Veterinary Sciences and Medicine

RESEARCH ARTICLE

# Bayesian Analysis for Bovine and Bubaline Herpes Virus Cross-Reaction in Bubaline Serum Sample

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## Abstract

**Background and Objectives**: Considering the possibility of false-positives in the bovine herpes virus diagnostic tests, this study evaluated how probable to occur a cross-reaction among bovine herpes virus BoHV1, BoHV5 and BuHV1 is and how this can impact in a prevalence estimation.

**Methods**: 101 bubaline serums were sampled from a slaughterhouse located in Boituva City-SP (Brazil). The search for anti-BoHV1 and anti-BoHV5 was carried out by virus-neutralization technique (adapted from OIE protocol). On the other hand, the evaluation of the presence of anti-BuHV1 was developed by ELISA technique (commercial indirect ELISA kit). From the results of the diagnostic, the conditional probabilities were calculated. For those calculation, each virus was considered reference and compared with the other two species separately.

**Results**: The condition of higher probability of cross-reaction was P(BoHV1+|BuHV1+) = 0.97, which means the probability of an animal is positive for BoHV1, given it is infected for BuHV1. On the other hand, the condition of lower probability was P(BuHV1+|BoHV5+) = 0.86, which indicates the probability of an animal is positive for BuHV1, given it is infected for BoHV5. Moreover, if the prevalence of BuHV1 is higher than 7.8%, at least half of positive cases detected for BoHV5 may indeed be BuHV1. The inverse thinking is considered if the investigator is interested to estimate the prevalence of BuHV1 where there are cases of BoHV5. In this case, the "threshold" prevalence of BoHV5 is 5.3%.

**Conclusions**: The prevalence estimation of BuHV1 is the most influenced by the previous prevalence of BoHV1. In opposition, the prevalence estimation of BoHV1 is the less influenced by the previous prevalence of BuHV1.

**Keywords:** Bayesian analysis; Bovine and bubaline herpes virus; Cross-reaction; Diagnostic test

## Introduction

In Brazil, buffalo farming has been expanding. This fact reflects the advantageous characteristics that buffaloes have and investments in this sector, such as genetic improvements, high fertility, low morbidity and mortality, greater capacity in fiber digestion, productive longevity and great adaptability in the most adverse conditions [1].

The buffalo herd in Brazil is estimated at 1,405,654 animals, with the state of São Paulo owning 107,995 of them, according to the last census published by the Ministry of Agriculture, Livestock and Supply [2].

Bovine alpha herpes virus types 1 (αBoHV-1) and 5 (αBoHV-5) are etiological agents that cause economic losses due to reproductive and/or neurological losses in cattle herds [3]. Both viruses are economically important pathogens and are recognized for their ability to establish latent infections in neuronal ganglia [4]. Despite the fact that cattle are naturally infected with BoHV-1, and that serological studies indicate a high spread in herds throughout the country, studies on the spread of this disease in the buffalo species are scarce [5] It is important to note that viral latency, the presence of asymptomatic animals, the lack of diagnosis and of control

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measures are factors that contribute to the permanence of the virus in herds [4].

In the buffalo species there are reports of BoHV-1. However, information about the frequency is still scarce [5], as well as BoHV-5 [5], showing that these viruses probably are not species-specific, as it has been attributed to the Herpesviridae family. According to [6], the buffalo alpha herpes virus (BuHV-1) (which causes subclinical infection in buffaloes) is associated with abortion, although the pathogenicity is unclear, especially in relation to loss of productive and reproductive efficiency. Another aspect that must be considered is the role of the buffalo in the epidemiological chain of alpha herpes viruses in cattle, since some properties adopt crop-Livestock System [7].

In Italy, the presence of bubaline alpha-1 herpes virus type-1 (BuHV-1) has also been reported, in contrast to cross-reactions with BoHV-1 and BoHV-5 [8]. In a preliminary research carried out by the team of the Laboratory of Bovine Viruses of Biological Institute of São Paulo State – Brazil (LBV/BI), it were detected buffalo with antibodies against bovine Herpesvirus type 5 and bubaline type 1, from Vale do Ribeira Region and Southwest of the State of São Paulo (unpublished data).

Considering the possibility of false-positives in the bovine herpes virus diagnostic tests, this study evaluated how probable to occur a cross-reaction among bovine herpes virus BoHV1, BoHV5 and BuHV1 is. In addition, the impact of such cross-reaction in a prevalence investigation is discussed.

## Material and Methods

### Sampled Animals and Collected Material for Analysis

101 bubalines were sampled from a slaughterhouse located in Boituva City (São Paulo State, Brazil). Those animals were sent from 7 cities of this region, distributed as following: Salto de Pirapora (6 animals); Capão Bonito (33); Itapetininga (14); Pariquea-açu (18); Cerquilho (9); Tapirai (8); Cerqueira César (13). Those animals had their serum collected September to November 2019.

The dataset considered in this study had obtained from usual work routine of our laboratory. In this case, since the dataset was acquired from services provision of the laboratory, the Biological Institute periodically evaluates the adequation of the laboratory activities in order to provide the ethical approval. In this case, the ethical approval was registered according to the formal letter LVB 013/20, dated in 2020 June 08th.

