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Investigations on Infectious Bovine Rhinotracheitis in Egyptian Cattle and Buffaloes

[1]M.A. Mahmoud, [2]Nahed A. Mahmoud and [1]A.M. Allam

[1]Department of Parasitology and Animal Diseases,
Veterinary Researsh Division, National Research Center, Dokki, Giza, Egypt
[2]Animal Health Research Institute, Dokki, Giza, Egypt

Abstract: Infectious bovine rhinotracheitis-Infectious pustular vulvovaginitis (IBR/IPV) virus was isolated and identified from a herd of cattle and buffaloes, suffering from respiratory and genital disorders during May 2007. These animals were located at Giza Governorate with a total number of 350, (200 in open farms and 150 in closed farms). Vaginal, nasal and ocular swabs were collected (136 samples were collected from the open farm and 55 samples were collected from the closed farm). By inoculation on MDBK, 23 suspected viral isolates gave clear cytopathic effect (CPE) and by fluorescent antibody technique (FAT) ten IBR viral isolates were identified, showing clear intranuclear inclusion bodies. Serologically, a total number of 86 serum samples were collected from cattle, buffalo calves aged from 3 – 12 months and adult dairy cattle and buffaloes and were tested for IBR / IPV antibodies by ELISA. ELISA demonstrated all types of antibodies to the virus. The highest percentage of positive reactors was reported for acute convalescent cattle (85.7%) in closed farms followed by acute convalescent adult cattle (66%) in open farms. While the incidence in apparently healthy cattle in closed farms was 80% and in open farms were 62.5%. The percent of positive reactors in buffaloes were 40%, which is less than percent of cattle present in contact with them in the same open farm. The positive reactors in cattle and buffaloe calves were 62.5 and 40%, in 3-6 months of age and 50 and 33% in 6-13 months of age, respectively. ELISA is considered the most rapid, reliable, inexpensive and simple test and is particularly well suited to the analysis of a large number of samples.

Key words: Infectious bovine rhinotracheitis · BHV-1 · Isolation · Fluorescent antibody technique · ELISA · Cattle · Buffaloes

INTRODUCTION

Infectious Bovine Rhinotracheitis/ Infectious pustular vulvovaginitis (IBR/IPV) is a contagious disease of cattle and buffaloes caused by Bovine Herpesvirus Virus type 1 (BHV-1) which is a member of Alphaherpesviridae subfamily Herpesviridae. BHV-1 can be differentiated on DNA basis into three subtypes using restriction endonuclease [1 -3].

Reisinger and Reimann [4] succeeded in isolation of bovine herpesvirus –1 (BHV-1) for the first time in Germany.

The virus had been associated with a wide range of clinical symptoms including rhinotracheitis [4] abortion [5] infertility [6], conjunctivitis [7] and encephalitis in calves [8].

BHV-1 initially replicates in epithelial mucosa of respiratory or genital tract. It establishes long life latency in ganglionic neurons of peripheral nervous system. There is evidence that the long-term persistence and reactivation occurs in the germinal centers of pharyngeal tonsils [9]. In Egypt, detection of neutralizing antibodies against IBR/IPV virus for the first time in cattle and buffaloes suffering from respiratory syndrome was achieved by Hafez and Frey [10]. The first isolation and characterization of IBR/IPV in Egypt was by Mohamed [11]. Hafez *et al*.[12] isolated IBR/IPV virus from Egyptian cattle with respiratory manifestation in Abou Hamad district, Sharkia Governorate, while another author, Hafez *et al*. [13] isolated the virus from Friesian cattle suffering from genital form of the disease.

Studies in Egypt indicated that BHV-1 infection play a role as stress factor in cattle of Sakha farm and buffaloe calves of Mahalet Mousa farm during the first 3 months of ages. Also, these studies revealed the occurrence of single or mixed infection with BVDV or PI3V in cattle and buffalo calves. While studies by Nakashly [15] revealed that 11.4% of tested sera have neutralizing antibodies against BHV-1 virus in non-vaccinated cattle.

