# Comparative Diagnosis and Identification of Bovine Herpes Virus -1

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**ABSTRACT**

Bovine herpes virus type 1 (BHV-1) occur world wide and cause serious economic losses due to loss of animals, infertility, abortions, decreased milk production and loss of body weight. Therefore, the present study was undertaken for comparing the sensitivity, specificity, and efficacy of the different diagnostic techniques of BHV-1 as well as detection of valuable diagnostic tool which is very helpful for BHV-1 surveillance studies. In turn, eradication campaigns and control programs could be done successfully. The relative sensitivity and specificity of virus isolation compared to polymerase chain reaction (PCR) was found 67.74% and 97.98%, respectively. The over all agreement between these two methods was found to be 92.77%, while in comparison direct fluorescent antibody technique (FAT) with PCR showed a relative sensitivity and specificity with a percentage of 77.4% and 99.3%, respectively. The overall agreement between the two techniques was 95.6% for detection of BHV-1. In comparison of indirect FAT with that of indirect enzyme linked immunosorbent, assay (ELISA) for detection of BHV-1 specific antibodies, a relative sensitivity and specificity was found to be 88.89% and 100% respectively. The overall agreement between both tests was 93.33%. Our results, reveal that, PCR is superior to both immunodetection using direct FAT and virus isolation, as well as ELISA is a rapid, inexpensive, more sensitive and specific in serodiagnosis of BHV-1.

## INTRODUCTION

BHV-1, is an enveloped double stranded DNA virus, and currently classified as a member of the genus *varicellovirus* of the subfamily *Alphaherpesvirinae* within the family *Herpesviridae*, *(1)* . BHV-1 genome encoding at least 70 proteins, of which 33 structural and up to 15 non structural proteins *(2)*.

Cattle of all ages and breeds are susceptible to infection with BHV-1, but the disease is more common in animals over 6 months of age *(3)*. The virus infects mucosal surfaces (nasal, laryngeal, tracheal or genital) with primary replication to high titers in epithelial cells then, disseminates to conjunctiva. Infected cells may be destroyed causing severe damage in respiratory and genital tracts or they may become latently infected surviving the viral DNA for life time. Upon stress such as transport and parturition, reactivation of latent infection occurs with the risk of intermittent virus shedding *(4)*.

Specific antibodies to BHV-1 can be first detected at 7 to 10 days post infection. After the acute phase and during latency, BHV-1 infected cattle are mainly detected by the presence of specific antibodies to BHV-1*(5)*.

Several laboratory methods are available for BHV-1 detection including virus isolation, direct fluorescent antibody examination of the infected tissue, antigen and antibody detection by ELISA & FAT and viral nucleic acid detection by PCR *(6)*. Although there are too many researches done on BHV-1 in Egypt, it still one of the greatest problems that threaten livestock and causes heavy losses among susceptible animals. Therefore the present study was undertaken for comparing the sensitivity, specificity and efficacy of the previously assessed diagnostic technique of BHV-1 as well as detection of valuable diagnostic tool which is very helpful for BHV-1 surveillance studies. In turn, eradication campaigns and control programs could be done successfully.

## MATERIAL AND METHODS

**Animal and samples:** A total of 180 clinical specimens include (60 nasal swabs, 60 vaginal swabs, and 60 buffy coats) and 120 paired serum

samples (two weeks apart) were collected from diseased animals( 30 cow and30 buffaloes) from different governorates between October 2007 and February 2008. The specimens were used for isolation, antigen and nucleic acid detection of BHV-1 as well as detection of its specific antibodies.

**Samples preparation:** The swabs were soaked in sterile tubes containing maintenance Eagle's minimal essential media (EMEM) with antibiotics. Consequently, shaked well, squeezed and centrifuged at 4°C at 3000 rpm/20 minutes. The supernatants were collected in eppendorf tube and kept at -70°C till used. Whole blood samples were collected into heparin coated tubes. Consequently, centrifuged at 2000 rpm for 20 minutes at $4^{\circ}_{C}$, the plasma were discarded and buffy coat were gently aspirated, washed, transferred to small cryovials containing maintenance medium, labeled and kept at $-70^{\circ}_{C}$ till used. Blood samples were allowed to clot then centrifuged at 3000 rpm for 10 minutes for collection of sera.