### Laboratory Analysis

The collected serums were analyzed for presence of antibodies anti-BoHV1, anti-BoHV5 and anti-BuHV1. The search for anti-BoHV1 and anti-BoHV5 was carried out by virus-neutralization technique (VN). On the other hand, the evaluation of the presence of anti-BuHV1 was developed by ELISA technique (Commercial indirect ELISA kit).

### Virus Neutralization for Abohv-1 and Abohv-5

The search for anti-BoHV-1 and anti-BoHV-5 antibodies was carried out using the VN technique, according to OIE (2018) protocol adapted by LBV/BI (Fig. 1). This protocol is briefly presented as follow.

Prior to the reaction, the serum samples were subjected to complement inactivation, in a water bath at 56ºC for 30 minutes.

The VN reaction for αBoHV-1 or αBoHV-5 was performed on independent plates. In general, the serum samples were diluted in minimum essential medium (MEM), pH 7.0, in the proportion 1: 2, 96 well cell culture plate. Thus, the dilution was from 2 to 2048. The suspension of each viral strain (αBoHV-1 or αBoHV-5) was added to its corresponding plate in the volume of 50μL, in the concentration of 100 TCID50 / 50μL.

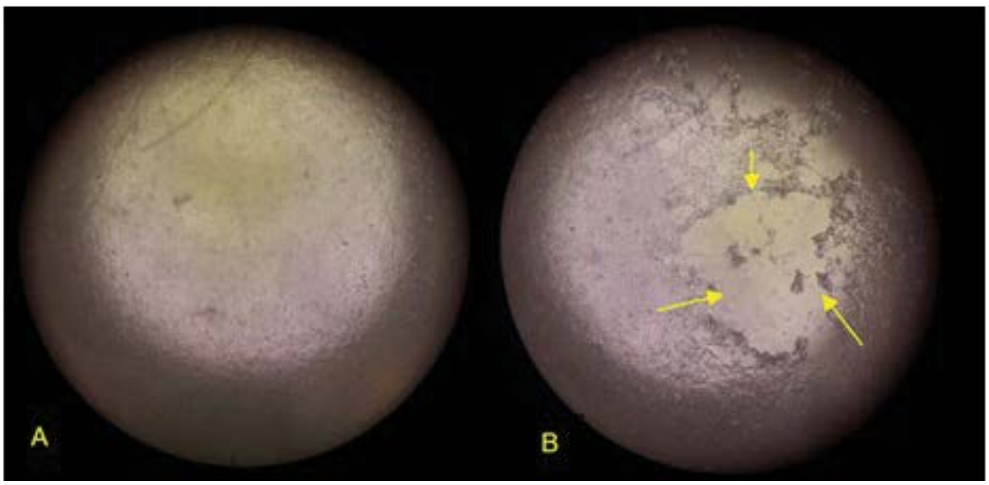


**Figure1.** Examples of a Virus Neutralization Assay, where A illustrates a reactive result and B, a non-reactive result.

In order to promote viral adsorption, the plates were incubated at 37 ° C with 5% CO2 for 24 hours and 100µL of Madin Darbin Bovine Kidney (MDBK) cell suspension was added at a concentration of 3x105 cells/mL. The plates were incubated in the same conditions as before for up to 72 hours and the reading was performed under an inverted optical microscope.

The neutralizing titer was considered to be the reciprocal of the highest dilution of the serum capable of neutralizing viral replication. Antibody titers were determined using the Spearman-Karber method. The animal was considered reactive when it had an antibody titre equal to or higher than the cutoff point (log 0.3), demonstrated by the intact cell monolayer.

Samples that did not show neutralizing activity at the lowest dilution were considered non-reactive. In these cases, it was possible to observe the presence of a cytopathic effect (ECP), with refringent cells and in the shape of "grape clusters".

The VN reactions were valid when the infective dose control plates (100 to 3000 TCID50 / 50µL), viral re-titration (that is, the viral strain had the previously determined titer) and serum control (without cytotoxicity) showed the expected results.

**Commercial Indirect ELISA Kit**

The commercial kit Eradikit® BoHV-1 and BuHV-1 from IN3 Diagnostic was used for the research of anti-BuHV-1 antibodies. Such ELISA kit was developed by the Zooprofilattico Sperimentale del Piemonte, Liguria and Valle d'Aosta Institute (Italy), which was kindly donated by this Institution. This kit aims to differentiate anti-BoHV-1 antibodies from BuHV-1.

**The Bayesian Approach for Result Analysis**

The Bayes theorem is among the basis of Evidence-Based Medicine. This theorem considers two definitions. The first is *a priori probability* (P (event)), which refers to probabilities related to an event in the absence of any information explaining the presence or absence of some fact. The second is *a posteriori probability* (P (event|evidence)), which is the conditional probability of an event where there is some evidence related to these phenomena [9].

