Veterinary Microbiology 241 (2020) 108544

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Sero-prevalence, cross-species infection and serological determinants of prevalence of Bovine Coronavirus in Cattle, Sheep and Goats in Ghana

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ARTICLE INFO

Keywords:
Bovine Coronavirus
Africa
Ghana
Global health

ABSTRACT

Cattle, goats and sheep are dominant livestock species in sub-Saharan Africa, with sometimes limited information on the prevalence of major infectious diseases. Restrictions due to notifiable epizootics complicate the exchange of samples in surveillance studies and suggest that laboratory capacities should be established domestically. Bovine Coronavirus (BCoV) causes mainly enteric disease in cattle. Spillover to small ruminants is possible. Here we established BCoV serology based on a recombinant immunofluorescence assay for cattle, goats and sheep, and studied the seroprevalence of BCoV in these species in four different locations in the Greater Accra, Volta, Upper East, and Northern provinces of Ghana. The whole sampling and testing was organized and conducted by a veterinary school in Kumasi, Ashanti Region of Ghana. Among sampled sheep (n = 102), goats (n = 66), and cattle (n = 1495), the seroprevalence rates were 25.8 %, 43.1 % and 55.8 %. For cattle, seroprevalence was significantly higher on larger farms (82.2 % vs 17.8 %, comparing farms with > 50 or < 50 animals; p = 0.027). Highest prevalence was seen in the Northern province with dry climate, but no significant trend following the north-south gradient of sampling sites was detected. Our study identifies a considerable seroprevalence for BCoV in Ghana and provides further support for the spillover of BCoV to small ruminants in settings with mixed husbandry and limited separation between species.

1. Introduction

Cattle, goats and sheep are among the major livestock species in Ghana. The present numbers in 2017 based on the Food and Agriculture Organization (FAO) animal production database are estimated to range around 1.76, 6.4, and 4.6 million cattle, goats and sheep in the country, respectively. Among livestock, only chicken outnumber these species (74 million). While disease surveillance is in place, there are knowledge gaps concerning the laboratory-based prevalence of some major livestock diseases. Among these is Bovine Coronavirus (BCoV) that affects cattle and other livestock species including horses and camels.

BCoV is an enveloped plus strand RNA virus that belongs to the

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https://doi.org/10.1016/j.vetmic.2019.108544
Received 27 September 2019; Received in revised form 2 December 2019; Accepted 2 December 2019

genus *Betacoronavirus* (Yang and Leibowitz, 2015; Oma et al., 2016; Pfefferle et al., 2009). While different strains may have some antigenic variability, all strains elicit cross-reactive seropositivity and thus form a single serotype (Clark, 1993; El-Ghorr et al., 1989). The virus is an important livestock pathogen causing effects on animal welfare as well as the economy (Lathrop et al., 2000a). It causes diarrhea and respiratory disease in calves, as well as winter dysentery in adult cattle (Boileau and Kapil, 2010; Ksiazek et al., 2003). Transmission of BCoV is mainly through respiratory or fecal-oral routes (Clark, 1993), infecting the respiratory (nasal, tracheal, and lung) and intestinal (villi and crypts of the ileum and colon) epithelial cells (Park et al., 2007). When infected with BCoV, within-herd transmission is generally rapid and infected animals display diverse clinical signs including diarrhea with or without blood, fever, and respiratory signs, which range from none to severe (Clark, 1993; Boileau and Kapil, 2010).

In many African countries including Ghana, livestock species live in close contact and animals serve diverse purposes such as transportation, draught power, fuel, clothing and as a source of meat and milk. Husbandry practices do not involve the same standards of species separation and hygiene as in other parts of the world. Close and sustained interaction between different animals as well as between animals and humans pose a risk of interspecies spillover of pathogens.

BCoV is characteristically a cattle virus. However, reports indicate BCoV infections also occur in small ruminants. Previous studies in Australia (Pass et al., 1982), New Zealand (Durham et al., 1979), Chile (Reinhardt et al., 1995), and Scotland (Snodgrass et al., 1980) reported BCoV infection in small ruminants. Eisa and Mohamed, 2014 also detected BCoV antigens in goats (Eisa and Mohamed, 2004) whereas Tråvén et al., detected BCoV antibodies in sheep (Tråvén et al., 1999). Recently, Gumusova et al., have also detected BCoV antibodies in goats (Gumusova et al., 2007).