**Reference BHV-1:** Reference BHV-1(Abou-Hammad strain) used in this study was kindly supplied by Rinderpest Like Diseases Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia, Cairo.

**Cell culture:** Madin Darby bovine kindney (MDBK) cells were supplied by Rinderpest Like Diseases Department, (VSVRI), Abassia, Cairo. The cells were grown and propagated using EMEM and used for virus isolation, propagation and identification of virus isolates.

**Isolation of BHV-1 on MDBK cells:** MDBK cells counted $1x10^{6}$ cell/ml were distributed into the plates by 200 ul/ well and incubated at 37°C for the cell confluency 90%. The growth medium was decanted and the cells were inoculated with supernatants from swabs and buffy coat by 100 ul/ well into triplicates for each sample. Cell and virus controls were included. The plates were incubated at 37°C/ 1 hr in 5 % $CO_2$ with frequent rotation every 15 min. The excess inoculum was decanted and replaced with maintenance media containing 2% horse serum then incubated as above for 3-5 days. The plates were examined daily under inverted microscope for the development of cytopathic effect (CPE). Three further blind passages were performed for each sample, according to the methods of *(7)*.

**Detection of BHV-1 antigen by direct FAT:** A 90% confluent sheet of MDBK cells were inoculated with supernatants from swabs and buffy coat by 100 ul/ well into duplicates for each sample. Cell and virus controls were included. The plates were incubated at 37°C/ 36 hr and fixed in 80% cold acetone at 4 °C for 15 minutes. The fixed plates were stained with 100 ul of bovine anti BHV-1 antiserum conjugated with fluorescent isothiocyantes( FITC)/ each well, incubated at 37°C in a humid chamber, and washed with phosphate buffer saline( PBS), mounted with 50% buffered glycerol. Cells were examined for specific fluorescence using fluorescent microscope *(8)*.

**Detection of BHV-1 genome by PCR** *(9)*

DNA was extracted from cell harvests, reference BHV-1, normal MDBK cells. The PCR was performed in a total volume of 50 ul using a thermocycler .The reaction mixture was prepared containing; 25 ul of 2X RED*Taq* Ready mix (1.5 units *Taq* DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 0.2 mM $dNTP_S$, Stabilizers), 1 ul forward primer (10 PMol) **5' CACGGACCTGGTGGACAAGAAG 3'**, 1 ul of reverse primer (10 PMol) **5' CTACCGTCACGTGAGTGGTACG 3'**, 5 ul of template DNA, and 18 ul of PCR reagent. The optimized PCR reaction conditions was performed as following : Amplification was started with 5 minutes at 96 °C, to obtain full denaturation of DNA, followed by 35 cycles of (94 °C for 50 seconds (denaturation), 56 °C for 50 seconds (annealing), and 72 °C for 1 minutes (extension). A final extension time of 10 minutes at 72 °C was included at the end of last cycle. The amplified PCR products were detected by agarose gel electrophoresis at 100 volts/30 minutes. The amplified product was visualized as a single compact band of expected size by ethidium bromide staining

under ultraviolet light according, then photographed by paloroid camera.

**Detection of BHV-1 specific antibodies using indirect FAT:** A 90% confluent sheet of MDBK cells were inoculated with reference BHV-1 by 100 ul/ well. The plates were incubated at 37°C/ 36 hr, washed with PBS (pH 7.2) and fixed in 80% cold acetone at 4 °C for 15 min.The plates were filled with 100 ul of tested serum samples. Positive, negative and blank control sera were included in each plate. The plates were incubated at 37°C/ 1 hr in humid chamber, washed and stained with 100 ul FITC labeled anti bovine IgG / well, incubated at 37 °C / 1hr in a humid chamber, washed with PBS, mounted with 50% buffered glycerol, and examined for specific fluorescence using fluorescent microscope *(10).*

**Detection of BHV-1 specific antibodies using indirect ELISA:** ELISA microtiter 96 well plates were coated by 100 µl/well of previously titrated BHV-1 antigens diluted 1/10 in coating buffer, incubated at 4°C overnight. After overnight incubation, the plate was washed 4x with PBS, were blocked by 100 µl/well of blocking buffer (1-3% bovine serum albumin), and incubated at 37°C/ hr, then washed once. Test sera diluted 1: 10 in PBS were distributed in duplicates by 100 µl/well and incubated at 37°C for 1 hr then washed with PBS. A 100 µl of Anti-bovine, horse radish peroxidase labelled IgG, diluted in PBS, was added to all wells and incubated at 37°C for 1 hour then washed. A 100 µl of OPD substrate was dispensed into each well and incubated for 15 min at room temperature in dark place. The reaction was stopped by adding 50 µl of 1.25 M $H_2SO_4$ / well. *(11).* The plates were read using an ELISA reader (Behring EL 311) at wave length 450 nm.