The application of Bayesian theory can be explained by the following illustration. Suppose that an investigator needs to estimate the prevalence of positive individuals for virus A. However, there is a possibility of cross-reactivity among virus A and virus B. In this case, the question is what could be the probability of detect the virus B although he is detecting virus A. In other words, this refers to *a posteriori probability* $P(B+|A+)$. Consider the following information:

- The probability of a diagnostic test detects a positive result for virus A, given the individual has already been positive for virus B, is $P(A+|B+) = 0.90$;
- The probability of a diagnostic test detects a positive result for virus A, given the individual has already been negative for virus B, is $P(A+|B-) = 0.02$;
- The investigator expects the prevalence of positive results for virus B is $P(B+) = 0.05$ (the *a priori probability*) and, consequently, $P(B-) = 0.95$.

$$P(B+|A+) = \frac{P(A+|B+) \times P(B+)}{P(A+)} = \frac{P(A+|B+) \times P(B+)}{P(A+|B+) \times P(B+) + P(A+|B-) \times P(B-)} \quad (1)$$

The estimation of $P(B+|A+)$ is given by Bayes equation (Eq. 1).

Substituting the hypothetical values in Eq. 1:

$$P(B+|A+) = \frac{0.90 \times 0.05}{0.90 \times 0.05 + 0.02 \times 0.95} \cong 0.70 \quad (2)$$

Finally, $P(B+|A+) \cong 0.70$. This means that the probability of the diagnostic test has detected virus B among the results positive for A is 70%. Observe that $P(A+|B+) \cong 0.90$ and $P(A+|B-) \cong 0.02$ were values that characterize the diagnostic test and, therefore, they were parameters for this calculation. On the other hand, the expected prevalence $P(B+)$ was the source of variability of this analysis. The value from $P(B+)$ was provided before any evidence or previous information, being considered a subjective information in some cases. Thus, the prevalence of the virus (in this example, the virus B) which may react with the investigated virus (the virus A) can influence on the final result of the investigation (that is, the estimation of prevalence of virus A, $P(A+)$).

Once the sampled animals provided serum for diagnostic test for BoHV1, BoHV5 and BuHV1, the conditional probabilities were calculated. For that calculation, each virus was considered reference (the same idea as the virus B in the example before) and compared with the other two species separately (similar to the virus A). Thus, 6 contingency tables were elaborated and the analysis of prevalence of each virus was discussed.

## Results

At total, 101 animals were sampled from 9 cities in São Paulo State. All collected serums were tested for BoHV1, BoHV5 and BuHV1. In the case of BoHV1 and BoHV5, all virus neutralization tests provided conclusive results. However, 3 results from ELISA test for BuHV1 were considered inconclusive and, therefore, excluded from this study.

The following contingency tables (Table 1 to 3) present the results of cross-reaction among bovine herpes virus. From such tables, it was calculated the conditional probabilities of an animal that is already infected for a specific bovine herpes virus presenting positive result for a different bovine herpes virus.

The contingency tables above provided the parameters for Bayesian analysis. Thus, considering the Eq. 1 and assuming that the *a priori* probability is the source of variability for the *a posteriori* probability, the probability of the diagnostic test detecting the virus reference among the positive results of the virus of interested is presented in the figures above (Fig. 2 to 4).

From Fig. 2 to 4, all curves presented the same dynamics, that is, they showed fast increase for low values of *a priori* probabilities and such increase slow down when it reaches

**Table 1:** Contingency tables of the results for BoHV5 and BuHV1, considering the condition of BoHV1 as reference. In grey, there is the number of tests that were positive for both viruses. Under each table, there is the conditional probability for each case.

| | | BoHV1 (VN) | | |
|---|---|---|---|---|
| | | + | - | Total |
| BoHV5 (VN) | + | 34 | 3 | 37 |
| | - | 3 | 61 | 64 |
| | Total | 37 | 64 | 101 |

| | | BoHV1 (VN) | | |
|---|---|---|---|---|
| | | + | - | Total |
| BuHV1 (ELISA) | + | 32 | 1 | 33 |
| | - | 3 | 62 | 65 |
| | Total | 35 | 63 | 98 |

Probability of an animal BoHV1+ being BoHV5+:

$$P(BoHV5+|BoHV1+)=\frac{34}{37}=0.92$$

Probability of an animal BoHV1– being BoHV5+:

$$P(BoHV5+|BoHV1-)=\frac{3}{64}=0.05$$

Probability of an animal BoHV1+ being BuHV1+:

$$P(BuHV1+|BoHV1+)=\frac{32}{35}=0.91$$

Probability of an animal BoHV1– being BuHV1+:

$$P(BoHV5+|BoHV1-)=\frac{1}{62}=0.02$$

**Table 2:** Contingency tables of the results for BoHV1 and BuHV1, considering the condition of BoHV5 as reference. In grey, there is the number of tests that were positive for both viruses. Under each table, there is the conditional probability for each case.

