Cattle-associated risk factors for human tuberculosis in rural livestock keeping communities, Uganda

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Abstract

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Tuberculosis (TB) is a leading infectious cause of human death worldwide. TB can also infect cattle, resulting in productivity losses, trade barriers, and zoonotic transmission via milk, meat, or direct contact. While the majority of TB cases are non-zoonotic, an unknown proportion are acquired from cattle; in Africa, this proportion is estimated to be 0.4% to 10%. We conducted a cross-sectional study in rural communities in southeastern and northwestern Uganda between 2014 and 2016 to evaluate the association between tuberculosis skin test (TST) positivity in humans and cattle-associated risk factors. Human and cattle skin testing was performed in communities followed by a survey of household practices. TST data are available on 493 humans, 250 men and 243 women; 184 individuals in total—111 men and 73 women—tested positive. Separate log binomial models were fit to estimate relative risks (RR) for herd TST positivity stratified on gender and for raw milk consumption, using generalized estimating equations. Having at least one TST positive bovid in the household's herd was significantly associated with decreased risk of TB among men (PR 0.61, 95% CI 0.47, 0.79) but was not

significantly associated with TB among women (PR 1.26, 95% CI 0.80, 1.97). This was contrary our *a priori* hypothesis of higher effect of exposure among men—the primary caretakers of cattle—than women. This apparent protective effect may be the result of residual confounding by socioeconomic status: wealthier individuals may be less likely to be TB positive, but more likely to have TST positive herds by virtue of larger herd sizes, ability to purchase new and possibly infected stock, and propensity to keep more TB-susceptible European breeds. For raw milk consumption, effect estimates were close to one and not statistically significant; adjustment for confounders or pathways mediated by exposure to non-zoonotic TB did not change the size or magnitude of effect estimates. The importance of cattle-associated risk factors for human TB burden may be setting-specific, as suggested by the lack of consensus reached by prior research. In settings where bovine TB prevalence is low, such as Uganda, cattle-associated zoonotic transmission may be rare.

Introduction

Tuberculosis (TB)—caused by infection with members of the *Mycobacterium tuberculosis* (MTB) complex—is a leading infectious cause of death worldwide, with an estimated annual cost of over US$12 billion [1]. Most members of this complex are zoonotic pathogens, resulting in animal morbidity and mortality in addition to human illness, and thereby compromising human livelihood and access to animal-source protein [2]. While the majority of human TB cases are non-zoonotic, an unknown proportion are acquired from cattle; in Africa, the WHO estimates this proportion to be between 0.4% and 10% [3].

The preponderance of research effort on zoonotic tuberculosis has been devoted to *M. bovis*, the most common causal agent of bovine tuberculosis (bTB). This is largely because bTB is a 'listed disease' by the World Organization for Animal Health (OIE) and as such trade restrictions on animals and animal products originating from bTB-endemic regions are justified in accordance with the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures [4]. Bovine tuberculosis is endemic in many low- and middle-income countries, resulting in trade losses and hampering economic growth, in addition to causing human and animal illness [3].

Members of the MTB complex cannot be distinguished on the basis of clinical or pathological manifestation; speciation requires sophisticated and costly laboratory methods and is thus rarely performed in resource-limited settings [5]. Extrapulmonary TB is often used as a proxy indicator for zoonotic TB, as zoonotic transmission occurs primarily through consumption of unpasteurized contaminated milk, resulting in gastrointestinal and other extra-pulmonary lesions [6]. However, estimates of zoonotic transmission based on incidence of extrapumonary

TB in the absence of speciation, as well as estimates based on incidence of *M. bovis* when speciation is performed, may not accurately estimate the true burden of zoonotic tuberculosis.

First, close contact with infected animals can result in aerosol transmission and subsequent pulmonary tuberculosis[7]. In sub-Saharan Africa, where livestock production systems frequently result in close contact with cattle and bTB is poorly-controlled, zoonotic-origin pulmonary TB (PTB) is likely to occur. Secondly, *M. tuberculosis*, and, more recently, *M. africanum* infection have been documented in cattle, though the dynamics of anthroponotic and zoonotic transmission of these pathogens have not been well-established [8-10]. Lastly, minimal research effort has been focused on the other members of the *M. tuberculosis* complex, including *M. canettii* and *M. microti.* Thus, despite decades of human and animal research on the topic, the importance of zoonotic transmission of tuberculosis remains an unsettled debate.

To explore this issue, we used data from a cross-sectional survey administered in rural Uganda from 2014-2016 to evaluate the association between cattle-related exposure variables and tuberculosis skin test (TST) positivity in humans, above and beyond risk resulting from exposure to other humans. The primary aim was to evaluate TST positivity in the cattle herd as the exposure of interest, with TST positivity in humans as the outcome. As a sub-aim, we evaluated whether this relationship differed by sex, as men are the primary keepers of the cattle herd and thus will have more sustained contact with infected cattle.

The tuberculin skin test (TST)—also called the Mantoux skin test in humans—is the mainstay of TB screening in humans [11] and cattle [12]. In both species this test involves intradermal injection of tuberculin purified protein derivative (PPD), followed by evaluation at 48-72 hours for local reaction. While in cattle the validity of this test varies with administration

method, the sensitivity of the commonly-used caudal fold test (CFT)—in which PPD is administered to a tail fold—is estimated to be as low as 53.6% [13].

To address this potential low sensitivity of our measure of TB in cattle, as a secondary aim we studied the relationship between human TST positivity and consumption of raw milk as the exposure of interest. As most milk consumption in rural Uganda is from one's own herd, in the absence of TB infection in the cattle herd consumption of raw milk should not increase one's risk of TB; thus, increased risk of TB associated with raw milk consumption would suggest the presence of bTB in the cattle herd.

By not restricting the definition of the outcome to confirmed *M. bovis* cases, this study ensures cases of zoonotic-origin *M. tuberculosis* will not be overlooked. While several prior studies [6, 14-18] have investigated the association between human TB and cattle-associated risk factors without distinguishing between members of the *M. tuberculosis* complex, the findings of these studies do not reach a consensus: among those that evaluated bTB positivity in the cattle herd as a risk factor [14, 15, 17], odds ratios for TB positivity in humans ranged from close to one and non-significant (OR 1.2, 95% confidence interval 0.6, 2.4 [17]) to strongly positive (OR 8.32, 95% CI 2.82-24.6 [15]), and among those that evaluated raw milk consumption as a risk factor [6, 14, 15, 17, 18], odds ratios ranged from less than one and non-significant (OR 0.3, 95% CI 0.5-1.8 [17]) to, again, strongly positive (OR 8.8, 95% CI 2.6-29.81 [15]). These studies differed both from each other and from this study in terms of study setting—country, hospital- vs. population-based—control population, outcome definition—latent TB infection vs. TB disease with—and measurement, and other factors.

We hypothesize that individuals with TST positive cattle—commonly called TB reactors—in their herd are at a higher risk of being TST positive than individuals without TB

reactors in their herd, with this risk being greater in men than in women. Additionally, we hypothesize this risk will be even higher when consumption of raw milk is considered as the exposure rather than TB reactors in the herd, as the low sensitivity of the TST in cattle likely results in frequent misclassification of this exposure, attenuating the estimated association.

Methods

STUDY DESIGN

These data are a subset of a larger cross-sectional study administered by Veterinarians Without Borders, an NGO engaged in livestock and nutrition development projects in Africa. In addition to tuberculosis, the parent study also studied zoonotic transmission of brucellosis and trypanosomiasis and the nutritional status of livestock-keeping communities in Uganda.

