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Epidemiology of Brucella infection and cost of reproductive disorders in dairy animals in Assam and Bihar, India

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Cover: Photograph of a heifer in a dairy farm near Guwahati, Assam, India
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Abstract

Brucellosis is one of the most important zoonotic diseases in the world and one of the most important causes of reproductive disorders in livestock. The disease is endemic in India. The This study aimed to assess seroprevalence, risk factors, and clinical predictors of *Brucella* infection in dairy animals, along with the relevant knowledge and practices of farmers and also the cost of reproductive disorders in dairy farming. A cross-sectional study was conducted in the states of Assam and Bihar, India through a primary survey of 534 randomly selected dairy farming households and serological investigation of 740 blood samples collected from their dairy animals (cattle and buffalo).

From laboratory analysis, animal-level *Brucella* seropositivity was 15.9% in Assam and 0.3% in Bihar. Three identified risk factors for *Brucella* seropositivity in Assam were geographical location (district) (p<0.001), age of dairy animals (p=0.008) and mating system (p=0.07). Occurrence of retained placenta was the most important clinical symptom (OR 20.7) for predicting *Brucella* infection. Only a small percentage of farmers (3.4%, n=18) knew about brucellosis. Actions to prevent *Brucella* infection by the farming community were negligible. The estimated cost of reproductive disorders was USD 36.1 per dairy animal per year which represented approximately 4.1% of the mean value of dairy animals (USD 877). Reproductive disorders caused an estimated annual economic cost of USD 59.0 million in Assam and USD 453.9 million in Bihar.

The findings help in identifying future research priorities and limitations and designing effective *Brucella* control programme in dairy farming in India and beyond.

Keywords: *Brucella*, bovine, risk factors, prevalence, predictors, knowledge, reproductive problems, economics, India

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Dedication

I dedicate this thesis to all my family members without whose blessings, help, support, and encouragement this would not have been possible to produce.

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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Deka, R.P.; Shome, R.; Dohoo, I.; Magnusson, U.; Grace, D.; Lindahl, J. (2021). Seroprevalence and risk factors of *Brucella* infection in urban and rural areas in the state of Assam and Bihar, India; *Microorganism*, 9 (4). Pp.1-15

II. Deka, R.P.; Magnusson, U.; Grace, D.; Shome, R.; Lindahl, J. (2020). Knowledge and practices of dairy farmers relating to brucellosis in urban, peri-urban and rural areas of Assam and Bihar, India; *Infection Ecology and Epidemiology,* 10(1).Pp.1-8

III. Deka, R.P.; Magnusson, U.; Randolph, T.; Grace, D.; Shome, R.; Lindahl, J. Estimate of the economic cost caused by five major reproductive disorders of dairy animals in Assam and Bihar, India (submitted).

IV. Deka, R.P.; Magnusson, U.; Grace, D.; Lindahl, J. (2018). Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India- a review; *Infection Ecology and Epidemiology*, 8 (1). Pp.1-7

V. Deka, R.P.; Shome, R.; Dohoo, I.; Magnusson, U.; Grace, D.; Lindahl, J. Clinical symptoms as predictors of *Brucella* infection in dairy cattle (submitted).

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Abbreviations

AI	Artificial insemination
AGPT	Agar gel plate test
BCE	Before common era
BCR	Benefit cost ratio
BPAT	Buffer plate agglutination test
CD	Cattle demography
CDB	Community development block
cELISA	Competitive enzyme-linked immunosorbent assay
CFT	Complement fixation test
CI	Confidence interval
DAHD	Department of Animal Husbandry and Dairying
DCS	Dairy cooperative society
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agricultural Organisation
FBAT	Febrile antigen *Brucella* agglutination test
FC	Farm characteristic
FMD	Foot and mouth disease
FM	Farm management

FGD	Focus group discussion
FPA	Florescence polarization assay
GDP	Gross domestic product
GIS	Geographic information system
GPS	Global positioning system
ICAR	Indian Council of Agricultural Research
iELISA	Indirect enzyme-linked immunosorbent assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INR	Indian Rupees
IRR	Internal rate of return
IREC	Institutional Research Ethics Committee
ILRI	International Livestock Research Institute
MRT	Milk ring test
NDDB	National Dairy Development Board
NIVEDI	National Institute of Veterinary Epidemiology and Disease Informatics
NPV	Net present value
OIE	World Organisation for Animal Health
OD	Optical density
OR	Odds ratio
OPS	O-polysaccharide
PBP	Payback period
PCR	Polymerase chain reaction
PD	Producer demographics
PUO	Pyrexia of unknown origin
RBPT	Rose Bengal plate test

RB51	Rough *Brucella* 51
RLPS	Rough lipopolysaccharide
SAT	Serum agglutination test
SLPS	Smooth lipopolysaccharide
STAT	Standard tube agglutination test
TEC	Total economic cost
WHO	World Health Organisation
USD	United States Dollar

1. Introduction

Brucellosis is a transboundary bacterial zoonotic disease of high economic importance. It is prevalent in most of the low and medium income countries (Franc et al., 2018). The geographical distribution of the disease is constantly changing with emergence or re-emergence of new foci of infection (Seleem et al., 2010).

The disease causes huge economic losses to farming families by reducing productive and reproductive performances, increasing the expenses of livestock management and reducing the productive life of domestic animals. International trade of livestock and livestock products, food safety and food security are also affected by brucellosis (Song et al., 2018; Adetunji et al., 2020; Khurana et al., 2021).

In humans, the disease causes a variety of non-specific signs and major costs in terms of loss of person-days, expenditure for long term treatment and decline in socioeconomic status of the infected persons (Singh et al., 2018; Franc et al., 2018). It is considered the most common laboratory-acquired pathogen and is a potential biological weapon (Seleem et al., 2010; Owowo et al., 2019).

Because of the difficulty in diagnosis, poor diagnostic infrastructure and capacity, lack of vaccine for several species of animals as well as humans, inadequate awareness among different stakeholders, lack of an integrated approach by animal and human health professionals and inadequate investment in disease control, the disease is yet to be controlled in most low and medium income countries in Asia and Africa (McDermott et al., 2013; Dadar et al., 2021). Because both human and animal health are affected, a One Health approach to control brucellosis is emphasized by several studies (Franc et al., 2018; Lindahl et al., 2020).

The study of brucellosis in India is especially important because of its large livestock population, endemic nature of brucellosis, dominance of smallholder farming system, and, poor hygiene practices in livestock production and product trading (Lindahl et al., 2018) as well as high human and animal interaction.

An attempt has been made in this study to better understand Brucella infection in large ruminants in two Indian states to help identify future research needs and design future brucellosis control programmes.

1.1 Dairying in India

India, a country with a projected population of 1.36 billion in 2021 (MHFW, 2019), is the seventh largest country in the world and the third largest economy after US and China (FAO, 2021b). It is a diverse country in terms of religion, culture, tradition, language, cuisine, topography, climate, environment, and so on. India is the world's largest milk producer (FAO, 2021b). The estimated milk production in the country was about 194.8 million tons in 2020 in comparison to the global production of 906.0 million tonnes which means India produces about 21.5% of global milk (FAO, 2021a). India's bovine population is also the largest in the world, comprising 193.5 million cattle and 109.8 million buffaloes (DAHD, 2019). The Indian dairy sector grew by 6.7% in 2018-2019 in comparison to global growth of 1.4% during the same period (DAHD, 2020; FAO, 2020). The dairy sector in India contributed about 4.9% of the total Gross Domestic Product (GDP), which is the highest among all the agricultural commodities (including paddy rice and wheat) in the country. This contribution remained almost static although contribution of grains reduced over the years (DAHD, 2020).

This high contribution of dairy sector to the country's economy is due to more dairy animals rather than higher productivity (FAO, 2021a). India's average production per dairy animal per day is much lower than many developed and developing countries (only 2.5 kg per day in case of indigenous cows and 11.7 kg per day in case of exotic cows) (Haile et al., 2014; DAHD, 2020). Annual milk yield of dairy cows in India is one tenth of that in the USA, and one fifth of the yield in New Zealand (FAO, 2003). In India, along with cattle, buffaloes play an important role as dairy animals and contribute about 49% of the country's total milk production which is much higher than global average contribution of 15% from buffaloes (FAO, 2020). About 34.6% dairy cows are improved (exotic breed imported from abroad or their crossbred) and remaining are indigenous (native breeds or non-descript indigenous) (DAHD, 2019). All buffalo are either indigenous breed or non-descript indigenous.

Dairying in India has a long history. Domestication of indigenous zebu cattle probably started about 8000 years ago (Singh, 2012). Domestication of riverine buffalo also started about 7000-5000 years ago (Prasad, 2017). In ancient India, among the semi-nomadic people, cattle pastoralism was the mainstay of livelihood and agriculture used to play a secondary role (Jha, 2002). By the beginning of the Indus Valley civilization (3300 - 1300 Before Common Era (BCE), zebu cattle were fully domesticated, and their milk was used. Rearing of cattle and use of milk and milk products such as curd, cream were well explained in different ancient epics such as the *Rigveda*, *Ramayana*, *Mahabarata* of Hindu religion (Jha, 2002). During the *Vedic* Period (1500- 500 BCE), milk played an important role in the human diet (Prasad, 2017); perhaps because of this millions of people in India are still vegetarian and cattle still play an important role in life of millions of Hindu people in the country, many of whom consider the cow as a holy animal.

The trend of growth of the dairy sector in India is also remarkable. A largely milk deficit country up to the late 1960s (with total production of 21 million tonnes), India became the world's largest producer of milk in 1998. This significant improvement of dairy sector in India has increased the per capita availability of milk from about 112 gm/day in the late sixties to 394 gm/day in 2019 (DAHD, 2020) although the global per capita availability of milk declined after 1980 (FAO, 2005). The credit for such impressive growth of the dairy sector in India goes to the Operation Flood Programme launched by India's National Dairy Development Board (NDDB) in 1970. The programme introduced a three-tier cooperative system consisting of primary Dairy Cooperative Societies (DCS) at village level, Milk Unions at district level and Milk Federations at state level for organised production, procurement, processing, and marketing of milk. Under Operation Flood, popularly known as "White Revolution", cross-bred breeding programme coupled with access to farm inputs and market were highly emphasised (FAO, 2021a). However, the dairy farming infrastructure and services have not transformed significantly from a traditional to a modern scientific approach. One of the reason behind such dismal picture is that about 86% of the country's dairy farmers belongs to small or marginal category (less than two hectare of landholding per farmer) (DAHD, 2020). In India, there are around 70 million dairy farming households in 2012 (Douphrate et al., 2013) which is almost half of the estimated farming households in the world (150 million) (FAO, 2021b). About 80% of dairy farming households in India reared only 2-8 animals (FAO, 2003). These households have limited resources to invest in housing, good feed, and better livestock technologies and have limited access to veterinary, training, and extension services (Kumar et al., 2007).

In regards to milk marketing, about 20.4% of produced milk in the country is consumed within the farming families by themselves, while 45.4% milk is traded through informal milk market agents which include milk vendors, sweet makers and cottage processors, and the remaining 34.3% is traded through formal sector which includes dairy cooperatives societies and processing plants (Kumar et al., 2018). The higher share of informal milk market is partly because of the higher price of milk paid by informal milk market agents compared to cooperatives or processing plants (Kumar et al., 2018). Handling large volumes of unpasteurized milk by informal milk market actors poses significant risk to human health as several zoonotic pathogens such as *Brucella*, *Leptospira, Coxiella, Mycobacterium* etc. are in circulation in the country (Lindahl et al., 2019; Shome et al., 2019; Leahy et al., 2021). Further, there is poor knowledge of farmers, market actors and consumers about zoonotic diseases in India (Rajkumar et al., 2016; Lindahl et al., 2018; Singh et al., 2019). Hence, there is need for studying zoonotic diseases like brucellosis in the India context.

1.2 Brucellosis - the disease

Brucellosis is one of the most common but neglected zoonotic diseases in the world (Franc et al., 2018). The disease in livestock occurs worldwide, except in some high-income countries such as Denmark, Finland, Netherlands, United Kingdom, Norway, Belgium, Switzerland, Iceland, Germany, Luxembourg, Sweden, Austria, Canada, Japan, Australia, and New Zealand, which are reported to be brucellosis free (Pappas and Papadimitriou, 2006; OIE, 2009). In low- and middle-income countries, the disease is often underreported and there is little or no effective control, resulting in major health, economic and livelihood burdens (McDermott et al., 2013). Brucellosis is reported to be endemic in parts of Africa, Central and South America, Middle East and Asia in both humans and animals

(Pappas et al., 2006). In India, it was first reported in 1942 and is now known to be endemic.

The disease in bovines is called bovine brucellosis which effect both cattle and buffalo. In sexually mature female bovines, *Brucella* infection localizes in the reproductive tract and causes placentitis followed by abortion. The most frequently reported symptom is abortion, especially in the last trimester (OIE, 2009; Ul-Islam et al., 2013). Most infected animals abort only once in their lifetime, but may remain infective for their entire life (Godfroid et al., 2010). Even in the absence of abortion, profuse excretion of the organism may occur from the placenta, foetal fluid, and uterine and vaginal discharges. The mammary gland and associated lymph nodes may be affected, and the organism may be shed in milk. The disease is often asymptomatic in non-pregnant female bovines and after the first abortion. Adult male bovines may develop orchitis, and brucellosis may cause infertility in both sexes. Hygromas can occur in leg joints and are a common manifestation of brucellosis in some tropical countries (OIE, 2009). Bovine brucellosis can also occur in bison and yak and clinical manifestations in these animals are similar to those in cattle (OIE, 2009).

Different reported clinical symptoms of brucellosis/ *Brucella* infection by different researchers, open the avenues for studying them further to see their association with brucellosis/ *Brucella* seropositivity and to use them as predictor of the same.

1.3 *Brucella*- the causative agent

Brucellosis is caused by bacteria of the genus *Brucella.* A physician named David Bruce identified the bacterium *Brucella* in 1887 in the spleen of a British soldier. In 1918, Alice Evans, an American microbiologist, suggested the term “brucellosis” to acknowledge David Bruce (Pradeepkiran et al., 2021). *Brucella* are intracellular, gram-negative, non-spore forming, non-motile, coccobacilli. There are diverse opinions in the scientific community as to the different species or sub-species of *Brucella*; however, it is generally accepted that there are about twelve species (Rajala, 2016). These include *B. abortus* (preferred natural host - cattle and other bovines including buffalos), *B. melitensis* (sheep and goat), *B. canis* (dog), *B. suis* (swine, wild rodents), *B. ovis (*sheep)*, B. neotomae* (desert wood rat), *B. microti* (common vole, fox), *B. ceti* (cetaceans), *B. inopinata* (unknown, and *B. pinnipedialis* (seals, pinnipeds) (Godfroid et al., 2010; Scholz and Vergnaud, 2013; Rajala, 2016). The most recently described species are *B. vulpis* and *B. papionis,* but the preferred host is unknown for these species (Whatmore and Davison, 2014; Hofer et al., 2016). Most species of *Brucella* can infect multiple species of animals, including humans (Godfroid et al., 2010). In cattle, infection is predominantly caused by *B. abortus,* less frequently by *B. melitensis* and occasionally by *B. suis* (OIE, 2016). In humans, *B. melitensis* is the most commonly responsible for causing brucellosis, but *B. abortus* and *B. suis* are also important. *B. abortus* has been isolated from a variety of wildlife species including wild buffalo and bison, and *B. suis* has been isolated from wild boars (Godfroid et al., 2013). Therefore, transmission of *Brucella* infection from wild species to domestic species and from them to humans cannot be overlooked.

All species of *Brucella* contain smooth lipopolysaccharide (SLPS) in their outer cell wall except *B. ovis* and *B. canis* that contain rough lipopolysaccharide (RLPS). Smooth lipopolysaccharide contains a lipid and an immunodominant O-polysaccharide (OPS). Other microorganisms, for example *Yersinia enterocolitica* O:9, that contain antigens with epitopes similar to those of OPS and immunoglobulin M (IgM) antibodies may cross-react, therefore measurement of IgM antibody may result in a false positive reaction in serological test (Muñoz et al., 2005; Nielsen and Yu, 2010). False positive reactions may occur among vaccinated animals if the vaccine includes antigen containing SLPS (e.g. *Brucella* strain 19 vaccine) (OIE, 2009). Eliminating false positive reactions is a major challenge for serological tests, and if the vaccination history of the animals is not clear it may be difficult to conclude whether the animal has been infected.

There are several biotypes of *Brucella* organism. Bio-typing of *Brucella* provides important epidemiologic information that allows better tracing of infections to their sources in areas where several biotypes circulate (Godfroid et al., 2010). In India, 7 biotypes (1-6 and 9) of *B. abortus*, 3 biotypes (1-3) of *B. melitensis* and 5 biotypes of (1-5) of *B. suis* are reported (Gall et al., 2000; Renukaradhya et al., 2002; OIE, 2009; Godfroid et al., 2010; Nagalingam et al., 2012). *B. abortus* biotype 1 appears to predominate in India, followed by biotype 3, 9, 4 and 6.

1.4 Brucellosis in humans

Brucellosis in humans is called relapsing or undulant fever (caused by *B. abortus*), Malta fever or Mediterranean fever (caused by *B. melitensis*). It may present as an acute or sub-acute febrile illness accompanied by anorexia, sweating, fatigue, weight loss, headache and joint pain persisting for weeks to months (WHO, 2006b; OIE, 2009; Godfroid et al., 2010). The epidemiology of human brucellosis in the world has been changing with time. Several areas traditionally considered as endemic (e.g., France, Israel, and Latin America) have controlled the disease while some other new areas of infection have emerged (e.g., Central Asia) and in some areas (e.g., Syria) the situation deteriorating. It is estimated that more than 500,000 new cases of human brucellosis are reported globally each year (Pappas et al., 2006). Human disease is mostly reported from Syria, Mongolia, Kyrgyzstan, Iraq, Iran, Turkey, Former Yugoslav Republic of Macedonia, Albania, Georgia, Algeria, Tunisia, Peru, and Mexico (Pappas et al., 2006).

A study in India found 4.9% seroprevalence among 352 people professionally exposed to animals, and a markedly higher seroprevalence (17%) among field veterinarians (Thakur and Thapliyal, 2002). Brucellosis has been reported as a major cause of pyrexia of unknown origin (PUO) (Sen et al., 2002). A much higher seropositivity (27%) was recorded in a study in Ludhiana in a purposively sampled population (Yohannes et al., 2011). However, a study conducted in Kashmir, India reported only 0.8% seropositive among a larger group of PUO patients (3532 nos.) (Kadri and Rukhsana, 2000). The seroprevalence seems to vary with sample size, level of farm intensification and human-animal interaction. No study report has been found on seroprevalence in a randomly selected study population in India and therefore it is difficult to comment on the true seroprevalence in the general human population.

A study reported 4% seropositivity in patients for whom fever was not the major complaint, but joint pain, headache and lower backache were the major complains (Agasthya et al., 2012). This is in agreement with the findings of a study conducted in Bikaner, India, that has reported joint pain (83.0% patients reported), fever (78%) and backache (58%) as the three most common symptoms exhibited by brucellosis affected people (Kochar et al., 2007).

Regarding risk factors in humans, seropositivity in men is higher than in women, possibly because of more occupational exposure of men to livestock and their products. Relatively higher prevalence is recorded in the age group of 20-36 years compared to older and younger age groups (Yohannes et al., 2011) but another study has suggested that persons having long associations with livestock are more likely to suffer from brucellosis (Proch et al., 2018). Among the occupational groups, veterinarians are the most affected, followed by farm workers (Yohannes et al., 2011). On the contrary, another study has suggested that trained veterinarians are less likely to suffer from brucellosis than veterinary pharmacists and animal handlers (Proch et al., 2018), and two studies suggest that abattoir workers are more seropositive than other workers (Awah-Ndukum et al., 2018; Igawe et al., 2020). History of ingestion of raw milk is reported as an important risk factor (Kochar et al., 2007; Migisha et al., 2018; Dadar et al., 2019). Other studies have reported that contact with aborted foetus, aborted materials and retained placenta are strongly associated with *Brucella* seropositivity in human (Igawe et al., 2020; Getahun et al., 2021). Poor hygiene in milk handling and poor knowledge on hygienic practices and poor access to potable water are also reported as important risk factors for human brucellosis (Onyango et al., 2021). Further, a systematic review suggests that human-to-human transmission is very rare, but there have been reports of such cases (Tuon et al., 2017).

As symptoms of brucellosis in humans are quite complex and non-pathognomonic, more systematic investigations are required to differentiate from other diseases such as malaria, typhoid or venereal disease that might show similar symptoms, before starting any treatment against brucellosis (Bano and Ahmad Lone, 2015). For human health professionals, taking a thorough history may give some indication about the prevalence of the disease. The disease has various presentations and stages; therefore, identification of the best diagnostic test is challenging. Every test has some advantages and disadvantages, and as such correct interpretation of each test result is important. Because of complexities in diagnosis of the disease, brucellosis is termed as a 'disease of mistake' (Araj, 2010). Many human health professionals simply mark the disease as PUO and treat it as a fever. This delays diagnosis and correct treatment.

In India, true incidence might be much higher than reported because of misdiagnosis or under-reporting (Boral et al., 2009). Conversely, in some areas, there appears to be a problem of over-diagnosis because of reliance on tests (e.g. febrile antigen *Brucella* agglutination test- FBAT) with poor specificity (Glanville et al., 2017; Karlsson et al., 2021). However, the accuracy of modern assay has significantly improved the diagnosis (Poester et al., 2010).

1.5 Laboratory diagnosis of *Brucella* infection in dairy animals

Symptoms of bovine brucellosis are not pathognomonic and therefore laboratory examination is needed for a definitive diagnosis of *Brucella* infection/brucellosis. Laboratory tests can either be direct or indirect. Direct tests identify the pathogen through bacteriological cultures or the genome through molecular tests such as polymerase chain reaction (PCR) while indirect tests identify *Brucella* infection by detecting antibodies in serum or milk or allergic reaction in the skin (Godfroid et al., 2010). Positive indirect tests do not necessarily mean animals have current or active infections, confirmatory diagnosis of clinical cases can only be obtained through isolation and identification of the *Brucella* organism. Therefore, isolation and identification of the organism is regarded as the gold standard (Mukherjee et al., 2007; Godfroid et al., 2010; Nielsen and Yu, 2010; OIE, 2016). However, isolation and identification of the *Brucella* organism is difficult and poses risk to human health because of its zoonotic nature. Therefore, cheaper, safer, and easier serological tests are commonly used for assessing *Brucella* infection.

The World Health Organization (WHO) laboratory bio-safety manual classifies brucellosis in risk group-III (OIE, 2009). Laboratory workers may acquire *Brucella* infection through accidental inhalation, ingestion, or skin or mucosal contact. Therefore, biosafety level-III laboratories and skilled personnel are required for laboratories working with *Brucella* organism (WHO, 2004). Due to this, and the fact that indirect tests (serological, milk and skin) are often cheap, rapid, and easy to perform, these are more commonly used for screening of *Brucella* infection (OIE, 2009).

Serological tests, that identify antibodies, are broadly classified in two groups: a) conventional tests and b) primary binding assays (Nielsen and Yu, 2010). Conventional serological tests include standard tube agglutination test (STAT), standard agglutination test (SAT), Rose Bengal plate test (RBPT), agar gel plate test (AGPT), buffer plate agglutination test (BPAT) and complement fixation test (CFT). Binding assays include radioimmunoassay, indirect immunoassay, competitive immunoassay, and florescence polarization assay (FPA). There is also a skin test that relies on a hypersensitivity reaction at the skin site of inoculation. Other commonly used tests are milk ring test (MRT) and milk immunoassay (OIE, 2016).

Serological tests do not distinguish between the different *Brucella* species and no single serological test is appropriate in all epidemiological situations. All tests have some limitations and different sensitivity and specificity depending on the efficiency of detecting immunoglobulin- IgM, IgG1, IgG2 and IgA (OIE, 2009, 2016), as well as the underlying prevalence. The production and disappearance of the immunoglobulin isotypes during infection determines the results of serological tests. For instance, IgM isotypes are rapidly induced 5-15 days after exposure (may be delayed further) and may disappear after a few months, while IgG1 isotypes are induced 3-4 weeks after exposure and remain detectable over a long period of time (up to several years) (OIE, 2009).

For preliminary screening of *Brucella* infection, conventional serological tests are commonly used. RBPT is a very simple, cheap, and rapid test that does not require a sophisticated laboratory to perform. The World Organisation for Animal Health (OIE) recommends this test for screening *Brucella* infection. BPAT is a highly sensitive test, but false positive reactions may occur. CFT has good sensitivity and specificity and is widely used and accepted for international trade although it is complex to perform and needs good laboratory facilities and skilled staff (Godfroid et al., 2010; OIE, 2016). SAT is most commonly used in absence of alternative techniques but not considered reliable enough for screening animals for international trade (OIE, 2016). MRT is an efficient test conducted in bulk milk samples for screening *Brucella* infection at herd level. False positive reactions may arise in dairy animals under certain conditions such as animals vaccinated less than four months prior to testing, in milk samples containing colostrum, in mastitis milk or in late lactation cycle milk (Nielsen and Yu, 2010). If the MRT is positive, all the cows contributing milk to the bulk tank should be tested individually (OIE, 2016). The skin test is a highly specific test, but its weak sensitivity makes it a better test for herds than for individual animals (Godfroid et al., 2010).