| | | BoHV5 (VN) | | |
|---|---|---|---|---|
| | | + | - | Total |
| BoHV1 (VN) | + | 34 | 3 | 37 |
| | - | 3 | 61 | 64 |
| | Total | 37 | 64 | 101 |

| | | BoHV5 (VN) | | |
|---|---|---|---|---|
| | | + | - | Total |
| BuHV1 (ELISA) | + | 30 | 3 | 33 |
| | - | 5 | 60 | 65 |
| | Total | 35 | 63 | 98 |

Probability of an animal BoHV5+ being BoHV1+:

$$P(BoHV1+|BoHV5+)=\frac{34}{37}=0.92$$

Probability of an animal BoHV5– being BoHV1+:

$$P(BoHV1+|BoHV5-)=\frac{3}{64}=0.05$$

Probability of an animal BoHV5+ being BuHV1+:

$$P(BuHV1+|BoHV5+)=\frac{30}{35}=0.86$$

Probability of an animal BoHV5– being BuHV1+:

$$P(BoHV1+|BoHV5-)=\frac{3}{60}=0.05$$

**Table 3:** Contingency tables of the results for BoHV1 and BoHV5, considering the condition of BuHV1 as reference. In grey, there is the number of tests that were positive for both viruses. Under each table, there is the conditional probability for each case.

| | | BuHV1 (ELISA) | | |
|---|---|---|---|---|
| | | + | - | Total |
| BoHV1 (VN) | + | 32 | 3 | 35 |
| | - | 1 | 62 | 63 |
| | Total | 33 | 65 | 98 |

| | | BuHV1 (ELISA) | | |
|---|---|---|---|---|
| | | + | - | Total |
| BoHV5 (VN) | + | 30 | 5 | 35 |
| | - | 3 | 60 | 63 |
| | Total | 33 | 65 | 98 |

Probability of an animal BuHV1+ being BoHV1+:

$$P(BoHV1+|BuHV1+)=\frac{32}{33}=0.97$$

Probability of an animal BuHV1– being BoHV1+:

$$P(BoHV1+|BuHV1-)=\frac{3}{65}=0.05$$

Probability of an animal BuHV1+ being BoHV5+:

$$P(BoHV5+|BuHV1+)=\frac{30}{33}=0.91$$

Probability of an animal BuHV1– being BoHV5+:

$$P(BoHV5+|BuHV1-)=\frac{5}{65}=0.08$$

values close to 1.00. However, the P(BoHV1+ | BuHV1+) (the dashed line from Fig. 2) crossed the *a posteriori* probability of 0.50 with the lower value of *a priori* probability, when compared with the other *a posteriori* probabilities. On the other hand, the P(BuHV1+ | BoHV5+) (the thick line in Fig. 4) presented the opposite result compared to P(BoHV1+ | BuHV1+), that is, it was the *a posteriori* probability that crossed the value of 0.50 with the greater value for *a priori* probability. The Table 4 presents those values in details.

From Table 4, two sceneries deserve some attention. First, when the prevalence of BoHV1 is 0.017, the probability P (BoHV1+ | BuHV1+) = 0.50. Second, if the prevalence of BuHV1 is 0.078, the probability P(BuHV1+ | BoHV5+) = 0.50. Comparing the both cases, the first one presented a lower prevalence than the second one, in order to obtain the same *a posteriori* probability. Thus, a low circulation of BoHV1 is need to cause an "aleatory" detection of the test between BoHV1 or BuHV1 (since the *a posteriori* probability is 0.50,

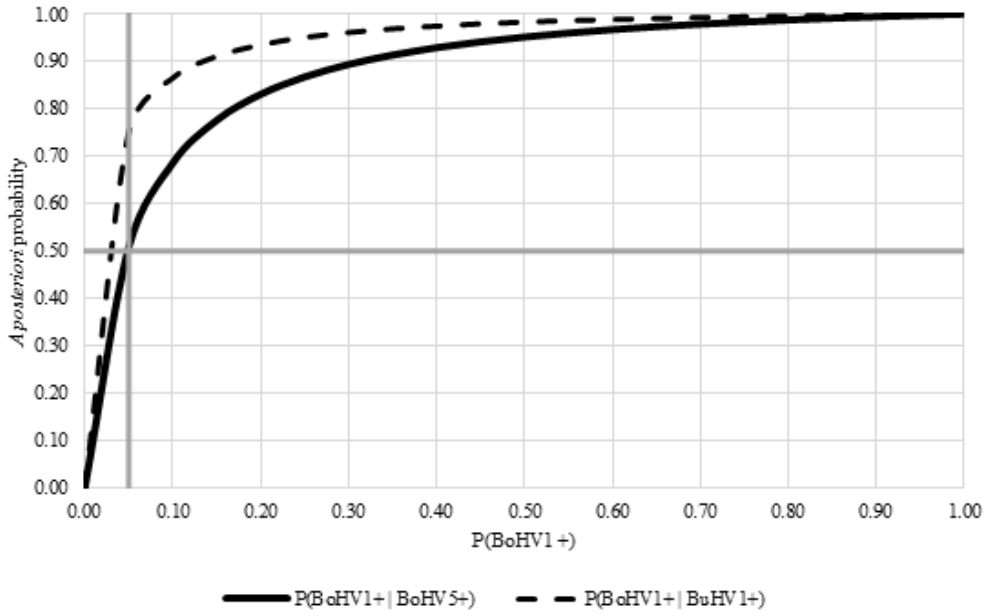


**Figure2.** *A posteriori* probability, considering BoHV1 as reference. Note that the probability of obtaining results positive for BoHV1 among the positive results for virus of interested (in this case, BoHV5 or BuHV1) increases according to the BoHV1 prevalence. The vertical grey line indicates P(BoHV1 +) = 0.05. The horizontal grey line indicates the *a posteriori* probability of 0.50.

