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Seroprevalence of Antibodies against Bovine Herpesvirus-1(Bohv-1) in Zebu Cattle in the Vina Division, Cameroon

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Abstract

Infectious Bovine Rhinotracheitis (IBR) which is caused by Bovine Herpesvirus-1 (BoHV-1) is poorly documented in Sub-Saharan Africa. In Cameroon, there is no previous report available for prevalence of IBR infections in cattle. In the present study, the serosurveillance of IBR infection was carried out in 252 randomly selected zebu cattle in small holder livestock farms from 7 subdivisions of the Vina Division using an indirect ELISA (Bio-X Diagnostics kit; Belgium). Antibodies against BoHV1 were present in the Zebu Gudali cattle indicating the past or present infection of the animals with BoHV-1. The seropositivity rate varied from 11.11 ± 1.1% in Ngaoundere to 22.22 ± 2.2% in Ngangha sub-division, with the highest prevalence rate of 38.89 ± 3.9% in Nyambaka and an overall relative prevalence rate of 16.1 ± 1.6%. The older animals (>3-10 years and >10 years) had a significantly higher ($p < 0.05$) seroprevalence than those in the younger age group (1-3 years). No significant difference ($p > 0.05$) could be observed in prevalence between male and female animals. These epizootiological values need to be considered in the planning of cattle disease control programmes in this predominantly cattle producing area.

Keywords:

Infectious Bovine Rhinotracheitis; Serosurveillance; Antibodies

Abbreviations:

IBR: Infectious Bovine Rhinotracheitis

BoHV-1: Bovine Herpesvirus-1

ELISA: Enzyme-Linked Immunosorbent Assay

IPV: Infectious Pustular Vulvovaginitis

IBP: Infectious Balanoposthitis

CBPP: Contagious Bovine Pleuropneumonia

Introduction

Infectious bovine rhinotracheitis infectious pustular vulvovaginitis (IBR/IPV), is an important emerging viral disease of livestock caused by bovine herpe svirus 1 (BoHV-1). It affects domestic cattle throughout the world with varying prevalence rate [1]. The bovine herpesvirus-1 (BoHV-1) belongs to the Subfamily Alpha-herpesviridae, genus Varicellovirus. IBR/IPV is one of the most economically important diseases of farm animals. It has been eradicated in some countries such as Austria and Denmark.

IBR is characterized by clinical signs of the upper respiratory tract such as purulent nasal discharges and conjunctivitis. The signs of general illness are fever, depression, inappetence, abortions and reduced milk yield. Although mortality due to IBR is low, the virus can also infect the genital tract and cause infectious pustular vulvovaginitis (IPV) in female and infectious balanoposthitis (IBP) in bulls. It also causes immuno-suppression and increased susceptibility to other infections. Secondary bacterial infections can lead to more severe respiratory infections and fatal cases are found at neonatal periods and in calves unlike adults [2].

In many countries, it is estimated that about 50% of adult livestock were infected with this virus [3]. In 1992, 34% of the farms in the United Kingdom had antibodies against BoHV-1 [4]. In 1996, 70% of the 360 tested dairy herds were positive for BoHV-1 infection as determined by the presence of antibodies against BoHV1 in milk. It has been speculated that the virus may be widespread in Cameroon, the Central African Republic and Nigeria

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[5], but no proven data is available. There is a paucity of reports on the prevalence of IBR in Sub-Saharan Africa, a region where livestock species abound. In these countries, no strategy is put in place by governments to control the disease.

A few studies have reported IBR in some countries of the region including Tchad, Zambia and South Africa [6]. Cameroon counts about 5.5 million cattle and about 38% of these cattle are found in the Adamawa region [7]. In central Africa, there is a paucity of data on the disease and also, no strategy is put in place by governments to control the disease. Attempts to increase the competitiveness of Cameroon beef in international trade should be accompanied by the provision of disease data on such notifiable diseases.

The present study is the first report of prevalence of antibodies against BoHV-1 in cattle of Cameroon and considers the seroprevalence of IBR as well as the influence of age and sex on the occurrence of the disease.

