CHAPTER 3

EVALUATION OF NEWLY DEVELOPED IMMUNOPEROXIDASE MONOLAYER ASSAYS FOR DETECTION OF ANTIBODIES AGAINST BOVINE HERPESVIRUS 4

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Abstract

This study describes the evaluation of immunoperoxidase monolayer assays (IPMAs) for detection of antibodies against bovine herpesvirus 4 (BHV4) DN-599 or BHV4 LVR140 in sera of cattle. We compared the quality of these IPMAs with the quality of a BHV4 indirect enzyme-linked immunosorbent assay (ELISA). In addition, a preliminary serological survey on BHV4 antibodies was carried out to estimate the seroprevalence of BHV4 in Dutch cattle at different ages. The specificities of both BHV4 IPMAs were 1.00. The geometrical mean titers (detection limit) of the BHV4 IPMAs were twice as high as that of the BHV4 indirect ELISA. In experimentally infected cattle, BHV4 antibodies were detectable by IPMAs 16 to 18 days post-infection, which was almost 2 weeks earlier than in the indirect ELISA. The reproducibility of the BHV4 DN-599 IPMA ($\kappa$D-value: 0.92) and of the BHV4 LVR140 IPMA ($\kappa$D-value: 0.87) were good. For field sera the overall agreement between the BHV4 indirect ELISA and the two BHV4 IPMAs, DN-599 and LVR140, was 95% and 96%, respectively. The serological-survey study showed that the estimated seroprevalence of BHV4 in Dutch cattle was 16-18%, and that the percentage of BHV4-positive animals varied by age category (between 6% and 43%). In summary, the two BHV4 IPMA formats have several advantages that make IPMA a useful alternative to the BHV4 indirect ELISA for detecting BHV4 antibodies in cattle.
1. Introduction

Bovine herpesvirus 4 (BHV4), a group of virus strains belonging to the *Gammaherpesvirinae* (17), is distributed worldwide, and BHV4 strains have been isolated from cattle with a variety of clinical symptoms (8, 12, 14, 20, 23, 24). For antibody detection, a sensitive test is required because the humoral immune response of cattle to BHV4 infections is characterized by a low neutralizing antibody response, or even by absence of neutralizing antibodies (21). Other methods have been developed to detect BHV4 antibodies in serum, such as complement fixation (9), indirect fluorescent-antibody test (IFAT) (18), and indirect enzyme-linked immunosorbent assay (iELISA) (7). According to Guo et al. (9), the complement fixation test was less sensitive than the iELISA for the detection of immunoglobulin G (IgG) to BHV4. The IFAT and the iELISA are the most common tests to be used for detection of BHV4 antibodies, but the IFAT is laborious, particularly if quantitative results are required (7). Also cross-reactions between BHV4 and other herpesviruses have been recorded by IFAT (11, 16). Edwards and Newman (7) described that the iELISA appeared to be as sensitive as the IFAT, but these results were only based on sera collected from experimentally infected cattle. Until now, no thorough validation of any BHV4 ELISA has been published. Because there is a need for an easy test to detect BHV4 antibodies, we developed an immunoperoxidase monolayer assay (IPMA). IPMAs have proven to be an easy method and a valuable tool for the diagnosis of several infectious diseases (19), for instance it is a common method for the detection of antibodies against porcine reproductive and respiratory syndrome virus in pigs (10).

The purpose of this study was to evaluate newly developed BHV4 IPMAs which are based on the American BHV4 reference strain, DN-599 (13), and on LVR140, the Belgian BHV4 reference strain (26), which belongs to the European Movar 33/63-like group (22). The results of the IPMAs were compared with the results of a BHV4 iELISA. Data on the prevalence of BHV4 antibodies in Dutch cattle are be presented.
2. Materials and methods