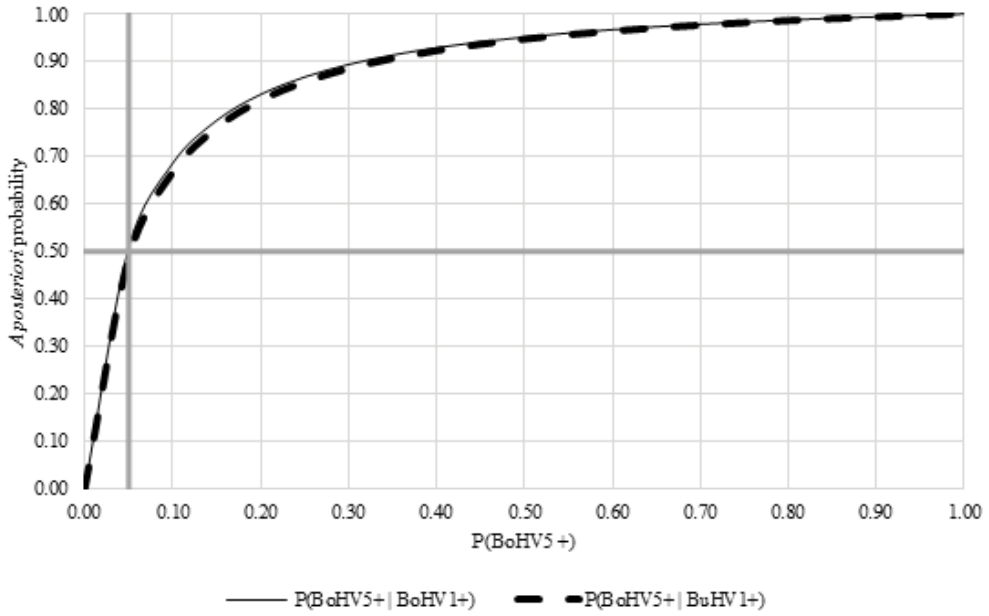


**Figure3.** *A posteriori* probability, considering BoHV5 as reference. Note that the probability of obtaining results positive for BoHV5 among the positive results for virus of interested (in this case, BoHV1 or BuHV1) increases according to the BoVH5 prevalence. The vertical grey line indicates P(BoHV5+) = 0.05. The horizontal grey line indicates the *a posteriori* probability of 0.50.

there is an equilibrium about the probability occurrence of cross-reaction). The same idea is observed in the second case, but it is necessary a higher prevalence of BuHV1 in order to make the same effect with BoHV5.

The Table 4 above also presents the conditions in which it is possible to minimize the probability of cross-reaction. For instance, suppose an investigator is interested to estimate the prevalence of BoHV5 in an area where there also are cases of BuHV1. If the expected prevalence of BuHV1 is higher than 7.8%, the probability of the investigator indeed detects BuHV1 instead of BoHV5 will be at least 50.0%. In practical terms, if the prevalence of BuHV1 is 7.8%, half of positive cases detected for BoHV5 may be BuHV1. The inverse thinking is considered if the investigator is interested to estimate the prevalence of BuHV1 where there are cases of BoHV5. In this case, the "threshold" prevalence of BoHV5 is 5.3%.

