



Serological evidence of Brucella infections in dairy cattle in Haryana, India

Johanna F. Lindahl, Naresh Goyal Kumar, Ram Pratim Deka, Rajeswari Shome & Delia Grace

To cite this article: Johanna F. Lindahl, Naresh Goyal Kumar, Ram Pratim Deka, Rajeswari Shome & Delia Grace (2018) Serological evidence of Brucella infections in dairy cattle in Haryana, India, *Infection Ecology & Epidemiology*, 8:1, 1555445, DOI: [10.1080/20008686.2018.1555445](https://doi.org/10.1080/20008686.2018.1555445)

To link to this article: <https://doi.org/10.1080/20008686.2018.1555445>



© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 08 Jan 2019.



Submit your article to this journal [↗](#)



Article views: 1059



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 2 View citing articles [↗](#)

Serological evidence of *Brucella* infections in dairy cattle in Haryana, India

Johanna F. Lindahl ^{a,b,c}, Naresh Goyal Kumar^d, Ram Pratim Deka^e, Rajeswari Shome^f and Delia Grace ^g

^aDepartment of Biosciences, International Livestock Research Institute, Southeast Asia Regional Office, Hanoi, Vietnam; ^bZoonosis Science Centre, Uppsala University, Uppsala, Sweden; ^cDepartment of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden; ^dDairy Microbiology Division, National Dairy Research Institute, Karnal, India; ^eDepartment of Biosciences, International Livestock Research Institute, Guwahati, India; ^fNational Institute for Veterinary Epidemiology and Disease Informatics, Bangalore, India; ^gDepartment of Biosciences, International Livestock Research Institute, Nairobi, Kenya

ABSTRACT

Background: In India, milk production is important for both the economy and the provision of nutritious food. However, the productivity of the livestock is affected by circulating infectious diseases, and some zoonotic diseases, such as brucellosis, may cause a heavy impact on the farm as the disease cause abortions and reproductive failures in bovines, with chronic febrile illness in humans.

Methods: 249 dairy farms in the state of Haryana, India, were interviewed, and collected raw milk from 81 were analyzed for antibodies towards *Brucella abortus*.

Results: More samples were positive using milk ring test (MRT) (55.6%, 45/81) than using enzyme-linked immunosorbent assay (ELISA) (29.6%, 24/81), with all ELISA positive samples also positive in MRT. The ELISA results were used for risk factor analyses. Seropositive farms were significantly ($p = 0.015$) larger than seronegative, with an average 7.9 cattle, compared to 4.9. Seropositive farms were more likely to report stillbirth occurring the last year, and a significantly higher proportion of seropositive farms reported retained placenta (odds ratio 5.2).

Conclusion: This study showed that *Brucella* seroprevalence is high among farms in Haryana, and a control program is needed to ensure improved human and animal health, as well as improved livestock productivity.

ARTICLE HISTORY

Received 31 October 2018
Accepted 28 November 2018

KEYWORDS

Brucellosis; zoonosis; dairy production; South Asia; OneHealth

Introduction

Milk consumption is increasing in low and middle-income countries, and this is likely to continue with the increasing demand for animal-source food following urbanization and growing middle-income classes [1,2]. India, with a population of more than one billion people, is since 2001 the world's leading milk producer and has the world's largest dairy herd at around 300 million, both buffalos and cows [3]. In 2013 the total milk production was over 135 mega tonnes [3,4]. The majority of the population still live in rural areas, where the dairy sector is an important source of income, especially for the poor, and there are estimates that around 70 million Indian households are engaged in dairy production [4]. The milk provides consumers a source of protein and calcium, which in a country with a large vegetarian population like India [5], is of great importance; for many, dairy products are the sole source of animal proteins.

In India, the health of livestock, humans and their economic welfare are closely linked [6]. Zoonotic diseases create a double burden on the households with both human and animal morbidity, causing reduced incomes, and reduced resilience towards disease in a vicious cycle [7]. Brucellosis is a severe disease for

dairy farmers since the disease is associated with abortions and reproductive failures in the livestock, but it also cause serious chronic disease in humans [8].

While there are multiple species capable of causing brucellosis, the infection in cattle is predominantly caused by *Brucella abortus*, and sometimes by *B. melitensis* (which is more common in small ruminants) or by *B. suis* (which is more common in swine) [9–11].

In India, awareness of brucellosis is low among livestock-keepers and healthcare staff, and because of the general symptoms and the limited availability of laboratory facilities in many rural hospitals, diagnosis is not easy [12,13].