Description of the study area

The study was undertaken in the Adamawa region of Cameroon, a high plateau stretching across the middle of the country lying between latitudes 60and 80 North of the equator and longitude 110 and 150 east. The mountainous region has a Sudano-Guinean climate with two seasons: the rainy season which extends from April to October and the dry season which runs from November to March. The temperature oscillates between 20°C-25°C and rainfall is about 1500 mm for 102 to 150 days per year [8]. Most of the plateau lies between 1000 and 2000 meters (average of 1000-1100 meters above the sea level) and narrows down to 500 meters in the valleys of Djerem and Mbere [9]. This region is generally made up of two types of vegetation, trees mostly Daniella Oliveri and Lophira Lanceolata. The grass cover is quite thick and made up particularly of Hyparrhenia sp. and Panicum sp. [10]. There are about 7000 inhabitants and the principal economic activity is cattle rearing. Other economic activities seen in the region are agriculture, apiculture and local fishing [11].

This Vina division is made up of 8 administrative units, namely: Ngaoundere I; Ngaoundere II; Ngaoundere III; Martap; Nyambaka; Ngangha; Belel and Mbé (Figure 1). Each of them is a veterinary center which is managed by a veterinary chief of center who takes care of the animal health when the need arises.

Animal sampling method

During blood collection from the animals, information recorded for each animal included the farming system, age, sex, reproductive history and whether the animal presented with some clinical signs of IBR. All herds were said to have been vaccinated against major epizootics such as contagious bovine pleuropneumonia (CBPP), pasteurellosis and black quarter diseases.

Blood samples were collected from 14 herds in seven of the eight subdivisions. Blood samples w rd size. Sampling was then undertaken with the consideration that the seroprevalence of IBR was 10%, 90% probability of finding the antibodies against BoHV-1. This was to give all the animals equal chances of being selected [12]. Blood was collected from 18 animals per herd by Jugular vein puncture and a total of 252 male and female Zebu cattle were involved (Table 1). The blood samples were kept in the veterinary research laboratory at IRAD Wakwa, Ngaoundere and allowed to clot overnight at 8°C and then centrifuged at 3000 rpm for 15 minutes. The serum was then separated and stored in coded sterile Eppendorf tubes.

The kit consisted of 96-well microtitration plates containing BoHV-1 virus and control antigen (Bio-X Diagnostics, Belgium). All the reagents were brought to room temperature (21°C ± 3°C) 30 minutes before they were used. The odd columns (1,3 5,6,7,9and11) contained the BoHV-1 antigen, whereas the even columns (2,4,6,8,10and12) contained a control antigen. The serum samples as well as positive and negative serum samples provided in the kit were diluted 1:100 using the dilution buffer provided in the kit that was prepared according to the manufacturer's instructions.

The diluted serum samples dispensed as follows -100 µL positive serum was placed in wells A1 and A2, negative serum was placed in wells B1 and B2, while test serum samples were placed in wells C1 and C2 and D1 and D2 wells and so on. The plate was covered with a lid and then incubated at 21°C ± 3°C for one hour. The microtitration plates were emptied of their content by flipping them sharply above the wash hand basin sink and taped against a piece of clean absorbent paper to remove all the liquid. The wells were then filed with the 1x washing solution provided in the kit using a squeeze bottle and the plates were emptied once more by flipping it sharply above the sink. The washing operation was repeated two more times, avoiding the

