Research Article:

INFECTIOUS AND NON-INFECTIOUS FACTORS ASSOCIATED WITH INFERTILITY IN CROSS-BRED DAIRY COWS IN MID TERAI REGION OF NEPAL

Gokarna Gautam[a]*, Binod Bahadur Kathayat[a], Madhav Prasad Acharya[b] and Bhuminand Devkota[a]

[a]Faculty of Animal Science Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Chitwan, Nepal

[b]National Animal Health Research Centre, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal

*Corresponding author: ggautam@afu.edu.np

Received date: 23 April 2023, Accepted date: 29 November 2024

DOI: https://doi.org/10.3126/jafu.v6i1.78163

ABSTRACT

Infertility refers to the decrease of normal fertility, and it can be associated with various infectious and non-infectious factors. Brucellosis and neosporosis are important infectious causes of infertility in cattle. This study evaluated the association of brucellosis, neosporosis and some non-infectious factors with infertility in cross-bred dairy cows in mid terai region of Nepal. A questionnaire survey was conducted in 100 households from three districts of mid terai region to collect information related to infertility. The cows that did not conceive at least until five month postpartum were considered infertile. Serum samples obtained from 162 infertile cows (Chitwan-62, Nawalparasi-50, Rupendehi-50) were examined using ELISA kits to detect antibodies against *Neospora caninum* and *Brucella abortus*.These infertile cows were clinically examined for anemic condition (using eye's mucous membrane)and body condition score (BCS). Overall, 4 (2.5%) and 9 (5.6%) cows were serologically positive for brucellosis and neosporosis, respectively. There was no association of brucellosis with abortion. Abortion cases were significantly higher in neosporosis positive cases (33.3%) than in neosporosis negative cases (9.8%). There was no association of brucellosis, neosporosis, breed and parity with repeat breeding. However, repeat breeding was more prevalent in cows having good nutritional status indicating that there might be other unknown factors causing repeat breeding. Likewise, there was no association of brucellosis, neosporosis and breed with anestrus. However, primiparity, low BCS, anemia, no drenching history since last calving and season of calving (spring and summer) were associated with anestrus in cross-bred dairy cows in mid terai region of Nepal.

Key words: Abortion, anestrus, brucellosis, neosporosis, repeat breeding

INTRODUCTION

Cows are the major livestock species in Nepal having 7.2 million heads, out of which there were 13% cross-bred and rest were indigenous dairy cattle. Dairy cattle contribute 34.7% to total milk production in the country and cow milk production is in expanding trend (MoLD, 2017). There is increasing trend of commercialization of dairy farming for the last few decades. With this commercialization of dairy industry, the cross bred dairy cows have been introduced and their number has been expanding during last few decades. However, such cross bred exotic dairy cows have been suffering from various health problems including infertility. In Nepal, infertility problem in crossbred and exotic cattle has been reported to be the most prioritized problem in dairy pocket areas in the country (Jha, 2000).

Infertility denotes a degree of reduced fertility which results in failure to produce or delay in producing the normal live calf (Noakes et al., 2009). Repeat breeding and anestrus are the two major forms of infertility (Chakurkar et al., 2008; Khanal et al., 1996; Moellers & Riese, 1988). In Nepal, about two decades ago, the incidence of repeat breeding in dairy cattle was reported to be 27% in Kathmandu valley (Khanal, 1996) and 36% in Pokhara (Sankhi, 1999); incidence of anestrus was 45% in Kathmandu (Khanal, 1996) and 21% in Pokhara (Sankhi, 1999) and the incidence of abortion was 5% in Kathmandu (Khanal, 1996).

