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Bovine ownership and reduced pulmonary tuberculosis risk in Nepal: A case–control study

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Abstract

This case–control study sought to confirm and investigate in more depth protective associations previously found of bovine (cattle and water buffalo) ownership with reduced risk of both pulmonary tuberculosis (PTB) and latent tuberculosis infection (LTBI) in humans. The study recruited male and female PTB cases from a diagnostic centre and a frequency-matched community-based control group in Kaski District, Nepal. Controls were tested for LTBI status and a separate nested case–control study was conducted based on LTBI status. Data were collected on participant household animal ownership. Using logistic regression, animal ownership was investigated for associations with both PTB and LTBI. Data were obtained from 570 PTB cases and 1,224 controls, the latter group providing 396 LTBI-positive and 692 LTBI-negative subjects. Results provided evidence of decreased odds of both PTB and LTBI positivity associated with owning bovines. The evidence was strongest for protection against infection, rather than activation of infection to PTB. Effects were strongest in women, who usually manage the animals in Nepal, and there were exposure-response relationships with numbers of bovines owned. Results suggest that exposure to bovines is protective against LTBI and PTB. A possible mechanism involves boosting the effect of BCG (Bacillus Calmette-Guerin) vaccination, particularly in protecting against tuberculous infection. Additional studies with more extensive data collection are needed to confirm the observed associations.

KEYWORDS

Bacillus calmette-guerin, cattle, latent tuberculosis infection, pulmonary tuberculosis, water buffalo

1 | INTRODUCTION

There is some evidence that living closely with cattle may be a risk factor for pulmonary tuberculosis (PTB) (Mfinanga et al., 2003; Waziri et al., 2014); however, other evidence suggests that close contact with *Mycobacterium bovis*-infected cattle may have a protective association with TB in cattle herders (Berg et al., 2015; Meisner et al., 2019).

Some illnesses attributed to *M. tuberculosis*, particularly in high TB burden countries, are caused by *M. bovis*. Although the natural host of *M. bovis* is bovines, including cows and buffaloes, the organism can infect a wide range of animal species, including humans (Michel et al., 2010). *M. bovis* infection in humans is mostly extrapulmonary, via ingestion of unpasteurized milk; however, pulmonary infections can occur from close contact with the exhalations of infected animals (Michel et al., 2010). Most diagnoses of PTB involve sputum examination after staining, in which the *M. tuberculosis* and

M. bovis organisms are microscopically indistinguishable. Thus, the *M. bovis*-attributable proportions of PTB in humans are usually unknown, but probably mostly small.

We conducted a PTB case–control study in Nepal, the primary objective of which was to examine associations with use of household fuels for cooking, heating and lighting (Bates et al., 2019). Controls were tested using an interferon-gamma release assay (IGRA) for evidence of latent tuberculosis infection (LTBI) status. The PTB controls were used to examine the association between household fuels and LTBI, as a separate case–control study nested in the parent study (Albers et al., 2019). In these analyses, we observed protective associations with ownership of cattle and water buffalo. Such associations were present for TB disease and for LTBI.

The investigation described here was to explore these observations in more detail, hypothesizing that they would stand up to closer scrutiny. If so, they might offer insights informative for vaccine development.

Impacts

- About 40% of the world's population, mainly in low- and middle-income countries (LMICs), is infected with mycobacteria that cause tuberculosis (TB). Those infected that do not have active tuberculosis are described as having latent tuberculosis.
- Typically in LMICs, babies receive the Bacillus Calmette-Guerin (BCG) vaccine to protect against TB. BCG was originally derived from a form of bovine tuberculosis.
- We found that Nepali women whose families owned bovines (cattle or water buffalo) appeared to be protected from *M. tuberculosis* infection, apparently further protecting against pulmonary tuberculosis.
- We interpret this finding as possibly representing a boost to the original BCG vaccine, the TB-protective effects of which generally wear off after childhood. It is conceivable that this could provide additional off-target protection against the SARS-CoV-2 virus, which causes COVID-19 disease. This could partially accounting for the unexpectedly low incidence and mortality from this disease in Nepal and other LMICs.