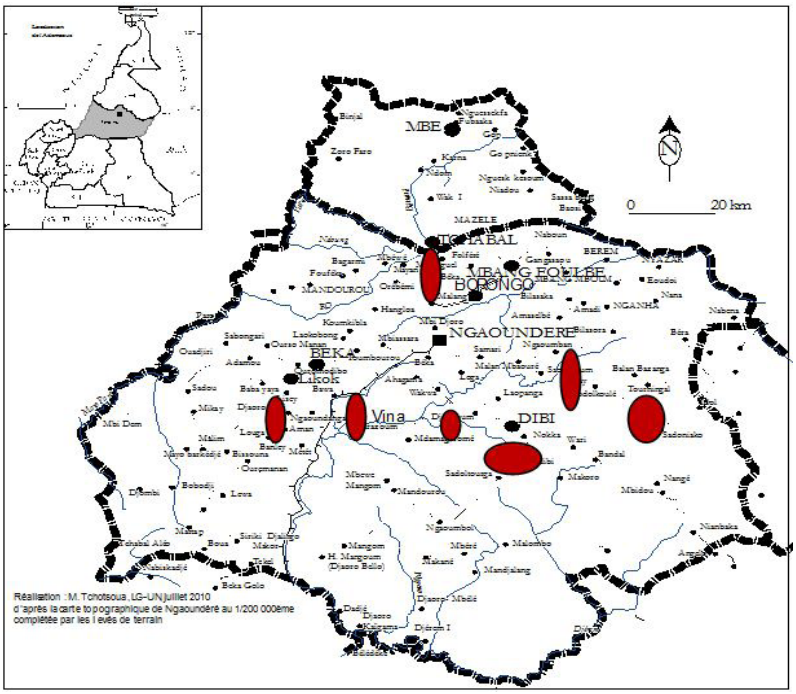

Figure 1: Map of the Vina Division: sampling areas are shown in red

Sub division	Animal age range			Total number of animals per subdivision
	(1-3)years	(above 3 - 10) years	(above 10) years	
Ngaoundere 1	13	5	0	18
Ngaoundere II	15	17	4	36
Ngaoundere III	19	17	0	36
Ngangha	22	14	1	37
Martap	22	14	0	36
Nyambaka	17	33	4	54
Belel	12	20	3	35
Total	120	120	12	252

Table 1: Number of cattle sampled per subdivision with age wise data

Sub division	Animal age range		Total number of animals per subdivision
	(1-3)years	(>3 - >10) years	
Ngaoundere 1	13	5	18
Ngaoundere II	15	21	36
Ngaoundere III	19	17	36
Ngangha	22	15	37
Martap	22	14	36
Nyambaka	17	37	54
Belel	12	23	35
Total	120	132	252

Table 2: Percentage positivity of antibodies against BoHV-1 in cattle sera in the Vina Division

formation of bubbles in the micro plates. The plates were incubated at 21°C ± 3°C and subsequently washed with 1x washing solution. 100 µL of the conjugate, protein G peroxidase-labelled was added to the wells. The plate was incubated at 21°C ± 3°C followed by three times washing with the 1x washing solution as in the above step. The Substrate solution was prepared as per the kit instructions and 100 µL dispensed into each well. The incubation and washing step was repeated as described above. 100 µL/well of the Chromogen (tetramethylbenziden) (prepared with 12 ml of Na $H_2PO_4.2H_2O$ PH 5.5 in Dimethyl Sulfoxide and 12 µL of H_2O_2) was added and the plate was incubated for 10 minutes at 21°C ± 3°C whilst being protected from the light and covered. At this stage, if BoHV-1 specific immunoglobulins were present in the test sera, the conjugate would remain bound to the microwell that contained the viral antigens combined with antibody and the enzyme catalyses the transformation of the colorless chromogen into a pigmented compound. After this stage, there was the appearance of a sky blue colour in wells in odd columns indicating a successful binding reaction between the antibodies in the serum and the virus forming virus-antibody complex while micro wells in the even columns (cell antigen control) remained unchanged as there was no virus-antibody complex formed. The intensity of the resulting blue color is proportionate to the titre of the specific antibody present in the sample and this was measured as the optical density. After 10 minutes of incubation at 21°C, the reaction was stopped using 50 µL per well of a stop solution (1 M H_2SO_4) and the sky blue colour of the solution then changed into a yellowish solution. The Optical Density (OD) was measured using an OPSYS MR (Dynex Technologies) USA S/N:1MRA-1653 ELISA readWer machine.

Evaluation of ELISA results

For each OD value recorded in the odd columns, the signal of the corresponding negative well in even columns was subtracted to obtain result X. In performing this calculation, it was necessary to allow for any negative values that could exist. The same calculations were undertaken for the column corresponding to the positive and negative control. The test was validated only if the positive serum produced a difference in optical density at 10 minutes that was greater than the one given in the kit quality control data sheet. Thus, a sample was considered positive if it produced a result that was greater than or equal to one plus sign (+) according to the kit quality control reference table instructions (Bio-X Diagnostics, Belgium).

Statistical analysis

CHI Square was used to compare the prevalence within age groups and sexes using SPSS 12.0 operational system and sigma plot was used to draw the histograms.

$$\text{Prevalence rate} = \frac{(\text{Number of IBR seropostive animals})}{\text{Total number of animals examined}} \times 100$$

Results

Animals showing clinical signs of the disease were characterized by (mucopurulent nasal discharge from the upper respiratory tract, conjunctivitis, hyperaemia of the muzzle (red nose disease), depression, abortions, fever, inappetence and reduced milk yield. Six of these examined animals had developed balanoposthitis (two) or pustular vulvovaginitis (four).