The second group of serological tests comprises primary binding assays such as enzyme-linked immunosorbent assays (ELISAs) and FPA. Of the several tests, ELISA is the most commonly used and recommended serological tests for assessing *Brucella* infection in cattle and buffalo (OIE, 2009; Londhe et al., 2011; Kushwaha et al., 2016). Two types of ELISAs are commonly used: indirect ELISA (iELISA) and competitive ELISA (cELISA). Most iELISAs use purified smooth lipopolysacharide antigen, and are thus highly sensitive for distinguishing *Brucella* antibodies in bovines but unable to distinguish B. abortus S19 vaccinal antibody and cross-reacting antibody (OIE, 2009; Godfroid et al., 2010). It is reported that cELISA is little more successful in distinguishing antibodies produced in response to vaccine but cannot distinguish all (OIE, 2009, 2016; Nielsen and Yu, 2010). A joint report of WHO, Food and Agriculture Organisation (FAO) and OIE suggests that there is no test currently available that is able to distinguish vaccinated from infected animals (WHO, 2006a). Because of higher sensitivity of iELISA, it is more commonly used than cELISA. OIE also recommend the use of iELISA over that of cELISA for detection of *Brucella* antibody in individual animal or in herd (OIE, 2016). The diagnostic sensitivity and specificity of the FPA are almost identical to that of cELISA (OIE, 2009).

The sensitivity and specificity of various serological tests used for detection of *Brucella* infection are presented in Table 1.

Unlike the indirect methods discussed above, the direct bacteriological and molecular methods detect live bacteria, the genome or antigens. For diagnosis of *Brucella* infection by bacterial culture, the choice of samples depends on the clinical signs. The most useful samples include aborted foetus, foetal membranes, vaginal secretions, milk, semen and arthritis or hygroma fluid. As the number of *Brucella* organisms is likely to be lower in milk, colostrum and tissues than aborted materials, enrichment of these materials is useful (OIE, 2009). Polymerase chain reaction (PCR) is also used to detect *Brucella* in culture, tissues and animal products (Nagalingam et al., 2012). The PCR can detect deoxyribonucleic acid (DNA) from both living and dead *Brucella* organisms. Depending on the protocol and sequencing used, this test can potentially differentiate all *Brucella* species including the species found in marine mammals and S19, RB51 and the Rev.1 vaccine strains (López-Goñi et al., 2008). However, the sensitivity of PCR could be affected by the DNA extraction procedure, which requires specialized skills and time, and even with this, quality control may be difficult. Therefore, combining PCR with a primary binding assay could improve diagnostic accuracy (Gall and Nielsen, 2004).

Table 1. Sensitivity and specificity of the main tests used for screening *Brucella* infection

Name of the test	**Sensitivity**	**Specificity**
Rose Bengal plate test (RBPT)	81.2	86.3
Standard agglutination test (SAT)	81.5	98.9
Milk ring test (MRT)	88.5	77.4
Complement fixation test (CFT)	90.0-91.8	99.7-99.9
Milk CFT	89.0	86.0
Indirect enzyme linked immunosorbent assay (iELISA)	97.2	97.1-99.8
Competitive enzyme linked immunosorbent assay (cELISA)	95.2	99.7
Milk indirect enzyme linked immunosorbent assay (iELISA)	98.6	99.0
Florescence polarization assay (FPA)	96.6	99.1
Milk FPA	76.9	100
Skin test (cellular test)	78.0-93.0	99.8

Source: Compiled by Godfroid et al. (2010)

Based on the above review, it can be stated that use of cheaper, easier, and safer serological tests with good sensitivity and specificity (e.g., RBPT and iELISA) are more appropriate for conducting serological studies in developing countries context.

1.6 Seroprevalence of *Brucella* infection in dairy animals in India

To assess *Brucella* seroprevalence in a population, it is important to consider sample size, sampling frame, and selection of proper serological tests. Developing an appropriate sampling frame for epidemiological studies is of primary importance.

A recent large study using random sampling method found a *Brucella* seropositivity of 8.3% in dairy cattle and 3.6% in buffaloes (Shome et al., 2019). Another earlier large epidemiological study found 5% seroprevalence in cattle and 3% in buffalo in India (Renukaradhya et al., 2002); however, this study used biological samples collected for sero-monitoring of rinderpest. A study in Karnataka (India) reported 6% prevalence by iELISA and 5% by PCR in non-randomised samples of different sizes of cattle and buffalo farms (Shome et al., 2014).

Two studies using probabilistic sampling in Punjab reported an overall seroprevalence of 21% and 18% respectively (Aulakh et al., 2008; Ul-Islam et al., 2013). Another two studies in Punjab (based on random sampling and non-random sampling), reported 12% seroprevalence (Gill et al., 2000; Dhand et al., 2005). Similar prevalence was reported in Assam (14% in cattle and 10% in buffalo, based on non-random sampling), Gujarat (12%, based on random sampling), Bihar (12%, based on random sampling), Andhra Pradesh (12%, based on non-random sampling) and Chhattisgarh (14.2%) (Bhattacharya et al., 2005; Trangadia et al., 2012; Patel et al., 2014; Pandian et al., 2015; Jain et al., 2018). A relatively lower seroprevalence (8%) was reported in both organised and smallholder dairy farms in Uttar Pradesh (no mention of sampling strategy) (Kumar et al., 2009).

Other studies (Chand and Sharma, 2004; Dalvi et al., 2007; Aulakh et al., 2008; Londhe et al., 2011; Jagapur et al., 2013; Ul-Islam et al., 2013; Patel et al., 2014; Neha et al., 2014; Shome et al., 2015; Pathak et al., 2016) reported much higher seropositivity (20%-60%) in cattle and buffalo in different parts of India but most were conducted in farms with a history of bovine brucellosis/abortion/retained placenta or in a small number of farms selected purposively or from particular dairy belts. Therefore, these seropositivity rates might not reflect the overall seroprevalence in a general population.

In contrast to reported higher seroprevalence of *Brucella* infection by several studies, the country's government reported only four outbreaks of brucellosis in cattle and buffalo with 46 reported cases in 2016 (DAHD, 2017), indicating under-reporting of the disease.

This review indicates the importance of conducting seroprevalence study using random sampling method for assessing the prevalence in a general population.

1.7 Risk factors and clinical symptoms as predictors of *Brucella* infection

In epidemiology, risk factors are referred to those determinants that are associated with the increased risk of disease/ infection. Regarding brucellosis/ *Brucella* infection, several risk factors have been identified by various researchers which can be broadly classified in to four groups: host factors, agro-ecological factors, farmer factors and management factors (Fig.1).

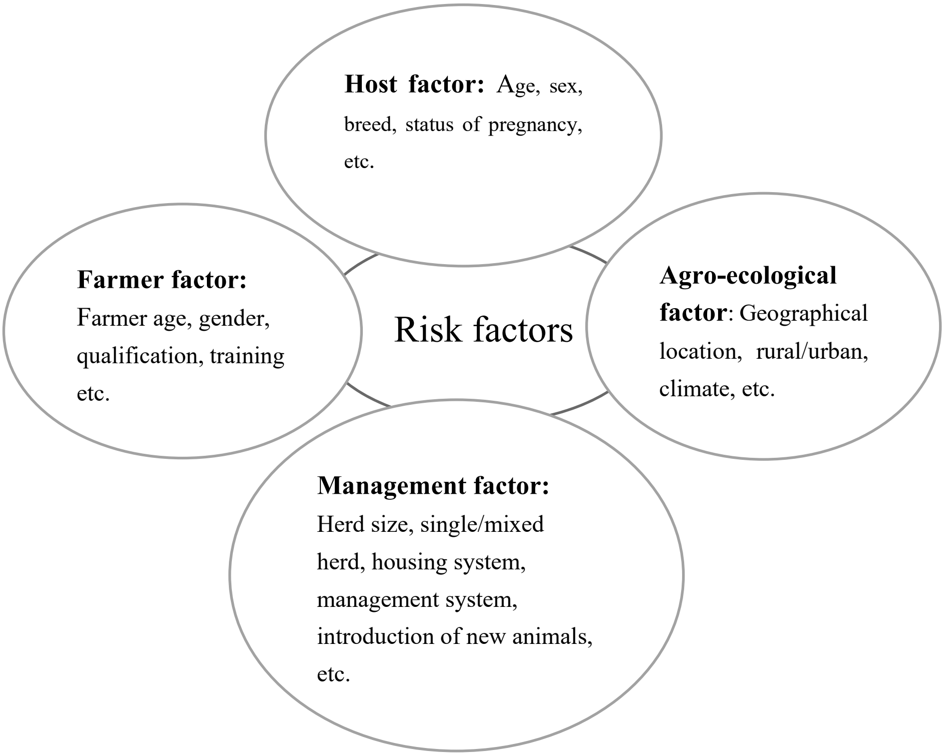

Figure 1. Risk factors of *Brucella* infection in dairy animals classified in four groups

Many researchers found significant associations between species, sex, breed and age of animals and seropositivity (Makita et al., 2011; Chand and Chhabra, 2013; Patel et al., 2014; Shome et al., 2014; Mugizi et al., 2015). Other risk factors reported include: insufficient manure removal and cleaning, poor management of aborted materials, introduction of new animals from herds that were not free from brucellosis or of unknown status, herds kept in close confinement, and mixed herds (Bhattacharya et al., 2005; Dhand et al., 2005; Aulakh et al., 2008; Calistri et al., 2013; Soomro et al., 2014). Animals bred using natural mating are reported to be more likely seropositive for *Brucella* infection than animals bred with artificial insemination (AI)(Shome et al., 2014). Farmer knowledge and awareness of brucellosis is significantly associated with lower seropositivity of *Brucella* in animals (Chand and Chhabra, 2013; Shome et al., 2014; Pathak et al., 2016). Inadequate floor space has also been reported as a the risk factor for *Brucella* infection (Pathak et al., 2016). Reports suggest that younger cows are less likely to be seropositive than older cows (Lindahl et al., 2014) and pregnant cows are more likely to be seropositive than non-pregnant cows (Amin et al., 2004; OIE, 2009). Higher prevalence of *Brucella* infection in females than males is also reported (Dhand et al., 2005).

There are reports that prevalence in large dairy farms is higher than smallholder farms (Chand and Sharma, 2004; Kumar et al., 2009, 2016; Trangadia et al., 2010; Jagapur et al., 2013). This higher prevalence may be due to higher prevalence of the disease in exotic and cross-bred animals, compared to indigenous animal (Pandian et al., 2015), transmission of disease during breeding, physical contact because of close confinement, and, exposure to diseased animals (Kumar et al., 2009; Jagapur et al., 2013). On the contrary, a few studies have reported significantly lower seroprevalences in large farms than smallholder farms, possibly because of proper management, good sanitation and disinfection, proper disposal of placenta, better animal health awareness and management and vaccination; however, the number of farms where studies were conducted were few and not representative of organised farms overall (Gill et al., 2000; Bhattacharya et al., 2005). While large herds have been reported to be more prone to *Brucella* infection, large herds may be owned by farmers who have more resources and are more knowledgeable and this may result in less disease. In this case wealth and education may be confounding factors that mask the positive relation between large herd size and occurrence of brucellosis. Another study has reported higher prevalence in medium size farms (26-100 animals) compared to small or large size farms (Shome et al., 2014). This might be because of indiscriminate bovine replacement from unknown sources or poor hygiene and management in medium sized farm compared larger sized farms. In addition, significantly higher prevalence in cattle than buffalos were reported in some studies (Gill et al., 2000; Bhattacharya et al., 2005; Aulakh et al., 2008; Ul-Islam et al., 2013), but contradictory findings are also not uncommon (Dhand et al., 2005; Trangadia et al., 2010; Jagapur et al., 2013; Soomro et al., 2014). Higher herd prevalence than individual prevalence was also reported (Patel et al., 2014), which is expected when multiple animals are sampled per farm. One study found no significant association between large ruminants (dairy animals) reared along with other domestic species of livestock (Arif et al., 2019). Based on the above discussion, the risk factors and predictors are summarised in Table 2 under four different categories.

Almost all studies have reported one or more symptoms of brucellosis as risk factors, although these are more correctly interpreted as predictors of brucellosis/*Brucella* infection. The symptoms which can predict brucellosis include abortion, repeat breeding, retained placenta, stillbirth, metritis/purulent vaginal discharge and carpal hygroma (Makita et al., 2011; Lindahl et al., 2014; Patel et al., 2014; Nguna et al., 2019; Getahun et al., 2021).

The above review indicates the needs for assessing the risk factors and predictors (clinical symptoms) separately as both are two different groups of variables and can be utilised for two different purposes. Assessment of risk factors may help in reducing the *Brucella* infection while assessment of predictors may help in identifying seropositive animals.

Table 2. Risk factors and predictors of *Brucella* infection/ brucellosis reported by various researchers in dairy animals

	Risk factors and predictors	Association with brucellosis/*Brucella* infection	Reference
Host factors	Species	Cattle are more likely to be seropositive than buffalo	Kumar et al., 2016
	Age of animal	Older animals are more likely to be seropositive than calves	Ntivuguruzwa et al., 2020
	Sex	Female dairy animals are more likely to be seropositive than male	Ali et al., 2017
	Breed	Purebred animals are more likely to be seropositive than indigenous	Kumar et al., 2017
Management factors	Farming system	Fully stall-fed (organised) farms are more likely to be seropositive than partly stall-fed (unorganised) farms	Jain et al., 2018
	Mixed herd	Cattle housed with goat and/or sheep are more likely to be seropositive than if housed alone	Calistri et al., 2013
	Herd size	Larger herds are more likely to be seropositive than smaller herds	Terefe et al., 2017
	Distance between herds/ density of herds	Herds located close to one another are more likely to be seropositive than herds located away from each other	Soomro et al., 2014
	Breeding method	Breeding by natural mating is more likely to be seropositive than artificial insemination	Cárdenas et al., 2019
	Introduction of new animal from unknown source	Introduction of new animals from unknown sources is more likely to be seropositive than known source	Cárdenas et al., 2019
	Biosecurity	Farms with proper biosecurity are less likely to be seropositive than farms without biosecurity	Wolff et al., 2017
	Routine milk diagnosis	Herds that are not routinely tested for *Brucella* infection are more likely to be seropositive than herds tested routinely for *Brucella* infection	Shome et al., 2014
Farmer factors	Age of owner	Cattle and buffalo belonging to older farmers (above 40 years) are more likely to be seropositive than those belonging to younger farmers	Patel et al., 2014

	Knowledge and awareness of farmers	Cattle and buffalo belonging to farmers having knowledge about brucellosis are less likely to be seropositive than farmers who do not have knowledge about brucellosis	Ntivuguruzwa et al., 2020
	Education of farmers	Cattle and buffalo belonging to educated farmers are less likely to be seropositive than uneducated farmers	Assenga et al., 2016
	Agro-ecological factors		
	Region of origin	Seropositivity differs in different parts of a country	Mugizi et al., 2015
	Animals reared close to wildlife	Animals reared closed to areas inhabited by wildlife are more likely to be seropositive than those away from wildlife	Ntivuguruzwa et al., 2020
Risk predictors	History of abortion	Dairy animals with a history of abortion are more likely to be seropositive than animals which do not have such history	Khan et al., 2021
	History of repeat breeding	Dairy animals with a history of repeat breeding are more likely to be seropositive than animals which do not have such history	Ismail, 2018
	History of retained placenta	Dairy animals with a history of retained placenta are more likely to be seropositive than animals which do not have such history	Lindahl et al., 2019
	History of metritis/ endometritis	Dairy animals with a history of metritis/ endometritis are more likely to be seropositive than the animals which do not have such history	Patel et al., 2014
	History of stillbirth	Dairy animals with a history of stillbirth are more likely to be seropositive than the animals without such history	Yanti et al., 2021
	History of carpal hygroma	Dairy animals with a history of carpal hygroma are more likely to be seropositive than the animals without such history	OIE, 2009

1.8 Knowledge, attitude, and practice regarding bovine brucellosis

Prevalence of brucellosis in both humans and animals appears to be increasing because of a dearth of awareness, policies and resources (Pappas et al., 2006). A study from India suggests that poor knowledge of brucellosis is significantly associated with prevalence of the disease (Govindaraj et al., 2016), suggesting a vicious cycle between underreporting/under-diagnosis and less awareness of farmers (Mahmoodabad et al., 2008). Similarly, a study in Pakistan found that about 61.3% and 87.3% farmers are not aware of bovine brucellosis and its zoonotic importance respectively (Hussain et al., 2021). A study in Sudan found that farmers in the country had moderate level of knowledge, attitude and practices in regards to bovine brucellosis while they had very poor knowledge and poor understanding about the zoonotic nature of brucellosis (Ismail et al., 2019). A meta-analysis of 79 studies conducted in 22 countries suggest that the average awareness level of farmers about brucellosis is 55.5%, while the awareness level of the zoonotic nature of brucellosis, mode of brucellosis transmission and sign of human brucellosis are 37.6%, 35.9% and 41.6% respectively. The analysis did not find any significant difference in the knowledge level of high-risk people in Asia and Africa. The study emphasised building awareness and knowledge of farmers in Asia and Africa (Zhang et al., 2019). A study in Egypt suggest that knowledge, attitude and practices of dairy farmers could be effectively improved by providing animal health information (Ibrahim and Elsherbeny, 2019). Therefore, assessment of knowledge and practices among the farming community is important for designing a customised knowledge dissemination programme. Studies suggest that awareness building of communities about bovine brucellosis and its effect on public health is critical, along with making them aware about the safer consumption of food of animal origin (Bifo et al., 2020).

This review indicates the importance of studying knowledge, attitude and practices of people about brucellosis/ *Brucella* infection in given country context to design appropriate knowledge generation programme.

1.9 Vaccination and control in dairy animals

A control programme is designed to reduce the impact of a disease in a population, but not to eliminate the disease from the population. Hence, some acceptable level of infection may remain in the population even after successful implementation of a control programme. On the other hand, eradication means the complete elimination of a pathogenic agent from a country or a zone. Eradication needs a highly organised effort. Effective surveillance system coupled with laboratory support is critical for success of an eradication programme (WHO, 2006a; Verma, 2013). Low and middle-income countries find it difficult to launch eradication programmes because of low public investment on veterinary and health services and weaker surveillance and operational capacity (McDermott et al., 2013).

Control or eradication of brucellosis could be achieved by vaccination, surveillance, testing, reporting, elimination of infected animals from the herd, and generating awareness among stakeholders. Mass vaccination of the animals with approved vaccines can bring down the incidence of brucellosis in areas of high prevalence. When used routinely to cover more than 80% of population, vaccination may produce herd immunity and reduce the incidence of brucellosis gradually. In case of an eradication programme, vaccination should be stopped when incidence falls below 0.2% and then infected animals should be eliminated from the herds (Boral et al., 2009). Regular testing, reporting and quarantine of affected animals are essential for brucellosis control programmes. Educating all relevant stakeholders, especially the farmers and animal health professionals and all the value chain actors involved in livestock product trading including consumers is important to control the disease in humans and animals. They should not only be made aware to protect themselves but also to guide them to protect the animals and other humans. However, eradication can only be achieved by test and slaughter method, combined with vaccination and restriction of animal movement (WHO, 2006a).

In low and middle-income countries, the classical approach of test and slaughter, vaccination and movement restriction coupled with compensation policies is reported to be less successful and less feasible (Pal et al., 2017). More targeted control measures may be more realistic and useful (McDermott et al., 2013). In countries like Uganda, where brucellosis is endemic, vaccination was considered as the most appropriate control measure and stamping out might be too economically burdensome (Makita et al., 2011). Test and slaughter would not be an easy option because of the huge economic loss it causes. In India, it is even more difficult because of the socio-religious factors, which make slaughter of cattle unacceptable.

Cattle slaughter is even officially banned in some parts of India, and it is therefore important to mass-vaccinate animals for control of brucellosis (Renukaradhya et al., 2002). An action research project in Uttar Pradesh, India, has found periodic testing of all animals and then segregating the seropositive animals (test and segregation method) in a specialized farm away from the main farm reduced the seropositive animals from 12.4% to 1.2%. The study also found that the test and segregation method coupled with better housing, proper hygienic disposal of aborted materials and calf vaccination could help in reducing prevalence of brucellosis (Kollannur et al., 2007). In another study in Punjab, India, the researchers found that B. abortus S19 vaccine reduced the rate of abortion from 8% to 1%, and 3% to 1%, in cows and buffalo cows respectively (Gill et al., 2000).

Brucella abortus S19 is the most widely used and the first effective vaccine (described in 1930) against brucellosis in bovines (Schurig et al., 2002). It is also the reference vaccine to which all other vaccines are compared (OIE, 2009). It is a live vaccine given to female calves aged between 3-8 months as a single subcutaneous dose. Vaccination is recommended at young age so that it does not induce abortion (WHO, 2006a). A reduced dose could be administered subcutaneously to adult animals, but some animals may show persistent antibody titres and may abort and excrete the vaccine strain in milk. Alternatively, it could be administered to any age group of animals as one or two doses through a conjunctival route (OIE, 2009). This way, it does not induce abortion in pregnant animals, which is the major advantage of the conjunctival route over subcutaneous route (Chand et al., 2015). The S19 vaccine induces reasonable protection against brucellosis caused by *B. abortus* but does not protect against brucellosis caused by *B. melitensis* (WHO, 2006a).

The problem of positive reactions in serological screenings of *Brucella* infection is partly overcome by the development of a live vaccine devoid of SLPS having O-polysaccharide. *B. abortus* live attenuated RB51 vaccine (a rifampicin-resistant mutant, ('R' stands for rough; 'B' stands for *Brucella*) that contains no O-polysaccharide on its cell surface can reduce the false positive rate and therefore unnecessary further test and slaughter of animal. *B. abortus* RB51 strain has proved safe and effective in the field against bovine brucellosis and exhibits negligible interference with diagnostic serology (Schurig et al., 2002; Singh et al., 2012). RB51 has been successfully used in USA since 1996 (OIE, 2009) for prevention and eradication of bovine brucellosis and has been suggested for use in India (Singh et al., 2012). Both *Brucella* S19 and RB51 vaccines are recommended by OIE but it is advised that vaccine efficacy may be limited in the event of heavy exposure (WHO, 2006a). *B. melitensis* Rev 1 vaccine is used in goat and sheep to protect them from brucellosis but use in cattle is not well reported (WHO, 2006a). There was no state-run vaccination programme against brucellosis in large ruminants in India until 2018. Only in September, 2019, the government of India launched a new programme called "National animal disease control programme on foot & mouth disease (FMD) and brucellosis" (DAHD, 2020).

Safe and effective vaccines against brucellosis for humans, pigs and wildlife are not commercially available (Godfroid et al., 2010). However, *B. abortus* strain 19BA vaccine was used in humans in former USSR from 1952 onwards. It provided protection up to one year with maximum efficacy up to 5-6 months after vaccination. The main disadvantage of the vaccine was that it induced hypersensitivity with repeated doses (WHO, 2006a). In China, *B. abortus* 104 M vaccine was used in humans and *B. suis* S2 in animals (Deqiu et al., 2002) but the efficacy and availability of these vaccines were questionable. Some efforts were also made to produce *B. melitensis* M15 vaccine in France, and a *Brucella* chemical vaccine in Russia but with limited success (WHO, 2006a; Abt, 2020).

In absence of effective and readily available vaccine for humans, efforts are generally made to control brucellosis through hygiene in farms, laboratories, slaughterhouses, and markets along with pasteurizing or boiling milk and milk products and adequate cooking of meat and meat products. In addition, movement restriction, and education and sensitization of susceptible groups including medical professionals is also essential. Control of brucellosis in wildlife has been proved more challenging especially because of movement of animals and difficulties in vaccination (Boral et al., 2009).

1.10 Economic cost of reproductive disorders in dairy animals

The causes of reproductive disorders may be infectious or non-infectious. Several studies in India have indeed found a number of infectious reproductive diseases circulating in dairy farms (Lindahl et al., 2019; Shome et al., 2019) as well as non-infectious diseases such as nutritional deficiencies (Velladurai et al., 2016; Balamurugan et al., 2017; Assadulla et al., 2019).

Dairy animals in India, like elsewhere, frequently suffer from various reproductive disorders which have important bearing on productive and reproductive performances and farm economics. Among the reproductive disorders, repeat breeding, anoestrus, retained placenta, dystocia, abortion, stillbirth, vaginal discharge and uterine prolapse are commonly reported (Haile et al., 2014; Mekonnin et al., 2015). These reproductive disorders reduce fertility, prevent conception, create problems in delivery of healthy calves, lead to postpartum complications, increase inter-calving periods, reduce milk yield and reduce overall lifetime productivity (Zadeh, 2013; Abdisa, 2018). Further, to manage these problems, farmers need to spend money for treatment (medicine and veterinarian's fee) and to manage the animals for longer inter-calving/unproductive periods, result in higher management cost. Often, treatments fail, and farmers may be compelled to sell the animals (distress selling) at reduced prices before the end of their productive life resulting further economic loss. Cow slaughter is the subject of legal prohibitions and restrictions in several states in India because of socio-religious reasons. Slaughtering of cows for meat purpose is restricted in Assam under the "Assam Cattle Preservation Act, 1950" and in Bihar under the "Bihar Preservation and Improvement of Animal Act, 1955". Therefore, unproductive cows do not carry much value either in Bihar or in Assam. The unproductive cattle are mainly smuggled to Bangladesh where demand for beef is very high (Bhattacharjee, 2013).

In estimating the cost of reproductive disorders, a critical issue is assessing the physical effects of reproductive disorders on dairy animals and expressing them in terms of economic cost. It is difficult to quantify the exact costs as their effects are not always obvious and may be influenced by other factors (e.g. breed, feed, healthcare, management, stage of occurrence, severity of disease etc.) or may manifest with other diseases (Dijkhuizen et al., 1997). Also, effects may extend from days to years which makes the cost estimation more difficult. Absence of data recording systems, especially in smallholder dairy farms, further increases difficulties as getting insights of reproductive health is not easy (Lindgren, 2017).

Because of the complexity of the subject and lack of accessible farm data, there is paucity of economic studies on reproductive health of dairy animals in India, but this type of study is very important to help in understanding the depth of the problem and to guide investment decisions for disease control by policy makers based on evidence.

