Seroepidemiological Assay of Water Buffalo (Bubalus Bubalis) Enzootic Pneumonia Agents (BVDV, BHV-1, bPI3V) in Khuzestan Province of Iran

Mohsen A. Lotfi, Kamalzadeh B. Morteza, and Shahrokh C. Navidpour
Razi Vaccine Serum Research Institute, Karaj, Iran
Email: {M.Lotfi, M.Kamalzadeh, S.Navidpour}@rvsri.ac.ir

Sholeh D. Javadi
Department of Biology, Centeral Payame Noor University, Tehran, Iran
Email: sholeh javadi@yahoo.com

***Abstract*—In most cases, bacterial pneumonia is a secondary infection, frequently prefaced by more common viral infections in the upper respiratory tract, such as bovine herpes virus 1 (BHV1); the causative agent of Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea Virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV), and parainfluenza type 3 (PV-3). In this study we evaluated seroprevalence of three main viral agents of bovine enzootic pneumonia in southwestern of Iranian buffalo. 513 sera sample from nine region of Khuzestan province were investigated. Sera were tested by ELISA for bPI3V and BHV-1, indirect Immunofluorescence Assay Test (IFAT) for BVDV antibody detection. Seroprevalences for PI3V, BHV-1 and BVDV was 45.2%, 5.5% and 32.6% respectively. This result was confirmed and completed computed study about buffalo enzootic pneumonia causatives in Khuzestan province.**

***Index Terms*—buffalo, enzootic pneumonia, bovine respiratory disease, seroepidemiology, PI3V, BHV-1, BVDV**

I. Introduction

Water buffaloes (Bubalus bubalis) are mainly distributed in tropical and sub-tropical regions, such as Asia, North Africa, Australia and Central America [1]. They are raised and kept as economically important animals because they provide milk, meat and hides of good quality and also their dung is good as fertilizer. Enzootic pneumonia or Bovine Respiratory Disease (BRD) complex is a major disease, classically in cattle and buffalo. The etiopathogenesis of BRD is multifactorial and complex. In this complex etiology an equivalent role plays both the infectious agents as well as the environmental factors which are called also as environmental stressors. The most significant pathogens which are involved in the etiopathogenesis of Enzootic pneumonia, i.e. suitable species of viruses such as (Bovine Respiratory Syncytial Virus (BRSV), parainfluenza virus type 3 (PI3V), Bovine Herpes Virus type 1 (BHV1), Bovine Viral Diarrhea Virus (BVDV), are usually associated with concurrent bacterial infections [2]-[5]. These viral pathogens infect the upper respiratory tract, resulting in rhinitis, tracheitis, and bronchitis but have little direct relationship to pulmonary disease. The BRSV is a pneumovirus in the family Paramyxoviridae. BRSV is an enveloped non-segmented negative-stranded RNAvirus [3], [6], [7]. BRSV is recognized as one of viral agents of lower respiratory tract and lung infections in beef and dairy calves. Respiratory clinical signs of BRSV are in feeder calves commonly [6]-[13]. Infectious bovine rhinotracheitis, caused by BHV-1, is a disease of domestic and wild cattle. BHV-1 is a member of the genus Varicellovirus in the subfamily Alphaherpesvirinae, which belongs to the Herpesviridae family [9]. The BHV-1 causes respiratory disease, abortion, conjunctivitis, and other clinical forms of disease complex. Genetic analyses of various clinical isolates have found at least three distinct BHV-1 subtypes; a respiratory subtype, a genital subtype, and an encephalitic subtype designated as BHV-1.1, BHV-1.2, and BHV-1.3, respectively [2], [6], [11]. The BVDV is classified in the virus family Flaviviridae and is a member of the genus Pestivirus. This virus is detected in most clinical cases that are investigated early after the onset of clinical signs [2], [6]. BPI-3V is in the genus Respirovirus of the subfamily Paramyxovirinae, order Mononegavirales, of the family Paramyxoviridae [14]. This virus causes clinical signs in the respiratory system of cattle and sheep. Antibodies of BPI-3V have been demonstrated in humans, cow, buffalo, sheep, and other animals. Like BHV-1, BPI-3V is a viral agent of shipping fever [2]. Seroepidemiological studies can define seroprevalence and distribution of bovine major respiratory viruses. Diagnosis of major viral causes of bovine respiratory infections has been based largely on the serological testing by VNT, ELISA, IFA, HI, and etc.