Major causes of infertility in dairy cows can be infectious (bacterial, viral, fungal and mycoplasmal infection) and non-infectious i.e. nutrition, stress and genetics etc. (Christine et al., 2010). Among infectious causes of infertility, brucellosis is one of the most important and widespread zoonotic diseases caused by *Brucella* spp. resulting significant economic losses due to decrease in calving percentage, delayed in calving, culling for infertility, decrease in milk production, abortions, stillbirth, birth of weak calves and loss of labors spent in infected animal cost of treatment, and can infect people too (Adamu, 2009; Ocholi et al., 2004; Poester et al., 2002). Neosporosis (caused by *N. caninum*) is also one of the major infectious causes of infertility in dairy cattle causing epidemic or endemic abortions (Wouda et al., 1999). Neosporosis has emerged as a serious disease of cattle and dogs worldwide (Dubey et al., 2007) and seropositive cows are more likely to abort than seronegative cows (Dubey, 2003). There is no effective treatment to completely eliminate *N. caninum* from infected cows. Thus *N. caninum* cows are not only infertile, but also pose risk of transmission to other cows.

In addition to infectious causes, there are various non-infectious causes of anestrus and repeat breeding in dairy cattle (Christine et al., 2010). Nutritional status as expressed in terms of body condition score (BCS), breed, anemic condition, history of deworming against internal parasites, season of calving and parity might be risk factors causing repeat breeding and anestrus in dairy cows.

Several studies have reported the seroprevalence of brucellosis and neosporosis in dairy cattle from different parts of Nepal. Also, various studies indicated infertility as one of the major problems in cattle of Nepal. However, it has not been reported that whether infertility was associated with brucellosis and neosporosis or non-infectious factors. Therefore, this study was conducted to determine the association of brucellosis and neosporosis and non-infectious factors (breed, parity, BCS, anemic condition, history of deworming against internal parasites and season of calving) with infertility in cross-bred dairy cows in mid terai region of Nepal.

MATERIALS AND METHODS

Study area

This study was conducted in different parts of Chitwan, Nawalparasi and Rupandehi districts which are located in the mid terai region of Nepal. These areas are considered to be the pocket areas of Nepal for milk production. There were very few large commercial farms and many small holder dairy farms in those regions. This study was conducted in cows both from commercial farms and small holder farms.

Questionnaire survey and data collection

A total of 100 households were purposively selected from dairy pocket areas of three districts where outreach research activities from National Cattle Research Program (NCRP, NARC) were carried out. A questionnaire survey was done to collect information on breed, parity, date of last calving, history of drenching since last calving, history of abortion, history of repeat breeding, history of anestrus, presence of dog at home etc. These data were recorded by interviewing with the owner of each household in the study.

Defining infertility, repeat breeding and anestrus

The cows that did not conceive at least until five months postpartum were considered as infertile cows (Gautam et al., 2010). In case of heifers, those animals that did not conceive at least until 2.5 years of age were considered infertile. Cows which failed to conceive even after three or more number of services were considered as repeat breeder. Likewise, the cows which did not show estrus signs since last calving were defined as anestrus cows.

Clinical examination of cows

The cows in the study were examined clinically to assess the body condition score (BCS) and color of eye's mucous membrane. The BCS was measured on 1-5 scale with 0.25 increments (Ferguson et al., 1994). The color of mucous membrane of eye was observed and recorded as normal or anemic.

Serological diagnosis of brucellosis and neosporosis

The plan of the study was to include at least 50 infertile cows from each district assuming the incidence of infertility being approximately 10%. Thus, blood samples were collected from 162 infertile cows from 100 households of three districts (Chitwan-62, Nawalparasi-50 and Rupendehi-50) via jugular vein puncture. The serum was harvested after centrifugation of blood sample at 1000×g for 10 min and stored at −20°C until ELISA. The serum samples were examined using ELISA kits to detect antibody against *Neospora caninum* (IDScreen®*Neospora caninum* indirect multi-species; ID-Vet, France) and *Brucella abortus* (IDScreen®Brucellosis serum indirect multi-species; ID-Vet, France).

Statistical analyses

Seroprevalence of brucellosis and neosporosis was calculated as percentage of the positive cases considering all the subjects under study. The collected data were analyzed by using MS- Excel 2007 and IBM SPSS Statistics Version 20. Association of brucellosis and that of neosporosis with history of abortion was compared using Chi-square test. Similarly, the association of various factors with repeated breeding and that with anestrus and the association of dog present at household with neosporosis in cows was also analyzed using Chi-square test. If the expected frequency was <5 in any of cells, the Fisher Exact Probability test was used. Probability value ≤0.05 was considered as significant whereas 0.05<P≤0.1 was considered to have tendency effect.

