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Variations in the prevalence of antibody to brucella infection in cattle by farm, area and district in Kenya

M. KADOHIRA[1]*, J. J. McDERMOTT[1,2]†, M. M. SHOUKRI[1] AND M. N. KYULE[2]

[1] *Department of Population Medicine, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*
[2] *Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya*

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SUMMARY

Variations in the sero-prevalence of antibody to brucella infection by cow, farm and area factors were investigated for three contrasting districts in Kenya: Samburu, an arid and pastoral area; Kiambu, a tropical highland area; and Kilifi, a typical tropical coastal area. Cattle were selected by a two-stage cluster sampling procedure and visited once between August 1991 and 1992.

Schall's algorithm, a statistical model suitable for multi-level analysis was used. Using this model, older age, free grazing and large herd size ($\geqslant 31$) were associated with higher sero-prevalence. Also, significant farm-to-farm, area-to-area and district-to-district variations were estimated. The patterns of high risk districts and areas seen were consistent with known animal husbandry and movement risk factors, but the larger than expected farm-to-farm variation within high risk areas and districts could not be explained. Thus, a multi-level method provided additional information beyond conventional analyses of sero-prevalence data.

INTRODUCTION

Bovine brucellosis, whose main manifestation is abortion, is caused by *Brucella abortus* [1, 2]. Brucellosis is prevalent throughout Africa [3–5]. Variations in the prevalence of brucellosis have been attributed, without quantitative analysis, to regional (ecological) [4] and farming system [6] differences. Influences on the transmission of infection by herd management factors have also been discussed [7–9].

The main limitation in the analyses cited above is that area and farm-level influences are often confounded (e.g. rainfall influences grazing system and herd size). For example, variations in prevalence attributed to climate may be due to husbandry factors since the probability of disease transmission within and among herds increases with the frequent animal movements and larger herd sizes found in semi-arid areas where extensive pastoralism is practised. To adjust for such confounding, formal methods to partition variation among area, farm and individual-animal levels at each stratum need to be conducted. Such information is required to plan brucellosis control programmes, by identifying the highest risk populations and the most important risk factors in those populations.

In this paper, data on the prevalence of antibody to *Br. abortus* infection and individual-animal, farm and area risk factors from three distinct districts in Kenya were analysed using a multi-level generalized linear mixed model. The objective was to assess the pattern of sero-positive reactions for antibody to *Br. abortus*

* Current address is Department of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia.
† Corresponding author at the University of Guelph address.

infection, both the relative variability attributable to farm, area and district and associations with potential risk factors at each of these levels.

METHODS

Study areas and data collection

Cattle were sampled in three ecologically distinct districts in Kenya. Samburu District, in northern Kenya, is an arid and pastoral area, which except for two slightly higher and wetter areas (Maralal Township and the Lerroki plateau) is covered with thornbush/acacia tortillas and sparse grassland. Kiambu District, adjacent to Nairobi in the central highlands of Kenya, has a tropical highland climate with two rainy seasons per year. The district is densely populated and most of the land is intensively cultivated. The main cattle-rearing activity is mixed (crop-livestock) small-scale (2–5 cattle on 1–3 ha) dairy production. Kilifi District, on the Indian Ocean coast, has a typical tropical lowland climate. Most farms are mixed and of small to medium size. Livestock are primarily kept for meat production.

In each of the study districts, areas, farms and cattle were sampled by two-stage cluster sampling. First, a list of areas (sublocations in Samburu, divisions in Kilifi and dairy cooperative societies in Kiambu) within district was compiled and between four and six areas randomly selected. In each randomly selected area 3–15 farms (3–5 in Samburu; 5–10 in Kilifi and 15 in Kiambu) were then randomly sampled depending on cost and logistic constraints. All cattle on selected farms were sampled.