Manuscript received June 6, 2015; revised January 12, 2016.

doi: 10.18178/joaat.3.3.213-216

of serum from all or parts of the affected dairy herds [15]-[20]. Detecting and controlling bovine viral respiratory diseases can decrease economic losses due to these infections [2], [6]. The presence of several viruses, causing bovine Enzootic pneumonia, in Iran has been previously demonstrated by some studies, separately [16], [17], [19], [21], [22]. The aim of this study was to determine the seroprevalence of BHV-1, BPI3V and BVDV and likely their mixed infections in water buffalo in southwestern region of Iran (Khuzestan province).

II. Method and Material

Khuzestan province is one of the most water buffalo raising area in the country. There are approximately 25.7% (134 thousand) out of a total of this ruminant in this area [16]. Buffalo farming system is traditional and semi-industrial (Fig. 1). The climate of Khuzestan is generally hot and occasionally humid, particularly in the south, while winters are much more cold and dry.

Figure 1. Picture of Iranian buffalo (Bubalus bubalis). IRNA Press.

Summertime temperatures routinely exceed 40 degrees Celsius and in the winter it can drop below freezing. This area is irrigated by some rivers that greatest one is Karoun (850 Kilometers long). This climate is suitable for buffalo farming [23]. About 513 water buffalo serum sample with some data like age, gender, site of bleeding, and season of sampling per case, was obtained and in freezed condition (-20 ℃) transferred to Razzi vaccine serum research institute Karaj – Iran. Sampling was randomly and buffaloes not vaccinated against BoHV-1, BVDV and bPI3 in history, also they hadn't any clinical signs of disease in sampling time. All sera were investigated for antibody against three above viruses as following [24]: BVDV antibody was detected by Indirect Immunofluorescence Assay (IFA). Bovine viral diarrhea virus-free Razi Bovine Kidney (RBK) cell line (Established in Razi institute) was culture with DMEM medium and 10% fetal serum (Gibco) in a 25cm^2 flask and incubated in 37 ℃ for 2 days. 0.1MOI of NADL strain BVDV was inoculated to RBK cell line, maintained in DMEM with 2% fetal serum (Gibco) and 100IU/ml penicillin, and then incubated in 37 ℃ for 3 days. When 10-15% Cytopathic Effect (CPE) was observed in cell culture, flask was tripsinized, washed once in DMEM. The cells were collected by centrifugation, resuspended in 2ml DMEM and then were transferred to micro spot slides and finally fixed by cold acetone in -20 ℃ for 15 min. After fixation slides were stored at -20 ℃. Diluted buffalo sera (at least 1/8) with Phosphate Buffer Saline (PBS) were added to each micro well (20 µl/well) and allowed to incubate for 60 min. in humid chamber at room temperature.

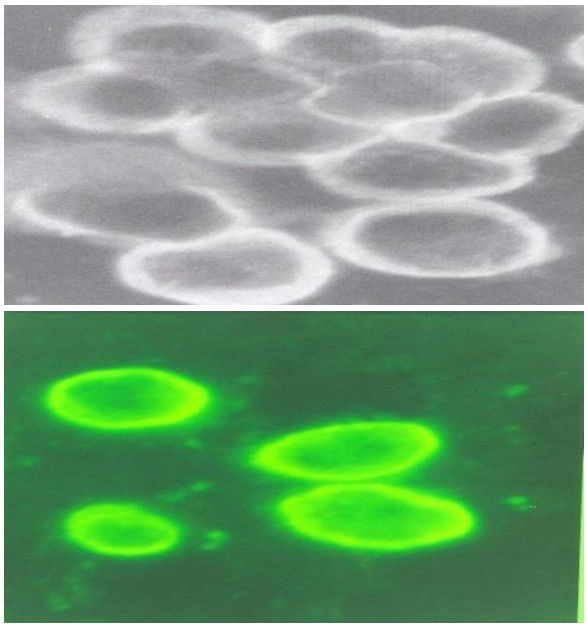

Figure 2. Microscopic pictures of indirect immunofluorescence assay for detection of BVDV antibody in water buffalo sera sample. Above picture is negative sample and down is positive sample.