Studies regarding the prevalence of BCoV and its associated risk factors are however limited in Africa, and none have been conducted in Ghana. This study evaluated the sero-prevalence of BCoV infection and assessed its associated risk factors among cattle, sheep, and goats in Ghana.

2. Materials and methods

2.1. Study design and area

This study employed a cross-sectional design and was conducted between January 2015 to December 2018 in five districts in four regions of Ghana. Ghana is located in the west of Africa, sharing borders with Togo to the east, Cote d'Ivoire to the west, Burkina Faso to the north and the Gulf of Guinea, to the south and lies on latitude 7.9528 and longitude -1.0307. Ghana has a tropical climate with an average annual temperature of about 26 °C and the annual rainfall of 736.6 mm/29″. Agriculture dominates the economy of Ghana and extensive farming practices in the country increase the livestock-wildlife-human interface

2.2. Ethics approval

This study was approved by the Wildlife division of the Ghana Forestry Commission (Approval number: AO4957).

2.3. Study population, sampling strategy and data collection

A total of 1498 animals aged ≥ 6 months, comprising 1328 cattle, 104 sheep and 66 goats were included in the study. Animals aged < 6 months were excluded due to the possibility of detecting maternal antibodies.

Sampling was done using a simple two-stage cluster sampling technique. The Regional Veterinary Officers of the Ministry of Food and Agriculture (MOFA), Ghana in the selected regions were contacted for information on animal populations in their respective regions prior to the study. The list provided served as the sampling frame. Prior to the study, a survey was carried out and an inclusion criteria of animal population (cattle, sheep and goats) ≥1000 for districts to be eligible for selection for the study was upheld. As a result, five (5) districts that fulfilled the criteria were randomly selected. Secondly, farms within these districts with herd size ≥100 animals were randomly selected. All cattle, sheep, and goats within the selected farms were included in the study. If a district meets the first criteria, but the individual farms fail to meet the second criteria, farms which were very close to each other were pooled together.

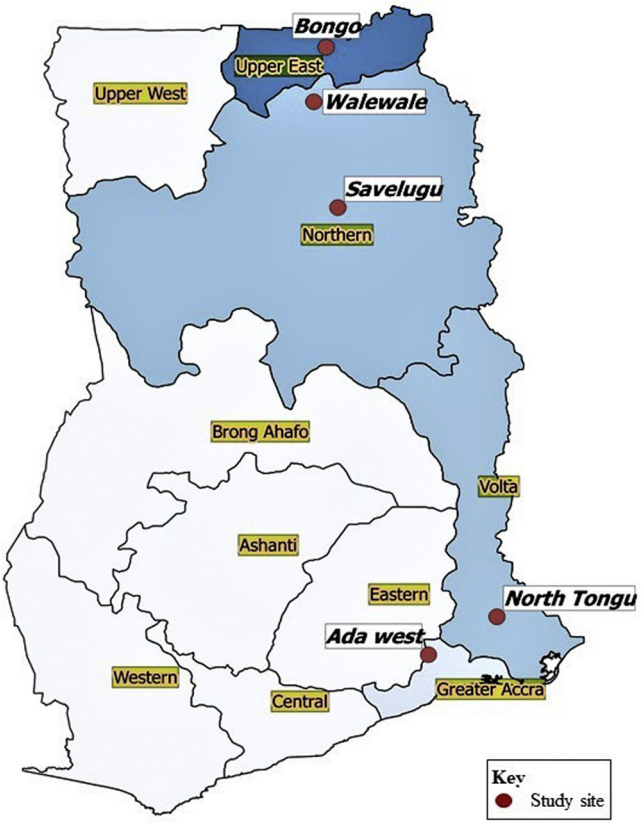

Fig. 1. The map showing the locations for sampling.

Sites included were: Bongo district in the Upper East; Savelugu and Wale wale in Northern; Ada West in Greater Accra, and North Tongu in Volta. The map with the locations for sampling in shown in Fig. 1.

A validated questionnaire was used to obtain data on possible risk factors of BCoV. Data collected include: age, sex, dietary changes, parturition, and lactation status of female animals. Additionally, the body score, presence of ectoparasites, and signs of infections such as fever, diarrhoea, respiratory distress, neurological disorder, and icterus were also assessed.