2.1 BHV4 IPMA

2.1.1 Preparing BHV4 IPMA plates

Trypsinized embryonic bovine trachea cells (EBTr) were resuspended in Earle’s minimal essential medium (EMEM) cell culture medium, containing 10% horse serum and 0.5% antibiotic mix (antibiotic stock solution containing: 10^7 IU of penicillin G, 8.6 x 10^6 IU of streptomycin, 1% kanamycin and 5 x 10^6 IU of nystatin per liter aquadest), and seeded into wells of a 96-wells cell culture plate (Greiner). Each well contained 100 µl of an EBTr cell suspension of approximately 7.5 x 10^4 cells/ml. The plates were placed in a humidified incubator at 37°C with 5% CO_2. After 3 to 4 days, when the monolayer was 100% confluent, the cell culture medium was discarded and the cells were infected with 25 µl (containing 10^2 50% tissue culture infective doses/ml) of the BHV4 DN-599 strain (ATCC VR631) or the BHV4 LVR 140 strain (kindly provided by E. Thiry). After 1 hour at 37°C (5% CO_2), a volume of 75 µl EMEM (containing 2% horse serum and 0.5% antibiotic mix) was added to each well. When cytopathogenic effect started to appear the incubation was stopped. The virus-cell culture medium was discarded and a volume of 150 µl of 4% formaldehyde in phosphate-buffered-saline (PBS) was added to the wells for 10 minutes at 18 to 25°C. The fluid was poured off, and again a volume of 150 µl of 4% formaldehyde in PBS (fixative) was added to the wells. Plates were sealed and stored at 4°C.

2.1.2 Performance BHV4 IPMA

Prior to use, the fixative was removed and the plates were washed once with 100 µl/well of a 2% Triton X-100 solution (Sigma) in PBS. To reduce nonspecific reactions, the plates were first incubated with a volume of 100 µl of 2% Triton X-100 in PBS for 60 minutes at 37°C. After the Triton X-100 solution was discarded, plates were incubated with 75 µl of PBS (containing 10% horse serum) for 60 minutes at 37°C. Serum test samples were diluted 1:20 in IPMA buffer (38.5 g of NaCl, 2% Tween-80, 0.1% NaN₃ and 10% horse serum per 1 liter of PBS). After the pre-incubation, the PBS-horse serum solution was discarded and a volume of 75 µl of the 1:20 pre-diluted serum sample were added to the wells and incubated at 37°C for 1 h. The plates were washed six times in wash solution (PBS, containing 1% Tween-80 [Merck]). A volume of 75 µl of the conjugate solution was added to the wells (1:200 dilution of horseradish peroxidase labeled rabbit anti-bovine immunoglobulin
IPMAs to detect BHV4 antibodies

[Dakopatt] in IPMA buffer [without NaN$_3$], and incubated at 37°C for 1 h. The plates were washed five times as described above, and 75 µl of substrate-chromogen (H$_2$O$_2$/3-amino-9-ethylcarbazole) was added and incubated at 18 to 25°C for 30 min. Without stopping the reaction, the results were read under a light microscope by at least two persons, independently from each other. The serum samples were retested if the interpretations of the two persons were not the same. Positive reactions were characterized by an intense red-brown staining of mainly the nucleus of the EBTr cell, indicating binding of antibodies to BHV4 DN-599 or BHV4 LVR 140 antigens (Fig. 1). The results were interpreted as either negative (a titer of < 20) or positive (a titer of ≥ 20).

![A](image1.png)  ![B](image2.png)

**Fig. 1.** (A) BHV4 IPMA positive staining reactions, which are characterized by red-brown staining mainly of the nuclei of BHV4 infected EBTr cells, indicate the binding of serum antibodies to BHV4 antigens. (B) Negative reactions are characterized by the absence of color.

### 2.2 BHV4 iELISA

De BHV4 iELISA (BIO-X, Brussels, Belgium) was performed as described by the manufacturer; it follows the principle of an iELISA. Briefly, the odd wells (antigen wells) of the ELISA microplate are coated with a BHV4 LVR 140-cell suspension lysate, while the even wells (control wells) are coated with a lysate of cell suspension. Volumes of 100 µl (each) of 1:100 diluted serum samples, including the positive reference serum sample of the kit, were added to the antigen and the control well and incubated at 18 to 25°C for 1 h. The plates were washed six times, and a volume of 100 µl of conjugate (horseradish peroxidase-labeled anti-bovine IgG1 monoclonal antibody) was added to each
wells. Plates were incubated at 18 to 25°C for 1 h, and washed again (six times). After the washing, a volume of 100 µl of the substrate-chromogen (H₂O₂-tetramethylbenzidine) was added to each well. After 10 min the color reaction was stopped by the addition of 50 µl of 1M phosphoric acid. The optical density (OD) value was determined by an ELISA plate reader at 450 nm. The interpretation of the results was as follows: the OD value of the control well was subtracted from the OD value of the antigen well. The net OD value was compared with the net OD value of the positive reference serum sample by dividing the net OD value of the serum sample by the net OD value of the positive reference serum sample. Finally, this value was multiplied with the coefficient of the iELISA batch, as noted for each BHV4 iELISA batch. Serum samples with values < 0.2 were classified as negative, and serum samples with values ≥0.2 were classified as positive for BHV4 antibodies.