The slides were washed and diluted (1/150) FITC-labelled rabbit anti-cow IG (DAKO, Denmark) were added to the wells (20 µl/well) and incubated for 30 min. after washing, the slides were examined by fluorescent microscope. In positive samples, Cells were observed brilliant green (Fig. 2). The antibodies to BoHV-1 and bPI3V were detected using a commercially available ELISA kit developed by Svanova Biotech (Uppsala, Sweden). According to the procedure provided in the kit, briefly, each diluted serum sample was added to the antigen-coated micro plates and incubated at 37 ℃ for 1h. After washing, the rabbit anti-cow HRP conjugate was added for 1h at 37 ℃. The reaction was revealed by adding a ready to use substrate solution after washing and tapping.

The reaction was stopped by a stop solution after 30 min. Plates were read at 450nm.

Results are expressed as sample to positive (S/P) ratios, as recommended by the manufacturers. Serum samples with an S/P ratio greater than or equal to the cut off were classified as positive for antibodies. Data were described using descriptive statistics and analysis by Chi-square and One-way ANOVA tests. The statistical program SPSS version 22 was used for the statistical analysis.

III. Result

From 513 samples, 230 samples were for male buffaloes and 283 samples for female. Relative frequency

of seroprevalence to BVDV, BHV-1, and bPI3V was 29.1%, 3.9% and 48.7% respectively for male and 35.3%, 6.7% and 42.4% respectively for female buffaloes.

Relative frequency of antibodies to BHV-1, BVDV, and bPI3 in Khuzestanian water buffaloes were shown in Table I. Plenty of co-seropositivity to above viruses so is listed. The most is concern to BVDV and bPI3 co-seroprevalence with 13.5%. Seroprevalence of these viral agents was determined in several groups (Table II). No significant difference was observed in age groups for each above viruses with oneway ANOVA analysis P>0.05, but there was some significant difference within groups in Tukey method.

TABLE I. RELATIVE FREQUENCY OF SEROPOSITIVE WATER BUFFALOES TO BVDV, BHV-1 AND BPI3 IN KHUZESTAN PROVINCE

Agents	Sample	Positive	
		F	%
BVDV	513	167	32.6
BHV-1	513	28	5.5
bPI3	513	232	45.2
BVDV and BHV-1	513	11	2.1
BVDV, BHV-1 and bPI3	513	4	0.8
BHV-1 and bPI3	513	8	1.6
BVDV and bPI3	513	69	13.5

TABLE II. AGE GROUPED RELATIVE FREQUENCY OF SEROPOSITIVE WATER BUFFALOES TO BVDV, BHV-1 AND BPI3 IN KHUZESTAN PROVINCE

Agents / Age (month)	BVDV F. (%)	BHV-1 F. (%)	bPI3V F. (%)
<6	39 (35.8)	5 (4.6)	48 (44.0)
6-12	68 (33.0)	12 (5.8)	94 (45.6)
12-24	45 (27.3)	9 (5.5)	75 (45.5)
>24	15 (45.5)	2 (6.1)	15 (45.5)
Total	167 (32.6)	28 (5.5)	232 (45.2)

Plenty of seropositivity to above viruses in different city of Khuzestan province was shown in Fig. 3. There was no significant difference for distribution of BVDV and bPI3V between Khuzestan cities by Pearson chi-square analysis, but Scattering for BHV-1 seroprevalence was differs from city to city (P<0.05).