RESULTS

Prevalence of brucellosis and neosporosis in infertile cows

Out of 162 infertile cows tested, 4 (2.5%) were serologically positive for brucellosis and 9 (5.6%) were serologically positive for Neosporosis (Table 1).

Table 1. Prevalence of brucellosis and neosporosis in infertile cows in mid terai region of Nepal

Disease	Number of cows tested				Number of positive cases				Overall prevalence (%)
	Location*				Location*				
	C	N	R	Total	C	N	R	Total	
Brucellosis	62	50	50	162	3	1	0	4	2.5
Neosporosis	62	50	50	162	4	2	3	9	5.6

*C-Chitwan, N-Nawalparasi, R-Rupandehi

Incidence of abortion

To determine the incidence of abortion and its association with brucellosis and neosporosis, only parous cows (n=144) were included in the analysis. Out of 144 cows, 18 (12.5%) cows had history of abortion.

Association of brucellosis and neosporosis with the history of abortion

There was no association (P=0.58) of brucellosis with the history of abortion. However, there was an association between neosporosis and history of abortion (Table 2). There was a tendency (P=0.08) that the abortion cases were higher in neosporosis positive cases (33.3%) than in neosporosis negative cases (11.1%).

Table 2. Association of brucellosis and neosporosis with the history abortion in infertile cows

Diseases	Level	Number of cows	Aborted cases		P- value
			Number	Percentage (%)	
Brucellosis	Positive	4	0	0	0.58
	Negative	140	18	12.9	
Neosporosis	Positive	9	3	33.3	0.08
	Negative	135	15	11.1	

Association of brucellosis, neosporosis and non-infectious factors with repeat breeding

To analyze repeat breeding, only the parous cows were included (n=144). Out of 144 parous cows, 88 (61.1) cows had the history of repeat breeding. Table 3 shows the association of various factors with repeat breeding in cross-bred dairy cows. There was no association of brucellosis, neosporosis, breed and parity on the incidence of repeat breeding. However, there was significant (P<0.05) association of BCS, anemic condition and deworming history with the incidence of repeat breeding. Likewise, there was a tendency (P = 0.07) that the season of calving influenced the incidence of repeat breeding.

Table 3. Association of brucellosis, neosporosis and non-infectious factors with repeat breeding in infertile cows

Variables		Number of Cows(n=144)	Incidence of repeat breeding %	P-value
Brucellosis	Positive	4	2 (50%)	0.5
	Negative	140	86 (61.4%)	
Neosporosis	Positive	9	6 (66.7%)	0.51
	Negative	135	82 (60.7%)	
Breed	Jersey cross	91	56 (61.5%)	0.51
	Holstein cross	53	32 (60.4%)	
Parity	Primiparous	23	11 (47.8%)	0.2
	Pluriparous	121	77 (63.6%)	
BCS	Low (<2.75)	65	32 (49.2%)	0.01
	Good (≥2.75)	79	56 (70.9%)	
Anemic condition	Yes	73	38 (52.1%)	0.03
	No	71	50 (70.4%)	
Drenching since last	Yes	109	72 (66.1%)	0.02
Calving	No	35	16 (45.7%)	
Season of calving	Autumn	35	21 (60.0%)	0.07
	Winter	40	19 (47.5%)	
	Spring	23	11 (47.8%)	
	Summer	46	37 (80.4%)	

Association of brucellosis, neosporosis and non-infectious factors with anestrus

Out of 162 cows, 39 (24.1%) cows had history of anestrus. Table 4 shows the association of various factors with anestrus in cross-bred dairy cows. There was no significant association of brucellosis, neosporosis and breed with the incidence of anestrus in cross-bred dairy cows. However, there was significant ($P<0.05$) association of parity, BCS and season of calving with anestrus. Likewise, there were tendencies that there was an association of anemic condition and drenching since last calving with anestrus.