2.4. Sample collection, processing, and analysis

Ten milliliters (10 ml) of blood was collected through jugular puncture from each animal after disinfection of the site with 70 % using vacutainer tubes (Becton Dickinson, NJ, USA) with needles (18 gauge). In the field, the blood was allowed to clot before transportation to the Veterinary laboratory in the district. At the district laboratory, the samples were spun for 10 min at 1500 rpm to obtain the sera. The sera were transferred into three separate aliquots in cryotubes for each animal. The tubes were subsequently placed in liquid nitrogen to minimize antibody degradation. These processes were undertaken under sterile conditions. Upon obtaining representative samples per district,

the frozen samples were transported in a cold-chain to the Kumasi Centre for Collaborative Research (KCCR) for long term storage at −70 °C prior to laboratory analysis. Sample collection and preparation in each district and transportation to the KCCR lab took an average of 5–7 days.

During laboratory analysis, frozen sera were thawed at room temperature, vortexed and aliquots of 100 µl of each sample were prepared. The aliquots were incubated at 56 °C for 30 min in warm water prior to recombinant immunofluorescence assay (IFA) as previously described (Hoye et al., 2010; El-Duah et al., 2019; Reusken et al., 2013a). Briefly, Vero B4 cells were co-transfected with pCG1 plasmids bearing Human coronavirus OC43 spike proteins. After overnight transfection, cells were harvested by treatment with trypsin to detach them in a cell culture incubator at 37 °C and re-suspended in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, USA) in 10 % Foetal Calf Serum (FCS). Aliquots of cells were pelleted at 300 x g for 5 min and washed twice with 1 ml phosphate buffered saline (PBS). Fifty microliters (50 µl) of cell pellets were spotted on 12 well slides by dispensing and immediately aspirating, allowing 2 s interval between spotting. The cells were fixed using ice cold acetone/methanol (1:1), dried at ambient temperature, and kept at 4 °C after drying for 20 min. To conduct the assay, 45 µl of protein-free blocking solution (Roti®-Block, Carl Roth, Karlsruhe, Germany) was first added to each of the 12 spotted fields on the slide and incubated at room temperature in a humid box for 30 min, followed by rinsing with 1X tween-free PBS. After inactivation at 56 °C for 30 min, sera to be tested were diluted 1:100 in a 1X concentration of the protein-free blocking solution. Subsequently, 30 µl of the diluted sera was applied on each of the spotted area and incubated at 37 °C for 1 h in a humid chamber, followed by rinsing with 0.1 % tween in 1X PBS. Secondary antibody detection was done by the Alexa488 fluorescent reporter-conjugated goat anti-bovine, donkey anti-sheep, and donkey anti-goat IgG antibodies for cattle, sheep, and goat BCoV IgG respectively.

Test evaluation was done by microscopic examination under a fluorescent microscope and a positive outcome was determined by bright green cytoplasmic fluorescence as shown in Fig. 2.

2.5. Statistical analysis

Data were presented as frequencies (percentages) and Chi square test was used to test for association where applicable. Univariate and multivariate logistic regression analysis were performed to determine the possible factors associated with BCoV sero-positivity for cattle, sheep, and goats. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) software 25 (SPSS Inc., Chicago, IL, USA), and GraphPad Prism 7 version 7.04 (GraphPad Software, Inc., La Jolla, California USA).

3. Results

The proportions of sheep, goat, and cattle were 4.4 %, 6.8 %, and 88.8 % respectively. There were more adults than weaners (74.9 % vs 25.1 %) and more female animals (71.2 %) compared to male animals (28.8 %), with 22.5 % of the females being lactating females at the time of the study. Majority of the animals had high rectal temperature (95.6 %) and 4.4 % had physical signs of disease of which 2.7 % was diarrhea, 1.7 % was respiratory distress, and 0.1 % of the animals were icteric. None of the animals had neurological disorders. Additionally, a higher proportion of the animals had ectoparasites (93.9 %), were thin (76.5 %) and have not had any dietary change (98.9 %) (Table 1).

The sero-prevalence of BCoV in the entire animal population was 53.6 %. Upon stratification by sheep, goats, and cattle, the prevalence was 25.8 %, 43.1 % and 55.8 %, respectively. Cattles had significantly higher prevalence of BCoV compared to sheep and goats. Among the entire animal population, sero-positivity of BCoV was significantly associated with farms with ≥ 50 animals (75.9 % vs 24.1 %, $p < 0.0001$). Upon stratification by type of animal, this effect seemed to be explained by cattle (82.2 % vs 17.8 %, $p = 0.027$) but not sheep and goats that are normally kept in smaller groups (Table 2). The sero-prevalence of BCoV was highest in the Northern region followed by the Volta region (Table 3). Even though our sampling sites formed a north-south gradient, there was no latitude-dependent trend in seroprevalence.

