A Serological Investigation on Parainfluenza –3 (PI–3) and Bovine Adenovirus (BAV) Infections in Dairy Cow Enterprises in Aydın Province [1]

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Summary

In this study, presence and seroprevalence of Parainfluenza-3 (PI-3) virus and adenovirus, two major viruses causing serious economic losses and their distribution in Aydın Province were investigated. For this purpose, 288 serum samples from different age groups of dairy cattle were collected from 4 different dairy cow enterprises and the presence of specific antibodies to PI-3 virus and Bovine adenovirus (BAV) serotypes 1, 2, and 3 in these samples were investigated using microneutralization test. Specific antibodies for PI-3 and BAV Type 1-2-3 were detected at a rate of 38.2%, 75.7%, 39.9% and 62.2%, respectively. Out of all sampled animals, the rates of seropositive animals for one, two, or three serotypes of BAV were 28.1%, 45.1%, and 19.8%, respectively. Based on the test results, no age effect was noted on the presence of these two viral diseases. .

Keywords:PI-3, BAV, Virus, Dairy cattle, Serological investigation, Aydın

Aydın Yöresindeki Süt Sığırcılığı İşletmelerinde PI–3 ve BAV 1–2–3 Enfeksiyonlarının Serolojik Olarak Araştırılması[1]

Özet

Bu çalışmada, önemli ekonomik kayıplara neden olan PI-3 ile bovine adenovirus (BAV) enfeksiyonlarının Aydın yöresindeki varlığı ve yaygınlığı araştırıldı. Bu amaçla 4 farklı süt sığırcılığı işletmesindeki değişik yaşlardan 288 sığırdan alınan kan serumu örneklerinde PI-3 virusu ve BAV 1, 2 ve 3 serotiplerine karşı spesifik antikor varlığı mikronötralizasyon testi ile araştırıldı.

Test sonucunda, PI-3 ve BAV Tip 1-2-3 için sırasıyla %38.2, %75.7, %39.9 ve %62.2 oranlarında spesifik antikor varlığı tespit edildi. Araştırmada örneklenen sığırların %28.1'inde BAV'larının sadece bir serotipine, %45.1'inde virusun iki serotipine ve %19.8'inde de virusun her üç serotipine karşı spesifik antikor varlığı tespit edildi. Sığırların %7'sinin bütün BAV serotiplerine karşı negatif olduğu saptandı. Test sonuçlarına göre, enfeksiyon oranları ile hayvanların yaş guruplarının arasında istatistiksel olarak anlamlı bir farklılık göstermediği belirlendi.

Anahtar sözcükler: PI-3, BAV, Virus, Sığır, Serolojik Araştırma, Aydın

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INTRODUCTION

Parainfluenza-3 (PI-3) virus and bovine adenoviruses (BAV) are two major respiratory tract disease agents in cattle and cause enzootic bronchopneumonie in calves. Although these viruses can be the primary agent for the diseases, they can also act as predisposing agents for other bacteria or virus induced respiratory diseases [1-5].

The percentages and geometric mean anti-body titer of positive serum samples against these viruses increase by the animal age [1]. In general, these infections cause enzootic pneumonia and shipping fever in calves while in older animals they are in sub-clinic form [1,4].

Previous serological and virological inves-tigations indicate the presence of these infections all around the world, including various parts of Turkey [1,4,6-9]. Studies conducted in various countries have reported that the prevalence of PI-3 virus infections is between 12% and 97%[9]. Sero-epidemiologic studies indicated that a big portion of adult cattle (75%) has neutralizing antibodies against BAV-3 [4]. In animal production, these viruses induced diseases are considered breeding diseases. Since these diseases result in mortality, weight loss or a reduction in production, they have economical importance [3,7,8].

This study was conducted to determine the prevalence and distribution of PI-3 and BAV serotypes 1-2-3 infections by determining the presence of specific antibodies at different age groups in non-vaccinated cattle in Aydın province.

MATERIALS and METHODS

Blood samples used in the study were obtained from 4 dairy cattle enterprises in Aydın province. A total of 288 serum samples were obtained from dairy cows at various age groups (Table 1). Sampled cows were not vaccinated against PI-3 virus and BAV. None of cows had shown clinical signs of disease at the time of blood sampling.

Blood samples were first drawn into caolinated tubes, centrifuged at 3000 rpm for 10 minutes and then serum samples were separated. These serum samples were then inactivated at 56°C for 30 minutes and stored at -20°C until further analysis.

In this study, SF-4 reference strain of PI-3 and BAV 1-2-3 serotypes were used in the test. Virus titration was determined as described by Kaerber. Viruses were diluted at 100 DKID50 /0.1 ml for virus neutralization test.

Madin Darby Bovine Kidney (MDBK) cell culture was used for virus propagation, titration and micro-neutralisation test. As cell producing media, Eagle's Minimum Essential Medium (EMEM, Biochrom T-031-10) containing 10% fetal calf serum (FCS, Biochrom S-0113) were used.