The antibody titer of each serum sample was calculated from the following formula:

$$\frac{\textit{Average OD of test serum} - \textit{Average OD of negative control serum}}{\textit{Average OD of positive control serum} - \textit{Average OD of negative control serum}}$$

**Statistical analysis:** To compare the sensitivity, specificity and overall agreement between the various tests. The statistical analysis was done according the formula given by *(12)*

## RESULTS

**Isolation of BHV-1 on MDBK cells:** The inoculated cells were examined daily for the development of CPE. The CPE as shown in **(Photo 1),** grapes like appearance and cell rounding were detected in both of the positive virus control and tested samples, where no CPE was observed in the negative control **(Photo2).** The results of isolation trial of BHV-1 on MDBK cells are shown in **(Fig.1).**

**Detection of BHV-1 antigen by direct FAT:** BHV-1 antigens were detected in 25 samples (13.89%). The positive samples showed bright greenish yellow intranuclear fluorescence, while absence of this bright fluorescence considered as negative **(Photo 3 and 4).** In cows, the greenish yellow fluorescence was observed in 19 (21.11%), distributed as (18/30) of nasal swabs, (1/30) of buffy coat, but not detected in vaginal swabs. In buffaloes, the greenish yellow fluorescence was observed in 6 (6.67%), distributed as (6/30) of nasal swabs, but no detected in vaginal swab and buffy coat samples **(Fig.1).**

**Detection of BHV-1 genome using PCR:** BHV-1 genome was detected by gB based primer. Reference BHV-1 as well as 31 clinical specimens out of 180 samples (17.22%), produced 468 bp amplicon. **(Photo 5).** In cows, BHV-1 genome was detected in only 22 (24.44%), and their was distributed (21/30) of nasal swabs, (1/30) of buffy coat, but not detected in vaginal swabs, where in buffaloes, BHV-1 genome was detected in 9 (10%), distributed as (9/30) of nasal swabs, but not detected in vaginal swab and buffy coat samples **(Fig.1).**

**Comparative efficacy of virus isolation, direct FAT and PCR for detection of BHV-1 in different clinical specimens of the same animal:** A total of 180 clinical specimens were tested with the mentioned methods. A total of 21 clinical specimens were positive by

the three methods, where 145 were negative by the same methods. While, 7 samples were found positive by PCR and found negative by both virus isolation and direct FAT. In the mean while, 3 samples were found positive by both direct FAT and PCR were found negative by virus isolation. Interestingly, only 1 sample was found positive by direct FAT and was negative by virus isolation and PCR, while 3 samples were positive by virus isolation and negative by both direct FAT and PCR.

**Comparison of virus isolation with PCR:** In cross tabulation of virus isolation and PCR, 21 samples were found positive and 146 samples negative by both tests. However, 3 samples which were found positive by virus isolation turned out to be negative by PCR. Similarly 10 samples were found positive by PCR turned out to be negative by virus isolation. The relative sensitivity and specificity of virus isolation compared to be PCR was found 67.74% and 97.98%, respectively. The overall agreement between these two methods was 92.77% **(Table 1).**

**Comparison of direct FAT with PCR:** In cross tabulation of direct FAT and PCR, 24 samples were found positive and 148 samples negative by both the tests. However, one sample which was found positive by direct FAT turned out to be negative by PCR. Similarly, 7 samples were found positive by PCR turned out to be negative by direct FAT. The relative sensitivity and specificity of direct FAT compared to be PCR was found 77.4% and 99.3%, respectively. The overall agreement between these two methods was 95.6% **(Table 2).**

**Detection of BHV-1 specific antibody using indirect FAT:** A total of 120 serum samples were collected from 60 animals (30 cow and 30 buffalo) were bleeded during the clinical symptoms and after two weeks, the samples were screened by indirect FAT for detection of BHV-1 specific antibodies. In first collected serum samples, BHV-1 specific antibodies were detected in 29samples (48.33%). While after two weeks, were detected in 35 samples (58.33%). Six seronegative animals detected in1$^{st}$ serum sample and become seropositive in 2$^{nd}$ serum sample.