1.11 Economic cost of bovine brucellosis

Economic impacts can include direct (e.g., reduced milk yield, increased mortality) and indirect (e.g. vaccination, culling) impacts. Direct impacts may further be classified as visible (e.g. abortion, repeat breeding), invisible (e.g. lower fertility), additional costs (e.g. treatment, vaccination) and revenue forgone (e.g. distress selling) (Oseguera Montiel et al., 2014). Loss may comprise only those parameters that reduce benefits (e.g. reduced milk yield, reduced weight gain, reduced fertility, increased replacement cost, increased mortality etc.) while cost would comprise amounts spent for treatment and control (e.g. biosecurity, vaccination, movement control, disease surveillance, research etc.) of the disease (McDermott et al., 2013; Oseguera Montiel et al., 2014). Most economic estimates have not taken into consideration the cost caused by distress selling, feeding and management during extended calving interval, additional person-day costs for treating animals, cost of antiseptic or detergents, cost of transportation related to treatment, cost of diagnosis etc. Often studies extrapolate the economic figures based on limited epidemiological information and assumptions developed in the given country or elsewhere. There is dearth of studies that have estimated the economic cost based on rigorous epidemiological data collected from a randomly selected population. Because of lack of uniformity in measuring economic cost, and the fact that these are highly context specific, estimates vary widely.

A study in India (Panchasara, 2012) reported that economic losses caused by brucellosis were mainly due to reduction in milk production followed by cost of treatment and loss of the aborted calf. It was further stated that there was an average loss of 231 litres and 177 litres of milk (10% of total lactation yield) in *Brucella* positive cows and buffalo cows respectively, causing an economic loss of around USD 40 per cow. The average costs of treatment following abortion, repeat breeding and retention of placenta of dairy cattle were estimated at USD 4, USD 5 and USD 7 respectively (Panchasara, 2012). A study in Gujarat, India, reported the highest quantified losses (46%) caused by reduced milk yield followed by extended calving interval (18%), treatment cost of abortion (14%), and treatment cost of metritis/endometritis

(8%), out of the total loss caused by brucellosis to peri-urban dairy farms of cattle and buffalo (Patel et al., 2014). This estimation was based on simple economic calculation of losses obtained from the primary data. A study in India estimated an economic loss of USD 58.8 million per year based on an active surveillance program (Kollannur et al., 2007) on bovine brucellosis, but the paper did not explain how they arrived at the figure. Another study estimated that an abortion caused a loss of USD 89 per animal (Dhand et al., 2005) but with no mention of other losses. Outside India, a study conducted in Brazil, found a loss of USD 78.3 per animal. The study further estimated that for every 1% increase or decrease in prevalence of brucellosis was expected to increase or decrease the economic burden of brucellosis by USD 28.9 million for the country (Santos et al., 2013). However, a study in Sudan found that, on average, each seropositive animal caused an economic loss of USD 29.8 (Angara et al., 2016). This might be because of lower value of animals and cost incurred for its management and treatment in Sudan than in Brazil.

The above review suggests that estimation of the cost of reproductive disorders is not easy and straight forward. There are lot of lapses in the present studies which open up more opportunities for research on the subject.

1.12 Economics of bovine brucellosis control programme

Several trials are going on around the world to control brucellosis and to assess the cost-benefit of such programmes. A study conducted in Spain evaluated the cost-benefit of a brucellosis control programme based on information obtained from published literature on epidemiological information (i.e., abortion, infertility, perinatal mortality, milk losses, meat losses, mortality and replacement requirement) and government data on control programme (i.e., compensation for slaughter, hired labour, material used, laboratory cost and administrative cost). The epidemiological information helped assess the benefits (reducing the losses are the benefits), while government data helped work out the cost of implementing the control programme. The study found that cost of the control programme was the highest in the first year and reduced gradually, while benefits showed an opposite tendency (Bernués et al., 1997). Another study in Brazil estimated the net present value (NPV) and payback period (PBP) of a brucellosis control programme. The benefit was calculated based on surplus of milk, meat and animal sale revenue and reduction of replacement cost while the cost of control programme was worked out based on cost of vaccines, cost of travel, depreciation of vaccination gun and vaccine (Alves et al., 2015). The same study assessed the losses caused by brucellosis using the parameters associated with losses in a simulation model as suggested by Bernués et al. (1997) as mentioned above. The economic analysis suggested that higher vaccination rates (90% of calves in comparison to 70% and 80% of calves) reduced the time to reach expected minimum brucellosis prevalence, resulting in shorter vaccination programme and less cost. The PBP was also shorter if vaccination rates were higher, especially when the impacts of the diseases were maximal (Alves et al., 2015). A study conducted in Mexico on the economics of brucellosis control in goat suggested that control based on vaccination alone was predicted to be economically profitable for goat farmers. The analysis indicated that control based on test-and-slaughter was not economically profitable, yet was more effective in reducing brucellosis prevalence (Montiel et al., 2014). An economic cost-benefit analysis of the brucellosis eradication programme in New Zealand found a benefit cost ratio (BCR) and internal rate of return (IRR) of 1.7% and 10.3% respectively when 80% calves were vaccinated in calfhood (Shepherd et al., 1979). Economic analysis of different control strategies in Turkey suggested that annual vaccination of the entire population applied for four years was a more rational strategy than test and slaughter method or a combination of vaccination and test and slaughter method applied for three years (Can and Yalçin, 2014). The study further estimated that the NPV of a brucellosis control programme in three different scenarios viz. pessimistic, expected and optimistic scenarios was e USD 3.1 million, 29.2 million and 41.9 million respectively and the BCRs were USD 0.9, 2.3 and 2.8. The study worked out the cost based on cost of vaccination, testing, diagnosis, compulsory slaughter, transportation, and workforce expenditure. The benefit was based on reduction of disease due to brucellosis. An economic estimate on disease control in India suggest that by implementing a suitable vaccination programme in the country, *Brucella* seroprevalence could be reduced to less than 2% after 20 years which will generate a benefit cost ratio ranged from 3.2 – 10.6 in case of cattle and 3.8- 21.3 in case of buffalo(Singh et al., 2018).

In light of the above discussions, it can be interpreted that there is already enough knowledge on prevalence, distribution, diagnosis, epidemiology, risk factors, knowledge, practices and economic cost and control options of *Brucella* infection/ brucellosis. However, much of the information available is very context specific, and varies largely depending on location, time, prevailing farming system, size of farms, economic status of the country, knowledge and capacity, access to resources and technologies, socio-cultural issues etc. Therefore, more systematic investigations are required, particularly in the areas where there is lesser evidence about the disease and where the disease is endemic.

Based on the above review, this study aimed to assess *Brucella* infection in two Indian states, where there is not enough evidence on *Brucella* infection/brucellosis, but the disease is reported to be endemic, to generate new knowledge on the subject, to identify limitation/s and scope/s for future research and to contribute in designing an effective brucellosis control programme.

2. Aims of the thesis

The aims of this thesis were the followings

- To assess seroprevalence of *Brucella* infection in dairy animals in the state of Assam and Bihar, India.
- To identify risk factors for *Brucella* infection in dairy animals.
- To identify clinical symptoms that can predict brucellosis in dairy farming.
- To assess knowledge and practices of dairy farmers regarding brucellosis.
- To assess the economic cost caused by reproductive disorders among dairy animals.

3. Materials and methods

3.1 Data and sampling procedure

3.1.1 Sampling plan

The Republic of India has 28 states and seven union territories. Each state comprises several districts and each district several Community Development Blocks (CDB). Each CDB comprises several villages, a village being the smallest administrative unit. The cross-sectional study was conducted in two Indian states (Assam and Bihar) (Figure 2). Both states are in eastern India. Assam is located between 22°19' - 28°16' north latitude and 89°42' - 96°30' east longitude while Bihar is located between 24°20' - 27°31 north latitude and 83°19' - 88°17' east longitude.

Assam has a tropical monsoon climate, with an average summer temperature of 35ºC - 38ºC and a winter temperature of 6ºC - 8ºC, high rainfall (average annual rainfall 2,135 mm) and high humidity (average annual relative humidity 76.6%). In Bihar, the climate is humid subtropical with two distinct seasons (summer and winter). Average temperature during summer ranges from 25ºC - 31ºC while in winter it varies from 16ºC – 19-ºC. Average annual rainfall is 1,099 mm (Guhathakurta et al., 2020) while average relative humiity is 62%. Both states are among the poorest in India in terms of per capita income (Kumar and Rani, 2019).

Figure 2. Map of India highlighting the state of Assam and Bihar

Studies using random sampling to assess *Brucella* seroprevalence are lacking in both states, therefore both were considered for this study. Since prevalence of brucellosis and its possible risk factors may vary between urban or peri-urban and rural areas, the study was conducted in both. Here, urban areas are those under the administrative division of town committee or municipal corporation or council (the local administrative body of urban areas), while rural areas are administered by a village *panchayat* (local village level administrative body); the peri-urban areas are administered by a village *panchayat* but adjoin urban areas.

The cross-sectional study started with a survey of 534 dairy farming households during 2015-2016. Assuming a 15% household level of the seroprevalence of brucellosis, a 95% level of confidence, and 5% precision in the estimates, as well as using a one-sample binomial calculation, we needed a sample size of at least 200 households (Naing, 2006) from each state. To account for a small design effect, because of clustering, more households (242 in Assam and 292 in Bihar) were targeted.

Multistage sampling was used to select households. In the first stage, three districts were purposefully selected in each state. Selection was guided by the district's potential for dairy development (low, medium, and high), availability of primary laboratory support, safety and security of the study team, and the districts' potential as major milk consumption centre (districts having the largest city of each state). Accordingly, Kamrup (Metropolitan), Golaghat, and Baksa districts were selected from Assam while Patna, Nalanda, and Vaishali districts were selected in Bihar.

In the second stage, two CDBs from each district (one rural and one urban CDB) were selected. In the urban areas, since 'CDB 'and 'village' are not used as designations, the entire town or city was considered as equivalent to one CDB, and different dairy clusters were considered as equivalent to villages. In the Patna district of Bihar, one peri-urban CDB was also selected because of its emerging commercial dairy farming. For analytical purposes, that peri-urban CDB was considered an urban CDB. Therefore, in total, seven CDBs in Bihar and six CDBs in Assam were covered.

In the third stage, four villages were selected randomly from the list of villages in each CDB. At the fourth stage, 10 households were selected randomly from the list of households with dairy animals (cattle and/or buffalo) in each selected village. Key informants, such as people from local non-governmental organizations, and leading farmers, including the village headmen, and local veterinary officers helped in preparing the list of households with dairy animals and informed the selected households in advance about the survey. Random selection was done by assigning computer-generated random numbers. In total, 292 farming households belonging to 28 villages of Bihar and 242 households belonging to 24 villages of Assam were selected. Of the total farming households, 46.4% (n = 248) were from rural areas, and the remaining 53.6% (n = 286) were from urban or peri-urban areas.

The primary surveys were carried out by a team led by the author. Members of the farming families mainly responsible for management of dairy animals were interviewed in local language but the responses in the questionnaire were made in English.

3.1.2 Framing the questionnaire

The primary survey was carried out using a systematically designed structured questionnaire. The questionnaire covered the location of the farm (e.g. state, district, CDB, village, rural/urban, etc.); farmer demography (e.g. age, gender, education, and training completed etc.), farming system details (e.g. herd size, breed, breeding system, rearing system, feeding system, movement of animals, introduction of new animals, quarantine, vaccinations and cleanliness of the farms and animals etc.), reproductive disorders that could be predictors of brucellosis (e.g. history of abortion, repeat breeding, retained placenta, vaginal discharge, carpal hygroma, stillbirth, etc.), different brucellosis preventive practices (e.g. proper disposal of placenta and aborted materials, clean and hygienic practices, vaccination, etc.) and cost of production and economics (e.g. milk production, milk sold, price of selling, calf loss, cost of calf, cost of breeding/feeding/health care, and different losses caused by brucellosis in terms of reduced milk yield, reproductive month loss, cost of treatment, etc.). Knowledge about brucellosis was assessed by asking the farmers if they had heard about the disease called 'brucellosis' (there is no local term for the disease, the English word "brucellosis" is the term used by veterinarians). Those who responded positively, were asked additional questions related to symptoms, mode of transmission and preventive practices they observed. In addition, the instrument required direct observation of selected factors (e.g., cleanliness of the farms, cleanliness of the animals, type of roof, type of floor etc.).

The questionnaire was pre-tested by conducting mock interviews with some farmers in the field in both states to understand if the questions were correctly framed, easily understandable, relevant, easy to respond to, and not excessively time-consuming. Based on the experience of the field testing, necessary changes were made before going to the actual survey. Minor modifications were made in the questionnaire after completing the survey in Bihar and before starting in Assam. Global positioning system (GPS) coordinates of every surveyed household were recorded using a hand-held GPS (made by Garmin Ltd., Olathe, Kansas, United States), and were imported to a geographic information system (GIS) platform to produce GIS maps.

3.2 Informed consent and ethical approval

Ethical approval for the study was received from the Institutional Research Ethics Committee (IREC) of the International Livestock Research Institute (ILRI) on 21 September 2015 vide authorization letter no. ILRI-IREC2015-12. At the beginning of the interview, farmers were told the purpose of the study and their written consent were obtained using a customised consent form.

3.3 Biological sample collection and laboratory analysis

From each selected household, blood samples were collected from up to three female dairy animals of reproductive age. If there were more than three in the herd, three were randomly selected. Animals were handled carefully with the support of the owners to avoid any unnecessary excitement or injury to the animals. The blood collection tubes and cryovials were marked with specific code number to align with the household number and animal number of the respective household and animal. About 10 ml blood sample was collected from each sampled dairy animal through puncturing the jugular vein by use of disposable needle and syringe. Before and after inserting the needle, the punctured site was disinfected with alcohol. Appropriate precautions were taken to prevent transmission of blood borne zoonosis by wearing of disposable gloves, apron and use of hand sanitizer. Blood samples were immediately transferred to collection tubes (pre-numbered) and allowed to coagulate. All biological wastes were carried back in biohazard bags to the laboratory and disposed off properly. Serum was separated after centrifugation and stored in cryovials at −20 °C for shipment to the Indian Council of Agricultural Research (ICAR)-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bangalore, India, where serological analyses was conducted. Blood samples were collected from 829 female cattle and buffaloes from the two states. However, only 740 samples were found in good condition for analysis, of which 364 samples were from Assam and 376 samples were from Bihar. Of the total from Bihar, 354 samples belonged to cattle and the remaining 22 samples belonged to buffalo. No buffalo was found in the sampled households in Assam.

In addition to collection of biological samples, an animal history sheet was also completed against each animal selected for collection of biological samples. The animal history sheet had information about species, breed, age, number of calving and stage of lactation of dairy animals and incidence of abortion, repeat breeding, retained placenta, stillbirth and carpal hygroma of the animal in the previous 12 months from the date of survey. Biological sample number marked in the blood collection tube was also included in the animal history sheet for merging the data at a later stage for analysis.

The serum samples were initially tested by RBPT. The RBPT antigen with known positive and negative control serum were procured from the Institute of Veterinary Biological, Bangalore, India. The RBPT test was conducted by placing one drop of antigen on a glass slide and one drop of serum sample using 0.1 ml micro pipette. Thereafter, with the help of a spreader the mixture was slowly mixed within an area of 2.5 cm of the slide and then the slide was rotated manually for about 4 minutes. Development of any agglutination observed in bright light and the result was compared with the positive control and negative control serum (Figure 3). Development of agglutination was considered as positive for *Brucella* antibody and no development of agglutination was considered as negative for *Brucella* antibody.

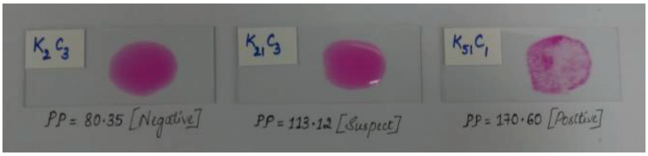

Figure 3. Pictures of RBPT smears with different samples

Thereafter, all the samples were tested by indirect ELISA (iELISA) test kit (produced by IDEXX Laboratories Inc., Westbrook, Maine, United States) to assess the presence of anti-*Brucella* antibodies in serum samples, following the test protocol and calculation method of sample-to-positive (S/P) ratio as recommended by the manufacturer.

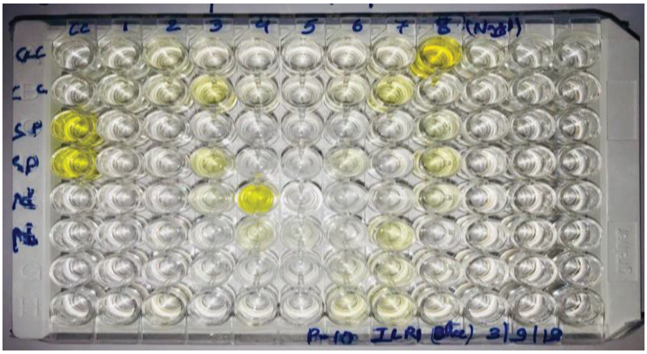

Figure 4. iELISA coated plate with microwell showing the reactions

All iELISA tests were run in duplicate. Optical density (OD) value of the samples and control were measured and recorded (Figure 4). For calculating the S/P ratio, all the OD values were entered in Microsoft excel. OD value of positive control was calculated by using the formula:

(OD value of positive control A + OD value of positive control B)/2

Similarly, OD value of negative control was calculated by using the formula:

(OD value of negative control A + OD value of negative control B)/2

The S/P ratio was calculated by using the following formula:

S/P % = (OD value of the sample - OD value of negative control) x 100/ (OD value of positive control – OD value of negative control)

Following the manufacturer's protocol, iELISA results were considered positive if the S/P ratio was found ≥120, questionable if the S/P ratio was found in between 110 and 120, and negative if S/P ratio was found ≤110. In the serological testing by iELISA, two samples were found questionable, and both the samples were considered as negative for analytical purposes. Because of consideration of the doubtful results as negative it was unlikely to have biased result on the overall outcome of the study, as both the samples belonged to Kamrup (Metropolitan) district, in which *Brucella* seropositivity was significantly higher than other two districts anyway. Furthermore, one of the suspected samples belonged to Kamrup (rural CDB), while another suspected sample belonged to Kamrup (urban CDB). This indicates that the result might not have any effect on the significance of seropositivity between rural and urban areas as well. Furthermore, both suspected samples belonged to medium-category farms. This may be noted here that although RBPT and iELISA were used for assessing *Brucella* seroprevalence, for analytical purpose, only iELISA results were considered for its higher sensitivity and specificity than RBPT (Table 1).

As no state-run *Brucella* vaccination programme was initiated in the year of survey, and no history of a *Brucella*-vaccinated animal was found in response to our question on vaccination in the field survey, the possibility of a false positive reaction that might occur because of *Brucella* vaccination was unlikely.

3.4 Statistical analysis for seroprevalence and risk factors

Data were entered in Microsoft Excel and data cleaning was performed before going to data analysis. In Bihar, only one *Brucella* seropositive sample was found, and therefore the state was excluded from the risk factor analyses. Descriptive statistics was completed by producing frequency tables. Data were analysed using Stata, version-14 (STATA Corp Ltd., Texas, United States). The farms were categorized into "small" (1–3 dairy animals), "medium" (4–10 dairy animals), and "large" (more than 10 dairy animals), according to the classification made by FAO in the Indian context (Sharma et al., 2003). The district classification was reorganised, as farm size was highly colinear with the districts. Large- and medium-size farms were found mainly in the Kamrup (Metropolitan) district. In the other two districts (Golaghat and Baksa), only three medium size farms were found, there was no large size farm. Therefore, by combining farm size and district, the districts were reclassified into the following: Kamrup (large farm), Kamrup (small farm), Golaghat, and Baksa.

Statistical analyses were conducted to assess risk factors for *Brucella* seropositivity at the animal level. Initially, univariable analyses was conducted to study the associations between *Brucella* seropositivity and all independent variables. A Pearson Chi-square test or Fisher's exact test (when assumptions for the Pearson Chi-square test were not met) was employed for analysing two binary or categorical variables (nominal or ordinal), while mean difference test (*t*-test) was employed for analysing continuous variables. Regression coefficients and odds ratio (OR) were obtained from multi-level mixed effect logistic regression between the outcome variable and respective independent variables considering household as random effect. Multivariable assessment of risk factors was carried out in a series of multilevel logistic regression models. The outcome of interest was seropositivity by iELISA at the cow level.

3.4.1 Causal diagram

A systematic process was followed for multivariable model building. Initially, a causal diagram was developed (Figure 5) to show the possible relationships between groups of potential risk factors and the outcome. To simplify the diagram (and the subsequent analyses), potential risk factors were grouped into four categories: producer demographics (PD; e.g., gender, education, training received by farmers, interaction with veterinarians, etc.), farm characteristics (FC; e.g., location of the farms, farm size, floor type, dairy animal in contact with goat, etc.), cow demographics (CD; e.g., age, breed, etc.) and farm management (FM; e.g., animal movement, introduction of new animals, artificial insemination, use of disinfectant in cleaning the farms, etc.).

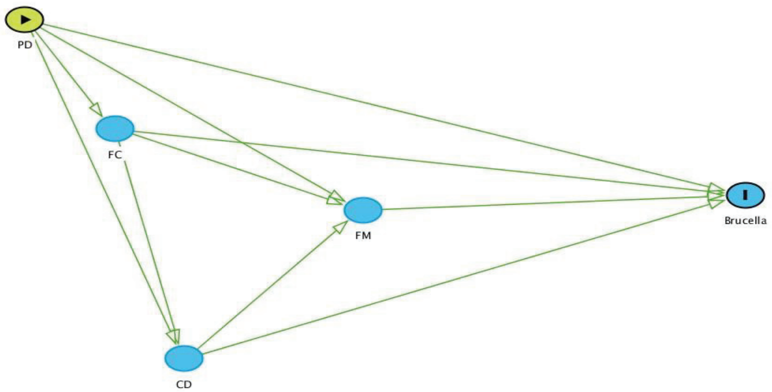

Figure 5. Causal diagram of potential risk factors of *Brucella* infection. PD: producers' demography, FC: farm characteristics, CD: cattle demography, FM: farm management. Here arrows indicate the probability of causation of the outcome (*Brucella* seropositive) by risk factors group

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3.4.2 Selection of variables for multivariable analysis

To select suitable variables for model building, associations between the variables within each group of variables were studied using the following methods:

For all pairs of variables within a group (e.g., FC, FM, etc.) in which both variables in the pair were either continuous or dichotomous, correlations were computed using simple correlation command in the Stata software.

For all pairs of variables within a group (e.g., FC, FM, etc.) in which one variable was continuous and the other was categorical (the continuous variable was considered as the outcome and the categorical variable was converted to a set of dummy variables), R^2 and a p-value were computed using linear regression.

For all pairs of variables within a group (e.g., FC, FM, etc.) in which one variable was categorical and the other variable was either categorical or dichotomous, a p-value was computed using a Chi-square test.

Thereafter, a few selection criteria were followed as stated below for identifying suitable variables for multivariable models:

A variable that did not have a plausible biological relationship to the outcome variable was excluded.

A variable that did not have substantial variability (e.g., species of dairy animals) was excluded.

A variable that had more than 10% missing values was excluded.

If the correlation between two variables was greater than 0.7 or $R^2 > 0.5$ (as computed above), the following selection criteria were used to exclude one variable in favour of the other: (a) the variable that had a weaker association with the outcome variable was excluded, (b) the variable that had more missing values was excluded, (c) the variable for which it was relatively difficult to collect accurate data was excluded.

A variable that had no significant association with the outcome variable ($p > 0.3$) was excluded.

3.4.3 Model building for multivariable analyses

After selection of the candidate variables, the following steps were followed for each multivariable analysis:

Separate models were built for each group of variables (i.e., PD, FC, CD, and FM).

Following the causal diagram, a multivariable regression analysis was conducted using variables in the PD group. This was followed by similar analysis for FC, CD, and FM groups separately in sequence. For each group, significant variables from the antecedent groups (i.e., to the left of the group of interest) were retained to see if there was any confounding effect. No variables from subsequent groups were included, as these would be intervening (intermediate) variables. For example, when analysing variables in the CD group, significant variables from the PD and FC models were retained, but all variables in the FM group were excluded.

During analyses, variables were manually eliminated one at a time. The variable for elimination was selected based on its *p*-value, absence of evidence of confounding effects (i.e., changes in the magnitude of other coefficients), and the plausibility of its causal association with *Brucella* seropositivity.

A variable was dropped from the analysis if it had strong collinearity with another variable that had more plausible association with *Brucella* seropositivity.

In the summary model, only the significant variables ($p < 0.1$) identified through multivariable regression analysis of each group were kept, along with the pre-selected confounders, if any.

A cut off point of $p < 0.1$ was chosen in light of the relatively small sample size and low prevalence of *Brucella* seropositivity.

The hierarchical structure of the data was accounted for by forcing the combined district/farm size variable as a fixed effect in all models and including village and household as random effects. For each group of variables, the final model showed the total effect of the selected variables in that model. Their direct effect (after adjustment for intervening variables) was determined from the final model (FM group). Regression diagnostics were carried out on all final models. These primarily consisted of evaluating the normality and homoscedasticity of the random effects at the household and village levels and looking for outlying observations.

3.5 Assessment of symptoms as predictors of *Brucella* infection

Each selected household was surveyed with a structured questionnaire having questions related to farming system and potential symptoms of brucellosis exhibited by the dairy animals as indicated in section 3.1.1, 3.1.2 and 3.3. The potential symptoms studied include abortion, repeat breeding, retained placenta, purulent vaginal discharge, stillbirth and carpal hygroma. All six were assessed for the previous 12 months from the date of survey.

Conducting the *Brucella* seroprevalence study, only one sample was found seropositive in Bihar. Because of very low *Brucella* seropositivity (0.03%) in Bihar, the state was excluded from predictors analysis in this manuscript, similar to the approach that was used for analysis of risk factors. Data were analysed using Stata, version-14 (STATA Corp Ltd., Texas, United States). Statistical analyses were conducted to assess the predictors for *Brucella* seropositivity at the animal level. Initially, univariable analyses were conducted to study the associations between *Brucella* seropositivity and the potential predictors using unconditional logistic regressions with village and households included as random effects.