There was no statistically significant association between the possible risks factors assessed and BCoV sero-positivity among all animals with the exception of dietary change, where a significantly lower odd of BCoV was observed among cattle with recent dietary change [OR = 0.08, 95 % CI (0.01-0.61), $p = 0.015$] (Table 4). Multivariate logistic regression identified both effect to be independent (farm size [OR = 1.39, 95 % CI (1.04–1.87), $p = 0.025$]; dietary change [OR = 0.07, 95 % CI (0.01-0.58), $p = 0.013$]) in cattle (Table 5).

4. Discussion

BCoV is a ubiquitous infection and BCoV-specific antibodies have been detected in cattle populations in numerous countries (Hasoksuz et al., 2002; Kapil et al., 1990; Lathrop et al., 2000b; Yavru et al., 2016). BCoV shares recent common ancestry with human coronavirus OC43 (HCoV-OC43) (Vijgen et al., 2006) and the two are serologically closely related to the extent that HCoV-OC43 is often used as a proxy in serological testing as previously described where specific proteins of BCoV were not available for serological testing or when National legislation restricts the use of certain livestock pathogens (Reusken et al., 2013b; Meyer et al., 2014). In most African countries such as Ghana, diverse farm animals live in close contact that poses a risk of cross-species infection. Indeed, we found seropositivity against BCoV not only among cattle (55.8 %) but also among sheep and goats at 25.8 % and 43.1 % prevalence rates, respectively. This prevalence was predominant among

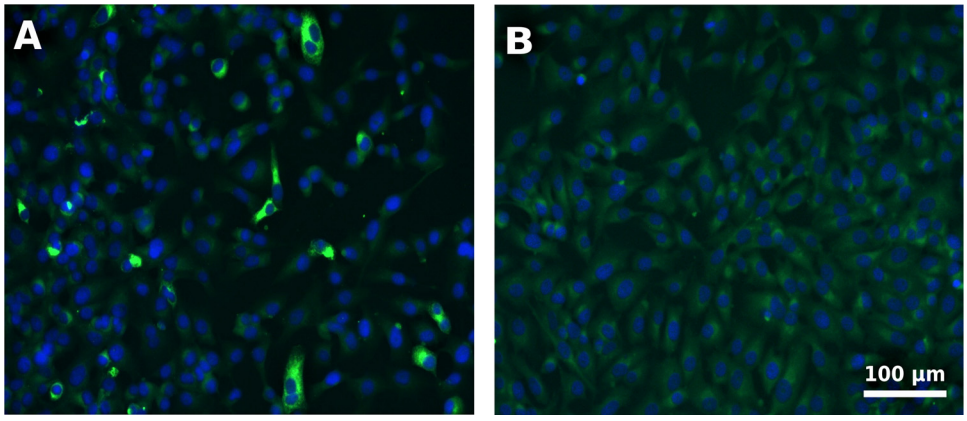

Fig. 2. Depiction of typical BCoV IgG positive outcome (Panel A) against a negative outcome (Panel B). Cell nuclei were stained with DAPI and are shown as dark blue and the bright green impressions around the nuclei represent fluorescent antibody-antigen complexes (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Baseline characteristics of the entire animal population.

