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SEROLOGICAL EVALUATION OF VIRAL INFECTIONS IN BOVINE RESPIRATORY TRACT

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In this study, a total of 254 blood sera samples taken from cattle of different age, sex and breed at Meat and Fish Association Slaughter House in Konya were tested against neutralizing antibodies for Bovine Adenovirus type 1, 2 and 3 (BAV-1, 2 and 3), Infectious Bovine Rhinotracheitis / Infectious Pustular Vulvovaginitis Virus (IBR/IPV), Parainfluenza type 3 (PI-3) Virus, Bovine Respiratory Syncytial Virus (BRSV) and Bovine Viral Diarrhea Virus (BVDV) by microneutralization test (mNT).

At the end of the serological control by mNT, neutralizing antibody presence was detected against BAV-1 in 56 (22.04%), BAV-2 in 38 (14.96%), BAV-3 in 51 (20.07%), PI-3 in 137 (53.93%), IBR/IPV in 145 (57.08%), BRSV in 117 (46.06%) and BVDV in 112 (44.09%) animals.

In the cattle tested for antibodies, 14.7% were positive for only one virus, 36.22% of sera had antibodies to two viruses, 29.92% of sera had antibodies to three viruses, 14.56% of sera had antibodies to four viruses, 3.93% of sera had antibodies to five viruses, 1.57% of sera had antibodies to six viruses and 0.39% of sera had antibodies to seven viruses. However antibodies were not detected in 3.15% of the 254 sera.

Key words: multiple viral infection, serology, neutralization test, cattle.

INTRODUCTION

Infectious agents implicated in bovine respiratory disease include viruses, bacteria, mycoplasma and chlamydia. The most common viruses implicated in the respiratory complex of cattle include Bovine Adenovirus type 1, 2 and 3 (BAV-1, 2 and 3), Infectious Bovine Rhinotracheitis / Infectious Pustular Vulvovaginitis Virus (IBR/IPV), Parainfluenza type 3 (PI-3) Virus, Bovine Respiratory Syncytial Virus (BRSV), Bovine Viral Diarrhea Virus (BVDV) and Mammalian Reovirus types 1 and 2 (Reo-1, 2) (Radostits *et al*. 1994).

According to the original source of tissue (adenoid) in which the prototype viral strain was discovered, these agents were named adenoviruses. Though they are frequently isolated from asymptomatic animals, in certain cases, severe, sometimes fatal infections or disease outbreaks have been described, specially if

other immunosuppressive factors, such as accumulation of weaned calves and lambs, crowding or other viral or bacterial infections were observed (Lemkuhl 1979).

Numerous reports on bovine PI-3 virus activity have been presented for herds of young cattle with respiratory diseases such as enzootic calf pneumonia and shipping fever. PI-3 virus infection may be accompanied by concurrent infection of the respiratory tract by other viruses such as respiratory syncytial virus, adenovirus or BVDV (Suzan *et al*. 1983).

Bovine Rhinotracheitis infection of the upper respiratory tract is present in almost all herds, but causes illness in unexposed animals or in those with lowered levels of immunity. This agent is commonly implicated with bacterial agents as the cause of shipping fever or other severe cases of pneumonia (Kahrs 1981).

Bovine Respiratory Syncytial, Virus a well recognized infectious agent, is now identified in respiratory infections all across the country. It is mainly a problem in weaner and feedlot animals (Baker 1985).

Bovine Virus Diarrhea is present in almost all herds. It has profound deterimental effects on the immune system. BVDV's role in respiratory disease is primarily due to immunosuppression and synergism with other pathogens of the respiratory disease complex (Akhtar and Asif 1996).

The aim of this study was to investigate the presence of antibodies against BAV-1, 2 and 3, IBR/IPV, PI-3, BRSV and BVDV by microneutralization test (mNT) in blood samples collected from cattle slaughtered at Meat and Fish Association Slaughter House in Konya, Turkey.

MATERIALS AND METHODS

Animals: A total of 254 blood samples were collected from various cattle breeds in Konya Meat and Fish Association Slaughter House. All animals were healthy and not previosly vaccinated against viral respiratory diseases (table 1).