**Detection of BHV-1 specific antibody using indirect ELISA:** A total of 60 serum samples were collected from animal during clinical symptoms, a total of 35 out of 60 (58.33%) were positive. While, after two weeks, a total of 37 out of 60 tested serum samples were found positive (61.67%). Two seronegative animals were observed in 1$^{st}$ serum samples and become seropositive in 2$^{nd}$ serum sample. While four seropositive cattle were observed low titer of antibodies in 1$^{st}$ serum sample and high titer of antibodies in 2$^{nd}$ serum samples.

**Comparison of indirect FAT with indirect ELISA:** In cross tabulation of indirect FAT and indirect ELISA, 64 samples were found positive and 48 samples negative by both tests. However, there is no sample which was found positive by indirect FAT turned out to be negative by indirect ELISA. Similarly 8 samples were found positive by indirect ELISA turned out to be negative by indirect FAT. The relative sensitivity and specificity of indirect FAT compared to indirect ELISA was found 88.89% and 100%, respectively. The overall agreement between these two methods was found to be (93.33%) **(Table 3).**

**Comparison of PCR and indirect ELISA in diagnosis of BHV-1:** While comparing indirect ELISA and PCR, 28 animals revealed the presence of both antibodies and viral genome, respectively, in serum and clinical specimens, where 23 cattle did not reveal the presence of both antibodies and genome. Further, 7 seropositive cattle could not reveal viral genome in clinical specimens, while 2 seronegative cattle in 1$^{st}$ serum sample, revealed presence of viral genome as well as they become seropositive in 2$^{nd}$ serum samples (seroconversion).

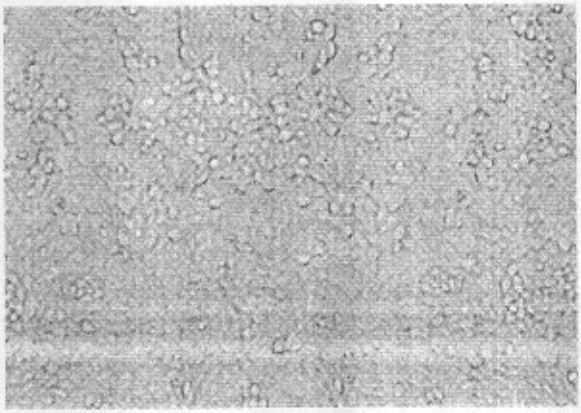

Photo 1. Normal MDBK cells, showing confluent monolayer sheet of cell. (X 100)

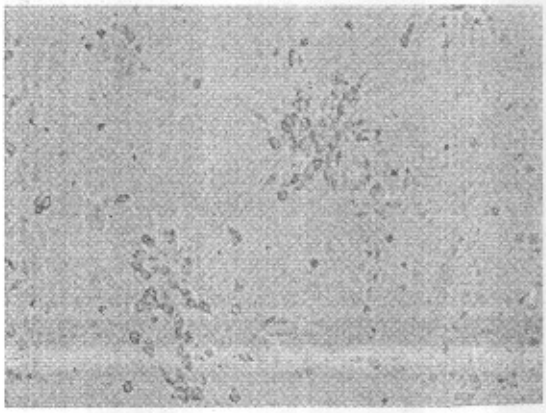

Photo 2. MDBK monolayer cells, inoculated with BHV-1 isolates showing CPE as grapes like appearance and cell rounding. (X 100)

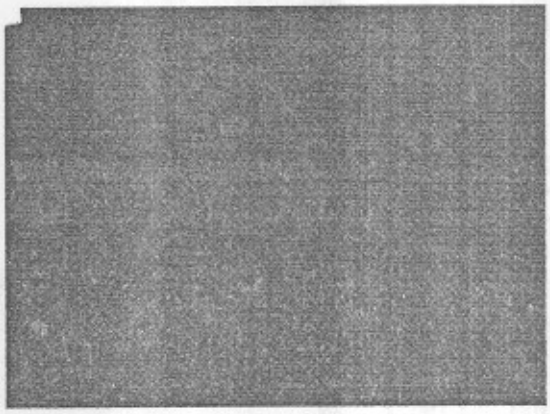

Photo 3. Normal MDBK cell monolayer stained with FITC, showing no illumination (X 400).