2.3 Controls

To guarantee the quality of the performed tests, control serum samples were incorporated into ELISA test plates and into each IPMA test run. Therefore a strong positive, a dilution of positive (weak-positive) and a negative serum sample were used (15). The BHV4-positive serum samples were kindly provided by E. Thiry and by G. Czaplicki (Belgium), while the negative control was provided by E. VanOpdenbosch (Belgium). The results of test runs were accepted if the controls were correct.

2.4 Specificity

The specificities of the BHV4 tests were determined by testing monospecific serum samples containing high antibody levels directed against BHV1 (number of serum samples; [n] = 5), BHV2 (n = 1), bovine respiratory syncytial virus (n = 3), parainfluenza virus type 3 (n = 3), bovine leukemia virus (n = 2), bovine immunodeficiency virus (n = 2), bovine viral diarrhea virus (n = 3), rotavirus (n = 4), and coronavirus (n = 4). The specificities of the BHV4 IPMA's and the BHV4 iELISA were also determined by testing 69 individual serum samples collected from 69 specified-pathogen-free (SPF) cattle (cattle born by caesarean section, held in isolation stables, and raised colostrum free).

2.5 Detection limit

For the determination of the detection limits for both BHV4 IPMAs, and the BHV4 iELISA, the
following sera were used; the positive bovine BHV4 antiserum of the Central Veterinary Laboratory (Weybridge, England), one serum sample collected from one experimentally infected SPF calf (4797), and randomly chosen positive serum samples of cattle from the field study (n = 8). The sera were titrated in serial twofold dilutions, starting at a dilution of 1:20. The detection limit was defined as the reciprocal of the highest dilution giving a positive reaction.

2.6 Experimental infection

Two SPF calves (4797 and 4798), 3 weeks of age, were intranasally inoculated with 10 ml of $10^{6.5}$ 50% tissue culture infective doses of BHV4 DN-599 (ATCC VR631; bovine viral diarrhea virus and Mycoplasma free)/ml. Pre- and post-inoculation serum samples were collected and tested for BHV4 antibodies. The serum samples were tested in the BHV4 IPMAs and the BHV4 iELISA in a 1:20 dilution. Positive serum samples were titrated in serial twofold dilutions, starting at a dilution of 1:20.

2.7 Reproducibility

The reproducibility of the BHV4 tests was calculated from the results of duplicate tests by use of the Kappa test (2). Kappa ($\kappa_D$) was defined as the quotient of the observed proportion of agreement beyond chance and the maximal proportion of agreement. Therefore, serum samples (n = 150) of six randomly chosen BHV4-positive herds of the field study were tested twice on two different occasions, at least 2 months apart, using new IPMA reagents and ELISA kits with different lot numbers.

2.8 Field sera

Field sera were used to compare the newly developed BHV4 IPMAs with the iELISA and to estimate the BHV4 seroprevalence in Dutch cattle. For that purpose, a total amount of 750 serum samples were collected at random from 30 randomly chosen Dutch herds (25 serum samples per herd). These herds had participated in a field study for BHV1 marker vaccine efficacy (3). The date of birth of each animal was recorded to estimate the BHV4 seroprevalence in Dutch cattle at different ages.

2.9 Statistical analyses and data processing

Statistical analysis was performed on the data in Table 1 using the Friedman nonparametric two-way analysis of variance test, followed by the Wilcoxon signed-rank test (one-sided test) for pairwise comparison of both IPMAs with the iELISA. The data were processed with Statistix for Windows,
version 2.0.
3. Results

3.1 Evaluation of the BHV4 IPMAs

3.1.1 Specificity

No positive reactions were observed in the BHV4 IPMAs and the BHV4 iELISA with the monospecific serum samples containing high antibody levels directed against BHV1, BHV2, bovine respiratory syncytial virus, parainfluenza virus type 3, bovine leukemia virus, bovine immunodeficiency virus, bovine viral diarrhea virus, rotavirus and coronavirus. In addition, all 69 serum samples collected from the SPF cattle reacted negative in the three BHV4 tests, indicating test specificities of 100%.

