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Full Length Research Paper

Seroprevalence of Herpes virus-1(BoHV-1) antibodies in Zebu cattle in the Vina Division, Cameroon

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Infectious bovine rhinotracheitis (IBR) which is caused by Herpesvirus-1 (BoHV-1) is poorly documented in Sub-saharan Africa. In Cameroon there are no previous estimates of IBR infections rates in cattle. A serosurveillance by an indirect ELISA test for detecting BoHV-1 antibodies using the Bio-X Diagnostics kit (Belgium), was undertaken with 252 randomly selected zebu cattle in small holder livestock farms from 7 subdivisions of the Vina Division. Herpesvirus -1 antibodies were present in the zebu Gudali cattle indicating past or present infection of the animals with Herpes virus-1(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 point prevalence of 38.89±3.9% and an overall relative prevalence rate of 16.7±1.7%. Older animals (>3-10years) had a significantly higher ($p < 0.05$) sero prevalence of Herpesvirus -1 antibodies than those in the 0-3 years age group. No significant difference ($P>0.05$) in Herpesvirus -1 antibodies prevalence was found between males and female cattle. 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

INTRODUCTION

Infectious bovine rhinotracheitis also called infectious pustular vulvovaginitis, is an important emerging viral disease of livestock caused by bovine Herpes virus 1 (BoHV-1). It affects domestic cattle and other ruminants in all the continents even though the incidence and prevalence varies (Ackermann & Engels, 2006). Herpesvirus-1 (BHV-1) belongs to the Subfamily Alphaherpesvirinae and IBR is one of the most economically important diseases of farm animals. It has been eradicated in some countries of the world like Austria and Denmark. The world organization for Animal health (Office International des Epizooties; OIE) lists IBR as list B - notifiable disease (OIE, 2000). OIE list B diseases are transmissible diseases that are of socio-economic and/or

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public health importance and that are noteworthy in international trade.

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 pustular vulvovaginitis and balanoposthitis in bulls. It also causes immune-suppression and increased susceptibility to other infections. Secondary bacteria infections can lead to more severe respiratory infections and fatal cases are found at neonatal periods and in calves unlike adults (Patel, 2005).

In many countries throughout the world it is estimated that about 50% of adult livestock were infected with this virus (Seal, 2007). In 1992, 34% of the farms in the United Kingdom had one or more calves with antibodies against BoHV-1 (Hogg, 1992). In 1996, some 70% of the 360 tested dairy herds were positive for BoHV-1 antibody in milk (Hogg, 2000). It has been speculated that the virus may be wide spread in Cameroon, the Central African Republic and Nigeria (Rweyemamu, 1970), but no hard data is available. There is paucity of reports on the prevalence of IBR in Sub-Sahara Africa, a region where millions of ruminant livestock species abound. In these countries no strategy is put in place by governments to control the disease.

A few studies have reported Infectious bovine rhinotracheitis in some countries of the region including Tchad, Zambia and South Africa (Maré *et al*., 1961). Cameroon counts about 5.5 million cattle and about 38% of these cattle are found in the Adamawa region (MINEPIA, 2003). 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 provision of disease data on such notifiable diseases.

The present study, which appears to be the first report of IBR in Cameroon, examined the seroprevalence of IBR as well as the influence of age and sex on the occurrence of the disease.

MATERIALS AND METHODS

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 6° and 8^0 North of the equator and longitude 11^0 and 15^0 East. The mountainous region has a Sudano-Guinean climate with two seasons: the rainy season which starts from April to October and the dry season which runs from November to March. The temperature oscillates between 20^{0}C-25^{0}C and rain fall is about 1500 mm for 102 to 150 days per year (Mbahe, 1989). Most of the plateau lies between 1000m to 2000m (average of 1000-1100m above sea level) and narrows down to 500m in the valleys of Djerem and Mbere (Letouzey, 1968). 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 Panicium sp* (Reppstein, 1985). 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 (Mope, 1997).

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 peripneumonia (CBPP), pasteurellosis and black quarter.

Blood samples were collected from 14 herds in seven of the eight subdivisions. Blood samples were not collected from Mbe; since it was considered as a purely farming zone in the rainy season. The sample frame was drawn by numbering animals from 01 to 50 or more depending on the herd size. Sampling was then undertaken with the consideration that the sero-prevalence of IBR was 10%, 90% probability of finding the antibodies to BoHV-1. This was to give all the animals equal chances of being selected (Putt *et al*., 1987). 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 centrifuge at 3000 rpm for 15 minutes. The serum was then separated and stored in coded sterile eppendorf tubes.

Indirect ELISA test for detection of BoHV-1 antibodies in bovine sera.

The kit, which consisted of 96-well micro titration plates sensitized with purified BoHV-1 virus (Bio-X Diagnostics, Belgium), and all the reagents were brought to room temperature (21°c±3°c) at least half an hour before use. The odd columns (1, 3, 5, 6, 7, 9 and 11) contained the virus, whereas the even columns (2, 4, 6, 8, 10 and 12) contained a control antigen. The serum was diluted 1:100 using the kit dilution buffer solution that was prepared according to the manufacturer's instructions.

