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Risk factors associated with human brucellosis in the country of Georgia: a case-control study

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SUMMARY

Human brucellosis occurs when humans ingest or contact *Brucella* spp. from shedding animals or contaminated environments and food. In Georgia animal and human brucellosis is endemic, but the epidemiology has not been fully characterized. A case-control study was conducted in 2010 to identify risk factors for human brucellosis. Using multivariable logistic regression, the following risk factors were identified: animal-related work [odds ratio (OR) 77·8, 90% confidence interval (CI) 4·7–1278], non-animal-related work (OR 12·7, 90% CI 1·1–149), being unemployed or a pensioner (OR 13·1, 90% CI 1·7, 101), sheep ownership (OR 19·3, 90% CI 5·1–72·6), making dairy products (OR 12·4, 90% CI 1·4–113), living in eastern Georgia (Kakheti) (OR 278·1, 90% CI 9·5–8100), and being aged >44 years (OR 9·3, 90% CI 1·02–84·4). Education of at-risk groups about risk factors and control of disease in sheep may reduce the human disease risk. This is the first study of its kind in Georgia since the collapse of the Soviet Union.

Key words: Animal pathogens, brucellosis, *Brucella*, zoonoses.

INTRODUCTION

Brucellosis is an infectious disease of ruminant livestock, swine, dogs, rats, horses, and humans. There are six *Brucella* spp., four of which are zoonotic. The zoonotic species in order of decreasing virulence in humans are: *B. melitensis*, *B. suis*, *B. abortus* and *B. canis*. *Brucella* spp. can remain latent within the host's macrophages and cause chronic illness [1, 2]. Each *Brucella* spp. has a preferential host but can infect others either as a dead-end or incidental host. Humans are a dead-end host and very rarely infect other humans. *B. melitensis* is commonly found in sheep and goats. It can also infect cattle and dogs, and cattle can shed the organism in their milk [3]. *B. abortus* is commonly found in cattle and can infect sheep and dogs. Sheep can also shed the bacteria [3]. *B. suis* is commonly found in swine, but can be transmitted to cattle and horses which are dead-end hosts [3].

Human brucellosis is under-reported worldwide, but is most prevalent in Mediterranean countries, Central Asia, the Caucasus, Latin America and sub-Saharan Africa [3, 4]. *B. melitensis* is the most

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common cause of human brucellosis in the Mediterranean and Central Asia. *B. abortus* is most common in Latin America and sub-Saharan Africa [3, 4]. Reasons for under-reporting of human brucellosis include: lack of access to medical care, vague clinical signs and symptoms resulting in misdiagnosis, and the need for complicated laboratory techniques [5, 6]. Cases of chronic brucellosis can significantly reduce the quality of life. A decrease in productive life years can result from sequelae of chronic infections, including endocarditis, osteomyelitis, arthritis and meningitis [2].

Brucellosis in animals is most symptomatic in primary infections. *Brucella* spp. have a propensity for the pregnant uterus and in initially infected animals this results in a late-term abortion. Animals with recurrent infections do not repeatedly abort, but all infected animals have tissues, including aborted material, afterbirth and vaginal discharge, that are laden with bacteria. The bacteria are also shed in milk for up to 2 months post-parturition in sheep and for the duration of lactation in cattle. Infected tissues and milk are the main source of environmental contamination that spread the disease horizontally to other members of the flock or herd, and to humans [3].

Human infection is associated with factors and behaviours related to exposure to contaminated food products and shedding animals. Occupational exposure for shepherds, animal caregivers, veterinarians and milkers occurs when the individual is exposed to vaginal discharges, milk, aborted fetuses and infectious afterbirth tissues [3, 7]. Consumption of raw milk and contaminated dairy products also cause disease [5, 7]. Individuals who own livestock, have family members with the disease, consume raw dairy products or have a greater occupational exposure to the disease are at greater risk of contracting brucellosis [5, 6, 8–10]. In addition, preventive measures at the human level can include education [11].

