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Predictive modelling of the occurrence of Bovine Herpesvirus type 1 and 4 infections in Cameroon

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ABSTRACT

Background: Bovine Herpesvirus types 1 (BoHV-1) and 4 (BoHV-4) contribute to reproductive failure and respiratory disease in cattle, yet their prevalence in Cameroon remains under-reported. Given the lack of vaccination programmes and the economic reliance on livestock in the Sudano-Sahelian zones, understanding the subclinical circulation of these viruses is essential for regional food security.

Methods: To assess past exposure to these viruses, a cross-sectional study was conducted on 938 cattle at regional slaughterhouses in the Adamawa, North and Far North regions of Cameroon. Ante-mortem data (breed, sex, age, BSC, weight, temperature, physiological status) were recorded from randomly sampled cattle. Serum samples were analysed using indirect ELISA for BoHV-1 and BoHV-4 IgG. Biochemical profiles by absorbance reading and hormonal assays by Enzyme-Linked Fluorescent Assay were performed at the National

Veterinary Laboratory (LANAVET), Garoua. Data collected were analysed using multinomial logistic regression to identify significant predictors of positive IgG titres and calculate odds ratios (OR).

Results: High seroprevalence was observed: 92.7% for BoHV-1, 74.9% for BoHV-4, and 71.7% for co-infection. Breed emerged as the primary intrinsic risk factor for BoHV-1, with Akou (OR = 2.1 and OR = 2.4) having a significantly greater influence on Herpesvirus types 1 and 4, respectively. Geographic location, such as the Far North (OR = 9.3) and Akou (OR = 2.3), was the leading factor affecting seroprevalence in cases of co-infection ($P < 0.01$). Biochemical markers served as significant predictors for co-infection; ASAT levels < 13 IU/L and glucose levels between 2–2.59 g/L doubled the risk of co-infection. ALAT levels between 20–22.5 IU/L were associated with a significant reduction in BoHV-4 risk.

Conclusion: To our knowledge, these findings provide the first insights into BoHV-4 seroprevalence and its associated risk factors in one of Cameroon's main livestock hubs. The predictive model indicates that specific breeds and biochemical changes can signal a risk of exposure, offering a tool for targeted veterinary surveillance and farmer education.

Keywords: Cattle, BoHV-1, BoHV-4, Co-infection, Indirect ELISA, Multinomial Logistic Regression, Cameroon.

1. INTRODUCTION

In 2009, the Ministry of Livestock, Fisheries and Animal Industries set itself the goal of substantially increasing national meat consumption in Cameroon. However, the cattle population accounts for 12.83% of meat production in Cameroon, with the north accounting for 81.85% of the country's cattle population [1]. Cattle farming consists mainly of zebu (*Bos indicus*: Goudali, Akou, Djafoun, Peuhl) [2]. Despite the diversity of livestock breeds in Cameroon, productivity remains low and fails to meet the population's needs, primarily due to zootechnical and health issues [3] and, in particular, the lack of control over reproductive disorders [4].

Among the main reproductive problems having a direct impact on reproductive and production performance in cows are abortion, retained placenta, slow uterine involution, and a prolonged calving-to-calving interval, resulting in increased medication costs, a decline in calving rates

and the premature culling of initially high-performing animals [5]. These reproductive performance disorders are also thought to result from the action of numerous infectious agents, whether of bacterial, parasitic, fungal or even viral origin [6]. This condition affects both dairy and beef cattle populations indiscriminately [7]. In some African countries, abortion without clinical signs is a common sequela of numerous abortive infections, with a notable seroprevalence in cases of brucellosis, salmonellosis, leptospirosis is (54.5%), neosporosis (27.3%), Q fever (84.8%) and infection with Bovine Herpesvirus type 4 (90.9%) [8]. Numerous studies conducted outside the African continent have shown that Bovine Virus Disease (BVD) and Infectious Bovine Rhinotracheitis (IBR) are two of the most well-known viral causes of abortion in cattle, with rates reaching 25–60% in a herd [9].

Bovine herpesvirus type 1 (BoHV-1) has attracted global attention since it was first described as a cause of infectious bovine rhinotracheitis (IBR) in dairy cattle in California in 1953. BoHV-1 infection has been associated with Infectious Pustular Vulvovaginitis (IPV) in cows and Infectious Pustular Balanoposthitis (IPB) in bulls [10]. Indeed, IBR was first isolated in Cameroon in 1964 from 36 sera of young cattle from the Northern Region [11] ; subsequently, [12] demonstrated the circulation of anti-BoHV-1 antibodies in the Vina Department (Adamaoua Region) with an overall prevalence of 16.7% and 38% in Nyambaka. Females aged 3–10 years were more susceptible. This figure rises to 72.9% in Bénoué [13].

As for the BoHV-4 virus, it is genetically quite distinct from other bovine Herpesviruses, particularly the infectious bovine rhinotracheitis virus (BoHV-1). The role of BoHV-4 in bovine abortions is a current topic of study, although it is poorly documented [6]. In most cases, it has been associated with infections of the reproductive tract in cattle, particularly during the postpartum period [14].

In view of the above, this study is justified by the lack of data on the circulation of Herpesviruses in Cameroon. Indeed, several diseases have attracted the attention of researchers following numerous cases of reproductive failure in cattle in Cameroon. Diseases such as Brucellosis, Q fever, Campylobacteriosis, etc. have been diagnosed in livestock farms in sub-Saharan Africa. However, other diseases such as Herpesviruses have been implicated in reproductive failures in genetic improvement programmes in most countries worldwide, with a high prevalence of up to 95% [15].

2. MATERIALS AND METHODS

2.1.Type, period, population and study area

This cross-sectional study was conducted in the northern region of Cameroon (Adamaoua, North and Far North) from May 2020 to June 2023.

The choice of this study area is justified by the fact that 83% of Cameroon's cattle population is found in the three regions of the North, Far North and Adamaoua [16]. The livestock and fisheries sectors recorded a growth rate of 2.7% in 2008, with a cattle population of five million head (38% in Adamaoua) according to [17]. Today, the cattle population has risen from 6,859,359 head in 2015 to 10,202,369 head in 2020. Adamaoua alone accounts for 61% of the total livestock population of the three regions (1,546,288 cattle) [18].

The study area falls within two of Cameroon's five agro-ecological zones, namely:

- The Sudano-Sahelian zone (Zone I): which extends beyond 10° north latitude and corresponds to the North and Far North regions. It has a dry season lasting seven to nine months, and low rainfall ranging from 300 to 900 mm/year from north to south. The vegetation here is as varied as the terrain and climate, ranging from equatorial forest (which covers 40% of the country) in the south to savannah and then steppe in the north. It is a zone of transhumant livestock farming for cattle (approximately 38% of the national herd), sheep and goats (INC, 2016).
- The Guinean high savannah zone (Zone II): consisting largely of a vast plateau at altitudes between 900 and 1,500 metres, with peaks reaching 1,800 metres. This is a high-altitude savannah zone with a tropical climate characterised by two seasons per year, occupying 28% of Cameroon's total area and covering mainly the Adamaoua region, the departments of Mbam (in the Central region) and Lom and Djerem (in the Eastern region), with an average annual rainfall of around 1,500 mm. Temperatures are moderate, with a monthly average of 20–26°C (40% of the national cattle herd) (INC, 2016).