Corresponding Author: M.A. Mahmoud, Department of Parasitology and Animal Diseases, Veterinary Researsh Division, National Research Center, Post code: 12622, Dokki, Giza, Egypt

BHV-1 precipitating antigen was detected in 43.2% of bronchial and 68.3% of prescapular lymph nodes from apparently healthy native breed cattle, slaughtered at El-Kanater Abattoir [16].

Moussa *et al.* [17] revealed that the incidence of BHV-1 positive serum reactors were 19, 15.4, 17, 19.4 and 13.5% in the tested sera of cattle, buffaloes, sheep, goats and camels, respectively. BHV-1 was isolated from a dairy herd showing the genital form of infection in El_fayoum Governorate by Madbouly and Hussein [18]. Ahmed [19] detected neutralizing antibodies in 53% of the tested serum samples of cattle (53%) and buffalo (45%) calves. Gaber *et al.* [20] detected BHV-1 antibodies in sheep sera by using indirect ELISA test in two governorates. The prevalence of positive serum reactors were 5.7% in Beni Suef and 14.8% in Quena. Obando *et al.* [21] used a Commercial indirect ELISA kits and found that ELISA is more specific for seroprevalence than virus-neutralisation (VN) test. In addition, Bastawecy *et al.* [22] developed indirect ELISA for detection of antibodies against BHV-1. Abd El-moniem *et al.* [23] concluded that ELISA is more sensitive than agar gel precipitation test (AGPT) and virus neutralization test (VN).

This investigation was carried out to clarify the incidence of BHV-1 in a herd suffering from respiratory and reproductive disorders. Also, comparison of the three methods of laboratory diagnosis of this disease (ELISA, isolation and FAT) was another aim of this study.

MATERIALS AND METHODS

Materials

Samples:

- Vaginal, nasal and ocular swabs were collected from a farm at El-Kata region (Giza Governorate). 136 samples were collected from the open farm (the animals were reared in open yard). 55 samples were collected from the closed farm (the animals were housed in partitions and not allowed to get out). Samples from apparently healthy and diseased animals with symptoms of nasal, lacrimal discharges, abortion and opacity of eyes were collected as shown in Table (1).
- A total of 86 serum samples were collected for detection of antibodies against BHV-1 virus by using of Indirect-ELISA kits as shown in Table (1).

Tissue Culture: Madin Derby Bovine Kidney(MDBK) cell line were obtained from Virology Department, Animal Health Research Institute, Giza, Egypt and used for isolation of BHV-1 grown in Eagle's MEM supplemented with 10% fetal calf serum.

Specific Antisera: Specific anti-IBR hyperimmune serum conjugated with fluorescine isothiocynate were also, supplied by Virology Department, Animal Health Research Institute, Giza, Egypt.

ELISA Kit: A comercially available Screening/Verification blocking ELISA wit was obtained from HerdChek, Idexx, (USA),

Methods

Isolation of the Virus: Collected and prepared nasal and ocular swabs from cattle, buffaloes, semen of bull and vaginal swabs from dams were subjected to virus isolation via propagation on MDBK as described by Wellenberg *et al*. [24]. Inoculated cells were incubated at 37°C and examined daily for 5 days post inoculation for three successive blind passages. CPE changes began to appear at the fourth passage on the second day post inoculation (PI).

Table 1: Description of the collected samples

				Swabs				
Farms	Animal case	Species	Age	Total	Ocular	semen	Nasal	Vagina
Open farm	Apparently healthy	Cattle calves	3-6 month	15	8	-	7	
	Apparently healthy	Buffaloe calves	6-13 month	17	8	-	9	
	Apparently healthy	Dairy buffaloe	adult	42	16	-	13	13
	diseased	Dairy cattle	adult	62	24	-	41	42
	Total			136	56	-	43	37
Closed farm	Apparently healthy	Dairy cattle	adult	10	4		4	2
	Previously infected and treated	Dairy cattle	adult	17	8	-	6	3
	diseased	Dairy cattle	adult	26	12	-	7	7
	diseased	bull	adult	2	1	1	-	-
	Total			55	25	1	17	12

Virus Identification: Trypsinized MDBK infected with the isolates were dispersed on microscopic slide and fixed with chilled acetone. Fluorescent antibody technique (FAT) was applied according to Moore et *al*. [25]. Fixed cells were mounted by anti-BHV-1 fluorescine isothiocynate conjugated serum and incubated for one hour at 37°C with 70% humidity. Then, cells were washed with phosphate buffer saline, mounted with 50% glycerol buffer saline and examined under fluorescent microscope.