Variables were collected on the basis of individual animal, farm and area. Age ($\geqslant 4$ years (AGE1), 1–4 years (AGE2), or $\leqslant 12$ months), sex, breed (zebu or taurine) and body condition (thin or satisfactory) were recorded as individual-animal factors. Herd size (large $\geqslant 31$ (SIZE1), medium 11–30 (SIZE2), or small $\leqslant 10$), grazing pattern (free or restricted), frequency of disease control application (regular (at least yearly), sporadic (not every year), or never), breeding method (natural only versus any artificial insemination), type of feeds (concentrates and others, salt only, or none) were recorded as farm factors. The presence of tsetse flies, camels and wildlife, and the human population density, Normalized Difference Vegetation Index (NDVI) and agro-ecological zone were recorded as area factors. The NDVI is a unitless quantity for evaluating vegetation conditions [10, 11]. The four agro-ecological zones represented in this study were lower highland (LH), upper midland (UM), lower midland (LM) and lowerland (L) [12].

Serological test

The brucellosis enzyme-linked immunosorbent assay (ELISA) kit used was prepared by the Joint Food and Agriculture Organization/International Atomic Energy Agency (FAO/IAEA), Division of Nuclear Techniques in Food and Agriculture [13]. The kit contains antigen (smooth lipopolysaccharide (SLPS)), control sera (strong and moderate antibody positives and antibody negative), and standardized buffers, conjugates and chromogens. The assay procedures followed were described in the bench protocol manual version BRA 1.1 [13] based on a standard indirect ELISA technique [14].

Microplate readings were interpreted in two ways; percent positive (PP)* values for quality assurance acceptance and percent positive (PP)† values for diagnostic interpretation [13]. For acceptance of microplates, three of four replicates of each control were required to fall within established limits [13]. For diagnostic interpretation, test sera with mean PP equal to or greater than a threshold PP were considered positive if the variation between two replicates was acceptably low. Calculations were performed using the EDI (ELISA Data Information) software provided by FAO/IAEA.

Data analysis

The main objective was to estimate statistical associations between fixed (regression) and random (component of variation attributable to a level of organization) effects at farm, area and district levels with positive ELISA test results. The statistical method used was a mixed generalized linear model procedure developed by Schall [15] and executed in an SAS IML macro [16]. The estimates from Schall's method were compared to estimates from standard but more restricted techniques including ordinary logistic regression, generalized estimating equation [17] estimated using an SAS IML macro GEE1 [18], and Jackknife (JK) [19, 20] procedures at the farm-level of aggregation; and random-effect models fit in SAS

* $PP_{ij} = (OD_{ij}/(\text{mean OD value of strong-positive control})) \times 100$, where i = type of control 1, 2, 3 for moderate-positive, negative and conjugate; j = replicate 1, 2, 3, 4.

† $PP_{ij} = (OD_{ij}/(\text{mean OD value of strong-positive control})) \times 100$, where i = serum sample from cow; j = replicate 1, 2.

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VARCOMP [21] at one, two and three-levels of aggregation.

Fixed effects were first screened in ordinary logistic regression using a forward stepwise procedure ($P < 0{\cdot}05$). These selected fixed effects were then included in all subsequent models to be compared.

RESULTS

Table 1 lists the number of farms, median and range of herd sizes and prevalence of antibody to *Br. abortus* by area and district. Prevalence varied between districts ($P < 0{\cdot}001$) with Kiambu having the lowest ($8/374 = 2\%$), Kilifi an intermediate ($13/132 = 10\%$) and Samburu the highest ($94/640 = 15\%$). The prevalence of antibody was homogeneous in Kiambu District, but area within district heterogeneity was noted in Samburu (between sublocations; $P < 0{\cdot}001$) and Kilifi (between divisions; $P = 0{\cdot}003$).