Microneutralization test was performed to determine the specific neutralizing antibodies against BAV type 1-2-3 and PI-3 (SF-4) [18]. Test results were evaluated based on the visibility of cytopathogenic effect (CPE) by inverted microscope in 2-4 days for BAV and 3-5 days for PI-3.

To determine the mean antibody titer (SN50), serum samples were diluted in 2 fold increments starting from 1/10 for BAV 1-2-3 and 1/5 PI-3 respectively. All the PI-3 samples with presence of antibodies were considered positive. Antibody titer of 1/10 or higher were considered for BAV serotypes positive as suggested by Bibrack and Mckercher [16].

RESULTS

Out of 288 serum samples collected from dairy cow enterprises in Aydın, the rate of seropositivity for PI-3 and BAV 1-2-3 were 38.2%, 75.7%, 39.9% and 62.2%, respectively (Table 1).

Antibody titers were determined against either one or two of PI-3 virus and BAV serotype 1-2-3 in 11 serum samples obtained from 1 to 6 month-old calves whose mothers were seronegative for respective viruses. Antibody titer was found 1/40 in one of the samples obtained from a calf whose mother was seronegative against PI-3 virus. Out of 3 serum samples obtained from calves whose mothers were seronegative for BAV serotype 1, two samples had antibody titers at a rate of 1/80 and one sample had antibody titer at a rate of 1/160. Out of 3 serum samples obtained from calves whose mothers were sero-

Age, year	Number of tested samples (n)	PI-3 Seropositive samples (%)	BAV-1 Seropositive samples (%)	BAV-2 Seropositive samples (%)	BAV-3 Seropositive samples (%)
≤6 months	78	28 (35.9%)	56 (71.8%)	36 (46.1%)	51 (65.4%)
7 m-1 year	5	4 (80%)	4 (80%)	1 (20%)	1 (20%)
2	17	10 (58.8%)	13 (76.5%)	7 (41.2%)	12 (70.6%)
3	53	22 (41.5%)	36 (67.9%)	20 (37.7%)	31 (58.5%)
4	38	13 (34.2%)	31 (81.6%)	15 (39.5%)	28 (73.7%)
5	38	16 (42.1%)	30 (78.9%)	12 (31.6%)	17 (44.7%)
6	20	8 (40%)	16 (80%)	7 (35%)	13 (65%)
7	19	6 (31.6%)	16 (84.2%)	11 (57.9%)	13 (68.4%)
8	11	2 (18.2%)	9 (81.8%)	3 (27.2%)	7 (63.6%)
≥9	9	1 (11.1%)	7 (77.8%)	3 (3.33%)	6 (66.7%)
Total	288	110 (38.2%)	218 (75.7%)	115 (39.9%)	179 (62.2%)

negative for BAV serotype 2, two samples had antibody titers at a rate of 1/80 and one sample had antibody titer at a rate of 1/40 against the virus. Out of 8 serum samples obtained from calves whose mothers were seronegative for BAV 3, one sample had antibody titer at a rate of 1/40, two samples had antibody titers at a rate of 1/80, and five samples had antibody titers at a rate of 1/160 against this serotype.

Based on the age of sampled animals, the rates of antibody-positive samples for PI-3 virus and BAV serotypes 1-2-3 are presented in Table 1. Out of 78 blood samples obtained from calves aged 6 months or younger, antibody prevalence against PI-3, and BAV serotypes 1-2-3 were 28 (35.9%), 56 (71.8%), 36 (46.1%) and 51 (65.4%), respectively. Out of 210 samples obtained from cattle aged 7 months- 9 years, 82 samples (39%) had antibodies against PI-3 virus, 162 samples (77.1%) had antibodies against BAV serotype 1, 79 samples (37.6%) had antibodies against BAV serotype 2 and 128 samples (60.9%) had antibodies against BAV serotype 3. No statistical difference was detected between age and seropositivity rate of animals against all viruses using chi-square test (p>0.05).

Out of 288 sampled animals, 268 (93%) animals were seropositive against either one or more serotypes of BAV. Only 20 animals (7%) did not have antibodies against any of BAV serotypes. Out of 268 antibody-positive animals against either one or more BAV serotypes, number of animals against one, two or three BAV serotypes were 81 (28.1%), 130 (45.1%), and 57 (19.8%) respectively. Numbers of serum

Table 2. Distribution of 288 sampled animals for BAV serotypes 1-2-3 in the study.
Tablo 2. BAV serotiplerinin test edilen 288 hayvanda görülme sıklığı.

Serotypes	Number of positive samples against only one serotype (%)	Number of positive samples against a combination of two serotypes (%)			Number of positive samples against only one serotype (%)	Total
		BAV-1	BAV-2	BAV-3		
BAV-1	48 (16.7%)			83 (28.8%)	57 (19.8%)	268 (93%)
BAV-2	11 (3.8%)	30 (10.4%)				
BAV-3	22 (7.6%)		17 (5.9%)			
Total	81 (28.1%)	130 (45.1%)			57 (19.8%)	

samples, which were antibody-positive against one or more BAV serotypes, are presented in Table 2.

DISCUSSION

Current serological study was conducted to investigate the presence and distribution of PI-3 virus and BAV infections in Aydin province.