2 | METHODS

Methods have previously been fully described (Albers et al., 2019; Bates et al., 2019) and are only summarized here.

The parent study was a case–control study of PTB, with cases and controls recruited mainly in Kaski District, Nepal. Controls provided blood samples for determining LTBI status.

Prior to commencement of field work, ethical approvals were obtained from the Center for Protection of Human Subjects at the University of California, Berkeley, and the Nepal Health Research Council. Informed consent was obtained from all subjects before their participation in the study.

Eligible for recruitment were males and females, aged 18–70 years. Excluded was anyone with known immunosuppressive conditions (e.g., HIV/AIDS) or taking immunosuppressive drugs, and anyone with a history of TB.

2.1 | Participant recruitment

Cases were recruited mainly from the Western Regional Tuberculosis Center (WRTC), Pokhara, Kaski District, Nepal, and controls mainly from the same district. A population-based control group was obtained. The aim was to have an approximately 2:1 ratio of controls to cases, with controls reflecting the anticipated geographic residential distribution of PTB cases. Recruitment of participants was from May 2013 to February 2017.

Controls were recruited on a geographic frequency basis, with a 2:1 ratio of males to females, the expected ratio among PTB cases, based on the ratio of diagnoses obtained in the previous 3 years at the WRTC. Recruitment of controls was made by visits to selected residences, also based on the geographic distribution of residences of pulmonary TB cases diagnosed by the WRTC during the previous 3 years. Recruitment procedures have previously been described in detail (Bates et al., 2019). Blood samples from controls and participant interview data were collected during visits to the residences.

2.2 | The questionnaire

As previously described (Bates et al., 2019), modules in the questionnaire covered sources of household air pollution, and personal exposure and demographic data. A series of questions asked about household animal ownership, including of cows and bulls, water buffalo, goats, chickens, pigs, sheep and ducks. Participants were asked about the number of each type of animal owned.

2.3 | Control LTBI testing

From each control, a 3 mL blood sample was collected by venipuncture for LTBI testing using the QuantiFERON-TB Gold test (Cellestis Ltd)(Albers et al., 2019). Results were interpreted as LTBI positive, LTBI negative or indeterminate, per manufacturer instructions.

2.4 | Data analysis

Statistical analysis was performed using Stata 14 (StataCorp LLC, College Station). Initial descriptive analysis compared distributions, by sex, of key variables across PTB cases and controls, and for controls by LTBI-negative and LTBI-positive status, after excluding indeterminants. Bivariate odds ratios and confidence intervals were calculated using conditional logistic regression for TB cases with all controls (matched by geographic area) and unconditional logistic regression to compare LTBI-positive with LTBI-negative control subgroups.

Multivariate analysis was carried out with potential confounders selected for inclusion if they altered the association with bovines (cattle or water buffalo) by 10% or more, after excluding possible colliders or variables on the causal pathway.

The representations of household-owned animals in the five final models were: (1) ownership of any animal (cattle, water buffalo, goats or chickens); (2) ownership of any bovine; (3) ownership of at least one of each of the 4 main animal species represented by separate terms in the model; (4) number of bovines owned in 4 categories; and (5) number of bovines owned as a continuous variable for 1, 2, 3 and 4+ animals.

3 | RESULTS

We obtained study data from 570 PTB cases and 1,224 controls, after excluding 13 subjects (11 cases and 2 controls) with self-reported HIV infection. As previously described in more detail, participation rates were 98.3% and 97.3% for cases and controls, respectively (Bates et al., 2019). Of the controls, LTBI test results were available for 1,195 (97.5%). Of these, 396 (33.1%) tested positive and 692 (57.9%) tested negative. The 107 (9.0%) participants with indeterminate LTBI results were excluded from that analysis, which used the 1,088 participants with clearly positive or negative LTBI test results.

Substantial descriptive data have already been published for our study population (Albers et al., 2019; Bates et al., 2019) and are not repeated here. Tables 1 and 2 show basic descriptive data for animal ownership, for both PTB cases and controls (Table 1) and LTBI-positive and LTBI-negative groups (Table 2), with bivariate odds ratios (OR) and 95% confidence intervals (CI). There are a number of associations in a protective direction for both PTB and LTBI with animals—particularly cattle, buffalo and goats—with confidence intervals excluding the null.