The two variables with unconditional associations with a p-value <0.1 were included in a multivariable model along with their interaction. Age of dairy animals was also included as it had possible confounding effect on *Brucella* infection and its symptoms. However, the association of *Brucella* seropositivity and age of animals was not linear and therefore age was grouped in four quartiles (≤3 year, 4-years, 5-years and ≥6 years age) and was included in the multivariable model along with random effects for village and households. The regression model with four categories of age and four categories of repeat breeding and retained placenta was considered as the final model. To investigate the predictive ability of the final model, three different cut-off points for predicted mean (i.e., predicted probability of seropositivity of 0.1, 0.2 and 0.3) were used to classify cows as positive or negative. These were compared to their observed serostatus to compute the sensitivity and specificity of the model.

For comparison purposes, the predictive ability of the model containing only retained placenta and repeat breeding was compared to that of a model with all five predictors by comparing the log likelihood of the two models.

3.6 Assessment of knowledge and practices of farmers regarding brucellosis

The statistical analysis to assess the knowledge and practices of dairy farmers were conducted on the data collected for this purpose in the same survey for assessing *Brucella* seroprevalence, risk factors and predictors as explained above. However, the statistical method used for this purpose was relatively simpler than the method used for risk factor and predictors analysis because of smaller number of respondents who knew about brucellosis. Initially, knowledge of farmers about different aspects of brucellosis in Assam and Bihar was computed and unconditional associations between two binary or categorical variables (nominal or ordinal) were assessed by using Chi-square test. To build the multivariable model for assessing factors that influenced Brucella preventive practices by farmers, initially, correlations among the variables were assessed through correlation matrix. Thereafter, a multilevel mixed effects logistic regression model was built to assess the factors considering districts, blocks and villages as random effects and states as a fixed effect. Initially, all the possible variables based on plausible association with *Brucella* preventive practices were included in the regression model and nonsignificant variables were dropped one after another based on level of insignificance and its effect on the coefficients/ OR of other variables. In the final model, only the significant variables (p<0.05) were kept.

3.7 Assessment of the cost of reproductive disorders

We had intended to assess the economic cost of brucellosis, but the idea was dropped as seropositivity of *Brucella* infection does not confirm the occurrence of the disease brucellosis. As reproductive disorders have other causes, we extended the scope of the study to the economic cost of reproductive disorders without specifying the aetiology. No confirmatory diagnosis was conducted to ascertain the cause-and-effect relationship, therefore assessing the economic cost based on aetiology was not possible.

The methodology of the field study, sampling and questionnaire survey have already been explained in the sections 3.1.1 and 3.1.2. Data on reproductive disorders were based on farmers' reports of problems and expenditure incurred for treatment or management based on farmers' recollection of the problems in the previous 12 months from the date of the survey. Therefore, only the five most common clinical reproductive disorders, namely, abortion, repeat breeding, retained placenta, purulent vaginal discharge and stillbirth, were included since the farmers could easily report their occurrence without the need for a clinical examination.

The cost of treatment of abortion, the problem of highest interest, was collected in the primary survey, but the costs of four other reproductive disorders were collected using participatory methods, focus group discussion (FGD) was organised in each selected CDB. In each FGD, about 15-20 dairy farmers (both male and female) who encountered the reproductive disorders participated. FGD was conducted by two persons, while one initiated the discussion, other one noted the agreed points. The discussion covered topics like common dairy farming system, breeds and breeding, feeds and fodder, labour and electricity, major reproductive disorders, history of the reproductive disorders (e.g., abortion, repeat breeding, retained placenta, purulent vaginal discharge and stillbirth) in the previous 12 months from the date of survey, cost incurred for treatment of the problems (both medicine and veterinarians' fee), milk yield loss (if any), volume loss, duration of milk yield loss etc. Also, the FGD covered the cost of managing a dairy animal per day or per month including all cost components.

As smallholder farmers in Assam and Bihar hardly maintained any farm record, they responded to questions based on their memories. To validate the information/data provided, supplementary questions were asked when responses were inconsistent during the interview. Further, thorough data cleaning was done to remove obvious erroneous responses. If any inconsistency was observed at this stage, the respondent was telephoned to gather correct information. As reproductive disorders were not found in every farming household, there were some absent data or non-applicable data. At the time of data entry, the cell for absent data/ non applicable data was left blank (i.e., not recorded as zero) to avoid counting of the cell in the statistical analysis. As such, no questionnaire was fully rejected based on one or more absent/non applicable datum. The total number of farming households who responded to relevant questions and who did not respond/found not applicable to respond were calculated.

The collected data were entered in Microsoft Excel and were analysed using Stata 14 version (STATA Corp Ltd., Texas, United States). The categorical variables were analysed by using chi-square test while the continuous variables were analysed using t-test. For categorical variables, the percentage of responses out of the corresponding total were also calculated.

Finally, the costs of managing reproductive disorders were estimated by using the economic model as indicated in the following section.

3.7.1 Estimating the cost of reproductive disorders

The total economic cost of the reproductive disorders was estimated in terms of two distinct components, loss and expenditure, as explained by McInerney et al. (1992). A loss (L) implies a benefit that is taken away, alternatively, it represents a potential benefit that is not realized (such as when disease causes milk yield to fall). On the other hand, expenditure (E) represents resources that has to be allocated to unplanned or non-preferred uses (such as treatment of diseased animals, feeding for extra days etc.). The term economic cost (C) is used to represent the sum of both loss and expenditure. The economic model was worked out in light of previous studies (Panchasara, 2012; Patel et al., 2014; Angara et al., 2016) with various changes as these studies estimated the cost of only brucellosis while we estimated the cost of five reproductive disorders, with unknown etiology.

The following definitions were used to estimate the total economic cost caused by reproductive disorders in the surveyed households. This cost estimation was based on all the dairy animals that suffered from one or more reproductive disorders in the surveyed households.

3.7.2 Explanation of the key terminology used

Before conducting the field study, the key terminologies used in the study were decided to avoid any ambiguity at the time of recording the response of the surveyed households. The key terminologies used in this study are:

Repeat breeding: If a cow cycled normally with no clinical abnormalities but failed to conceive after at least two successive inseminations, this was regarded as repeat breeding.

Abortion: A foetus lost between the age of 42 days and approximately 260 days of pregnancy was considered as an abortion. Pregnancies lost before 42 days were considered early embryonic deaths.

Stillbirth: If a calf was born dead between 260 days and full term (280 days), this was considered as stillbirth.

Purulent vaginal discharge: Presence of purulent or mucopurulent exudates (cloudy, whitish, yellow, greenish, or bloody discharge) through the female genital tract as a result of infection was considered as purulent vaginal discharge.

Retained placenta: If a cow failed to expel foetal membrane within 24 hours after calving, this was considered as retained placenta**.**

Calving interval: The time period between two successive calving was considered as the calving interval.

Extended calving interval: The time period in a calving interval beyond the normal calving interval period in the given dairy animal population was considered as the extended calving interval.

Expenditure of treatment: This included the expenditure on medicine and veterinarian's fees that were incurred for treating a dairy animal for reproductive disorders.

Large ruminant dairy animals: These included both cattle and buffalo of sexually mature age (heifer, lactating, or dry).

Loss of milk production: If a milking cow, which aborted, produced less milk for some days/ months this was considered as loss of milk production.

Salvage sale: A dairy animal with reproductive disorder/s sold at lower price than its normal market value was regarded as salvage sale.

Reproductive month loss: After occurrence of a reproductive disorder like abortion or repeat breeding, a dairy animal lost days or months during which the animal did not gain anything in terms of reproductive cycle, but the farmer continued to spend money on feed, labour, electricity, etc. for management of the animals. The loss incurred during this period was considered as reproductive month loss.

3.7.3 Estimations of loss and expenditure incurred

Economic model used for estimating the loss and expenditure incurred for reproductive disorders are stated below:

Total economic cost caused by reproductive diseases (TEC) = Losses caused by reproductive disorders + Expenditure caused by reproductive disorders.

TEC= (LMP+LSS) + (ETA+ETRB+ETRP+ETPVD+ETSB+ EEI+ERMLA+ERMLRB)

Where:

Loss of milk production (LMP): (Number of animals with reduced milk yield after abortion) x (Mean milk loss per animal in liter) x (Number of days with reduced milk yield) x (Mean price of milk per liter)

Loss caused by salvage sale (LSS): (Number of animals sold in salvage) x {(Mean price of animal without disease) – (Mean price of animals with disease)}

Expenditure for treatment of abortion (ETA)/ repeat breeding (ETRB)/ retained placenta (ETRP)/ purulent vaginal discharge (ETPVD)/ stillbirth (ETSB) = (Number of animals treated) x (Mean expenditure of treatment)

Three other expenditures that are incurred by farmers for reproductive disorders include:

Expenditure for extra insemination of repeat breeders (EEI) = (Number of repeat breeder animal) x (Mean number of extra inseminations required per repeat breeder) x (Mean expenditure for artificial insemination)

Expenditure for animal management during reproductive month loss for abortion (ERMLA) = (Number of animals that lost reproductive months because of abortion) x (Mean number of months lost for abortion) x (Mean expenditure of rearing per dairy animal per month)

Expenditure for animal management during reproductive month loss for repeat breeding (ERMLRB) = (Number of animals that lost reproductive months because of repeat breeding) x (Mean month loss for repeat breeding) x (Mean expenditure of rearing per dairy animal per month)

To calculate the mean expenditure of rearing per dairy animal per month, the following formula was used:

Mean expenditure of rearing per dairy animal per month = {(Quantity of concentrate feed consumed/animal/day x price/kg) + (Quantity of fodder consumed/animal/day x price/kg) + (Average expenditure of labour/animal/day) + (Other miscellaneous expenses/animal/day) + (Average expenditure of electricity/animal/day)} x 30 days

The initial estimates were made following the calculation method explained above to represent the total aggregate cost incurred by the surveyed households in the study locations of Assam and Bihar. We extrapolated the cost from the sample to the whole state by assuming the same unit cost and same percentage of dairy animals that suffered from reproductive disorders in our study sites in Assam and Bihar for the respective state. To extrapolate the cost, secondary data of the numbers of mature female dairy animals (in milk, dry and heifer) available in Assam and

Bihar were taken from the National Livestock Census held in 2019 (DAHD, 2019).

The cost of reproductive disorders of indigenous (either of a known indigenous breed or non-descript cattle/buffalo) and improved (cross-bred/exotic) dairy animals were estimated separately to account for the different shares of indigenous dairy animal population since significantly ($p<0.001$) fewer reproductive disorders were reported among indigenous dairy animals than among improved dairy animals in both the states. In India, pure bred dairy animals that are imported from abroad, or descended from these (e.g. Jersey, Holstein Friesian etc.) are considered as exotic breed, while indigenous animals that are characterized and notified as a breed by the National Bureau of Animal Genetics Resources (NBAGR), Govt. of India, are called indigenous breed and those native animals that are not characterized and notified as a breed called non-descript indigenous

Dairy animal population data included the total dairy animals irrespective of farm category or rearing system and therefore this estimate did not address bias, if any, associated with these factors. Further, it was considered that the dairy farming prevailed in the three selected districts of each state were largely representative of the whole state and therefore the districts were not weighted. In addition, although prices of dairy animals varied based on breed, age, milk yield, etc., only the mean prices were considered. Similarly, the mean expenditure of treating reproductive disorders of indigenous and improved dairy animals was applied to both groups of dairy animals.

4. Results

4.1 Demographic features of the dairy farmers

Out of the total interviewed households (n=534), 46.3% (n=247) were from rural areas and the remaining 53.7% (n=287) were from urban areas (including peri-urban). The demographic features of the farming households are presented at Table 3. The mean household number of people in Bihar (7.9±0.3) was significantly (p=0.009) higher than in Assam (5.6±0.2). Most of the surveyed farm owners were male: only 7.5% were female. Around half of the farmers (49%) in both Bihar and Assam were aged 41-60 years, the next biggest category was 21-40 years followed by above 60 years. Similarly, the most common educational category in both the states was high school educated (42%) (class VI-X), followed by class XI-XII educated, and then class I-V educated. We found a significantly (p=0.02) higher number of farmers had obtained training in urban areas (7.0%, n=17) than in rural areas (2.5%, n=6), and significantly (p<0.001) more large-scale farmers (32.5%) had received training than medium or small-scale farmers (4.9%). Furthermore, significantly (p<0.001) more urban farmers (88.4%) consulted veterinarians than rural farmers did (64.5%) and more large-scale (95.4%) farmers consulted veterinarians than medium (88.6%) and small-scale (71.9%) farmers. However, the questions related to training and consultation with veterinarians were not incorporated in our questionnaire in Bihar, where the first survey was conducted.

Table 3. Demographic features of the farmers with level of significance (p<0.05) showing difference between Bihar and Assam states

Variable	**Observation/ corresponding total**			***p*- value**
	Bihar	**Assam**	**Total**	
Gender of farmers (male)	262/292 (89.7%)	232/242 (95.9%)	494/534 (92.5%)	0.007
Gender of farmers (female)	30/292 (10.3%)	10/242 (4.1%)	40/534 (7.5%)	
Age of farmers (≤20 years)	16/292 (5.5%)	2/242 (0.8)	18/534 (3.4%)	<0.001
Age of farmers (21- 40 years)	104/292 (35.6%)	51/242 (21.1%)	155/534 (29.0%)	
Age of farmers (41- 60 years)	129/292 (44.2%)	133/242 (55.0%)	262/534 (49.1%)	
Age of farmers (≥60 years)	43/292 (14.7%)	56/242 (23.1%)	99/534 (18.5%)	
Education of farmers (no education)	15/292 (5.1%)	36/242 (14.9%)	51/534 (9.6%)	<0.001
Education of farmers (class I-V)	27/292 (9.2%)	32/242 (13.2%)	59/534 (11.0%)	
Education of farmers (class VI-X)	127/292 (43.5%)	102/242 (42.1%)	229/534 (42.9%)	
Education of farmers (class XI-XII)	73/292 (25.0%)	51/242 (21.1%)	124/534 (23.2%)	
Education (graduation and above)	50/292 (17.1%)	21/242 (8.7%)	71/534 (3.2%)	

4.2 Profile of dairy farming system and dairy animals

As regards farm size, small farms (1–3 dairy animals) were the most common both in Bihar (78.8%) and Assam (76.4%), followed by medium (4–10 dairy animals) and large farms (>10 dairy animals). The mean herd size of dairy farms in Assam was significantly (p=0.01) higher (4.1) than in Bihar (2.8) and was higher in urban areas (5.5) than in rural areas (2.9). Further, herd size was almost uniform across the surveyed districts in Bihar, while in Assam, it largely varied among the surveyed districts and large farms were mainly observed in Kamrup (Metropolitan) district. In Assam, a significantly (p<0.001) higher percentage of farming households (72.3%) reared dairy animals under partly stall-fed condition, while in Bihar, only a small percentage of households (14.8%) reared dairy animals under partly stall-fed condition. Fully stall-fed system (zero grazing) of rearing was more common in Bihar (85.3%) than in Assam (27.7%). In addition, improved (exotic or exotic crossbreed) dairy animals were predominant in the study areas of Bihar (91.8%), while in Assam little more than half of the dairy animals in the study areas were improved, and the remaining were indigenous (native breed/ non-descript indigenous) (Table 4). In the studied households of Bihar, both cattle and buffalo were used for milk production, whereas in Assam only cattle were used for milk production. In the surveyed farming households of Assam, only about half of the households (52.9%) adopted artificial insemination (AI) practices while in Bihar majority of the households (91.1%) adopted AI for breeding.

Table 4. Profile of dairy animals in the surveyed households, with *p*-values showing difference between Bihar and Assam states

Variables	**Particulars**	**Bihar**	**Assam**	**Total**	***p*-value**
Mean herd size per farm (± standard error (SE))		2.8 ± 0.2	4.1 ± 0.4	3.4 ± 0.2	0.009
Species of the animals	Cattle	354/376 (94.1%)	364/364 (100.0%)	718/740 (97.0%)	
	Buffalo	22/376 (5.9%)	0/364	22/740 (2.9%)	<0.001
Breed of the animals surveyed	Improved	345/376 (91.8%)	186/364 (51.1%)	531/740 (71.8%)	
	Indigenous	31/376 (8.2%)	178/364 (48.9%)	209/740 (28.2%)	<0.001
Mean age of animals surveyed (± SE)		4.7 ± 0.1	6.2 ± 0.1	5.4 ± 0.1	<0.001
Breeding method followed by households	Followed AI	266/292 (91.1%)	128/242 (52.9%)	394/534 (73.8%)	<0.001

As risk factor analysis under this study focused only on Assam data (because of very low *Brucella* seroprevalence in Bihar), the variability of the dairy farming system under three different geographical districts of the state was studied further and have been presented at Table 5. It was observed that mean herd size of the dairy farms in Kamrup (Metropolitan) (8.8 ± 1.1) district was significantly (p=0.01) larger than Baksa (1.6 ± 0.1) and Golaghat (1.7 ± 0.1) districts. Further, more farms in Kamrup (Metropolitan) district had improved cattle, used stall-fed system, adopted AI, used disinfectants, had more trained owners, and used more veterinary services than farms in the other two districts. Only a few (3) farms in Kamrup (Metropolitan) district had any quarantine shed or other biosecurity infrastructure. Although about 29.5% farms in the district reported using disinfectant, none of them used disinfectant regularly. The case was the same with regard to other biosecurity practices followed during milking, disposal of aborted materials, etc.

Table 5. District-wise profile of the dairy animals with *p*-values showing differences between the three districts in Assam

Variables	**Particulars**	**Observation/ corresponding total (%)**			***p*-value**
		Kamrup (Metropolitan)	**Golaghat**	**Baksa**	
Rearing system	Fully stall-fed	141/178 (79.2%)	9/105 (8.6%)	6/94 (6.4%)	
	Partly stall-fed	37/178 (20.8%)	96/105 (91.4%)	88/94 (93.6%)	<0.001
Breed	Improved (exotic/ cross)	148/176 (84.1%)	18/96 (18.7%)	20/92 (21.7%)	
	Indigenous (native breed/ non-descript	28/176 (15.9%)	78/96 (81.2%)	72/92 (78.3%)	<0.001
Adoption of AI	Yes	152/178 (85.4%)	45/105 (42.8%)	34/94 (36.2%)	<0.001
Animal movement	Yes	39/178 (21.9%)	96/105 (91.4%)	90/94 (95.7%)	<0.001
New animal introduced	Yes	71/178 (39.9%)	6/105 (5.7%)	4/94 (4.2%)	<0.001
Animals belonging to trained farmers	Yes	41/178 (23.0%)	5/105 (4.8%)	5/94 (5.3%)	<0.001
Animals belonging to farmers who had consulted a veterinarian	Yes	158/178 (88.8%)	77/105 (73.3%)	70/94 (74.50%)	0.001
Use of disinfectants in cleaning the farms	Used	52/176 (29.5%)	2/96 (2.1%)	4/92 (4.3%)	<0.001

4.3 Laboratory results of seroprevalence

The results of laboratory study conducted through RBPT and iELISA are stated in Table 6.

Table 6. Results of *Brucella* seropositivity by RBPT and iELISA (2 x 2 table)

RBPT	iELISA			
	Positive	**Suspect**	**Negative**	**Total**
Positive	35	1	0	36
Negative	24	1	679	704
Total	59	2	679	740

In total, 59 samples were found seropositive, and two samples were suspicious for Brucella infection using iELISA while using RBPT only 35 samples were seropositive. Of the two iELISA suspicious samples, one was positive by RBPT and other was negative. For analytical purpose, both samples suspected by iELISA were considered as seronegative. Only iELISA results were considered for analysis because of the higher sensitivity and specificity of iELISA. Of seropositive samples by iELISA, 58 belonged to Assam and only one sample belonged to Bihar. The single positive sample in Bihar came from the rural area of Nalanda district. No buffalo sample was found seropositive. Apparent animal level seropositivity was 15.9% in Assam and 0.3% in Bihar while herd level seropositivity was 16.9% in Assam and 0.3% in Bihar. Among Brucella seropositive farms in Assam (n= 40), 23 farms had only one positive animal, 15 farms had two positive animals, and another two farms had three positive animals. Within Assam, the highest animal level seropositivity was recorded in Kamrup (Metropolitan) district (29.5%), followed by Baksa (4.3%) and Golaghat (2.1%) districts. Furthermore, higher Brucella seropositivity was recorded in urban areas (18.7%) than in rural areas (12.4%) but the difference was not found statistically significant.

The locations of sampled households are shown in Figure 6 and Figure 7.

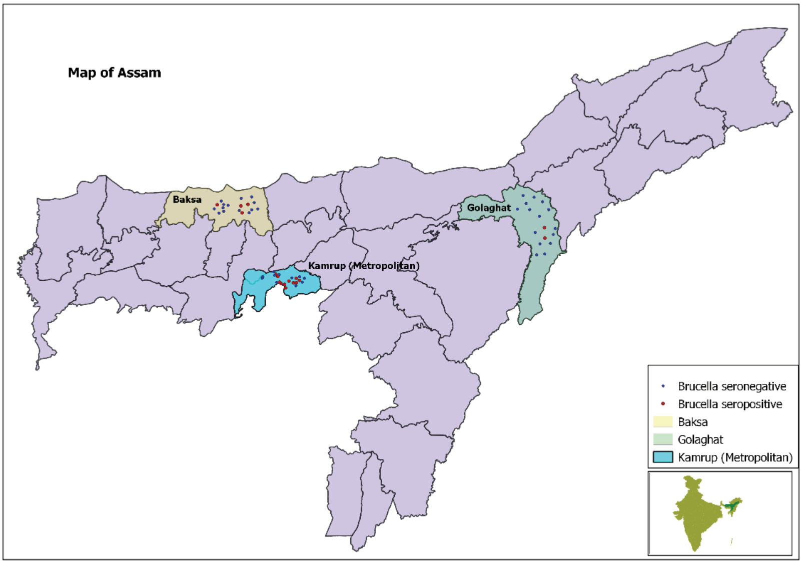

Figure 6. Map of Assam showing the project districts in different colours

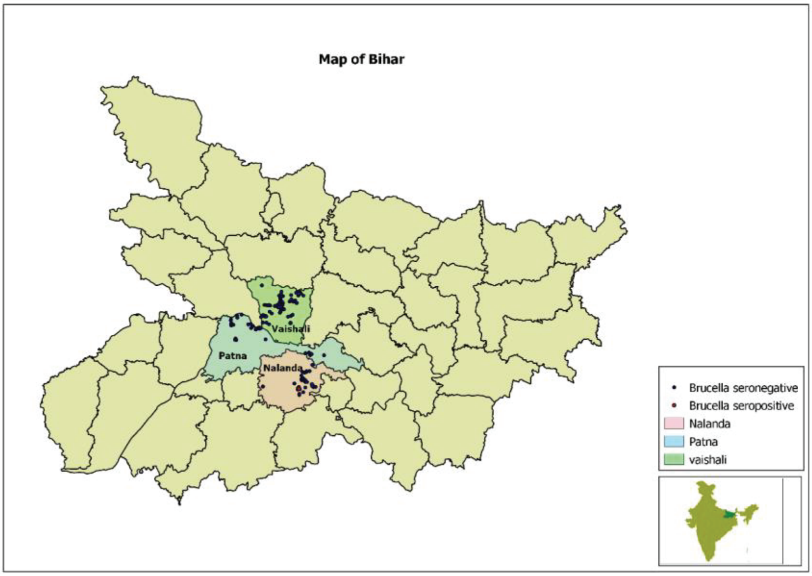

Figure 7. Map of Bihar showing the project districts in different colours

4.4 Analysis of risk factors

4.4.1 Univariable analysis

About 30 variables were studied for identifying potential risk factors under this study. Of these, only 15 variables were selected for multivariable analysis, using the selection criteria as described in the Methodology section. Details of the selected variables are presented in Table 7, and a complete list of all 30 variables is presented in the Appendix-I. Significant ($p<0.001$) differences were observed between *Brucella* seropositivity and reclassified districts. Animals belonging to large farms in Kamrup (Metropolitan) district were more likely to suffer from *Brucella* infection than the animals belonging to other two districts or to small farms in Kamrup (Metropolitan).

Under FC, *Brucella* seropositivity was found to be significantly higher in dairy animals belonging to urban areas, large sized farms, dairy animals not in contact with goats, and those belonging to farms having concrete or other types of floors (e.g., brick, earthen etc.).

Under FM, *Brucella* seropositivity was found to be significantly higher in dairy animals belonging to the farms that introduced new animals or using AI. Contrary to the plausible biological association, *Brucella* seropositivity was found significantly higher in case of animals belonging to farms that did not move their animals and animals belonging to farms that used disinfectant.

Under PD, *Brucella* seropositivity was significantly higher in dairy animals belonging to uneducated farmers and younger farmers (20–40 years old). Animals belonging to farmers who completed training on dairy cattle management and who interacted with veterinarians suffered more from *Brucella* infection.

Under CD, significantly more animals belonging to improved breeds and animals in higher age groups suffered more from *Brucella* infection.

