Serosurveillance and factors associated with the presence of antibodies against bluetongue virus in dairy cattle in two eco-zones of Nepal

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Summary

Cattle play an important role in the epidemiology of bluetongue (BT) by acting as reservoir hosts. However, the status of BT virus (BTV) in dairy cattle in Nepal is unknown. The objective of this study was to estimate the prevalence of BTV antibodies in dairy cattle in two eco-zones of Nepal, and to identify the factors associated with virus exposure. The authors conducted a cross-sectional serosurvey from March 2012 through February 2013 by sampling 131 dairy cattle from

seven clusters (villages) in the Chitwan district in the Terai region (southern lowlands) and the Lamjung district in the Hills region (the middle part of Nepal). Of the 131 serum samples tested, 29.3% (95% confidence interval [CI]: 21.5–37.2) were positive for BTV antibodies. Herd-level seroprevalence was 45.7% (95% CI: 30.9–61.0). Bivariate analysis indicated a positive association between seroconversion to BTV and age, and an association with breed of cattle after controlling for clustering of animals within herds. Based on this model, cattle were more likely to become seropositive as they aged. Crossbred cattle were more likely to be seropositive than those of exotic breeds (odds ratio [OR] = 4.6; 95% CI: 1.5–14.1). The results indicate widespread exposure of dairy cattle to BTV in Nepal. The authors suggest that dairy cattle should be included in the surveillance plan for BTV infection in Nepal and that it is important to educate farmers about the possible impacts of this disease.

Keywords

Antibody – Associated factor – Bluetongue virus – Competitive enzyme-linked immunosorbent assay (c-ELISA) – Dairy cattle – Nepal – Seroprevalence.

Introduction

Bluetongue (BT), an infectious but non-contagious viral disease of sheep, goats, and wild ruminants (1), is transmitted by *Culicoides* spp. midges (2). Bluetongue virus (BTV) belongs to the genus *Orbivirus* in the family *Reoviridae* and is considered endemic in Africa, the Middle East, Asia, Australia, and parts of the northern hemisphere (3). BTV causes clinical disease predominantly in sheep, whereas cattle act as reservoir hosts and play an important role in the transmission to *Culicoides* and in the epidemiology of BT because they show prolonged viraemia (4). The subclinical infection in cattle may be associated with reduced milk yield, infertility, teratogenesis and abortion (5, 6). In Nepal, BTV infection was first reported in sheep in 2008 (7) and subsequent studies revealed that 5–28.4% of the sheep from 11 districts were seropositive to BTV (8). Additionally, BTV was identified in Nepal's two neighbouring countries, India and

China, many decades ago. India first reported BT in 1964 in sheep (9), and infection with BTV has subsequently been detected in sheep, goats, cattle and buffaloes (10). Similarly, China first recognised the disease in 1979 (11) and antibodies to BTV have been detected in sheep, goats, cattle and buffaloes (11, 12).

Considering the high seroprevalence in surrounding countries and transmission of BTV by insects, combined with transhumance, loose borders and large numbers of cattle, it is not surprising that cattle in Nepal have BT infection. However, as far as the authors are aware, the status of BT in cattle in Nepal has not been defined. The lack of baseline data on the seroprevalence in domestic ruminants other than sheep has resulted in a poor overall understanding of the epidemiology of this disease in Nepal. The objective of this study was to estimate the seroprevalence of BTV antibodies in dairy cattle in two eco-climatic zones of Nepal. In addition, factors associated with seropositivity were evaluated, including age, herd size, breed, and the abortion history of cattle with BTV seropositivity.

Methods

Study area

Nepal is surrounded by India to the east, west and south, and China to the north. Nepal is divided into three eco-zones: Terai (the southern lowlands, mostly bordering India and with an altitude up to 700 m), Hills (the middle area, with an altitude ranging from 700 m to 3,000 m) and Mountains (mostly bordering China and with an altitude greater than 3,000 m). Administratively, Nepal is further divided into five development regions, 75 districts, 99 municipalities and nearly 3,900 village development committees (VDCs). The study was conducted in two of the different eco-zones: the Terai zone and the Hills zone, from March 2012 to February 2013 (Fig. 1). The Chitwan district (27° 35′ 0″ N, 84° 30′ 0″ E) is part of the Terai lowlands in Central Nepal, and the Lamjung district (28° 14′ 0″ N, 82° 25′ 0″ E) is part of the Mid-Hills of Nepal. Three VDCs in Chitwan (Gitanagar, Padampur and Patihani) and four VDCs in Lamjung (Ghermu, Bahundanda, Bhulbhule and Besisahar) were included in the study.