Table 1. Distribution of cattle sera by breed and sex

Breed	Calves		Adults		Total
	♀	♂	♀	♂	
Holstein	8	18	36	80	142
Montaphon	2	4	45	26	77
Hereford	1	2	20	12	35
Total	11	24	101	118	254

Blood samples: Peripheral blood was aseptically obtained by jugular venepuncture with vacutainer systems (Becton Dickson, UK).

Cell culture: Madin Darby Bovine Kidney (MDBK) was used for propagation and titre determination of BAV-1, 2 and 3, IBR/IPV, PI-3 and BRSV. However,

specific neutralizing antibodies were detected by mNT in these cells. Fetal Calf Kidney (FCK-BVDV-Ag^{-})was used for the detection of BVDV.

Virus: In this study BAV-1, 2 and 3, IBR/IPV, PI-3, BRSV and BVDV were used for mNT.

Virus titration: Viruses titrations were done according to Frey and Liess (1971). On the 5th day cytopathologic changes were studied tissue culture microscopy and the results were calculated according to Kaerber (1964).

Microneutralization test (mNT): Microneutralization test (mNT) was done according to Frey and Liess (1971). Except for IBR/IPV, all inactivated sera samples were diluted BAV-1, 2 and 3 to 1 : 10, PI-3 and BVDV to 1 : 5 and BRSV to 1 : 2. On the 5th day cytopathologic changes seen in cells were studied by tissue culture microscopy and the results were evaluated.

Serum Neutralization $_{50}$ (SN_{50}): In mNT, positive sera were subjected to SN_{50} test. Results were calculated according to Kaerber (1964).

RESULTS

In this study, BAV-1, 2 and 3, IBR/IPV, PI-3, BRSV and BVDV's titres were determined (table 2).

Table 2. Virus titres

Tested Viruses	$DKID_{50}$ / 0.05 ml	Tested Viruses	$DKID_{50}$ / 0.05 ml
BAV-1	$10^{-5.20}$ / 0.05 ml	IBR/IPV	$10^{-6.45}$ / 0.05 ml
BAV-2	$10^{-5.70}$ / 0.05 ml	BRSV	$10^{-4.70}$ / 0.05 ml
BAV-3	$10^{-4.95}$ / 0.05 ml	BVDV	$10^{-4.20}$ / 0.05 ml
PI-3	$10^{-5.45}$ / 0.05 ml		

Out of the 254 serum samples tested positive: BAV-1 56 (22.04%), BAV-2 38 (14.96%), BAV-3 51 (20.07%), PI-3 137 (53.93%), IBR/IPV 145 (57.08%), BRSV 117 (46.06%) and BVDV 112 (44.09%). Distributions of seropositive cattle were determined by breed, age and sex (table 3, 4, 5).

Sera samples from 8 (3.15%) cattle were not positive by means of neutralization test to antibodies against respiratory viruses. Sera samples from 36 (14.17%) cattle were detected positive by neutralization test for only one respiratory virus. Further, distribution of seropositive cattle was determined by neutralization test on antibodies against more than one respiratory virus.

SN_{50} test was applied to sera of the animals detected as positive. SN_{50} values of positive sera were detected between 1/10.0-1/25.2 in BAV-1, 1/11.9-1/94.4 in BAV-2, 1/12.6-1/63.1 in BAV-3, 1/11.3-1/168 in PI-3, 1/14.8-1/200 in IBR/IPV, 1/22.4-1/126 in BRSV, 1/6.68-1/26.6 in BVDV.

Table 3. Distribution of seropositive cattle by breed

Breed	Samples	BAV-1	BAV-2	BAV-3	PI-3	IBR/IPV	BRSV	BVDV
H	142	36	24	27	76	84	65	57
M	77	12	10	15	44	40	36	41
Hr	35	8	4	9	17	21	16	14
Total	254 % 100	56 % 22.04	38 % 14.96	51 % 20.07	137 % 53.93	145 % 57.08	117 % 46.06	112 % 44.09

H: Holstein M: Montaphon Hr: Hereford

Table 4. Distribution of seropositive cattle by age

Age	Samples	BAV-1	BAV-2	BAV-3	PI-3	IBR/IPV	BRSV	BVDV
Calves	35	7% 2.75[a]	5 % 1.96	10 % 3.93	20 % 7.87	21 % 8.26	30 % 11.81	15 % 5.90
Adults	219	49 %19.29	33 % 12.99	41 % 16.14	117 % 46.06	124 % 48.81	87 % 34.25	97 % 38.18
Total	254	56	38	51	137	145	117	112

