

# Zoonoses and Food Hygiene News

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**Late Dr. Durga Datt Joshi, Founder of NZFHRC**

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## **CONTENTS:**

- ☞ **Assessment of biosecurity level in farm and estimation of seropositivity of Brucellosis in milking animals of two districts (Kathmandu and Kavre) of Nepal**
- ☞ **NEWS**

### **Assessment of biosecurity level in farm and estimation of seropositivity of brucellosis in milking animals of two districts (Kathmandu and Kavre) of Nepal**

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#### **Abstract**

#### **Introduction**

Brucellosis is a bacterial zoonotic disease with worldwide distribution. It causes serious economic losses to farmers and the government through direct production losses as well as additional costs for control and eradication program. In Nepal, brucellosis is endemic and remains a significant problem for cattle and human health. The study was conducted with aimed to assess the biosecurity level in farm and estimation of seropositivity of brucellosis in two districts of Nepal.

#### **Methods**

This cross-sectional study was conducted from 14<sup>th</sup> June 2017 to 21<sup>st</sup> July 2017 in Kathmandu and Kavre districts of Nepal. Fifty four farms were selected from Kathmandu and Kavre districts. Semi-structured questionnaires based on biosecurity level, knowledge, attitude and practice about brucellosis were administered among the farmers. A total of 93 blood samples were collected from their milking animals (cows) and tested for brucella antibody by ELISA method.

#### **Results**

In this study, no positive brucellosis cases were detected in the milking animals (cows). Intensive rearing system and traditional type of husbandry practice had been found to be adopted in two district of Nepal. All respondents were engaged in at least on practice at their farm and household conducive to transmission of brucellosis to other animals and human. Knowledge and awareness about brucellosis among the farmers was minimal. Only one respondent had heard about brucellosis.

#### **Conclusion**

Poor understating of the disease, presence of multiple risky practice on farms and households level suggest the need of one health education related to zoonotic diseases in local communities in order to change practice in households and farm level.

**Key words:** Brucellosis, cattle, ELISA test, Kathmandu, Kavre

#### **Introduction**

Brucellosis is a bacterial zoonotic disease with worldwide distribution caused by Gram-negative bacteria of the genus *Brucella* (Abernethy et al., 2011; Abdussalam and Fein, 1984). In cattle, the main pathogenic species are *B. abortus*, responsible for bovine brucellosis; *B. melitensis*, the main etiologic agent of small ruminant brucellosis and *B. suis* responsible for swine brucellosis (Godfroid et al., 2005; Sanogo et al., 2012). Infertility and abortion in females, and orchitis and epididymitis in males are the main characteristic features of this disease (Anka et al., 2013). Brucellosis causes serious economic losses to farmers and the government through direct production losses as well as additional costs for control and eradication programs (Mekonnen et al., 2010). Although brucellosis has been eradicated in most of the developed nations, it remains a significant problem for both cattle and human health in developing countries (Apan et al., 2007; Lee et al., 2009).

As in most Southeast Asia countries, bovine brucellosis has been a problem among livestock for many years in Nepal. The first evidence of the disease was reported in 1977 (Pyakurel and Mishra, 1977). Since then it has been reported in various parts of Nepal (Joshi, 1983; Pradhan, 1996; Lefkowitz et al., 2003; Rana, 2005; Paudel, 2006). Although brucellosis has been reported to be endemic in Nepal, nationwide distribution, economic and public health impact of this disease is unknown. Large scale brucellosis control program targeting the eradication of the disease has not been adopted in Nepal. Financial constraints, inadequate laboratory facilities, lack of compliance by farmers, and religious taboos may impede the success of *Brucella* eradication in Nepal. Few publications have focused only on sero-prevalence. None have attempted to assess the farm level biosecurity and identify the risk factors for *Brucella* seropositivity among cattle. In this study, we assessed the biosecurity level in farm and estimated seropositivity in cattle. This study will suggest how these factors can be modified to reduce the risk of the infection.

#### **Methods**

This cross-sectional study was conducted from 14<sup>th</sup> June 2017 to 21<sup>st</sup> July 2017 in Kathmandu and Kavre districts of Nepal. Kavre is mid-hilly with a subtropical climate and elevation range of 280 meters to 3018 meters. Agriculture and livestock sector is the major contributor for livelihood. Kathmandu district is the centre for several industries (carpets, garments, finance and tourism, health and educational services). The agriculture sector represents only 9% of its finances. For the study, 54 farmers were enrolled in this study. Fifteen farmers were selected from Kavre and 39 were from Kathmandu district. Twenty-two blood samples were collected from farm of Kavre and 71 samples were collected from farm of Kathmandu respectively.