2.2 Administrative authorisations

Before commencing field data collection, authorisations were sought from the ESMV and signed by the Rector of Ngaoundere University following the decision: 2020/0164/UN/R/VRE-PDTIC/DAAC/D-ESMV/DAACRS dated 21 April 2020.

2.3 Method and sample size

For sampling, a non-probabilistic method was used (all cattle meeting the selection criteria). The minimum sample size was calculated using [19] formula with a 95% confidence level and a 5% margin of error.

2.4 Characterisation of slaughtered cattle

To have access to the animals, with the help of veterinarians responsible for veterinary health inspections, we entered into physical contact with the owners of the animals to find out the origin of the subject and obtained verbal agreement to carry out *ante-mortem* examinations and *post-mortem* samples from their subject. The latter having found no problem and gave us free access to handle. According to the verbal exchange with the owners in the study, all animals came from local breeders and markets. The phenotypic characteristics of the subjects were noted, namely BSC on a scale of 0 to 5, weight based on chest circumference, age by examination of the dentition or horns, sex by visual inspection (male or female) and physiological status by examination of the uterus (pregnant or not).

2.5 Sample collection

A blood sample of 3–5 ml was taken from the jugular vein immediately after slaughter of the animals in the various abattoirs within the study areas. The method used is Muslim slaughtering called "*Halal*". After physical immobilization of animals without use of anaesthesia, bleeding is carried out by cutting the two carotids and jugulars using a knife sharp. The incision usually used is the section of the vessels in a retrograde manner following a first incision in the neck practices with a knife. A dry, additive-free Vacutainer-type tube was used for collection blood. Each tube was labelled with a number to identify the animal. The blood samples were then stored at 4°C in an insulated cooler packed with dry ice and transported to the laboratory for analysis. Each animal underwent a *post-mortem* examination to determine whether or not macroscopically visible lesions were present in the various organs.

2.6 Sample processing and storage

In the laboratory, the samples were homogenised using a vortex mixer prior to analysis, and the blood samples were centrifuged at 3,500 rpm for 5 minutes. The serum obtained was collected using a micropipette and stored in pre-labelled cryotubes, with the entire sample stored at -20°C until the time of serological analysis.

2.7 Determination of the seroprevalence of bovine herpesviruses types 1 and 4

The kits used to detect anti-BoHV-1 IgG antibodies were the ID Screen® IBR Indirect in bovine serum (REF: IBRS-5P, batch J32) (Appendix 1) and the ID Screen® *BoHV-4* Indirect (REF: BHV4S, batch J34) (Appendix 2). The serological analysis for the detection of anti-BoHV-1 and anti-BoHV-4 IgG antibodies in serum (indirect ELISA) was carried out at the LANAVET in Garoua. This indirect ELISA method involves a number of steps. Interpretation was carried out after calculating the percentage of inhibition (S/P %) for each sample based on the mean optical densities (OD). The interpretation of the , and the qualitative and quantitative assessment for each case were carried out in accordance with the instructions provided in the package leaflet.

2.8 Analysis of biochemical and hormonal parameters

Biochemical and hormonal analyses were carried out at the IRAD laboratory in Wakwa using the BIOREX® Diagnostic kit. The experimental protocol described by the manufacturer was followed. The aim was to assess markers such as total protein, albumin, cholesterol, glucose, creatinine and urea, calcium, phosphorus, and liver enzymes (ASAT/TGO and ALAT/TGP). The assays were of a colorimetric nature and absorbance readings were taken using a spectrophotometer.

As for the hormonal parameters, these were progesterone, oestradiol and testosterone, which were measured using the competitive enzyme-linked immunosorbent assay (ELISA) method with the FINECARE® kit.

2.9 Predictive modelling of the occurrence of bovine Herpesvirus types 1 and 4

Multinomial logistic regression modelling was performed using a selection of variables to estimate the characteristic *odds ratios*. A dispersed distribution would have a negative impact on the modelling, thereby reducing the accuracy of predicting the type of Herpesvirus infection. Consequently, a new classification based on observable categories was proposed. Once the variables had been recoded, the summary of the distributions of the new nominal variables was reconstructed and is presented in Table 2. At the end of each analysis, the determining factors were statistically identified, as were the risk factors for an increase or decrease in the occurrence of a type of herpes virus infection.

Several steps were taken to formulate a predictive equation for positivity to a type of herpes virus infection:

- ✓ We simultaneously selected variables using the Wald criterion, thereby maximising the ability to predict the type of herpes virus infection.
- ✓ The confusion matrix (allows comparison of positive prediction results against actual observations);
- ✓ The accuracy is the model's ability to correctly predict that a positive result is positive and a negative result is negative. The model performs well when scores range from 80% to 90%;
- ✓ The equation using the logistic regression model formula has been written. It involves evaluating a linear combination based on the observed parameters and calculating a probability.

2.10 Statistical analysis s

The data were analysed using IBM SPSS® Statistics software (version 25.0). A one-way analysis of variance (ANOVA) combined with Duncan's post hoc test was performed to compare the prevalence of biochemical and hormonal parameters and animal characteristics according to factors comprising at least three variables. Pearson's correlation was used to determine the strength of the relationship between certain parameters. The Hosmer-Lemeshow test was used to test the significance of the model and the coefficients. It also allowed us to assess whether the probabilities predicted by the model corresponded well with the observed frequencies. For parameters significantly associated with prevalence and considered as potential risk factors, multiple logistic regression was performed to quantify the strength of each association by determining the *odds ratios* (OR). These indicate the increased 'chance' of testing positive for the type of bovine Herpesvirus studied. A prediction model was obtained with good fit and statistical significance ($p\text{-value} < 0.05$) for the selected variables.

- $OR > 1$: factor increasing (risk of) disease occurrence.
- $OR < 1$: factor reducing (protective against) the occurrence of the disease.
- $OR = 1$: No association. Exposure does not influence the likelihood of the disease occurring. The variables that allow for a significant prediction of the type of Herpesvirus infection were selected.

As hypotheses:

- H_0 (Null hypothesis): there is no significant difference between the observed and expected values; the model does not fit well.
- H_1 (Alternative hypothesis): there is no significant difference between the observed and expected values; the model fits well.