Since a family owning one type of animal is likely to own other types of animals, confounding among animal species is plausible. The following analyses were limited to consideration of cattle (cows and bulls), water buffalo, goats and chickens. Other animals (e.g. sheep, pigs and ducks) were owned by few families and were excluded from the models (Table 3).

Table 3 shows sex-stratified results for the five logistic regression models, all adjusted for age, caste, house ownership and household crowding. Results are presented both for TB cases with all controls, and for the comparison of LTBI-positive controls with LTBI-negative controls.

The adjusted results show that ownership of any farm animal (Model 1) has protective associations, but those associations are all stronger when limited to bovines (Model 2). These protective associations are strongest in women, for whom the OR for PTB was 0.54 (95% CI:0.32, 0.93) and for LTBI, 0.55 (0.38, 0.82). Corresponding ORs for males were 0.80 (0.51, 1.24) and 0.67 (0.44, 1.01), respectively.

TABLE 1 Animal ownership by households of pulmonary tuberculosis cases and controls

	Females			Males		
Animal ownership	Cases *N* (%)	Controls *N* (%)	OR (95% CI)[a]	Cases *N* (%)	Controls *N* (%)	OR (95% CI)[a]
Cows						
No	174 (93%)	488 (75%)	1.00	310 (81%)	427 (74%)	1.00
Yes	14 (7%)	160 (25%)	0.38 (0.19, 0.76)	72 (19%)	149 (26%)	0.61 (0.43, 0.88)
Buffalo						
No	157 (84%)	410 (63%)	1.00	293 (77%)	383 (66%)	1.00
Yes	31 (16%)	238 (37%)	0.45 (0.27, 0.73)	89 (23%)	193 (34%)	0.57 (0.35, 0.91)
Goats						
No	155 (82%)	437 (67%)	1.00	294 (77%)	398 (69%)	1.00
Yes	33 (18%)	211 (33%)	0.55 (0.29, 1.03)	88 (23%)	178 (31%)	0.54 (0.36, 0.81)
Chickens						
No	134 (71%)	366 (56%)	1.00	227 (59%)	317 (55%)	1.00
Yes	54 (29%)	282 (44%)	0.62 (0.36, 1.06)	155 (41%)	259 (45%)	0.77 (0.56, 1.06)
Pigs						
No	181 (96%)	614 (95%)	1.00	361 (95%)	538 (93%)	1.00
Yes	7 (4%)	34 (5%)	0.93 (0.32, 2.69)	21 (5%)	38 (7%)	0.69 (0.40, 1.19)

Abbreviations: CI, confidence interval; *N*, number of participants; OR, odds ratio.
[a]Calculated using conditional logistic regression, matching for geographic area.

TABLE 2 Animal ownership and latent tuberculosis infection (LTBI) status of control households

	Females			Males		
Animal ownership	LTBI-positive *N* (%)	LTBI-negative *N* (%)	OR (95% CI)	LTBI-positive *N* (%)	LTBI-negative *N* (%)	OR (95% CI)
Cows						
No	151 (77%)	284 (74%)	1.00	152 (76%)	225 (73%)	1.00
Yes	45 (23%)	98 (26%)	0.86 (0.58, 1.29)	48 (24%)	85 (27%)	0.84 (0.55, 1.26)
Buffalo						
No	140 (71%)	226 (59%)	1.00	140 (70%)	198 (64%)	1.00
Yes	56 (29%)	156 (41%)	0.58 (0.40, 0.84)	60 (30%)	112 (36%)	0.76 (0.52, 1.11)
Goats						
No	144 (73%)	246 (64%)	1.00	138 (69%)	214 (69%)	1.00
Yes	52 (27%)	136 (36%)	0.65 (0.45, 0.96)	62 (31%)	96 (31%)	1.00 (0.68, 1.47)
Chickens						
No	108 (55%)	219 (57%)	1.00	110 (55%)	171 (55%)	1.00
Yes	88 (45%)	163 (43%)	1.09 (0.77, 1.55)	90 (45%)	139 (45%)	1.01 (0.70, 1.44)
Pigs						
No	185 (94%)	361 (95%)	1.00	190 (95%)	286 (92%)	1.00
Yes	11 (6%)	21 (5%)	1.02 (0.48, 2.17)	10 (5%)	24 (8%)	0.63 (0.29, 1.34)

Abbreviations: CI, confidence interval; *N*, number of participants; OR, odds ratio.