STUDY SETTING

We performed data collection in rural communities in Iganga District in southeastern Uganda and Arua and Moyo districts in northwestern Uganda between 2014 and 2016. Within targeted districts (n=3), a total of 13 subcounties, 18 parishes, and 71 villages were selected for sampling by local government officials, typically Animal Health Officers (AHOs) and/or District Veterinary Officers (DVOs). The same government official or a colleague then mobilized households within the study villages, with all livestock-owning households being eligible for participation. Several of the communities sampled are polygamous, with all polygamous households in this dataset comprising one husband and two wives. As each wife typically lives with her children separately from the other wives and with distinct household practices, for the purposes of the survey and human sampling, households were defined by wife. As cattle ownership is by husband, cattle herd was defined by husband. These households were linked by

assigning the husband's household a number, and identifying each wife's household as a lettered addendum to this number.

The first consenting households up to a pre-determined number were selected to be the study sample for each village. This number varied over time (mean 5.0 households per village, range 1-49) based on budget and timeline considerations. Survey administration and skin testing of human subjects was performed at each participant's home, and skin testing of cattle was performed at kraals if cattle were not kept at home.

STUDY SUBJECTS

All consenting and present adult household members were eligible for tuberculosis skin testing. Because BCG vaccination is commonly administered in Uganda and may lead to false positive TST results in children and teenagers, these analyses were restricted to individuals 18 years of age or older. No further eligibility criteria were applied. While 2,435 individuals were sampled in total, the majority of these were either too young for TB skin testing, declined TB testing, or could not be located to read test results; complete TB skin testing data were available on 796 individuals from 505 wives' households.

DATA COLLECTION

Sampling typically took place over a two day period. On day one, cattle keepers were mobilized to a kraal—an enclosure for domestic animals, common in sub-Saharan Africa—and TST was performed on 50-60 cattle in total, corresponding to five households. Tested cattle represented a convenience sample of each household's herd, with on average 84.7% (range 4.4-100%) of a household's herd being tested. The first cattle caught from each household up to a

pre-determined number comprised the study sample, with this number being determined as for household sampling.

On day two, the study team visited the households corresponding to the previous day's cattle herds. The team administered human PPD to all eligible household members, and one member of each household—typically the head of the household—completed the household survey. Surveys were developed and printed in English and administered as in-person interviews. Survey translation into the local language was simultaneous with interview administration, with both being performed by a trained and bilingual local member of the study team.

For both cattle and humans, TST interpretation was performed by a trained member of the study team 48 to 72 hours after PPD administration; this was conducted at kraals for cattle and for human subjects accompanying their cattle herd on the day in question, and at households for all other study subjects.

MEASURES

The primary outcome was TST positivity in humans, measured by the Mantoux skin test. As prior BCG vaccination and exposure to environmental mycobacteria can result in false positive TST results, we defined a positive response as a 15 mm or greater reaction, to improve specificity and minimize misclassification[19]. We defined cattle herd positivity as the presence of at least one TB reactor in the cattle herd, with positivity being defined as a 10mm or greater reaction to PPD administration.

We also evaluated the relationship between TST positivity and raw milk exposure, as self-reported by the household survey respondent. Two variables—modeled as individual covariates—comprise this exposure: sometimes versus never consumption of raw milk in the

household, and frequency of milk consumption in the household. Consumption of raw milk was defined as “sometimes” if the question “How is milk prepared for personal use?” was answered as “boiled and unboiled” or “unboiled”, and “never” if the answer was “boiled”. Frequency of milk consumption was defined by the response to the survey question “How many days in the past week the household has consumed milk?” with answers ranging from zero to seven and without distinction between raw and boiled milk.

Lastly, to evaluate whether there was a cumulative effect of both cattle herd exposure and raw milk consumption, we explored a four-level categorical joint exposure variable in a separate model: exposure to both raw milk (sometimes consumption within household) and TB reactors (at least one TB reactor in the household’s cattle herd); exposure to raw milk but no exposure to TB reactors; exposure to TB reactors but not raw milk; exposure to neither raw milk or TB reactors.

DATA ANALYSIS

Characteristics of individuals who had at least one TB reactor in their cattle herd were compared to those of individuals with no TB reactors in their cattle herd using descriptive statistics. Similar comparisons were made for individuals with positive versus negative TSTs, and for individuals residing in households that reported raw milk consumption versus households reporting no raw milk consumption.

Multivariable analyses were conducted using relative risk regression—log binomial regression—using a generalized estimating equation (GEE) to account for clustering by wife’s household, assuming an exchangeable working correlation structure. A GEE model was selected over a multi-level model as we were interested in estimating the marginal association between

cattle positivity and human TST positivity—"averaged" across wives' households— rather than the conditional association—that is, the association specific to each household. Household identifiers were used as clustering variables; in polygamist households, the wife's household was the clustering variable. This selection was based on the *a priori* hypothesis that the most important level of outcome clustering would be the household in which the individual resides. Missing data were addressed using complete case analyses, where regression models included only observations that had no missing data in any modeled covariates.

We identified confounders to be adjusted for in the final regression models in three steps: first, we defined *a priori* risk factors for TB based on prior studies. Second, we constructed a directed acyclic graph (DAG) using DAGitty.net to clarify causal hypotheses, identify the minimal adjustment set, and avoid over-adjustment bias (Figure 1). Last, we evaluated associations with each exposure of interest—TB reactors in the cattle herd, and raw milk consumption—and the variables in this set qualitatively, on the basis of bivariate frequency tables. Effect modification by sex was judged on the basis of statistical significance testing of an exposure*effect modifier interaction term. Where statistically significant effect modification was found, we presented sex-stratified results.

Characteristics identified as risk factors for TB based on prior evidence were: inadequate ventilation [16], approximated by "Number of windows"; crowding within the household [18], approximated by "Number of people sleeping together in a single room"; knowledge of TB, judged to be "yes" if the response to the household survey question "Have you heard of [zoonotic] diseases before," was "TB" or "yes, TB" [20]; exposure to human TB cases, as determined by TST results in other household members; and socioeconomic status [21], as

approximated by four separate variables: house structure, number of rooms in the home, cattle herd size, and educational attainment of household survey respondent.

The minimal sufficient adjustment set for the effect of herd positivity on human TST positivity was cattle herd size and knowledge of TB ("total" effect). To isolate the effect of bTB exposure that does not go through exposure to other TST positive individuals, we repeated analyses with adjustment for the presence of TB in other household members ("direct" effect). Both total and direct effect models were fit for all analyses, however as the estimate of interest was the direct effect, a full mediation analysis was not performed.

For the exposure to TB reactors, effect modification by sex was evaluated on the basis of statistical significance testing of a gender*exposure interaction term, at an alpha level of 0.05. For the exposure to raw milk, two models were fit; first, TST positivity was regressed on sometimes vs. never consumption of raw milk and potential confounders; next, TST positivity was regressed on frequency of milk consumption and potential confounders, restricting the analysis to individuals residing in households that reported sometimes consumption of raw milk.

The joint exposure variable, as described above, was modeled as a four-level categorical variable without adjustment for any covariates.

The STROBE checklist was used to guide the reporting of this manuscript [22]. The parent study was approved by the Mildmay Uganda Research and Ethics Committee (REC REF 0406-2015) and registered with the Uganda National Council for Science and Technology (approval #1830). Data received for this analysis did not contain any participant identifiers, thus the Human Subjects Division of the University of Washington determined this activity to constitute 'non-engagement' with human subjects.

Results

PARTICIPANTS

A total of 505 wives' households (representing 494 husbands' households and 2,435 individuals) were enrolled. After excluding duplicate observations (N=70) and those with missing exposure data (N=14 missing household survey data, N=678 missing cattle TST data), 1,673 individuals from 295 wives' households and 284 husbands' households remained. After exclusion of individuals with missing TST data (N=1,174) and those under 18 years old (N=6), 493 individuals from 272 wives' households and 262 husbands' households were available for analyses (Figure 2). For the purpose of describing patterns of missingness, descriptive statistics for an additional 293 individuals over 18 years of age with non-missing exposure data but with missing TST data are provided in the supplementary materials (Table S1).