PI-3 virus and BAV serotypes are among the most important causing-agents of respiratory tract diseases with pneumonia and can be seen particularly in young animals. Enzootic pneumonia, bovine flu and shipping fever are among these diseases. Pneumonia causes weight loss and loss in productivity and the animals with pneumonia requires treatments, which results in delayed marketing. Thus, these diseases are considered the major economically important diseases [3,7,8]. PI-3 virus and BAV infections are common all around the world and they have been reported in various parts of Turkey. Studies indicated that distribution of these viruses in various regions and provinces of Turkey are different. It has been reported that the seroprevalance for PI-3 virus and BAV serotypes 1-2-3 in different parts of Turkey was within the range of 11-97% [12-17].

This study is the first investigation for demonstrate the presence and distribution of PI-3 and BAV infections in Aydın region. The study revealed that, seropositivity rate for PI-3 virus and BAV serotypes 1-2-3 were 38.2%, 75.7%, 39.9% and 62.2%, respectively. Based on the results, it can be concluded that PI-3 and BAV serotype 1-2-3 infections are also common in Aydin province as in other parts of the country.

An increase in the percentage of serum samples positive for PI-3 virus antibody and the geometric mean titer was noted with increasing age1. Some studies reported that, antibody positivity rate against BAV infections is increased in animals older than two years [11,16,18]. However, findings in this study indicated that seropositivity rate for a specific virus within each age group was similar to the rate for the same virus in all age groups. Based on the results of this study, it can be said that no relationship within age groups in the antibody-positivity rate against PI-3 and BAV serotypes 1-2-3 infections could be observed.

Animals with PI-3 and BAV serotype 1-2-3 infections display serious clinical symptoms and therefore calves aged from 0 to 6 months are potentially under high risc. These animals are suspicious to these diseases even if they are antibody-positive. Antibodies only moderate the heavy clinical symptoms of the diseases but can not avoid the spread of the diseases by the animal. In older animals, the diseases are in either sub-clinic or latent form and these animals act as carriers for the diseases [3].

In this study, out of 78 blood samples obtained from calves aged 6 months or younger, seroprevalence of PI-3, and BAV serotypes 1-2-3 were found to be 35.9%, 71.8%, 46.1% and 65.4%, respectively. While evaluating this data, it should be considered the possibility of these antibodies having maternal-origin. Out of 210 samples obtained from calves aged 12 months or older, 82 samples (39%) had antibodies against PI-3 virus, 162 samples (77.1%) had antibodies against BAV serotype 1, 79 samples (37.6%) had antibodies against BAV serotype 2 and 128 samples (60.9%) had antibodies against BAV serotype 3. Since this data is obtained from 12 months or older animals, these antibodies are considered to be produced against postnatal infections by PI-3 and BAV serotypes 1-2-3. It means these infec-tions are widely spread and animals from all age gro-ups are exposed to the disease agents.

High titers of antibodies were detected in 11 calves 1 to 6-month-old, whose mothers were seronegative for the antibodies indicating that they were exposed to the disease agents after their birth. Based on this fact, the viruses, particularly BAV-3 serotype were already present in these dairy enterprises at least 6 months prior to sampling and the infection was unceasing. Based on the results, calves with no maternal antibodies are under high risk against PI-3 virus and particularly BAV diseases.

Seropositivity rates against PI-3 virus and BAV serotypes 1-2-3 were different in four enterprises. Based on the data, it was concluded that BAV serotypes 1 and 3 were more common then PI-3 virus and BAV serotype 2.

Out of 288 sampled animals, 268 (%93) had antibodies against either one or more serotypes of BAV. The rates of animals against one, two or three BAV serotypes were 28.1, 45.1, and 19.8% respectively. These rates were higher than the BAV seropositivity rates reported by Karaoğlu et al.[15]. Yavru et al.[17] reported a higher seropositivity rate against one BAV serotype (35.90%), however, seropositivity rates against two (10.64%) or three (2.83%) were lower than the rates found in present study.

A successful animal production requires training producers on production environment, good management skills, common infectious diseases in surrounding area and preventive measurements to avoid these diseases. Stress factors such as changes in ambient temperature, farm hygiene, ventilation, transportation, sudden changes in diet, population density and some other factors have an important effect on activating the respiratory tract disease agents such as PI-3 virus, BAV, bovine respiratory syncytial virus (BRSV), infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhoea virus (BVDV) and rhino-virus2,4,5. For this reason, protective programs must be adapted and high priority should be given to pre-

vent respiratory tract diseases. Recommended vaccination programs are either not practiced properly or totaly ignored in Aydın and Turkey and this is considered the main reason why these diseases are very common.

Serological or virological studies in Aydın province also revealed the presence of major virus-origin respiratory tract disease agents such as infectious bovine rhinotracheitis (IBR) and bovine viral diarrhoea (BVD) infections [19,20].

Based on the current and previous studies, it can be estimated that virus-origin respiratory tract diseases can cause important economic losses in all around Turkey, including Aydın. Therefore, all stress and predisposing factors should be eliminated and all preventive measurements need to be taken into account to avoid further economic losses.

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