The country of Georgia lies in a region of the world with a history of brucellosis. It is estimated that the annual human incidence is 27·6 cases per 1 million persons [12]. Georgian society is heavily dependent upon milk and meat from cattle and sheep. In addition, sheep exports to countries in Southwest Asia are growing; if infected these exports could spread brucellosis throughout that region.

To be effective, brucellosis control programmes need to be tailored to the country in which they are applied. Control of brucellosis in animals in Georgia may be challenging because after the collapse of the Soviet Union, all active animal health programmes were discontinued and have yet to be fully re-initiated. Thus, minimizing human brucellosis in the country of Georgia requires prevention of spread from animals to humans and understanding which risk factors for human brucellosis are most important. To identify these risk factors, further study on the disease ecology of brucellosis and the risk it poses to humans in Georgia is needed.

The purpose of this study was to identify potential risk factors associated with human brucellosis infection in the country of Georgia. Since this is the first study of its kind, the results can also be used as a guide to further research in the area of human brucellosis in Georgia. More specifically, this study explored animal-related, occupational, ethnic and regional diversity factors. The goal is to use the collected data and available information about the country to propose practical and effective control measures to reduce the spread of the disease in the country, to highlight needs for further research and to aid local medical professionals in understanding the epidemiology of brucellosis.

METHODS

Study design

We conducted a case-control study. Cases and controls were interviewed using a standard questionnaire. The online OpenEpi unmatched Case Control Sample Size calculator (http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm) was used to calculate the necessary sample size for 80% power and 95% confidence for the smallest risk difference of 20% as estimated by expert opinion at the Georgian National Center for Disease Control and Public Health and the Institute of Parasitology and Tropical Medicine (IP) [13]. This yielded a sample size of 83 cases and 83 controls. The sample size was rounded to 100 in each group to ensure adequate numbers in case some questionnaires were incomplete.

Case and control definitions

Both cases and controls were included from the IP in Tbilisi, Georgia from February to September 2010. Cases were incident cases of brucellosis and were defined as patients referred to the IP who upon examination presented clinically for brucellosis and were positive on both the plate and tube agglutination tests. Clinical cases were those that had at least six

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clinical symptoms of brucellosis. The IP is the only centre in Georgia that provides definitive diagnosis and treatment for human brucellosis. Therefore, it is necessary for individuals to travel from their home location to Tbilisi for care if brucellosis is suspected. In addition, recurrent brucellosis infections were excluded. Controls were defined as incident cases of parasitic infestation diagnosed at the IP from February to September 2010. Parasitic infestations include malaria, amoebiasis, trichinellosis, leishmaniasis, ascariasis, enterobiasis, fasciolosis, etc. Individuals who were previously diagnosed with brucellosis were excluded from the controls. The IP is the primary diagnostic and treatment centre for parasitic infections for Georgia. Only ascariasis and enterobiasis patients can be treated at other medical facilities in Georgia. Brucellosis is the only non-parasitic infection treated in the hospital. Patients come from all over Georgia for treatment of these diseases. The purpose of using hospital controls was to reduce the bias associated with subjects being able to travel to Tbilisi for treatment; therefore they are more representative of the population from which cases would arise, but are probably not representative of all Georgians [14, 15].

Questionnaire and interviewing

Upon diagnosis, physicians at the IP interviewed the cases and controls using a standard form. Questions included age, sex, ethnicity, region and municipality of residence and work, income level, education, occupation, travel, assisting in livestock births, livestock ownership (specifically sheep, cow, goat and/or swine ownership), whether other family members had brucellosis (if so, what was their age and sex and the frequency of consumption of raw milk), consumption of dairy products from raw milk, production of dairy products, milking and slaughtering. The questionnaires were reviewed for clarity and translated into Georgian by the collaborators at the National Center of Disease Control and Public Health in Georgia. Clinicians were subsequently trained to use the questionnaire. The study design obtained ethical approval following a review by the Colorado State University's Institutional Review Board.

Statistical analysis

Individual factors were summarized using either proportions with 90% confidence intervals or means with their standard deviations. In addition, frequencies and characteristics of the variables of interest were compared between cases and controls for differences in proportions or means using the *t* test, χ^2 test or Fisher's exact test where appropriate.

