A study on investigation of occurrence of some virus infection in Buffaloes in Turkey*

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SUMMARY

In this study, occurrences of Parainfluenza Virus 3 (PIV3), Bovine Respiratory Syncytial Virus (BRSV), Bovine Adenovirus type 1, 2, and 3 (BAV1,2,3) and Enzootic Bovine Leukosis (EBL) infections were investigated in Turkey buffalo population.

For this purpose, serum samples collected from 452 buffaloes housed in 8 different provinces in Turkey were used and analysed with virus neutralisation technique (PIV3, BRSV, BAV1, 2 and 3) and with agar gel immunodiffusion for EBL. Seropositivity rates were 11 % for PIV3, 28 % for BRSV, 49 % for BAV1, 56 % for BAV2 and 55 % for BAV type 3. None serum showed any evidence of precipitation activity against gp51 BLV antigen. As a result, the occurrence of viral infections have been detected firstly in buffaloes in Turkey, and might be compared with seroprevalence in cattle, for testing the possibilities of interspecies transmission.

KEY-WORDS : BAV 1,2,3 - BLV - BRSV - PIV3 - buffalo - seroprevalence - Turkey.

RÉSUMÉ

LES INFECTIONS VIRALES CHEZ LE BUFFLE EN TURQUIE. Par Y. AKÇA, I. BURGU, S. GÜR et S. BILGE DAGALP.

Dans cette étude, l’existence d’infections par les virus PIV3 parainfluenza virus 3), RSV (bovine respiratory syncytial Virus), BAV1, 2 et 3 (Adenovirus 1, 2 et 3) et EBL (ou BLV) (Enzootic Bovine Leukemia) a été recherchée sur la population de Buffles en Turquie. Pour cela, les séra de 452 buffles localisés dans 8 provinces turques ont été collectés et analysés par des techniques de séronutralisation (pour les virus PIV3, BRSV et BAV1, 2, et 3) et d’immuno-diffusion en gel d’Agar (pour le BLV).

Les taux de séropositivité obtenus ont été de 11 % pour PIV3, 28 % pour BRSV, 49 % pour BAV1, 56 % pour BAV2 et de 55 % pour BAV3. Aucun sérum n’a montré d’activité précipitante de la gp51 du BLV.

En conclusion, les fréquences de ces infections ont été pour la première fois explorées en Turquie chez le Buffle et devraient être comparées aux séroprévalences obtenues chez les autres animaux de rente afin d’évaluer les possibilités de transmissions inter-espèces.

MOTS-CLÉS : BAV 1, 2, 3 - BLV - BRSV - PIV3 - buffle - séroprévalence - Turquie.

Introduction

Buffalo breeding has economical importance for some provinces in Turkey. But the number of the population showed dramatic decrease at last 20 years because of breeding is out of economic value. Some infections and low milk yield are prominent reasons of it. Buffalo meat and milk are used at locally producing some special kind of products. Buffalo meat is used to produce for meat products at different percentages and milk using for delight, cheese and «kaimak». Today, buffalo breeding having mostly at the small family farms with cattle in Central Anatolia and Blacksea coastal region.

The prevalence and importance of any viral infections were not investigated in buffaloes until now in Turkey. But different researchers [13, 14, 18, 23, 24] reported that infections as Bovine herpes Virus 1 (BHV1), Bovine Viral Diarrhoea Virus (BVDV), Parainfluenza Virus 3 (PIV3), Enzootic Bovine Leukosis (EBL), Bovine RotaVirus (BRV) can be present and cause economical losses in both cattle and buffalo population. PIV3, Bovine Respiratory Syncytial Virus (BRSV) and Bovine Adenoviruses (BAVs) are important respiratory pathogens and they are often detected in animals as sheep, goat, cattle suffering from respiratory disease [8, 20]. These agents may cause an acute or subacute viral disease of animals characterized by pyrexia, nasoocular discharge and pneumonia. Animal may suffer from respiratory infection caused by any respiratory agent alone or by combinations with bacterial or other viral pathogens [9].