In Model 3, where animals were specified separately, the protective associations with goats are no longer present, but cattle and buffalo remain associated in a protective direction. Models 4 and 5, which investigate relationships with numbers of bovines owned by the household, provide evidence of exposure-response relationships, strongest for women.

4 | DISCUSSION

As earlier mentioned, this work emerged from a study that was primarily focused on identifying associations of household fuel use with pulmonary TB. That study found that, relative to use of LPG for cooking, use of biogas had protective associations with both PTB and LTBI (Albers et al., 2019; Bates et al., 2019). Typically, biogas is produced in Nepal from household digesters, utilizing mainly animal dung. We considered whether this protective association could be because a household usually needs to maintain cattle or buffaloes to produce sufficient dung for digester operation. In investigating this, we found protective associations with animal ownership, independent of the protective associations with biogas use.

Our results show that ownership of farm animals, particularly cattle and water buffalo may be protective against both PTB disease and *M. tuberculosis* infection. Since the odds ratio for PTB disease conflates the odds of infection and the odds of activation of the infection, it appears that in our study population most of the TB protection is actually against infection, rather than against activation of infection to PTB. However, since the strengths of the associations are sometimes greater for PTB than for LTBI (Table 3), there may be some additional protection against activation of infection to PTB, although the observed differences may represent random variation. Exposure-response relationships strengthen the evidence. However, since these findings were not *a priori* hypothesized, but arose fortuitously in the data analysis, confounding, information bias and selection bias are important considerations.

It is unlikely that information bias, either of outcome or exposure, explains our results: PTB cases were confirmed by sputum examination or by GeneXpert; latent *M. tuberculosis* infection status of controls was confirmed by IGRA testing and indeterminants excluded from the analysis. Although we excluded from the study anyone with a prior history of TB and any potential control with self-reported symptoms suggestive of PTB, we did not actually test controls to ensure they were TB-free. As TB is a rare disease at a population level, if some controls were undiagnosed PTB cases, they could have been no more than a small proportion of the controls. The resulting bias would be towards the null and could not explain our findings. Although interviews were carried out at participant residences, no systematic attempt was made to visually confirm animal ownership. Nonetheless, inaccuracies in animal reporting seem unlikely, as in Nepal there is little awareness of the possibility of zoonotic PTB infection (Pandey et al., 2012).

In regard to selection bias, the participation rates of both cases and controls were very high (>97%) and efforts were made to ensure both groups represented the same population (Bates et al., 2019). In particular, controls were selected to match the expected case distribution at the residential levels of rural village development committee and city ward, using a random selection method within those geographic entities (Albers et al., 2019; Bates et al., 2019). Adjustment was carried out by matching at this geographic level in

TABLE 3 Adjusted[a] odds ratios and 95% confidence intervals for animal ownership, separately for females and males