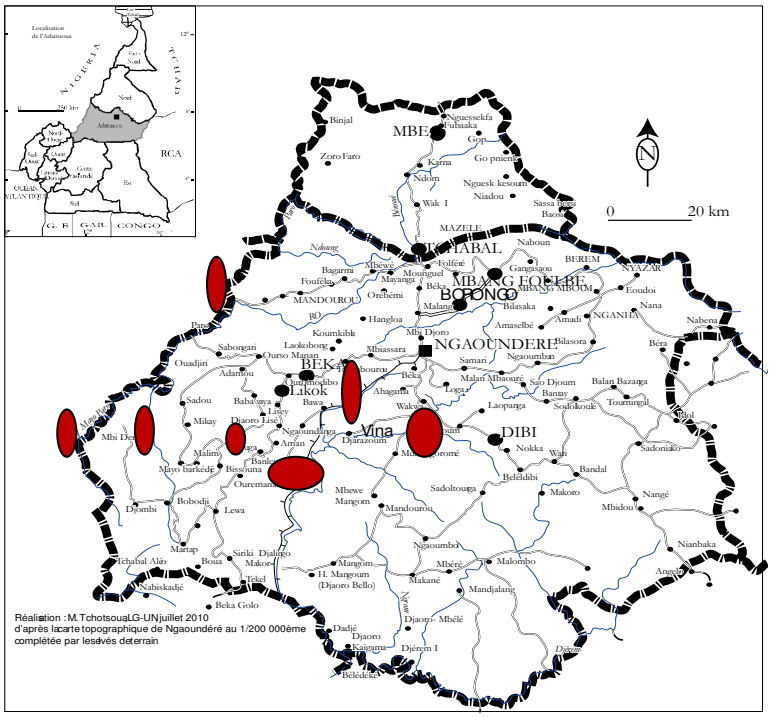

Figure 1: Map of the Vina Division: sampling areas are shown in red

Table 1: Number of cattle sampled per sub division with respect to age group.

Sub Division	Animal Age range		Total number of animals per sub division
	(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**
	120	**132**	**252**

Known positive and negative sera were similarly diluted in a 1/100 ratio in dilution buffer before use. The positive and negative sera were distributed in the kit micro ELISA plates which were previously coated with isolated bovine herpesvirus1 in odd columns and control antigens in even columns as follows: 100 µl positive serum was placed in wells A1 and A2, negative serum was equally placed in wells B1 and B2, while test serum was placed in wells C1 and C2 and D1 and D2 wells and so on. The plate was covered with a lit and then incubated at 21°C±3°C for one hour.

The micro plates were emptied of its content by flipping them sharply above the wash hand basin sink. The micro plates were 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 Bio-X diagnostics (Belgium) 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 formation of bubbles in the microplates.

The plates were incubated at 21°C±3°C and subsequently washed with 1x washing solution. 100ul of

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

Area	Total tested	Infection status of animals		Site specific % positivity
		no infection	Positive cases	
Ngaoundere 1	18	16	2	11.1
Ngaoundere II	36	32	4	11.1
NgaoundereIII	36	28	8	22.2
Ngangha	36	30	8	22.2
Martap	36	30	6	16.7
Nyambaka	54	45	9	16.7
Belel	36	31	6	16.7
Total	252	210	42	16.7

the conjugate, protein G peroxidase-labelled, was added to the wells. The plate was incubated a second time at 21°C±3°C. This was followed by another three times washing with the 1x washing solution and 100 µl of the substrate solution prepared as per the instructions of the kit manufacturer, was added to each well and incubated, for 1h at 21°C ±3°C with a lit. Thereafter, the plate was again washed three times as described above.

100 µl of the kit Chromogen (tetramethylbenziden) solution prepared with (12 ml of Na $H_2PO_4.2H_2O$ PH 5.5 in Dimethyl Sulfoxide and 12 µl of H_2O_2 for 96 wells) was added to each well of the plate and incubated for 10 minutes at 21°C±3°C protected from the light and covered. At this stage if specific BoHV-1 immunoglobulins were present in the test sera, the conjugate would remain bound to the micro well that contained the viral antigens combined with antibody and 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 the micro wells in the even columns remained unchanged as there is no virus antibody complex since they act as negative control. The intensity of the resulting blue color is proportionate to the titre of the specific antibody present in the sample and this when measured represents the optical density. After 10 minutes of incubation at 21°C the reaction was stopped using 50 µl per well of the kit 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 obtained using an OPSYS MR (Dynex Technoloies) USA S/N:1MRA-1653 ELISA reader machine.

Evaluation of ELISA results

For each OD value recorded in the odd columns, the signal of the corresponding negative well 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

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

khi^2 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.

RESULTS

Animals showing clinical signs of the disease were characterised by (muco- purulent 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 animals examined had developed Balanoposthitis or pustular vulvovaginitis.