An opened and closed type questionnaire was drafted, including questions about general information, farm management, animal feeding and housing practices, biosecurity and Brucellosis knowledge. It was written in English and translated orally in Nepali language. Before collecting data, questionnaires were pre-tested.

After obtaining a verbal informed consent, information was collected from farmers. Blood samples were drawn by vein puncture of the jugular vein from cows that could be restrained. Holder with needle (22 G) or 21G syringes were used. Samples were kept in dry vacutainers in a cool box during the collect. After collecting samples, blood was centrifuged 3500 rpm at the zoonosis centre and sera was extracted by using 100 µL micro-pipette and put in Eppendorf tubes stored at 4°C.

Anti-Brucella ELISA Bovine kits (EUROIMMUN) were used to detect antibodies of the immunoglobulin class IgG against Brucella in bovine serum which had 99% sensitivity and specificity. ELISA was performed using protocol. Data achieved by the questionnaire were coded and analysed with Statistical Package of Social Sciences (SPSS.16.0). Descriptive analysis in form of frequencies and percentages was used to summarize the data.

## Result

### 1. ELISA test result

In this study, no positive brucellosis cases were detected by ELISA method.

### 2. Socio-demographic characteristics

A total of 54 farmers were involved in the study. The participants' aged ranged from 26 to 81 years with a median age of 43 years. Most of them 21 (42%) belonged to the 40-50 years age groups. The majority of interviewed farmers were female 61 (61%). Out of 54 farmers, 40 (74%) farmers had sole occupation as an agriculture and 14 (26%) were involved with other occupations besides agriculture. Only 1(2%) of farmers had up to 5000 NRPS income, 13 (25%) had between 5000 and 10000 NRPS, 18 (35%) had 10000 to 20000 NRPS, and 20 (38%) had more than 20000 NRPS. (Table 1)

**Table 1: Descriptive socio-demographic characteristic of participants**

Characteristics	Frequency
<b>Gender</b>	
Female	33(61)*
Male	21(39)
<b>Age (years)</b>	
20-30	3(6)
30-40	13(26)
40-50	21(42)
50-60	8(16)
>60	5(10)
<b>Occupation</b>	
Agriculture	40(74)
Agriculture and others	14(26)

\*parenthesis indicates percentage

In interviewed farms, (61%) farms had intensive rearing system, 21(33%) were semi-intensive and 3(6%) were extensive system. Regarding types of sheds, 37(69%) farm had traditional sheds, 16(30%) had semi-modern and only one had modern farm. Half of farmers responded that they sold their milk production to people, either door to door or directly from the farm. Twenty-six per cent sold their milk production with local dairy or milk agent. Twenty per cent kept their production for their own consumption. For the distribution of food and water, 48 (89%) farmers responded that they used separate trough, whereas 6 (11%) farmers used common trough for several or all animals. Several sources were used to provide water for their livestock. Only 1(2%) farms were using a river/pond whereas 31(57%) supplied with municipal water and 20(37%) with tube well. The last 2 (4%) had other water supply source such as tanker. Artificial insemination 52 (96%) was common. Regarding herd composition, 113(62%) of cows were improved and 70 (38%) were local breeds. In order to replace the herd, the majority 31 (61%) farmers responded that they bought and sold animals from other households. Commercial farms supplied in 6(12%) farms while 12 (24%) farms were involved from self-renewal. Only 2(4%) were involved through middle men. The majority of farmers (78%) called their vet in case of disease when only 6% called him rarely and 17% called him frequently. Farms were really close to each other- 38% were next door and 38% were separated by less than 100m. A majority of farmers 34(64%) responded that they had vaccinated their cattle against Foot and Mouth Disease (FMD). None had declared

having their cows vaccinated against Brucellosis. However, some farmers did not know against which diseases their herds were vaccinated. Regarding cattle genital symptoms, 12 (22%) farmers responded that at least one retention of placenta encountered in their farms and 10 (19%) farms had at least one abortion. These genital issues could be evocative of Brucellosis, but other disease can also provoke them. Eighty per cent of farms dispose waste materials close to animals. Likewise, most of residences were separate but very close to the farm 43 (87%) & only 7(13%) were clearly separate (Table 2).