Table 4. Association of brucellosis, neosporosis and non-infectious factors with anestrus in infertile cows in mid terai region of Nepal

Variables		Number of cows	Percentage of anestrus	P-value
Brucellosis	Positive	4	0 (%)	0.25
	Negative	158	39 (24.7%)	
Neosporosis	Positive	9	2 (22.2%)	0.89
	Negative	153	37 (24.2%)	
Breed	Jersey cross	103	29 (28.2%)	0.11
	Holstein cross	59	10 (16.9%)	
	Nulliparous	18	7 (38.9%)	
Parity	Primiparous	23	10 (43.5%)	0.01
	Pluriparous	121	22 (18.2%)	
BCS	Low (<2.75)	70	24 (34.3%)	0.01
	Good (≥2.75)	92	15 (16.03%)	
Anemic condition	Yes	80	24 (30%)	0.08
	No	82	15 (18.3%)	
Drenching since last calving	Yes	124	26 (21%)	0.09
	No	38	13 (34.2%)	
Season of calving (n=144)*	Autumn	35	8 (22.9%)	0.03
	Winter	40	11 (27.5%)	
	Spring	23	9 (39.1%)	
	Summer	46	4 (8.7%)	

* In 'season of calving', only parous cows were included in analysis.

Association of dog at household with neosporosis in cow

Association of dog at household with neosporosis in cross-bred dairy cows has been shown on Table 5.There was no association of whether the dog presence at household or not with incidence of neosporosis in cows.

Table 5. Association of dog at home with neosporosis in infertile cows

Dog at home	Number of cows	Neosporosis positive	P-value
Yes	25	2 (8%)	0.63
No	137	7 (5.1%)	

DISCUSSION

This study was conducted in order to characterize infertility cases, determine the seroprevalence of brucellosis and neosporosis and their association with infertility as well as to determine the association of other non-infectious factors with main two forms of infertility (repeated breeding and anestrus) in cross-bred dairy cows in mid terai region of Nepal. Overall, the incidence of

abortion, repeat breeding and anestrus was 12.5%, 61.1% and 24.1%, respectively. Incidences of abortion and repeat breeding in this study were higher than that reported by previous studies (Khanal, 1996; Sakhi, 1999). Likewise, incidence of anestrus in this study was lower than that reported by Khanal (1996) but higher than that reported by Sankhi (1999).

Seroprevalence of brucellosis in the present study (2.5%) was similar to that (1.5% i.e. 2/131) reported in Chitwan and eastern terai of Nepal (Khanal et al.,1998) but lower than that (10.86% i.e. 10/92) reported in Chitwan (Subedi et al., 2016). Out of 162 cows 18 cows had the history of abortion and of these, no animal (0%) was positive for brucellosis. On the other hand, four brucellosis positive cows had no history of abortion. This means brucellosis in this study was not associated with abortion. Similar finding was reported by Mahajan et al. (2012) in bovines of organized and unorganized dairy farms of Jammu, India. It can be assumed that the abortion was either due to other causes than brucellosis or the antibodies were not at detectable level because of long period after abortion.

Seroprevalence of neosporosis in infertile cows was 5.6% which was almost similar to that (4.84%) reported by Yadav et al., (2016) in Chitwan but lower than that reported from other countries such as 8.2 % in Punjab state in northern India (Meenakshi et al.,.2007) and13.3 % in China (Wang et al., 2010). Present study indicated that the history of abortion was higher in neosporosis positive cases than in neosporosis negative cases. This was in agreement with previous studies in which the abortion risk was higher in neosporosis seropositive animals than in sero-negative animals (Jenkins et al., 2002; López-Gatius et al., 2005; Schares et al., 2004).

There was no significant association of brucellosisand neosporosis with the incidence of repeat breeding which was in agreement with previous studies(Mahajan et al., 2012; Yadav et al., 2016). However, the cows having good BCS, history of drenching and non-anemic condition showed higher incidence of repeat breeding which reflect that the such cows came into estrus normally or become cyclic but did not conceive even with three or more breeding and became repeat breeders; that mean there were various unknown factors (other than we included in the study) that caused repeated breeding which was beyond the scope of this study. Likewise, repeat breeding was more prevalent in cows that calved during summer/autumn than in those that calved during spring/winter. Cows those calved during summer and autumn are eligible to be bred in late summer, autumn and winter. It has been reported that the cows inseminated during summer and fall months were less likely to become pregnant than those inseminated during winter and spring (Santos et al., 2009). Likewise, Gustafsson and Emanuelson (2002) found that the risk of becoming repeat breeding was higher in cows inseminated during winter compared to those from other seasons, showing that external factors such as climate can influence the repeat breeding in a herd.