Table 7. Variables with the details of univariable analysis and the decision to include for multivariable analysis

Variable	**Particulars**	**Seropositive/ total**	**Unconditional association with *Brucella* seropositivity**			**Missing value**	**Selected for multivariable analysis**
			Coefficient	**OR#**	***p*-value**		
Identifiers							
District	Kamrup (large farm)	49/135 (18.9%)	Ref.*	Ref.			
	Kamrup (small farm)	3/42 (7.3%)	-2.2	0.1			
	Golaghat	2/96 (2.1%)	-2.8	0.1			
	Baksa	4/92 (4.3%)	-3.6	0.03	<0.001	0	Yes
Farm characteristics (FC)							
Location of the farms	Rural CDB	20/161 (12.4%)	Ref.	Ref.			
	Urban CDB	38/203 (18.7%)	0.4	2.8	0.16	0	Yes
Category of farms	Small (1- 3 dairy animals)	8/223 (3.6%)	Ref.	Ref.			
	Medium (4- 10 dairy animals)	19/81 (23.4%)	2.2	8.9			
	Large (> 10 dairy animals)	31/60 (51.7%)	3.6	36.0	<0.001	0	Yes
Dairy animals in contact with goat	Yes	9/106 (8.5%)	-1.2	0.3			
	No	49/258 (19.0%)	Ref.	Ref.	0.03	0	Yes
Type of floor	Concrete	20/67 (29.8%)	Ref.	Ref.			
	Earthen	9/202 (4.4%)	-2.4	0.1			
	Other	29/95 (30.5%)	0.1	1.1	<0.001	0	Yes
Farm management (FM)							

Variable	Particulars	Seropositive/ total	Unconditional association with *Brucella* seropositivity			Missing value	Selected for multivariable analysis
			Coefficient	OR#	*p*-value		
Adoption of AI	Yes	46/225 (20.4%)	1.2	3.2			
	No	12/ 139 (8.6%)	Ref.	Ref.	0.02	0	Yes
Introduction of new animal	Introduced	22/ 79 (27.8%)	1.5	4.7			
	Not introduced	36/285 (12.6%)	Ref.	Ref.	0.006	0	Yes
Animal movement	Animal moved	9/213 (4.2%)	-2.7	0.1			
	Not moved	49/151 (32.4%)	Ref.	Ref.	<0.001	0	Yes
Use of disinfectant in cleaning farms	Used disinfectant	35/160 (21.9%)	1.1	2.9			
	Not used disinfectant	23/204 (11.3%)	Ref.	Ref.	0.02	0	Yes
Farmer demographic (PD)							
Education of farmers	No education	17/66 (25.7%)	Ref.	Ref.			
	Class I-V	11/49 (22.4%)	-0.2	0.9			
	Class VI-X	18/149 (12.1%)	-1.2	0.3			
	Class XI and above	12/100 (12.0%)	-1.1	0.3	0.14	0	Yes
Age of farmers	20-40 years	21/ 89 (23.6%)	Ref.	Ref.			
	41-60 years	26/191 (13.6%)	-1.2	0.3			
	>60 years	11/84 (13.1%)	-1.2	0.3	0.10	0	Yes
Training completed by farmers	Completed	15/50 (30.0%)	1.5	4.6			
	Not completed	43/ 314 (13.7%)	Ref.	Ref.	0.02	0	Yes
Interaction had with veterinarians	Had interaction	55/297 (18.5%)	1.9	6.3			
	No interaction	3/67 (4.5%)	Ref.	Ref.	0.005	0	Yes

Variable	Particulars	Seropositive/ total	Unconditional association with *Brucella* seropositivity			Missing value	Selected for multivariable analysis
			Coefficient	OR#	*p*-value		
Cow demographic (CD)							
Breed of animals	Indigenous (descript/ non-descript)	7/178 (3.9%)	Ref.	Ref.			
	Improved (cross/ exotic)	51/186 (27.4%)	2.7	14.3	<0.001	13	Yes
Age of animals	With *Brucella* seropositive	6.83±0.33	0.2	1.2			
	With *Brucella* seronegative	6.09±0.13	Ref.	Ref.	0.003	13	Yes

Note: Last one year means previous 12 months from the date of survey. * Reference level. #Odds ratio

4.4.2 Multivariable analysis

The selected variables were analysed using logistic regression by sub-group, and the outcome of analysis are presented in Table 8.

Under the PD group, no significant association was observed between *Brucella* seropositivity and the age of farmers, training completed by farmers, consultation with veterinarians, and the category of education of farmers.

From the sub-group level regression analysis on FC, the likelihood of dairy animals being *Brucella* seropositive in Kamrup (small farms), Baksa, and Golaghat was reduced by approximately 86%, 93%, and 97%, respectively, compared to Kamrup (large farms). No significant association was observed between *Brucella* seropositivity and floor type or contact with goats, so both the variables were dropped during the process of regression analysis. Strong collinearity was observed between *Brucella* seropositivity and the farm category.

Among the variables under CD, for every additional year of age of the dairy animal, there was approximately a 20% increase in the chance of being *Brucella* seropositive. There was no collinearity observed between age of animals and breed. However, breed and mating system (i.e., AI and natural mating) were found to be highly associated.

Under the FM group, the likelihood of being *Brucella* seropositive was associated with a reduction of approximately two-thirds when AI was used, although this effect was only borderline significant ($p = 0.07$). No significant association was observed between *Brucella* seropositivity and use of disinfectants in cleaning the farms and introduction of new animals in regression analysis. Animal movement was found to be strongly collinear with the district variable.

The model, in which all the sub-models were considered together, identified the districts (to which animals belonged), age of dairy animals, and mating system as the important risk factors for being *Brucella* seropositive (Table 9). The OR was found to be almost the same with the sub-group level regression analyses, indicating that the effects mediated through intervening variables were quite weak. In all the regression analysis, the random effect of villages and households were considered. The random effects at the village and household levels were fairly small. Estimates of the intra-class correlation coefficient for each of these effects were 0.10 (village) and 0.12

(household), indicating only a low to moderate level of clustering at these levels.

Table 8. Results of the four sub-models used in the multivariable analysis with odds ratio (OR), *p*-values, and 95% confidence interval (CI)

Variables	**OR**	***p*-value**	**95% CI**
Producer Demographics (PD)			
Kamrup (large farms)	Ref*.		
Kamrup (small farms)	0.1	0.007	0.03–0.6
Baksa	0.07	<0.001	0.02–0.3
Golaghat	0.03	<0.001	0.006–0.2
Cow Demographics (CD)			
Age of the dairy animals, in years	1.2	0.008	1.05–1.4
Farm Management (FM)			
AI adopted	0.3	0.07	0.1–1.1
Not adopted AI	Ref.		

* Reference level.

Table 9. Models with the identified risk factors from all sub-group model with odds ratio (OR), *p*-values, and 95% confidence interval (CI)

Variables	**OR**	***p*-value**	**95% CI**
District			
Kamrup (large farms)	Reference		
Kamrup (small farms)	0.1	0.007	0.02–0.5
Baksa	0.03	<0.001	0.00–0.2
Golaghat	0.01	<0.001	0.002–0.1
Age of dairy animals (in years)	1.2	0.007	1.06–1.4
AI adopted	0.3	0.072	0.1–1.1
Random effect of villages	0.4		
Random effect of households	0.5		

4.5 Analysis of clinical symptoms as predictors of *Brucella* infection

Considering potential clinical symptoms among the surveyed dairy animals, repeat breeding was the most common, followed by abortion, retained placenta, carpal hygroma and purulent vaginal discharge (Table 10). Stillbirth was the least common. A higher percentage of dairy cattle below three years of age and above 6 years of age exhibited potential clinical symptoms than the fourth or fifth year of age.

Table 10. Distribution of potential clinical symptoms according to age group of dairy animals and *p*-values of age group differences

Potential clinical symptom	Total animals exhibiting the symptoms	No. of animals exhibiting symptoms according to age group				*p*-value
		< 3 years	4 years	5 years	>6 years	
Abortion	78/377 (20.7%)	9/25 (36.0%)	7/51 (13.7%)	10/87 (11.5%)	52/201 (25.9%)	0.005
Repeat breeding	120/372 (32.3%)	14/25 (56.0%)	16/50 (32.0%)	16/84 (19.0%)	74/200 (37.0%)	0.002
Retained placenta	75/377 (19.9%)	10/25 (40.0%)	9/51 (17.6%)	9/87 (10.3%)	47/201 (23.4%)	0.006
Purulent vaginal discharge	42/369 (11.4%)	7/25 (28.0%)	6/50 (12.0%)	4/83 (4.8%)	25/198 (12.6%)	0.02
Carpal hygroma	63/377 (16.5%)	7/25 (28.0%)	8/51 (15.7%)	11/87 (12.6%)	36/201 (17.9%)	0.35
Stillbirth	8/377 (2.1%)	2/25 (8.0%)	0/51	1/87 (1.1%)	5/201 (2.5%)	0.25

Further, about 58 dairy animals that were found aborted belonged to 29 households. Of these, 86.2% (n=50) aborted in third trimester, 10.3% (n=6) aborted in second trimester and only 3.4% (n=2) aborted in first trimester. Of the aborted foetuses, 75.9% (n=44) looked fresh and moist while remaining looked leathery and dry.

4.5.1 Univariable analysis

In the univariable analyses, all clinical symptoms appeared to have a positive association (OR>1) with seropositivity. However, only repeat breeding and retained placenta had *p*-values <0.1 (Table 11). *Brucella* seropositivity was the highest among the animals that exhibited retained placenta in terms of percentage (62.7%) followed by animals that exhibited abortion (37.2%), repeat breeding (32.7%) and purulent vaginal discharge (30.9%).

Table 11. Univariable analysis of potential clinical symptoms as predictors of *Brucella* infection in dairy animals in Assam with odds ratio (OR), *p*-values, and 95% confidence interval (CI)

Variable	Particulars	Seropositive/total of respective variable	OR	95% CI	*p*-value
Occurrence of abortion	Yes	29/78 (37.2%)	1.9		
	No	29/286 (10.1%)	Ref.*	0.6-5.8	0.27
Occurrence of repeat breeding	Yes	39/120 (32.5%)	3.2		
	No	18/239 (7.5%)	Ref.	0.9-11.6	0.08
Occurrence of retained placenta	Yes	32/75 (62.7%)	4.7		
	No	26/289 (9.0%)	Ref.	1.3-16.2	0.02
Occurrence of purulent vaginal discharge	Yes	13/42 (30.9%)	1.0		
	No	43/314 (20.2%)	Ref.	0.3- 3.5	0.95
Occurrence of carpal hygroma	Yes	22/62 (13.7%)	2.0		
	No	36/302 (11.9%)	Ref.	0.6- 6.2	0.24
Occurrence of stillbirth	Yes	4/8 (50.0%)	2.6		
	No	54/356 (15.2%)	Ref.	0.3 -20.3	0.37

*Reference level

4.5.2 Multivariable analysis

In the multivariable analysis, it was found that there was a significant interaction between *Brucella* seropositivity and repeat breeding and retained placenta. Animals exhibiting the symptoms of retained placenta had the highest odds of *Brucella*-infection, (OR=20.7), followed by animals exhibiting symptoms of both repeat breeding and retained placenta (OR =16.59) and only repeat breeding (OR=6.2).

The log likelihood of the final model was -124.6, while that of a model with all five clinical symptoms was -122.1. A likelihood ratio test comparing the two models was completely non-significant (*p*=0.87) indicating no gain in predictive ability by adding other clinical symptoms as predictors.

The final model correctly predicted *Brucella* infection in 48 dairy animals out of 57 *Brucella* seropositive animals at cutoff point 0.1. Similarly, the final model correctly predicted 221 *Brucella* seronegative dairy animals out of total 302 seronegative animals. It was observed that with higher cutoff points of predicted mean, the sensitivity gradually reduced, and specificity increased.

Table 12. Results of multivariable analysis of potential predictors of *Brucella* infection with odds ratio (OR), *p*-values, and 95% confidence interval (CI)

Variable	OR	*p*-value	95% CI
Age < 3 years	Ref.*		
Age 4 years	0.6	0.56	0.1 - 3.3
Age 5 years	1.2	0.79	0.3 - 5.5
Age > 6 years	1.5	0.55	0.4 – 5.5
Negative for both repeat breeding and retained placenta	Ref.		
Positive for repeat breeding but negative for retained placenta	6.2	0.002	2.01 - 19.2
Negative for repeat breeding but positive for retained placenta	20.7	0.001	3.5 - 122.4
Positive for both repeat breeding and retained placenta	16.6	<0.001	4.6 - 59.7
Variable for random effect	Variance		CI
Village	0.2		0.005 - 8.5
Household	0.9		0.1 - 5.8

*Reference level

Table 13. Predictive ability of *Brucella* infection of the final model at three different cut-off points

Cut-off points of predicted mean	Sensitivity	Specificity
0.1	48/57 (84%)	221/302 (73%)
0.2	45/57 (79%)	255/302 (84%)
0.3	41/57 (72%)	278/302 (92%)

4.6 Farmers' knowledge about brucellosis

It was found that only 3.4% farmers in both the states reported that they knew something about brucellosis, while another 4.7% farmers had heard the term brucellosis but did not know anything about it, and the remaining 91.9% farmers had not even heard about brucellosis. We did not observe any significant difference between Bihar and Assam in respect of knowledge about brucellosis (Table 14). For further analysis, all the farmers who knew something about brucellosis and those who heard the term brucellosis were considered as one group called - who knew about brucellosis.

Table 14. Frequency distribution of farmers' knowledge about brucellosis with p-values between Bihar and Assam

Knowledge of farmers on brucellosis	**No. of farmers positively responded/ total farmers**			***p*-value**
	Bihar	**Assam**	**Total**	
Know what brucellosis is	7/292 (2.4%)	11/242 (4.6%)	18/534 (3.4%)	0.30
Heard the term brucellosis but don't know what it is	12/292 (4.1%)	13/242 (5.4%)	25/354 (4.7%)	
Don't know anything about brucellosis	273/292 (93.5%)	218/242 (90.1%)	491/534 (91.9%)	

The farmers who reported that they knew about brucellosis were asked some additional questions related to brucellosis with multiple options. The results suggest that most common knowledge among farmers was that brucellosis affects cattle and it causes abortion in pregnant animals. Less than 1% of the farmers knew that brucellosis could transmit from animals to humans. It was observed that the level of knowledge in regard to brucellosis was almost the same even in sub-group level analysis between both the states (Table 15).

Table 15. Farmers' knowledge about brucellosis in Bihar and Assam

Knowledge of farmers on brucellosis	No. of farmers positively responded/ total farmers		
	Bihar	Assam	Total
Farmers who knew something about brucellosis	7/292 (2.4%)	11/242 (4.6%)	18/534 (3.4%)
Farmers who knew *Brucella* affects cattle	7/292 (2.4%)	11/242 (4.6%)	18/534 (3.4%)
Farmers who knew *Brucella* affects buffalo	4/292 (1.4%)	0	4/543 (0.8%)
Farmers who knew *Brucella* affects human	3/292 (1.0%)	1/242 (0.4%)	4/543 (0.8%)
Farmers who knew human symptoms	4/292 (1.4%)	0	4/543 (0.8%)
Farmers who knew animal symptoms	7/292 (2.4%)	9/242 (3.7%)	16/534 (3.0%)
Believe *Brucella* vaccine available	3/292 (1.0%)	4/242 (1.7%)	7/534 (1.3%)
Know *Brucella* can transmit animals to human	1/292 (0.3%)	3/242 (1.2%)	4/534 (0.8%)
Know *Brucella* can transmit animal to animal	3/292 (1.0%)	0	3/534 (0.6%)
Know *Brucella* transmit through milk	4/292 (1.4%)	4/242 (1.7%)	8/534 (1.5%)
Brucella been diagnosed in the farm	0	2/242 (0.8%)	2/534 (0.4%)

Under multivariable analysis, brucellosis knowledge was significantly associated with medium ($p=0.05$) and large sized ($p<0.001$) farms comparing with small sized farms (OR 2.4 and OR 6.7 times higher respectively) (Table 16). Fully stall-fed system of rearing was also significantly ($p=0.04$) associated with the knowledge about brucellosis (OR 2.9 times higher in case of fully stall-fed rearing).

Table 16. Results of multivariable analysis of determinants having association with farmers' knowledge about brucellosis with odds ratio (OR), *p*-values, and 95% confidence interval (CI)

Variables	**Particulars**	**OR**	**95% CI**	***p*-value**
Herd size	Small size (1-3 dairy animals)	Ref.*		
	Medium size (4-10 dairy animals)	2.4	1.0- 5.7	0.05
	Large size (>10 dairy animals)	6.7	2.2-20.3	<0.001
Rearing system	Partly stall-fed system	Ref.		
	Fully stall-fed system compared	2.9	1.0-7.9	0.04

*Reference level

4.7 Farmer practices relevant for transmission of brucellosis

The univariable analysis indicated that knowledge about brucellosis was significantly associated with a few practices that are relevant for brucellosis transmission. The practices include consumption of raw milk (p=0.001), use of disinfectant in cleaning the farm (p<0.001), animal movement (p<0.001) and introduction of new animal (p=0.03). However, no significant association was observed between farmers' knowledge about brucellosis and practice of cleaning udder before milking (p=0.59), throwing away placenta outside the farms (p=0.16), burying the placenta (p=0.26), washing of hands after handling aborted materials (p=0.86), taking bath after handling aborted materials (p=0.77), introduction of new animal (p=0.11) and quarantine practice followed (p=0.61). Only one farmer reported to use protective clothing like gloves while handling aborted materials.

Practices having significant association with knowledge about brucellosis were further studied by using multilevel mixed effects logistic regression model to see their association with some of the farms/farmers' characteristics (Table 17).

Table 17. Farmers' practices relevant for transmission of brucellosis

Variable	Particulars	Odds ratio (95% confidence interval)			
		Consumption of raw milk	**Use of disinfectant**	**Movement of animals**	**Introduction of new animal**
Knowledge about brucellosis	Know	3.3 (1.2-8.6)*	2.4 (1.2-5.0)*	Non-significant	Non-significant
	Don't know	Ref.***	Ref.		
State	Assam	0.08(0.02-0.3)	Non-significant	Non-significant	0.2 (0.1-0.3)**
	Bihar	Ref.			Ref.
Location of farms	Urban	Non-significant	Non-significant	0.3(0.2-0.8)**	Non-significant
	Rural			Ref.	
Category of farms	Small (1-3 dairy animals)	Non-significant	Non-significant	Non-significant	Ref.
	Medium (4-10 dairy animals)				6.2 (0.1-0.3)**
	Large (>10 dairy animals)				18.8 (7.1-49.0)**
Rearing system of dairy animals	Fully stall-fed	Non-significant	2.6 (1.6-4.2)**	0.01 (0.0-0.03)**	Non-significant
	Partly stall-fed		Ref.	Ref.	

*$p<0.05$, ** $p<0.001$ *** Reference level

Contrary to what may be expected, it was found that significantly more farmers who knew about brucellosis consumed raw milk than those who did not know (OR 3.3 times higher in case of those who knew about brucellosis) (Table 17). Significantly more farming households consumed raw milk in Bihar (15.1%) than in Assam (2.1%).

It was observed that 39.4% farmers in Bihar and 36.1% farmers in Assam used disinfectants while cleaning the farms. Out of the total farmers who used disinfectant, only a small percentage of farmers (2%) in Assam used disinfectant daily, the remaining used it seldom. Significantly more farmers who knew about brucellosis (65.1%) used disinfectant than who did not know about the disease (35.6%). It was also found that significantly more dairy farmers who reared dairy animals under fully stall-fed system (46.8%) used disinfectant than those who reared under partly stall-fed system (25.2%).

More farmers moved their animals in Assam (43.1%) than in Bihar (12%) for grazing, selling etc. It was found that significantly more rural farmers (49.4%) moved their animals than urban (35.6%) or peri-urban (5%) farmers as grazing is a common practice in rural areas, more particularly in Assam. In addition, significantly more farmers who reared dairy animals under partly stall-fed system (89.5%) moved their animals than those who reared under fully stall-fed system (5.4%).

Significantly more farmers in Bihar introduced new animals (35.6%) compared to Assam (14.9%), and more large-sized farms (67.7%) and medium-sized farms (54.5%) introduced new animals than small-sized farms (17.1%).

4.8 Assessment of the cost of reproductive disorders

The total dairy animals kept by surveyed households (n=534) were 2,302 of which 1,348 were in Assam and the remaining 954 were in Bihar.

4.8.1 Reproductive disorders at farm level

With regard to the reproductive disorders at households level, it was found that the problems of interest (repeat breeding, abortion, retention of placenta, purulent vaginal discharge and stillbirth) were significantly higher (Table 18) in the dairy farms located in urban areas than in rural areas, in large (>10 dairy animals) and medium (4-10 dairy animals) sized farms compared to small sized (1-3 dairy animals) farms, under fully stall-fed (zero grazing) system compared to partly stall-fed system, in the farms that reared improved animals compared to farms with indigenous animals.

Table 18. Reproductive disorders at household levels under different context and level of significance between the groups

Farming system characteristics	Categories	No. of households that reported the problem/corresponding total				
		Repeat breeding	**Abortion**	**Retained placenta**	**Purulent vaginal discharge**	**Stillbirth**
State	Assam	59/242 (24.4%) **	68/242 (28.1%)	9/ 34/242 (14.0%)	26/242 (10.7%)	3/242 (1.2%) *
	Bihar	132/292 (45.4%)	151/292 (51.7%)	39/292 (13.3%)	23/292 (7.9%)	12/292 (4.1%)
Location of the farms	Rural	42/247 (17.0%) **	23/247 (9.3%) **	21/247 (8.5%) **	16/247 (6.5%) *	3/246 (1.2%) *
	Urban	89/286 (31.1%)	50/285 (17.5%)	52/286 (18.2%)	33/286 (11.5%)	12/282 (4.3%)
Farm size	Small	59/305 (19.3%) **	18/305 (5.9%) **	25/305 (8.2%) **	10/305 (3.3%) **	5/304 (1.6%) *
	Medium	40/178 (22.5%)	27/177 (15.2%)	23/178 (12.9%)	19/178 (10.7%)	6/176 (3.4%)
	Large	32/50 (64.0%)	28/50 (56.0%)	25/50 (50.0%)	20/50 (40.0%)	4/48 (8.3%)
Rearing system	Fully stall-fed	115/315 (36.5%) **	64/315 (20.3%) **	65/315 (20.6%) **	43/315 (13.6%) **	14/310 (4.5%) **
	Partly stall-fed	16/218 (7.3%)	9/217 (4.1%)	8/218 (3.7%)	6/218 (2.7%)	1/218 (0.5%)
Breed of the animals kept	Indigenous	12/154 (7.8%) **	3/154 (1.9%) **	2/154 (1.3%) **	2/154 (1.3%) **	0/154 (0%)
	Improved	179/380 (47.1%)	70/380 (18.4%)	71/380 (18.7%)	47/380 (12.4%)	15/380 (3.9%)

*Significant ($p \leq 0.05$) difference within the same category **Highly significant ($p \leq 0.001$) difference within the same category

In Assam, the percentage of dairy farming households with reproductive disorders were significantly (p<0.001) higher in Kamrup (metropolitan) district (65.4%) than Baksa (11.3%) and Golaghat (7.4%) districts while, in case of Bihar, the percentage of households with reproductive disorders was almost similar in Patna (58.3%), Nalanda (46.5%) and Vaishali (48.2%) districts.

4.8.2 Basic data used for estimating the cost

The summary of five selected reproductive disorders of dairy animals of surveyed households and the parameters essential to estimate the cost of the problems are stated in Table 19. All the cost estimations in the subsequent tables were worked out based on the figures in Table 19. It was found that 32.9% of dairy animals, belonging to 28.1% of farming households, in Assam and 43.1% of dairy animals, belonging to 51.9% of dairy farming households, in Bihar suffered from one or more of the selected reproductive disorders. Of the affected households, 92.6% households in Assam treated reproductive disorders while in Bihar only 72.8% households treated reproductive disorders. In terms of the percentage of animals treated, about one fifth of affected animals left untreated.

The most common of the five reproductive disorders reported in the study area was repeat breeding (23.2%) followed by retained placenta (6.1%), abortion (4.9%), purulent vaginal discharge (2.9%) and stillbirths (1.0%). Among the reproductive disorders, percentages of dairy animals experiencing repeat breeding, abortion and stillbirth were significantly higher in Bihar than in Assam while the percentage of dairy animals experiencing retained placenta and purulent vaginal discharge were significantly higher in Assam than in Bihar. In Assam, a larger share of dairy animals aborted in third trimester than in Bihar.