Bovine leukemia is a cancer of lymphatic tissue, and the most common form is caused by bovine leukemia virus

(BLV). The leukemia caused by BLV occurs in cattle over 3 years old. Clinical signs are varied and depend on the site of tumor localisation. When bovine leukemia was first recognized in Europe in the early 1900s, the disease was seen to spread from herd to herd and from one geographic area to another [18]. It is reported that these infections have been detected serologically and virologically in cattle and sheep in Turkey [2, 4, 5]. Also, a study on occurrence of BVDV and BHV-1 infection in buffaloes has been just completed [12].

The purpose of the present study was to investigate the occurrence of PIV-3, BRSV, BLV and BAV type 1, 2 and 3 in buffaloes in Turkey.

Material and method

MATERIAL

Sampled animals

Buffalo breeding having mostly in Central Anatolia and Blacksea coastal region placed in north of Turkey. Also, materials of this study were obtained from animals breeding these parts of Turkey (Table I). In this study, sera samples were randomly collected from 452 healthy, 1-4 year old buffaloes without vaccination from a state farm (intensive breeding) and from several private herds having between 5 to 10 buffaloes breeding extensively in 8 different parts of Turkey. All the buffaloes in herds used in this study were sampled. In these herds, buffaloes have been housed together with cattle, except when they were housed in the state farm.

Cell culture

Viruses used in this study were propagated and titrated in Madin Darby Bovine Kidney (MDBK) cells. MDBK cells were also used in a microtitre serum neutralization test. Cells were grown in Eagle’s minimal essential medium (EMEM) containing 10 % heat inactivated foetal bovine serum and EMEM containing 2 % serum. In the microtiter serum neutralization test, EMEM was used with 10 % foetal bovine serum.

Viruses

The viruses used in this study were grown at 37°C in MDBK cell cultures. PIV3 (SF4, German strain), BRSV, BAV type 1 (strain 11/66), 2 (strain 12/66), 3 (strain 13/66) stock viruses contained $10^{5.0}$, $10^{3.25}$, $10^{5.0}$, $10^{5.5}$, $10^{4.75}$ median Tissue Culture Infectious Dose 50 (TCID$_{50}$) of virus/ml, respectively. BRSV was obtained from Moredun Research Institute, Scotland, UK. BAVs was obtained from Vien Veterinary Institute.

METHODS

Virus neutralisation technique

For screening purposes as well as for quantitative determination of antibodies against PIV3, BRSV and BAV type 1, 2, 3, the simultaneous technique of microtitre neutralization test was applied according to FREY and LIESS [11] using MDBK cell culture in microneutralization plates. A microtitre serum neutralization test applied for the detection of serum antibodies against BRSV were considered to be positive at the presence of antibodies in dilutions of 1:2 or higher as described BAKER et al., [3]. RICHER et al. [21] also were considered a result as positive at the presence of antibodies in dilutions of 1:5 or higher.

The agar gel immunodiffusion test (AGID)

For the detection of antibodies against Bovine Leukosis Virus (BLV), the AGID test was performed as described earlier [10]. BLV antigen containing BLV gp51 and positive control serum was obtained from Seromed, Germany.

RESULTS

All serum samples were determined as negative for antibodies against gp51 BLV antigen. Seropositivity rates for other viruses were: 11 % for PIV3, 28 % for BRSV, 49 % for BAV-1, 56 % for BAV-2 and 55 % for BAV-3 (Table I). Also, rates of seropositivity for each infections determined in the 8 province were shown in Table I. PIV3 and BRSV seropositivity were similar in all the explored provinces except for Samsun country, in which a greater proportion of buffaloes