Indirect-ELISA for Detection of Antibodies Against IBR Virus: The serum samples were tested for presence of antibodies against BHV-1 with a kit specific for BHV-1 glycoprotein B (gB) [26]. All samples were tested according to the manufacturer's instructions. Samples with S/NHC ratio greater than or equal to 1.80 were considered verified positive for IBR antibodies, while ratios less than 1.80 were classified as negative [27-28].

S/P = 100 (OD sample-OD negative/OD positive-OD negative)

RESULTS

Table 2 indicates the type and number of samples, animal case, age, closed or open farms, number of samples that show CPE in monolayer cells and their positivity for perinuclear yellowish green fluorescent granules detected by FAT. Ten samples showed characteristic CPE by propagation on monolayer of MDBK cells. CPE became more pronounced and appearance after a short time by excessive propagation on the cells and perinuclear yellowish green fluorescent granules was detected by FAT.

Table (3) reveals that the highest percentage of positive reactors was reported for acute convalescent cattle (85.7%) in closed farms followed by acute convalescent adult cattle (66%) in open farms. While the incidence in apparently healthy cattle in closed farms was 80% and in open farms were 62.5%. The percent of positive reactors in buffaloes were 40%, which is less than percent of cattle present in contact with them in the same

Table 2: Results of BHV-1 isolation and identification

				Positive	
Farms	Species	Type of swabs	No. of samples	CPE in T.C.	FAT
Open farm	Cattle calves	Ocular	8	2	1
		Nasal	7	-	-
	Buffaloe calves	Ocular	8	3	1
		Nasal	9	2	1
	Dairy buffaloe	Ocular	16	1	1
		Nasal	13	- ve	- ve
		Vaginal	13	- ve	- ve
	Dairy cattle	Ocular	24	5	2
		Nasal	14	2	1
		Vaginal	24	4	1
Closed farm	Dairy cattle	Ocular	4	- ve	- ve
		Nasal	4		
		Vaginal	2		
	Dairy cattle	Ocular	8	- ve	- ve
		Nasal	6		
		Vaginal	3		
	Dairy cattle	Ocular	12	3	1
		Nasal	7	- ve	- ve
		Vaginal	7	- ve	- ve
	bull	Ocular	1	1	1
		semen	1	- ve	- ve
	Total		136	23	10

Table 3: Distribution of antibodies to BHV-1 by using Institute Pourquier ELISA kit

Farms	Animal case	Age and Species	No. of samples	+ve	% of positive samples
Open farm	App. healthy	Cattle calves 3-6 m	8	5	62.5%
	App. healthy	Buffaloe calves 3-6 m	5	2	40%
	App. healthy	Cattle calves 3-16 m	6	3	50%
	App. healthy	Buffaloe calves 3-16 m	3	1	33%
	App. healthy	adult Dairy cattle	16	10	62.5%
	diseased	adult Dairy cattle	12	8	66%
	App. healthy	adult Buffaloes	5	2	40%
Closed farm	App. healthy	adult Dairy cattle	10	8	80%
	diseased	adult Dairy cattle	21	18	85.7%
		Total	86	47	

open farm. The positive reactors in cattle and buffaloe calves were 62.5 and 40%, in 3-6 months of age and 50 and 33% in 6-13 months of age, respectively.