Table 19. Attributes used for estimating the cost of selected reproductive disorders

Particulars	**Farms that responded to the question**	**Farms that did not responder/ not applicable**	**Assam**	**Bihar**	**Combined**	**p-value of state variation**
			Observation/ total, or mean ±SE	**Observation/ total, or mean ±SE**	**Mean± SE**	
Mean number of dairy animals per farm	534	0	5.6±0.6	3.3±0.2	4.3±0.3	<0.001
Farms with reproductive disorders	219	315	68/242 (28.1%)	151/292 (51.7%)	219/534(40.0)	<0.001
Total reproductive disorders	219	0	444/ 1348 (32.9%)	411/954(43.1%)	855/2302 (38.0)	<0.001
Mean reproductive disorders in affected households	219	0	6.5±0.7	2.7±0.2	3.9±0.3	<0.001
Abortion cases						
Farms with history of abortion in dairy animals	73	315	35/242 (14.5%)	38/292 (13.0%)	73/534 (13.8)	0.64
Animals aborted in 1st trimester	8	0	4/62 (6.5%)	4/50 (8.0)	8/112 (7.3)	
Animals aborted in 2nd trimester	23	0	9/62 (14.5%)	24/50 (48.0%)	33/112 (31.3)	
Animals aborted in 3rd trimester	42	0	49/62 (79.0%)	22/50 (44.0%)	71/112 (61.5)	0.04

Particulars	Farms that responded to the question	Farms that did not respond er/ not applica ble	Assam	Bihar	Combined	p-value of state variation
			Observation/ total, or mean ±SE	Observation/ total, or mean ±SE	Mean± SE	
Total aborting animals of surveyed farms	73	0	62/1348 (4.6%)	50/954 (5.2%)	112/2302 (4.9)	0.05
Aborting animals treated out of total abortion cases reported	63	10	49/62(79.0%)	41/50 (82.0%)	90/ 112 (80.5)	0.03
Mean treatment** expenditure* of treated abortions	63	0	1475.7±100.4	1182.1±79.6	1319.9±65.4	0.02
Repeat breeding cases						
Farms with repeat breeding	191	343	59/242 (24.4%)	132/292 (45.2%)	191/534 (34.9)	<0.001
Total repeat breeding cases in surveyed farms	191	0	241/1348 (17.9%)	272/954 (28.5%)	513/2302 (23.2)	<0.001
Repeat breeding treated out of total cases reported	142	49	206/241 (85.5%)	197/272 (72.4%)	403/513 (79.0)	<0.001
Mean extra insemination required per repeat breeder	142	0	2.8±0.1	2.5±0.1	2.6±0.1	0.09
Mean expenditure of inseminating/natural mating per time	414	120	200.5±6.8	128.0±2.8	150.0±0.4	<0.001

Particulars	Farms that responded to the question	Farms that did not responder/ not applicable	Assam	Bihar	Combined	p-value of state variation
			Observation/ total, or mean ±SE	Observation/ total, or mean ±SE	Mean± SE	
Mean treatment*** expenditure of treated repeat breeders	7	7	1750.1±61.9	1542.8±52.8	1638.5±48.7	0.02
Retained placenta cases						
Farms with retained placenta	73	461	34/242(14.0%)	39/292(13.3%)	73/534 (13.7)	0.05
Total retained placenta cases in surveyed farms	73	0	95/1348 (7.0%)	49/954(5.1%)	144/ 2302 (6.1)	0.005
Mean retained placenta cases per farm reporting the problem	73	0	2.7±0.5	1.2±0.2	1.9±0.3	0.005
Retained placenta cases treated out of total retained placenta cases reported	61	12	79/95(83.1%)	37/49(75.5%)	116/144 (79.3)	0.03
Mean treatment**** expenditure of treated retained placenta cases	7	0	825.0±44.2	707.1 ±35.2	761.5±31.6	0.06
Purulent vaginal discharge cases						
Farms with purulent vaginal discharge cases	49	485	26/242 (10.7%)	23/292 (7.9%)	49/534 (9.3)	0.26

Particulars	Farms that responded to the question	Farms that did not responder/ not applicable	Assam	Bihar	Combined	p-value of state variation
			Observation/ total, or mean ±SE	Observation/ total, or mean ±SE	Mean± SE	
Total purulent vaginal discharge cases in surveyed farms reporting the problem	49	0	41/1348 (3.0%)	26/954(2.7%)	67/2302 (2.9)	0.01
Total purulent vaginal discharge cases treated	41	8	31/41(75.6%)	22/26(84.6%)	53/67 (80.1)	0.03
Mean purulent vaginal discharge cases per farm reporting the problem	49	0	1.6±0.1	1.1±0.1	1.4±0.1	0.01
Mean treatment[*****] expenditure of treated purulent vaginal discharge cases	7	0	1216.7±70.3	1064.3±44.6	1134.6±44.3	0.08
Stillbirth cases						
Farms with stillbirth cases	15	519	3/242(1.2%)	12/292 (4.1%)	15/534 (2.7)	4.01
Total stillbirth cases in the surveyed farms reporting the problem	15	0	5/1348 (0.4%)	14/954(1.5%)	19/2302 (1.0)	0.09
Stillbirth cases treated out of stillbirth cases reported	9	6	4/5(80.0%)	7/14(50.0%)	11/19 (65.0)	0.62
Mean stillbirth cases per affected farm	15-	0	1.7±0.3	1.2±0.1	1.3±0.1	0.62

Particulars	Farms that responded to the question	Farms that did not responder/ not applicable	Assam	Bihar	Combined	p-value of state variation
			Observation/ total, or mean ±SE	Observation/ total, or mean ±SE	Mean± SE	
Mean treatment[******] expenditure of treated stillbirth cases	7	0	1066.7±49.4	957.1±57.1	1007.7±40.0	0.18
Reproductive month loss						
Mean calving interval (months)	52	482	15.5±0.1	14.9±0.1	15.2±0.1	<0.001
Mean reproductive month loss because of abortion (months)	52	0	5.7±0.5	4.9±0.3	5.3±0.3	0.02
Mean reproductive month loss because of repeat breeding (months)	191	0	1.9±0.1	1.7±0.6	1.8±0.1	0.09
Expenditure of managing dairy animals						
Mean expenditure of concentrate feed consumption/dairy animal/day	139	395	70.8±6.1	59.3±2.8	64.6±3.4	0.09
Mean expenditure of fodder consumption/dairy animal/day	128	406	20.0±2.9	15.4±1.0	17.5±1.5	0.14

Particulars	**Farms that responded to the question**	**Farms that did not respond er/ not applica ble**	**Assam**	**Bihar**	**Combined**	**p-value of state variation**
			Observation/ total, or mean ±SE	**Observation/ total, or mean ±SE**	**Mean± SE**	
Mean expenditure of other miscellaneous items (medicine, breeding, detergents etc.)/dairy animal/day	121	413	12.0±0.5	10.6±0.4	11.2±0.5	0.18
Mean labour expenditure/animal/day	121	413	13.7±1.3	12.3±0.5	12.9±0.7	0.30
Mean electricity expenditure/animal/day	121	413	1.7±0.4	1.9±0.2	1.8±0.2	0.68
Total expenditure of management/animal/day	121	413	118.2±11.5	99.4±4.0	108.1±6.1	0.12
Reduced milk yield of aborted animals						
Farms with reduced milk yield	67	0	20	27	23.5	0.41
No. of animals suffered milk yield loss	47	20	34±0.2	37±0.8	35.5	0.52
Mean volume of milk loss per animal/day (litre)	47	0	1.8±0.2	1.3±0.1	1.6±0.1	0.07

Particulars	Farms that responded to the question	Farms that did not responder/ not applicable	Assam	Bihar	Combined	p-value of state variation
			Observation/ total, or mean ±SE	Observation/ total, or mean ±SE	Mean± SE	
Mean days with reduced milk yield loss/aborted animal	47	0	104.0±5.5	73.3±2.3	88.6±4.50	0.04
Mean milk price/ litre at the farm gate	389	145	41.3±0.4	29.8±0.4	33.8±0.4	0.001
Salvage selling						
Farms that sold dairy animals under salvage	58	476	32/242(13.2%)	26/292 (8.9%)	58/534 (11.0)	0.11
Total animals that were sold under salvage	58	0	57/1348(4.2%)	35/954(3.7%)	92/2302 (3.9)	0.10
Mean price of healthy animal	58	0	65468.7± 4401.5	50961.5± 4132.6	58,965.5 ±3175.5	0.40
Mean price of animals with reproductive disorders	58	0	14281.2± 1335.9	12846.1± 929.3	13637.9±845.1	0.02
Mean salvages selling loss/ animal	58	0	51,187.5± 3849.1	38,115.4± 3640.5	45327.6±2791.5	0.02

*All financial figures are in Indian Rupees (INR). Conversation rate USD 1 = INR 67.2 (average conversion rate of the year 2016, the year of study. Source- https://www.exchangerates.org.uk/USD-INR-spot-exchange-rates-history-2016.html)
**Treatment of abortion included antibiotic/ hormone/ vitamin/others depending on the complexity of the problem.

***Treatment of repeat breeding included antibiotic/ hormone/ dewormer/ mineral mixture/ irrigation of uterus with antiseptic solution/ others depending on the possible cause of repeat breeding.

****Treatment of retained placenta included hormone/ manual removal of placenta/irrigation of uterus with antiseptic solution/antibiotic/others depending on the complexities.

*****Treatment of purulent vaginal discharge included antibiotic/ irrigation of uterus with antiseptic solution/others depending on the complexities.

******Treatment of stillbirth was almost similar to abortion.

4.8.3 Estimating the cost of reproductive disorders

Based on the data presented in Table 19, the economic costs of reproductive disorders in the surveyed areas were estimated and presented in Table 20. As prevalence of reproductive disorders significantly varied between indigenous and improved dairy animals, the costs of all reproductive disorders were worked out according to herd composition (indigenous or improved dairy animals).

It was observed that extra reproductive month loss (for repeat breeding and abortion) was the single most important reproductive disorders that contributed nearly half of the total cost of all reproductive disorders. This was mainly because dairy animals were to be fed, watered and managed in every passing day even though these animals did not produce milk or did not gain anything in terms of reproductive performance. Reproductive months were lost mainly because of abortion followed by repeat breeding. Both the losses were significantly ($p<0.05$) higher per animal in Assam than in Bihar, however, the total number of animals that suffered from abortion and repeat breeding were higher in Bihar than in Assam because of larger size of total population. In addition, the cost of managing dairy animals per day was also higher in Assam than in Bihar.

The second most important cost of reproductive disorders was salvage selling loss. The per unit cost of salvage selling was higher in Assam than in Bihar because of higher price of dairy animals in Assam than in Bihar (Table 19). Most of the dairy animals in Assam sold for salvage belonged to Kamrup (Metropolitan) district where most of the dairy farmers reared larger herds of dairy animals under fully stall-fed condition compared to the other two districts.

Average loss of milk per animal, and number of animals with milk loss were found higher in Assam than in Bihar. Mean price of milk per liter was about 35% higher in Assam than in Bihar. Same was in case of extra insemination requirement per repeat breeder and per unit expenditure of insemination. All the treatment expenditures were also significantly higher in Assam than in Bihar.

Table 20. Estimated cost of reproductive disorders among the surveyed households in Bihar and Assam

Item of cost of reproductive disorders	Total estimated cost in Indian Rupees (INR) of the affected animals in surveyed households (all figures are in thousands)						Cost per animal (INR)		Percentage of the cost	
	Assam			Bihar			Assam	Bihar	Assam	Bihar
	Indigenous	Improved	Total	Indigenous	Improved	Total				
Treatment of aborted animals	2.9	69.4	72.3	1.2	47.3	48.5	54	51	1.0%	1.2%
Extra inseminations of repeat breeders	3.4	131.9	135.3	3.8	83.2	87.0	100	91	1.9%	2.1%
Treatment of repeat breeding cases	10.5	350.0	360.5	9.3	294.7	303.9	267	319	5.1%	7.3%
Treatment of retained placenta cases	2.5	62.7	65.2	0	26.2	26.2	48	27	0.9%	0.6%
Treatment of purulent vaginal discharge cases	1.2	36.5	37.7	0	23.4	23.4	28	25	0.5%	0.6%
Treatment of stillbirth cases	0	4.3	4.3	0	6.7	6.7	3	7	0.1%	0.2%
Management of animal for extra reproductive month loss because of abortion	40.4	1,212.7	1,253.1	14.6	716.7	731.3	930	767	17.9%	17.6%
Management of animal for extra reproductive month loss because of repeat breeding	42.5	1,666.6	1,709.2	64.5	1,397.0	1,461.4	1,268	1,532	24.4%	35.2%

Item of cost of reproductive disorders	Total estimated cost in Indian Rupees (INR) of the affected animals in surveyed households (all figures are in thousands)						Cost per animal (INR)		Percentage of the cost	
	Assam			Bihar			Assam	Bihar	Assam	Bihar
	Indigenous	Improved	Total	Indigenous	Improved	Total				
Loss of milk production	15.5	448.4	463.9	2.8	127.8	130.6	344	137	6.6%	3.1%
Loss of salvage selling	51,187	2,866.5	2,917.7	38.1	1,295.9	1,334.0	2,164	1,398	41.6%	32.1%
Total cost among surveyed animals (in INR)	170,138	6,849.0	7,019.1	134.3	4,018.8	4,153.1	5,207	4,353	100%	100%
Total cost among surveyed animals (in USD)*	2,532	101.9	104.4	2.0	59.8	61.8	78	65		

* Per animal cost among the surveyed animals in Assam (n=1348) and Bihar (n=954)

Note: USD to INR conversation rate was considered as USD 1= INR 67.2 which is the average of the year in 2016, Source- https://www.exchangerates.org.uk/USD-INR-spot-exchange-rates-history-2016.html

4.8.4 Extrapolating the cost of reproductive disorders

To extrapolate the cost of reproductive disorders to the whole state of Assam and Bihar, the affected dairy animal population for both Assam and Bihar were estimated (Table 21) following the approach explained in the methodology section. The affected dairy animals in each state were estimated based on the total number of dairy animals (in milk, dry and heifer) in Assam (n=3,738,775) and Bihar (n= 10,473,230) as per the 20th livestock census conducted in 2019. Of the total dairy animals in Assam, 92.2% (n=3,445,483) were indigenous and the remaining 7.8% (n= 293,292) were improved, while in case of Bihar, 80.4% (n=8,419,959) were indigenous and the remaining 19.6% (n=2,053,271) were improved. In Bihar, the total dairy animal population was 73.7% higher than in Assam and improved dairy animal population was 87.5% higher than in Assam.

Because of higher unit cost of each reproductive disorders per animal in Assam than in Bihar, as observed in Table 19, the cost per affected animal in the surveyed areas was found higher in Assam than in Bihar (Table 20). Despite the fact, the extrapolated cost of reproductive disorders per dairy animal per year (Table 22) was found about three times higher in Bihar (INR 2,912/ USD 43.3) than in Assam (INR 1,060.0/ USD 15.8). The overall cost per dairy animal per year was INR 2,424.9/USD 36.1. The total cost of reproductive disorders in Assam was just 13% of the total cost in Bihar. Little more than half (52.4%) of the cost of reproductive disorders in Assam was contributed by indigenous dairy animals while in Bihar, about 61.9% of the cost was contributed by indigenous dairy animals.

Table 20. Affected dairy animals in surveyed areas (in %) and estimated numbers of affected dairy animals in the whole state of Assam and Bihar

Item of cost of reproductive disorders	Percent animals affected in the surveyed areas				Estimated total number of affected dairy animals in the states					
	Assam		Bihar		Assam			Bihar		
	Indigenous	Improved	Indigenous	Improved	Indigenous	Improved	Total Assam	Indigenous	Improved	Total Bihar
Animal treated for abortion	0.7 %	4.4 %	1.9%	4.4%	24,436	12,931	37,367	158,867	91,155	250,022
Repeat breeders bred through extra AI	2.1%	22.1%	22.6%	28.9%	73,308	64,656	137,964	1,906,406	592,509	2,498,915
Animals treated for repeat breeding	2.1%	18.8%	11.3%	21.2%	73,308	55,027	128,335	953,203	435,266	1,388,469
Animals treated for retained placenta	1.1%	7.1%	-	4.1%	36,654	20,910	57,564	-	84,319	84,319
Animals treated for purulent vaginal discharge	0.3%	2.8%	-	2.4%	12,218	8,254	20,472	-	50,135	50,135

Item of cost of reproductive disorders	Percent animals affected in the surveyed areas				Estimated total number of affected dairy animals in the states					
	Assam		Bihar		Assam			Bihar		
	Indigenous	Improved	Indigenous	Improved	Indigenous	Improved	Total Assam	Indigenous	Improved	Total Bihar
Animals treated for stillbirth	-	0.4%	-	0.8%	-	1,101	1,101	-	15,952	15,952
Animals managed for extra reproductive months caused by abortion	0.7%	5.6%	1.9%	5.4%	24,436	16,508	40,944	158,867	111,665	270,532
Animals managed for extra reproductive months caused by repeat breeding	2.1%	22.1%	22.6%	28.9%	73,308	64,656	137,964	1,906,406	592,509	2,498,915
Animals faced reduced milk yield	0.7%	5.4%	1.9%	5.0%	24,436	15,958	40,394	158,867	102,550	261,417
Animals sold under lower salvage value	0.3%	5.3%	1.9%	3.8%	12,218	15,407	27,625	158,867	77,482	236,349

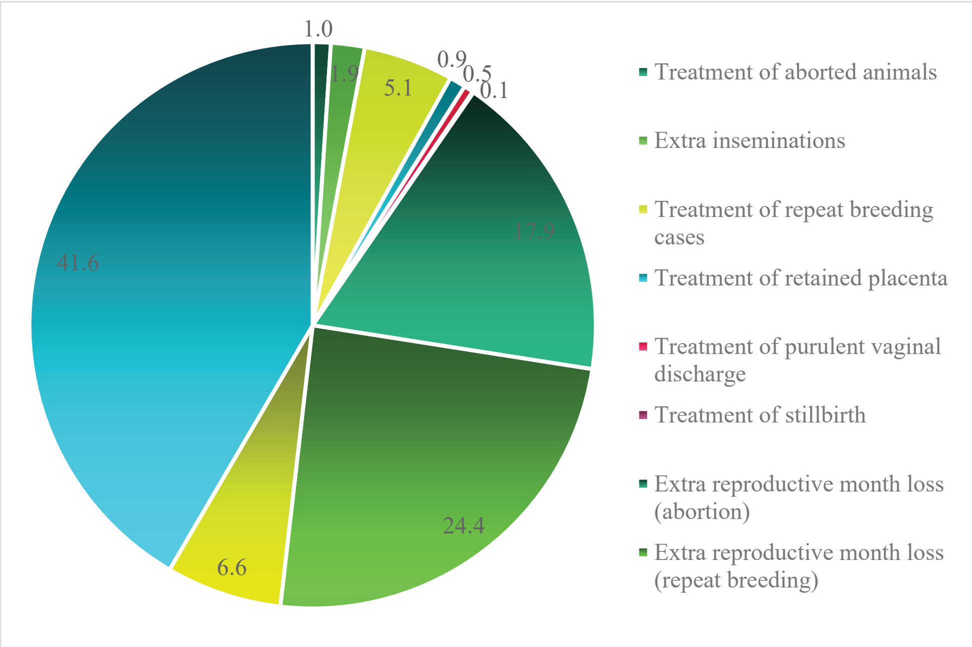

Figure 8. Contribution of different cost components to the total reproductive cost in Assam

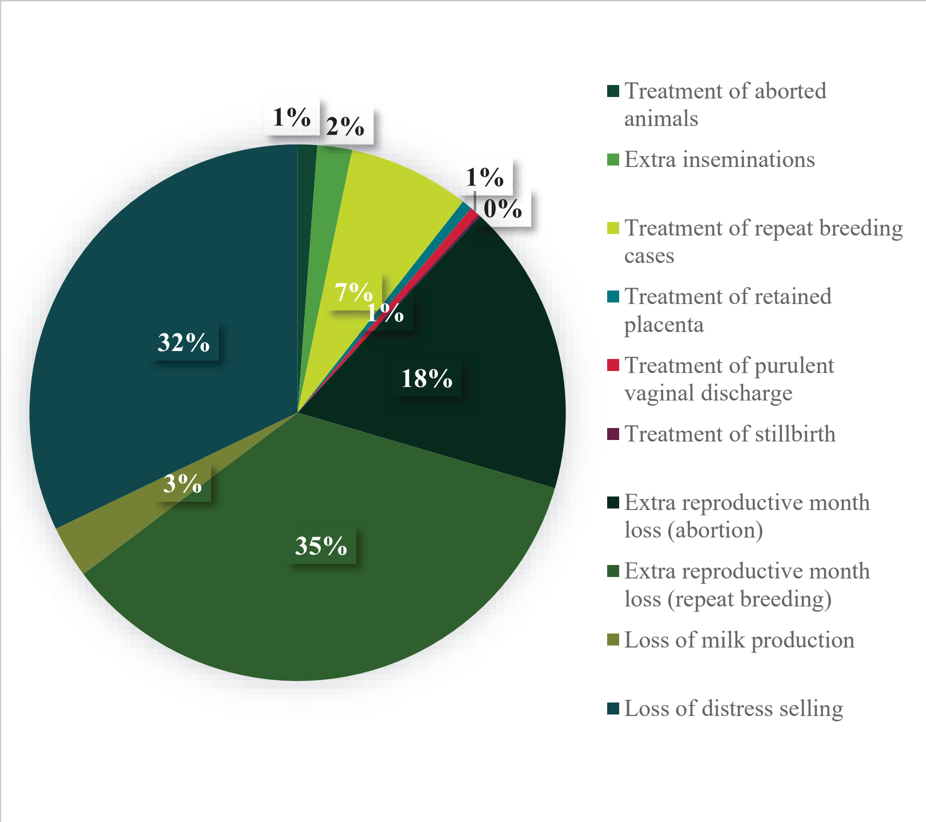

Figure 9. Contribution of different cost components to the total reproductive cost in Bihar

The estimated cost INR 2,424.9/ USD 36.1 per dairy animal of the total dairy animal population represents approximately 4.1% of the mean value of dairy animals (INR 58,966/ USD 877).

Table 21. Estimated cost of selected reproductive disorders in large ruminant dairy animals in the states of Assam and Bihar (all figures in Million INR or USD)

Item of cost of reproductive disorders	Estimated cost in Assam			Estimated cost in Bihar			Total of Assam and Bihar
	Indigenous	Improved	Total	Indigenous	Improved	Total	
Treatment of aborted animals	36.1	19.1	55.1	187.8	107.8	295.6	350.7
Extra inseminations of repeat breeders	41.2	36.3	77.4	61.0	189.6	799.6	877.1
Treatment of repeat breeding cases	128.3	96.3	224.6	1470.6	671.5	2,142.1	2,36.7
Treatment of retained placenta cases	30.2	17.2	47.5	0	59.6	59.6	107.1
Treatment of purulent vaginal discharge cases	14.9	10.0	24.9	0	53.4	53.4	78.3
Treatment of stillbirth cases	0	1.2	1.2	0	15.3	15.3	16.4
Management of animal for extra reproductive month loss because of abortion	493.9	333.7	827.7	2,323.6	1,633.2	3,956.8	4,784.4
Management of animal for extra reproductive month loss because of repeat breeding	519.9	458.5	978.4	10,243.1	3,183.5	13,426.7	14,405.1
Loss of milk production	188.9	123.4	312.3	451.2	291.2	742.4	1,054.7
Loss of salvage selling	625.4	788.7	1,414.1	6,055.2	2,953.2	9,008.4	10,422.5

Item of cost of reproductive disorders	**Estimated cost in Assam**			**Estimated cost in Bihar**			**Total of Assam and Bihar**
	Indigenous	**Improved**	**Total**	**Indigenous**	**Improved**	**Total**	
Total cost among surveyed animals (in INR)	2,078.7	1,884.4	3,963.1	21,341.6	9,158.4	30,500.0	34,463.1
Total cost (in USD)***	30.9	28.0	59.0	317.6	136.3	453.9	512.8

*Indigenous, **Improved ***Conversation rate USD 1 = INR 67.2 (average of the year in 2016, Source- https://www.exchangerates.org.uk/USD-INR-spot-exchange-rates-history-2016.html)

5. Discussion

5.1 Farmers and farming system

In this study, there were similarities as well as differences in farmers and dairy farming systems between the states of Assam and Bihar. Average household size was found significantly higher in Bihar than in Assam. This was possibly because of the lower ranking of Bihar in human development index, income index and education index (Mishra and Chaudhary, 2014). In both the states, male-headed households were predominant as women generally become the household head only in absence of any adult male (father/husband/son) in India. More farmers were found within the age group of 41-60 years than other age groups (Table 3). This might be because of reluctance of young people to take up farming as a source of income and employment because of poor income in farm activities compared to non-farm activities and poor sustainability of smallholder farms (Sharma and Bhaduri, 2009). Further, more farmers were found poorly (Class VI-X) educated probably because they have limited options to join the service sector. The illiterate group of farmers perhaps started farming operation several years ago when percentage of literacy was poorer than at present owing to which we could see correlation between higher age group and poor literacy.

Further, the farming systems in both Bihar and Assam had significant differences in terms of herd size, farm category, rearing system, and mating system, even though the smallholder farming system was predominated in both the states (Table 4 and Table 5). This might be influenced by the traditional food habits of the people. In Assam, most people are non-vegetarian and people generally do not have the habit of drinking milk, except children and elderly people. People use milk mainly as tea whitener and as milk sweets and milk products like curd, cottage cheese or cream. In Bihar, there is a sizable vegetarian population who are habitual milk drinkers and therefore there is demand of milk across the districts. In respect of dairy animals, Assam does not have any indigenous cattle or buffalo breed that is a high milk yielder. The average productivity of indigenous dairy animals in Assam is only about one litre per day (ILRI, 2007) while in Bihar, apart from a sizable cross-bred cattle population, the state has also a large buffalo population (3.7 million mature female buffalo in Bihar) that are good milk yielders. In Assam, the buffalo population (0.1 million mature female buffalo) are good for draught power (NDDB, 2019) but are poor milk producers. Perhaps because of the low milk yield of dairy animals, investment on housing, feeding, management etc. is poor in Assam and therefore, majority of dairy animals in the state are reared under partly stall-fed condition.

Further, milk production in Assam is very much dependent on local market demand of unpasteurized milk in nearby urban consumption centres. As a result the farming system is mostly unorganised and 97% of the milk in Assam is marketed through informal dairy value chain actors (milk vendors and sweet makers) (ILRI, 2007). A higher concentration of large and medium sized farms in Kamrup (Metropolitan) districts, particularly in and around Guwahati city where about one million people live (Directorate of Census Operation, 2011), was observed possibly to meet the good demand of fresh milk in the city. No large farm was found in the other two studied districts of Assam, likely because of an absence of major urban consumption centres like Guwahati in both the districts. In Bihar, dairy cooperative societies (DCS) constituted among dairy farmers as a part of organised production, procurement, processing, and marketing of milk under three tier cooperative system was much stronger. There were about one million DCS members in Bihar compared to only 15,817 DCS members in Assam (DAHD, 2018). This strong cooperative system facilitates procurement of milk from producers residing in villages, irrespective of distance from major urban centres. Such procured milk is processed, pasteurised, and marketed by the dairy plants of the Milk Union or Milk Federation. Therefore, presence of local market demand of milk and ease of access to organised market, make the dairy farming operation more uniformly distributed across the districts in Bihar. Possibly because of this, it was not necessary for Bihar to have so many large-size farms near the big urban consumption centres, as is the case in Assam, to meet the local demand of milk. Furthermore, every DCS generally provides access to AI services to its farmers, which explains the more common adoption of AI in Bihar than in Assam.

5.2 Seroprevalence of *Brucella* infection

Fewer serum samples were positive for *Brucella* infection by RBPT than iELISA in our study (Table 6). Studies suggest that serological tests that can detect IgM antibody (e.g., RBPT) may show positivity at the initial stage but may show negativity at the later stage. On the other hand, serological tests that can detect IgG antibody (e.g. iELISA) may show negativity at the initial stage but show positivity at a later stage (Godfroid et al., 2010; Nielsen and Yu, 2010). Since *Brucella* infection remains in the body of the affected animals for a longer period of their life, there is more possibility of finding the animals seropositive by iELISA that detect IgG antibody produced in later stage of infection than RBPT that detect IgM antibody produced at the initial stage. Further, studies suggested that ELISA is more accurate and better test than RBPT for assessing *Brucella* infection because of higher sensitivity and specificity (OIE, 2009; Godfroid et al., 2010; Hassan et al., 2020). A study in Pakistan reports that there is substantial agreement between the iELISA seropositive results and RBPT results (Khan et al., 2021). Our finding is partly in agreement with this as all the 35 samples found seropositive by RBPT was confirmed by iELISA although there was no agreement on remaining 24 samples.