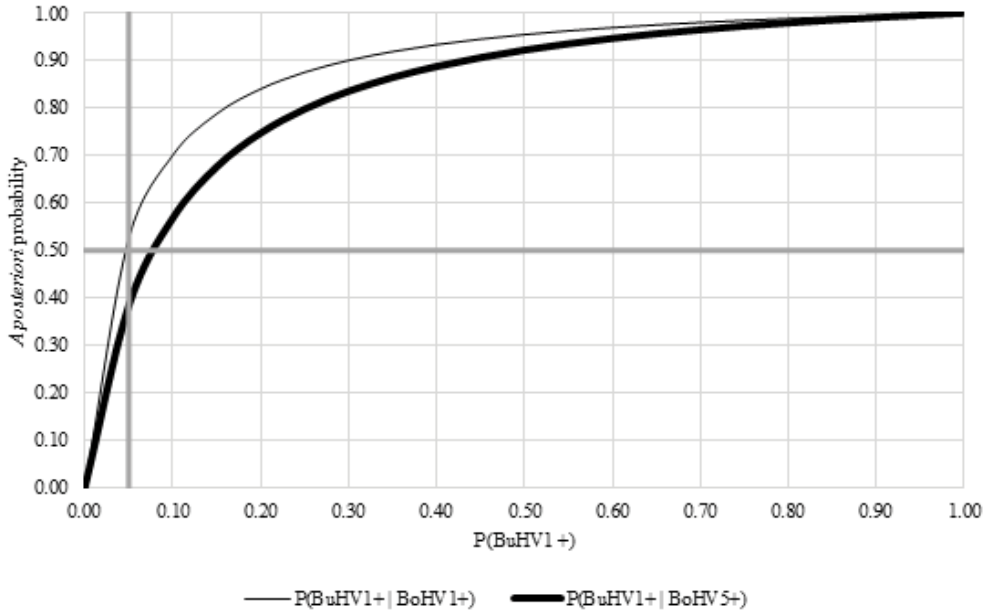


**Figure4.** *A posteriori* probability, considering BuHV1 as reference. Note that the probability of obtaining results positive for BuHV1 among the positive results for virus of interested (in this case, BoHV1 or BoHV1) increases according to the BoHV1 prevalence. The vertical grey line indicates P(BuHV1 +) = 0.05. The horizontal grey line indicates the *a posteriori* probability of 0.50.

**Table 4:** Values of the estimated prevalence P (Infec +) for each of the investigated virus, for which the probability of a positive test (Test +) detecting the virus of previous infection being equal to 0.50 (that is, P(Infec + | Test +) = 0.50).

| | Prevalence of previous infection of the cross-reactive virus. (Infec +) | | |
|---|---|---|---|
| Positive test for the investigated virus. (Test +) | BoHV1 | BoHV5 | BuHV1 |
| BoHV1 | - | 0.049 | 0.046 |
| BoHV5 | 0.049 | - | 0.078 |
| BuHV1 | 0.017 | 0.053 | - |

Since the threshold prevalence of BuHV1 is higher than the BoHV5's one, it is more probable to detect BoHV5 when there is BuHV1 than in an inverse situation (to detect BuHV1 when there is BoHV5). Thus, in order to minimize the probability of cross-reaction, the prevalence of previous infection should be lower than the values presented in Table 4.

## Discussion

In this study, it was evaluated the probability of cross-reaction among three species of herpes virus (BoHV1, BoHV5 and BuHV1), considering a sample of bubaline serum. For this purpose, 101 bubaline serums were sampled from a slaughterhouse in Boituva City (São Paulo State, Brazil) and they were tested for BoHV1 and BoHV5 by virus neutralization technique and for BuHV1 by indirect ELISA technique. From those tests results, 6 contingency tables were considered (each one standing an analysis between two viruses, one as reference and the other as the investigated one). In addition, it was discussed the impact of those cross-reactions possibilities on the prevalence investigations of such herpes viruses.

The analysis presented here allowed a probabilistic approach about those three viruses in terms of serological cross-reaction, considering bubaline individuals. [10] Presented the importance of improve the knowledge about those bovine herpes viruses, since the infection on alternative species of ruminants may work as source of infection. In terms of disease control strategies and economic development, the lack of knowledge about the alternative sources of infection is an important limitation.

For the best of our knowledge, the work developed here is the first one that evaluate the probability of cross-reaction among BoHV1, BoHV5 and BuHV1 in bubaline individuals. There are works that refers to co-infection of two or more specie of virus in the cattle, as the study published by [3], in which he has presented a prevalence study about the co-infection of BoHV1 and BoHV5 in Uruguayan beef cattle. In other study, [10] found that there is evidence of horizontal transmission of BuHV1 from infected buffaloes to cattle housed with them. In terms of diagnostic performance for BoHV1, BoHV5 and BuHV1, the present study deserves some highlight, since it focused on bubaline individuals. Most of the studies have concentrated their efforts of cross-reaction studies on cattle, like [11, 12 and 13].

The results obtained in this study indicated that not only the cross-reaction among the BoHV1, BoHV5 and BuHV1 may occur in diagnostic tests for bubaline individuals, but also this fact may lead to wrong decisions for strategy controls. According to the work developed by [14], the water buffalo can

be susceptible to both BuHV1 and BoHV1 infections, rising concern on the role of water buffaloes in the epidemiology of BoHV1 infection. This finding support the result presented in this present work, where a prevalence of 0.017 of BoHV1 infection on buffalo population is enough to generate a posteriori probability P(BoHV1+ | BuHV1+) = 0.50 (which means that the positive result of a test for BuHV1 in bubaline individuals may be due to reactivity to BoHV1 with 0.50 of probability). For this reason, the need of reliable diagnostic tools is highly desirable especially in countries where IBR is under control program and water buffalo farms has gained increasing interest [14]

In terms of frequency, the number of tests that were simultaneously positive between two viruses were very similar (30 tests positives for BuHV1 and BoHV5; 32 for BuHV1 and BoHV1; and 34 for BoHV1 and BoHV5). Considering the total of tests (101 for BoHV1 and BoHV5; 98 for BuHV1), the frequency of being positive for two viruses was between 0.31 to 0.34. Assuming that there are no co-infections, the diagnostic test may mistake in one of each three performed tests.