STUDY SAMPLE

Sample characteristics are presented in Tables 1a, 1b, 2a, and 2b. The study population (N=493) was on average middle-aged and tended to reside in a hut with a mean of 2.5 rooms, 4.5 windows, and 3.6 persons sharing a room (Table 1a). Participants tended to reside in a household with the respondent reporting milk consumption 2.5 times per week (Table 1b), attained primary education, knowledge of TB, and—among those that reported herd size—a cattle herd size of just under 24 head. On average, just under one person per household was found to be TST positive. Relative to those without TST data, those with TST results were more likely to have a slightly larger cattle herd (23.9 head vs. 19.6 head) and tended to have slightly smaller household sizes (mean 8.1 vs. 9.3). There were no other substantial differences (Table S1).

Characteristics of individuals with TB reactors in the cattle herd

Individuals with TB reactors in their cattle herd were more likely to be male (56.8% vs. 49.4%) (Table 1a). They also tended to have larger household sizes (mean 8.8 vs. 7.9 individuals); were more likely to be Anglican (19.7% vs. 11.3%) and less likely to be of other Christian religion (64.8% vs. 70.3%) or Muslim (15.5% vs. 18.4%); and were more likely to have purchased new stock in the past year (35.4% vs. 20.3%), keep all of their animals together (40% vs. 26.6%), live close to the used kraal (70.9% close or very close vs. 57.7%) and co-house with livestock at night (10.7% vs. 4.4%) (Table 1a).

With respect to TB and TB exposures, individuals with TB reactors in their cattle herd were more likely to consume raw milk (30.9% vs. 27.9%) and less likely to be TST positive (33% vs. 38.3%) (Table 1b). Individuals with and without TB reactors in their herd did not appreciably differ in other TB related variables studied.

Characteristics of individuals reporting raw milk consumption

Compared with individuals that never consume raw milk, individuals that sometimes consume raw milk were more likely to live in a hut (100% vs. 86.6%), less likely to have recently purchased new stock (14.8% vs. 26.3%) or co-house with their livestock at night (5.7% vs. 2%), and tended to have fewer windows (3.35 vs. 5.41) and cattle (mean 10.44 head vs. 28.28 head) (data not shown).

Characteristics of individuals with positive TST

Compared with TST negative individuals, TST positive individuals were more likely to be male (60.3% vs. 45%) and tended to have slightly larger cattle herds (mean 28.1 vs 21 head) (Table 2a). Additionally, TST positive individuals were less likely to be Anglican (9.9% vs. 14.2%) or Muslim (14% vs. 20.3%), more likely to use a communal grazing management system

(48.3% vs. 35.5%) versus tethering or both systems, less likely to house all animals together (25% vs. 30.6%), and more likely to live far from the used kraal (45.6% vs. 36.5%).

TST positive individuals were less likely to consume raw milk (22.8% vs. 32.1%) and somewhat less likely to have at least one TB positive animal in the cattle herd (15.8% vs. 19.1%) than TST negative individuals (Table 2b). Individuals with and without TST positivity did not appreciably differ in other TB related variables studied.

PRIMARY ANALYSES

Exposure to bovine tuberculosis

The frequency of TST positivity among those with one or more TB reactors in their cattle herd was 33%, versus 38.3% among those with no TB reactors in their cattle herd. As none of the potential confounders identified—cattle herd size and knowledge of TB as reported by the household's survey respondent—were associated with exposure to cattle TB reactors, none were included in the final model.

In unadjusted analyses, individuals with a TB reactor in their herd had a 15% lower prevalence of a positive TST than individuals without any TB reactors in their herd, but this was not statistically significant (PR 0.85, 95% CI 0.62-1.17). Sex was a significant effect modifier of this association; among men, the prevalence ratio was 0.61 (95% CI 0.47, 0.79), corresponding to an absolute prevalence of 0.49 among unexposed men and 0.30 among exposed men, versus 1.25 (95% CI 0.80, 1.98) among women (Table 2), corresponding to an absolute prevalence of 0.29 among unexposed women and 0.36 among exposed women.

After controlling for pathways mediated through exposure to other TST positive individuals—via adjustment for TST positivity in the household—the overall and sex-specific

prevalence ratio for exposure to cattle reactors was unchanged (Table 3). in exploratory analyses varying the TST cutpoint to 5mm and 10mm, effect estimates were found to be greater than one, but still close to one and non-significant (Tables S2a and S2b).

Exposure to raw milk

Among those who reported on raw milk consumption (n=439), those who sometimes consume raw milk had a TST prevalence of 31.2% (n=39), versus 42% (n=132) among those who never consume raw milk. Although cattle herd size and self-reported knowledge of TB were potential confounders of this association, cattle herd size was not adjusted for in the primary analysis due to the large amount of missing data (169 out of 493 observations missing).

After adjustment for knowledge of TB, those that ever consumed raw milk had a 27% lower prevalence of TST positivity, however this association was not statistically significant (PR 0.73, 95% CI 0.54, 1.00) (Table 3). After controlling for both TB knowledge and exposure to other TST positive individuals, the association was marginally statistically significant but the strength of the association was largely unchanged (PR 0.71, 95% CI 0.51, 0.98). Among those who reported raw milk consumption, there was no association between frequency of milk consumption and TST prevalence after adjustment for knowledge of TB (PR 1.01, 95% CI 0.93, 1.09). Exploratory analyses varying the TST cutpoint did not appreciably change effect estimates (Tables S2a and S2b).

Joint exposure

The group with exposure to neither raw milk nor TB reactors in the cattle herd served as the reference group (n= 258, 42.6% TST positive). One hundred individuals had raw milk exposure without exposure to TB positive cattle, of whom 32 (32%) were TST positive,

corresponding to a prevalence ratio of 0.75 (95% CI 0.55, 1.03). Fifty six individuals had exposure to TB positive cattle without raw milk exposure, of whom 22 (39.3%) were TST positive, corresponding to a prevalence ratio of 0.92 (95% CI 0.70, 1.22). Lastly, 25 individuals had both exposures, of whom 7 (27%) were TST positive, corresponding to a prevalence ratio of 0.66 (95% CI 0.29, 1.51) (Table 3).

EXPLORATORY AND SENSITIVITY ANALYSES

Effect estimates were largely unaffected by inclusion of individuals less than 18 years of age (Table S3) and exclusion of polygamous households (Tables S4a and S4b). Multi-level models clustering on village and using binary exposure to cattle TB reactors and village-level bovine TB prevalence as the predictor—in two separate models—also did not produce appreciably different effect estimates (Table S5). In an analysis of potential risk factors for individual-level cattle TST positivity, only cattle herd size and management system were statistically significant predictors; no significant associations were found for the purchase of new stock in the past 12 months or co-housing with humans at night (Table S6).

Discussion

Rates of TST positivity are high in this population, being almost 40% overall. In contrast, although herd positivity rates were moderate—15% of cattle herds tested contained at least one reactor—individual bovid positivity rates were low at 3.5%. Contrary to study hypotheses, exposure to bTB—defined as presence of at least one TB reactor in the cattle herd—was associated with a slightly lower prevalence of TST positivity, although this was not statistically significant. Also contrary to prior hypotheses, in sex-specific analyses bTB exposure was associated with a significant decrease in risk of TST positivity in men, but with a somewhat

increased risk in women, although the latter was not statistically significant. Over one quarter of the study sample consumed raw milk at least some of the time. However—again contrary to prior hypotheses—consumption of raw milk was associated with a nearly 30% lower risk of TST positivity after adjusting for possible TB in other household members and knowledge of TB; frequency of milk consumption was not found to modify this association. Adjustment for pathways going through exposure to human-origin TB did not appreciably change results of either analysis; while marginal statistical significance achieved for sometimes consumption of raw milk after adjustment for this variable, this may be an artefact of multiple testing.