Livestock farming is an important source of income generation in all of the VDCs investigated.

Insert Figure 1

Study design, sample collection and testing

A cross-sectional serosurvey was conducted from March 2012 to February 2013 to determine the prevalence of BTV antibodies in dairy cattle and possible factors associated with infection. In total, 131 dairy cattle were sampled, 92 from Chitwan and 39 from Lamjung. These samples were taken from 46 herds (30 herds in Chitwan and 16 herds in Lamjung). Given that there is no official definition for commercial dairy herds in Nepal, herds with more than five dairy cattle were considered as commercial farms, after discussion with staff from the District Livestock Services Office (DLSO). According to estimations made by the DLSOs in Chitwan and Lamjung, the combined population of commercial cattle and buffaloes is about 5,000 and 1,500, respectively, in these districts (personal communication with DLSO officers). The larger sample size obtained from Chitwan was due to the availability of more dairy farms than in Lamjung. For the selection of herds, commercial dairy farmers from dairying areas in Chitwan and Lamjung were contacted initially by field veterinarians and technicians working in the field. Herds whose owners agreed to participate in the study were included. When selecting individual animals within selected herds, the authors tried to ensure that there were proportionate numbers of cattle of different ages. More individuals per herd were selected from larger herds than from smaller herds. A 5 ml sample of blood was collected from the jugular vein of each selected individual using sterile evacuated tubes. Livestock owners were informed about the study objectives and procedures and gave permission to collect blood from their animals. The research advisory committee of the Institute of Agriculture and Animal Science in Chitwan approved the study protocol.

The samples were transported to the National Avian Laboratory, Bharatpur, Chitwan, Nepal in a cool box containing ice packs at 4°C. Serum was separated by centrifugation and stored at –20°C until

testing was performed. The samples were tested for the presence of BTV-specific immunoglobulin (Ig)G antibodies using a competitive enzyme-linked immunosorbent assay (c-ELISA) (Veterinary Medical Research and Development Inc., Pullman, Washington, USA), using the cut-off value recommended by the manufacturer to classify animals as positive or negative. Specifically, samples were considered positive if they produced an optical density greater than or equal to 50% of the mean of the negative controls. According to the manufacturer, the sensitivity and specificity of this test are 100% and 99%, respectively. The c-ELISA-positive samples were also tested with agar gel immunodiffusion (AGID) tests obtained from the same manufacturer as the c-ELISA.

During the sample collection process, farmers were interviewed to gather information on factors such as the herd size and the age, breed and abortion history of the individual cattle.

Statistical analysis

The independent variables used in this study were age, breed, herd size, and history of abortion in the cattle. Among the independent variables, age (in months) and herd size were continuous variables, whereas breed and history of abortion were used as binary variables. The dependent variable was the result of the c-ELISA test (positive/negative) for BTV seropositivity. Logistic regression was performed to assess the relationship between individual independent variables and the dependent variable using the generalised estimating equation (GEE) approach ('proc genmod') to account for the clustering effect at the farm level. An odds ratio (OR) was calculated to assess the association between the dependent variable and each binary independent variable. The apparent seroprevalence was calculated by dividing the number of positive samples by the total number of samples tested. The true prevalence was then estimated using the formula: true prevalence = (apparent prevalence + specificity – 1)/(sensitivity + specificity – 1) (13). Statistical analyses were conducted using SAS 9.3 software (SAS Institute Inc., North Carolina, USA). *P*-values less than 0.05 were considered significant.

Results

The mean and median age of the sampled cattle was 30.9 months and 32 months (range: 3–60 months), respectively. The mean and median herd size of the sampled farms was 15.3 cattle and 15 cattle (range: 2–30), respectively.

