# A Serological Study of Human Herpesvirus Simplex 1 and Bovine Herpesvirus 1, 2, and 4 in Kansas Cattle

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**Summary**

A total of 589 beef cattle and 466 dairy cattle were tested for antibodies to Herpes Simplex Virus-1 (HSV-1) and Bovine Herpes Virus-1, 2, and 4 (BHV) using the indirect fluorescent antibody test (IFAT). In the beef cattle, prevalence of antibodies to HSV-1, BHV-1, 2, and 4 was 34.8%, 67.9%, 36.5% and 12.4% respectively, and in the dairy cattle prevalence of antibodies to these viruses was 35.4%, 45.3%, 38.2%, and 24.4%, respectively. There were no cross reactions between the four viruses using an IFAT fluorescent antibody test with monospecific polyclonal antiserum to BHV-1, 2, and 4, and an IFAT with two separate monoclonal antibodies to HSV-1. Statistical analysis indicated that the presence of one or more viruses in an animal enhanced the chance of infection with other herpesviruses.

## Introduction

The human Herpes Simplex Virus-1 (HSV-1) was isolated from adult dairy cattle with a respiratory disease. This virus was identified using the serum neutralization test, direct fluorescent antibody test (DFAT), restriction enzyme analysis of DNA, and electron micropsy.[1] This work examines the incidence of HSV-1 in dairy and beef cattle in Kansas. There was a concern that a cross reaction would occur between HSV-1 and bovine herpesviruses. Therefore, antibody titers to bovine herpesviruses (BHV) 1, 2, and 4 were determined and used to check for possible cross reactions. In order to make an accurate comparison, the IFAT was used to measure all the antibody responses.

## Materials and Methods

*Viruses and Cells*

BHV-1, 2, and 4 were received from the National Veterinary Services Laboratory at Ames, Iowa. BHV-1 was produced in MDBK cells and BHV-2 and 4 were grown in embryonic bovine kidney cells (EBK). HSV-1 isolated in our laboratory was produced in VERO cells. All cells were maintained in minimum essential medium supplemented with 10% fetal calf serum and antibiotics (100 iu of penicillin, 100 ug of streptomycin sulfate, and 100 ug of kanamycin sulfate/ml).

*IFAT*

The IFAT was done in EBK cells for all of the viruses tested using standard procedures. Briefly, 250,000 EBK cells per ml were planted using 0.1 ml of cells per well in 10-well slides. Then the viruses were added at a multiplicity of infection of 0.025. The viruses and cells were incubated in a humidified incubator at 37°C for 18–24 hours. A slide was washed with PBS, fixed in acetone for 10 minutes, and examined by the direct fluorescent antibody test (DFAT) prior to use. When at least 50 cells were fluorescing per well, the slides were washed in PBS, dried, fixed in acetone, then frozen at –70 degrees centigrade until used. Test serums were added to the fixed infected cells starting at a 1:40 dilution. Then slides were incubated at 37°C for 30 minutes, washed twice in PBS pH 7.6 (5 minutes each), and rinsed in distilled water. The slides were dried and then flooded with anti-bovine immunoglobulin G fluorescein-conjugate goat F(ab) and incubated at 37°C for 30 minutes. After incubation, the slides were washed as before, dried, and mounted in borate-buffered glycerin for examination by fluorescent micropsy.

*Serology*

Reference serums for BHV-1, 2, and 4 were provided by the National Veterinary Service Laboratories of Ames, Iowa. Monoclonal antibodies to HSV-1 were purchased from a commercial source and kindly supplied by J.B. Mahony of St. Joseph's Hospital Regional Virology Laboratory, Hamilton, Ontario, Canada.[2] Two convalescent serums that were collected from cows in the original case were used to determine the titers of cows recently infected with HSV-1[1]. The test serums were kindly provided by the state and federal brucellosis laboratory, Topeka, Kansas.

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There were 466 serums collected from dairy cattle located in 44 different Kansas counties. There were 589 serums collected from beef cattle located in 54 different Kansas counties. The number of serums taken from each herd varied from 5 to 30, with 60 herds providing 10 serums each.

*Statistical Analysis*

Our interest in analyzing the data was to determine if the four herpesviruses in dairy and beef cattle were independent of one another. The four viruses constitute a four-dimensional contingency table, in which each virus is classified as present or absent. As such, there are 16 combinations or cells in the table (see Tables 1 and 2). The analysis of such a contingency table can be accomplished with "log-linear" models[3,4]. A "log-linear" model refers to a procedure in which the log of the expected cell frequency is expressed as a linear function of the grand means and the marginal effects and their interactions. A "log-linear" model may be used to test for independence among the four viruses. Likewise, hypotheses of partial independence and conditional independence may be tested using appropriate "log-linear" models.[3] Conditional independence of BHV-1, 2, and 4 would imply that the three viruses are independent in each level of virus HSV-1.