[a] According to total number of animals

Table 5. Distribution of seropositive cattle by sex

Sex	Samples	BAV-1	BAV-2	BAV-3	PI-3	IBR/IPV	BRSV	BVDV
Female	112	40 % 15.74[a]	20 % 7.87	17 % 6.69	85 % 33.46	78 % 30.70	61 % 24.01	53 % 20.86
Male	142	16 % 6.29	18 % 7.08	34 % 13.38	52 % 20.47	67 % 26.37	56 % 22.04	59 % 23.22
Total	254	56	38	51	137	145	117	112

[a] According to total number of animals

DISCUSSION

Öztürk and Toker (1988) determined the presence of antibodies against BAV-1, 2 and 3 in blood serum of 214 cows by mNT. These researchers found seropositivity rates of cow blood serums as 71%, 84% and 89% for BAV-1, 2 and 3, respectively. Yavru and Öztürk (1990) collected 150 blood serums from the slaughter house in Konya and found the seropositivity against BAV-1 to be 16.87%. Alkan *et al*. (1997), collected samples from 7 different sites, and reported

that seroprevalence of BAV infections was less than it has been previously reported. In this research seropositivity rates for BAV-1, 2 and 3 was 22.04%, 14.96% and 20.7, respectively.

The values in our research were lower than those previously reported. On the other hand, the results can be compared to those of Alkan et al. (1997), and Yavru and Öztürk (1990). According to our findings, seroprevalence of BAV in Turkey tends to be lower.

Incidence of PI-3 virus in Turkey was determined previously (Öztürk *et al*., 1988b; Öztürk and Yavru 1988). Öztürk and Yavru (1988) reported 45.6% neutralizing antibodies against PI-3 from serum samples of 1032 cows collected from a slaughter house in Konya. Again Öztürk *et al*. (1988b) stated a presence of neutralizing antibodies against PI-3 as 49.57% from serum samples collected from the Institute of Animal Research Center in Konya.

In the current research, neutralizing antibodies determined by mNT were 53.93%, being in agreement with previous reports which indicate that the rate of PI-3 infection is high in both open and close farming systems in Turkey.

Öztürk et al. (1988a) tested 238 serum samples, collected from Konya Institute of Animal Research Center, against IBR/IPV virus by microneutralization test and reported that 56.3% of the samples were positive to IBR/IPV. Alkan *et al*. (1997) reported that in every herd they checked against IBR/IPV the rate of seropositivity was 59.7%.

In our research, the seropositivity rate for IBR/IPV was 57.08%. Thus, all these researches indicate that prevalence of IBR/IPV is very high in Turkey.

The first research on BRSV infections in Turkey was reported by Burgu et al. (1990). The neutralizing antibody rate in animals on both government and family owned farms was 46.12% in the study. Alkan *et al*. (1997) reported 44.66% seropositivity for BRSV in cow herds. Baker *et al*. (1985), in 559 blood serum samples, found BRSV antibody prevalence to be 65.5%. Although our finding for BRSV in the current study was lower (46.06%) than stated by Baker *et al*. (1985), the result was similar to the ones reported by Alkan *et al*. (1997) and Burgu *et al*. (1990).

It is known that BVDV causes infectious respiratory diseases in cows (Key and Derbyshire, 1984). The presence of BVDV antigens and the immunosuppressive nature of BVDV cause secondary viral and bacterial infections in cows infected with respiratory diseases.

Alkan *et al*. (1997), found neutralizing antibodies to be 21.4-100% positive for BVDV in blood serum samples of cows in sampled dairy farms.

Durham and Hassard (1990) tested blood serum samples of 1745 healthy cows from 295 farms for specific antibodies against IBR/IPV, PI-3, BRSV and BVDV by ELISA, and the rates for IBR/IPV, PI-3, BRSV and BVDV were 37.8%, 93.9%, 78.5% and 40.6%, respectively. The same researchers also reported that antibodies against IBR/IPV and BVDV were lower among males, young and unvaccinated animals. In addition, the antibody rate of IBR/IPV was lower in Herefords and vaccinated animals had higher antibodies levels than unvaccinated cows.