### 3. Farm management system

**Table 2: Descriptive data about farm management**

Characteristics	Frequency
<b>Rearing system</b>	
Intensive	30(61)*
Semi-intensive	21(33)
Extensive	3(6)
<b>Types of Shed</b>	
Modern	1(2)
Semi-modern	16(30)
Tradition	37(69)
<b>Milk sale</b>	
Direct sale	27(50)
Sell at local dairy/milk agent	14(26)
Self	11(20)
Others	2(4)
<b>Food and water distribution</b>	
Separate trough	48(89)
Common trough	6(11)
<b>Water supply</b>	
Pond/river	1(2)
Tube well	20(37)
Municipal water supply	31(57)
Other	2(4)
<b>Breeding system</b>	
Artificial Insemination (AI)	52(96)
Natural breeding	2(4)
<b>Breed</b>	
Local	70(38)
Improved	133(62)
<b>Buy and sell animals</b>	
Farm production	12(24)
Other household	31(61)
Commercial farm	6(12)
Middle men	2(4)
<b>Vet visit</b>	
Frequently	9 (17)
In case of disease	42 (78)
Rarely	3 (6)
<b>Vaccination</b>	
Yes	34(64)
No	19(36)
<b>Farm encounter retention</b>	
Yes	12(22)
No	42(78)
<b>Farm encounter abortion</b>	
Yes	10(19)
No	44(81)
<b>Dispose waste materials</b>	
Close to animals	43(98)
Far from animals	11(20)
<b>Farm distance from house(meter)</b>	
Next door	20(38)
<100	20(38)
Between 100-500	11(21)
>500	1(2)

\*parenthesis indicates percentage

#### 4. Bio security level of farm

More than half farmers 29 (55%) responded that they changed clothes and shoes during delivery whereas only 17(31 %) wore gloves. Forty six per cent of farmers responded that vet/technician wore gloves during the examination. Only 21(38%) farmers had separate clothes and shoes for farm work activities. Consumption of raw milk seemed to be decreasing practice. Fifty farmers (94%) responded that they consumed boiled milk and milk products (Table 3).

**Table 3: Descriptive study of bio-security level of farms**

Parameters	Response	
	Yes	No
Change clothes/shoes during calving	29(55)*	24(45)
Wear gloves during calving	17(31)	37(69)
Vet/technicians wear gloves for examination	25(46)	29(54)
Use separate shoes and clothes for entering the shed	21(39)	33(61)
Consumption of raw milk/milk products	3(6)	50(94)

\*parenthesis indicates percentage

#### 5. Assessment of brucellosis knowledge and clinical symptoms in human

Only one farmer had heard about brucellosis but was not able to tell any things more about the diseases and its transmission mode. Some of the farmers responded that their families experienced symptoms which could be related to brucellosis infection, such as muscle pain (16%) and joint pain (2%).

#### Discussion

In this study, no sero-positive brucellosis cases were detected in milking animals in Kavre and Kathmandu districts. However, previous studies show that brucellosis cases do exist in Nepal. A cross sectional study conducted in Kailali district showed that seroprevalence was found to be 12% (Pandey, 2013). Seroprevalence of brucellosis infection in buffaloes of Balkot VDC of Bhaktapur district revealed 15% (Mishra and Joshi, 2008). Similarly, a sero-prevalence of brucellosis conducted by Poudel(2006) in small ruminants of Nepal revealed 29.26% (12 out of 41) goats from Makawanpur, 5.12% (2 out of 39) from Kavrepalanchowk and 5.4% (2 out of 37%) from Kathmandu. The result found in this study is inconsistent with previous findings. The reasons might be due to several factors such as the level of hygiene on the farm, the herd size, age of the cattle, sex, system of production, the presence of wildlife, multiple livestock species within the herd, variability in diagnostic procedures and geographical reasons.

In our study, intensive rearing system had been practiced in Kathmandu and Kavre district. Majority of farmers had traditional type of sheds and separate trough had been found to be used to provide feed and water to their livestock. Restriction on animal movement and sanitary measures might have been reduced the risks of transmission. A study conducted in Malaysia found a significant association between the cattle production system and *Brucella* seropositivity. The cattle in an extensive system were found to be 13 times more likely to be exposed to *Brucella* infection compared to cattle in an intensive system (Anka et al., 2014). Livestock farming is increasing trends in urban and peri-urban areas. Farmers rear livestock for their livelihood. Seventy four percent farmers had sole occupation as agriculture. Majority of farmers had improved breed and Artificial Insemination (AI) was common in practice. In Nepal, National livestock breeding centre screens all bulls involving AI against some diseases including brucellosis. Replacements of livestock from other households were in common in practice. Purchasing and selling animals without screening brucellosis enhances the risk of transmission. Previous studies suggest that purchasing of cattle from commercial farms without screening for brucellosis increases the chances of contact with infected herds (Muma et al., 2007; Asmare et al., 2013).