3.1.2 Detection limit

The geometrical mean titers (reciprocal of the mean logarithmic titers) of the BHV4 DN-599 IPMA, the BHV4 LVR 140 IPMA, and the BHV4 iELISA were 640, 686, and 343, respectively. Hence, the detection limits of both BHV4 IPMAs were twice as high as that of the BHV4 iELISA (Table 1). Statistical analyses by the Friedman test showed that the differences in BHV4 antibody titers in both BHV4 IPMAs and those in the BHV4 iELISA were statistically significant (P value, 0.048). The one-sided P value, calculated by the Wilcoxon signed-rank test, was 0.031 for BHV4 DN-599.

3.2 Experimentally infected cattle

The BHV4 IPMAs first demonstrated a BHV4 antibody response around 16 to 18 days after experimental infection; the iELISA first demonstrated a response around 30 days after experimental infection (Table 2). In the commercial BHV4 iELISA, serum samples of experimentally infected cattle were screened for BHV4 antibodies in a 1:20 dilution, instead of a 1:100 dilution. For the screening of serum samples for BHV4 antibodies, a 1:100 serum dilution has been recommended by the manufacturer. At this dilution, no BHV4 antibody responses were detectable at day 37 (calf 4797), or even at day 44 (calf 4798), post-infection.

3.3 Reproducibility
The reproducibilities of the BHV4 tests were good. The overall agreement of duplicate test results were as follows: BHV4 DN-599 IPMA, 0.96 [(83θ + 62θ)/150], BHV4 LVR 140, 0.94 [(75θ + 66θ)/150]; and BHV4 iELISA, 0.97 [(87θ + 59θ)/150]. Based on these results the κD-values of the BHV4 tests were as follows: DN-599 IPMA, 0.92; BHV4 LVR 140 IPMA, 0.87; and BHV4 iELISA, 0.96.

Table 1. Titer of antibody against BHV4 in sera of cattle as determined in the BHV4 IPMAs and the BHV4 iELISA (n = 10)

<table>
<thead>
<tr>
<th>Serum</th>
<th>BHV4 DN-599 IPMA</th>
<th>BHV4 LVR 140 IPMA</th>
<th>BHV4 LVR 140 iELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVL serum</td>
<td>320</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>4797 24/5</td>
<td>320</td>
<td>320</td>
<td>40</td>
</tr>
<tr>
<td>Herd A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow 1</td>
<td>640</td>
<td>640</td>
<td>320</td>
</tr>
<tr>
<td>Cow 2</td>
<td>1280</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td>Herd B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow 1</td>
<td>640</td>
<td>1280</td>
<td>640</td>
</tr>
<tr>
<td>Cow 2</td>
<td>640</td>
<td>1280</td>
<td>160</td>
</tr>
<tr>
<td>Herd C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow 1</td>
<td>640</td>
<td>640</td>
<td>160</td>
</tr>
<tr>
<td>Cow 2</td>
<td>1280</td>
<td>1280</td>
<td>1280</td>
</tr>
<tr>
<td>Herd D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow 1</td>
<td>640</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>Cow 2</td>
<td>640</td>
<td>1280</td>
<td>640</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>640</td>
<td>686</td>
<td>343</td>
</tr>
</tbody>
</table>

*a CVL, Central Veterinary Laboratory

3.4 Field sera

Of the 750 field serum samples, 120 (16.0%), in the BHV4 DN-599 IPMA and 135 (18.0%) in the
BHV4 LVR 140 IPMA scored positive for BHV4 antibodies, while in the BHV4 iELISA, 134 (17.9%) of the 750 field serum samples scored positive (Table 3). For field sera, the overall agreement of the BHV4 DN-599 IPMA and the BHV4 LVR 140 IPMA with the BHV4 iELISA were 95% and 96%, respectively. The overall agreement between the BHV4 DN-599 IPMA and the BHV4 LVR 140 IPMA was 98% (n = 750).

**Table 2.** Antibody responses against BHV4 in experimentally infected calves as determined in the BHV4 IPMAs and the BHV4 iELISA.