There was no association of brucellosis and neosporosis with the incidence of anestrus. It has been reported that Holstein had higher incidence of anestrus than Jersey cows (Khan et al., 2016). However, in the present study none of the cows were of purebred; they were classified into Jersey cross and Holstein cross based only on phenotypic characters and thus, exact blood level of both breeds was not known. This might be the reason not to have association of breed with anestrus in this study. However, parity had significant association with incidence of anestrus in cross-bred dairy cows. Primiparous cows had higher incidence of anestrus than pluriparous cows which was similar to the findings of previous study (Kamal et al., 2014) where primiparous cows were more likely to be true anestrous than multiparous in the first 60–70 days postpartum. Higher incidence of anestrus in primiparous cows might be linked to greater level of negative energy balance (NEB). It has been stated that cows in the first parity suffer

from NEB in more extent than the pluriparous cows (Yehia et al., 2020) because in primiparous cows, in addition to lactation they require more energy for their growth as well. Similarly, in this study 38.9% nulliparous cows (heifers) those were more than 2.5 years old were anestrus. This reflects that heifers were also in poor nutritional status or were not managed properly.

In the present study, there was significant association of BCS with the incidence of anestrus in cross-bred dairy cows. Cows with poor BCS were more likely to remain in anestrus than the cows with good BCS. BCS was important factor regulating the resumption of ovarian cyclicity after calving (Santos et al., 2009).Underfeeding and poor BCS led to high incidence of true anestrus (Opsomer et al., 2000). Similarly, anemic condition is one of health problems in the body, which can be assessed to some extent in the field by observing eye mucous membranes of the animals. In this study, the cows which were in anemic condition had higher incidence of anestrus than the non-anemic cows. It was reported that the hemoglobin level decreased in anestrus than in cycling buffaloes and cows (Dhoble et al., 1981; Nadiu & Rao, 1982). Likewise, incidence of anestrus was higher in cows with no history of deworming than in cows with history of deworming. Similar finding was reported by Nishi et al. (2018) in Bangladesh where they observed that the prevalence of anestrus was higher in cows with no deworming (56.96%) than in cows with history of regular deworming (29.64%). It can be assumed that poor BCS, anemic condition and no history of regular deworming might have impaired the nutritional status of the cows leading to anestrus in such cows.

In this study, the season of calving had significant association with the incidence of anestrus in cross-bred dairy cows. Cows calving in the spring season had the highest incidence of anestrus followed by winter, autumn and summer. It has been reported that cows calving in spring would suffer from heat stress during subsequent summer months leading to delayed resumption of ovarian cyclicity and thus, anestrus (Santos et al., 2009). It was also reported that the cows calving in the winter seasons were prone to delayed ovarian function (Bulman et al., 1978) that might cause anestrus. In Nepal, spring and winter seasons are dry periods leading to severe scarcity of green grasses. Hence, feeding problem might be cause of anestrus in cows those calved in spring and winter seasons.

Dogs are considered as the definitive hosts for *N. caninum* and they shed oocytes in their feces in pasture. The cattle pick up the infection by ingestion of contaminated fodder (Dubey et al., 2007). In the present study, the presence or absence of dog at household had no association with neosporosis in cows. Conversely, Yadav et al., (2016) reported that the cows in the household having the presence of dog were at 4.16 times more risk of neosporosis than the cows in households without dogs. Since, there were several stray dogs in study areas there might be equal chances of contamination of pastures/grasses by feces of such dogs, irrespective of either presence or absence of dog at household. This might be the reason why the incidence of neosporosis was not different between cows in household having dogs and those in household not having dogs.