According to the results from contingency table, there were a real possibility of diagnostic tests generate some mistaken in their results, due to such cross-reactivity. Because of this, the use of those diagnostic tests for a prevalence investigation may be influenced by the estimated prevalence of the virus that can cross-react (defined in this work as the "reference virus") with the investigated one (see Fig. 2 to 4). When the prevalence of the reference virus is low, the probability of a positive result of an investigated virus also being positive for the reference one (*a posteriori* probability) is low too. However, once the prevalence of the reference virus increase, the *a posteriori* probability also increases, due to the fact that more reference virus can be mixed in the total virus population. In the case of the scenario where BoHV1 (the reference virus) and BuHV1 (the investigated virus) are considered (Fig. 2, a little increase on BoHV1 prevalence make the *a posteriori* probability much higher. In practical terms, the investigation of BuHV1 prevalence where the BoHV1 prevalence is high can results in a high probability of mistaken results.

Comparing the BuHV1 (reference virus) with BoHV5 (investigated virus), it was observed the lowest *a posteriori* probability among the evaluated sceneries (Fig. 4). In this case, even though the prevalence of BuHV1 may increase, the *a posteriori* probability would not do it in the same rate as the other sceneries. In practical terms, when the prevalence of BoHV5 is investigated where BuHV1 is also present, the diagnostic tests mentioned in this work are more probable to differentiate them, when compared with the other sceneries.

Few studies have investigated on the cross-reaction among those three herpes virus species, in particular regarding to buffalo population [14]. Thus, studies about the performance of those diagnostic tests are important to understand what the possibilities to obtain mistaken results are and what should be done in terms of improving them. Since the buffalo farm production has been increasing in Brazil, it is necessary to enhanced technologies that aim to optimize such production.

The analyzed data in this paper were provided by serological tests. Therefore, none molecular test, neither investigation about genetic/antigenetic similarities, was considered. Because of this, this work did not approach such topic and the results provided by the serological test should be interpreted according to different prevalence values for these three investigated viruses. It is recommended future works in respect to understand how the genetic/antigenetic similarities can be related to the results presented in this paper. In addition, In Brazil, there are recombinant enzyme immunoassay kits against antibodies for BoHV1, when considering Glycoprotein B. However, in the case of BoHV5, there are no kits available. If available, it would be possible to clarify the level of cross-reactivity between those two virus specimens.

Finally, it is important to clarify that the sample of animals considered in this study was obtained from a convenience sampling. Thus, such sample may not be representative of bufallos' population. For instance,[15] alerted in their study that diagnostic metrics (as sensitivity, specificity, positive and negative predictive values) should be carefully considered in an individual approach. In this case, [15] concluded that populations may present specific values of those metrics for each subpopulation which composed it. In other words, studies where bufallos' additional characteristics may be considered for evaluate how the level of population's heterogeneity can interfere in Bayes theorem application.

## Conclusions

The results of this study indicated that the condition of higher probability of cross-reaction was $P(BoHV1 + | BuHV1 +) = 0.97$ , which means the probability of an animal is positive for BoHV1, given it is infected for BuHV1. On the other hand, the condition of lower probability was $P(BuHV1 + | BoHV5 +) = 0.86$ , which indicates the probability of an animal is positive for BuHV1, given it is infected for BoHV5. The other conditions and sceneries of cross-reaction presented probability values between those two probabilities above.

As consequence of those probabilities of cross-reaction, the prevalence estimation of BuHV1 is the most influenced by the previous prevalence of BoHV1. In opposition, the prevalence estimation of BoHV1 is the less influenced by the previous prevalence of BuHV1.

## References

1. Bernardes O (2017) Produção de búfalas leiteiras. In: IV Simpósio Nacional de Bovinocultura de Leite. Porto Alegre; Brazil. Accessed 2018 May 16th. [View Article]

2. Brasil, Ministério da Agricultura, Pecuária e Abastecimento (2019) Available in. Accessed 2019 April 10th. Only in Portuguese. [View Article]

3. Junqueira JRC, Alfieri AA (2006) Falhas da reprodução na pecuária bovina de corte com ênfase para causas infecciosas. Semina: Ciências Agrárias 27: 289-298. doi: 10.5433/1679-0359.2006v27n2p289. [View Article]

4. Puentes R, Campos FS, Furtado A, Torres FD, Franco AC, et al. (2016) Comparison between DNA Detection in Trigeminal Nerve Ganglia and Serology to Detect Cattle Infected with Bovine Herpesviruses Types 1 and 5. PLoS One. 11: e0155941. doi: 10.1371/journal.pone.0155941. [View Article]

5. Medeiros DM, Campos FS, Lima M, Hubner SO, Vargas GDA, et al. (2019). Infecção latente pelo herpesvírus bovino tipo 1 em búfalos (Bubalus bubalis) no Rio Grande do Sul. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 71: 1236-42. doi: 10.1590/1678-4162-10293. [View Article]