<table>
<thead>
<tr>
<th>Day post-infection</th>
<th>BHV4 antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IPMA DN-599</td>
</tr>
<tr>
<td></td>
<td>4797</td>
</tr>
<tr>
<td>0</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>7</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>11</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>14</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>16</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>21</td>
<td>80</td>
</tr>
<tr>
<td>23</td>
<td>160</td>
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<tr>
<td>30</td>
<td>320</td>
</tr>
<tr>
<td>37</td>
<td>640</td>
</tr>
<tr>
<td>44</td>
<td>640</td>
</tr>
</tbody>
</table>

*Calf number

### 3.5 Serological survey

#### 3.5.1 Dutch seroepidemiological field study
Based on the results of the three BHV4 tests (Table 3), 16 to 18% of the 750 Dutch field sera tested reacted positive for BHV4 antibodies. In 17 of the 30 herds examined (57%), BHV4 antibodies were detected in the BHV4 IPMAs. The herd prevalence of BHV4 antibodies varied between 0 and 76%, and within positive herds the seroprevalence of BHV4 antibodies varied by age category (Fig. 2). Cattle 5 years old and older showed the highest BHV4 seroprevalence: 43% of the sera contained antibodies to BHV4. The lowest level of BHV4 antibodies (6 to 7%) was found in the group of 0.5- to 2-year-old cattle. In young calves (between 0 and 6 months old) the BHV4 seroprevalence was 31%. Figure 2 shows only the results of the BHV4 LVR 140 IPMA for BHV4 seroprevalence in Dutch cattle at different ages; the same patterns were observed with the BHV4 DN-599 IPMA and the BHV4 iELISA.

**Fig 2.** BHV4 seroprevalence in Dutch cattle at different ages as assessed by the BHV4 LVR 140 IPMA
4. Discussion

Our study shows that the BHV4 DN-599 and the BHV4 LVR 140 IPMAs can be used for the screening of herds for BHV4 antibodies as an alternative to the BHV4 iELISA. Based on the specificity, detection limit, results from early-infection sera collected from calves, and reproducibility, the newly developed BHV4 IPMAs are reliable practical tests for the detection of BHV4 antibodies. In this evaluation study, the BHV4 IPMA specificities (100%) are comparable with those of the BHV4 iELISA (100%). The data on the detection limits (analytical sensitivity) and the experimental infection sera (diagnostic sensitivity) of both BHV4 IPMAs are even better than those of the BHV4 iELISA. The geometrical mean antibody titers (detection limits) of the BHV4 IPMAs were twice as high as the geometrical mean antibody titer of the BHV4 iELISA, and the differences between the BHV4 antibody titers of both BHV4 IPMAs and those of the BHV4 iELISA were statistically significant (P value, <0.05). The BHV4 IPMAs were able to detect BHV4 antibody responses 16 to 18 days post-experimental infection. Even when sera were tested in the commercial BHV4 iELISA in a 1:20 dilution, instead of a 1:100 dilution, no BHV4 antibody response was detectable within 30 days post-infection.

Table 3. Concurrence of BHV4 IPMAs and BHV4 iELISA results for field sera (n = 750)

<table>
<thead>
<tr>
<th>IPMA</th>
<th>Results^a</th>
<th>BHV 4 iELISA^b</th>
<th>BHV4 DN-599 IPMA^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHV4 DN-599^c</td>
<td>+</td>
<td>107</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>27</td>
<td>603</td>
</tr>
<tr>
<td>BHV4 LVR 140^c</td>
<td>+</td>
<td>119</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>15</td>
<td>600</td>
</tr>
</tbody>
</table>

^a +, positive; -, negative; ^b Titer of <100 was considered negative; titer of ≥100 was considered positive
^c Titer of <20 was considered negative; titer ≥20 was considered positive
One of the explanations for the inefficient detection of BHV4 antibodies could be that the commercial BHV4 iELISA detects only bovine IgG1. The immunoglobulin-specific conjugate, used in the BHV4 IPMAs to detect antibodies in the bovine serum samples, is known to have affinity for all bovine immunoglobulin subclasses (IgM, IgG1, IgG2, etc). The iELISA described by Edwards and Newman (7) was based on the detection of bovine IgG (IgG1 and IgG2) and detected BHV4-specific antibodies in experimentally infected calves 21 days after primary infection. The calves (infected with Movar 33/63) showed a response to the primary inoculation, which consisted mainly of IgG1. However, the detection of IgG2 and IgM could have been underestimated due to competitive inhibition by high levels of IgG1 (7). Although other parameters, e.g., buffer solutions and substrate, could also influence the difference in sensitivity of IPMA versus iELISA, the choice of the anti-bovine Ig conjugate is probably the most relevant factor.