The distribution of individual-animal and farm risk factors investigated is listed by district in Table 2. Of these, the main effects selected in the ordinary logistic regression model were grazing, large farm size, and age (adult) (Table 3). Interaction terms among these three main effects were not significant ($P > 0{\cdot}05$). An individual cow over 4 years old, kept on a large farm and grazed on a community pasture was 49 times more likely to be sero-positive than a young cow (< 4 years), kept in restricted grazing from a small farm. The risk of sero-conversion estimated by the ordinary logistic regression model increased in an additive manner on a logit scale. These parameter estimates were not adjusted for clustering.

To determine if clustering of sero-positives by farm was important, regression effects were compared between the ordinary logistic regression model and three models (GEE1, Jackknife method and Schall's algorithm) which adjust for clustering at the farm level (Table 3). Coefficients for each variable were similar among all methods. As expected, standard error estimates from the Jackknife, Schall's algorithm and GEE1 procedures for the parameter 'SIZE1' were approximately three, two, and one and a half times larger, respectively, than those from the ordinary logistic regression model. However, all three parameters (grazing, SIZE1, AGE1) from the ordinary logistic regression model were significant ($P < 0{\cdot}05$) in these models.

The analysis at one level of nesting was extended to a multi-level analysis using Schall's algorithm. This mixed effect model, included fixed effects selected in the ordinary logistic regression model and three (farm, area and district) random effects. Farm variation was the largest component of variation, with the area and district level variance components being approximately half and two-thirds of the farm variance component, respectively (Table 4). The regression effect estimates varied depending on the number, one (farm), two (farm and area) or three (farm, area and district), of random effects estimated (Table 4). The parameter estimate of 'AGE1' was virtually unchanged for all models. But the size of the two farm parameter estimates (grazing and farm size (SIZE1)) decreased and standard error estimates increased as the number of random effects increased. The parameter 'grazing' became non-significant ($P > 0{\cdot}05$) when 'district' was included as the third random effect. The other two parameters (AGE1 and SIZE1) remained significant in Schall's algorithm with three random effects. When measured proportionally, relative to total variation, the farm variation decreased as the number of random effects increased, but farm was the largest variance component estimate in all models.

A major limitation in modelling the effects of herd size and grazing system on the prevalence of sero-conversion was that these two risk factors were unevenly distributed. In Samburu District, there was a mixture of farm sizes (although the majority were large) but there was only free grazing. In Kiambu and Kilifi Districts, there was a mixture of grazing patterns but most farms were small. Thus, four restricted models – one using pooled data from Kiambu and Kilifi Districts and three with data from each District separately – were estimated for grazing system and farm size respectively. The result of the model restricted to Kilifi and Kiambu Districts was that sero-prevalences were similar between free and restricted (zero-grazed) grazing cows. In the district-specific mixed models, herd size was significant ($P = 0{\cdot}03$) only in Samburu District where large farms had higher sero-prevalences compared to small and medium sized farms.

DISCUSSION

Sero-prevalence surveys for antibody to *Br. abortus* in cattle have been commonly conducted in Africa to estimate infection risks for different areas, farms and animals and to investigate associations between risk factors and sero-conversion [3–8, 23–26]. An important constraint in the analysis of these surveys has been the difficulty in separating the effects of ecological

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Table 1. *Descriptive results: number of farms, number of cattle and sero-prevalence of brucella antibodies by area and district in Kenya*

District/area	Number of farms	Cattle per farm median and (range)	Sero-prevalence cattle positive/ cattle tested
Samburu			
Maralal	6	26·5 (15–68)	52/191 (27%) a*
Losurukoi	3	14 (5–23)	7/42 (17%) b
Lonyangatan	9	8 (5–132)	14/210 (7%) c
Nkaroni	3	30 (21–38)	15/89 (17%) b
Lpus	6	17·5 (8–29)	6/108 (6%) c
Kiambu			
Chania	15	2 (1–3)	1/30 (3%)
Kiambaa	15	4 (1–14)	2/74 (3%)
Kikuyu	15	4 (1–21)	1/81 (1%)
Lari	15	4 (1–10)	2/67 (3%)
Limuru	15	3 (1–11)	1/61 (2%)
Nderi	15	3 (1–18)	1/61 (2%)
Kilifi			
Bahari	5	5 (3–6)	0/24 (0%) b
Kaloleni	6	5 (4–8)	1/35 (3%)
Ganze	6	6·5 (3–8)	3/37 (8%) b
Malindi	8	4·5 (2–7)	9/36 (25%) a