Some stratified sample characteristics are of note. Firstly, the two exposures were associated; that is, those with bTB in their cattle herd were slightly more likely to consume raw milk than those without bTB in their cattle herd. Despite this, there was no evidence of association between joint exposure and TST prevalence. This may be explained by the negative association between raw milk consumption and co-housing with livestock at night—no individuals were observed to have all three risk factors for zoonotic transmission.

Secondly, while the sex distribution of the study population was representative of that of Uganda generally (51.3% female in the fully study population, 51% female across Uganda per the 2014 census[23]), TST positive individuals were more likely to be male (60.3%), consistent with prior literature suggesting the burden of TB is greater among men than among women[24]. Furthermore, individuals with TB reactors in their cattle herd were more likely to be male (56.8%), while individuals with raw milk exposure were more likely to be female (56%). This apparent association between sex and the two exposures under study may be the result of individual- or household-level selection bias, or to differential patterns of exposure among

female-predominant vs. male-predominant households. However, sex distribution was not found to differ between polygamous vs. monogamous households.

Individuals—and thus their corresponding households—with TB reactors in their cattle herd appeared to be of greater socioeconomic status (SES) than those without TB reactors in their herd, as indicated by slightly larger herd sizes and higher reporting of recent stock purchases. Conversely, those reporting raw milk consumption seemed to be of lower SES than those who never consume raw milk, evidenced by greater residence in huts as opposed to permanent houses, fewer windows in the home, lower reporting of recent stock purchases, and smaller cattle herds. Finally, compared with the study sample as a whole, individuals reporting consumption of raw milk were far more likely to be Muslim, consistent with a 2010 research report which found a preference for raw milk among Muslims in Bangladesh[25].

This study is limited by low sensitivity. First, the high baseline prevalence of TST positivity makes any additive increase in risk small on a relative scale, and thus difficult to detect. Second, the high prevalence of childhood BCG vaccination in this population may result in false positive TST results; we attempted to mitigate this source of bias by excluding individuals under 18 years of age and using a strict criterion for TST positivity—15mm—but cannot rule-out the possibility of false positive TST results. Third, there is a potentially large degree of misclassification of the exposure of interest—bTB exposure—arising from the low sensitivity of the caudal fold test (estimated to be as low as 53.5% [13]) and low herd testing coverage in some households. At the 25th percentile of the distribution, only 33% of the household's herd was tested; assuming sensitivity is 53.6%, the likelihood of a bTB-positive bovid in this herd being both tested and testing positive is only 17.7%. Given the low sensitivity of CFT, it was not possible to evaluate the relationship between the number of individual cattle

with bTB and TST positivity, despite the greater statistical power and approximate exposure dose afforded by a continuous measure. Furthermore, sensitization by non-tuberculous mycobacteria (NTM)—commonly detected in Uganda[26]—may result in false positive CFT results [27], compromising specificity of this test. Finally, even among truly positive cattle, only a small proportion may be shedding: Romero Tejeda *et al*. (2006) found only 26% of CFT positive cattle to be excreting *M. bovis* in nasal sections [28]. Thus, exposure to CFT positive cattle is, at best, a rough approximation of exposure to bovine tuberculosis. All of the mechanisms are expected to result in non-differential misclassification of exposure, thus attenuating estimated prevalence ratios.

We attempted to overcome this limitation by studying consumption of raw milk as an exposure, but did not find appreciably different results. This may also be the result of compromised sensitivity; for the sake of statistical power we collapsed "sometimes" and "always" raw milk consumption into a single "sometimes" category, thus treating individuals with heterogeneous exposure to raw milk as equivalent. While we did not find frequency of milk consumption to modify this association, frequency of raw milk consumption was not measured, and may indeed modify this association. Lastly—as with all studies of TB—sensitivity of this study is compromised by the inability to restrict our study sample to individuals with genetic susceptibility to TB, as genetic factors are known to confer susceptibility and resistance to this disease [29].

Inherent to observational studies is the threat of confounding. Most notably, it was not possible to adjust for HIV status in this analysis, as these data were not available. HIV is a known risk factor for tuberculosis, and while it is not expected to be directly associated with bTB in one's cattle herd, it may be indirectly associated via SES, or by sampling variability (chance).

The direction of bias induced by this residual confounding would depend on the direction of the HIV-bTB association. Additionally, we did not adjust for age or religion, nor did we adjust for sex in the raw milk analyses. Sex is a known risk factor for TB as described above; TB presentation is known to differ in elderly adults versus younger adults, and it is reasonable to assume risk of acquiring TB may differ between these populations [30]; finally, a greater risk of TB among religious minorities has been documented in other countries [31]. We did not adjust for these variables as we did not *a prori* hypothesize an association with the exposures of interest, and thus did not include them in the constructed DAG. However, association by chance with the exposures of interest would result in residual confounding by these variables. Sensitivity of these results to adjustment for these variables will be explored in future analyses.

Our choice of model may also have introduced bias into the results. The use of a GEE model clustering on household assumes outcomes are independent between households, which may be violated by practices common to livestock management in rural communities—communal grazing, shared husbandry tasks across households, transfer of animals between households as gifts or transactions, close proximity of households, milk pooling, etc.—that bring individuals into contact other households' herds. However, when we applied a multi-level model with clustering at the village level, our effect estimates did not change appreciably, and we expect such 'spillover' to be minimal across villages.

While the use of population-based sampling generally minimizes selection bias, convenience sampling may counteract these gains. Selection bias may arise if those in the study population differ from the underlying population with regards to their distribution of important covariates. This study did not include individuals whose household was not selected for sampling or did not consent, those who declined to participate or consented only to collection of

morphometric data, or those that were lost to follow-up for TST interpretation. In this sample, the sex distribution of the overall study population was similar to that of Uganda as a whole, suggesting study participation was similar between men and women. However, those with complete TST data were more likely to be male than those with missing TST data, suggesting men were more likely to consent to TST than women, or were more readily located for test interpretation than women were.

All variables were measured at the household-level with the exception of age, sex, and TST. We do not expect milk preparation or frequency of milk consumption to vary within a household or for self-reported measurement of this outcome to be subject to substantial error. Knowledge of TB was assessed at the household level by the survey respondent (typically—but not strictly—the male head of household in the case of polygamist households, and the female head of household otherwise), and the question did not distinguish between familiarity with TB generally versus zoonotic TB. Assuming that knowledge of TB—and, consequently, behaviors to reduce the risk of transmission—is shared within a household, minimal misclassification is expected. The number of other household members with positive TST results is subject to misclassification due to missingness in the TST variable. In households owning more than one housing structure, exposure to TST positive individuals may not be homogeneous across household members. Furthermore, the variables selected to approximate SES may not adequately capture this latent construct. Finally, TB disease may result in false negative TST results due to anergy, resulting in misclassification of the outcome [32].

Of the studies that have evaluated the association between animal-associated risk factors and human TB infection or disease, four performed cattle testing for bTB [14-17], although one of these studies [16] did not further utilize these data. Two case-control studies conducted in

Ethiopia found a strong increased risk of TB disease in humans among those exposed to bTB positive cattle: an OR of 8.32 (95% CI 2.82, 24.60) for herd prevalence after adjustment for confounders[15], and an OR of 3.31 ($p < 0.001$) for individual cattle prevalence without adjustment [14]. Conversely, a cross-sectional study from Ethiopia did not find having at least one bTB reactor in the herd to be a significant risk factor for human TB (OR 1.2, 95% CI 0.6, 2.4), without adjustment for confounders [17].