Multivariable logistic regression was used to estimate the associations between potential risk factors and the odds of brucellosis. Income was not used in the analysis because the responses varied between individual and household level information, making individuals non-comparable. Nominal categorical variables (e.g. consumption of raw milk products) were modelled as both 'yes/no' variables, and in their original categorical form. The assumption of linearity in the log odds was assessed for the continuous variable (age) by plotting the midpoints of the quartile *vs.* the logit and assessing for a linear trend ($R^2 > 0{\cdot}8$). If the assumption of linearity was not met, the variable was modelled using linear splines. In a separate analysis, age was analysed as a categorical variable to estimate risks for socio-demographically relevant age categories: 0–17 years (referent group) to represent school-aged children, who culturally have less animal contact than adults; 18–44 years to represent adults; and >44 years to represent the middle-aged and elderly.

Variables eligible for inclusion into a multivariable model were those with a *P* value $\leqslant 0{\cdot}25$ in univariable analysis. Multivariable model building was a backward selection process. Variables were retained in the model if removal significantly affected model fit (likelihood ratio $\leqslant 0{\cdot}10$). Variables with a *P* value $< 0{\cdot}10$ were discussed if considered biologically and culturally relevant. Categorical variables divided into dummy variables were evaluated using a partial likelihood ratio test and binomial categorical variables were evaluated with the Wald test. Factors that were not statistically significant during the backwards selection process were evaluated for confounding by adding them back into the model singly and factors that changed any of the model's coefficients by >15% were considered confounders. In addition, biologically and culturally significant factors were evaluated as effect modifiers as multiplicative interactions and were retained in the model if the *P* value was $\leqslant 0{\cdot}10$. The Hosmer–Lemeshow goodness-of-fit test was used to evaluate the overall fit of the model. Stata (StataCorp, USA) and Excel software (Microsoft, USA) were used for all analyses.

Some variables were condensed due to the low numbers within some categories. The occupation

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Fig. 1. Map showing the regions of Georgia [20].

category was collapsed into animal-associated employment, housewives, non-animal-associated employment, student or child, and unemployed or pensioner. The student and child group was the reference group because it best represented individuals with little or no animal contact. The provinces were categorized as western Georgia (reference), Kakheti, Kvemo Kartli, Mtskheta-Mtianeti and Shida Kartli, and Tbilisi. Western Georgia was comprised of patients from Samtskhe-Javakheti, Ajaria, Guria, Imereti, and Samegrelo and Zemo Svaneti (Fig. 1). Ethnicity was categorized as Georgian and non-Georgian. Finally, two of the 100 cases were missing data on variables that were significant to the analysis and had to be dropped from the analysis in order to be able to conduct the likelihood ratio test. The final sample size used in the study was 98 cases and 100 controls.

RESULTS

For each case and control diagnosed at the IP from February to September 2010, a questionnaire was completed, giving a 100% response rate. Evaluation of the differences in frequency and percentage of the descriptive characteristics of the cases and controls used in multivariable evaluation was performed and cases and controls differed in the distribution of gender, age category, occupation, province of residence, and ethnicity (Table 1).

Univariable analysis

Univariable analysis further assessed associations between odds of brucellosis and gender, livestock ownership (specifically cattle and sheep), consuming dairy products made from raw milk, making dairy products, milking, assisting in livestock births, slaughtering, having family members with disease, ethnicity, occupation, and province of residence (Table 2).

The frequency at which the variables of interest occurred for those who performed the activity among cases and controls was also studied. The frequency per week that cases made dairy products was significantly higher compared to controls (Table 2). Cheese was the most frequently consumed raw milk dairy product and was close to being statistically significant between cases and controls. The frequency of consuming raw milk dairy products was significantly higher in cases compared to controls (Table 2).