* Areas within district prevalences which varied significantly ($P < 0{\cdot}05$) from each other have different letters (multiple-proportion comparison described in [22]).

variables such as rainfall and vegetation from herd variables such as grazing pattern. This information could be used to better target resources (such as vaccine to high-risk herds in high-risk areas) and to improve hypotheses on brucella transmission.

Among serological methods to detect *Br. abortus* infections, ELISA is considered well suited for serological surveys [14, 27, 28]. Estimates of the sensitivity [27, 29] and specificity [29] of brucella antibody ELISA tests have been reported to be above 99% and 97% respectively. The FAO/IAEA brucella ELISA kits used in this study have been designed to standardize materials and procedures and thus minimize laboratory variations. Although performance parameters have not been estimated for these kits, we believe that the sensitivity probably exceeds 90% and the specificity 95%. The low and homogeneous 2–3% prevalence estimated within Kiambu District, a low-risk zone for clinical brucellosis, provides an indirect specificity estimate of 97%. The effect of these test mistakes on statistical analyses of the data will be to increase the relative size of the error variance, thus, biasing risk estimates toward the null.

The brucella sero-prevalence estimates were within the range of estimates from other Kenyan studies. Oomen & Wegener [23] reported antibody sero-prevalence of 2·4% (5/208), 10·7% (74/690) and 4·1% (28/682) in Kiambu, Kilifi and Samburu respectively, using the serum agglutination test (SAT). The estimates for Kiambu and Kilifi were similar to this study but their estimate for Samburu was much lower. Their sampling method was not described, but it was presumed that samples were not collected randomly and may not well represent the cattle population. Another survey in Samburu [24], estimated the prevalence at 14·5% (107/736) using the Rose Bengal Plate test (RBPT). This was close to the present estimate. These samples, originally collected for a rinderpest sero-survey, were also not randomly sampled but may have been collected from a wider variety of areas in Samburu.

Previous attempts to attribute variation in brucella antibody sero-prevalence to ecological [4] and herd [7–8] risk factors have been done qualitatively. Multi-level statistical models provide an opportunity to quantify this by partitioning variation between different levels. In this study, variation was estimated between districts, areas within district, and farms within areas. In accord with other studies [6, 30], cattle in the predominately pastoral herds of Samburu

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Table 2. *Percentage of sampled cattle or farms within potential cattle or farm risk categories by district in Kenya*

	Samburu	Kiambu	Kilifi	Overall
Animal risk category (*n*)	640	374	132	1146
Age				
⩾ 4 yr	45	52	70	50
13 months to 4 yr	33	28	18	30
⩽ 12 months	22	20	11	20
Sex				
Female (*vs.* male)	68	82	92	75
Breed				
Exotic (*vs.* native)	0	65	7	22
Body condition				
Poor (*vs.* good)	4	1	6	4
Farm risk category (*n*)	27	90	25	142
Farm size				
⩾ 31	18	0	0	4
11–30	52	8	0	15
1–10	30	92	100	81
Disease control				
Regular	30	75	84	68
Sporadic	55	23	16	28
None	15	2	0	4
Breeding				
Natural (*vs.* any AI)	100	46	56	58
Suppl. feed				
Concentrate	4	93	32	65
Salt	55	7	20	18
None	41	0	48	17
Grazing pattern				
Open (*vs.* zero-grazing)	100	30	72	51

Table 3. *Estimates obtained from the ordinary logistic regression model and from generalized estimating equation 1* (*GEE1*), *Jackknife* (*JK*), *Schall's algorithm adjusting for clustering at farm level*