Although 64% farmers responded that they had vaccinated their cattle against the diseases, vaccination status regarding to brucellosis was unknown. Foot and Mouth Disease vaccination was common in practice. Regarding to farm management, tradition type of husbandry practice was found to be adopted. Majority of farmers (64%) called the veterinarian in case of diseased animals. All respondents were engaged in at least one practice at their farm and household conducive to transmission of *Brucella* to other animals and most importantly to humans. Eighty seven percent farmers' houses were attached with their cowshed. Sharing accommodation with animals is an important risky practice for contracting brucellosis in humans (Corbel, 2006). Abortion and retention were also reported in some farms. These are the important signs associated with Brucellosis (Tolosa, 2004). In the 83% farms, disposed waste was very close to animals. *Brucella* can survive in a humid environment (manure and soil) for several months; this may also represent a risk for disease transmission in animals (Abubakkar et al., 2011).

Regarding to the assessment of biosecurity level in the farms, sanitary measures were suboptimal. Only 31% farmers responded that they used gloves during calving and 39% farmers had separate shoes and clothes for working in cowshed. Calving is one of the most critical times for the brucellosis transmission because a lot of bacteria are concentrated in genital mucus. Previous studies reported that brucellosis occurrence in humans was associated with contact with domestic animals, exposure to aborted animals and assisting animal parturition and or sharing of water sources with animals (Copper, 1992; Alballa, 1995; Kozukeev, 2003).

This study revealed that the knowledge and understanding about brucellosis among the farmers was minimal. Only one respondent had heard about brucellosis and some farmers had shared their clinical symptoms related to brucellosis infection such as muscle pain and weakness. The low awareness of brucellosis in this study could be attributed to the lack of health education (particular zoonotic diseases) and the low proportion of farmers receiving formal education in the study. Previous studies conducted in Kyrgyzstan and Iran demonstrated that good knowledge about the disease transmission routes for brucellosis among farmers had a precautionary effect for brucellosis (Kozukeev et al., 2006; Sofia et al., 2008).

#### Conclusion

In Nepal, Brucellosis in livestock is considered as a threat for public health as well as economic burden. This study revealed that knowledge regarding Brucellosis among farmers is minimal and that public awareness program is essential. Although no positive cases were detected in this study, previous studies confirmed the occurrence of the disease in several regions of the country. Bio-security level in farm has to be adopted in order to mitigate risky practices. A regular surveillance of herd is highly recommended, and vaccination protocol is essential for Brucellosis control.

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**NEWS:**

**EAT Asia-Pacific Food Forum on 30<sup>th</sup> – 31<sup>st</sup> October 2017:**

Ms. Minu Sharma, Program Coordinator of NZFHRC participated in the EAT Asia-Pacific Food Forum on the 30<sup>th</sup> – 31<sup>st</sup> October 2017 in Jakarta, the Republic of Indonesia.

**DDJ Research Award:**

*Please kindly submit your research work on zoonotic diseases for consideration of DDJ Research award for the year 2016 by the end of December 2017 to NZFHRC office Chagal, G.P.O. Box 1885, Kathmandu, Nepal, Phone: 4270667, 4274928 and Fax 4272694. This award was established by DDJ Research Foundation in 2071 B.S. (2014) in the memory of Founder of this Foundation, Late, Dr. D.D. Joshi. The award includes a grant of NCRs. 25,001/- (Rs. Twenty Five Thousand and One) with a certificate.*

**K.D.M.A. Research Award:**

*Please kindly submit your research work paper on allergy award for the year 2015 for the consideration by the end of December 2017 to KDMART office Chagal, G.P.O. Box 1885, Kathmandu, Nepal, Phone: 4270667, 4274928 and Fax 4272694. This award was established by Late Dr. Durga Datt Joshi in 2049 B.S. (1992) on the memory of his wife, the late Mrs. Kaushilya Devi Joshi. The award includes a grant of NCRs. 15,001/- (Rs. Fifteen Thousand and One) with certificate.*

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**TO:**

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