Variables	Total (n = 1495)	Sheep (n = 66)	Goat (n = 102)	Cattle (n = 1327)
Type of animals				
Sheep	66 (4.4)	–	–	–
Goat	102 (6.8)	–	–	–
Cattle	1327 (88.8)	–	–	–
Age of animals				
Weaner	375 (25.1)	7(10.6)	15(14.7)	353(26.6)
Adult	1120 (74.9)	59(89.4)	87(85.3)	974(73.4)
Sex of animals				
Male	430 (28.8)	4(6.1)	13(12.7)	413(31.1)
Female	1065 (71.2)	62(93.9)	89(87.3)	914(68.9)
Lactating females	*336 (22.5)*	*1(1.5)*	*8(7.8)*	*327(24.6)*
Rectal temperature				
Normal	204 (13.6)	16(24.2)	32(31.4)	204(13.6)
High	1291 (86.4)	50(75.8)	70(68.6)	1291(86.4)
Signs of disease				
No	1430 (95.6)	61(92.4)	92(90.2)	1276(96.2)
Yes	66 (4.4)	5(7.6)	10(9.8)	51(3.8)
Diarrhea	41 (2.7)	0(0.0)	7(6.9)	34(2.7)
Respiratory distress	25 (1.7)	4(6.1)	3(2.9)	18(1.4)
Icterus	1 (0.1)	1(1.5)	0(0.0)	0(0.0)
Neurological disorder	0 (0.0)	0(0.0)	0(0.0)	0(0.0)
Presence of ectoparasites				
No	91 (6.1)	10(15.2)	10(9.8)	71(5.4)
Yes	1404 (93.9)	56(84.8)	92(90.2)	1256(94.6)
Body scoring				
Emaciated	15 (1.0)	1(1.5)	0(0.0)	14(1.1)
Thin	1144 (76.5)	65(98.5)	93(91.2)	986(74.3)
Normal	330 (22.1)	0(0.0)	9(8.8)	321(24.2)
Moderately fat	6 (0.4)	0(0.0)	0(0.0)	6(0.5)
Dietary changes				
No	1479 (98.9)	65(98.5)	98(96.1)	1316(98.9)
Yes	16 (1.1)	1(1.5)	4(3.9)	16(1.1)

Normal temperature for cattle: 37.8–39.5 °C; Normal temperature for sheep and goats: 38.5–40.5 °C.
For cattle, sheep and goats: Age of weaner: 6 months to 1 year; Age of adult: > 1 year.

Table 2
Sero-prevalence of BCoV and its association with farm density.

Variables	Sero-prevalence	Farm with < 50 animals	Farm with ≥ 50 animals	p-value
Total				< 0.0001
Positive	801(53.6)	193(24.1)	608(75.9)	
Negative	694(46.4)	241(34.7)	453(65.3)	
Sheep				NA
Positive	17(25.8)	17(100.0)	0(0.0)	
Negative	49(74.2)	49(100.0)	0(0.0)	
Goat				NA
Positive	44(43.1)	44(100.0)	0(0.0)	
Negative	58(56.9)	58(100.0)	0(0.0)	
Cattle				0.027
Positive	740(55.8)	132(17.8)	608(82.2)	
Negative	587(44.2)	134(22.8)	453(77.2)	

Table 3
The sero-prevalence of BCoV among the entire animal population stratified by regions.

Region	Negative	Positive	p-value
Upper East	191(54.7)	158(45.3)	0.002
Greater Accra	142(47.3)	158(52.7)	
Volta	198(43.3)	259(56.7)	
Northern	163(41.9)	226(58.1)	

animals from the Volta region of Ghana. Cattle presented with significantly higher prevalence of BCoV compared to sheep and goats.

Varying prevalence rates of BCoV have been reported in different countries. A study by Alkan et al. reported prevalence ranging from 4.4 to 100.0% among cattle in Turkey (Alkan et al., 2003). Another study by Gumusova et al. in northern Turkey reported a sero-prevalence of 98.43 % in cattle. Yavru et al. (2016) and O'Connor et al. (O'Connor et al., 2001) reported a sero-prevalence of 94 % among 184 calves and their mothers in Burdur, Turkey and 90 % among 852 animals from 3 Ontario feedlots, respectively based on enzyme linked immunosorbent assay (ELISA) method.

Bidokhti et al. (2009); Hasoksuz et al. (2005), and Yildirim et al. (2008) also reported a sero-prevalence of 82–86 %, 54.5 %, and 26.3 %, respectively among cattle. The discrepancies in the prevalence rates compared to that of this present study could be attributed, at least in part, to differences in geographical location, different management systems, source population size, method employed for BCoV antibody detection, and the samples size used in the different investigations. In addition, the higher prevalence rate among cattle could be due to the fact that a higher proportion of the animals were cattle as well as the tropism of BCoV to cattle

Few studies have been conducted to evaluate the prevalence of BCoV among small ruminants. The most recent study was conducted by Gumusova et al. in 2007 in Turkey. In their study, they evaluated the sero-prevalence of BCoV in goats by employing commercially available competitive ELISA kits and reported a BCoV sero-prevalence of 41.1 % (Gumusova et al., 2007). In a previous study, Eisa and Mohamed reported detection of BCoV antigens in goats (Eisa and Mohamed, 2004). Additionally, Tråvén et al., in a study among 218 sheep from 40 flocks in different parts of Sweden, reported that 19 % of the sheep were positive for BCoV antibodies (Tråvén et al., 1999). Prior to these recent studies, there had been reports of BCoV infection in small ruminants in Australia (Pass et al., 1982), New Zealand (Durham et al., 1979), Chile (Reinhardt et al., 1995), and Scotland (Snodgrass et al., 1980). Our finding in sheep and goats, thus, provides update information of spillover of BCoV from cattle.