Effect of study sites on level of IBR infection status

Of the 252 animals examined, 42 animals had antibodies against BoHV-1 wheras 210 were found to be negative for this antibody. Therefore, the seroprevalence of IBR in Zebu cattle was 16.7 ± 1.7% of the 252 cattle examined (Table 2).

The analyzed data indicates that of the 6 males that showed possible clinical signs of the disease only 3 showed positive reaction in the test while 7 males that did not show any of the clinical signs also showed positive reaction. Similarly, among 33 females with clinical signs only 11 showed positive results while 19 females without clinical signs were also found positive.

Sub Division	Herd name	Infection status of animals		% positive within herd
		Number of animals examined	Number of animals positive	
Ngaoundere I	Market	18	2	11.11
Ngaoundere II	Ali	18	4	22.22
	Ou	18	0	0
Ngaoundere III	Tchabal A	18	2	11.11
	Tchabal C	18	5	27.78
Ngangha	Laphia A	18	4	22.22
	Laphia B	18	3	16.67
Martap	Likok E	18	3	16.67
	Likok F	18	3	16.67
Nyambaka	Nyambaka A	18	7	38.89
	Nyambaka B	18	0	0
	Nyambaka C	18	0	0
Belel	Tournigal C	18	5	27.78
	Tournigal D	18	3	16.67
Total		252	42	16.67

Table 3: Point prevalence of IBR in the Vina division

Effect of study sites on level of IBR infection status

The highest prevalence (38.89%) was detected in one of the farms/herds examined in Nyambaka (Table 3) whereas BoHV-1 specific antibodies could not be detected in three herds: One in Ngaoundere II and two in Nyambaka.

Influence of sex of animal on IBR infection rate

Out of 252 animals tested, 3.9% and 12.69% of males and females respectively, had contact with the virus in Vina division whereas 24.2% and 59.1% of males and females, respectively, were not infected. However, this difference was non-significant ($p > 0.05$); Chi square: P = 0.287 chi square Pearson's value=1.133b

149 Females not infected

61 Males not infected

10 Males infected

32 Females infected

Effect of age of animal on the IBR infection rate

IBR seropositive rates were significantly different ($p<0.05$) between the age groups (1-3 and >3-10 years). Meanwhile there was no significant difference between the age groups 1-3 and >10 years; similarly there was a non significant difference between the age groups >3 to 10 and above 10 years. The age group which is the most actively involved in reproduction is the >3-10 years group (Figure 2).

Discussion

The importance of BoHV-1 infection as a reproductive pathology and respiratory disease syndrome is well established and IBR is of enormous importance financially to the cattle industry. However, very little attention has been paid to this disease in Sub-Saharan Africa and its potential role as an important co-infection causing immuno-suppression.

Using a commercial indirect ELISA kit (Bio-X Diagnostics, Belgium), antibodies to BoHV-1 was assessed in all but one of the subdivisions of Vina Division. Durham and Sillars [13] confirmed that ELISA tests are reliable and that the most important points using ELISA kits in serological studies are that, the tests are less time consuming, more specific, more sensitive and well suitable for reproducibility.

From the test carried out, a relative prevalence of 16.7 ± 1.7% for IBR or overall prevalence rate was detected. The highest herd prevalence detected was 38.88 ± 3.9%. This is in accordance with the proposition made by Rweyemamu [5] about the probable occurrence of antibodies to BoHV 1 in Central Africa and Cameroon in particular. The present results are similar to the prevalence range from 12 to 38% reported in Sudan [14]. A high prevalence rate was also reported in Sudan in camels that were in daily contact with cattle in grazing-land and at water points [15]. Although a few semi-intensive production systems are emerging in the region, cattle rearing are mainly very traditional and could be described as an extensive production system which involves communally grazed herds that are usually moving on transhumance across borders each dry season in search of pasture [16]. In such a production system, animals or herds frequently come in contact with other herds thus perpetuating the transmission of the disease leading to the presently detected high prevalence rate of antibodies to BoHV-1.