CONCLUSION

There was no association of brucellosis with any forms of infertility in cross bred dairy cows. However, neosporosis was associated with the history of abortion but not with repeat breeding and anestrus. Similarly, among non-infectious factors, BCS, anemic condition and deworming history were associated with infertility. The highest incidence of anestrus in spring calving and the highest incidence of repeat breeding in summer calving suggest that the calving in spring and summer months was one of the risk factors causing infertility in cross-bred dairy cows in mid terai region of Nepal.

ACKNOWLEDGEMENTS

The authors are thankful to the National Cattle Research Program (NCRP, NARC) for its financial and laboratory support. We are also grateful to the participating farmers for allowing us to use their cattle and providing valuable information for this research work.

REFERENCES

Adamu, N. B. (2009). Epidemiology of brucella infection in ruminants and humans and its public health implications in Borno state, Nigeria. *PhD Thesis*, Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, 231.

Bulman, D. C., & Lamming, G. E. (1978). Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. *Reproduction, 54*(2), 447-458.

Chakurkar, E. B., Barbuddhe, S. B., & Sundaram, R. N. S. (2008). Infertility in farm animals: Causes and remedies. *Technical Bulletin, (15)*, 1-14.

Dhoble, R. L., & Gupta, S. K. (1981). Total plasma protein and hemoglobin status during estrous cycle and anestrus in post-partum buffaloes. *Indian Veterinary Journal, 58*, 544-547.

Dubey, J. P. (2003). Review of Neospora caninum and neosporosis in animals. *The Korean Journal of Parasitology, 41*(1), 1.

Dubey, J. P., Schares, G., & Ortega-Mora, L. M. (2007). Epidemiology and control of neosporosis and Neospora caninum. *Clinical Microbiology Reviews, 20*(2), 323-367.

Ferguson, J. D., Galligan, D. T., & Thomsen, N. (1994). Principal descriptors of body condition score in Holstein cows. *Journal of Dairy Science, 77*, 2695-2703.

Gautam, G., Nakao, T., Yamada, K., & Yoshida, C. (2010). Defining delayed resumption of ovarian activity postpartum and its impact on subsequent reproductive performance in Holstein cows. *Theriogenology, 73*(2), 180-189.

Gustafsson, H., & Emanuelson, U. (2002). Characterisation of the repeat breeding syndrome in Swedish dairy cattle. *Acta Veterinaria Scandinavica, 43*(2), 115.

Jenkins, M., Baszler, T., Björkman, C., Schares, G., & Williams, D. (2002). Diagnosis and seroepidemiology of Neospora caninum-associated bovine abortion. *International Journal for Parasitology, 32*(5), 631-636.

Jha, V. C. (2000). Study on infectious causes of infertility and its management in crossbred and exotic cattle in Nepal. *Nepalese Veterinary Journal, 28*, 137-141.

Kamal, M. M., Bhuiyan, M. M. U., Parveen, N., Momont, H. W., & Shamsuddin, M. (2014). Risk factors for postpartum anestrus in crossbred cows in Bangladesh. *Turkish Journal of Veterinary and Animal Sciences, 38*(2), 151-156.

Khan, M. H., Manoj, K., & Pramod, S. (2016). Reproductive disorders in dairy cattle under semi-intensive system of rearing in North-Eastern India. *Veterinary World, 9*(5), 512.

Khanal, D. R. (1996). Study on clinical cervicitis and metritis in cross bred cattle of Kathmandu valley. *Proceedings of First National Workshop on Livestock and Fisheries Research in Nepal, May 7-9, Khumaltar, Lalitpur*, pp. 212-214.

Khanal, D. R., & Jha, V. C. (1998). Study on Bovine abortion and repeat breeding due to Brucellosis in Chitwan and Eastern Terai. *Annual Report 1997/98 NARC, AHRD*, Tripureshwor, pp. 12-17.

López-Gatius, F., García-Ispierto, I., Santolaria, P., Yániz, J. L., López-Béjar, M., Nogareda, C., & Almería, S. (2005). Relationship between Rainfall and Neospora caninum-associated Abortion in Two Dairy Herds in a Dry Environment. *Journal of Veterinary Medicine, Series B, 52*(3), 147-152.