The reproducibilities of the BHV4 DN-599 IPMA (κD value, 0.92) and the BHV4 iELISA (κD value, 0.96) are good, while the reproducibility of the BHV4 LVR 140 is somewhat lower but still acceptable (κD value, 0.87).

The seroepidemiological field study shows that the estimated BHV4 seroprevalence in Dutch cattle is 16 to 18%. The BHV4 seroprevalences in cattle determined in countries surrounding the Netherlands are comparable with those in Dutch cattle. In the Northern part of Belgium 15%, and in Wallony (Belgium) 29%, of the cattle older than 1 year were BHV4 seropositive (24). In former West Germany, the BHV4 seroprevalence in cattle was 18.4%, while 38% of the bulls used for artificial insemination contained BHV4 antibodies (23). Young calves (< 6 months) had antibodies directed against BHV4. These antibodies are probably of maternal origin and not the result of a BHV4 infection. According to VanOpdenbosch et al. (25), 38% of veal calves in Belgium (< 3 months) had maternal antibodies against BHV4, and only 3% of the BHV4-negative veal calves seroconverted within a period of 3 months. In this study, an increase in BHV4-seropositive animals was recorded within the group of 2- to 3-year-old cattle. Hence, it is likely that most cattle become infected after introduction in the dairy herd. Whether there is a relation between this finding and that of Czaplicki and Thiry (4), who reported an association between BHV4 seropositivity and abortion in cows, is unknown.

Although the group of BHV4 comprises a collection of antigenically closely related isolates (1), and no major antigenic differences between BHV4 isolates have been demonstrated by cross-serological analysis with polyvalent antisera (5), the evaluation of the newly developed BHV4 IPMA was based on two different BHV4 strains. The IPMAs were performed with the American BHV4 DN-599 reference strain, and the Belgian LVR 140 reference strain, which belongs to the European Movar 33/63-like group (22), because the use of monoclonal antibodies showed some differences in the
antigenic patterns of BHV4 isolates (6), and BHV4 strains can be isolated in many countries all over
the world. Dubuisson et al. (6) confirmed the close antigenic relationships between BHV4 by
comparison of field isolates with BHV4 monoclonal antibodies, but some monoclonal antibodies
recognized all BHV4 isolates, while others allowed differentiation among them. Our data support the
finding that there could be some minor differences in the antigenic patterns of BHV4 isolates, because
the results, obtained with field sera, showed some slight differences between the BHV4 DN-599
IPMA and the BHV4 LVR 140 IPMA. Fifteen field serum samples reacted positive in the BHV4 LVR
140 IPMA, while these sera did not react in the BHV4 DN-599 IPMA. Thirteen of these 15 sera also
reacted positive in the BHV4 iELISA, in which the plates were coated with a BHV4 LVR 140-cell
suspension lysate. Differences in affinity of certain immunoglobulins of cattle for the different BHV4
strains could be an explanation, but another explanation could be the differences in antigenic
expression or differences in the exposure of certain BHV4 antigens. Probably the antigenic expression
and antigen exposure of Dutch BHV4 strains are more related to BHV4 LVR 140 than to those of
BHV4 DN-599.

The BHV4 IPMA has several advantages compared to the BHV4 iELISA, e.g., this study shows
better analytical sensitivity results and the IPMA can be more easily adapted to the BHV4 strain of
choice (for example, the BHV4 strain which has been isolated in the area of interest) while the
commercial BHV4 iELISA is based on the use of BHV4 LVR 140. This study also shows that, in
comparison to the BHV4 iELISA, the BHV4 IPMA, which is based on the detection of all bovine
immunoglobulin subclasses, is the test of choice for the early detection of BHV4 antibodies in bovine
serum samples. A disadvantage of the IPMA is that it depends on subjective readings of test results
whereas the iELISA is more objective because of the use of automatic readers. IPMA test results need
to be interpreted by at least two trained persons to reduce the subjectivity of readings of test results (as
indicated in Materials and Methods).

In conclusion, the BHV4 IPMA is a reliable practical test for the screening of cattle for BHV4
antibodies, and it is a useful alternative to iELISA for detecting BHV4 antibodies in cattle.
Chapter 3

Acknowledgements

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