There has been a number of studies looking at seropositivity in different parts of India, but there is a lack of probabilistic studies evaluating seroprevalence, and this study aimed to study the prevalence in Haryana, a state close to New Delhi with a rapidly growing dairy sector.

Material and methods

Selection of participating households were used using multistage random sampling, using the random function

in Ms Excel. The district containing the National Dairy Research Institute, Karnal was selected, and in addition, two more districts were randomly selected. Within each district, one urban and one rural block was randomly selected, and within each block, four villages were randomly selected, and within each village ten households were selected. Selected farmers were contacted the day before the visit by a local non-governmental organization, and asked for the consent to participate, and asked if they could save some milk from the morning milking. At the time of visit, the farmers were informed about the study and if they gave a signed consent, they were interviewed using a questionnaire, which had been approved by the Institutional Research Ethics Committee (IREC) at International Livestock Research Institute (ILRI) (IREC 2017–05), and a bulk milk sample was brought to the laboratory where it was kept at -20°C until analysis.

The milk samples were tested using Milk Ring Test (MRT) and an indirect Milk-ELISA (IDEXX Laboratories, Inc.). The ELISA was conducted according to the manufacturer's instructions, and the recommended cut-offs used for judging results as positive or negative.

Farmers were interviewed as to the number of cattle at the farm, their handling of milk as well as the incidence of specific diseases the last year, and if they perceived this incidence as increasing or decreasing. Disease incidence was normalized by dividing with the number of animals. Reproductive symptoms (mastitis, abortion, stillbirth, repeat breeding, retained placenta and vaginal discharge), all common symptoms of brucellosis in livestock were normalized by dividing with the total number of adult females, while symptoms such as fever, carpal hygroma and lameness, which may also be symptoms of brucellosis, were divided by the total number of animals.

Data was entered in Excel and imported into STATA 14.0 (STATA Corp Ltd). For analysis as to symptoms and the association with serological results, the ELISA results were used. Chi-square tests were used to test for associations between categorical data.

Results

Out of the 249 interviewed farms, 99 kept both buffaloes and cows, 57 had only cows and 93 had only buffaloes. The mean number of adult female cows at the farms with cows was 1.6, and ranged from 0 to 12, while the mean number of adult female buffaloes was 1.5, but ranged from 0 to 11. The mean number of milking animals on a farm (both cows and buffaloes) was 1.9, but ranged from 1 to 12.

68.7% of the respondents were men and 31.3% were women. Only 4 farmers had ever participated in a training about livestock diseases. Only 2 farmers used machine milking, and one of them also hand milked. Only 8.4% of the farmers would throw away

Table 1. Results from the milk ring test (MRT) and enzyme-linked immunosorbent assay (ELISA) tested on milk from 81 farms in Haryana, India.

	ELISA negative	ELISA positive	Total
MRT negative	36	0	36
MRT positive	21	24	45
Total	57	24	81

Table 2. Incidence of cattle diseases in *Brucella* seropositive and seronegative farms in Haryana, India.

Herd incidence per female cattle and year	<i>Brucella</i> seronegative herds	<i>Brucella</i> seropositive herds
Mastitis	0.15 (0–1)	0.20 (0–1)
Repeat breeding	0.21 (0–1)	0.12 (0–2)
Abortion	0.05 (0–1)	0.07 (0–0.5)
Retained Placenta	0.07 (0–1)	0.13 (0–1.5)
Stillbirth	0 (0–0)	0.06 (0–1)
Vaginal discharge	0.03 (0–0.7)	0.00 (0–0.1)
Herd incidence per cattle and year		
Fever	0.14 (0–1)	0.13 (0–0.6)
Lameness	0.03 (0–1)	0.02 (0–0.3)
Carpal hygroma	0.02 (0–1)	0.01 (0–0.1)

milk from a sick animal, 88% would consume it themselves, and 3.6% would sell it.

More samples were positive using MRT (55.6%, 45/81) than using the ELISA (29.6%, 24/81) (Table 1). There were no samples that were positive in ELISA that was negative in the MRT. Of the farms with positive ELISA results, 8.3% had experienced stillbirth last year, which was higher ($p = 0.08$) than farms with negative results, which had no stillbirths. Similarly, more positive farms experienced retained placentas (33.3%) compared to negative farms (8.8%) ($p = 0.006$), and the odds ratio was 5.2 (95% CI 1.5–18.2). For the other disease symptoms, no differences were detected (Table 2). *Brucella* positive farms were significantly larger (average 7.9 heads of cattle, standard deviation 1.3) than negative farms (average 4.9 heads of cattle, standard deviation 0.6) ($p = 0.015$).