Interpretation:

- P-value > 0.05 : the test is not significant. The null hypothesis (H_0) is retained;
- P-value < 0.05 : the test is significant. We reject the null hypothesis (H_0). This indicates a good fit of the model.

3. Results

3.1 Characterisation of the animals

Table 3 shows that the Bororo breeds (Akou 39.9% and Djaffoun 32.3%) are significantly more numerous and predominantly aged 4–7 years across the three regions. 73.8% of the animals were females with NEC 3 (63.1%) and weighing between 201 and 401 kg. Table 4 shows that temperature varies according to region, lesions and physiological status, whilst NEC and weight varied according to region, breed, sex, lesions and physiological status. Table 4 shows that the most commonly observed lesions in the study areas were bronchopneumonia, dermatitis, splenomegaly, mastitis and metritis in Adamaoua; vaginitis in the North; and orchitis. Regarding ocular lesions, cases of conjunctivitis were the most frequently recorded.

3.2 Prevalence of bovine herpesvirus types 1 and 4

Nearly 93% of subjects tested positive for Type 1, whilst 75% of the same subjects tested positive for Type 4 (Table 5). This significant difference suggests that there are two distinct predispositions leading to positivity for either Type 1 or Type 4. The Akou breed was twice as at risk as the Goudali breed in terms of seroprevalence for BoHV types 1 and 4 (Table 6). In cases of co-infection, seroprevalence varied significantly according to breed and region (Table 7).

3.3 Analysis of the animals' biochemical and hormonal parameters

According to Table 8, there is a positive correlation between creatinine and ALAT, creatinine and oestradiol, and oestradiol and progesterone in subjects positive for type 4. As for type 1, as the creatinine level increases, so do the levels of ALAT and total protein; the same applies to the relationship between ALAT and total protein (Table 9). Its progesterone level is strongly correlated with oestradiol. As it increases, the oestradiol level increases positively (Table 10).

In cases of co-infection, the mean testosterone value varies in male subjects according to NEC and weight in the different regions ($P < 0.05$) (Table 11).

3.4 Predictive modelling of the occurrence of bovine herpesvirus types 1 and 4

3.4.1 Modelling of positivity for type 1

Table 12 of the coefficient model shows that being of the Bokolo and Djafoun breeds increases the risk of being diagnosed with type 1 by 4.2 times and 1.1 times respectively. Similarly, another factor increasing the risk is the cow's weight: the higher the weight, the greater the likelihood of the cow being diagnosed with type 1. The lower the weight, the less likely the cow is to be of type 1. The remaining factors reduce the likelihood of being of type 1, but do not eliminate it.

The model equation: formulated as a probability calculation (P).

If we write:

$$T1 = 1.178 + 0.956 * \text{CALCIUM from 2.45 to 2.65} - 2.396 * \text{CALCIUM Less than 2.44} - 0.221 * \text{GLUCOSE from 2 to 2.59} - 1.374 * \text{GLUCOSE Less than 2} - 1.212 * \text{CREATININE from 0.8 to 1} - 0.367 * \text{CREATININE Less than 0.8} + 0.009 * \text{WEIGHT} + 0.078 * \text{RACE_Djafoun} + 1.447 * \text{RACE_Bokolo} - 4.782 * \text{RACE_Akou}$$

$$P+ (\text{BoHV1=positive}) = \frac{1}{1 + \text{EXP}^{-T1}}$$

$$P- (\text{BoHV1=negative}) = 1 - P+ (\text{BoHV1=positive})$$

The cow will be positive for type 1 if $P+ > P-$ and negative for type 1 if $P+ < P-$

Where P is the probability

$P+$ is the probability of being positive

$P-$ probability of being negative

3.4.2 Modelling of BoHV4 positivity

An ALAT level of less than 20 U/L increases the risk of being diagnosed with type 4 by a factor of 2.2, whereas an ALAT level of 20 to 22.5 U/L significantly reduces it.

The same applies to an AST level below 13 U/L versus an AST level of 13 to 14 U/L. Furthermore, a glucose level below 2.59 g/L is also a risk factor predisposing to a type 4

diagnosis. A calcium level of 2.45 to 2.65 mmol/l increases the risk of being diagnosed with type 4 by 1.6 times, whereas a calcium level below 2.44 mmol/l significantly reduces it. Another risk factor is the subject's phosphorus level: the higher it is, the greater the likelihood of being diagnosed with type 4, increasing the risk by a factor of 2.6. The lower the level— the less likely the animal is to be type 4 (Table 13). The remaining factors reduce the likelihood of being type 4, but do not eliminate it.

The model equation: formulated as a probability calculation (P).

If we write:

$$T4 = 9.390 + 0.805 * ALAT < 20 - 0.761 * ALAT \text{ from } 20 \text{ to } 22.5 + 1.017 * ASAT \text{ less than } 13 - 0.444 * ASAT \text{ from } 13 \text{ to } 14 + 0.213 * GLUCOSE \text{ Less than } 2 + 0.997 * GLUCOSE \text{ 2 to } 2.59 - 0.811 * TOTAL \text{ PROTEIN less than } 4.7 - 1.371 * ALBUMIN \text{ less than } 6.8 - 1.280 * CALCIUM \text{ less than } 2.44 + 0.509 * CALCIUM \text{ 2.45 to } 2.65 - 1.055 * UREA \text{ less than } 6.65 + 0.941 * PHOSPHORUS - 0.050 * CHOLESTEROL$$

$$P+ (\text{BoHV4=positive}) = \frac{1}{1 + \text{EXP}^{-T4}}$$

$$P- (\text{BoHV4=negative}) = 1 - P+ (\text{BoHV4=positive})$$

The cow will be type 4 if $P+ > P-$ and negative for type 4 if $P+ < P-$

3.4.3 Modelling of co-infection (positive for BoHV-1 and BoHV-4)

A glucose level of less than 2 g/l up to 2.59 g/l is also a risk factor predisposing the animal to being diagnosed as infected with types 1 and 4 simultaneously. Furthermore, an ALAT level below 20 U/L increases the risk of an animal having a co-infection with types 1 and 4 by 1.5 times, whereas an ALAT level of 20 to 22.5 U/L significantly reduces it. An ASAT level of less than 13 U/I increases the risk of an animal having a co-infection with types 1 and 4 by 2.4 times, whereas an ASAT level of 13 to 14 U/I significantly reduces it (Table 14). Whilst the remaining factors reduce the likelihood of a Type 1 and Type 4 co-infection, they do not eliminate it.

The model equation: formulated as a probability calculation (P).