DISCUSSION

BHV-1 as a viral disease of cattle and buffaloes is of an extreme importance and it is responsible for sever economic losses [29]. These losses are due to respiratory and reproductive disorders and mortalities caused by secondary bacterial infection resulting in pneumonia and death [30]. This work aimed to determine the prevalence of BHV-1 in cattle and buffaloes through viral isolation and testing viral specific antibodies. This was done at a farm in El-Giza Governorate at El-Kata region. In the present investiagation, 10 samples showed characteristic CPE by propagation on monolayer of MDBK cells. CPE became more pronounced after a short time by excessive propagation on the cells and perinuclear yellowish green fluorescent granules was detected by FAT.

As all Herpesvirdae, BHV-1 remains latent in infected animals and may recur under certain stress condition. Shedding of the virus may or may not be accompanied with clinical signs [31], which explain the positive results of CPE and FAT for the samples from apparently healthy animals. Latency allows the virus to persist and the introduction of a latently infected carrier into a noninfected herd is the best way to spread the disease [32]. ELISA test is usually used in detecting antibodies against BHV-1 in enzootic regions and potentially respective extension zone [29, 32 and 33]. Durham and Sillers [34] mentioned that one of the most important points for using ELISA is reliability for examination of serum samples with cytotoxic nature which can not examined by serum neutralization test. The use of Institute Pourquier ELISA kit is rapid, reliable and well suitable for its high reproducibility and inexpensive. The test consumes less than the half of a day.

The demonstration of high percentage of positive reactors, beside the clinical symptoms of respiratory and reproductive manifestation or recent recovering from these signs, were indicative for active virus exposure [19, 35]. The stock density and mixing of the animals in closed farms allow the virus to spread [36]. In addition, due to the virus latency that is normal criteria of BHV-1, the identification of serologically positive apparently healthy animals, provide a useful indicator of infection status [33].

The percent of positive reactors in buffaloes were lower cattle in contact with them in the same open farm. This finding agrees with the results recorded by Moussa *et al*. [17] in some farms in Egypt.

The high incidence of positive reactors in young cattle and buffalo calves in this study may be explained in light of the presence of high percentage of seropositive dams in endemically infected herds, despite some authors claimed that colostrum derived antibodies in young calves have protective effect [37, 38].

In conclusion, results of this study clearly established that BHV-1 is subclinical prevalent virus in cattle and buffaloes in Egypt. A quick and reliable test for diagnosis of BHV-1. ELISA is required to solve this problem, which must be cheap, highly sensitive and could be used on large scale for screening and eradication programs. Buffaloes are more resistant to the BHV-1 infection if compared with the cattle. BHV-1 produces latent infection and persists in closed herds, under stress condition like. Infection with Bovine viral diarrhea virus (BVDV) and / or Border disease virus (BDV), large doses of corticosteroids and transportation, the latent virus can be reactivated resulting in recurrent clinical disease or asymptomatic viral shedding.

REFERENCES

1. Edwards, S.R., H. Newman and H. White, 1991. The virulence of British isolates of BHV-1 in relationship to viral genotype. Brit. Vet. J., 147: 216-231.
2. Kilari, S., S.N. Singh, A.V. Buche, S. Sawarkar and S. Ghosh, 2000. Evaluation of immunogenecity of infectious bovine rhinotracheitis vaccine in Indian tropical climate. Indian Vet. J., 77: 185-188.
3. D'Arce, R.C., R.S. Ameida, T.C. Silva, A.C. Franco, F. Spiki,, P.M. Roehe and C.W. Arns, 2002. Restriction endonuclease and monoclonal antibody analysis of Brazilian isolates of herpes virus type 1 and 5. Vet. Microbiol., 88: 315-324.
4. Reisinger, L. and H. Reimann, 1928. Beitrag Zur Atiologic des lachenausschlages des Rindes. Wine Tirrarztl, Mch., 15: 249-261.
5. Kendrick, J.W., J.H. Gillespic and K. Mcentee, 1958. Infectious pustular vulvovaginitis of cattle. Cornell Vet., 48: 458-495.
6. Mare, C.J. and S.J. Van Rensburg, 1961. The isolation of virus associated with infertility in cattle. Aprelimanry report. J.S. Afr. Vet. Med. Ass., 32: 201-210.

