhe, E., Muma, J.B., Oloya, J., Madekurozwa, R.L., Lund, A., Skjerve, E., 2011. Seroprevalence of brucellosis and its risk factors in cattle from smallholder dairy farms in Zimbabwe. Tropical Animal Health and Production, 43:975-979.

Abstract

A cross sectional study was conducted to investigate seroprevalence of brucellosis and the associated risk factors in cattle from smallholder dairy farms in Gokwe, Marirangwe, Mushagashe, Nharira, Rusitu and Wedza areas of Zimbabwe. A total of 1440 cattle from 203 herds were tested serially for *Brucella* antibodies using Rose Bengal test (RBT) and the competitive ELISA (c-ELISA). Weighted seroprevalence estimates were calculated and risk factors in individual cattle investigated using logistic regression analysis. The overall individual animal brucellosis seroprevalence was low, with mean of 5.6 % (95 % CI: 4.4 %, 6.8 %). Gokwe had the highest individual (12.6%; 95 % CI: 3.9 %, 21.4 %) and herd-level (40.0%; 95 % CI: 22.1%, 58.0 %), while Wedza had the lowest individual (2.3 %; 95 % CI: 0 %, 5.3 %) and herd-level (8.0%; 95% CI: 0.0 %, 18.9 %) brucellosis seroprevalence, respectively. In individual cattle, the area of origin, age and history of abortion were independently associated with brucellosis seroprevalence. While the seroprevalence was independent of sex, it decreased with increasing age. Cattle 2-4 years old had higher odds (OR = 3.2; 95 % CI: 1.1, 9.1) of being seropositive compared to those > 7 years. Cows with a history of abortion were more likely to be seropositive (OR= 7.9; 95 % CI: 3.1, 20.1) than controls. In conclusion, the area-to area variation of brucellosis may be linked to ecological factors and differences in management practices. The implementation of stamping out policy, bleeding and testing animals before movement and promoting the use self-contained units are likely to significantly reduce the public health risks associated with *Brucella* infections in cattle.

Key words: Brucellosis, cattle, seroprevalence, smallholder dairy, Zimbabwe

Abbreviations used in the text

c-ELISA	competitive enzyme-linked immunosorbent assay
CI	confidence intervals
DDP	dairy development programme
OR	odds ratio
RBT	Rose Bengal test

Introduction

Bovine brucellosis is usually caused by *Brucella abortus* and occasionally by *Brucella melitensis* where cattle are kept together with infected sheep or goats (OIE, 2008). The disease has existed since antiquity and causes significant economic loss in cattle production in many regions of the world. Brucellosis is endemic in most Sub-Saharan African countries including Zimbabwe (Faye et al. 2005; Karimuribo et al. 2007; McDermott and Arimi, 2002; Mohan et al. 1996; Muma et al. 2007b; Omer et al. 2000). Brucellosis is amongst the 'neglected zoonoses' (WHO, 2009) largely due to lack of public awareness and yet it is one of the most important zoonotic infections, especially in pastoral and mixed crop-livestock farming systems in Africa (McDermott and Arimi, 2002).

In Zimbabwe, cattle farming is broadly divided into large scale commercial (beef and dairy) and smallholder sectors, with between 60 to 80% found in the latter. In some areas of the country, smallholder dairies were established between 1980 and 1991 by the Dairy Development Programme (DDP), in order to improve the availability of milk to these communities (Matope et al. 2010). Cattle of mainly *Bos taurus* breeds were purchased from commercial dairy farms and brought to these smallholder household herds where they were later cross-bred with the indigenous Sanga (Mashona and Tuli) cattle and kept as small semi-independent herds (Matope et al. 2010). The brucellosis control regulations prescribe that commercial dairy farms regularly vaccinate calves between the ages of three to 10 months (Anon., 1995), but the vaccination status of the purchased animals could not be ascertained at the time of the study.