6. Barcelos LS, Almeida RB, Peter CM, Botton NY, Rodrigues PRC, et al. (2019) Estudo retrospectivo (2018 – 2019) da presença de anticorpos neutralizantes contra o alphaherpesvirus bovino 1 em rebanhos no sul do Brasil. XXVIII Congresso de Iniciação Científica UFPEL 2019. Accessed 2020 May 16th. Only in Portuguese. [View Article]

7. Scicluna MT, Caprioli A, Saralli G, Manna G, Barone A, et al. 2010 Should the domestic buffalo (Bubalus bubalis) be considered in the epidemiology of Bovine Herpesvirus 1 infection? *Vet Microbiol* 143: 81-88. [View Article]

8. Amoroso MG, Cerrone A, Natale A, Guarino A, Galiero G 2012 Isolamento di bovine herpesvirus 1 (BOHV1) nel bufalo mediterraneo (Bubalus bubalis) in un allevamento del sud Italia. In: Proceedings of the XIVth SIDILV. Sorrento; Italy: 41–42. [View Article]

9. Bertolotti L, Muratore E, Nogarol C, Caruso C, Lucchese L, et al. (2015) Development and validation of an indirect ELISA as a confirmatory test for surveillance of infectious bovine rhinotracheitis in vaccinated herds. BMC Veterinary Research 11: 300. doi: 10.1186/s12917-015-0612-5. [View Article]

10. Massad E (2004) Métodos Quantitativos Em Medicina. Manole. Only in Portuguese.[View Article]

11. Maidana SS, Delgado F, Vagnoni L, Mauroy A, Thiry E, Romera S (2016) Cattle are a potential reservoir of bubaline herpesvirus 1 (BuHV1). Vet Rec Open 3 (1): e000162. doi:10.1136/vetreco-2015-000162. [View Article]

12. Mohanty SB, Rockemann DD, Snyder DB (1984) Serologic Cross-Reaction Between Bovine Herpesviruses 1 and 4 by the Enzyme-Linked Immunosorbent Assay. Microbiologica 7:179-86. PMID: 6087088. [View Article]

13. de Wit JJ, Hage JJ, Brinkhof J, Westenbrink F (1998) A comparative study of serological tests for use in the bovine herpesvirus 1 eradication programme in The Netherlands. Veterinary Microbiology 61:153-163. doi: 10.1016/s0378-1135(98)00166-7. [View Article]

14. Nogarol C, Bertolotti L, De Carlo E, Masoero L, Caruso C, et al. (2014) Expression and antigenic characterization of bubaline herpesvirus 1 (BuHV1) glycoprotein E and its potential application in the epidemiology and control of alphaherpesvirus infections in Mediterranean water buffalo. J Virol Methods 207: 16-21. doi: 10.1016/j.jviromet.2014.06.023. [View Article]

15. Hedayat N, Haji Hajikolaei MR, Seyfi Abad Shapouri MR, Ghadrdan Mashhadi AR, Izadnia H, et al. (2020) Isolation and identification of bubaline herpesvirus 1 (BuHV-1) from latently infected water buffalo (Bubalus bubalis) from Iran. Trop Anim Health Prod 52: 217–226. doi: 10.1007/s11250-019-02007-9. [View Article]

16. Moons KG, van Es GA, Deckers JW, Habbema JD, Grobbee DE (1997) Limitations of sensitivity, specificity, likelihood ratio, and bayes' theorem in assessing diagnostic probabilities: a clinical example. Epidemiology 8 (1): 12-17. doi:10.1097/00001648-199701000-00002 x.[View Article]

17. Caruso C, Prato R., Ingravalle F, Vecchio D, Sciarra A, et al. (2016) Prevalence of antibodies against bubaline herpesvirus (BuHV-1) among Mediterranean water buffalo with implications in buffalo trade. Veterinary Quaterly 36: 184-188. doi: 10.1080/01652176.2016.1205236. [View Article]

18. Fusuma MM (2014) Epidemiological surveillance of bovine central nervous system disease: diagnosis of bovine herpesvirus. Instituto Biológico. Accessed 2020 May 16th. Only in Portuguese. [View Article]

19. Halfen DC, VIDOR T (2001) Infecções por herpesvírus bovino 1 e herpesvírus bovino 5. In: Riet-Correa F, Schild AL, Méndez MDC, Lemos RAA (2001) Doenças de Ruminantes e Eqüinos. Editora Varella. Only in Portuguese.[View Article]

20. International Committee Taxonomy of Viology (2016) Family Herpesviridae. [View Article]

21. Thiry J, Keuser V, Muylkens B, Meurens F, Gogev S, et al. (2006) Ruminant alphaherpesviruses related to bovine herpesvirus 1. Vet Res 37 (2): 169-190. doi: doi.org/10.1051/vetres:2005052 [View Article]

22. Furtado A, Campos FS, Llambí S, Maisonnave J, Roehe PM, et al. (2014) Detection of bovine herpesvirus 1 and 5 in trigeminal ganglia of beef cattle in Uruguay. Arch Med Vet 46 (3): 451-455. doi: 10.4067/S0301-732X2014000300016. [View Article]


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