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of samples</th>
<th>PIV3 (PS%)</th>
<th>BRSV (PS%)</th>
<th>BAV-1 (PS%)</th>
<th>BAV-2 (PS%)</th>
<th>BAV-3 (PS%)</th>
<th>EBL (PS%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afyon</td>
<td>145</td>
<td>10 (6)</td>
<td>42 (28)</td>
<td>78 (53)</td>
<td>94 (64)</td>
<td>66 (45)</td>
<td>-</td>
</tr>
<tr>
<td>Konya</td>
<td>10</td>
<td>2 (20)</td>
<td>5 (50)</td>
<td>9 (90)</td>
<td>6 (60)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Elazığ</td>
<td>8</td>
<td>1 (12)</td>
<td>2 (25)</td>
<td>4 (50)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sivas</td>
<td>21</td>
<td>4 (19)</td>
<td>14 (66)</td>
<td>11 (52)</td>
<td>9 (42)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ankara</td>
<td>38</td>
<td>4 (10)</td>
<td>23 (60)</td>
<td>19 (50)</td>
<td>14 (36)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amasya</td>
<td>84</td>
<td>7 (8)</td>
<td>49 (58)</td>
<td>42 (50)</td>
<td>53 (63)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tokat</td>
<td>31</td>
<td>6 (19)</td>
<td>22 (70)</td>
<td>28 (90)</td>
<td>24 (77)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Samsun</td>
<td>115</td>
<td>24 (20)</td>
<td>46 (40)</td>
<td>31 (26)</td>
<td>48 (41)</td>
<td>75 (65)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>452</td>
<td>51 (11)</td>
<td>129 (28)</td>
<td>222 (49)</td>
<td>253 (56)</td>
<td>251 (55)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table I. — Seroprevalence of PIV3 (Parainfluenza virus 3), BRSV (Bovine Respiratory Syncytial Virus), BAV 1, 2 and 3 (Bovine Adenoviruses type 1, 2, 3) and EBL (Enzootic Bovine Leucosis) infections. Results are expressed as number of positive serum (PS) and percentages in parenthesis.
(40%) showed a BRSV positive result. We have also noticed that animals were less often exposed to BAV1 and 2 in this particular country (27% and 42% in Samsun province against 49% and 56% in all the Turkey respectively). For each virus, Serum Neutralisation Index 50 (SN50) rates were also determined by microneutralisation test. SN50 values of PIV3, BRSV, BAV-1,2 and 3 ranged between 1:5 to 1:320, 1:2 to 1:32 and 1:10 to 1:320, respectively. Additionally, the distribution of geometric mean antibody titers against test viruses were presented in Figure 1. Data were evaluated in respect with single or multiple seropositivity. Thirteen% (59/452) of the animals were negative for antibodies against selected viruses. In 20.5% (93/452) of animals, antibodies for a single virus were detected. In 29.4% (133/452) aniamls were seropositive for two disease, and in 27.2% (123/452) they were positive for 3 virus infections. In 9.7% (44/452), antibodies were simultaneous found for 4 -5 viruses (Figure 2 and Table II). The principal viral combinations have frequently associated BAV (1, 2 and 3) with BRSV or PIV3 (Table II).

**Discussion**

There have been reports [13, 14, 17, 22, 23] of natural infection in buffalo population with viruses associated with respiratory tract and other systemic infections of cattle and other ruminants. For some of these viruses, little is known about epizootic character in buffalo population. In addition, there have been serosurveys used to detect these infections in buffalo in countries other than Turkey. Occurrence of infections with PIV3, BRSV, BLV, BAV-1, 2 and 3 in cattle were reported by different authors [2, 4, 5] but report on these viral infections in buffaloes in Turkey were absent. In the present study, the occurrence and seroprevalence of PIV3, BRSV, BLV and BAV type 1, 2 and 3 infections were detected in Turkey, for the first time. Seropositivity rates for each virus were 0% for BLV, 11% for PIV3, 28% for BRSV, 49% for BAV1, 56% for BAV2, 55% for BAV-3 in the sampling population. Reports of PIV3 infection in buffalo from various parts in the world were based on antibody surveys. ULBRICH [23] reported occurrence of antibodies against IBR-IPV (72.2%), BVD-MD (70%) and PI3 (100%) viruses in Vietnamese buffaloes. In another study [24] conducted on buffalo population in Egypt, antibodies against IBR and PIV3 were detected in 45% and 53%, respectively. In our study, antibodies against PIV3 were detected in 11% of sampling buffaloes, that indicating a lower prevalence of this viral infection in buffaloes in Turkey.