The livelihood of smallholder farmers is heavily dependent on cattle, which apart for milk production, they are used for drought power, meat, income, transport and manure, and other social or cultural activities. However, cattle productivity in smallholder farms is primarily affected by diseases, in addition to lack of adequate grazing, poor husbandry practices and lack of adequate veterinary services. Among the infectious diseases, brucellosis has been shown to be widely spread in Zimbabwe, with a higher prevalence in commercial compared to smallholder farming sectors (Madsen, 1989; Mohan et al. 1996; Swanepoel et al. 1976). The variation in the prevalence of the disease may be influenced by the characteristics of animal populations, management factors and other biological features such as herd immunity, persistence of infection in calves and vaccination status that largely determine the epidemiology of brucellosis (Faye et al. 2005; McDermott and Arimi, 2002; Salman and Meyer, 1984). The establishment of the smallholder dairies, and most recently, the introduction of the agrarian reform programme in the year 2000 brought about increased movement of cattle between the commercial and smallholder sectors. This has created a unique cattle management system with the potential of changing the epidemiology of brucellosis and other infectious diseases. While brucellosis continues to be closely monitored in the commercial farming sector, there is lack of information on its seroprevalence and the risk factors associated with the disease in smallholder cattle. Therefore, this study was conducted to estimate the seroprevalence of brucellosis and associated risk factors in individual cattle from smallholder dairy farms in Zimbabwe.

Materials and methods

Study areas

The study was conducted in Gokwe, Marirangwe, Mushagashe, Nharira, Rusitu and Wedza smallholder dairy cattle farms of Zimbabwe from September 2004 to November 2005. These areas were specifically selected because they; (1) represented the different agro-ecological regions of Zimbabwe, (2) kept mixed cattle breeds of *Bos taurus* (originally from commercial farms) and *Bos indicus* (indigenous Sanga) origin, (3) had smallholder dairy farms, (4) were not using *B. abortus* S19, *B. abortus* S45/20 and *B. melitensis* Rev1 vaccines. The geographical locations, climatic and the predominant agricultural activities of these study areas are described in detail in the previous report by Matope et al. (2010).

The cattle management type as prescribed by DDP was generally similar for all the study areas. This involved grazing of cattle on separate pastures with own supplies of drinking water. Therefore, unlike other smallholder farms in communal areas where there is a lot of commingling of cattle both within and between villages, making the definition of a herd difficult under these conditions of management, the type of cattle management in these smallholder dairies permitted us to regard the individual farms as independent herds. A farm was classified as a piece of land allocated to a single household for farming purposes and was demarcated from others by perimeter fencing.

Study design and sampling of individual animals

A cross sectional study was carried out using a stratified sampling procedure to select herds and then individual cattle per herd. The details of the study design, sampling of herds and individual animals have been described previously (Matope et al. 2010). In each study area, the approximate number farms were listed with the assistance of local veterinary/ agricultural office. Herds that were co-grazed were grouped together and considered as one and only herds with a minimum of 10 cattle ≥ 2 years were included in the study. The sample sizes of herds in each area were predetermined as described by Dohoo et al. (2003), by assuming that brucellosis existed at 25% inter-herd and 15% intra-herd seroprevalence (Madsen, 1989). All the eligible herds from each study area were identified by numbers (written on small cards) and then study herds randomly chosen from a bowl without replacement. The sample sizes of individual animals were estimated as described (Jordan, 1995) using the diagnostic sensitivity (*Se*) and specificity (*Sp*) of Rose Bengal test (RBT) of 90% and 75%, respectively and for the competitive enzyme-linked immunosorbent assay (c-ELISA) 98% and 99%, respectively based on previous validation studies (McGiven et al. 2003; Nielsen et al. 1995). Therefore, at individual animal level, the combined sensitivity and specificity for the RBT and the c-ELISA using a serial interpretation were calculated to be 88.2% and 99.8%. To balance on the resources available in the project, at least eight cattle from each herd were sampled and a 25% sampling fraction from herds with > 40 cattle. This resulted in herd *Se* and *Sp* of 86.6% and 98.4%, respectively, when herds were classified as brucellosis seropositive if at least a single positive reactor animal was detected. For bleeding, cattle were selected by systematic random sampling by taking every fourth animal in the pen. Where random sampling was not possible, eight animals were selected from those present in the herd and blood samples taken.

Epidemiological data collection

Information on individual animal variables (age, sex and history of abortion for cows) was recorded separately on sample data sheets. Herd level data that included: herd structure, size, history of purchases of animals and farm management practices were collected by interviewer-administered questionnaire. This herd data was envisaged for further use in studying the herd-level risk factors for brucellosis.