Multivariable logistic regression

Variables eligible for inclusion in a multivariable model included: gender, family members with disease, livestock ownership, cattle ownership, sheep ownership, milking, assisting in livestock births, slaughtering, occupation, age groups and age, ethnicity, and province of residence. Family members with disease fell out of the model because it was perfectly predictive. Cattle and sheep ownership were separately

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Table 1. *Comparison of characteristics between cases (n = 98) and controls (n = 100) from the Institute of Parasitology, Tbilisi, Georgia, 2010*

Factors	Cases (%)	Controls (%)	*P* value*
Gender			
Male	77 (79)	52 (52)	< 0·01
Female	21 (21)	48 (48)	
Age group (yr)			
0–17	14 (14)	66 (66)	< 0·01
18–44	52 (53)	27 (27)	
> 44	32 (33)	7 (7)	
Occupation			< 0·01
Student/child	14 (14)	70 (70)	
Animal related	33 (34)	1 (1)	
Housewife	7 (7)	8 (8)	
Non-animal related	30 (31)	15 (15)	
Unemployed/retired	14 (14)	6 (6)	
Province of residence			< 0·01
Western Georgia	2 (2)	31 (31)	
Kakheti	44 (45)	13 (13)	
Kvemo Kartli	30 (31)	24 (24)	
Shida Kartli/Mtskheta Mtianeti	8 (8)	20 (20)	
Tbilisi	14 (14)	12 (12)	
Ethnicity			< 0·01
Georgian	54 (55)	77 (77)	
Armenian	3 (3)	2 (2)	
Azerbaijani	38 (39)	19 (19)	
Kist	2 (2)	0	
Russian	1 (1)	0	
Ossetian	0	2 (2)	

* A χ^2 or Fisher's exact test of association was used where appropriate.

placed in the model instead of livestock ownership to provide more information than livestock ownership alone. In the model that analysed age as a categorical variable, milking and assisting in livestock births confounded the initial backwards-selection model and the odds ratios were calculated with and without adjusting for these variables. The assumption of linearity in the log odds was met for age ($R^2 = 0{\cdot}83$), it was therefore kept in its original form in the model that analysed age as a continuous variable. Interaction terms between owning sheep and making dairy products, owning sheep and assisting in livestock births, owning sheep and milking, milking and making dairy products, and making dairy products and consuming raw milk dairy products were investigated. No interaction terms significantly improved either of the two model fits.

For the model that assessed age as a categorical variable, the province of residence for Kakheti, Kvemo Kartli and Tbilisi had large odds ratios compared to western Georgia when all other variables were controlled (Table 2). Cases had 19·3 (90% CI 5–72·6) greater odds of being sheep-owners and 12·4 (90% CI 1·4–113) greater odds of being a dairy product producer (Table 2). Consuming dairy products appears protective with cases having 0·15 times smaller odds (90% CI 0·05–0·5) than controls of consuming dairy products. Compared to students and children, cases had 77·8 (90% CI 4·7–1278), 13·1 (90% CI 1·7–101) and 12·7 (90% CI 1·1–149) greater odds of working with animals, being unemployed or pensioners, and being employed in non-animal occupations, respectively. Finally, cases had 9·3 (90% CI 1·02–84·4) greater odds of disease when aged > 44 years compared to individuals aged 0–17 years than controls (Table 2). The Hosmer–Lemeshow goodness-of-fit *P* value was 0·28, indicating adequate model fit.

For the model that assessed age as a continuous variable, all of the same variables were significant except for age. The province of residence for Kakheti, Kvemo Kartli and Tbilisi had large odds ratios

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Table 2. *Descriptive, univariable and multivariable logistic regression results for risk factors for brucellosis with P values <0·10, Georgia, 2010*