Discussion

This study measured brucella seroprevalence on farm bulk milk and found that more than 20% of the randomly selected farms tested were seropositive. These results are similar to what has been found in the close-by state Punjab, where 21% and 18% seroprevalence was found by [14] and [15], but higher than some other studies in Punjab [16,17], and what has been found screening samples from 23 states of India [18]. Not surprisingly, the screening by MRT found a higher number of positives than the ELISA. Milk ring test is known to have a lower specificity than ELISA and may give false positives in different stages of lactation, or with mastitic milk [10,19,20].

Disease occurrence were all reported by the farmers, which can make it prone to memory bias, but since reproductive disorders are of such great importance to the farmers, the risk in incorrect remembrance is low.

Although there was only a small number of farms tested in this study, we found an association between serological status of *Brucella* and having experienced retained placenta the last year, and there was also a tendency of more positive farms experiencing stillbirths, but a study with higher power would be needed to further investigate this. This is in accordance with other studies and to the clinical picture often seen with *Brucella* infections [14]. Risk of *Brucella* has previously also been associated with herd size [17,21–23].

In conclusion, this high *Brucella* seropositivity and the fact that farmers still sell and consume milk from animals perceived sick, does give a cause of concern and indicates that a brucellosis control program may be warranted in Haryana, both to improve the dairy production and to safeguard human health.

Acknowledgments

The authors would like to thank the donors to the CGIAR system for their support. We would like to acknowledge all farmers for their participation, and Dr HR Rahman for his support to the project.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This project was supported by the ICAR ILRI collaboration funds and the CGIAR Research Program Agriculture for Nutrition and Health.

Notes on contributors

Johanna Lindahl is a veterinary epidemiologist working at International Livestock Research Institute (ILRI) in Nairobi Kenya, and adjunct at Swedish University of Agricultural Sciences as well as Uppsala University. Johanna graduated from Swedish University of Agricultural Sciences after doing her PhD working on Japanese encephalitis virus in Vietnam. Since her PhD she has been focusing her research on food safety, and vector-borne, zoonotic and emerging infectious diseases in developing countries, mainly East Africa and South and Southeast Asia. In addition to this, she is coordinating a number of aflatoxin projects within ILRI.

Naresh Kumar works at ICAR-NDRI, in Karnal, Haryana. He has a MSc degree in dairy bacteriology and a PhD in dairy microbiology. His main research interests include Quality Assurance & Food Safety with specialization in the field of biosensor development for pathogens and non-microbial contaminants in dairy industry.

Ram Pratim Deka is a scientist at ILRI based in the office in Guwahati, Assam, India. He has a MSc in veterinary medicine, and also a MBA degree. He is working in many projects including zoonotic diseases, food safety and accelerating the dairy and pig value chains in the Northeast India region.

Rajeswari Shome works at ICAR-NIVEDI and has her basic qualification in veterinary sciences and doctoral degree in microbiology. She has vast research work experience in different agroclimatic conditions like island ecosystem, north-eastern hilly regions and southern plain lands of India. While serving in different regions, and has gained hand on experience in investigation of infectious bacterial diseases of poultry (colibacillosis, salmonellosis), pig diseases (bordetellosis, pasteurellosis, salmonellosis and piglet diarrhoea), bovine diseases (Para TB, BQ, HS, bovine mastitis, brucellosis, listeriosis and leptospirosis), fish diseases (edwardsiellosis, vibriosis, pop eye disease and ulcerative syndrome) and exploration of marine mangrove microbes for bioactive compounds etc.

Delia Grace is an epidemiologist and leads the Health Program at ILRI and the Flagship on Food Safety in the CGIAR research program on agriculture and health. She has been a lead researcher in food safety in informal markets for several decades. She has led or contributed to evidence syntheses and investment advice for World Bank, DFID, USAID, ACIAR, BMGF, FAO, OIE, WHO, AUIBAR, OECD and others.