Laboratory tests

The clotted blood samples were centrifuged at 3000 x *g* for 15 minutes and 2 ml of serum were collected into cryo-tubes and stored at -20°C until laboratory tests were performed. The RBT, conducted as previously described (OIE, 2008) was used to screen sera for anti-*Brucella* antibodies.The buffered *B. abortus* antigens and control sera (positive and negative) used were obtained from VLA, Weybridge (UK). Since a serial testing was used (to increase on test specificity), then only the RBT positive (agglutinations visible by the unaided eye) were tested using the Svanovir^{TM} *Brucella*-Ab c-ELISA test kits (Svanova Biotech, Uppsala, Sweden) for confirmation. The c-ELISA was done according to the manufacturer's instructions and essentially as described elsewhere (Matope et al.2010; Muma et al.2006). Only animals positive on both RBT and c-ELISA were classified *Brucella* seropositive.

Statistical analysis

The epidemiological and animal bio-data were stored in a computer data base and statistical analysis was performed using Stata version SE 10.0 version (Stata Corp. College Station, TX, USA). In order to improve the estimation of brucellosis seroprevalence, individual animal level-data were weighted according to the inverse of the sampling fraction (Dohoo et al. 2003). A sampling weight was obtained as a product of the proportion of herds sampled against the total number of herds in each study area and the proportion of cows sampled in a herd. The Stata survey (svy) analysis which takes into account the sampling weights was used to calculate the seroprevalence estimates according to the study areas, sex and age categories. Herd-level data were not weighted and raw seroprevalence were estimated using the proportion command in Stata.

Logistics regression analysis

The association between individual animal-level factors and brucellosis seroprevalence was investigated using a logistic regression model. A two-sided Fisher's exact test was used for testing the unconditional association between brucellosis seropositive status of cattle (negative = 0, positive =1) and potential categorical risk factors, while a Kruskal Wallis test was used for age. Since age was skewed to the right, we categorised it into quartiles in order to correct for the linearity problem. The predictor variables were assessed for collinearity by cross tabulations using the two-sided Fisher's exact test. Only variables with *P*-values <0.25 in univariable analysis and having counts ≥ 5 in each cell were tested in the logistic regression model. The logistic regression model was constructed by a forward selection applying the iterative maximum-likelihood estimation procedure and the statistical significance of individual

predictors to the model ssessed using the Wald's test and likelihood ratio test (Dohoo et al. 2003). The interaction between variables was tested by constructing two-product terms for the significant main effect variables, forcing them into the model and examining changes of coefficients and *P*-values of the main effects. The logistic model was evaluated for goodness-of-fit using a Hosmer-Lemeshow test.

Results

A total of 1440 cattle from 203 herds from the six study areas were tested for presence of antibodies to *Brucella* spp. (Table 1). The brucellosis seroprevalence adjusted for sampling weights according to the study areas, age group, sex and origin of cattle (purchased or locally raised), are shown in Table 2. The mean number of individual animals that were positive for antibodies to *Brucella* spp. was estimated at 5.6% (81/1440; 95% CI: 4.4, 6.8%). Brucellosis seroprevalence ranged from 12.6 % (95% CI: 3.9, 21.4 %) to 2.3 % (95% CI: 0.0, 5.3 %), with Gokwe and Wedza recording the highest and lowest, respectively (Table 2). The mean number of *Brucella* seropositive reactor cattle were significantly higher ($P<0.05$) in Gokwe compared to the other five study areas. Weighting of seroprevalence estimates was perceived to be necessary in order to obtain proper population based estimates.

The association of individual animal-level factors (sex, age groups and the origin of the animals) with brucellosis seroprevalence is shown in Table 2. Brucellosis seroprevalence was observed to decrease with increasing age of cattle. There were significantly higher ($P<0.05$) numbers of seropositive cattle in the 2-4 years age group compared to those over 7 years. There was no difference ($P>0.05$) in seroprevalence between males and females, or locally raised and purchased cattle (Table 2). When only female animals were assessed using univariable analysis, the odds of testing seropositive were higher in animals with a history of abortion compared to those without (OR= 7.9, 95% CI: 3.1, 20.1). However, this variable was not run in the full model.