All six studies of risk factors for zoonotic TB evaluated raw milk consumption as an exposure. Two population-based cross-sectional studies, one conducted in Tanzania [16] and one in Ethiopia [17], found no association between raw milk consumption and TB risk, consistent with our findings. However, three case-control studies conducted in Ethiopia and one conducted in Nigeria did find a strong association: OR 8.8 (2.6-29.81) in Ethiopia, after adjustment for confounders [15]; OR 6.4 (95% CI 2.4-17.2) in Nigeria, after adjustment for confounders[18]; OR of 3.23 ($p < 0.001$) in Ethiopia, univariate analysis [14]; and OR of 1.9 (95% CI 1.2-2.8) in Ethiopia, after adjustment for confounders[6]. There are several potential explanations for this discordance between our findings and the prior literature, including poor generalizability across studies, differences in exposure ascertainment and case definition, differences in study design, and differences in methods of covariate selection in multivariable models.

While this study as well as most of the prior literature was conducted in rural, subsistence agriculture settings, the majority of these prior studies were conducted in Ethiopia; the only one conducted in Central East Africa was concordant with our findings[16]. This suggests poor generalizability across study settings—from Central East Africa to the Horn of Africa—may drive the inconsistency of results. While herd TB prevalence was similar between our study (15%) and the concordant Ethiopian study (12.4% [17]) this exposure was far more prevalent in

the studies that reported an association (23.6% [15] to 43.3% [14]). Raw milk consumption was also far less common in our study (28.5%) and the concordant Tanzanian study (18.8% [16]) than in the studies identifying an association (49.3% [15] to 87.1% [14]). However, raw milk consumption rates were also high in the Ethiopian study that did not report an association (68.5% [17]). Similarly, sharing of housing with livestock and absence of windows—potential mediators of the pathway under study—were far less commonly reported in our study than in all other studies. Thus, it is possible that the intensity of human-animal contact is greater in Ethiopia, explaining the discordance between our findings and several of the Ethiopian studies. Further research should explore differences in livestock management as possible explanations for differential risk of zoonotic TB.

Second, the prior literature that studied bTB positivity in cattle herds used the comparative intradermal tuberculin test (CIDTT) rather than the caudal fold test [14, 15, 17]. Unlike the CFT, the CIDTT can differentiate between animals infected with *M. bovis* and false positives due to exposure to other mycobacteria[12]. It is possible that the sensitivity losses incurred by using the CFT—a low specificity test—to ascertain exposure drove of the lack of association seen in this study.

Furthermore, the outcome definition varied between this study and prior research. While in this study outcome was defined as TST positivity at 15mm or greater—thus including both latent TB and TB disease—the prior research discussed here has been restricted to active TB disease. Several prior studies defined outcome at the household level [15-17] and relied on self-reporting [15-17], and several restricted the outcome definition to pulmonary tuberculosis [6, 18], or tuberculous lymphadenitis[17].

Both this study and the two studies that found no association between cattle exposure and human TST positivity [16, 17] were population-based cross-sectional studies, while all of the studies demonstrating increased risk were case-control studies, with three of the four being hospital-based [6, 14, 18]. Selection bias may arise in hospital-based studies if individuals attending these clinics differ in their distribution of the exposure of interest or potential confounders from the target population.

Finally, the covariates that each study adjusted for varied markedly across studies; several studies did not perform any adjustments, or only performed multivariable modeling on exposures found to be significantly associated with the outcome of interest on univariable analyses [14, 16, 17]. Two studies adjusted for a large number of covariates, including variables potentially on the causal pathway of interest (raw milk consumption and degree of contact with cattle) [15, 18]. Large effect estimates were produced despite this possible overadjustment, raising concerns about collider stratification bias, where unnecessary adjustment results in erroneous exposure-outcome association. Furthermore, of the studies that gave detailed description of methods of covariate selection for these models, several used backwards stepwise selection [16, 18]; such data-driven approaches to model building may compromise reproducibility.

Our findings suggest that females exposed to TST positive cattle have greater prevalence of TST positivity than unexposed females, however in males this association was reversed. This finding is the inverse of that hypothesized *a priori* and should be considered at best hypothesis-generating. As the prevalence of TST positivity was greater in males than in females generally, attenuation of the prevalence ratio would explain a stronger association in women than men, but not a protective effect in men. It is difficult to conceive of an explanation by which this exposure

could be protective, thus this finding may be a result of bias. Households with TST positive cattle appeared to be, on average, wealthier than households without TST positive cattle, and SES was likely not adequately captured by the covariates used to approximate it. Higher SES individuals may be not only less susceptible to TB, but may additionally be more able to hire workers to care for their cattle, and thus have less exposure to TB reactors in their herd. Similarly, our findings suggest that exposure to raw milk was protective against TST positivity; this may be the result of the residual confounding, by which risk factors for TST positivity are inversely associated with raw milk consumption.

Exploratory analyses provide evidence that our findings are robust to definition of TST positivity and a joint bivariate definition of exposure, inclusion of individuals under 18 years of age, exclusion of polygamous households, and modeling method. Furthermore, these analyses suggest that cattle herd size and management system, variables expected to be highly correlated with each other, may be risk factors for herd positivity.

While discrepancies with prior literature suggest generalizability to other rural communities is limited, generalizability to urban and peri-urban communities is likely limited further: management systems in these communities tend to be more intensive—facilitating more animal-to-animal transmission of bTB—and European cattle breeds, known to be more susceptible to bTB than African breeds, are more commonly kept [33]. Any broader generalization—outside of Africa—is difficult to justify, as bTB has been largely eradicated in industrialized countries and management practices in other low- and middle-income settings may differ markedly from those employed in sub-Saharan Africa.

While these findings suggest that bTB does not pose a human health threat to pastoralists in Uganda, such conclusions should be cautiously made. Beyond the apparently limited

generalizability across livestock keeping settings, information bias arising from an insensitive measure of exposure—application of a low-sensitivity diagnostic to an at-times small sample of the cattle herd, and collapsing "sometimes" and "always" raw milk consumption into one "sometimes" category—may explain the lack of a detectable exposure-outcome association in this dataset. Thus, it may be more accurate to conclude that in rural livestock-keeping communities with low bTB burden, in which milk is typically boiled before consumption, livestock uncommonly share housing with humans, and ventilation is subjectively adequate, exposure to TST positive cattle may not be a strong risk factor for TST positivity in humans. Nevertheless, even in the absence of a strong association with human disease, bTB still places a burden on human well-being via livestock morbidity and mortality.

The eradication of bTB has been achieved in much of the industrialized world through nationally- or regionally-coordinated control programs involving strict biosecurity, active surveillance, and culling of infected cattle [34]. As such programs are not feasible in many bTB endemic areas and an effective vaccine is not available, prevention of human disease in these areas will depend on minimizing zoonotic transmission. These findings provide evidence of a low-income, rural livestock-keeping setting in which bTB does not pose a strong risk for human TB infection, despite a lack of coordinated control efforts. Further research is needed to identify similar settings and define the factors that drive these disparities, and the social and cultural motivations for these factors.