Suzan *et al.* (1983) tested blood serum samples of dairy cows and beef cattle from 19 different states for BHV-1, PIV-3, BAV-7 and BVDV, and found seropositivity of these samples to be 57%, 75%, 23.4%, 70.5%, and 52%, 69.3%, 71.4%, 62.5% for dairy cows and beef cattle, respectively.

Ghirotti *et al*. (1991) found seroprevalence of BVD-MD, PI-3, IBR/IPV and BAV-3 to be 76%, 94.4%, 42.1% and 87.4%, respectively. They also reported that antibodies against BVD-MD and IBR/IPV viruses were higher in cows 1 year old or older and sex was not a very important factor for antibody rates.

In our research, in cow blood serum samples the presence of viral respiratory infections was checked by neutralizing antibodies and results were compared to other studies. The overall values we found were similar to the studies reported previously.

In the current research, the highest antibody occurrence against BRSV was in calves and this compares to the conclusion made by Kimman *et al.* (1989) stating that calves were more susceptible to BRSV infection.

Our results are in agreement to the findings reported previously that seropositivity was not affected greatly by sex and was similar in both males and females (Ghirotti *et al*., 1991).

In our research, of all the cows tested, 14.17% of the cows had only 1, 36.22% of the cows had 2, 29.92% of the cows had 3, 14.56% of the cows had 4, 3.93% of the cows had 5, 1.57% of the cows had 6, and 0.39% of the cows had 7 of the specific antibodies tested. On the other hand, 3.15% of the cows showed no antibody response against any of the viruses tested in this study.

As a result, this study showed again that a number of viral respiratory diseases can be simunltaneosly present in one animal. In addition, according to the survey with the owners of the farm, no animals in the study were vaccinated against the viruses examined in this study. Thus, we concluded that seropositivity reported here was not due to vaccination but was due to previous natural infection.

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REFERENCES

1. *Akhtar S, Asif M,* 1996, Epidemiologic association between antibody titres against bovine virus diarrhoea virus: Rinderpest disease virus and infectious bovine rhinotracheitis virus in a buffalo herd, *Trop Anim Hlth Prod*, 28, 207-12.
2. *Alkan F, Özkul A, Karaoglu MT, Bilge S, Akça Y, Burgu I, Yesilbag K et al,* 1997, Sigirlarda viral nedenli solunum sistemi enfeksiyonlarinin seroepidemiyolojisi, *A Ü Vet Fak Derg,* 44, 73-80.
3. *Baker JC, Ames TR, Markham JF,* 1985, Serological studies of bovine respiratory syncytial virus in Minnesota cattle, *Am J Vet Res*, 46, 891-2.