TABLE 1. Serological response of beef cattle to HSV-1, BHV-1, 2 and 4 using the IFAT.

| Viruses | Numbers of Cattle with Titers | % of all Beef Cattle |
|---|---|---|
| HSV-1 | 3 | 0.5 |
| BHV-1 | 125 | 21.3 |
| BHV-2 | 17 | 2.9 |
| BHV-4 | 0 | 0.0 |
| HSV-1, BHV-1 | 80 | 13.6 |
| HSV-1, BHV-2 | 29 | 4.9 |
| HSV-1, BHV-4 | 5 | 0.8 |
| BHV-1, 2 | 83 | 14.1 |
| BHV-1, 4 | 18 | 3.1 |
| BHV-2, 4 | 3 | 0.5 |
| HSV-1, BHV-1, 2 | 51 | 8.7 |
| HSV-1, BHV-1, 4 | 15 | 2.5 |
| HSV-1, BHV-2, 4 | 4 | 0.7 |
| BHV-1, 2, 4 | 10 | 1.7 |
| HSV-1, BHV-1, 2, 4 | 18 | 3.1 |
| Negative | 128 | 21.7 |

TABLE 2. Serological response of dairy cattle to HSV-1, BHV-1,2 and 4 using the IFAT.

| Viruses | Numbers of Cattle with Titers | % of all Beef Cattle |
|---|---|---|
| HSV-1 | 20 | 4.3 |
| BHV-1 | 44 | 9.4 |
| BHV-2 | 17 | 3.6 |
| BHV-4 | 10 | 2.1 |
| HSV-1, BHV-1 | 28 | 6.0 |
| HSV-1, BHV-2 | 27 | 5.8 |
| HSV-1, BHV-4 | 10 | 2.1 |
| BHV-1, 2 | 23 | 4.9 |
| BHV-1, 4 | 22 | 4.7 |
| BHV-2, 4 | 16 | 3.4 |
| HSV-1, BHV-1, 2 | 45 | 9.6 |
| HSV-1, BHV-1, 4 | 5 | 1.1 |
| HSV-1, BHV-2, 4 | 6 | 1.3 |
| BHV-1, 2, 4 | 20 | 4.3 |
| HSV-1, BHV-1, 2, 4 | 24 | 5.1 |
| Negative | 149 | 32.0 |

The "log-linear" analysis was performed using the catmod procedure in SAS.[5] The analysis utilized the maximum likelihood estimation procedure, and the goodness of fit statistic was the likelihood ratio test statistic in the analysis of variance table.

## Results

The reference serums were used to check for cross reactions between the different viruses. The IFAT was done using the anti-bovine IGG conjugate for the BHV reference serums and an anti-mouse IGG conjugate for the monoclonal antibodies to HSV-1. There were no cross reactions using these serums with the different viruses. All test serums with a titer of 1:80 to any of the four viruses was considered positive for that virus. The range of antibody titers was 1:80 up to 1:5120. The titers of the two convalescent serums from the cows infected with HSV-1 in the original case were 1:320 and 1:640, so cattle with titers of 1:320 or greater were considered to have been exposed to that virus in recent months. These higher titers are illustrated in Table 3. A summary of the antibody response virus is given in Table 4.

TABLE 3. Cattle with higher titers of 320 or greater.

| | HSV-1 | BHV-1 | BHV-2 | BHV-4 |
|---|---|---|---|---|
| Beef | 60 | 291 | 165 | 44 |
| Dairy | 50 | 112 | 37 | 60 |
| Total | 110 | 403 | 202 | 104 |
| Percent of total cattle tested | 10.4% | 38.2% | 19.1% | 9.9% |

TABLE 4. Antibody response of cattle to HSV-1 and BHV-1, 2, 4.

| | HSV-1 | BHV-1 | BHV-2 | BHV-4 |
|---|---|---|---|---|
| Beef Cattle | | | | |
| No. Tested | 589 | 589 | 589 | 589 |
| No. Positive | 205 | 400 | 215 | 73 |
| % Positive | 34.8% | 67.9% | 36.5% | 12.4% |
| Dairy Cattle | | | | |
| No. Tested | 466 | 466 | 466 | 466 |
| No. Positive | 165 | 211 | 178 | 113 |
| % Positive | 35.4% | 45.3% | 38.2% | 24.2% |
| Total Cattle Positive | 370 | 611 | 393 | 186 |
| Percent Cattle Positive | 35.1% | 57.9% | 37.2% | 17.6% |

There were 611 cattle positive to BHV-1 and 345 (56%) were negative for HSV-1 antibodies; 403 (65.9%) of the positive cattle had titers of 320 or greater. There were 266 cattle with titers to both BHV-1 and HSV-1, and the titers were within one dilution in 166 (62.4%). There was at least a twofold higher titer to BHV-1 in 81 cattle and a twofold or higher titer to HSV-1 in 19 cattle. There were 22 cattle with titers to HSV-1 of 320 or greater and no titer to BHV-1.

There were 393 cattle positive to BHV-2; 189 (48%) of these were negative to HSV-1 and 202 (51.5%) had titers of 320 or greater. There were 204 cattle positive to both BHV-2 and HSV-1, and the titer to both viruses was within one dilution in 149 (73%) of the cattle. There was a twofold or greater titer to BHV-2 in 43 of the cattle with titers to both viruses and a twofold or greater titer to HSV-1 in 12 of these cattle. There were 13 cattle with titers of 320 or greater to HSV-1 and no titer to BHV-2.