The logistic regression analysis showed that study area and age groups were independently associated with *Brucella* seropositive status of cattle (Table 3). The odds of *Brucella* seropositivity were lower in Wedza (OR = 0.14; 95 % CI: 0.03, 0.59) and Rusitu (OR = 0.27; 95 % CI: 0.13, 0.55) compared to Gokwe. There were moderate differences in odds of *Brucella* seropositivity between Gokwe and Marirangwe (OR = 0.38), Mushagashe (OR = 0.48) and Nharira (OR = 0.53). *Brucella* seropositivity was influenced by age, with the 2-4 years age group having higher odds (OR = 3.2, 95% CI: 1.1, 9.1) compared > 7 years-old. There were no significant interactions between the main effects and no evidence of confounding was detected in the regression model. The Hosmer-Lemeshow test showed that the model fit the data ($X^2 = 17.7$, d.f. 15, $P = 0.3$ (Table 3).

The median herd sizes and herd-level brucellosis seroprevalence are shown in Table 4. The median herd sizes were largest in Marirangwe (19) and least Wedza (12) smallholder areas. The highest herd brucellosis seroprevalence were from Gokwe (40.0%) and Marirangwe (40.0%), while the least (8.0%) was found in Wedza. Herd-level brucellosis seroprevalence was found to differ significantly ($P<0.05$) among some study areas (Table 4).

Table1. The distribution of herds (n = 203) and individual cattle (n = 1440) sampled in the study (2004 to 2005).

Study area	**Total number of herds sampled**	**Animals sampled**	**Ages and categories of animals sampled**	
			Age (years)	**No. of animals**
Gokwe	30	265	2-4	145
			4.5-5	46
			5.5-7	57
			>7	17
			Females	233
			Males	32
Marirangwe	28	305	2-4	64
			4.5-5	41
			5.5-7	109
			>7	91
			Females	245
			Males	60
Mushagashe	15	133	2-4	35
			4.5-5	30
			5.5-7	38
			>7	30
			Females	122
			Males	11
Nharira	40	272	2-4	102
			4.5-5	58
			5.5-7	79
			>7	33
			Females	254
			Males	18
Rusitu	65	354	2-4	136
			4.5-5	96
			5.5-7	82
			>7	40
			Females	338
			Males	16
Wedza	25	111	2-4	49
			4.5-5	27
			5.5-7	28
			>7	7
			Females	107
			Males	4
Total	203	1440		

Table 2. Brucellosis seroprevalence and univariable associations in cattle by study area, age group and sex, with data adjusted for sampling weights (2004-2005). Results are given as percent seroprevalence with 95% confidence intervals (CI). Categories with different letters have different (P<0.05) seroprevalence

Risk factor	**Level**	**Cattle tested**	**Percent individual animal sero-prevalence (95% CI)**
Study area[a]	Gokwe	265	12.6 (3.9, 21.4)[a]
	Marirangwe	305	3.6 (1.7, 5.5)[b]
	Mushagashe	133	5.7 (2.6, 8.7)[b]
	Nharira	272	6.1 (2.9, 9.3)[b]
	Rusitu	354	3.6 (1.4, 5.8)[b]
	Wedza	111	2.3 (0.0, 5.3)[b]
	Overall	**1440**	**5.6 (4.4, 6.8)**
Age category[a]	2– 4 years	531	6.7 (4.3, 9.0)[c]
	4.5 – 5 years	298	6.1 (2.0, 10.2)[c]
	5.5 – 7 years	393	5.5 (2.7, 8.4)[c]
	> 7years	218	1.3 (0.0, 2.7)[d]
Sex	Female	1291	5.4 (3.6, 7.2)[e]
	Male	149	7.4 (2.9, 11.8)[e]
Origin of animal[a]	Locally raised	1269	5.3 (4.0, 6.5)[f]
	Purchased	171	8.2 (4.1, 12.3)[f]

[a]These values had Fisher's exact P- value ≤ 0.25 in univariable analyses and were identified as possible risk factors and were further investigated in multivariable logistic regression analysis.

Table 3. The multivariable logistic regression model to predict the risk factors associated with brucellosis in individual cattle from smallholder farms in Zimbabwe (2004-2005)[a]. [b]Results given with beta (*b*), standard errors (S.E.), and odds ratio (OR) with 95% confidence intervals (CI).