Granted the contagious nature of BCoV, it is imperative that factors

Table 4
Possible risk factors for BCoV sero-positivity.

Variables	Sheep		Goat		Cattle	
Age of animals	OR(95 % CI)	p-value	OR(95 % CI)	p-value	OR(95 % CI)	p-value
Weaner	1		1		1	
Adult	2.23(0.25–20.02)	0.474	0.32(0.10–1.02)	0.054	1.18(0.93–1.51)	0.175
Sex of animals						
Male	1		1		1	
Female	0.32(0.04–2.47)	0.274	1.25(0.38–4.12)	0.716	1.16(0.92–1.46)	0.219
Lactating females	9.00(0.35–231.83)	0.185	2.35(0.53–10.42)	0.261	1.25(0.97–1.62)	0.080
Rectal temperature						
Normal	1		1		1	
High	0.47(0.14–1.58)	0.223	0.55(0.24–1.29)	0.171	0.91(0.65–1.28)	0.606
Signs of disease						
No	1		1		1	
Yes	0.70(0.07–6.77)	0.761	0.30(0.06–1.48)	0.138	1.14(0.65–2.01)	0.654
Diarrhea	–	NA	0.20(0.02–1.74)	0.145	1.14(0.57–2.27)	0.716
Respiratory distress	0.96(0.09–9.89)	0.972	0.65(0.06–7.42)	0.730	0.99(0.39–2.53)	0.986
Icterus	0.92(0.04–23.75)	0.962	–	NA	–	NA
Neurological disorder	–	NA	–	NA	–	NA
Presence of ectoparasites						
No	1		1		1	
Yes	0.27(0.07–1.10)	0.068	1.15(0.31–4.37)	0.833	0.86(0.53–1.40)	0.555
Body scoring						
Normal	–	NA	1		1	
Emaciated	1		–		1.06(0.36–3.12)	0.919
Thin	0.11(0.004–2.86)	0.185	0.94(0.24–3.74)	0.934	0.99(0.77–1.28)	0.098
Moderately fat	–	NA	–		1.59(0.29–8.79)	0.597
Dietary changes						
No	1		1		1	
Yes	9.00(0.35–231.83)	0.185	0.14(0.01–2.60)	0.185	0.08(0.01–0.61)	0.015

Table 5
Multivariate analysis of potential risk factors of BCoV sero-positivity in cattle.

Variables	aOR (95 % CI)	p-value
Farm density		
< 50 animals	1	
≥ 50 animals	1.39(1.04–1.87)	0.025
Body scoring		
Normal	1	
Emaciated	1.01(0.34–3.00)	0.983
Thin	0.91(0.70–1.20)	0.509
Moderately fat	1.78(0.32–9.97)	0.513
Dietary changes		
No	1	
Yes	0.07(0.01–0.58)	0.013
Lactating females	1.27(0.98–1.65)	0.067

that influence exposure and the determinants of BCoV infection be identified which would assist in the development of apt control and preventive measures against BCoV and other infectious diseases.

Though there was no statistically significant association between the possible risks factors assessed and BCoV sero-positivity, we found BCoV sero-positivity to be significantly associated with farms with higher cattle density. This finding is in harmony with studies by Beaudeau et al. (2010); Hägglund et al. (2006), and Ohlson et al. (2010) who reported that large herd size is a risk factor for BCoV infections in dairy cattle. This may be due to poor biosecurity especially among farms with larger herd size in Ghana, and also due to the close contact between animals in these farms which could potentiate the transmission of BCoV compared to farms with small herd size (Beaudeau et al., 2010; Ohlson et al., 2010).

Infectious diseases surveillance can be greatly enhanced by research studies, as often there is close collaboration between governmental and academic institutions. Restrictions in the movement of samples to prevent the spread of notifiable livestock diseases create a demand for domestic laboratory capacities. Through this study we hope to demonstrate the value of capacity building in field-based research.

Acknowledgement

This work was supported by Deutsche Forschungsgemeinschaft under a Grant to Y. A. S. and C. D. (DR 772/12-1).

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