Antibody to RSV have been detected in many domestic animals including cattle, horses, pigs, sheep and buffalo. SHALABY et al. [22] reported that seroprevalence of RSV infection detected by ELISA in buffaloes in Giza was 37.5%. YOUSSEF [24] detected that seropositivity rate against RSV was 7.5% in buffalo calves in Egypt. In our research, antibodies against BRSV were detected in 28% of sampling buffaloes and BRSV were considered to be positive at the presence of antibodies in dilutions of 1:2 or higher according to BAKER et al [3] but other researchers [21]
considered serum as positive at the presence of antibodies in dilutions of 1:5 or higher. Reference titre used in this study may be affected the seropositivity rate for RSV.

EBL was seen to spread from herd to herd and from one geographic area to another. WHILE HAMBLIN et al. [14] and HAFIEZ et al. [13] didn’t found specific antibodies to BLV in the 195 buffalo sampled from Tanzania. MEAS et al. [17] determined antibodies to BLV as 0.8 % in Pakistan. In Turkey, the presence and seroprevalence rate of EBL infection in cattle were described in several studies [1, 5]. Seroprevalence of the infection varied between 1.2 to 34.2 % in some state farms. However, there is not any other data about the occurrence of EBL infection in buffaloes in Turkey. Also, the absence of detection of antibodies to BLV in the 452 buffaloes used in this study may suggest that buffalo is not readily infected because of an eventual natural resistance or because there is weaker contacts between cattle and buffalo for allowing transmission by biological or mechanical routes. Additionally, western blot and ELISA methods are more sensible than AGID technique used in this study [6, 7]. The low sensitivity of the used technique could partially explain the negative result of our own study.

The seropositivity rates to BAV 1, 2 and 3 were found to be 49 %, 56 % and 55 % respectively. Seropositivity to every 3 viruses were detected with different rates from herd to herd. It is known that bovine adenoviruses- 1, 2 and 3 were serologically distinct from each other by microneutralisation test [15, 16, 19]. However, it is necessary to discuss the observed simultaneous seropositivity for BAV 1-3 according to the possibility of cross reaction between these serotypes. It was found that seropositivity rates of every serotype were similar and the presence of antibody against BAV 1 to 3 were higher than the other viruses. Data couldn’t be compared with any study because any study on adenoviruses in buffalo were not available. It was thought that adenovirus infections may be also common in buffalo as in cattle.

Although this study was conducted on a limited population of buffaloes, more particularly in some provinces (Elazığ, Sivas and Tokat), the frequency of seropositive results for the different tested viruses were quite similar in the 8 explored countries, except for Samsun country. In this province, BRSV infection seemed to be more frequent whereas BAV1 and BAV2 appeared less frequently than in the rest of the Turkey. As a result, data presented here have been indicated that infections, except EBL, are present within buffalo population in Turkey. Although this study was performed on a limited buffalo population, these infections have been already reported in Turkey by different researchers [1, 4] in cattle but not in buffaloes. The possibility of cross-infections between species must also be considered. For this purpose, firstly in herds which included together cattle and buffalo, the seroprevalence of these infections can be investigated in the two species simultaneously. Possibilities of interspecies transmission, routes and mechanisms of infections in buffalo, and the role of the affected buffaloes as a reserve for infection towards cattle need further investigations.

References