7. Abinanti, F.R and G.J. Plummer, 1961. "The Isolation of infectious bovine rhinotracheitis virus from cattle affected with conjunctivitis- observation on experimental infection." Am. J. Vet. Res., 22: 13-17.
8. French, E.L., 1962. Relationship between IBR, virus Isolated from calf with encephalitis. Aust. Vet. J., 38. 555-556.
9. Winkler, M.T., A. Doster and C. Jones, 2000. Persistence and reactivation of bovine herpes virus-1 in the tonsils of latently infected calves. J. Virol., 74. 5337-5346.
10. Hafez, S.M. and H.Y. Frey, 1973. Serological evidence for the occurrence of BVD-MD and Infectious Bovine Rhinotracheitis in Egypt. Bull. Epiz. Dis. Afric., 21: 5-10.
11. Mohamed, F.M., 1974. Characterization and identification of Infectious Bovine Rhinotracheitis virus of calves in Egypt. Master Thesis in Microbiology, Virology, Cairo Univ.
12. Hafez, S.M., T.G. Baz, M. Zahran and Y.A. Ahmed, 1974. Isolation and identification of infectious pustular vulvovaginitis from local cattle and buffaloes in Egypt. J. Egypt. Vet. Med. Ass., 38: 45-53.
13. Hafez, S., I. Mohsen, T.A. Mohsen and M.H. Zahran, 1976. Infectious bovinc rhinotracheitis in Egypt isolation and serological identification the virus. Egypt Vet. Med. Assoc., 36: 129-139.
14. Baz, T., M. Taha, M. Zahran, M.H. and A. El Dopeigy, 1982. Respiratory viral newborn calf Disease in Egypt. 1- studies on the Role of IBR infection and colostral antibodies. Agric. Res. Rev., 60: 105-121.
15. Nakashly, S.A.H., 1981. Studies on virus respiratory affection in calves. Thesis Ph.D inf.D Fac. Vet. Med. Cairo University.
16. Madbouly, H.M., 1984. Studies on bovine leukemia and other lymphogenic viruses of cattle and buffaloes in Egypt. Thesis M.V.Sc. Microbiology Fac. Vet. Med. Cairo University.
17. Moussa, A.A., M.S. Saber E. Nafie, M.A. Shalaby, W.N. Ayoub, S. EL Nakashly, A.Y. Mohsen, M.M. Madbouly, A.A. El Sanousi, M.M. Fathia, A. Sami, I. Allam and I.M. Reda, 1990. Serological survey on the prevalence of BHV1 in Domestic Animal in Egypt. Vet. Med. J. Giza, 38: 87-94.
18. Madbouly, H.M. and M.M. Hussien, 1997. Isolation of BHV-1, from Dairy herd Genital form of infection. Alex J. Vet. Scien., 13: 439-446.
19. Ahmed, N.M., 1997. prevalence of antibodies to bovine respiratory syncytial virus, infectious bovine rhinotracheitis and parainfluenza-3 in cattle and buffaloe calves. Egypt J. Agric. Res., 75: 1135-1145.
20. Gaber, A.S., M.M. Mahmoud, O.A. Sami and F.M. Ali, 2000. Serological and biochemical changes in sheep sera infected with either Parainfluenza-3 PI-3 or Infectious Bovine Rhinotracheitis IBR viruses. Egypt. J. Comp. Path. Clinc. Pathol., 13: 134-143.
21. Obando, R.C., M. Hidalgo, M. Merza, A. Montoya, B. Klingeborn and J. Moreno-LoÂpez, 1999. Seroprevalence to bovine virus diarrhoea virus and other viruses of the bovine respiratory complex in Venezuela Apure State. Preventive Veterinary Medicine, 41: 271-278.
22. Bastawecy, I.M., M.E. Eweis, Omayma, M. El-Desawy and S.A. Ahmed, 2005. Development of an indirect ELISA for the detection of antibodies against bovine herpes virus-1 BHV-1. J. Egypt Vet. Med. Assoc., 65: 121-130.
23. Abd El-moniem, M.I.I., Y.G.M. Abdel-Hafeiz and H.A. Hussein, 2006. Immunological diagnosis of BHV-1 in ruminants. Egypt J. Comp. Path. and Clinic. Path., 19: 17-26.
24. Wellenberg, G.J., E.R.A.M. Verstraten, F. Jongejan and J.T. Van Oirschot, 2002. Susceptibility of bovine umbilical cord endothelial cells to bovine herpesviruses and pseudocowpox virus. Vet. Res. Comm., 26(5): 407-417.
25. Moore, S., M. Gunn and D. Walls, 2000. A rapid and sensitive PCR-based diagnostic assay to detect bovine herpesvirus 1 in routine diagnostic submissions. Vet. Moicrobiol., 75(2): 145-53.
26. Kramps, J.A., J. Magdalena, J. Quak, K. Weerdmeester, M.J. Kaashoek, M.A. Maris-Veldhuis, F.A.M. Rijsewijk, G. Keil and J.T. Van Oirschot, 1994. Asimple, specific and highly sensitive blocking enzyme-linked mmunosorbent assay for detection of antibodies to bovine herpesvirus-1. J. Clin. Microbiol., 32: 2175-2181.
27. Herring, A.J., P.F. Nettleton and C. Burrells, 1980. A Micro-Enzyme-linked Immunosorbent Assay for the detection of antibodies to infectious bovine rhinotracheitis virus. Vet. Rec., 107: 155-156.
28. VanDonkersgoed, J. and L. Babiuk, 1991. Diagnosing and managing the respiratory form of infectious bovine rhinotracheitis. Vet. Med., 86: 86-94.
29. Boelaert, F., N. Speybroeck, A. de Kruif, M. Aerts, T. Burzykowki, G. Molenberghs and D.L. Berkvens, 2005. Risk factors for bovine herpesvirus-1 seropositivity. Prev. Vet. Med., 69: 285-295.