If we write:

$$Tco = 8.440 + 0.005 * WEIGHT - 0.040 * CHOLESTEROL + 0.441 * ALAT \text{ less than } 20 - 0.639 * ALAT \text{ from } 20 \text{ to } 22.5 + 0.892 * ASAT \text{ less than } 13 - 0.396 * ASAT \text{ from } 13 \text{ to } 14 + 0.135 * GLUCOSE \text{ less than } 2 + 0.985 * GLUCOSE \text{ from } 2 \text{ to } 2.59 - 0.696 * TOTAL$$

PROTEIN less than 4.7 - 1.176* ALBUMIN less than 6.8 - 1.245* CALCIUM less than 2.44
+ 0.584* CALCIUM From 2.45 to 2.65 - 0.948* UREA less than 6.65

$$P+ (\text{BohV1 and 4=positive}) = \frac{1}{1 + \text{EXP}^{-T_{co}}}$$

$$P- (\text{BohV1 and 4=negative}) = 1 - P+ (\text{BohV1 and 4=positive})$$

The cow will be type 1&4 if $P+ > P-$ and negative for type 1&4 if $P+ < P-$

4. DISCUSSION

This study was conducted in northern Cameroon (Adamaoua, North and Far North) with the aim of modelling the seroprevalence of Herpesviruses types 1 and 4 in relation to associated risk factors. The objective was to determine the prevalence and risk factors in the study, and to assess the influence of biochemical and hormonal parameters in subjects seropositive for Herpesviruses types 1 and 4.

4.1 Characterisation of the animals

The average age of the cattle was 5.4 ± 1.20 . This result differs from that of [20] and [4]. It is important to note that the study was conducted on all animals entering the abattoir, regardless of their age. According to these authors, cattle suffer from various pathologies, which have an impact on weight gain, resulting in low NEC; this could explain the average NEC of 2.63 ± 0.6 obtained. The Mbororo breed is found mainly in all study areas; however, the Goudali breed is virtually absent in the north and the far north; this could be explained by the fact that the Goudali breed originates from Adamaoua and is also known as the Adamaoua Fulbe zebu, a name that specifies both the traditional herders (Fulbe) and the locality (Adamaoua) [21]. The average weight of the cattle was 332.06 ± 66 kg; a result similar to that of [2] for zebu cows in Adamaoua.

The most commonly observed post-mortem lesions in the various study areas were bronchopneumonia affecting the respiratory system. The [22] report, as part of the surveillance of bovine diseases in France, states that 36.05% of animals subjected to post-mortem examination presented with respiratory lesions; similarly, this report shows that the age group most affected comprises animals under 2 years of age. Ante-mortem respiratory signs were reported in 24.3% of the case histories received. Half of these were associated with lesions visible at post-mortem.

4.2 Prevalence of Bovine Herpesvirus types 4, 1 and Coinfection

Bovine Herpesvirus type 4 is a ubiquitous disease found worldwide, responsible for cases of abortion in both dairy and beef cattle herds [8]. The overall seroprevalence of bovine Herpesvirus 4 was 74.8% in this study, a figure close to the 70% recorded in Zaire by [23]. In Cameroon, there appear to be no data available that could serve as a basis for assessing the distribution of the virus across different regions. At present, it is difficult to determine whether the circulation of the virus is increasing or decreasing. Nevertheless, in the Maghreb region, particularly in Morocco, a prevalence of 50% was found among dairy cows with a history of abortion on farms. Based on the existing data in this country, the circulation of the virus has been increasing over the years [8]. In several other countries, the prevalence of Herpesvirus type 4 was low compared to that of the present study. This is the case with the results obtained in Sudan by [24] at a rate of 47.2%, as well as in Turkey at 41.3% [25], 3.61% in Algeria [26], 6% and 7.9% respectively in the Quebec and Ontario regions of Canada [27], and 42% in Turkey [28] by real-time PCR using serum samples from cows with endometritis diagnosed by endoscopy. The prevalence rates in the North and the Far North, at 94.9% and 97.7% respectively, were significantly higher than that of Adamawa (56%) in this study. The difference in results is thought to be due to the fact that cattle in the North and Far North undergo significant migration in search of pasture, as well as cross-border movements to neighbouring countries. In contrast, the prevalence of BoHV-4 antibodies in the milk of 176 cows tested in Canada, from dairy herds, was 98.2% [15]. Similarly, a serological survey conducted in Colombia on a serobank of 959 samples from female animals of different ages reported a BoHV-4 seropositivity rate of 95.4% by immunofluorescence [29]. The technique used to diagnose seropositivity may influence the results obtained, as may the nature of the sample, whether it is milk, whole blood, aborted foetal secretions, serum, semen, follicular fluid, etc. [30]. The indirect ELISA test used in this study has also been employed in several other scientific research studies due to its high sensitivity, specificity, relative ease of use, speed of execution and capacity to process a large number of samples. Furthermore, its reagents are generally inexpensive, stable and easy to prepare [31].

Infectious Bovine Rhinotracheitis (IBR) and infection with Bovine Herpesvirus type 4 are highly contagious diseases with a global distribution. They cause considerable economic losses in cattle herds, particularly through induced abortions [32]. The prevalence of IBR was over 90% in the three regions of the study. Previous studies conducted in Cameroon reported prevalences of 72.9% in Benoué, 16.7% in Vina and 28% in the North by [13,12,11], respectively, thus confirming the endemic presence of the virus in Cameroon. In other countries

around the world, seroprevalence rates have also been determined, and the results obtained were lower than those of this study, namely 45.7% in Spain [33], 77.27% in Senegal [34] and 44.71% in Turkey [35]. The difference in the results obtained may therefore be due to variability in various parameters such as sample transport-given that these are viral diseases sensitive to thermal effects-herd health management practices on farms, vaccination programmes implemented or not in certain countries, climate, farming methods, feeding, geographical factors, number of pregnancies and disinfection [36]. Only the Akou breed was twice as at risk as the Goudali breed. However, in Belgium, a study showed that only increasing age is a risk factor for seropositivity in beef cattle [37].

The subjects co-infected with BoHV-1/BoHV-4 in this study had a seroprevalence of 71.7%. In contrast to certain studies conducted in Turkey, where it was 8.6% and 2.0% [38, 39], and 11.4% in the case of triple co-infection with BoHV-1/BVD/BoHV-4 in Turkey [38].

According to surveys conducted in the various regions, very few farmers observed quarantine procedures when new animals were introduced to the herd, which is thought to facilitate the spread of the virus. Similarly, stress factors such as transport during transhumance in search of pasture are a common practice observed in all three regions covered by the study. Indeed, these two viruses can become latent and be reactivated by various stressors such as transport, changes in environment, calving and intercurrent diseases in infected cattle [32]. The introduction of animals in the incubation phase or latent carriers onto farms without quarantine, as well as the expression of clinical signs in these animals upon reactivation, is most often non-apparent, making their clinical detection difficult despite the fact that they continue to shed the viruses and infect other cattle [32].