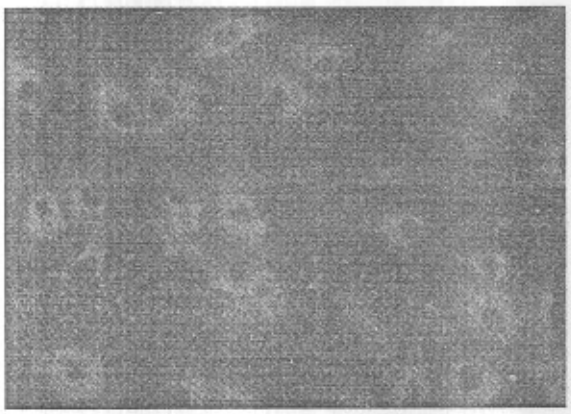

Photo 4. MDBK cell, inoculated with BHV-1 isolates and stained with FITC, showing bright intranuclear greenish yellow fluorescence (x400).

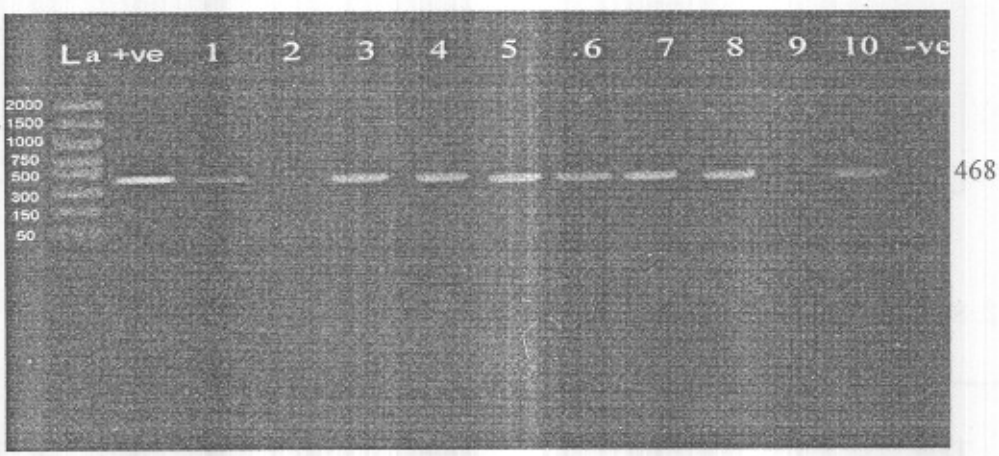


Photo 5. Agarose gel electrophoresis pattern of BHV-1 gB gene, 468 bp specific PCR Products amplified with primer gB1, gB2.Lane 2,9 are negative samples.Lane 1,3-8,10 are positive samples.