Laboratory results revealed that animal level seropositivity of *Brucella* infection in Bihar was only 0.3%, in contrast to 15.9% in Assam. No buffalo was found seropositive in this study, but due to the low number of buffaloes included in the study, this is not representative for the whole population. An earlier study conducted in 23 states of India reported an overall seroprevalence of 3.5% in Bihar (Renukaradhya et al., 2002). A few other studies have reported even higher seropositivity in Bihar: 12.2% by Pandian et al. (2015) and 9.3% by Kaushik et al. (2010). These findings were contrary to our study results. One possible reason could be that none of the above-mentioned studies were conducted on randomly selected samples. The lower seropositivity in Bihar could be explained by the fact that Bihar had a significantly lower mean herd size of dairy farms, higher adoption of AI, and lower mean age of dairy animals in comparison to Assam.

On the other hand, overall seropositivity found in the present study in Assam (15.9%) agreed with previously reported estimates of 14.3% (Bhattacharya et al., 2005) and 13.8% at the animal level (Gogoi et al., 2017) in Assam. Further, our district level seropositivity was in agreement with the findings of Gogoi et al. (2017) who reported the highest seropositivity in the Kamrup (Metropolitan) district (28.6%) among nine studied districts of Assam (Gogoi et al., 2017). Aulakh et al. (2008) reported significant variation of *Brucella* seropositivity in different districts of the Punjab state in India. They reported significantly higher *Brucella* seropositivity in the districts where there were larger herds with more introduction of new animals. This was possibly because, in those districts farmers used to sell *Brucella* infected animals as there was no system of screening of the animals for *Brucella* before selling/purchasing. In this study, it was found that *Brucella* seropositivity was significantly higher ($p = 0.006$) in the farms in which new animals were introduced than those where new animals were not introduced. This finding was in agreement with couple of other studies which reported that geographic location had a significant association with *Brucella* seropositivity (Muma et al., 2007; Mugizi et al., 2015; Lindahl et al., 2019). However, our finding of *Brucella* seropositivity in the Kamrup (Metropolitan) district was much lower than the reported positivity of 72.5% by Lindahl et al. (2019), who reported the herd-level positivity based on milk samples using a milk ELISA test, only including smallholder farms in the peri-urban belt. This was the highest reported *Brucella* positivity in Assam.

However, this study also observed significantly higher *Brucella* seropositivity in urban areas than in rural areas. This might be because of higher presence of large-sized farms in urban areas to meet the market need of fresh milk in nearby urban centres. In rural areas, farmers mainly rear smaller herds of dairy animals which are easy to manage and keep clean because of popularity of partly stall-fed system of rearing.

5.3 Risk factors of *Brucella* infection

Out of 15 potential risk factors used for multivariable regression analysis, only three factors were found significant in multivariable regression analyses (Table 9). The identified risk factors were (a) district to which animals belong, (b) age of the animals, and (c) mating system.

Regarding districts, significantly higher seropositivity in Kamrup (large farms) was observed, possibly because of the dominance of large sized farms in the district. This is in agreement with several other studies (Makita et al., 2011; Lindahl et al., 2014; Mugizi et al., 2015; Gogoi et al., 2017; Terefe et al., 2017; Khan et al., 2021) which reported that large-sized farms are more likely to suffer from *Brucella* infection than small-sized farms. This higher prevalence in large-sized farms may be due to the confinement of a higher number of animals leading to a greater chance of exposure to infected animals and aborted materials (Kumar et al., 2009; Jagapur et al., 2013; Lindahl et al., 2014; Khan et al., 2021). Furthermore, it has been found that districts have a significant correlation with rearing system and farm size. Larger farms mainly follow a fully stall-fed system of rearing. Several studies have suggested that fully stall-fed farms are more likely to suffer from *Brucella* infection than partly stall-fed farms (Jagapur et al., 2013; Anka et al., 2014; Kumar et al., 2016; Terefe et al., 2017), which is in corroboration with this study findings. It was found that large farms introduced significantly more new animals, some of which might be from *Brucella* infected herds. This is in corroboration with Coelho et al. (2007), who have reported that the introduction of new animals is an important risk factor. In addition, most large herds in Kamrup (Metropolitan) district were in small plots of hilly areas mainly belonging to the government, and were managed by traditional, dairy-farming community migrants from the neighbouring country Nepal. These larger herds available in the district seemed to have poor biosecurity practices that might result in higher *Brucella* seropositivity. Infectious diseases like brucellosis have been shown earlier to be reduced by following proper biosecurity practices (Wolff et al., 2017).

Using multivariable analysis, we found that the age of the animal was an important risk factor. It was found that with an increase in the age of the animals, likelihood of being *Brucella* seropositive increases. Increased susceptibility to clinical disease with age, could be more associated with sexual maturity due to the effects of sex hormone and placenta erythritol on the pathogenesis of brucellosis (Asmare et al., 2013). Studies in India, Tanzania, Tajikistan, Uganda and Pakistan have also reported that older dairy animals are more likely to be seropositive than younger animals (Lindahl et al., 2014; Mugizi et al., 2015; Jain et al., 2018; Sagamiko et al., 2018; Khan et al., 2021). Lower seroprevalence of brucellosis in young animals could be attributed to resistance of sexually immature cattle to infection, or to less time of risk of exposure. However, one study reported marginally higher seroprevalence of *Brucella* infection in calves (10%) than older dairy animals (9%) and the study suggested that age does not have positive correlation with seropositivity (Kumar et al., 2016). Similarly, some other studies have also reported that younger animals are more likely to be *Brucella* seropositive than older animals (Boukary et al., 2013; Shome et al., 2014).

Dairy animals bred using AI were less likely to be *Brucella* seropositive than animals bred through natural mating. This might be because AI is a well-established biosecurity measure, as no movement of bulls takes place and no physical contact between male and female dairy animals happens that eliminate the chances of transmitting *Brucella* infection. In addition, in village conditions, community bulls are more commonly used by farmers for breeding. If such a bull suffers from brucellosis, it could easily transmit the disease from one animal to the other. This is in corroboration with few other studies that have observed higher seropositivity in the animals bred through natural mating than AI (Shome et al., 2014; Ali et al., 2017; Cárdenas et al., 2019). Also some studies have not found any significant association between *Brucella* seropositivity and AI (Patel et al., 2014). Contrary to our findings, a study in India has found that dairy animals bred through AI are more likely to be seropositive than animals bred through natural mating in Kamrup (Metropolitan) district (Lindahl et al., 2019). This might be because of confounding effect of other factors; for example, larger herd size was highly significantly associated with *Brucella* seropositivity in our univariable analysis. Possibly because of this association, we also found positive association between *Brucella* seropositivity and AI in our univariable analysis (OR 3.2) but when we controlled for Kamrup (Metropolitan) district by bifurcating the district in three groups based on herd size (small, medium, and large) for multivariable analysis, instead of AI, natural mating become positively associated with *Brucella* seropositivity.

Further, by employing univariable analysis (Table 7), it was found that there was a greater likelihood of being *Brucella* seropositive if the animals belonging to farmers who completed training on dairy cattle management, who had consulted with veterinarians, if the animals were not moved and if the farms used disinfectants in cleaning the farms. These implausible associations were perhaps the results of confounding effect of district, and once the district was controlled by accounting for farm size, the variables became non-significant in multivariable analysis.

5.4 Clinical symptoms as predictors of *Brucella* infection

As noted in earlier studies, our study found that smallholder dairy farming system is the most predominant in the state of Assam. Dairy animals in the state had suffered from potential clinical symptoms of *Brucella* infection such as abortion, repeat breeding, retained placenta, purulent vaginal discharge, stillbirth and carpal hygroma (Table 10) and all have positive association (OR>1) with *Brucella* infection which suggest that all the potential symptoms may predict occurrence of brucellosis to some extent. However, significant associations were observed only with the retained placenta and repeat breeding (Table 11).

Occurrence of retained placenta was the most important predictor of *Brucella* infection. This is common in *Brucella* infection because brucellosis causes placentitis, which may induce abortion in the subsequent pregnancy (OIE, 2016). Further, if placenta is not fully expelled it may cause metritis or endometritis, and purulent vaginal discharge (OIE, 2016). Our finding agreed with some other researchers who reported significant association between *Brucella* seropositivity and retained placenta and repeat. However, another study did not find significant association between *Brucella* seropositivity and retained placenta (Mugizi et al., 2015).

A significant association between abortion and *Brucella* seropositivity has been reported by various researchers (Ahasan et al., 2010; Chand and Chhabra, 2013; Anka et al., 2014; Patel et al., 2014; Mugizi et al., 2015) but we did not find such association. Some other studies also did not find significant association between *Brucella* seropositivity and abortion (Asmare et al., 2013; Mugizi et al., 2015). They opined that acute brucellosis with abortion was mainly seen when cattle got infected with *B. abortus* for the first time, although they may remain seropositive for the rest of their life (Godfroid et al., 2010) therefore, there is every possibility that most of the animals that shows seropositivity at the later age may not exhibit abortion. Also, non-pregnant heifers would not exhibit abortion. In the case of *B. melitensis* infection in cattle, seroprevalence was lower and abortion occurred less frequently than in the case of *B. abortus* infection (Asmare et al., 2013). Another explanation to why it is difficult to detect an association between brucellosis and abortion is that many other conditions may also cause abortion, including other pathogens, which we have already shown circulate in the study population (Shome et al., 2019; Leahy et al., 2021).

Significant associations between *Brucella* seropositivity and stillbirth, purulent vaginal discharge, and endometritis were reported by some studies (Patel et al., 2014; Yanti et al., 2021). One paper suggests that hygromas can occur in leg joints and are a common manifestation of brucellosis in some tropical countries (OIE, 2009). However, we did not find significant association between *Brucella* seropositivity and stillbirth, or purulent vaginal discharge, or carpal hygroma.

The predictive ability of the final model suggests that the model could predict *Brucella* seropositive cases reasonably accurately, with a percentage of true positives (sensitivity) ranging from 72%-84% and true negatives (specificity) from 73%-92% depending on the cut-off points used (0.1, 0.2 and 0.3 of predictive mean). No such analysis was found in any previous literature. Using a predictive model for clinical symptoms in cattle could both be helpful for veterinarians to make a diagnosis, and for targeted efforts by *Brucella* control programs.

5.5 Knowledge and practices about bovine brucellosis

Our study found that farmers' knowledge about brucellosis in Bihar and Assam was very poor, as did other studies conducted in India and neighbouring countries. A study conducted in Puducherry, India reported that 4.8% farmers knew about brucellosis (Rajkumar et al., 2016). In Sri Lanka, only 2.6% farmers knew about brucellosis as a zoonotic disease (Kothalawala et al., 2018). Reported levels of farmer knowledge widely varied among countries outside South Asia. For instance, in Senegal, none of the farmers knew about brucellosis (Tebug et al., 2015). On the contrary, in Tajikistan, 15.0% farmers (Lindahl et al., 2015), in Ecuador, 30.0% of farmers (Perez and Aguayo, 2017), and in Ethiopia, 48.0% of farmers (Tschopp et al., 2013) knew about brucellosis.

We found that only 2.4% of the farmers knew that abortion occurs if animals suffer from brucellosis. This finding agrees with a study from Sri Lanka which found only 8.3% of the farmers were aware about abortions due to brucellosis (Kothalawala et al., 2018). Relatively more farmers (19.2%)

in South Africa were knew that abortions were indicative of brucellosis (Cloete et al., 2019). In our study, only 0.8% of the farmers mentioned that brucellosis is transmitted from animals to humans. This finding is in agreement with a study from Pakistan where 3.0% of the farmers were aware of transmission of brucellosis from animals to human (Arif et al., 2017). However, there have been reports from other countries with higher knowledge levels. In Portugal, 74.7% (Diez and Coelho, 2013) and in Egypt, 96.3% of the farmers (Holt et al., 2011) were aware about the zoonotic nature of brucellosis.

We did not find any significant association between knowledge of brucellosis and farmers' education, age, or gender. This agrees with a study conducted in Portugal (Diez and Coelho, 2013) that reported education, age and gender do not have any association with knowledge about brucellosis. A study in Tajikistan also did not find significant association between knowledge about brucellosis and gender (Lindahl et al., 2015). Further, our finding contrasts with the findings of Lindahl et al. (2015) where farmers with lower level of education were reported as less likely to have knowledge about brucellosis compared to higher educated farmers (Lindahl et al., 2015). Similar findings were reported from Pakistan (Arif et al., 2017), Kenya (Njuguna et al., 2017) and Ecuador (Perez and Aguayo, 2017).

In Assam, farmers' knowledge about brucellosis had a significant association with training obtained by farmers and interaction with veterinarians. This finding is in agreement with a study finding from South Africa that reported that the main source of knowledge among farmers about brucellosis was veterinary consultation (Cloete et al., 2019). Similarly, couple of other studies also reported that farmers consulting about animal health issues with veterinarians were more knowledgeable than others in regards to brucellosis (Diez and Coelho, 2013; Lindahl et al., 2015).

We observed that urban farmers were more aware of brucellosis than rural farmers. This is likely because in urban areas farmers got better access to veterinary hospitals, veterinarians, and other veterinary teaching, research and development organisation than did farmers in rural areas. This relationship could also partly be explained by the fact that location of the farms had significant correlation with farm size, rearing system, training, and consultation with veterinarians. We did not find any significant association between farmers' age and knowledge about brucellosis which is in contrast with the findings in Kenya that has found significant associations between them (Njuguna et al., 2017).

5.6 Farmers' practices relevant for transmission of bovine brucellosis

A study from Mongolia reported that preventive practices against brucellosis were significantly associated with gender, location of farms, use of veterinary services and knowledge of brucellosis (Bat-Erdene et al., 2018). Our study is partly in agreement with this. Four farming practices that include consumption of raw milk, use of disinfectants in cleaning the farms, movement of animals and introduction of new animals are reported as important risk factors by some researchers (Aulakh et al., 2008; Calistri et al., 2013; Soomro et al., 2014) and we have found that these practices are significantly associated with either one or more farm/ farmers characteristics (viz. knowledge about brucellosis, location of the farms, rearing system and category of farms).

Consumption of raw milk is considered an important risk factor for transmission of brucellosis from animal to human (Sofian et al., 2008; Adesokan et al., 2013). In our study, significantly more farmers who knew about brucellosis consumed raw milk, contrary to our initial hypothesis. This might be because farmers had poor knowledge about the zoonotic nature of brucellosis and therefore failed to avoid consuming raw milk. A study from Turkey reported that raw milk consumption had a significant association with location (rural or urban), age and economic condition of consumers (Celik and Ceylan, 2010); however we did not find such an association. Studies in Punjab and Sindh province of Pakistan found that about 66% of households consumed raw milk but only 3% were aware that brucellosis could be transmitted through milk (Arif et al., 2017). The percentage of farmers consuming raw milk is even higher in some African countries such as Senegal where 95% farmers reported to consume raw milk (Tebug et al., 2015).

It is reported that those farms who use disinfectant are at lower risk of being seropositive against *Brucella* (Musallam et al., 2015). Use of disinfectants in cleaning the farms were significantly associated with knowledge about brucellosis and rearing system of dairy animals in our study. This might be because significantly more urban farmers reared dairy animals under fully stall-fed system, and they might have used disinfectant in cleaning the farms, as this category of farms produce more farm waste. Besides, this group of urban farmers had more access to training and more consultation with veterinarians and some of them might as a result have obtained knowledge about brucellosis.

A study from Sri Lanka reported that free movement of animals was significantly associated with prevalence of brucellosis (Kothalawala et al., 2017). In our study, we found that significantly fewer farmers in urban areas moved their animals. This was possibly because more urban farmers reared dairy animals under fully stall-fed system because of scarcity of land for grazing or free movement.

Introduction of new animals was significantly associated with the category of farms and the states to which the farms belonged. This was possibly because medium sized farms which were more common in Bihar, introduced new animals to replace old, diseased, or less productive stock to keep the farms economically productive throughout the year. In case of Assam, large and medium-sized farms were available mainly in Kamrup (Metropolitan) district, while in the other two districts presence of such farms were negligible.

While the study aimed at a completely random sampling of farms and animals, it is possible that responses of farmers may be biased as farmers may report the practices that are desired but may not always follow, which may inflate the positive practices. However, given the lack of knowledge and lack of good practices reported, this is likely not a major bias in the manuscript.

5.7 Economic cost of reproductive disorders in dairy animals

5.7.1 Reproductive disorders and cost components

As noted in the result section, the prevalence of the five reproductive disorders (i.e., abortion, repeat breeding, retained placenta, purulent vaginal discharge, and stillbirth) of interest varied according to several factors. These factors are reviewed to provide insights how they may influence the associated economic costs.

The selected reproductive disorders were widely present in dairy animals of Assam and Bihar with prevalence varying within and between states. The prevalence of dairy animals with one of more of the selected reproductive disorders found in our study in Assam (32.9%) and in Bihar (43.1%) align well with an earlier study in Meghalaya, a neighbouring state of Assam, which reported that 33.8% of dairy animals in the state were affected by one or more reproductive disorders (Khan et al., 2016). A study in Bangladesh, which borders Assam, also reported a similar prevalence (39.4%) of reproductive disorders (Alam et al., 2015), as did studies conducted in Kashmir, India (41.8%) (Ishfaq et al., 2017) and in Ethiopia (43.1%) (Haile et al., 2014). An exception was a study in Afghanistan which reported a much a higher prevalence (55.6%) (Assadulla et al., 2019).

The prevalence of the selected reproductive disorders in improved dairy animals was significantly ($p<0.001$) higher than in indigenous animals. A similar finding was also reported in a study in Ethiopia (Mebrahtom and Hailemichael, 2016). Indigenous animals might be expected to suffer fewer reproductive disorders given their lower reproductive efficiency relative to improved dairy animals (Mebrahtom and Hailemichael, 2016; Demeke, 2020), lower use of artificial insemination reducing the chances of repeat breeding because of poor semen quality/improper timing/ faulty insemination, as well as being better adapted to local climatic conditions that might make them more tolerant/ resistant to various reproductive disorders. Further, this may also be attributed to farmers not recognizing or paying attention to the reproductive disorders in indigenous animals because of their generally poor productive and reproductive performances and because they are often kept in open grazing systems, especially in Assam.

The average number of reproductive disorders per farming household in Bihar was significantly lower than in Assam possibly reflecting the significantly smaller herd size in Bihar. A study from Ethiopia also reported better reproductive performance in smaller herds than larger herds (Yifat et al., 2009). This study found that larger herd size (> 10 dairy animals) was significantly associated with higher rates of the selected reproductive disorders. Larger herds have poorer sanitation and hygiene with large numbers of animals kept in small, confined places where disease transmission might be easier. Further, it was found that households rearing animals under a fully stall-fed system (zero grazing) experienced higher rates of the selected reproductive disorders than those who reared dairy animals under partly stall-fed condition. Haile et al., (2014) reported a contrary finding with higher reproductive disorders in partly stall-fed than in fully stall-fed conditions. Further, the selected reproductive disorders were relatively higher in urban than in rural areas possibly because of higher concentrations of large sized farms in urban areas. A couple of recent studies reported higher seroprevalence of both *Brucella spp.* and *Leptospira spp.* infection in urban/peri-urban areas than rural areas in Bihar and Assam (Gogoi et al., 2017; Lindahl et al., 2019; Leahy et al., 2021); these infections are considered as the main cause of the five selected reproductive disorders (Lindahl et al., 2014; Patel et al., 2014; Shome et al., 2019).

Different researchers have reported different rates of prevalence of reproductive disorders in different parts of the world, but most agree that the prevalence of the selected reproductive disorders ranges from 0.2% to 25.0%. These differences might be because of location of the farms (urban/ rural areas), rearing systems, herd size (small/medium/large), and breed of animals (indigenous/ improved) as discussed above. Regional differences in etiology also cannot be ruled out. It was found that repeat breeding (23.2%), retained placenta (6.1%) and abortion (4.9%) were the main reproductive disorders among the five problems we studied. Incidence of purulent vaginal discharge (2.9%) and stillbirth (1.0%) cases were relatively lower in our study areas. Our findings are fully or partly in agreement with studies conducted in Meghalaya (India), Bangladesh, Kashmir (India), Haryana (India) and Ethiopia (Lobago et al., 2006; Meena and Malik, 2009; Haile et al., 2014; Alam et al., 2015; Mekonnin et al., 2015; Khan et al., 2016).

We found that repeat breeding and abortion resulted in loss of reproductive months in both Assam and Bihar. The mean inter-calving period reported in our study (15.2 months) was in agreement with a study conducted in Bangladesh (15.3 months) (Paul et al., 2013) but slightly lower than studies conducted in Pakistan (16.8 months), Ethiopia (17.9 months) and Tanzania (16.7 months) (Sattar et al., 2005; Swai et al., 2005; Mebrahtom and Hailemichael, 2016) while much higher than in countries like Korea (13.8 months) (Do et al., 2013) or USA (13 months) (Hare et al., 2006). Higher mean reproductive month loss because of abortion was found in Assam than in Bihar, attributable to a larger proportion of abortions in the 3rd trimester. The different timing of abortion may be due to differing etiologies. Kamrup (Metropolitan) district of Assam, for example, has been reported to have a high prevalence of brucellosis (Gogoi et al., 2017; Lindahl et al., 2019), and studies suggest that dairy animals that suffer from brucellosis generally abort in third trimester (Aulakh et al., 2008; Abdisa, 2018).

Because of reproductive disorders, dairy animals become less productive and more expensive to rear. Therefore, farmers report being compelled to sell the animals at lower value before the end of their productive life. This contributed to significant economic loss to the farmers. A study in Michigan reported that 60.8% of the repeat breeders were culled (Lafi et al., 1992). Consistent with these results, half of the repeat breeders in our study were reported to be salvage-sold. Relatively lower salvage selling percentage (30.4%) of repeat breeders has been reported by another study from India (Saraswat and Purohit, 2016). In India, culling of diseased animals is not easy as slaughtering of cows is prohibited by act of law (DAHD, 2019). A strong sense of belongingness, socio-religious beliefs and policy environment also greatly influence dairy producers in reluctance to cull diseased animals and, perhaps, reluctance to report if they do sell. Poor knowledge of farmers (Table 14 and 15) about the infectious diseases like brucellosis, and their preventive measures may reduce the adoption of proper reproductive health management practices (e.g., proper feed, clean and hygiene, deworming, vaccination, timely breeding, timely treatment, culling, quarantine, salvage selling, etc.) in the studied states. Further, poor farm record keeping system followed by the farming community may limit their capacity to identify and judge the reproductive disorders correctly and to take appropriate corrective measures on time.

5.7.2 Estimation of the economic cost

The terms 'loss' and 'cost' are often used rather loosely, and even interchangeably by many researchers (McInerney et al., 1992) but in this document the terms are used consistently as per the definition stated in the methodology section. A study in US estimated the economic cost of clinical diseases of dairy animals using stochastic simulation model based on seven parameters which include veterinarians’ fee, expenditure for medicine, expenditure for labour, loss of milk, loss of culling, loss of management of dairy animals during extended calving interval and on farm death (Liang et al., 2017). In our study we also considered all the above expenditure and loss, although we did not find any case of morality of dairy animals because of reproductive disorders. Studies have highlighted significant economic costs in terms of reduced milk production, expenditure on medication, reduced calf production, prolonged calving interval and early depreciation of potentially useful cows with reproductive disorders (Britt, 1985; Abdisa, 2018). Our present study confirms that these five selected reproductive disorders lead to economic costs and that costs are substantial. Among them, the highest cost was due to management of animals during an extended calving interval period followed by low salvage returns for culled animals. This ranking corroborates the findings of Dijukizen et al. (1985), but differs from the findings of Patel et al. (2014) who reported that reduced milk yield caused the highest cost. We did not find such a high economic cost because of reduced milk yield. This may be explained by milk yield loss being observed mainly in dairy animals that aborted in first trimester and early second trimester, whereas cost of managing an extended calving interval applied to every aborted animal and every repeat breeder. A study from Tanzania also reported higher inter-calving period as an important source of larger economic loss (Swai et al., 2005).

The treatment expenditure reported by various researchers varies even within the country, possibly because of location of farms, time, access to services, quality of services, line of treatment and type of individual engaged in treatment (community animal health worker/ veterinary diploma holder/ veterinary graduate/ veterinary professor/ specialist) that have important bearing on the expenditure. Relatively lower expenditures for the treatment of abortion (INR 250/ USD 3.7), repeat breeding (INR 506/ USD 7.5) and retained placenta (INR 320/ USD 4.8) were reported from Gujarat, India (2003-2005) (Panchasara, 2012) compared to our reported expenditure of INR 1,319 (USD 19.6), INR 1,638 (USD 24.4) and INR 761 (USD 11.3), respectively, for the same conditions. Similarly, lower treatment expenditure for reproductive disorders (INR 750/ USD 11.2) was reported by another researcher (Patel et al., 2014) in Gujarat, India, but this relates to an earlier time period and is not adjusted for inflation.

In addition, the expenditure estimated for treatment of reproductive disorders in this study does not reflect the expenditure on treatment of all animals with the selected reproductive disorders. We found that about one fifth of the dairy animals with reproductive disorders were left untreated, which may be attributed to poor access to veterinary services, poor availability of veterinarians, especially at night, lack of funds to treat animals, lack of skill of farmers to detect some problems or lack of knowledge about the effects of the problem (Meena and Malik, 2009). A study from 2007 suggested that only about 32% of the farmers in India get access to services- mainly for curative rather than prophylactic treatment (Kumar et al., 2007). Moreover, of those farmers who availed of veterinary services, 36.7% were not satisfied with the veterinary and extension services (Chand et al., 2014). Others have emphasized the need for good veterinary service with strong infrastructure, quality bull/semen and need based training and extension services as important elements for addressing reproductive health problems in Haryana, India (Meena and Malik, 2009).