4.3 Analysis of biochemical and hormonal parameters in animals

Herpesviruses are more common in intensive systems, particularly during periods of dietary deficiency, vitamin A deficiency or a stressful environment, and farm size is a predominant factor [40]. There are no documented publications regarding biochemical and hormonal variations in response to BoHV-4. Nevertheless, the results demonstrated that the presence of BoHV-4 in animals leads to biochemical and hormonal changes. Indeed, the main factor influencing variation in total serum protein concentration is diet [41]. An overall decrease in serum concentrations of energy parameters, specifically glucose, cholesterol and total protein compared to their reference values, as well as in ASAT levels, was observed. Cholesterol levels in the study subjects were below the reference value (200–240 mg/dl, [42]. High seropositivity in cases of Herpesviruses types 1 and 4 could be linked to the fragility of the tissue membranes

of the cells targeted by the viruses. Indeed, cholesterol is one of the components of the cells' phospholipid membrane. When this membrane is weak, it is easily penetrated by herpes viruses. Research by [43] in China showed that when viral cholesterol, as in the case of BoHV-1, is reduced, viral replication is inhibited and the body's cells are not colonised. Glucose plays a role in several bodily mechanisms. Indeed, the plants consumed by the animals are rich in cellulose, which is converted into glucose by microorganisms. This drop in glucose levels may also be the cause of liver failure, leading to the liver's inability to produce and store glucose. Indeed, an energy deficiency impairs the follicles' ability to produce sufficient oestradiol to ensure ovulation in the cow. Follicles that begin their growth during the energy-deficient phase contain less IGF-1; they are recruited to a lesser extent and develop more slowly. When they reach the dominant follicle stage, they synthesise little oestrogen, their ovulatory capacity is low, and they produce lower-quality oocytes. It appears that the mean progesterone values fall within the confidence interval, whilst those for oestradiol are above the interval. The average oestradiol level is relatively higher (1.4 ng/ml) regardless of the stage of the oestrous cycle compared to reference values. This could correspond to a follicular phase in the oestrous cycle of the study subjects.

The serum concentration of ASAT also decreased compared to the reference value. As ASAT is a less specific marker of the liver than ALAT, this enzyme is present in the cytoplasm and predominantly in the mitochondria of hepatocytes and in almost all muscles; the decrease in this enzyme is not significant.

However, these results show an overall elevated albumin level compared to the reference value. Indeed, an increase in the albumin fraction indicates dehydration and must be confirmed by the haematocrit. This elevated albumin level could be explained by the fact that the animals were dehydrated prior to slaughter.

The average testosterone value is low (1.54 ng/l) compared to the reference value. However, it is significantly higher in older males. This result is lower than that of [44], who studied the changes in plasma concentrations of testosterone and *insulin-like growth factor 1* during the peripubertal period in Belgium and obtained a level of 2.9 ± 0.3 ng/ml. This result may be explained by the fact that our study was conducted on cattle of all ages; different locations of investigation; and/or even by various testicular conditions. A strong 99% correlation between several biochemical parameters such as ALAT and creatinine, with a positive correlation coefficient, means that when ALAT increases, creatinine increases. There is also a strong 99%

correlation between glucose and creatinine, but the correlation coefficient is negative, meaning that when one parameter increases, the other decreases

4.4 Predictive modelling of the occurrence of bovine Herpesvirus types 1 and 4

For each case of Herpesvirus infection, a model equation based on probability calculations was formulated for each type of Herpesvirus infection. The factors taken into account in these equations were solely those increasing the likelihood (risk factors) of the disease occurring. The other factors were classified as those reducing the likelihood (protective factors) of the disease occurring. All these different parameters must be monitored to ensure better epidemiological surveillance of bovine diseases.

5. CONCLUSION

To our knowledge, these findings provide the first insights into BoHV-4 seroprevalence and its associated risk factors in one of Cameroon's main livestock hubs. The predictive model highlights that specific breeds and biochemical shifts can signal exposure risk, providing a tool for targeted veterinary surveillance and farmer education.

LIST OF ABBREVIATIONS

ALAT	:	Alanine aminotransferase
ASAT	:	Aspartate aminotransferase
BSC	:	Body Score Condition
ESMV	:	School of Veterinary and Medecine Sciences
IgG	:	Immunoglobulin G
IRAD	:	Agricultural Research Institute for Development
LANAVET	:	National Veterinary Laboratory
REF	:	Reference
VET ID	:	Innovative Diagnostics Veterinary

Ethical Approval: A field work Authorization was obtained from the School of Veterinary Medicine and Sciences of the University of Ngaoundere, Cameroon as approval to allow us to

conduct the research (2020/0164/UN/R/VRE-PDTIC/DAAC/D-ESMV/DAACRS, valid until 21 April 2020).

Consent for publication: All the information and data provided in this manuscript have been collected by the various stakeholders. And as principal authors, all data resulting from this work can be used and exploited to advance science.

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Table 1: List of 16 quantitative variables.

Variables	Minimum	Maximum	Average	Standard deviation
AGE (years)	2.0	9.0	5.4	1.2
WEIGHT (Kg)	100.0	657.0	333.6	67.3
TEMPERATURE (°C)	28.0	41.3	37.8	0.8
CREATININE (mg/dl)	0.6	1.3	0.9	0.1
ALAT (u/l)	12.7	25.7	20.8	1.8
ASAT (u/l)	11.8	17.2	14.0	1.0
PHOSPHORE (mmol/l)	0.8	2.3	1.4	0.3
CHOLESTEROL (mg/dl)	120.2	221.1	190.2	8.9
GLUCOSE (g/l)	1.3	4.6	2.6	0.7
TOTAL PROTEIN (mg/dl)	3.9	5.8	4.7	0.2
ALBUMINE (g/l)	5.1	7.8	6.8	0.3
CALCIUM (mmol/l)	2.1	3.3	2.7	0.3
UREA (mg/dl)	3.4	7.4	6.5	0.7
PROGESTERONE (ng/ml)	0.0	2.5	0.8	0.5
OESTRADIOL (ng/ml)	0.0	2.3	1.0	0.8
TESTOSTERONE (ng/ml)	0.0	2.4	0.4	0.7

Table 2: Observation processing summary

		N	Percentage
CREATININE	Less than 0.8	201	21.7%
	From 0.8 to 1	408	43.8%
	More than 1	322	34.2%
ALAT	Less than 20	424	45.6%
	From 20 to 22.5	303	32.6%
	More than 22.5	202	21.7%
ASAT	Less than 13	163	17.5%
	from 13 to 14	196	21.1%