Among the 131 tested dairy cattle, 38 were seropositive by c-ELISA testing. The apparent seroprevalence, at the individual animal level, was 29% (95% confidence interval [CI]: 21.4–37.6). The true seroprevalence, after adjusting for the sensitivity and specificity of the test, was 29.3% (95% CI: 21.5–37.2). Out of the 38 c-ELISA-positive samples, 29 were found to be positive with the AGID test. Among the 46 herds sampled, 21 herds had at least one animal positive for BTV antibodies, which means that the herd seroprevalence was 45.7% (95% CI: 30.9–61.0). In Chitwan, 32 out of 92 cattle tested were positive (34.8%), while six out of 39 cattle tested (15.4%) were positive in Lamjung district.

Among the continuous independent variables, the age of the cattle was significantly associated with seropositivity ($p = 0.009$). The fact that age was positively associated with BTV seropositivity indicates that older cattle are more likely to be seropositive to BTV. Herd size was not found to be associated with BTV seropositivity in this study. Among the binary independent variables, breed was found to be significantly associated with BTV seropositivity ($p = 0.03$). The seroprevalence in crossbred dairy cattle was 44.3% (27 positive out of 61 cattle tested), and that in exotic dairy cattle was 15.7% (11 positive out of 70 cattle tested). The odds ratio of BTV seropositivity in crossbred cattle was found to be 4.6 (95% CI: 1.5–14.1) when compared with exotic cattle. Abortion history (analysed only among adult cattle) was not found to be significantly associated with BTV seropositivity in this study. Out of the 131 cattle included in this study, 59 were more than three years of age. Among the 59 adult cattle, nine (15.3%) had a history of abortion. Among the nine cattle with a history of abortion, five (55.6%) (four from Chitwan, one from

Lamjung) were positive for BTV antibodies, while the other four (44.4%) were negative for BTV antibodies.

Discussion

In this study, the overall seroprevalence of BTV antibodies in dairy cattle in two eco-zones of Nepal was found to be approximately 29%. This level of seroprevalence is much lower than that recorded in several states in India, where 70–89.8% of the cattle were found to be seropositive to BTV (10, 14). However, previous studies in Nepal have shown similar levels of seropositivity to BTV in sheep (7) and goats (15). In a study conducted in 11 districts of Nepal in 2009, 5–28.4% of sheep were seropositive to BTV (8), while 25% of sheep and 31.3% of goats were seropositive to BTV in a study conducted in the Lamjung and Chitwan districts of Nepal in 2014 (15). That approximately 29% of cattle were found to be seropositive in this study indicates that BTV seroprevalence in cattle, sheep and goats is comparable. It is known that *Culicoides* vectors are more likely to feed on cattle than on small ruminants, and that BTV is usually amplified initially in cattle and then transmitted to sheep and goats via *Culicoides* spp. (16).

Cattle were more likely to be seropositive as their age increased. This is not surprising, because older cattle have had more time for exposure to BTV than younger animals. Studies in other countries have also shown higher seroprevalence in older cattle (17, 18, 19, 20, 21).

In this study, crossbred cattle were significantly more likely to be seropositive than purebred exotic cattle, an effect that might be due to less exposure of purebred cattle to BTV via *Culicoides* spp. One reason may be that farmers raising exotic cattle are more likely to keep their herds' surroundings cleaner, which may lead to lower culicoidal activity in the environment of the herd.

The study reported here was conducted over a limited geographical area of Nepal and additional work is required to obtain country-wide appreciation of BT disease. Another important question not addressed

in this study was which serotypes of BTV are circulating in Nepal, again a topic for future research efforts.

In summary, the overall seroprevalence of BTV antibodies in dairy cattle in the study area was 29%. Age and breed of the dairy cattle were identified as factors significantly associated with seropositivity to BTV. This study revealed that dairy cattle in Nepal are frequently infected with BTV and are likely to play an important role in the epidemiology of the disease. The authors suggest that cattle be included in surveillance plans involved in the policies for control of BTV in Nepal.

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Fig. 1

Map of the study area showing the villages from which samples were collected

The grey area in the inset shows the locations of Lamjung and Chitwan districts in Nepal