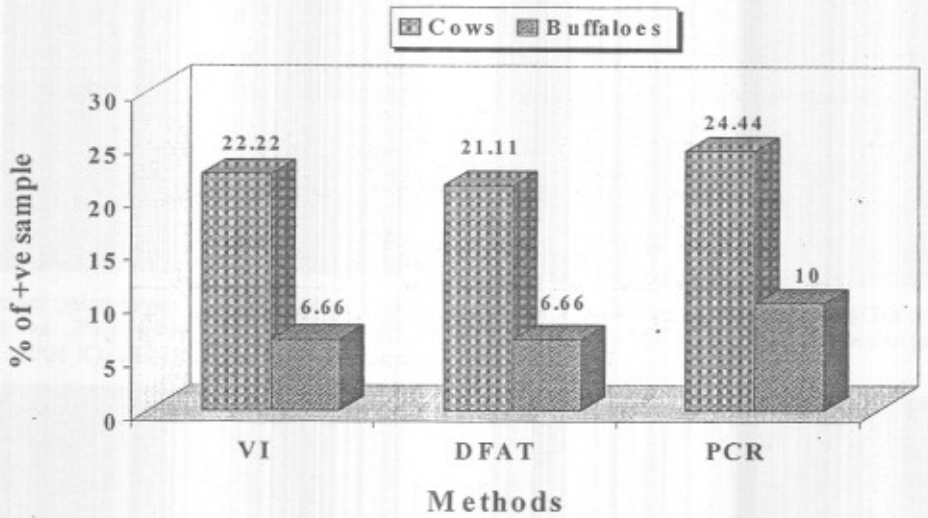


**Fig.1.** Virus isolation, direct FAT, and PCR for detection of BHV-1 infection.

**Table 1. Summarized the comparative efficacy of VI, direct FAT and PCR for detection of BHV-1 in different clinical specimens of the same animal.**

| Serial number | Method of BHV-1 diagnosis | | | Total |
|---|---|---|---|---|
| | Virus isolation | Direct FAT | PCR | |
| 1 | + | + | + | 21 |
| 2 | - | - | - | 145 |
| 3 | + | + | - | 0 |
| 4 | + | - | + | 0 |
| 5 | - | - | + | 7 |
| 6 | - | + | + | 3 |
| 7 | - | + | - | 1 |
| 8 | + | - | - | 3 |

**Table 2. Sensitivity and specificity of virus isolation by comparing with PCR in diagnosis of BHV-1.**

| Test | | PCR | | Total | Sensitivity (%) | Specificity (%) | Overall agreement (%) |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | |
| Virus isolation | Positive | 21 | 3 | 24 | (21/31) x 100 67.74 | (146 x 149) x 100 97.98 | (167/180) x 100 92.77 |
| | Negative | 10 | 146 | 156 | | | |
| | Total | 31 | 149 | 180 | | | |

**Table 3. Sensitivity and specificity of direct FAT by comparing with PCR in diagnosis of BHV-1.**

| Test | | PCR | | Total | Sensitivity (%) | Specificity (%) | Overall agreement (%) |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | |
| Direct FAT | Positive | 24 | 1 | 25 | (24/31) x 100 77.4 | (148 x 149) x 100 99.3 | (172/180) x 100 95.6 |
| | Negative | 7 | 148 | 155 | | | |
| | Total | 31 | 149 | 180 | | | |

**Table 4. Sensitivity and specificity of indirect FAT by comparing with indirect ELISA for detection of BHV-1 specific antibodies.**

| Test | | Indirect ELISA | | Total | Sensitivity (%) | Specificity (%) | Overall agreement (%) |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | |
| Indirect FAT | Positive | 64 | 0 | 64 | (64/72) x 100 88.89% | (48 x 48) x 100 100% | (112/120) x 100 93.33% |
| | Negative | 8 | 48 | 56 | | | |
| | Total | 72 | 48 | 120 | | | |

## DISCUSSION

BHV-1 is one of the most imported contagious viral pathogens of domestic and wild cattle. The virus is distributed world wide exerting an economic impact on live stock industry. BHV-1 is associated with abroad spectrum of multi-systemic clinical manifestations including; the upper respiratory tract (IBR), conjunctivitis, genital system (IPV & IBP), shipping fever and signs of general illness such as fever, abortion, inappetence, depression and reduced milk yield with predominance of respiratory manifestations *(13).*

Different diagnostic tools were used to detect the BHV-1 in clinical specimens. Our goal was to compare sensitivity and specificity of virus isolation, direct FAT and PCR as well as indirect FAT and indirect ELISA for detection of BHV-1 and its specific antibodies.

A total of 180 samples, including nasal swabs, vaginal swabs, and buffy coats were taken from cow and buffaloes with respiratory and genital disease using the routine methods of virus isolation and direct FAT and the results were compared with those obtained by PCR to determine sensitivity of three different techniques.