		Pulmonary TB						LTBI					
		Females			Males			Females			Males		
Model		OR	CI-	CI+	OR	CI-	CI+	OR	CI-	CI+	OR	CI-	CI+
1	Any Farm Animal												
	No	1.00			1.00			1.00			1.00		
	Yes	0.65	0.31	1.34	0.95	0.67	1.35	0.66	0.44	0.99	0.76	0.49	1.18
2	Any Bovine												
	No	1.00			1.00			1.00			1.00		
	Yes	0.54	0.32	0.93	0.80	0.51	1.24	0.55	0.38	0.82	0.67	0.44	1.01
3	Cows												
	No	1.00			1.00			1.00			1.00		
	Yes	0.50	0.23	1.06	0.83	0.55	1.25	0.96	0.61	1.51	0.77	0.47	1.27
	Buffalo												
	No	1.00			1.00			1.00			1.00		
	Yes	0.72	0.39	1.32	0.91	0.56	1.51	0.67	0.43	1.06	0.63	0.39	1.04
	Goats												
	No	1.00			1.00			1.00			1.00		
	Yes	1.10	0.54	2.26	0.90	0.57	1.42	0.74	0.46	1.19	1.29	0.76	2.19
	Chickens												
	No	1.00			1.00			1.00			1.00		
	Yes	0.58	0.31	1.10	1.00	0.70	1.42	0.96	0.63	1.48	0.95	0.61	1.49
4	Bovine Count												
	0	1.00			1.00			1.00			1.00		
	1	0.77	0.34	1.75	1.06	0.56	2.00	0.49	0.25	0.97	0.64	0.33	1.26
	2	0.51	0.23	1.12	0.68	0.38	1.21	0.62	0.37	1.03	0.52	0.28	0.97
	3+	0.34	0.14	0.83	0.77	0.45	1.32	0.55	0.33	0.92	0.75	0.45	1.27
5	Bovine (0–4+)												
	Continuous	0.75	0.59	0.95	0.89	0.77	1.04	0.84	0.73	0.97	0.89	0.77	1.02

Abbreviations: OR, odds ratio; CI-, lower confidence interval; CI+, upper confidence interval.
[a]Adjusted for age, caste, house ownership and household crowding.

the conditional logistic regression analysis comparing PTB cases and controls.

We consider that if the protective associations are a result of bias, the most likely source is uncontrolled confounding. For example, households owning one type of animal were likely to own other types of animal, with consequent confounding. Illustrating this, the originally apparent association with owning goats (Table 1) disappeared when we adjusted for other animals owned (Table 3). Apart from that, PTB is substantially a disease of poverty and ownership of a larger number of bovines may be indicative of greater wealth. Addressing this concern, we made extensive efforts to identify possible confounders, including socioeconomic indicators. In that regard, our final models are adjusted for caste, house ownership and household crowding, the variables that our analysis suggested might have confounded the associations with bovines. The possibility of some other, unrecognized confounding factor, however, cannot be ruled out, although we have no good hypothesis for what it might be.

A further question involves the mechanism of protective exposure. It is conceivable that the protective associations reflect a way that managing animals, particularly bovines, reduces contact of the people looking after the animals with people with active TB. However, another mechanism may involve exposure to animal-borne agents or antigens that boost the immune effects of BCG (Bacillus Calmette-Guerin) vaccine. That the protection we found was largely against infection is consistent with evidence about the TB-protective effect of BCG, which involves protection against infection, rather than against activation (Michelsen et al., 2014; Roy et al., 2014).

Most countries, including Nepal, that have a high incidence of TB routinely administer BCG vaccine, consistent with the current World Health Organization recommendation of administration to all healthy neonates at birth (World Health Organization, 2018). BCG contains an attenuated live *M. bovis* strain, which is believed to confer some protection for children, particularly against miliary TB and tuberculous meningitis (Trunz et al., 2006), but the protective effects generally wear off by adulthood (Dockrell & Smith, 2017; Mangtani et al., 2014). In some countries, a second BCG shot is given at a later age and may enhance TB prevention (Kashyap et al., 2010), but in Nepal no second dose is provided.

One possible BCG booster is exposure to *M. bovis*-infected bovines. The limited evidence suggests *M. bovis* is endemic in at least cows and buffalo in Nepal. Using genotyping, Jha et al. (2007) identified *M. bovis* in milk and/or faeces of 6 of 36 (17%) buffalo and 5 of 32 (16%) cattle identified as TB-positive using tuberculin skin testing. Another Nepalese study of bovines owned by tuberculosis patients found 15% TB positivity in the bovines (Pandey et al., 2012).