Tables

Table 1a: Sociodemographic characteristics and livestock practices of individuals with and without TB reactors in the household cattle herd

	Overall (N=493)	No TB reactors in cattle herd (N=405)	At least one TB reactor in cattle herd (N=88)
	n(%)	n (%)	n (%)
Male	250 (50.7)	200 (49.4)	50 (56.8)
Age*	40.8 (14.0)	40.8 (14.2)	40.7 (13.3)
Type of house individual resides in†			
Hut	218 (87.6)	184 (87.2)	34 (89.5)
Both	25 (10.0)	22 (10.43)	3 (7.89)
Permanent house	6 (2.4)	5 (2.37)	1 (2.63)
Household size (number of individuals)*	8.1 (3.8)	7.89 (3.67)	8.84 (4.02)
Number of rooms in house of residency*	2.5 (1.7)	2.52 (1.77)	2.29 (1.42)
Number of windows in house of residency*	4.5 (3.4)	4.43 (3.18)	4.93 (4.43)
Number of people sharing a room in house of residency*	3.6 (1.6)	3.56 (1.59)	3.77 (1.71)
Education of survey respondent in corresponding household			
None	49 (10.6)	42 (10.77)	7 (9.86)
Primary	304 (65.9)	260 (66.67)	44 (61.97)
Secondary	88 (19.1)	70 (17.95)	18 (25.35)
Tertiary	20 (4.3)	18 (4.62)	2 (2.82)
Religion			
Other Christian	314 (69.5)	268 (70.34)	46 (64.79)
Muslim	81 (17.9)	70 (18.37)	11 (15.49)
Anglican	57 (12.6)	43 (11.29)	14 (19.72)
Size of corresponding household's cattle herd*†	23.9 (36.5)	23.57 (35.69)	25.32 (40.49)
New stock purchased in the past year	109 (22.9)	80 (20.25)	29 (35.36)
All animals kept together	133 (28.9)	101 (26.58)	32 (40.0)
Distance to used kraal			
Very close	51 (10.7)	185 (47.44)	50 (58.14)
Close	235 (49.4)	40 (10.26)	11 (12.79)
Far	190 (39.9)	165 (42.31)	25 (29.07)
Co-house with livestock at night	26 (5.6)	17 (4.43)	9 (10.71)
Livestock management system			
Tethering	244 (51.4)	198 (50.38)	46 (56.10)
Communal grazing	191 (40.2)	160 (40.7)	31 (37.8)
Tethering and communal grazing	34 (7.2)	29 (7.38)	5 (6.10)
Fencing and communal grazing	4 (0.8)	4 (1.02)	0 (0)
Tethering and fencing	2 (0.4)	2 (0.51)	0 (0)

*Mean(SD)

†Missing more than 10% of observations (house type: missing 244 observations; cattle herd size: missing 170 observations)

Table 1b: TB knowledge and status of individuals with and without TB reactors in the household cattle herd

	No TB reactors in cattle herd (N=405)	At least one TB reactor in cattle herd (N=88)
	n (%)	n (%)
TB knowledge reported by survey respondent of corresponding household	379 (93.58)	85 (96.59)
Frequency of milk consumption per week*	2.5 (3.0)	0.18 (0.38)
Sometimes consume raw milk†	100 (27.9)	25 (30.9)
TST results in mms*	13.53 (15.1)	13.30 (15.1)
TST positive at 15mm cutoff	155 (38.3)	29 (33.0)
Total number TB positive individuals in household*	0.90 (0.96)	1.16 (1.26)

*Mean(SD)

†Missing more than 10% of observations (n= 54 missing)

Table 2a: Sociodemographic characteristics and livestock practices of individuals with and without TST positivity

	TST negative (N=309)	TST positive (N=184)
	n (%)	n (%)
Male	139 (45.0)	111 (60.3)
Age*	38.9 (14.7)	43.8 (12.4)
Type of house individual resides in†		
Hut	118 (87.4)	100 (87.7)
Both	15 (11.1)	10 (8.8)
Permanent house	2 (1.5)	4 (3.5)
Household size (number of individuals)*	8.0 (4.0)	8.2 (3.3)
Number of rooms in house of residency*	2.5 (1.7)	2.4 (1.7)
Number of windows in house of residency*	4.2 (3.2)	5.1 (3.7)
Number of people sharing a single room in house of residency*	3.6 (1.6)	3.7 (1.7)
Education of survey respondent in corresponding household		
None	32 (11.1)	17 (9.8)
Primary	188 (65.5)	116 (66.7)
Secondary	56 (19.5)	32 (18.4)
Tertiary	11 (3.8)	9 (5.2)
Religion		
Other Christian	184 (65.5)	130 (76.0)
Muslim	57 (20.3)	24 (14.0)
Anglican	40 (14.2)	17 (9.9)
Size of corresponding household's cattle herd*†	21.0 (29.5)	28.1 (44.7)
New stock purchased in the past year	67 (22.6)	42 (23.2)
All animals kept together	89 (30.6)	44 (26)
Distance to used kraal		
Very close	32 (10.8)	19 (10.6)
Close	156 (52.7)	79 (43.9)
Far	108 (36.5)	82 (45.6)
Co-house with livestock at night	17 (5.7)	9 (5.3)
Livestock management system		
Tethering	168 (56.2)	76 (43.2)
Communal grazing	106 (35.5)	85 (48.3)
Tethering and communal grazing	22 (7.4)	12 (6.8)
Fencing and communal grazing	2 (0.7)	2 (1.1)
Fencing and tethering	1 (0.3)	1 (0.6)

*mean(SD)

†Missing more than 10% of observations (house type: missing 244 observations; cattle herd size: missing 170 observations).

Table 2b: TB knowledge and risk factors in individuals with and without positive TST results

	TST negative (N=309)	TST positive (N=184)
	n (%)	n (%)
TB knowledge reported by survey respondent of corresponding household	292 (94.5)	172 (93.5)
Frequency of milk consumption per week*	2.3 (2.9)	2.8 (3.1)
Sometimes consume raw milk†	86 (32.1)	39 (22.8)
TST results in mms*	7.9 (15.5)	22.3 (8.1)
Total number TB positive individuals in household*	0.6 (0.9)	1.6 (0.8)
At least one TST bovid in corresponding household's herd	59 (19.1)	29 (15.8)

*Mean(SD)

†Missing more than 10% of observations (n= 54 missing)

Table 3: Association of cattle herd positivity and raw milk consumption with TST positivity.

	Crude prevalence ratio (95% CI)	Adjusted PR, total effect (95% CI)	Adjusted PR, direct effect (95% CI)
At least one TB reactor in the cattle herd	0.85 (0.62, 1.17)	-[a]	0.85 (0.62, 1.17)[c]
Male	0.61 (0.47, 0.79)	-[a]	0.62 (0.45, 0.81)[c]
Female	1.26 (0.80, 1.97)	-[a]	1.25 (0.79, 1.98)[c]
Sometimes consume raw milk	0.74 (0.55, 1.01)	0.73 (0.54, 1.00) [b]	0.71 (0.51, 0.98)[d]
Joint Exposure [e]			
No TB reactors in herd, never consume raw milk (n=258)		1.00 (REF)	
Sometimes consume raw milk, no TB reactor in herd (n=100)		0.75 (0.55, 1.03)	
TB reactor in herd, never consume raw milk (n=56)		0.92 (0.70, 1.22)	
TB reactor in herd and sometimes consume raw milk (n=25)		0.66 (0.29, 1.51)	

[a]No confounders identified
[b]Adjusted for self-reported knowledge of TB. Cattle herd size not adjusted for due to large amounts of missingness.
[c]Adjusted for TST positivity in other household members
[d]Adjusted for TST positivity in other household members and self-reported knowledge of TB. Cattle herd size not adjusted for due to large amounts of missingness.
[e]No confounder adjustment performed