BHV-1 was isolated from 26 samples out of 180 tested samples with a percentage of 14.44%. Similar percentage was recorded in semen *(14)* . The results revealed that BHV-1 was isolated with high percentage from cow than buffaloes and this fact was previously supported *(15)* Who isolated BHV-1 with a percentage of 16.1 and 7.3% from cow and buffaloes, respectively. This may be explained by the fact, buffalo is highly resistant to infectious diseases than cow. In addition, prevalence of BHV-1 in nasal swabs samples higher than in vaginal swab and buffy coat samples. It was established that BHV-1 infects primarily the exposed mucosal cells (respiratory and /or genital) and replicates in high titers then disseminates to elsewhere *(4).*Consequently, nasal swabs are the best

clinical specimens to be taken for diagnosis of BHV-1 respiratory infection (IBR). BHV-1 antigen was detected in 25 (13.89%) of the samples by direct fluorescent antibody technique with high prevalence in cow than buffaloes, as previously cited ***(16)*** .The direct FAT proved to sensitive, reproducible and more effective for detecting BHV-1 antigen and identifying isolates of BHV-1.

A total of 31 samples out of 180 clinical specimens with a percentage of (17.22%) produced 468 bp amplicon, while the remaining samples failed to produce the targeted amplification with high prevalence in cow (24.44%) than in buffaloes (10%). The amplification was higher in nasal swabs (50%) than buffy coat (1.67%) and vaginal swabs (0%) samples. BHV-1 genome has been recorded in 13.51% of the collected sample in Egypt ***(16).***

In comparison of virus isolation, direct FAT and PCR for diagnosis of BHV-1, we found that a total of 21 and 145 samples were positive and negative by all three methods respectively. While 7 samples were found positive by PCR and negative by virus isolation and direct FAT, three samples were found positive by direct FAT and PCR and negative by virus isolation. Only one sample was found positive by direct FAT negative by virus isolation and PCR and 3 samples were found positive by virus isolation and negative by direct FAT. There is no positive sample found positive by virus isolation and direct FAT and negative by PCR, also there is no any sample found positive by VI and PCR and negative by direct FAT. The results showed that the most of positive cases were diagnosed with a help of all three technique, either alone or in different combination, but PCR and direct FAT were found more effective than virus isolation.

The statistical analysis confirmed the relative sensitivity and specificity of virus isolation compared to PCR was found 67.74% and 97.98%, respectively. The overall agreement between these two methods was found to be 92.77%. The difference in sensitivity and specificity might be accounted for several factors, the PCR detects BHV-1 genomes of both viable and non infections viral particles since it identifies specific nucleotides sequence which are generally more stable in clinical specimens; where virus isolation detects only infectious particles, and depends on cell susceptibility to infection and monolayer sheet, also the ratio between the number of BHV-1 genomes and infectious particles was found to be 30 for a certain virus stock. These results are consistent with that recorded by several investigators were ***(17).*** The statistical analysis confirmed the relative sensitivity and specificity of direct FAT compared to be PCR was 77.4% and 99.3%, respectively. The overall agreement between two methods was 95.6%. This result revealed that PCR assay is more sensitive, independent of sample quality than FAT and the sample preparation method is simple and involving few steps ***(18).***

In this study, a total of 120 serum samples were collected from 60 animals (30 cow and 30 buffalo), these animals were bled during clinical symptoms and after 2 weeks. These samples were screened by indirect ELISA and indirect FAT for detection of BHV-1 specific antibodies.

The result of indirect FAT was revealed presence of BHV-1 specific antibodies in 29 samples (48.33%) during clinical symptoms and in 35 samples (58.33%) after 2 weeks. Similar recorded was found in Tehran ***(10).*** Six seronegative cattle were detected in 1$^{st}$ serum sample and become seropositive in 2$^{nd}$ serum sample, indicating the possibility of recent exposure to BHV-1 and thus getting sufficient period for development of antibody response.

Also, the results obtained by indirect ELISA revealed presence of BHV-1 specific antibodies in 35 samples (58.33%) and 37 (61.67%) during clinical symptoms and after 2 weeks respectively. The same finding was observed by pervious research ***(19).*** Two seronegative cattle were observed in 1$^{st}$ serum samples and become seropositive in 2$^{nd}$ serum sample. While four seropositive cattle were observed low titer of antibodies in 1$^{st}$ serum sample and high titer of antibodies in 2$^{nd}$

serum samples, indicating seroconversion and might be contributed to recent infection.