Although PTB is mostly caused by *M. tuberculosis*, it may also be caused by other organisms, including *M. bovis*. Most human tuberculosis caused by *M. bovis* has, historically, been extrapulmonary and occurring in infants and children (Thoen et al., 2006), but pulmonary disease can arise. The pulmonary disease caused by *M. bovis* is clinically and microscopically indistinguishable from the disease caused by *M. tuberculosis* (Olea-Popelka et al., 2017), so, it is hypothetically possible that the PTB risk reduction we observed reflected protection against pulmonary *M. bovis* disease. However, the IGRA test uses antigens specific to *M. tuberculosis*, arguing against a mechanism involving protection against *M. bovis* disease.

That there were exposure-response relationships for numbers of bovines owned by the participant household and the strongest evidence for protective effects was in women adds plausibility to the associations, since in Nepal it is mainly women who manage the farm animals (Paudel et al., 2009). Also, there is some evidence that women generally have stronger immune systems than men, likely because some of the immune-system genes reside on the X-chromosome, of which only women have two(Sarmiento et al., 2019).

Consistent with our findings, a study of cattle-owning families in Uganda found that having at least one TST (tuberculin skin test)-positive bovine in the household cattle herd was associated with a lower TST positivity among men (prevalence ratio (PR) = 0.66, 95% CI: 0.49, 0.87), but not among women (PR = 1.21, 95%CI: 0.76, 1.95) (Meisner et al., 2019). Traditionally, in Uganda, men look after the cattle.

There are several possibilities for exposure to bovines, including consumption of non-pasteurized milk, general daily close contact with the animals and/or their dung, and even sleeping in the same room as the animals. Any or all of these are possible exposure sources. In Nepal, it is widely known that milk should be boiled before consumption, but this often does not happen (Pandey et al., 2012) or the boiling process may be insufficient for milk sterilization.

Additional importance of our observations may lie in the fact that studies have shown that BCG has a more general 'off-target' effect on reduction of child mortality and viral diseases (Moorlag et al., 2019)(Goodridge et al., 2016). Although mechanisms are still somewhat speculative, they may involve induction of innate immune memory cells, which has been termed 'trained immunity' (O'Neill & Netea, 2020). It has even been suggested that BCG vaccination could be an effective response to viral pandemics for which there is no existing vaccine (Moorlag et al., 2019). Some ecologic studies have suggested that countries with BCG vaccination have lower rates of COVID-19 disease and/or mortality from the SARS-CoV-2 pandemic currently in progress (Akiyama & Ishida, 2020; Dayal & Gupta, 2020; Escobar et al., 2020; Ozdemir et al., 2020). This might account for the relatively low incidence of COVID-19 observed in low- and middle-income countries, such as Nepal, at the early stage of the pandemic. However, low levels of testing for the presence of the virus and under-reporting of sub-clinical cases in these countries may be more important reasons. Several recently started clinical trials, registered at ClinicalTrials.gov, are investigating whether BCG administration to adult health care workers protects against COVID-19 (O'Neill & Netea, 2020). Potentially, a bovine-related BCG co-factor, as suggested by our study, could enhance such an effect, if it is confirmed.

We recommend that any further investigation of a TB-protective effect associated with animal ownership incorporate several additional components:

1. Genotyping of all pulmonary TB organisms from sputum samples of TB cases.
2. Blood sampling of farm animals, especially cows and buffaloes, managed by participant families and IGRA testing of those samples for both *M. tuberculosis* and *M. bovis*. *M. bovis* IGRA test kits are now available (Bernitz et al., 2018).
3. Collection of data on the health history of the animals, how they are used (e.g. for milk supply or farm work), daily interactions of study participants with the animals (for cases, before TB symptoms), and where the animals spend the night.
4. Testing of animal dung and milk samples for mycobacterium contamination.
5. Collection of data on whether milk is consumed raw or after boiling. We did not obtain data on milk treatment before consumption.
6. Confirmation of participant BCG status, if possible.

In conclusion, we have found evidence that ownership of bovine species is associated with reduction in both *M. tuberculosis* infection and PTB disease risk in the owners. It cannot be excluded that these associations are caused by some unidentified confounder, but it is plausible that they are real. The associations may point to antigens potentially useful for development or boosting of vaccines—for TB, or even viral diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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