Effect of study sites on level of IBR infection status

Of the 252 animals examined, 210 of them were not infected with Herpes virus 1, while 42 animals were infected. Therefore, antibodies to Bohv-1 were detected in 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 for the disease only 3 of them were infected with the virus while 7 males that did not show any of the clinical signs were actually infected.

Table 3: Point prevalence of IBR in the Vina division

Area	Herd name	Number of animals examined	Number of animals Positive	% positive Within herd
Ngaoundere 1	Market	18	2	11.11
Ngaoundere II	Ali	18	4	22.22
	Ou	18	00	00
NgaoundereIII	Tchabal C	18	5	27.78
	Tchabal A	18	2	11.11
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	00	00
	Nyambaka C	18	00	00
Belel	Tournigal D	18	3	16.67
	Tournigal C	18	5	27.78
Total		252	42	16.67

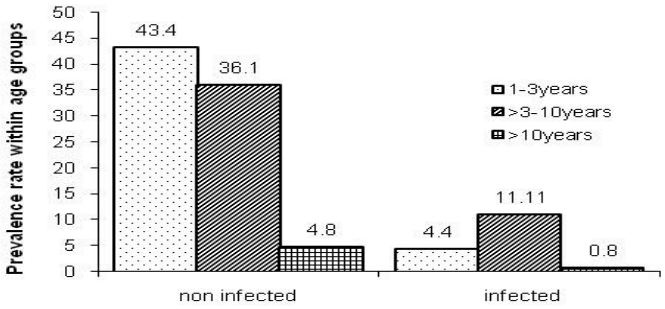

Figure 2. Influence of age on the seroprevalence of Infectious Bovine Rhinotracheitis virus antibodies in zebu cattle in the Vina Division.

Similarly, 33 females were observed with clinical signs but only 11 of them were infected with the virus and 17 females without clinical signs were also found infected.

Effect of herd on infection rate of IBR virus

The highest prevalence (38.89%) was detected in one of the farms/herds examined in Nyambaka (Table 3). No BoHV-1 antibodies were detected in examined animals from three herds: One in Ngaoundere II and two in Nyambaka.

Influence of sex of animal on IBR infection rate

24.2% and 59.1% of males and females, respectively, were not infected while 3.9% and 12.69% of males and females, respectively, had contact with the virus in Vina division. This difference was not significant ($P>0.05$); Khi^2: $P = 0.287$ Khi^2 Pearson's value=1.133[b]

Effect of age of animal on the IBR infection rate

IBR seropositive rates were significantly different ($P<0.05$) only between the animals of age group 0-3 and >3-10 years. This implied that animals within the age group of >3-10 years had the highest contact with the infectious bovine rhinotracheitis virus (11.11%) than the other two age groups defined in this study (Figure 2). The age group which is the most actively involved in reproduction is the >3-10 years group.

DISCUSSION

The importance of BoHV-1 infection as a reproductive pathology and respiratory diseases syndrome is well established and Infectious Bovine Rhinotracheitis 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 immune suppression.

Using indirect ELISA kits supplied by Bio-X Diagnostics in Belgium, antibodies to BoHV-1 were assessed in all but one of the sub divisions of Vina Division. Durham and Sillars (1986) 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 was detected. The highest herd prevalence detected was 38.88±3.9%. This is in accordance with the proposition made by Rweyemamu (1970) 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 (Hassan and Karrar, 1988). 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 (Instar *et al*., 2009). Although a few semi-intensive production systems are emerging in the region, cattle rearing is 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 (Bronsvoort *et al*., 2003). 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 BHV-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 (Whetstone & Evermann, 1988). 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*. (2005) isolated herpes-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 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 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 (2004) 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 (Rajkhowa et al, 2004). It has also been reported that in 50% of adult livestock, most of them had been in contact with IBR (Seal, 2007). Semen of an infected bull may contain BoHV-1, and the virus can thus be transmitted by natural mating and artificial insemination (Parsonson & Snowdon, 1975).

Previous field surveys in Vina Division and Adamawa region on a larger scale reported the occurrence of abortion in cattle (LSR, 1989). Five of the 33 females examined were seropositive for Bovine Herpes virus 1. This confirms the fact that Herpes virus 1 may have contributed to this abortion as indicated by Hassan and Khalda (1985). Hassan and Khalda (1985) actually isolated bovine herpes virus 1 from cattle with a history of abortion in Sudan.

Of all the cattle that indicated other clinical signs like ocular and nasal discharges in our study, 12 were found to be infected with bovine herpes virus 1. This also confirms the fact that bovine herpes virus 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 (Sakhaee *et al*, 2009). The role of Infectious Bovine Rhinotracheitis 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 herpesviridae-BHV-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 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 infectious bovine rhinotracheitis in Egyptian cattle and buffaloes (Mahmoud *et al*, 2009). A detailed molecular characterization of Boh V 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 BoHV-1 antibodies. 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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