Risk factor	**Level**	**Logistic regression**				
		b	**SE (b)**	***P*-value**	OR	**95% CI**
	Constant	-1.97	0.22	0.000	-	-
Area	Gokwe	-	-	-	1.0	-
	Marirangwe	-0.98	0.36	0.007	0.38	0.18, 0.77
	Mushagashe	-0.74	0.44	0.09	0.48	0.20, 1.13
	Nharira	-0.64	0.32	0.04	0.53	0.28, 0.98
	Rusitu	-1.3	0.35	0.000	0.27	0.13, 0.55
	Wedza	-1.98	0.74	0.007	0.14	0.03, 0.59
	Mushagashe	-0.74	0.44	0.09	0.48	0.20, 1.13
Age category	2-4 years	-	-	-	1.0	-
	4.5-5 years	0.03	0.3	0.93	1.03	0.57, 1.87
	5.5-7 years	-0.03	0.28	0.91	0.97	0.55, 1.69
	>7years	-1.17	0.55	0.03	0.31	0.11, 0.9

[a]Overall data of the model: Log likelihood = -296.3, LR chi2(8) = 31.1, P = 0.0001, number of observations = 1440. Hosmer-Lemeshow $X^2(15)$ = 17.7, P = 0.3

Table 4. Herd structure and herd-level *Brucella* seroprevalence by study area.

Study area	**Herd size**		**Herd seroprevalence**	
	Median	Range	Proportion (%)	95%
Gokwe	14	10, 38	40.0[a]	22.1, 58.0
Marirangwe	19	11, 78	35.7[a]	17.5, 53.9
Mushagashe	17	10, 42	40.0[a]	14.2, 65.8
Nharira	16	10, 74	35.2[a]	17.7, 47.3
Rusitu	13	10, 31	13.8[b]	5.3, 22.4
Wedza	12	10,22	8.0[b]	0.0, 18.9
Total	**14**	**10,78**	**25.6**	**19.6, 31.7**

Prevalence estimated using the proportion command in Stata. Results given as percent seroprevalence with 95% confidence intervals (CI). Seroprevalence with different superscripts are different (P<0.05).

Discussion

In this study, brucellosis seroprevalence and the associated risk factors were investigated in cattle from smallholder dairy farms selected from various agro-ecological regions of Zimbabwe. The study showed that brucellosis is present in all study areas with mean individual seroprevalence of 5.6% (95% CI: 4.4, 6.8%). The seropositive reactions were likely to be caused by field *Brucella* spp. because the c-ELISA which was used as a confirmatory test has a high specificity in individual animals which minimises false positive reactions caused by cross-reacting antibodies produced against other Gram-negative bacteria such as *Yersinia enterocolitica* O:9, *E. coli* O:157 and some *Salmonella* spp. (Nielsen et al. 2004). The observed brucellosis seroprevalence results agree with those of previous studies in Zimbabwe (Madsen, 1989; Mohan et al. 1996) and those from smallholder farming areas in other regions (Bayemi et al. 2009; Ibrahim et al. 2010; Karimuribo et al. 2007). However, higher brucellosis seroprevalence have been recorded in individual cattle from traditional smallholder herds in other areas (Chimana et al. 2010; Faye et al. 2005; Muma et al. 2006). The differences in seroprevalence is likely to be attributed to certain risk factors such as cattle management practices, population dynamics; and biological features, for instance herd immunity that largely influence the epidemiology of *Brucella* spp. (Al-Majali et al. 2009; McDermott and Arimi, 2002; Reviriego et al. 2000).

Our results showed higher individual animal seroprevalence in Gokwe (12.6%) compared to Wedza (2.3%) and other study areas. Similarly, herd-level brucellosis seroprevalence was highest in Gokwe (40.0%) and Mushagashe (40.0%) and lowest in Wedza (8.0%). The high seroprevalence highlights the economic and public health importance of brucellosis in these smallholder dairy farming systems, which often have limited resources to control the disease. Although there were no previous data on herd-level brcellosis seroprevalence in smallholder areas, our results are similar to what has been documented for commercial farms in Zimbabwe (Madsen, 1989). However, the continual movement of cattle from commercial to smallholder farming areas could present a risk of introducing brucellosis in the latter since the disease has been previously noted to be more prevalent in commercial farms compared to communal areas in Zimbabwe (Bryant and Norval, 1985; Swanepoel et al. 1975; Swanepoel et al. 1976). The movement of animals between herds has been established to be an important risk for *Brucella* spp. infection in other regions of the world (Al-Majali et al. 2009; Kabagambe et al. 2001; Muma et al. 2007b; Omer et al. 2000).