Parameter	Logistic	GEE1	JK	Schall
Intercept	−4·740	−4·698	−4·627	−4·739
	(0·529)*	(0·522)	(0·594)	(0·544)
Grazing	1·663	1·722	1·542	1·635
	(0·528)	(0·525)	(0·585)	(0·555)
SIZE1†	1·241	1·441	1·149	1·442
	(0·212)	(0·361)	(0·631)	(0·437)
AGE1‡	0·988	0·888	1·021	0·958
	(0·217)	(0·202)	(0·238)	(0·225)

* Standard error of parameter estimates is given within parentheses.
† Large farm size (⩾ 31 cattle in a farm).
‡ Adult (⩾ 4 yr).

had a higher prevalence of brucella infection than cattle in sedentary herds, presumably due to features of pastoral management, such as the movement of stock for grazing and the concentration of animals around water holes [31]. This transmission hypothesis was reinforced by the pattern of area variations within districts. Malindi in Kilifi District and Maralal in Samburu District, had much higher estimated prevalences (25 and 27%) than other areas in their districts. These are the trading centres of the district, so that greater livestock contacts are expected.

The largest variance component for brucella antibody sero-prevalence was between farms within areas and districts. This has been noted previously, without adjustment for district and area variability, by McDermott and colleagues [26], who found a large herd-to-herd variation (intra-herd correlation of 46%) for brucella antibody sero-prevalence in a pastoral area in the southern Sudan [32]. However, the reasons for this pattern were not obvious, since the sampled herds were grazed and tethered together and

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Table 4. *Regression coefficients and variance component estimates obtained for models incorporating one, two and three random effects*

Parameter	One (farm)	Two (farm and area)	Three (farm, area and district)
Intercept	−4·739	−4·567	−4·322
	(0·544)*	(0·578)	(0·658)
Grazing	1·635	1·442	1·140
	(0·555)	(0·590)	(0·620)
SIZE1†	1·442	1·139	1·049
	(0·437)	(0·423)	(0·434)
AGE1‡	0·958	0·957	0·964
	(0·225)	(0·227)	(0·227)
Variance component			
Farm	0·643	0·374	0·396
Area		0·306	0·215
District			0·257
V(e)§	0·792	0·837	0·826
Total	1·435	1·517	1·694

* Standard error of parameter estimates is given within parentheses.
† Large farm size (⩾ 31 cattle in a farm).
‡ Adult (⩾ 4 yr).
§ Variance due to error.

had essentially identical management. Crawford [9] categorized farm-level risk factors for brucella infection into between-farm (e.g. replacement of animals, grazing pattern and proximity to infected herds) and within-farm (e.g. vaccination level, herd size and stocking density) transmission factors. This classification provides a useful framework for subsequent investigations of potential farm-level influences causing these larger than expected differences in herd antibody sero-prevalence to *Br. abortus*.

The significant fixed effects found, age, herd size and grazing type, were expected and consistent with other studies [4, 6–9]. However, in surveys of this type, the estimation of fixed effects is difficult since desired contrasts may not be present in all districts and areas. For example, almost all herds in Samburu were of medium to large size and all were extensively grazed. Thus, additional analyses, stratified by district, were conducted. The effect of herd size was only seen in Samburu District, were larger (versus medium and small) herds had higher sero-prevalence.

In conclusion, multi-level models offer the opportunity to improve the planning, conduct and information obtained from sero-prevalence and other surveys. In this example, the multi-level analysis identified known risk factors for brucella infection and provided additional information on the relatively large farm-to-farm variation within high-risk pastoralist areas. Cattle production losses associated with brucella seropositives [26], and a high incidence of human brucellosis [33], have been estimated for pastoralist areas in eastern Africa. While eradication of brucellosis in such areas is unlikely, the most substantial and cost-effective reduction in its impact could be achieved by targeting control measures, such as vaccination, to herds with high seroprevalence or a history of high abortion rates.

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