In comparison indirect FAT with indirect ELISA, the statistical analysis confirmed the relative sensitivity and specificity of indirect FAT were 88.89% and 100%, respectively and overall agreement between these two methods was 93.33%. This result can be explained by the ability of antibody to bind with all accessible epitopes in antigen in indirect ELISA, while in indirect FAT, the antibody have to enter the cell and this depends on permeabilization efficacy. The epidemiological study in Dutch dairy herd showed nearly similar findings *(20).*

The presence of specific antibodies against BHV-1 in the tested animals' sera might be contributed to either infection, vaccination or latent infection.

A total of 60 cattle (30 cow and 30 buffalo) were such from those serum and clinical specimens were subjected, respectively, to indirect ELISA and PCR. The comparison was made, considering these 60 animals to know the presence of antibodies in serum as well as BHV-1 in clinical specimens of the same animal that help in diagnosis of BHV-1 infection.

While comparing indirect ELISA and PCR, 28 animals revealed the presence of both antibodies and viral genome, where as 23 animals did not reveal the presence of both. Thus, possibly indicating the presence of BHV-1 with previous exposure in 28 animals, while no infection and no previous exposure in 23 cattle. Further, 7 seropositive cattle could not reveal viral genome in clinical specimens. This might be due to the previous exposure and possibly attributed to the fact that the herpes viruses establish life-long latent infection with periodic reactivation and shedding of virus may be periodic or continuous. This is supported by the previous findings of *(21).* Interestingly, two seronegative cattle , revealed presence of viral genome in clinical samples indicating the possibility of recent exposure to BHV-1 and thus getting sufficient period for development of antibody response and become seropositive. 4 seropositive cattle revealed low titer of antibodies in 1st serum samples, after 2 weeks revealed high titer of antibodies (seroconversion), might be contributed to recent infection.

**In conclusion,** the findings of this study emphasized the utility of PCR depending on highly conserved sequences within the BHV-1 gB encoding region as efficient non conventional diagnostic tool for BHV-1 infection. It is superior to immunodetection using direct FAT in cell culture and virus isolation procedure which might have non specific reactivity so can react with the similar antigen, require higher viral concentration.

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## الملخص العربي

## التشخيص المقارن والتعرف على فيروس الهربس البقري -١

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ينتشر فيروس القوباء البقري -١ في جميع أنحاء العالم، ويسبب خسائر اقتصادية جسيمة بسبب ارتفاع نسبة الوفيات، وفقدان الخصوبة، والإجهاض، وكذلك انخفاض إنتاج اللبن واللحم فى الماشية. لذا تمت هذه الدراسة للتعرف على الوسيلة ذات اعلى حساسية وتخصيصية وكفاءة فى تشخيص هذا الفيروس وبالتالى القضاء علية. تم عمل مقارنة بين عزل الفيروس واختبار تفاعل انزيم البلمرة المتسلسل فى تشخيص فيروس القوباء البقرى-١ واظهر عزل الفيروس حساسية بنسبة ٦٧,٧٤% وتخصصية بنسبة ٩٧,٩٨%. مع وجود اتفاق بينهما بنسبة ٩٢,٧٧%. وبمقارنة اختبار الفلوروسنت المناعى المباشر مع اختبار تفاعل انزيم البلمرة المتسلسل، أظهر اختبار الفلوروسنت المناعى المباشر حساسية وتخصصية بنسبة ٧٧,٤% و ٩٩,٣% على التوالي، مع وجود اتفاق بينهما بنسبة ٩٥,٦%. بمقارنة اختبار الفلوروسنت المناعى غير المباشر باختبار الاليزا غير المباشر للكشف عن وجود الأجسام المضادة المناعية، أظهر اختبار الفلوروسنت المناعى غير المباشر حساسية وتخصصية بنسبة ٨٨,٨٩% و ١٠٠% على التوالي، مع وجود اتفاق شامل بينهما بنسبة ٩٣,٣٣%. وأخيرا ، تم التوصل إلى أن اختبار انزيم البلمرة المتسلسل افضل من اختبار الفلوروسنت المناعى المباشر والعزل الفيروسى فى تشخيص مرض فيروس القوباء البقرى-١ .وهكذا يعتبر اختبار انزيم البلمرة المتسلسل أداة تشخيصية لدراسة مدي تواجد الفيروس وبالتالى تصميم برنامج جيد لمكافحته .كما يعتبر اختبار الاليزا وسيلة ذو حساسية وتخصصية عالية فى التشخيص السيرولوجى لهذا الفيروس ايضا.