That sex had no influence on the prevalence of IBR suggests that, under the same circumstances, both male and female Zebu cattle have an equal chance of acquiring the BoHV-1 infection. Grazing of cattle in the Vina Division is frequently done along side with sheep and/or goats. Sheep and goats are known to be carriers of this enzootic disease [17]. Also, sampling for this study was carried out in the rainy season which is the period of animal herd concentration and the frequent contact in the reproductive season which provides more opportunity for virus transmission resulting in higher incidence of infection. Amira et al. [18] isolated BoHV-1 from bovine samples in Sudan collected in the rainy season. Most often some or all animals from a herd taken to the cattle market for sale are brought back

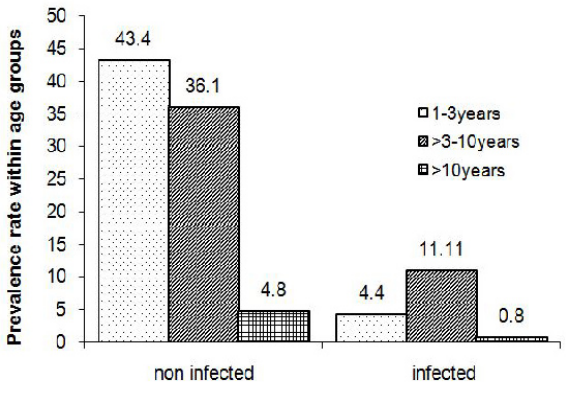

Figure 2: Influence of age on the seroprevalence of antibodies against BoHV-1 in Zebu cattle in the Vina Division, Camaroon

to their herds of origin without application of any health security measures. Such deficiencies in the production system or traditional management practices may facilitate disease transmission from infected cattle brought into contact with non-infected animals.

Animals within the age group >3-10 years old were significantly more ($P < 0.05$) infected than those of the 0-3 age group. Animals that fall in the age group of 0-3 years (younger animals) acquired passive colostral antibodies against the virus from their dams. The increase in the prevalence of IBR with increasing age of animal corroborates the finding of Rajkhowa et al [19] in India and could be due to the fact that as animals grow older, they are more likely to be exposed to the virus since they are more likely to come into contact with other animals which have recovered from the disease but remain carriers [19]. It has also been reported that in 50% of adult livestock, most of them had been in contact with IBR [3]. Semen of an infected bull may contain BoHV-1, and the virus can thus be transmitted by natural mating and artificial insemination [20].

Previous field surveys in Vina Division and Adamawa region on a larger scale reported the occurrence of abortion in cattle (LSR, 1989). In the present study, four of the 32 females examined were strongly seropositive for bovine herpesvirus 1. This confirms the fact that BoHV-1 may have contributed to this abortion as indicated by Hassan and Khalda [21]. Hassan and Khalda [21] actually isolated BoHV-1 from cattle with a history of abortion in Sudan. Of all the cattle that indicated other clinical signs such as ocular and nasal discharges, 12 were found to be infected with BoHV-1. This also confirms the fact that BoHV-1 is one of the causative agents of respiratory and ocular disorders in cattle. A serological survey of bovine respiratory diseases in dairy herds undertaken in Iran reported similar findings [22]. The role of IBR in co-infections in the West and Central Africa sub-region needs to be studied widely. However, some other cattle in the present study which were seropositive for IBR infections did not show any clinical signs. This may be explained by the fact that BoHV-1 remains latent in infected animals and may re-occur under stress conditions and virus shedding may or may not be accompanied by clinical signs. Latency allows for the virus to persist and the introduction of latently infected carriers into a non-infected herd is the best way to spread the disease. Similar results have been reported following investigations on IBR in Egyptian cattle and buffaloes [23]. A detailed molecular characterization of BoHV-1 circulating in cattle and other ruminants is of prime importance since the disease is a major limiting factor to livestock productivity.

Conclusion

The findings in the study area reveal that 16.7 ± 1.7% of cattle tested positive for antibodies against BoHV-1. This directly implies huge economic losses for the livestock breeder. Multiple risk factors in the local production systems such as irresponsible movement of animals, introduction of new animals that have not been tested negative into herds, poor feeding especially in the dry season and artificial insemination, without quality control of semen, help to sustain BoHV-1 infections in livestock farms. Livestock breeders should ensure a better herd management and the veterinary services should consider the inclusion of IBR vaccination, at least for cattle, during vaccination campaigns.

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