Figures

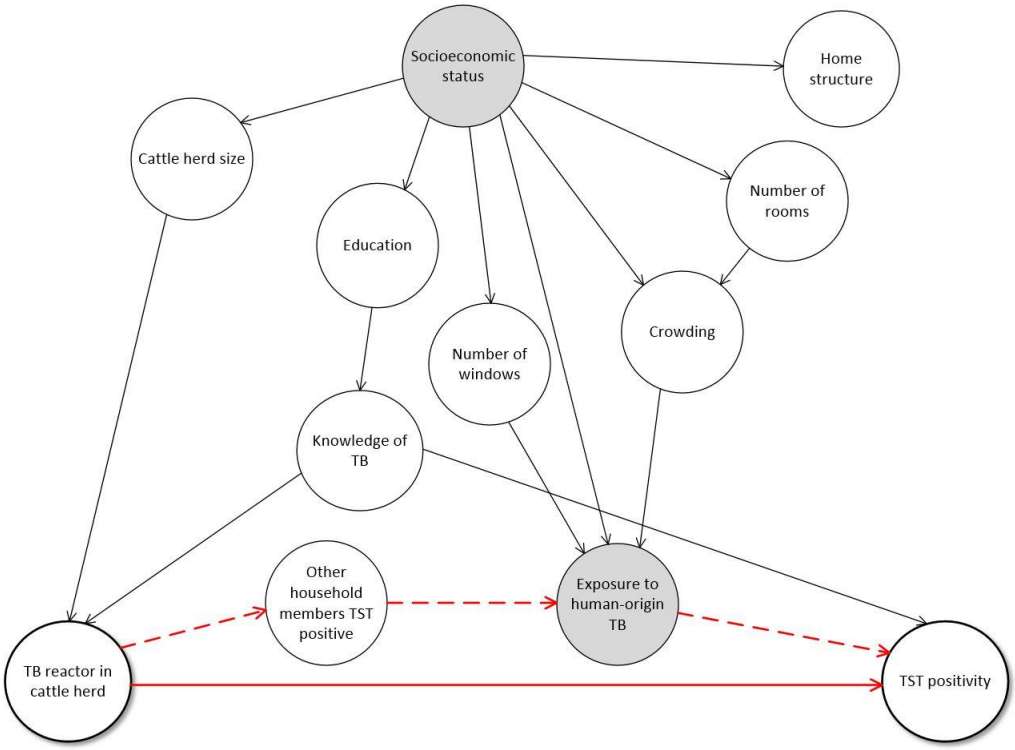

Figure 1: Directed acyclic graph. Solid red line denotes the "direct pathway", dashed red line the "indirect pathway". Grey notes denote unmeasured variables.

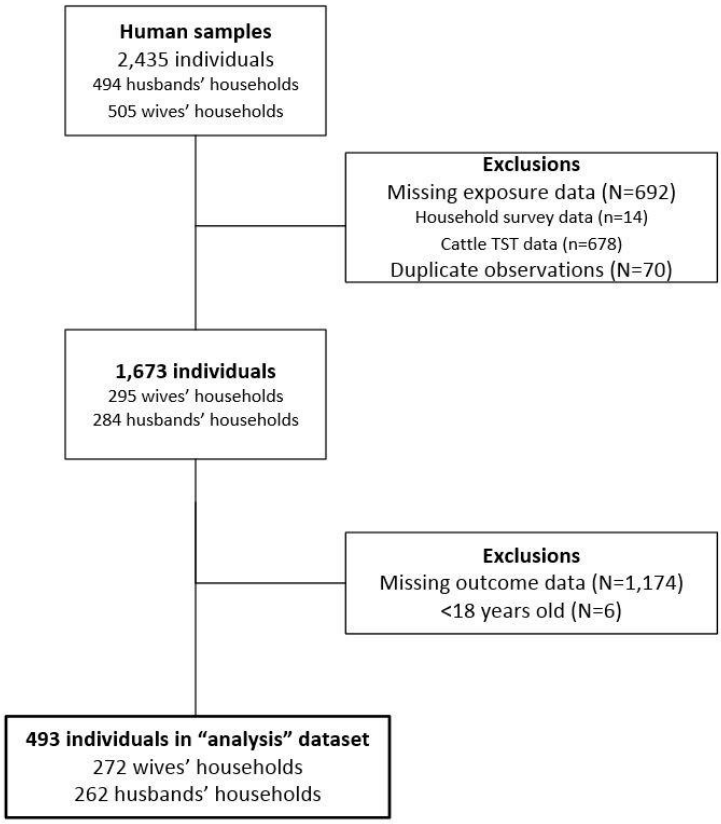

Figure 2: Flow diagram of individuals recruited into study and retained for final analyses

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Supplementary materials

Supplementary table 1: Descriptive statistics comparing the "full dataset" versus the "analysis dataset"

	Full dataset (N=787)[a]	Analysis dataset (N=493)[b]
	n(%)	n(%)
Male	383 (48.7)	250 (50.7)
Age*	38.4 (15.5)	40.8 (14.0)
Type of house individual resides in†		
Hut	282 (86.2)	218 (87.6)
Both	38 (11.6)	25 (10.0)
Permanent house	7 (2.1)	6 (2.4)
Household size (number of individuals)*	9.3 (5.3)	8.1 (3.8)
Number of rooms in house of residency*	2.6 (1.7)	2.5 (1.7)
Number of windows in house of residency*	4.3 (3.4)	4.5 (3.4)
Number of people sharing a room in house of residency*	3.7 (2.0)	3.6 (1.6)
Education of survey respondent in corresponding household		
None	63 (8.8)	49 (10.6)
Primary	458 (63.8)	304 (65.9)
Secondary	147 (20.5)	88 (19.1)
Tertiary	50 (7.0)	20 (4.3)
Religion		
Other Christian	544 (67.1)	314 (69.5)
Muslim	187 (23.1)	81 (17.9)
Anglican	80 (9.9)	57 (12.6)
Size of corresponding household's cattle herd*†	19.6 (32.1)	23.9 (36.5)
New stock purchased in the past year	181 (23.6)	109 (22.9)
All animals kept together	214 (28.9)	133 (28.9)
Distance to used kraal		
Very close	95 (12.5)	51 (10.7)
Close	399 (52.4)	235 (49.4)
Far	268 (35.2)	190 (39.9)
Co-house with livestock at night	43 (5.8)	26 (5.6)
Livestock management system		
Tethering	450 (59.1)	244 (51.4)
Communal grazing	261 (34.3)	191 (40.2)
Tethering and communal grazing	43 (5.7)	34 (7.2)
Fencing and communal grazing	4 (0.5)	4 (0.8)
Tethering and fencing	3 (0.4)	2 (0.4)
TB knowledge reported by survey respondent of corresponding household	750 (95.3)	464 (94.1)
Frequency of milk consumption per week*	2.3 (2.9)	2.5 (3.0)
Sometimes consume raw milk†	155 (24.8)	125 (28.5)
TST results in mms*	13.5 (15.0)	13.5 (15)
TST positive at 15mm cutoff	184 (37.3)	184 (37.3)
Total number TB positive individuals in household*	0.92 (1.04)	0.95 (1.0)

[a]Individuals over 18 years of age, with non-missing exposure data

[b]Individuals over 18 years of age, with non-missing exposure and non-missing outcome data

*Mean(SD)

†Missing more than 10% of observations (house type: missing 244 observations; cattle herd size: missing 170 observations)

Supplementary table 2a: Distribution of exposures across TST negative versus TST positive individuals with TST positivity at varying cut points

	TST negative	TST positive
	n(%)	*n(%)*
	5mm cut point[a]	
No TB reactors in cattle herd	145 (35.8)	260 (64.2)
At least one TB reactor in cattle herd	25 (28.4)	63 (71.6)
Never consume raw milk	97 (30.9)	217 (69.1)
Sometimes consume raw milk	52 (41.6)	73 (58.4)
	10mm cut point[b]	
No TB reactors in cattle herd	169 (41.7%)	236 (58.3%)
At least one TB reactor in cattle herd	31 (35.2%)	57 (64.8%)
Never consume raw milk	114 (36.3%)	200 (63.7%)
Sometimes consume raw milk	58 (46.4%)	67 (53.6%)

[a]TST negative individuals total 170, while TST positive individuals total 323
[b]TST negative individuals total 200, while TST positive individuals total 293

Supplementary table 2b: Modified Poisson regression analyses with varying TST positivity cut points

	Crude prevalence ratio (95% CI)	Adjusted PR, total effects (95% CI)	Adjusted PR, direct effects (95% CI)
	5mm		
At least one TB reactor in the cattle herd	1.09 (0.91, 1.31)	-[a]	1.09 (0.93, 1.28)[c]
Sometimes consume raw milk[a]	0.85 (0.72, 0.99)	0.83 (0.71, 0.97)[b]	0.85 (0.72, 0.99)[d]
	10mm		
At least one TB reactor in the cattle herd	1.10 (0.89, 1.35)	-[a]	1.09 (0.91, 1.30)[c]
Sometimes consume raw milk[a]	0.84 (0.71, 1.00)	0.83 (0.70, 0.99)[b]	0.86 (0.72, 1.01)[d]

Sex was not found to be a statistically significant effect modifier at these cutpoints.
[a]No confounders identified
[b]Adjusted for self-reported knowledge of TB. Cattle herd size not adjusted for due to large amounts of missingness.
[c]Adjusted for TST positivity in other household members
[d]Adjusted for TST positivity in other household members and self-reported knowledge of TB. Cattle herd size not adjusted for due to large amounts of missingness.