	More than 14	570	61.4%
GLUCOSE	Less than 2	273	29.4%
	From 2 to 2.59	90	9.7%
	More than 2.59	566	60.9%
TOTAL PROTEIN	Less than 4.7	557	60.0%
	More than 4.7	372	40.0%
ALBUMIN	Less than 6.5	103	11.1%
	from 6.5 to 6.86	592	63.7%
	More than 6.86	234	25.2%
CALCIUM	Less than 2.44	89	9.6%
	From 2.45 to 2.65	365	39.3%
	More than 2.65	475	51.1%
UREA	Less than 6.65	553	59.5%
	More than 6.65	376	40.5%
OESTRADIOL	Less than 1	490	52.7%
	More than 1	439	47.3%

Variables	Modality	Adamawa	North	Far North	Total	P value
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Table 3 : Distribution of zootechnical characteristics of animals according to regions

		N (%)	N (%)	N (%)		
Numbers		500 (53.3)	218 (23.2)	220 (23,5)	938 (100)	
Breeds	Akou	162 (32.4)	127 (58.3)	85 (38.6)	374 (39.9)	0.000
	Bokolo	33 (6.6)	36 (16.5)	3 (1.4)	72 (7.7)	
	Djaffoun	121 (24.2)	52 (23.9)	130 (59.1)	303 (32.3)	
	Gudali	184 (36.8)	3 (1.4)	2 (8.3)	189 (20.1)	
Sex	Male	127 (25.4)	64 (29.4)	55 (25)	246 (26.2)	0.987
	Femelle	373 (74.6)	154 (70.6)	165 (75)	692 (73.8)	
Age (years)	[0 – 3]	13 (2.6)	13 (6.0)	6 (2.7)	32 (3.4)	0.000
	[4 – 7]	454 (90.8)	203 (93.1)	199 (90.5)	856 (91.3)	
	[8 – 11]	33 (6.6)	2 (0.9)	15 (6.8)	50 (5.3)	
Temperatures (C)	< 37.9	360 (72.0)	172 (78.9)	138 (62.7)	670 (71.4)	0.001
	[38 – 39]	121 (24.2)	43 (19.7)	71 (32.3)	235 (25.1)	
	> 39	19 (3.8)	3 (1.4)	11 (5.0)	33 (3.5)	
BSC	1	27 (5.4)	16 (7.3)	4 (1 .8)	47 (5.0)	0.000
	2	142 (28.4)	77 (35.3)	53 (24.1)	272 (29.0)	
	3	314 (62.8)	123 (56.4)	155 (70.5)	592 (63.1)	
	4	17 (3.4)	2 (0.9)	8 (3.6)	27 (2.9)	
Weigth (kg)	[0 – 200]	3 (0.6)	10 (4.6)	7 (3.2)	20 (2.1)	0.001
	[201 –401]	445 (89.0)	196 (89.9)	197 (89.5)	838 (89.3)	
	[402 –602]	52 (10.4)	12 (5.5)	16 (7.3)	80 (8.5)	
Physiological statut	Pregnant	90 (24.1)	38 (24.7)	40 (23.8)	168 (24.2)	0.983
	Not pregnant	283 (75.9)	116 (75.3)	25 (76.2)	424 (75.8)	

Table 4: Characterization according to age, weight, NEC and temperature.

Variables	Modality	Age (years)	Weigth (kg)	NEC	Température (C)
		Moy±Ec	Moy±Ec	Moy±Ec	Moy±Ec
Regions	Adamaoua	5.5±1.29	337.3±69.45 ^b	2.64±0.63 ^b	37.8±0.88 ^a
	Nord	5.3±1.06	322.3±6.48 ^a	2.51±0.65 ^a	37.7±0.48 ^a
	Extrême-Nord	5.5±1.27	336.6±63.09 ^b	2.76±0.54 ^c	37.9±0.65 ^b
	P-value	0.093	0.018	0.000	0.006
Breed	Akou	5.4±1.09 ^b	334.1±58.40 ^b	2.6±0.62 ^b	37.8±0.93
	Bokolo	4.3±1.34 ^a	264.2±78.23 ^a	2,4±0.63 ^a	37.9±0.59
	Djaffoun	5.4±1.06 ^b	334.2±56.30 ^b	2.6±0.62 ^b	37.8±0.65
	Gudali	5.9±1.46 ^c	357.2±78.30 ^b	2.8±0.58 ^c	37.8±0.59
	P-value	0.000	0.0 00	0.000	0.883
Sex	Female	5.3±1.56	324.1±56.79	2.5±0.61	37.8±0.75
	Male	5.9±1.04	360.2±85.15	2.9±0.58	37.7±0.78
	P-value	0.000	0.000	0.000	0.071
Lesions	Absent	5.4±1.24	337.4±66.18	2.8±0.52	37.7±0.71
	Present	5.3±1.19	322.1±69.48	2.3±0.75	38.1±0.83
	P-value	0.376	0.003	0.000	0.000
Physiological statut	Pregnant	5.7±1.04	349.2±43.53	2.7±0.54	38.8±0.86
	Not pregnant	5.1±0.99	316.1±58.19	2.5±0.63	37.8±0.77
	P-value	0.000	0.000	0.000	0.000

Table 5: Overall prevalence of BoHV-1, BoHV- 4 and coinfection (BoHV 1 and 4) case according to study regions

	BoHV 1		BoHV 4		Coinfection (BoHV 1 and 4)	
Négative	69	7.3%	236	25.1%	266	28.3%
Positive	872	92.7%	705	74.9%	675	71.7%
Total	941	100.0%	941	100.0%	941	100.0%

Table 6 : BoHV-1 and BoHV-4 seroprévalence according to sociodemographics parameters

Factors	Variables	Data set					
		BoHV-1 Ni (%)	OR (IC 95%)	p-value	BoHV-4 Ni (%)	OR (IC 95%)	p-value
Regions	Adamawa	457 (91.6)	Ref	-	280 (56.0)	Ref	-
	Far -North	208 (94.5)	1.9 (0.9-3.9)	0.074	214 (95.4)	10.6 (6.1-18.5)	<0.01*
	North	208 (95.4)	1.5 (0.8-3.1)	0.170	208 (93.1)	16.5 (8.5-31.9)	<0.01*
Breeds	Gudali	170 (89.5)	Ref	-	113 (59.5)	Ref	-
	Akou	306 (95.6)	2.1 (1.1-4.1)	0.029*	262 (81.9)	2.4 (1.7-3.5)	<0.01*
	Bokolo	69 (95.8)	2.5 (0.7-8.6)	0.158	60 (83.3)	2.7 (1.4-5.2)	<0.01*
	Djaffoun	281 (93.7)	1.4 (0.7-2.6)	0.332	236 (78.1)	2.3 (1.5-3.4)	<0.01*
Sex	Male	232 (91.7)	Ref		199 (78.7)	Ref	-
	Female	641 (93.6)	1.2 (0.7-2.2)	0.425	503 (73.4)	0.8 (0.6-1.1)	0.176
Age (years)	[0 - 3]	27 (84.4)	Ref	-	28 (87.5)	Ref	-
	[4 - 7]	798 (93.2)	2.6 (0.9-6.9)	0.059	632 (73.8)	0.4 (0.1-1.1)	0.077
	[8 - 11]	48 (96.0)	4.4 (0.8-24.5)	0.087	42 (84.0)	0.6 (0.2-2.3)	0.508
BSC	1	46 (97.9)	Ref	-	32 (68.1)	Ref	-
	2	249 (91.5)	0.2 (0.0-1.8)	0.162	200 (73.5)	1.0 (0.5-2.1)	0.990
	3	551 (93.1)	0.3 (0.0-2.2)	0.240	447 (75.5)	1.1 (0.6-2.1)	0.765
	4	27 (100)	-	0.998	23 (85.2)	1.6 (0.5-5.3)	0.380