4. *Burgu I, Toker A, Akca Y, Alkan F,* 1990, A seroepidemiologic study of bovine respiratory syncytial virus (BRSV) in Turkey, *Dtsch Tierarztl Wochenschr*, 97, 88-9.
5. *Durham PJK, Hassard LE,* 1990, Prevalence of antibodies to infectious bovine rhinotracheitis, parainfluenza-3, bovine respiratory syncytial and bovine viral diarrhea viruses in cattle in Saskatchewan and Alberta, *Can Vet J,* 31, 815-20
6. *Frey HR, Liess B,* 1971, Vermehrungskinetik und verwendbarkeit eines stark zytopatogenen VD-MD virusstammes für diagnostische untersuchungen mit der mikrotiter-methode, *Zbl Vet Med Bul,* 18, 61-71.
7. *Ghirotti M, Semproni G, De Meneghi D, Mungaba FN, Nannini D, Calzetta G et al,* 1991, Sero-prevalences of selected cattle disease in the Kafue flats of Zambia, *Vet Res Com*, 15, 25-36.
8. *Kaerber G,* 1964, Diagnostic procedures for virus and ricketsial disease, Public Health Ass, New York, 3, 48-50.
9. *Kahrs FR,* 1981, Infectious bovine rhinotracheitis. The Iowa State University press, Iowa, 135-56.
10. *Key DW, Derbyshire JB*, 1984, Serological studies of parainfluenza type-3 virus, bovine adenovirus type-3 and bovine respiratory syncytial virus infection in beef calves, *Vet Mic*, 9, 587-92.
11. *Kimman TG, Sol J, Westenbrink PJ, Straver PJ,* 1989, A severe outbreak of respiratory tract disease associated with bovine respiratory syncytial virus probably enhanced by vaccination with modified live vaccine ,*Vet Quart*, 11, 250-3.
12. *Lehmkuhl HD, Smith MH, Gough PM,* 1979, Neutralizing antibody to bovine adenovirus serotype 3 in healthy cattle and cattle with respiratory tract disease, *Am J Vet Res*, 40, 580-83.
13. *Öztürk F, Toker A,* 1988, Konya Tarim isletmesi sigirlarinda sigir adenovirus tip 1, 2, 3'ün serolojik olarak saptanmasi, *S Ü Vet Fak Derg*, 4, 213-8.
14. *Öztürk F, Yavru S,* 1988, Konya bölgesi sigirlarinda parainfluenza virus-3 (PIV-3) enfeksiyonu üzerinde serolojik arastirmalar, *S Ü Vet Fak Derg,* 4, 135-41.
15. *Öztürk F, Toker A, Yavru S,* 1988a, Konya Hayvancilik Merkez Arastirma Enstitüsü sigirlarinda infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) üzerinde arastirmalar, *S Ü Vet Fak Derg*, 4, 53-64.
16. *Öztürk F, Toker A, Yavru S, Gökçay Y,* 1988b, Konya Hayvancilik Merkez Arastirma Enstitüsü sigirlarinda parainfluenza-3 (PI-3) virusuna karsi nötralizan antikor dagilimlari ve antikor titreleri üzerinde arastirmalar, *S Ü Vet Fak Derg,* 4, 183-88.
17. *Radostits O, Blood D, Gay C,* 1994, Veterinary medicine textbook of the diseases of cattle, sheep, pigs, goats and horses. 8th edition, Balliere Tindall, London, 749-70.
18. *Suzan VM, Onuma M, Aguilar RE, Murakami Y,* 1983, Prevalence of bovine herpesvirus-1, parainfluenza-3, bovine rotavirus, bovine viral diarrhea, bovine adenovirus-7, bovine leukemia virus and bluetongue virus antibodies in cattle in Mexico, *Jpn J Vet Res,* 31, 125-32.
19. *Yavru S, Öztürk F,* 1990, Konya bölgesi sigirlarinda sigir adenovirus tip 1 üzerinde nötralizasyon ve agar jel presipitasyon testi ile karsilastirmali arastirmalar, *Veterinarium*, 2, 28-32.

SEROLOŠKA PROCENA VIRUSNIH INFEKCIJA RESPIRATORNOG TRAKTA GOVEDA

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SADRŽAJ

U ovoj studiji su prikazani rezultati analiza krvnog seruma (dobijenih sa jedne klanice u Keniji) 254 goveda različite starosti, pola, rase, mikroneutralizacionim testom (mNT) na prisustvo antitela protiv goveđeg Adenovirusa, tip 1, 2 i 3 (BAV-1, 2 i 3), infektivnog goveđeg rhinotraheitisa, virusa infektivnog pustularnog

vulvovaginitisa (IBR/IPV), virusa parainfluence tipa 3 (IP-3), goveđeg respiratornog sincicijalnog virusa (BRSV) i virusa goveđe diareje (BVDV).

Prisutstvo antitela na BAV-1 utvrđeno je kod 56 (22.04%) jedinki, na BAV-2 kod 38 (14.96%), na BAV-3 kod 51 (20.07%), na PI-3 kod 137 (53.93%), na IBR/IPV kod 145 (57.08%), na BRSV kod 117 (46.06%) i na BVDV kod 112 (44.09%) grla.

Kod 14.7% goveda su utvrđena antitela na samo jedan virus, kod 36.22% na dva virusa, kod 29.92% na tri virusa, kod 14.56 % na četiri virusa, kod 3.93% na pet virusa, kod 1.57 % na šest virusa, a samo kod 0.39% na svih sedam virusa. Antitela protiv ispitivanih virusa nisu ustanovljena u 3.15% uzoraka od ukupno 254 ispitanih seruma.