Supplementary table 3: Inclusion of individuals less than 18 years of age; modified Poisson regression

	Crude prevalence ratio (95% CI)	Adjusted PR, total effects (95% CI)	Adjusted PR, direct effects (95% CI)
At least one TB reactor in the cattle herd	0.86 (0.63, 1.18)	-[a]	0.86 (0.62, 1.18)[c]
Male	0.62 (0.48, 0.80)	-[a]	0.61 (0.47, 0.79)[c]
Female	1.29 (0.82, 2.01)	-[a]	1.29 (0.82, 2.01)[c]
Sometimes consume raw milk[a]	0.75 (0.55, 1.00)	0.74 (0.54, 1.00)[b]	0.70 (0.51, 0.97)[d]

[a]No confounders identified
[b]Adjusted for self-reported knowledge of TB. Cattle herd size not adjusted for due to large amounts of missingness.
[c]Adjusted for TST positivity in other household members
[d]Adjusted for TST positivity in other household members and self-reported knowledge of TB. Cattle herd size not adjusted for due to large amounts of missingness.

Supplementary table 4a: Sample characteristics stratified on household type, full dataset

	Reside in monogamous households (N=734)	Reside in polygamous household (N=53)
	n (%)	n (%)
Male	357 (48.6)	26 (49.1)
Age*	38.5 (15.5)	36.2 (14.3)
Type of house individual resides in†		
Hut	34 (12.4)	4 (7.6)
Permanent house	233 (85.0)	49 (92.5)
Both	7 (2.6)	0 (0)
Number of rooms in house of residency*	2.7 (1.7)	1.3 (1.1)
Number of windows in house of residency*	4.0 (3.2)	8.2 (4.3)
Number of people sharing a single room in house of residency*	3.8 (2.1)	3.2 (0.6)
Education of survey respondent in corresponding household		
None	60 (8.8)	3 (7.9)
Primary	424 (62.4)	34 (89.5)
Secondary	147 (21.6)	0 (0)
Tertiary	49 (7.2)	1 (2.6)
TB knowledge reported by survey respondent of corresponding household	703 (95.8)	47 (88.7)
Size of corresponding household's cattle herd*†	16.3 (26.3)	50.6 (57.3)
Frequency of milk consumption per week *	2.3 (2.9)	2.8 (2.8)
Sometimes consume raw milk†	155 (27.1)	0 (0)
Presence of at least one reactor in cattle herd	130 (17.7)	17 (32.1)
TST results in mms*†	18 (19.7)	20.6 (21.0)
TB positive at 15mm cutoff†	169 (37.1)	15 (40.5)
Total number TB positive individuals in household*	0.91 (1.1)	0.96 (0.8)

*mean(SD)

†Missing >10% of observations (TST results: missing 294 observations; sometimes consume raw milk: missing 163 observations; house type: missing 460 observations; cattle herd size: missing 230 observations)

Supplementary table 4b: Analyses restricted to monogamous households, log binomial regression

	Crude prevalence ratio (95% CI)	Adjusted PR, total effects (95% CI)	Adjusted PR, direct effects (95% CI)
At least one TB reactor in the cattle herd	0.80 (0.56, 1.15)	-[a]	0.80 (0.56, 1.15)[c]
Male	0.57 (0.43, 0.76)	-[a]	0.58 (0.43, 0.77)[c]
Female	1.22 (0.74, 2.01)	-[a]	1.22 (0.74, 2.01)[c]
Sometimes consume raw milk[a]	0.74 (0.54, 1.01)	0.73 (0.54, 1.00)[b]	0.71 (0.51, 0.98)[d]

[a]No confounders identified

[b]Adjusted for self-reported knowledge of TB. Cattle herd size not adjusted for due to large amounts of missingness.

[c]Adjusted for TST positivity in other household members

[d]Adjusted for TST positivity in other household members and self-reported knowledge of TB. Cattle herd size not adjusted for due to large amounts of missingness.

Supplementary table 5: Results from three multi-level modified Poisson regression models, clustering on village

	Empty model	Random intercept	Random intercept and slope*
Fixed effects			
At least one TB reactor in the cattle herd (Model 1)	-	-	-
Male	-	0.63 (0.37, 1.10)	0.63 (0.37, 1.10)
Female	-	1.24 (0.68, 2.24)	1.26 (0.70, 2.27)
Sometimes consume raw milk (Model 2)	-	0.75 (0.50, 1.13)	0.78 (0.63, 0.98)
Village-level cattle TB prevalence (Model 3)	-	0.99 (0.94, 1.04)	1.00 (0.93, 1.070
Random effects			
At least one TB reactor in cattle herd (Model 1)	-	-	0.031
Sometimes consume raw milk (Model 2)	-	-	0.015
Village-level cattle TB prevalence (Model 3)	-	-	0.0024
Village-level variance	0.0329		
Model 1		0.033	0.048
Model 2		0.047	0.005
Model 3		0.026	0.055
Proportional change in village-level variance	REF		
Model 1		-0.003	-0.459
Model 2		-0.429	0.848
Model 3		0.207	-0.672

Supplementary table 6: Risk factors for cattle TST positivity, log binomial regression

	Crude prevalence ratio	Crude 95% confidence interval
New stock purchased in the past year	0.95	0.47, 1.92
Co-housing with humans	1.51	0.45, 5.10
Management system		
Communal grazing	REF	REF
Tethering	2.27	1.21, 4.25
Both	11.67	0.48, 5.83
Cattle herd size		
Missing	REF	REF
1-5	0.53	0.22, 1.26
6-20	0.51	0.26, 1.00
21-100	0.30	0.10, 0.86
>100	0.51	0.08, 3.14

Supplementary table 7: Sample characteristics stratified on missing versus recorded herd size

	Herd size recorded (N=324)	Herd size missing (N=169)
	n (%)	n (%)
Male	177 (54.6)	73 (43.2)
Age*	41.7 (13.9)	38.9 (14.2)
Type of house individual resides in†		
Hut	218 (87.6)	0 (0)
Permanent house	6 (2.41)	0 (0)
Both	25 (10)	0 (0)
Number of rooms in house of residency*	2.35 (1.92)	2.72 (1.19)
Number of windows in house of residency*	5.14 (3.84)	3.3 (1.96)
Number of people sharing a single room in house of residency*	3.37 (1.42)	4.01 (1.85)
Education of survey respondent in corresponding household		
None	15 (5.1)	34 (20.5)
Primary	210 (71.2)	94 (56.6)
Secondary	50 (16.9)	38 (22.9)
Tertiary	20 (6.78)	0 (0)
TB knowledge reported by survey respondent of corresponding household	315 (97.2)	149 (88.2)
Frequency of milk consumption per week *	2.16 (2.74)	3.09 (3.30)
Sometimes consume raw milk†	18 (6.52)	107 (65.6)4
Presence of at least one reactor in cattle herd	56 (17.28)	32 (18.9)
TB positive at 15mm cutoff	130 (40.1)	54 (32.0)
TST results in mms *	14.60 (15.28)	13.38 (14.4)
Total number TB positive individuals in household*	1.15 (1.12)	0.57 (0.66)

*mean(SD)

†Missing more than 10% of observations (sometimes consume raw milk: missing 54 observations; house type: missing 244 observations; cattle herd size: missing 170 observations).