Ni : Number of seropositive animals

Table 7 : Séroprévalence of BoHV-1 and BoHV-4 coinfection according to sociodemographics parameters

Factors	Variables	Seroprevalence of coinfection (BoHV-1 and BoHV-4) Ni (%)	OR (IC 95%)	p-value
Regions	Adamawa	275 (55.0)	Ref	-
	Far -North	202 (91.8)	9.3 (5.5-15.4)	< 0.01 *
	North	199 (91.2)	8.6 (5.2-14.3)	< 0.01 *
Breeds	Gudali	111 (58.7)	Ref	-
	Akou	289 (77.1)	2.3 (1.6-3.4)	< 0.01 *
	Bokolo	52 (75.4)	2.1 (1.2-4.0)	< 0.01 *
	Djaffoun	224 (73.4)	1.9 (1.3-2.9)	0.01 *
Sex	Male	190 (75.1)	Ref	
	Female	486 (70.9)	0.8 (0.6-1.1)	0.256
Age (years)	[0 - 3]	25 (78.1)	Ref	-
	[4 - 7]	609 (71.1)	0.6 (0.3-1.6)	0.386
	[8 - 11]	42 (84.0)	1.5 (0.5-4.5)	0.504
BSC	1	32 (68.1)	Ref	-
	2	194 (71.3)	1.2 (0.6-2.3)	0.652
	3	427 (71.9)	1.2 (0.6-2.3)	0.571
	4	23 (85.2)	2.7 (0.8-9.2)	0.113

Table 8: Correlation between biochemical parameters among themselves and between biochemical and hormonal parameters of BoHV-4 positive animals

parameters		CREATININ E	ALAT	ASAT	PHOSPH ORUS	CHOLESTE ROL	GLUCOS E	TOTAL _PROTEINE	ALBUMIN	CALCIU M	UREA	PROGESTERO NE	OESTRA DIOL	TESTOSTER ONE
CREATININE	Pearson Correlation	1												
	Sig. (2- tailed)													
	N	702												
ALAT	Pearson Correlation	.266**	1											
	Sig. (2- tailed)	.000												
	N	702	702											
ASAT	Pearson Correlation	-.072	-.060	1										
	Sig. (2- tailed)	.057	.110											
	N	702	702	702										
PHOSPHORUS	Pearson Correlation	-.052	-.076*	-.065	1									
	Sig. (2- tailed)	.166	.043	.083										
	N	702	702	702	702									
CHOLESTEROL	Pearson Correlation	.039	.064	-.025	-.004	1								
	Sig. (2- tailed)	.299	.093	.501	.912									
	N	702	702	702	702	702								
GLUCOSE	Pearson Correlation	-.325**	-.092*	.205**	-.030	.047	1							
	Sig. (2- tailed)	.000	.015	.000	.424	.212								
	N	702	702	702	702	702	702							

TOTAL_ PROTEINE	Pearson Correlation	.073	.162**	.000	-.258**	.126**	-.095*	1						
	Sig. (2-tailed)	.054	.000	.997	.000	.001	.012							
	N	702	702	702	702	702	702	702						
ALBUMIN	Pearson Correlation	.082*	.091*	-.078*	.102**	-.075*	-.178**	.070	1					
	Sig. (2-tailed)	.031	.016	.039	.007	.046	.000	.065						
	N	702	702	702	702	702	702	702	702					
CALCIUM	Pearson Correlation	-.271**	.019	.295**	-.277**	-.032	.367**	.027	-.195**	1				
	Sig. (2-tailed)	.000	.613	.000	.000	.397	.000	.478	.000					
	N	702	702	702	702	702	702	702	702	702				
UREA	Pearson Correlation	.117**	-.078*	-.177**	.183**	-.043	-.159**	-.413**	-.072	-.178**	1			
	Sig. (2-tailed)	.002	.038	.000	.000	.260	.000	.000	.056	.000				
	N	702	702	702	702	702	702	702	702	702	702			
PROGESTERONE	Pearson Correlation	-.077*	-.062	-.005	-.047	.115**	.018	.050	-.097*	.087*	-.036	1		
	Sig. (2-tailed)	.042	.103	.897	.214	.002	.629	.184	.010	.021	.343			
	N	702	702	702	702	702	702	702	702	702	702	702		
OESTRADIOL	Pearson Correlation	.138**	.009	-.061	-.049	.111**	-.159**	.111**	.017	-.075*	-.063	.768**	1	-.775**
	Sig. (2-tailed)	.000	.819	.104	.199	.003	.000	.003	.652	.048	.094	.000		.000
	N	702	702	702	702	702	702	702	702	702	702	702	702	702
TESTOSTERONE	Pearson Correlation	.034	.023	-.019	.007	-.074	-.011	-.017	.081*	-.071	-.016	-.890**	-.775**	1
	Sig. (2-tailed)	.371	.549	.617	.854	.051	.771	.648	.032	.062	.667	.000	.000	
	N	702	702	702	702	702	702	702	702	702	702	702	702	702

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 9: Correlation between the biochemical parameters of positive BOHV-1 animals.