Risk factor	Cases (*N*=98) *n* (%)	Controls (*N*=100) *n* (%)	Univariable OR (90% CI)	*P*	Multivariable (Model 1) OR (90% CI)	*P*	Multivariable (Model 2) OR (90% CI)	*P*	Multivariable (Model 3) OR (90% CI)	*P*	Multivariable (Model 4) OR (90% CI)	*P*
Own sheep	66 (67)	13 (13)	13·8 (7·5–25·2)	<0·01	22·4 (6·1–82·4)	<0·01	19·3 (5–72·6)	<0·01	20·3 (6–69)	<0·01	17·3 (4·9–60·4)	<0·01
Consume raw dairy products	59 (60)	49 (49)	1·7 (1·1–2·7)	0·06	0·14 (0·04–0·5)	0·01	0·15 (0·04–0·5)	0·01	0·13 (0·04–0·4)	<0·01	0·16 (0·05–0·5)	0·01
Make dairy products	49 (50)	7 (7)	13·3 (6·4–27·4)	<0·01	23·2 (4·8–111·5)	0·01	12·4 (1·4–113)	0·06	21·4 (4·7–97·1)	<0·01	11 (1·2–104)	0·08
Age (continuous)			1·07 (1·05–1·09)	<0·01								
Occupation						<0·01		<0·01		<0·01		<0·01
Students and children			Ref.		Ref.		Ref.		Ref.		Ref.	
Animal work			165 (29–938)	<0·01	119·1 (8–1771)	<0·01	77·8 (4·7–1278)	0·01	243·2 (25·6–2312)	<0·01	59·1 (4·7–744)	<0·01
Unemployed/pensioner			11·7 (4·6–29·7)	<0·01	13·4 (1·8–101·7)	0·04	13·1 (1·7–101)	0·04	30·3 (7·3–125·6)	<0·01	11·8 (2·2–63·5)	0·02
Non-animal work			10 (4·9–20·3)	<0·01	11·6 (1–130·8)	<0·10	12·7 (1·1–149·5)	0·09	60·2 (11·4–317)	<0·01	19·8 (2·4–162)	0·02
Age groups (yr)						0·06		0·08				
0–17			Ref.		Ref.		Ref.					
>44			21·6 (9·3–50)	<0·01	10·7 (1·2–95·4)	0·08	9·3 (1·02–84·4)	<0·10				
Province of residence						<0·01		<0·01		<0·01		<0·01
West Georgia			Ref.		Ref.		Ref.		Ref		Ref.	
Kakheti			52·5 (14·2–194)	<0·01	385·3 (13·1–11 313)	<0·01	278·1 (9·5–8101)	0·01	122·7 (8·4–1799)	<0·01	168·5 (8–3538)	<0·01
Kvemo Kartli			19·4 (5·4–69·8)	<0·01	174·2 (6·1–5003)	0·01	131·5 (4·7–3719)	0·02	54·5 (3·9–751·7)	0·01	76 (3·8–1505)	0·02
Shida Kartli/Mtskheta Mtianeti			6·2 (1·6–24·7)	0·03	44·5 (1·3–1487)	0·08						
Tbilisi			18·1 (4·6–70·7)	<0·01	994·1 (25·9–38 181)	<0·01	755·3 (20·3–28 096)	<0·01	310·8 (16·9–5720)	<0·01	450·4 (17·3–11 725)	<0·01

OR, Odds ratio; CI, confidence interval.

Multivariable model 1 uses age as a categorical variable without adjusting for confounders. Model 3 uses age as a categorical variable and adjusts for the confounders of milking and assisting in livestock births. Multivariable model 2 uses age as a continuous variable without adjusting for confounders. Model 4 uses age as a continuous variable and adjusts for the confounders of milking and assisting in livestock births.

The following variables were not found significant in the model: cow ownership, swine ownership, milking animals, assisting in livestock births, slaughtering, drinking raw milk, foreign travel, ethnicity (Georgian *vs*. non-Georgian), the 18–44 years age group, and working as a housewife.

compared to western Georgia when all other variables were controlled (Table 2). Cases had 17·3 (90% CI 4·9–60·4) greater odds of being sheep-owners and 11 (90% CI 1·2–103·6) times greater odds of being a dairy product producer (Table 2). Consuming dairy products appears protective with cases having 0·16 times smaller odds (90% CI 0·05–0·5) than controls of consuming dairy products. Compared to students and children, cases had 59·1 (90% CI 4·7–744·4), 11·8 (90% CI 2·2–63·5) and 19·8 (90% CI 2·4–161·8) greater odds of working with animals, being unemployed or pensioners, and being employed in non-animal occupations, respectively. Age, milking, and assisting in livestock births were identified as confounding variables and retained in the model. The Hosmer–Lemeshow goodness-of-fit *P* value was 0·57, indicating adequate model fit.