The reasons for the variations in brucellosis seroprevalence among the study areas could not be fully explained based on the available data, but may be related to cattle management differences. At the onset of the dairy schemes, farmers purchased *Bos taurus* cattle from commercial farms, but the screening of these for brucellosis was not done due to limited availability of veterinary services and this increases chances of contact with infected herds (Al-Majali et al. 2007; Muma et al. 2007b; Omer et al. 2000; Reviriego et al. 2000). Therefore, these management practices together with other agro-ecological factors could partly explain the observed area-level differences in seroprevalence. The fact that brucellosis was low in areas with small median herd sizes showed that the risk of transmission of *Brucella* spp. among cattle was low in small herds (Ibrahim et al. 2010). However, the observed results for Gokwe may be contributed by a high proportion of farms that shared facilities for grazing and watering of cattle compared to the other study areas which kept their herds as self-contained units (data not shown). The practice of mixing of cattle, either through grazing or sharing of watering points is an important risk factor for brucellosis (Al-Majali et al. 2009; Muma et al. 2007b). In Rusitu,

prominent geographical features like hills and mountains, separated by steep valleys help to prevent mixing of herds and possibly accounting for lower brucellosis seroprevalence in these sedentary cattle. Our results for Rusitu agree with those of previous studies for the area (Bryant and Norval, 1985; Madsen, 1989).

The lack of difference in seropositive reactors between males and females may indicate that the risk of infection with *Brucella* spp. is independent of sex of cattle. Similar findings have also been reported elsewhere (Bayemi et al. 2009). However, this relationship has been shown to vary with different cattle subpopulations (Chimana et al. 2010; Kubuafor et al. 2000; Muma et al. 2006). The preponderance of seropositive reactors in the 2 – 4 years age group may be related to the onset of sexual maturity, which is associated with increased risk of infection with *Brucella* spp., especially following abortions (Muma et al. 2007a). However, the age at which sexual maturity is attained varies with breeds of cattle and this is likely to influence the observed relationship between age and positive reactors in different sub-populations. Although our observations about age and brucellosis seroprevalence differ with other reports (Faye et al. 2005; Kebede et al. 2008; Muma et al. 2006; Silva et al. 2000), they corroborate those of previous findings (Omer et al. 2000). It is likely that in endemic areas, the risk of *Brucella* infection, and thus seroconversion is greater in younger naïve animals compared to older cows, some of which may not exhibit detectable antibody titres, possibly due to latency which is common in chronic brucellosis (Ficht, 2003).

When female animals were considered separately, the high odds of testing seropositive (OR= 7.9, 95% CI: 3.1, 20.1) in animals with a history of abortion suggested active *Brucella* spp. infection since some of these had very high antibody titres. This is consistent with the biology of *Brucella* spp. and supports earlier observations (Al-Majali et al. 2007; Berhe et al. 2007; Muma et al. 2007a; Schelling et al. 2003). However, since most cows usually abort once (OIE, 2008), this could distort the association between history of abortion and seropositivity.

We concluded that both individual animal- and herd-level brucellosis seroprevalence is low in Rusitu Valley and Wedza but relatively high in the other areas, especially Gokwe where the disease is likely to be endemic. Area level differences in brucellosis seroprevalence could be related to management practices. The seroprevalence did not differ between sexes of cattle but decreased with increasing age. Cows with a history of abortion were more likely to test seropositive for brucellosis. Considering the economic and public health importance of brucellosis, the introduction of control measures such as avoiding mixing of cattle without screening for brucellosis and promoting the use self-contained units instead of shared facilities could benefit these smallholder dairies.

Acknowledgements

This study was sponsored by the Norwegian Council for Higher Education and Development (NUFU), project vote: pro/2002/06. We are grateful for the contribution of all the stakeholders involved in the project. Our sincere appreciation goes to all the farmers who allowed us to use their animals for this research.

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