Cost estimation of reproductive disorders at state level is important to inform policy makers and to help them in making evidence-based decisions for designing control programme. This study estimated an economic cost of INR 3,963.1 (USD 59.0) million in Assam and INR 30,500.0 (USD 453.9) million in Bihar due to five selected reproductive disorders. An economic estimation from India reported a loss of USD 3.4 billion to the livestock sector for the single disease brucellosis, of which 95.6% (USD 3.2 billion) was incurred in the dairy sector alone (Singh et al., 2015). That study estimated that brucellosis caused a loss of USD 6.8 per cattle and USD 18.2 per buffalo which is less than the estimated cost of five reproductive disorders (INR 2,424.9/ USD 36.1) per animal presented here. Similarly, another recent study in India estimated a loss of INR 92,120 (USD 1370.8) million because of brucellosis (Bardhan et al., 2020) which is again seems lower than our estimate that considered only two states in the country out of 34. This difference seems quite obvious as they estimated the cost of reproductive disorders only caused by brucellosis, however, in our case, we estimated the cost of reproductive disorders irrespective of aetiology.

It may be mentioned here that we found a high incidence of reproductive disorders in both Assam (32.9% animals) and Bihar (43.1%) but our seroprevalence studies on *Brucella* infection found much higher seropositivity in Assam (16.5%) than in Bihar (0.3%), Further, our previous seroprevalence studies on, *Leptospira* spp. infection and *Coxiella burnetii* infection in both the states (based on the same sampling frame as used for this study and on the same biological samples), found that seroprevalence of *Leptospira* spp. infection in Assam was 1.2% in comparison to 4.5% in Bihar while seroprevalence of *C. burnetii* infection in Assam was 5.8% in contrast to 27.1% in Bihar (Shome et al., 2019; Leahy et al., 2021). All three infectious agents may cause reproductive disorders with a similar presentation in dairy animals (Loureiro and Lilenbaum, 2020; Yanti et al., 2021) and therefore it may not be wise to estimate the economic cost based on investigation of one infectious agent (say brucellosis). Further, any economic estimation of reproductive disorders based on a seroprevalence study without confirmatory diagnosis (based on historical, clinical, and laboratory investigation of the aetiology) could be highly misleading as seropositivity does not mean the disease is present. Therefore, in this work we preferred not to assess the economic cost of reproductive disorders based on specific pathogens but rather on syndromes.

The economic costs of reproductive disorders can be expected to increase as dairy systems intensify and the costs of inputs and animals increases. Two estimates of annual cost per dairy animal in the Netherlands USD 345 (Inchaisri et al., 2010) and USD 238 (Dijkhuizen et al., 1985) are indeed much higher than the estimate reported in the present study (INR 2,424.9/ USD 36.1). Here, they did not estimate the treatment expenditure of the five specific reproductive disorders or culling/ salvage selling loss, but estimated the economic cost of reduced milk yield, AI cost, calving management cost and increased calving interval cost. A more meaningful comparison is considering those costs relative to the gross production value of the animal, which equated to 2% in the case of the second estimate from the Netherlands, while the estimated costs of reproductive disorders in our study are estimated to represent 4.1% of the mean value of dairy animals.

The cost for an animal suffering of reproductive disorders among the sampled households was found to be lower in Bihar (INR 4,353/ USD 65) than in Assam (INR 5,207/ USD 77) mainly because of lower cost of production, expenditure of treatment and price of milk in Bihar than in Assam. This might be because of presence of a strong dairy cooperative system in Bihar in comparison to Assam (DAHD, 2018) that supports the farmers in getting access to farm inputs at reasonable prices and selling the milk in bulk quantity relatively at lower price. In Assam, higher expenditure for management and treatment of cattle by farmers was reported mainly attributable to dependence of farmers on external supply of farm inputs (e.g. feed, AI semen, medicine etc.), often from production sources more than 1000 km from away from Assam, but closer to Bihar. Further, cost of dairy animals is also higher in Assam than in Bihar because of the scarcity of improved animals owing to which, some dairy farmers in Assam import dairy animals from Bihar where the cost of dairy animals is relatively lower.

In case of the extrapolated cost for the states, per animal cost was found about three times higher in Bihar (INR 2,912.2/ USD 43.3) compared with Assam (INR 1,060.0/ USD 15.8). This was mainly because of much higher improved dairy animal population in Bihar (87.5%) than in Assam among which reproductive disorders were found significantly higher than in indigenous dairy animals. Again, the much higher dairy animal population in Bihar (73.7% higher dairy animal population to that of Assam) contributed to make the overall cost of reproductive disorders higher in Bihar than in Assam.

Under this study, the five most common reproductive disorders which were identified by farmers using a syndromic approach were included. There are several other reproductive disorders like anoestrus, dystocia, metritis, endometritis, uterine prolapse etc. that are present (Modi et al., 2011; Mekonnin et al., 2015; Assadulla et al., 2019) but these were not included in our study. The key reason for focusing on selected syndromes rather than the full list of problems was that some of the reproductive disorders (repeat breeding, retained placenta, metritis, endometritis, purulent vaginal discharge, anoestrus etc.) are interrelated (Zadeh, 2013) and the underlying cause may be manifested in any of these, so their individual economic cost would be difficult to assess without proper clinical or laboratory investigation and farm records. As a result, the economic cost estimates presented here should be considered a lower bound, though the other diseases not included in our analysis are not expected to dramatically increase the overall estimated cost. Further, it could be mentioned that there is lack of uniformity in the reproductive disorders included and items of cost estimation among different studies conducted by various researchers, therefore comparing the results of different studies is not straight forward. Again, in our study, we found lot of absent data/non applicable data because not all households encountered reproductive disorders. For respondents who did not report problems, many survey questions were non applicable. Further, missing data were also found related to the cost of management of dairy animals. This was mainly because farmers keep few management or day-to-day expenses record and often find it difficult to calculate out the cost as part of these expenses come from household feed resources or family labour.

6. Conclusion

This study has generated new knowledge, identified certain knowledge gaps, and assessed scope for improvement of future research on the epidemiology of *Brucella* infection and the economic cost of reproductive disorders of large ruminant dairy animals. The study concludes with the following key points.

- *Brucella* seroprevalence may significantly vary within and between states or districts based on different factors. The estimated seropositivity in Assam largely agrees with previous studies, but the result of seropositivity in Bihar was much lower than previous studies found.
- For proper assessment of *Brucella* seropositivity, it is critical that researchers use probabilistic sampling and accurate diagnostic methods.
- The likelihood that an animal is *Brucella* seropositive is lower in dairy animals in smaller-size herds than larger size herds, in younger dairy animals than older dairy animals, and in animals bred by AI than by natural mating. All three identified risk factors can partly explain the reason why *Brucella* seropositivity is lower in Bihar than Assam.
- Retained placenta is the most significant predictor of *Brucella* infection followed by both repeat breeding and retained placenta and repeat breeding alone. If an animal suffers from any of the above problem/s, there is reason to suspect that the animal may suffer from *Brucella* infection which may help the farmers to take early action for brucellosis diagnosis and control.

- Future brucellosis control program should use the knowledge of these risk factors and clinical predictors for prioritising high risk groups and identify infected farms.
- Risk factors identified by this study may inform designing appropriate control programme for reducing the risk of *Brucella* infection, while the identified clinical symptoms as predictors of *Brucella* infection may help in tentatively diagnosing *Brucella* infected animals.
- The study has shown that farmers' knowledge about brucellosis in both Bihar and Assam states is negligible. Farmers' knowledge about the disease is associated with category of farms, rearing system, location of the farms, training obtained on dairy animal management and consultation with veterinarians.
- Because farmer knowledge about brucellosis might help improve farmer practices, a customized awareness programme might be useful. However, more studies are required to have deeper understanding on the subject, including on farmers' attitude and incentives (moral, social, material) to change behaviour.
- Reproductive disorders in dairy animals like repeat breeding, retained placenta etc. are common in Assam and Bihar causing million-dollar costs to the dairy industry. By strengthening reproductive health management, the states can reduce this cost, estimated to account for approximately 4.1% value loss of dairy animals each year.
- The study has shown that overall cost of reproductive disorders could be even higher when the indigenous population gradually is replaced by improved breeds through crossbreeding programmes. Therefore, in future more comprehensive efforts are required to reduce these costs.
- The study found that prevalence of reproductive disorders significantly varies between indigenous (native breed or non-descript indigenous) dairy animals and improved (exotic or cross bred) dairy animals. Any economic estimation made without consideration of the breed type may lead to an incorrect economic estimation. It is important to make sure that both indigenous and improved animals are sufficiently represented to generate robust parameter estimates.

- Further, an economic estimation based on assessment of any single causative agent (say, brucellosis) is difficult and may be misleading as reproductive disorders can be caused by a range of infectious and non-infectious causes. In addition, economic estimation of reproductive disorders based on any seroprevalence study without confirmatory diagnosis of the aetiology, could lead to overestimation of the economic burden of the diseases as seropositivity does not necessarily mean the disease is present.
- Since one fifth of reproductive cases remained untreated and culling or other disease preventive practices are poorly followed, the study further suggests that there is need to increase awareness and capacity among the farming communities to adopt better reproductive health management practices and to keep proper farm records that will help them to address the problems in a more timely and efficient way.
- More studies are required to investigate the economic cost of the full range of reproductive disorders and to obtain additional representative samples to extrapolate the cost for other states and the country as a whole
- The significant differences between Assam and Bihar illustrates that the prevalence of reproductive disorder and costs incurred varies from state to state and therefore a blanket approach for controlling *Brucella* infections and reproductive disorders may not work.

Finally, it can be concluded that, in India, proper epidemiological investigation of *Brucella* infection is uncommon but essential. This should use adequate sample size and probabilistic sampling; conduct systematic investigation of risk factors and clinical symptoms (as predictors of *Brucella* infection) following more robust statistical methods; assess knowledge, attitude and practices of farming communities and other value chain actors and services providers; and estimate the economic cost more comprehensively by including all possible cost components in order to generate credible and useful evidence to influence policy makers and underpin disease control.

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8. Popular science summary

Globally, brucellosis is one of the most important bacterial diseases that can affect both humans and animals. It affects several species of animals including cattle, buffalo, sheep, goat, dog and pigs. The disease can transmit to humans through direct or indirect contact with affected animals, body fluids and aborted materials, or their products like milk and meat. The disease in animals mainly show reproductive disorders like abortion, repeat breeding, retained placenta, purulent vaginal discharge and stillbirth while in humans, the symptoms vary according to the organs affected. The most common symptoms of brucellosis in humans are intermittent fever (that may persist for weeks or months), fatigue, body ache, joint pain etc. The disease may cause huge economic cost in dairy production because of reduced milk yield, loss of calves, cost of management during extended calving interval, and cost of treatment. There are no effective vaccines for humans and several species of animals, except for ruminants. Diagnosis of the disease is difficult because of its zoonotic nature, since poor handling of laboratory samples may spread the disease to humans. Most low-income countries are not in a position to control the diseases because of lesser laboratory infrastructure, knowledge, technical capacities, socio-religious issues, and investment on disease control. Further involvement of humans and animals make the disease control difficult as multidisciplinary approach is required for the same.

The disease in India was reported for the first time in 1942 and now it is prevalent throughout the country. The disease in India's context is important because of large livestock population, high interaction of men and animals and low hygienic standards in the livestock farms. Therefore, an effort was made to investigate the epidemiology of *Brucella* infection in two of the poorest Indian state- Assam and Bihar- and to estimate the economic cost caused by the reproductive disorders in dairy animals. The objective of the study was to assess the prevalence of *Brucella* infections in the states, to identify the factors that may increase the risk of getting infection, to identify the symptoms that can help in predicting *Brucella* infected animals, to assess the knowledge and practices of farmers that are critical for controlling brucellosis and to estimate the economic cost caused by reproductive disorders.

The study was conducted by interviewing 292 farming households from 28 villages of three districts of Bihar and 242 farming households from 24 villages of three districts of Assam. The households were selected randomly without showing any favoritism to any household in selection process. Every farming households were interviewed using a structured questionnaire. Further, blood samples from 740 dairy animals were collected from the selected farming households of both the states (maximum three dairy animals from each household) to test the samples in the laboratory for presence of *Brucella* infection.

The data collected from the farming households and the laboratory results of *Brucella* infection were entered in computer and the data were analysed statistically to generate information to meet the objectives of the study.

From the study, it was found that small farms (1–3 dairy animals) were most predominant, both in Bihar (78.8%) and Assam (76.4%), followed by medium (4–10 dairy animals) and large farms (>10 dairy animals). Herd size was almost uniform across the surveyed districts in Bihar, while in Assam, it largely varied among the surveyed districts. Improved (exotic breed imported from abroad or their crossbreed) dairy animals were predominant in the study areas of Bihar (91.8%), while in Assam little more than half of the dairy animals were improved, and the remaining were native breed or non-descript indigenous animals. Fully stall-fed (zero-grazing) system of rearing was more common in Bihar than in Assam.

From the laboratory analysis, it was found that 15.9% of the sampled dairy animals in Assam had *Brucella* infection while only 0.3% selected dairy animals in Bihar had *Brucella* infection. More dairy animals in urban areas (18.7%) had *Brucella* infection than in rural areas (12.4%) of Assam. It was found that animals belonging to districts having smaller-sized herds were less likely to suffer from *Brucella* infection than animals belonging to districts having larger-sized herds. Chance of having *Brucella* infection increased with the increase in age of dairy animals but decreased with the adoption of artificial insemination (AI) for breeding. Further, it was found that retained placenta was the most important clinical symptom that can predict *Brucella* infection followed by presence of repeat breeding and retained placenta and repeat breeding alone.

Knowledge of farmers about brucellosis was found very poor. Only few farmers knew about brucellosis (3.4%) and its zoonotic importance (0.8%) in both Bihar and Assam. Knowledge about brucellosis was significantly higher among farmers who had larger herd size, managed dairy animals under fully stall-fed system of rearing, availed training on dairy cattle management, consulted with veterinarians, and farms belonged to urban areas.

In regard to the cost of reproductive disorders, it was found that 32.9% of dairy animals in Assam and 43.1% dairy animals in Bihar suffered from one or more reproductive disorder. The most common reproductive disorder reported in the study was repeat breeding (23.2%) followed by retained placenta (6.1%), abortion (4.9%), purulent vaginal discharge (2.9%) and stillbirths (1.0%). About one fifth of the reproductive disorders were left untreated. It was estimated that reproductive disorders caused an annual economic cost of USD 60.0 million in Assam and USD 462.1 million in Bihar in the year of survey with an average cost per animal at USD 36.7 in a year.

The study suggests that *Brucella* infection may significantly vary between states or districts based on different factors. In any future brucellosis control program, appropriate care should be taken to reduce the risk of *Brucella* infection by addressing the identified risk factors. Poor knowledge of farmers and inadequate adaptation of brucellosis preventive practices suggest that a thorough knowledge and capacity building programme among the farming community and other dairy value chain actors is critical to control brucellosis. In addition, by proper reproductive health management, the states can reduce the said cost which could be roughly considered as 4.1% value loss of dairy animals in each year. The study urges the need of adoption of good dairy animal management practices to reduce the incidence of the reproductive disorders. Since one fifth of reproductive disorders remained untreated, it suggests that there is need for increasing awareness among the farming community and increasing access to quality veterinary services.

9. Populärvetenskaplig sammanfattning

Brucellos är en viktig sjukdom i hela världen, men förekommer oftare i låg och mellaninkomstländer. Det är en zoonotisk sjukdom, vilket innebär att den kan spridas mellan djur och människor. Sjukdomen orsakas av olika bakterier, till exempel *Brucella abortus*, eller *Brucella melitensis*. Hos människa ger sjukdomen bland annat kronisk feber och ledsmärtor, och kallas ibland Maltafeber, eller undulantfeber. Många olika däggdjur kan drabbas, och vanliga symptom är feber, aborter, eller andra reproduktionsproblem. Brucellos är därför ett stort problem inom djurhållningen med både djurlidande och ekonomiska konsekvenser, och framförallt mjölkproduktion påverkas mycket negativt. Indien är ett land där brucellos förekommer i alla delstater, och eftersom landet har en mycket stor population av både människor, kor och bufflar så har sjukdomen stora ekonomiska och hälsomässiga konsekvenser. Många i Indien är vegetarianer, och mjölk är en viktig proteinkälla, och även en viktig inkomstkälla för den stora andelen av befolkningen som håller boskap.

Den här studien bedömer förekomst av sjukdomen i två indiska delstater, Assam och Bihar, genom att studera förekomsten av antikroppar, vilket indikerar att djuren har exponerats för bakterien tidigare. Dessutom så studerades vilka faktorer på gårdsnivå som var associerade med ökad exponering, och vilka symptom som förutspår vilka djur som troligen är infekterade. Även djurhållarnas kunskap utvärderades, och de ekonomiska konsekvenserna av reproduktionssjukdom uppskattades. Materialet samlades in under 2015-16 genom att besöka 534 gårdar med mjölkproduktion, och provta 740 kor och bufflar. Genom att använda två olika tester bedömdes vilka djur som hade antikroppar mot *Brucella* bakterier.

Studien visade att det vanligaste var att gårdarna hade 1-3 djur (79% i Bihar, och 76% i Assam). I Bihar var över 90% av djuren av exotiska raser, eller korsningar med exotiska raser, medan det i Assam endast var lite mer än 50%, och resten av djuren var inhemska raser. I Bihar var det vanligare att djuren hölls helt uppbundna, medan fler gårdar i Assam lät djuren beta.

I Assam hade 16% av korna antikroppar mot *Brucella*, medan endast 0.3% av korna i Bihar hade det. I Assam var förekomst av antikroppar vanligare i städer än på landsbygden, och det var mindre vanligt i distrikt med mindre gårdar, eller på gårdar som använde artificiell information jämfört med med gårdar som hade naturlig betäckning. Äldre djur var oftare positiva jämfört med yngre djur. Förekomst av kvarbliven efterbörd visade sig vara ett viktigt symptom för att förstå vilka djur som blivit infekterade, särskilt om det kombinerades med att kon hade problem att bli dräktig.

Endast 18 djurhållare hade hört talas om brucellos, och bara fyra av dem visste att det kunde smitta från kor till människor. Det var vanligare att djurhållare med stora gårdar och som tidigare hade fått träning i djurhållning kände till sjukdomen.

Studien visade att 33% av djuren i Assam och 43% i Bihar hade haft ett eller flera reproduktionsproblem senaste året. Det vanligaste var svårigheter att bli dräktig (23%), följt av kvarbliven efterbörd (6%) och abort (5%). Det var vanligare med reproduktionsproblem hos kor av exotiska raser eller korsningar. Kostnaderna (både utgifter och förluster) för reproduktionsproblem i Assam och Bihar uppskattades till 60 miljoner dollar respektive 462 miljoner dollar totalt per år, med en genomsnittlig kostnad på 36 dollar per djur, utslaget på alla djur i delstaterna.

Den här studien visar att förekomsten av *Brucella* infektion kan variera mycket mellan stater och distrikt beroende på flera olika faktorer. Resultaten för Bihar var lägre än tidigare studier, medan resultaten för Assam stämmer med tidigare resultat. De riskfaktorer som identifierats av studien kan användas i planeringen av framtida kontrollprogram i Indien. Eftersom kunskapen om brucellos var väldigt låg i studien är det också viktigt att öka kompetensen hos mjölkproducenter. Uppskattningsvis leder reproduktionsproblem till höga kostnader för producenterna, och det är viktigt att de lär sig förebygga och behandla detta, och det kan därför finns ett behov av bättre tillgång till veterinära tjänster.

Sammanfattningsvis är brucellos och reproduktionsproblem vanligt förekommande i dessa två indiska delstater, och både hindrar den ekonomiska utvecklingen och leder till sjukdom och lidande hos både människor och djur.

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11. Appendix

List of all the variables studied to assess risk factors of *Brucella*-infection with their particulars and our decision on using it for multivariable analysis

Variables	Description of the variables	Seropositive/ total (%)	Coefficient of unconditional association with *Brucella* seropositivity	*p*-value of association	Missing value	Kept for multivariable model
Outcome						
ELISA results of *Brucella*-infection	Positive	58			13	
	Negative	306				
Identifiers						
Districts	Kamrup (large farms)	49/135 (18.9)	Ref.*	<0.001	0	Yes
	Kamrup (small farms)	3/41(7.3)	-2.2			
	Golaghat	2/96(2.1)	-2.8			
	Baska	4/92(4.3)	-3.5			
Farm Characteristics (FC)						
Location of the farm in rural or urban areas	Rural CDB	20/161(12.4)	Ref.	0.16	0	Yes
	Urban CDB	38/203(18.7)	0.4			
Category of farms	Small (1-3 dairy animal),	8/223(3.6)	Ref.	<0.001	0	Yes

	Medium (4-10 dairy animals)	19/81(23.4)	2.2			
	Large (>10 dairy animals)	31/60(51.7)	3.6			
Dairy animals in contact with goat	Yes	9/106(8.5)	-1.2	0.03	0	Yes
	No	49/258(19.0)	Ref.			
Type of floor	Concrete	20/67(29.8)	Ref.	<0.001	0	Yes
	Earthen	9/202(4.4)	-2.4			
	Others	29/95(30.5)	0.1			
System of rearing	Fully stall-fed	50/155(32.2)	2.5	<0.001	0	No
	Partly stall-fed	8/209(3.8)	Ref.			
Dairy animal in contact with pig	Yes	2/14(14.3)	-0.1	0.86	0	No
	No	56/350(16.0)	Ref.			
Dairy animal in contact with dog	Yes	33/216(15.3)	-0.1	0.68	0	No
	No	25/148(16.9)	Ref			
Dairy animal in contact with wild animal	Yes	25/159(15.7)	-0.03	0.92	0	No
	No	33/205(16.1)	Ref.			
Type of roof	Thatch	9/46(19.6)	0.2	0.49	77	No
	Corrugated Tin/ asbestos	28/233(12.0)	0.1			
	Others	2/18(11.1)	Ref.			
Source of introduction	Known source	19/67(28.3)	0.2	0.80	285	No
	Unknown source	3/12(25.0)	Ref.			
Farm Management (FM)						
Adoption of AI	Yes	46/225(20.4)	1.2	0.02	0	Yes
	No	12/139(8.6)	Ref.			
Introduction of new animals	Introduced	22/79(27.8)	1.5	0.006	0	Yes
	Not introduced	36/285(12.6)	Ref.			
Movement of animal	Animal moved	9/213(4.2)	-2.8	<0.001	0	Yes
	Not moved	49/151(32.4)	Ref			
Use of disinfectant in cleaning farms	Used disinfectant,	35/160(21.9)	1.06	0.02	0	Yes
	Not used disinfectant	23/204(11.3)	Ref			
Quarantine of newly purchased animal	Yes	0/9	0	0	0	No
	No	58/355(16.3)				

Purchase of sick animal	Yes	4/18(22.2)	0.4	0.47	0	No
	No	54/346(15.6)	Ref			
Purchase of weak but cheap animal	Yes	3/10(30.0)	0.8	0.26	0	No
	No	55/354(15.5)	Ref.			
Vaccination (against any disease) followed	Yes	46/150(30.7)	2.0	<0.001	0	No
	No	12/214(5.6)	Ref.			
Cleanliness of animal	Very clean	20/118(16.9)	Ref.	0.73	0	No
	Clean	38/244(15.6)	-0.1			
	Dirty	0/2	0			
Aborted material buried	Yes	28/70(40.0)	1.2	0.23	288	No
	No	1/6(16.7)	Ref.			
Producers Demographic (PD)						
Education of farmers	No education	17/66(25.7)	Ref.	0.14	0	Yes
	Class I-V	11/49(22.4)	-0.2			
	Class VI-X	18/149(12.1)	-1.2			
	Class XI and above	12/100(12.0)	-1.1			
Age of farmers	20-40 years	21/89(23.6)	Ref.	0.10	0	Yes
	41-60 years	26/191(13.6)	-1.2			
	60 years and above	11/84(13.1)	-1.2			
Training availed by farmers	Availed	15/50 (30.0)	1.5	0.02	0	Yes
	Not availed	43/314(13.7)	Ref.			
Interaction had with the veterinarians	Had interaction	55/297(18.5)	1.9	0.005	0	Yes
	No interaction	3/67(4.5)	Ref.			
Gender of farmer	Male	58/351(16.5)	0	0	0	No
	Female	0/13				
Cow Demographic (CD)						
Breed of animal	Non-descript indigenous	7/178(3.9)	Ref.	<0.001	13	Yes
	Improved/CB/pure	51/186(27.4)	2.7			
Age of animals	With *Brucella* seropositive	6.83±0.33	0.1	0.03	13	Yes
	With *Brucella*	6.09±0.13	Ref.			

	sero-negative					
No. of lactation	With *Brucella* seropositive	3.24±0.23	0.2	0.009	0	No
	With *Brucella* seronegative	2.64±0.09	Ref.			
Species of animal	Cattle	58/364	0	0	0	No
	Buffalo	0				

*Reference level

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This thesis has an in-depth analysis and description of seroprevalence, risk factors and predictors of *Brucella* infection in dairy animals in two Indian states- Assam and Bihar. In addition, it provides a critical evaluation of the knowledge and practices of smallholder dairy farmers about brucellosis and the costs of reproductive disorders incurred by them in both the states. Overall, this thesis presents new knowledge on this important zoonotic disease and presents information that can help future intervention programs.

Ram Pratim Deka completed his bachelor's degree in veterinary sciences and animal husbandry (B.V.Sc.&A.H.) and master's degree in veterinary sciences (M.V.Sc.) from the College of Veterinary Science, Assam Agricultural University (AAU), Jorhat, Assam, India.

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