There were 186 cattle positive to BHV-4; 85 (45.7%) of these were negative to HSV-1 and 104 (55.9%) had titers of 320 or greater. There were 101 cattle with titers to both BHV-4 and HSV-1 and the titers to both viruses were within one dilution in 52 (51.5%) of these cattle. In 37 of the cattle positive to both BHV-4 and HSV-1, the titer to BHV-4 was higher by twofold or greater and in 12 cattle, the titer to HSV-1 was higher by twofold or greater. There were 69 cattle with titers to HSV-1 of 320 or greater and no titer to BHV-4. There were 370 cattle positive to HSV-1 and only 23 did not have titers to the bovine herpesvirus. The antibody response of all cattle to all four viruses is illustrated in Fig. 1. There were 277 (26.5%) negative to all four viruses, 42 (4%) positive to all four viruses, 156 (14.78%) positive to three viruses, 344 (32.6%) positive to two viruses, and 236 (22.36%) positive to one virus.

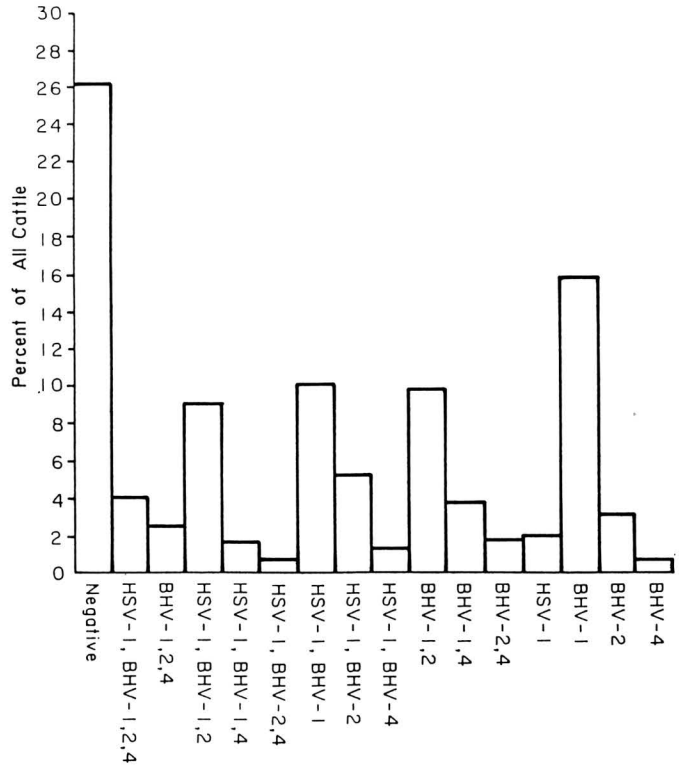


FIGURE 1. Antibody response of all cattle to bovine herpesviruses (BHV)-1, 2, 4 and Herpes Simplex Virus (HSV)-1.

Statistical analysis indicated that for both beef and dairy cattle, the four viruses were not independent of one another. There was no partial or conditional independence for any of the virus combinations. The data showed that the frequency or count for the occurrence of two, three, and four viruses together was significantly larger than what would be expected assuming independence. These positive associations suggest that the presence of one or more herpesvirus in the bovine enhances the chance of infection with other herpesvirus.

## Discussion

Studies have shown that BHV-1, 2, 3, 4, and 5 are not related immunologically, and serological cross neutralization is generally not demonstrated.[6] BHV-1 and the goat BHV-6 are exceptions and are neutralized by heterologous antiserum. A clear immunological relationship does exist between HSV-1, BHV-2, and the B virus. The antigens recognized by heterologous serums in HSV-1 and BHV-2 infected cells and virons share only partial identity and are located on a glycoprotein in HSV-1 and BHV-2. Cross neutralization experiments revealed that HSV-1 was inactivated by BHV-2 antiserum in the presence of complement, but HSV-1 antiserum did not neutralize BHV-2. The common antigen in HSV-1 is located in the membrane, and in BHV-2 it is located on the inside of the viral envelope.[6,7,8,9,10,11] These findings indicate that antibodies to BHV-2 would cross react with HSV-1 in cell cultures in the presence of added complement, and that HSV-1 antibodies would not cross react with BHV-2. In this work, added complement was not used, so cross reactions should not have occurred frequently between BHV-2 and HSV-1. Previous work has shown that complement-requiring antibodies to BHV are quite low in adult cattle.[12] Cross reactions did not occur between the viruses using the monospecific polyclonal antibodies to BHV-1, 2, and 4 and the monoclonal antibodies to HSV-1.

This study indicates a rather high incidence of HSV-1 in the bovine population, probably as the result of unapparent infections from HSV-1. The number of beef or dairy cattle infected with the different viruses was similar except for BHV-1 which had a significantly higher incidence in beef cattle and BHV-4, which had a higher incidence in dairy cattle. The study suggested that cattle infected with one of the herpesviruses would be susceptible to infection by other herpesvirus.

## References

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