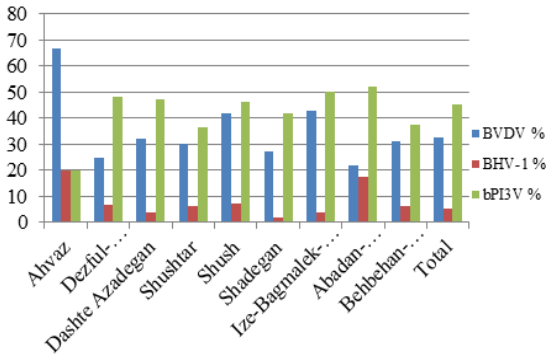

Figure 3. Relative frequency of seropositive water buffaloes to BVDV, BHV-1 and bPI3 in different city of Khuzestan Province.

Although seroprevalence frequency to BHV-1 in winter was higher than other season, but in oneway ANOVA analysis there was not any difference for each viruses.

IV. DISCUSSION

Infection with BVDV, BHV-1 and bPI3V has been shown to be widespread, with a worldwide distribution, but relatively small numbers of study was done for buffalo previously. According to several studies, prevalence of antibodies of BHV-1, BVDV and PI3V is approximately 10–50% or even higher, 60–80% and 40-50% respectively [2], [6], [12], [25]-[27] which demonstrated herd capacity as a very important risk factor for respiratory viruses. In recent years, seroprevalence of several viruses causing bovine respiratory diseases was studied in different bovine population in several regions of Iran. The average was 30-100%, up to 100% and 20-80% for BHV-1, PI3V and BVDV, respectively [16], [17], [19], [28], [29]. So in limited study in buffalo population in Iran, average seroprevalence for buffalo was 4-30%, 40-50% and about 30% for BHV-1, PI3V and BVDV, respectively [22], [30]. In the present study, we describe frequency of seropositivity to above viruses in Khuzestanian water buffalo, with attention to sex, age, site and season of sampling. In comparison to above studies about cattle, our result was shown lower rate for BHV-1 and BVDV. It may be result of viral imported by live cattle import to Iran and PI3V like the other region in the word is endemic in Iranian ruminants. Our result, confirm previously studies about frequency of above viruses. Although rate of seropositivity especially for PI3V in buffalo was high, there was no evidence of clinical disease in any of buffaloes at the time of sampling. The prevalence to the respiratory viruses selected for this study document the exposure of buffalo to bovine pathogens or closely related agents. To date, the epidemiology of these viruses in buffalo populations is poorly understood. Further research on these potential pathogens in this worthful animal is recommended.

ACKNOWLEDGEMENT

We would like to thank our colleague in the Razi institute in Ahvaz branch for help to sampling.

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Mohsen A. Lotfi was born in Kashan, Iran on November 22, 1967. His educational background is *DVM*, in Shahid Chamran University, Ahvaz, Iran (1987-1993) and PhD in Virology, Tehran University, Tehran, Iran (2002-2008). He is an assistant professor in QC Management of Razi Vaccine serum Research Institute, Karaj, Iran. His interests are Cell Culture, Monoclonal Antibody preparation, Practical Virology, Molecular technique (PCR, RT-PCR, Cloning, Protein Expression...) ELISA, SDS PAGE, Western blot, Handling of laboratory animals, Vaccinology.

Morteza B. Kamalzadeh was born in Karaj, Iran on March 2, 1972. His educational background is Bs in *Microbiology*, Islamic Azad University Karaj, Iran (2006-2009). He is working in QC Management of Razi Vaccine serum Research Institute, Karaj, Iran. His interests are Cell culture and Practical virology.

Shahrokh C. Navidpour was born in Ahvaz, Iran in January 13, 1967. His educational background is *DVM*, in Shahid Chamran University, Ahvaz, Iran (1987-1993) and PhD in *Parasitology*, Shahid Chamran University, Ahvaz, Iran (1997-2001). He is associate professor in Razi Reference Lab. of Scorpion Research (RRLS), Razi Vaccine and Serum Research Institute, Karaj, Iran.

Sholeh D. Javadi was born in Tehran, Iran on June 1, 1981. Her educational background is Bs in *Biological Science*, Islamic Azad University Karaj, Iran (2006-2009) and Master of *Biochemistry* in Payame Noor University Tehran, Iran (2010-2013). She is working in a laboratory for medical diagnostics, Karaj, Iran. Her interests are Cell culture, oncology, Biochemistry, Microbiology.