DISCUSSION

Both analyses identified living in Kakheti, Kvemo Kartli and Tbilisi, any occupation other than being a student or child, animal-related work, sheep ownership, and making dairy products as the most significant risk factors in this study. Consuming dairy products appeared protective.

Animal-related occupations included animal ownership, shepherd, milker and veterinarian, and the animal-related occupations were significant. This association of occupation with brucellosis infection is similar to other studies from former Soviet states and areas adjacent to the Caucasus [8, 10, 11]. Men and women take general care of the cattle, but the men milk and care for sheep, shepherd all livestock and slaughter animals. In this study, 96% of the subjects that were employed in animal-related work were male. Making dairy products is a female job. Gender was represented within these animal care roles and was not significant otherwise. Moreover, all groups of non-student occupations were identified as significant risk factors because they probably all owned livestock. Taking part in animal care occurred independently from animal-related employment. Animal ownership was therefore a more informative risk factor.

Those with brucellosis had 17·3–19·3 greater odds of owning sheep. Cattle ownership was not significantly associated with the risk of brucellosis. Sheep are the main reservoir for *B. melitensis* infections, but they can be infected with *B. abortus*. However, cattle, the *B. abortus* reservoir, are more prone to *B. melitensis* infection than sheep are to *B. abortus* [3, 7]. It is less likely that infected sheep carry *B. abortus* and more likely that infected cattle carry *B. melitensis*. Further, the majority of human patient cultures are *B. melitensis* and *B. melitensis* has been cultured from small ruminants [16, 17]. These findings indicate that *B. melitensis* is present in sheep and humans in the Georgia.

A cattle disease component is still possible. Cases had 11–12·4 times greater odds of being dairy product producers than controls. Further, dairy products are more commonly made from cow milk (88% of cases, 100% of controls) compared to sheep milk (18% of cases, 14% of controls). The increased disease odds associated with making dairy products combined with the high use of cow milk suggests a cow component to illness. This is strengthened by the fact that cattle are readily infected with *B. melitensis*, which also helps to explain why sheep ownership was significant – they are the primary reservoir for *B. melitensis* [3, 7].

The extremely high odds of brucellosis in individuals from Kakheti and Kvemo Kartli are probably associated with the large sheep populations in these regions. In 2009, Kakheti and Kvemo Kartli had 269 400 and 131 800 sheep, respectively, compared to Samtskhe-Javakheti in western Georgia with 87 400 sheep [18]. Sheep milk is used to make the Gouda cheese from this region. Moreover, predominately Muslim ethnic groups concentrate in these regions and include the Kists and Azerbaijanis. Their fat source is butter and rendered butter, which are made from raw milk. Butter can be contaminated with *Brucella* spp. Western Georgia is comprised primarily of ethnic Georgians, Kvemo Kartli has a large Armenian and Azerbaijani presence and Kakheti has a large presence of Muslims [19]. This ethnic distribution explains why ethnicity was not significant in the model; it was already represented by province of residence.

When age was analysed as a categorical variable, the odds of brucellosis in those aged >44 years appeared higher than in those aged $\leqslant 17$ years. However, when age was analysed as a continuous variable, it was not significant in the model, but did act as a confounder of the categorical occupational variable. Controls had a greater proportion of individuals aged <18 years compared to cases in this study. Because controls were patients diagnosed with intestinal parasitism, this potential association between brucellosis and age could possibly be due to bias. Children are more likely to exhibit pica behaviours and get intestinal parasitic infestations. Yet, if the apparent

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association between age and brucellosis was due to children being over-represented in the control group, then in theory brucellosis would have been similarly associated with both the 18–44 years and the >44 years age groups. Instead, only the >44 years age group approached significance in the model (OR 9·3, $P<0·1$); this may reflect a greater propensity for disease associated with ageing. Concurrent illness may make this age group susceptible to disease or disease recurrence. Of those involved in animal-related work, 79% were from the 18–44 years age group and 18% from the >44 years age group. The older age groups did not have a greater animal exposure. Therefore, the increased odds of being aged >44 years for cases is probably due to unmeasured confounders such as health status, healthcare access and nutrition.