30. Kramps, J.A., S. Quak, K. WeerDmesster and J.T. Oirschot, 1993. Comparative study on sixteen enzyme linked immunosorbent assays for detection of antibodies to BHV- I in cattle. Vet. Microbiol., 35. 11-21.
31. Muylkens, B., J. Thiry, P. Kirten, F. Schynts and E. Thiry, 2007. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. Vet. Res., 38: 181-209.
32. Pastoret, P.P., E. Thiry, B. Brochier, G. Derboven and Vindevogel, 1984. The role of latency in the epizootiology of infectious bovine rhinotracheitis. In G. Wittmann, R. M. Gaskell and H.-J. Rziha ed., Latent herpesvirus infections in veterinary medicine. Martinus Nijhoff Publishers, Boston, Mass. pp: 221-227.
33. OIE 2004. Manual of diagnostic tests and vaccines for terrestrial animals. 5th edition.
34. Durham, P.J. and H.M. Sillers, 1986. Evaluation of enzyme linked immunosorbent assay ELISA for serodiagnosis of IBR with results of preliminary survey. New Zealand Vet. J., 34: 27-30.
35. Adair, B.M., 1986. Parainfluenza type – 3 and respiratory syncytial virus infection in calves. Res. Vet. Sci., 41: 414.
36. Solis-Calderon, S.J., V.M. Correa, J.C. Correa and A.A. Islas, 2003. Seroprevalence and risk factor for IBR in Beef cattle herds of Ycatan Mexico" Prev. Vet. Med., 57: 199-208.
37. Mechor, G.D., C.G. Rousseaux, O.M. Radostitis, L.A. Babiuk and L. Petrie, 1987. Protection of new born calves against multisystemic IBR by feeding colostrums from vaccinated cows. Cand. J. Vet. Res., 51: 452- 459.
38. Belknap, E.B., J.K. Collins, V.K. Ayers and P.C. Schultheisss, 1994. Experimental infection of neonatal calves with neurovirulent BHV-1. J. Vet. Path., 31: 358-365.

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