		CREATININE	ALAT	ASAT	PHOSPHORUS	CHOLESTEROL	GLUCOSE	TOTAL PROTEIN	ALBUMIN	CALCIUM	UREA
CREATININE	Pearson Correlation	1									
	Sig. (2-tailed)										
ALAT	Pearson Correlation	0,235**	1								
	Sig. (2-tailed)	0.000									
ASAT	Pearson Correlation	-0.033	-0.066	1							
	Sig. (2-tailed)	0.330	0.052								
PHOSPHORUS	Pearson Correlation	-0.082*	-0.047	-0,073*	1						
	Sig. (2-tailed)	0.016	.0167	0.031							
CHOLESTEROL	Pearson Correlation	-0.076*	0.063	-0.037	0.031	1					
	Sig. (2-tailed)	0.024	0.061	0.273	0.359						
GLUCOSE	Pearson Correlation	-0346**	-0.038	0.147**	-0.041	0.108**	1				
	Sig. (2-tailed)	0.000	0.259	0.000	0.231	0.001					
TOTAL PROTEIN	Pearson Correlation	0.088**	0.182**	-0.020	-0.209**	0.101**	-0.079*	1			
	Sig. (2-tailed)	0.009	0.000	0.557	0.000	0.003	0.019				
ALBUMIN	Pearson Correlation	0.033	0.097**	-0.102**	0.161**	-0.060	-0.0150**	0.090**	1		
	Sig. (2-tailed)	0.336	0,004	0.003	0.000	0.078	0.000	0.008			
CALCIUM	Pearson Correlation	-0.325**	-0.015	0.262**	-0.260**	0.057	0.391**	-0.041	-0.235**	1	
	Sig. (2-tailed)	0.000	0.668	0.000	0.000	0.090	0.000	0.221	0.000		
UREA	Pearson Correlation	0.079*	-0.052	-0.194**	0.197**	-0.023	-0.117**	-0.401**	-0.073*	-0.131**	1
	Sig. (2-tailed)	0.020	0.125	0.000	0.000	0.493	0.001	0.000	0.031	0.000	
**. Correlation is significant at the 0.01 level (2-tailed).											
*. Correlation is significant at the 0.05 level (2-tailed).											

Table 10: Correlation between hormones in positives BoHV-1 females

		Progestérone	Œstradiol
Progestérone	Pearson correlation		1
	p-value		
Œstradiol	Pearson correlation	0.674**	1
	p-value	0.000	

Table 11: Variation of testosterone according to intrinsic and extrinsic factors of causes coinfectcd with BoHV -1 and BoHV -4.

Variables	Modalities	Testosterone level (Mean $\pm$ sd)	p-value
Regions	Adamawa	1.72 $\pm$ 0.33 ^b	0.000
	Far-North	1.70 $\pm$ 0.45 ^b	
	North	1.31 $\pm$ 0.53 ^a	
BSC	1	1.91 $\pm$ 0.54 ^b	0.014
	2	1.45 $\pm$ 0.58 ^a	
	3	1.56 $\pm$ 0.44 ^a	
	4	1.84 $\pm$ 0.35 ^b	
Weigth (kg)	[0 ; 3]	1.25 $\pm$ 0.64 ^a	0.001
	[4 ; 7]	1.57 $\pm$ 0.46 ^b	
	[8 ; 11]	1.83 $\pm$ 0.29 ^b	
Breeds	Akou	1.59 $\pm$ 0.51	0.149
	Bokolo	1.48 $\pm$ 0.46	
	Djaffoun	1.51 $\pm$ 0.49	
	Gudali	1.71 $\pm$ 0.36	

Table 12: The coefficients of the Bohv type 1 positivity model

	Coef	P- VALUE	OR	CI 95% of OR		Type of factor
				Lower limit	Upper limit	
Breed_Akou	-4.782	0.000	0.008	0.002	0.033	Reduction factor
Breed_Bokolo	1.447	0.055	4.252	0.967	18.700	Risk factor
Breed_Djafoun	0.078	0.843	1.082	0.498	2.347	Risk factor
Weigth	0.009	0.004	1.009	1.003	1.014	Risk factor
CREATININE less than 0.8	-0.367	0.563	0.693	0.200	2.403	Reduction factor
CREATININE from 0.8 to 1	-1.212	0.011	0.298	0.117	0.758	Reduction factor
GLUCOSE less than 2	-1.374	0.002	0.253	0.104	0.613	Reduction factor
GLUCOSE from 2 to 2.59	-0.221	0.685	0.801	0.275	2.336	Reduction factor
CALCIUM less than 2.44	-2.396	0.000	0.091	0.038	0.219	Reduction factor
CALCIUM from 2.45 to 2.65	0.956	0.090	2.601	0.860	7.861	Risk factor
Constant	1.178	0.279				

Table 13: The coefficients of the Bohv type 4 positivity model

	Coef	P- VALUE	OR	CI 95% of OR		Type of factor
				Lower limit	Upper limit	
ALAT less than 20	0.805	0.005	2.236	1.280	3.906	Risk factor
ALAT from 20 to 22.5	-0.761	0.007	0.467	0.269	0.811	Reduction factor
ASAT less than 13	1.017	0.001	2.765	1.488	5.137	Risk factor
ASAT from 13 to 14	-0.444	0.056	0.642	0.407	1.011	Reduction factor
GLUCOSE less than 2	0.213	0.462	1.238	0.701	2.185	Risk factor
GLUCOSE from 2 to 2.59	0.997	0.007	2.711	1.321	5.561	Risk factor
TOTAL PROTEIN less than 4.7	-0.811	0.001	0.444	0.281	0.702	Reduction factor
ALBUMIN less than 6.8	-1.371	0.000	0.254	0.162	0.397	Reduction factor
CALCIUM less than 2.44	-1.280	0.000	0.278	0.148	0.521	Reduction factor
CALCIUM from 2.45 to 2.65	0.509	0.069	1.663	0.962	2.876	Risk factor

UREA less than 6.65	-1.055	0.000	0.348	0.210	0.577	Reduction factor
PHOSPHORUS	0.941	0.013	2.563	1.218	5.393	Risk factor
CHOLESTEROL	-0.050	0.000	0.951	0.929	0.975	Reduction factor
Constant	9.390	0.000				

Table 14: The coefficients of the coinfection (Bohv type 1 and 4) positivity model

Variables	Coef	P- VALUE	OR	CI 95% of OR		Type of the factor
				Lower limit	Upper limit	
Weigth	0.005	0.000	1.005	1.002	1.007	Reduction factor
CHOLESTEROL	-0.040	0.000	0.961	0.940	0.982	Reduction factor
ALAT less than 20	0.441	0.088	1.553	0.936	2.578	Risk factor
ALAT from 20 to 22.5	-0.639	0.016	0.528	0.314	0.888	Reduction factor
ASAT less than 13	0.892	0.002	2.441	1.379	4.320	Risk factor
ASAT from 13 to 14	-0.396	0.062	0.673	0.444	1.020	Reduction factor
GLUCOSE less than 2	0.135	0.604	1.144	0.688	1.902	Risk factor
GLUCOSE from 2 to 2.59	0.985	0.004	2.677	1.370	5.229	Risk factor
TOTAL PROTEIN less than 4.7	-0.696	0.001	0.499	0.327	0.759	Reduction factor
ALBUMIN less than 6.8	-1.176	0.000	0.309	0.207	0.461	Reduction factor
CALCIUM less than 2.44	-1.245	0.000	0.288	0.160	0.517	Reduction factor
CALCIUM from 2.45 to 2.65	0.584	0.018	1.793	1.103	2.915	Risk factor
UREA less than 6.65	-0.948	0.000	0.388	0.245	0.612	Reduction factor
Constant	8.440	0.000				