There may be respondent bias regarding the results for dairy product consumption. Consuming raw milk dairy products was found to be protective, which is in contrast to other studies [8, 10, 11]. Georgians are aware of brucellosis and that un-aged cheese can carry disease. Therefore, individuals may not admit that they or their children became ill due to this well-known risk factor. Public awareness of the risk from contaminated cheese may create a bias that is more evident in the cases and causes cheese consumption to appear protective. It is also important to consider that our controls were patients with various forms of parasitic infestations and children are often over-represented in this population. If children also consume more dairy products, then this could also explain the protective effect seen in that category.

B. melitensis causes more severe disease in humans than *B. abortus*, so patients infected with *B. melitensis* may be more likely to seek treatment. This increased likelihood of seeking medical care could be a selection bias in the hospital-based sampling resulting in an overrepresentation of sheep ownership compared to cattle ownership.

Limitations

There are two significant limitations to this pilot study: the limited sample size and the difference in the distribution of age between cases and controls. As an initial study the size and scope of this project were limited and future studies would benefit from being multi-year studies that have sample sizes large enough to conduct analyses stratified by age or by conducting matching case-control studies on the appropriate variables made evident in this research.

The distribution of age differed in cases and controls. This difference could have affected estimates of risk of brucellosis for other variables including consuming dairy products. Dairy product consumption is a cultural component of Georgian life, but children may be more likely to consume raw milk or more dairy products in general. If that is the case, the preponderance of children in the control group could have biased our estimates for dairy product consumption.

Sample size was estimated based on expert opinion of the differences of exposures between brucellosis cases and controls, but the sample size selected did not allow for precision in the confidence intervals. For this reason statistical significance was set at 0·10 and confidence intervals of 90%, rather than 95%, were reported. Even with a low sample size and very wide confidence intervals, some variables were significant and other variables that approach significance should be considered for further study. Moreover, this study is representative of IP patients and may not be externally valid – a common concern with hospital-based samples because selection bias could result from the ability to access medical care. Ethnic minority populations are the primary inhabitants of some villages in Georgia and the language is not Georgian. They may not pursue treatment and medical care in the current system. The same bias is likely to be present for the controls as well. Nonetheless, the cases and controls are comparable.

CONCLUSIONS

The centrally provided diagnostics and medical care may prevent adequate access to all citizens and this inaccessibility prevents complete identification of brucellosis cases and their associated risk factors. The high odds ratios of certain provinces reflect that the majority of Muslims and sheep are also in these regions. Working with animals is a significant risk factor, but regular animal contact in general is important to consider because occupations did not delineate who did and did not have animal contact. The other key risk factors were being aged >44 years, making dairy products, and owning sheep. *B. melitensis* may be the causative agent since sheep are significantly associated with disease and are the reservoir for *B. melitensis*. However, it does not preclude human infections from *B. melitensis* and *B. abortus* from cattle. Methods to reduce disease in the human population should be focused on controlling disease

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at the sheep level. In addition, education of individuals who work with animals regarding routes of infection from shedding animals could also play a significant role in reducing disease [11].

Further study in this area is both warranted and required based on these results. This research reveals that animal contact is important, but does not clearly determine if there are different risks at different ages and how this is related to dairy product consumption. Future studies should be multi-year and/or matched for age to ensure a better precision in the risk factors. In addition, future studies may want to focus on regional differences that could exist between Kakheti, Kvemo Kartli, western Georgia, Tbilisi, and central Georgia (Mtskheta-Mtianeti and Shida Kartli).

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DECLARATION OF INTEREST

None.

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