Mahajan, S., Agrawal, N. P. R., Kalha, R. K., & Wazir, V. S. (2012). Bovine of organized and unorganized dairy farms of Jammu. *Indian Journal of Veterinary Medicine, 32*(1), 21-

23.

Meenakshi, K. S., Sandhu, S., Ball, M. S., Kumar, H., Sharma, S., Sidhu, P. K., ... & Dubey, J. P. (2007). Seroprevalence of Neospora caninum antibodies in cattle and water buffaloes in India. *Journal of Parasitology, 93*, 1374-1377.

Moellers, J., & Riese, R. (1988). Nutritional causes of infertility in dairy cows. *Iowa State University Veterinarian, 50*(2), 5.

MoLD. (2017). *Livestock Statistics of Nepal*. Government of Nepal, Ministry of Livestock Development (MoLD), Kathmandu, Nepal.

Nishi, S. A., Sarder, M. J., Islam, M. H., JosimUddin, M., & Sharmin, S. (2018). Managemental factors influencing anestrus problems in dairy cows. *Journal of Vaccines & Vaccination, 9*(393), 2.

Noakes, D. E., Parkinson, T. J., & England, G. C. W. (2009). *Veterinary Reproduction and Obstetrics* (9th ed.). Saunders Elsevier.

Ocholi, R. A., Kwaga, J. K. P., Ajogi, I., & Bale, J. O. O. (2004). Phenotypic characterization of Brucella strains isolated from livestock in Nigeria. *Veterinary Microbiology, 103*(1-2), 47-53.

Opsomer, G., Gröhn, Y. T., Hertl, J., Coryn, M., Deluyker, H., & de Kruif, A. (2000). Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: A field study. *Theriogenology, 53*(4), 841-857.

Poester, F. P., Gonçalves, V. S. P., & Lage, A. P. (2002). Brucellosis in Brazil. *Veterinary Microbiology, 90*(1-4), 55-62.

Sankhi, K. (1999). Infertility problem of dairy animals in western Nepal. *Workshop on Infertility in Dairy animals*, January 21, 1999.

Santos, J. E. P., Rutigliano, H. M., & Sá Filho, M. F. (2009). Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. *Animal Reproduction Science, 110*(3-4), 207-221.

Schares, G., Bärwald, A., Staubach, C., Ziller, M., Klöss, D., Schröder, R., ... & Conraths, F. J. (2004). Potential risk factors for bovine Neospora caninum infection in Germany are not under the control of the farmers. *Parasitology, 129*(3), 301-309.

Subedi, S., Prajapati, M., & Dhakal, B. (2016). Sero-Surveillance of Brucellosis in Cattle of Chitwan District, Nepal. *International Journal of Applied Sciences and Biotechnology, 4*(3), 365-371.

Thakur, S., Singh, M., & Vasishta, N. K. (2006). Study on etiology of repeat breeding in Himchal Pradesh. *Punjab Veterinary Journal, 4*, 27-29.

Wang, C., Wang, Y., Zou, X., Zhai, Y., Gao, J., How, M., & Zhu, X. (2010). Seroprevalence of Neospora caninum infection in dairy cattle in northeastern China. *Journal of Parasitology, 96*, 451–452.

Wiltbank, M. C., Gümen, A., & Sartori, R. (2002). Physiological classification of anovulatory conditions in cattle. *Theriogenology, 57*(1), 21-52.

Wouda, W., Bartels, C. J. M., & Moen, A. R. (1999). Characteristics of Neospora caninum-associated abortion storms in dairy herds in The Netherlands (1995 to 1997). *Theriogenology, 52*(2), 233-245.

Yadav, G. P., Manadhar, S., Karna, A. K., Acharya, Y. P. K., Maharjan, D., & Singh, D. K. (2016). Seroprevalence and risk factors associated with Neospora caninum in dairy cattle of western dairy pocket area in Chitwan District of Nepal. *Bangladesh Journal of Veterinary Medicine, 14*(2), 215-220.

Yehia, S. G., Ramadan, E. S., Megahed, E. A., & Salem, N. Y. (2020). Effect of parity on metabolic and oxidative stress profiles in Holstein dairy cows. *Veterinary World, 13*(12), 2780-2786.