ORCID

Johanna F. Lindahl  <http://orcid.org/0000-0002-1175-0398>

Delia Grace  <http://orcid.org/0000-0002-0195-9489>

References

- [1] Delgado CL. Rising consumption of meat and milk in developing countries has created a new food revolution. *J Nutr.* 2003 Nov;133(11):3907S–3910S.
- [2] Rae AN. The effects of expenditure growth and urbanisation on food consumption in East Asia: a note on animal products. *Agric Econ.* 1998;18:291–299.
- [3] FAOSTAT. Milk total production in India. [Online]. 2015 [cited 2015 Apr 12]. Available from: <http://faostat3.fao.org/browse/Q/QL/E>
- [4] Doupbrate DI, Hagevoort GR, Nonnenmann MW, et al. The dairy industry: a brief description of production practices, trends, and farm characteristics around the world. *J Agromedicine.* 2013 Jan;18(3):187–197.
- [5] Singh PN, Arthur KN, Orlich MJ, et al. Global epidemiology of obesity, vegetarian dietary patterns, and noncommunicable disease in Asian Indians. *Am J Clin Nutr.* 2014 Jul;100(suppl_1):359S–64S.
- [6] Thumbi SM, Njenga MK, Marsh TL, et al. Linking human health and livestock health: a ‘One-Health’ platform for integrated analysis of human health, livestock health, and economic welfare in livestock dependent communities. *PLoS One.* 2015 Mar;10(3):e0120761.
- [7] Grace D, Wanyoike F, Lindahl J, et al. Poor livestock keepers: ecosystem-poverty-health interactions. *Philos Trans R Soc B-Economy.* 2017;372:20160166.
- [8] McDermott JJ, Arimi SM. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Vet Microbiol.* 2002 Dec;90(1–4):111–134.
- [9] OIE. Infection with *Brucella abortus*, *Brucella melitensis* and *Brucella suis*. In: OIE terrestrial manual

2016. Rome: OIE (World Organisation for Animal Health); 2016. Chap 2.1.4; p. 1–44.
- [10] Godfroid J, Nielsen K, Saegerman C. Diagnosis of brucellosis in livestock and wildlife. *Croat Med J.* 2010;51(4):296–305.
- [11] Godfroid J, Scholz HC, Barbier T, et al. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Prev Vet Med.* 2011 Nov;102(2):118–131.
- [12] Omemo P, Ogola E, Omondi G, et al. Knowledge, attitude and practice towards zoonoses among public health workers in Nyanza province, Kenya. *J Public Health Africa.* 2012 Oct;3(2):22.
- [13] de Glanville WA, Conde-Álvarez R, Moriyón I, et al. Poor performance of the rapid test for human brucellosis in health facilities in Kenya. *PLoS Negl Trop Dis.* 2017 Apr;11(4):e0005508.
- [14] Aulakh HK, Patil PK, Sharma S, et al. A study on the epidemiology of bovine brucellosis in Punjab (India) using milk-ELISA. *Acta Vet Brno.* 2008;77(3):393–399.
- [15] Ul-Islam MR, Gupta MP, Filia G, et al. Sero-epidemiology of brucellosis in organized cattle and buffaloes in Punjab (India). *Adv Anim Vet Sci.* 2013;1(3S):5–8.
- [16] Kumar H, Sharma DR, Singh J, et al. A study on the epidemiology of brucellosis in Punjab (India) using survey toolbox. *Rev Sci Tech.* 2005 Dec;24(3):879–885.
- [17] Gill J, Kaur S, Joshi D, et al. Epidemiological studies on brucellosis in farm animals in Punjab state of India and its public health significance. *Proceedings of the 9th International Symposium on Veterinary Epidemiology and Economics; Breckenridge, CO; 2000.*
- [18] Isloor S, Renukaradhya GJ, Rajasekhar M. A serological survey of bovine brucellosis in India. *Rev Sci Tech - off Int Des Épizooties.* 1998 Dec;17(3):781–785.
- [19] Gall D, Nielsen K. Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Rev Sci Tech.* 2004;23(3):939–1002.
- [20] Nielsen K, Yu WL. Serological diagnosis of brucellosis. *Prilozi.* 2010;31(1):65–89.
- [21] Makita K, Fèvre EM, Waiswa C, et al. Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. *BMC Vet Res.* 2011;7(1):60.
- [22] Mugizi DR, Boqvist S, Nasinyama GW, et al. Prevalence of and factors associated with *Brucella* sero-positivity in cattle in urban and peri-urban Gulu and Soroti towns of Uganda. *J Vet Med Sci.* 2015 May;77(5):557–564.
- [23] Patel M, Patel P, Prajapati M, et al. Prevalence and risk factor's analysis of bovine brucellosis in peri-urban areas under intensive system of production in Gujarat, India. *Vet World.* 2014;7(7):509–516.