

SOME VIRAL AND BACTERIAL RESPIRATORY TRACT INFECTIONS OF DAIRY CATTLE DURING THE SUMMER SEASON

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In this research, dairy cattle with respiratory system problems that were brought to a private slaughterhouse in Burdur province were investigated for viral and bacterial infections present in the summer season. The blood samples were collected from 56 animals. The samples were tested for antibodies against bovine herpesvirus 1 (BoHV-1), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (BPIV-3) and bovine adenovirus 3 (BAV-3) by ELISA. Bacteriological cultivation was carried out from lung samples taken after cutting the same animals. The seropositivity rates which were determined for 5 viruses in cattle (BoHV-1, BVDV, BRSV, BPIV-3 and BAV-3) were 7.14%, 50%, 94.64%, 94.64% and 82.14% respectively. The presence of antibodies against the viruses was as follows; 5.36% of cattle had antibodies against only one virus, 14.29% against two, 30.36% against three, 44.64% against four and 5.36% against five viruses. A total of 36 bacterial agents were isolated from 30 out of 56 lung samples. From the lung samples, only one bacterium was isolated from 39.3% (22/56) samples, and more than one bacterium from 14.3% (8/56). Escherichia coli, Staphylococcus aureus and Streptococcus spp. were detected as the most often isolated agents. Compared to bacteria, the rates of viral infections associated with Escherichia coli (BRSV+BPIV-3+BAV-3+Escherichia coli; 8.92% and BRSV+BPIV-3+Escherichia coli; 5.35%) were higher.

As a consequence, it was thought that primary agents which were the viruses and bacteria may have attended as secondary factors in respiratory tract infections of dairy cattle.

Key words: bacteria, bovine respiratory infections, ELISA, isolation, summer season, viruses

INTRODUCTION

Respiratory system infections in cattle are economically important because they cause an increase in morbidity-mortality rates and precautions should be taken for their treatment and control of production losses (Irsik *et al.*, 2006). Respiratory system problems that can be seen in cattle are formed depending

upon stress, sensitivity, changes in the environment and diet, physiological changes, breeding conditions, animal transport, transfer of animals in and out of herds and various pathogens (Callan and Garry, 2002; Hodgson *et al.*, 2005). However, it was stated that respiratory problems caused by viruses and bacteria were the greatest (Autio *et al.*, 2007). Bovine herpesvirus 1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (BPIV-3) and bovine adenoviruses (BAV) generally cause respiratory problems (Hodgins *et al.*, 2002). However, in respiratory system infections, bovine coronavirus (BoCV), influenza A virus, bovine herpesvirus 4 (BoHV-4), malignant catarrhal fever virus, bovine rhinovirus, bovine enterovirus, bovine reovirus, bovine calicivirus and bovine parvovirus agents were also isolated (Bowland and Shewen, 2000; Gay and Barnouin, 2009). Many bacteria are facultatively present in the respiratory tract of healthy animals. These facultative bacteria may cause infections when the immune system is weakened or stress factors are present (Ayers and Los Olivos, 1992). Although more *Pasteurella spp.* are isolated as secondary agents from pneumonia cases, *Corynebacterium spp.*, *Staphylococcus spp.*, *Escherichia coli*, *Streptococcus spp.*, *Mycoplasma spp.* and *Acinetobacter spp.* isolations were also identified (Erdag *et al.*, 1993; Girgin *et al.*, 1989; Kilic, 2003). In studies, pneumonia in cattle had rather a mixed infection character (Kaya and Erganis, 1991).

It was reported that respiratory problems in cattle increase in autumn and winter (Van der Fels-Klerx *et al.*, 2001). During these seasons, viral infections increase because of insufficient hygiene in the stables, population density and harsh climate conditions (Ozdarendeli, 1997). In this study, the goal was to present some important viral and bacterial agents in dairy cattle with respiratory problems that were brought to slaughter in the summer, and to detect levels of their co-existence in the respiratory system.

MATERIALS AND METHODS

Animals: In Burdur province, blood and lung samples after slaughter were taken from 56 Holstein dairy cattle with respiratory problems. Animals were over 2 years of age, and were brought to slaughter at a private slaughter plant. The owners provided the necessary data that the sampled animals were not vaccinated against any of the investigated agents.

Blood serum: Blood was taken into sterile vacutainers from *v. jugularis* and centrifuged at 2500 rpm for 15 minutes. Serum samples were collected and placed into 1 mL sterile serum storage tubes. After they were inactivated for 30 minutes in a water bath at 56°C, they were controlled for sterility and stored frozen (-40°C) until testing.

Antibody presence in blood serum against BoHV-1, BVDV, BRSV, BPIV-3 and BAV-3 was investigated with Bio-X Respiratory ELISA Pentakit (Bio-X Diagnostics, Belgium).

BVDV-ELISA (Antigen): Detection of BVDV antigen in serum samples was done by BVD/MD Ag Mix Screening ELISA (Institut Pourquier, France).

Isolation and identification of bacteria: After slaughtering, lungs of cattle with respiratory problems were examined macroscopically. Lung samples collected from 56 cattle with pneumonia lesions were seeded onto 7% sheep blood agar (Oxoid) and MacConkey agar (Oxoid) and incubated for 24-72 hours at 37°C under aerobic conditions. For the identification of *Haemophilus* spp. seeding on chocolate agar was done followed by 3-4 days incubation at 37°C in a microaerophilic environment. From the same samples, seeding was done onto Mycoplasma agar base (Oxoid) with Mycoplasma supplement G (Oxoid) for *Mycoplasma* spp. isolates were left for incubation for two weeks in a microaerophilic environment. From the petri dishes with no isolated mycoplasma colonies, 3-4 blind passages were applied and those with no colonies were evaluated as negative. Colonies growing in Mycoplasma agar were examined under a light microscope (Baysal and Guler, 1992; Guler, 1993).

RESULTS

In 56 dairy cattle blood serum samples, seropositivity against BoHV-1, BVDV, BRSV, BPIV-3 and BAV-3 was detected at rates of 7.14%, 50%, 94.6% and 82.1% respectively. The presence of BVDV antigen was not detected (Table 1).

Table 1. Seropositivity levels against the virus in blood serum samples of dairy cattle with respiratory problems

Viruses	ELISA results			
	Seronegative		Seropositive	
	n	%	n	%
BoHV-1	52	92.9	4	7.14
BVDV	28	50	28	50
BRSV	3	5.4	53	94.6
BPIV-3	3	5.4	53	94.6
BAV-3	10	17.9	46	82.1

Viral multiple infection rates of animals detected as seropositive by means of BoHV-1, BVDV, BRSV, BPIV-3 and BAV-3 were detected for only one in 5.4%, two in 14.3%, three in 30.4%, four in 44.6% and five in 5.4% samples (Table 2).

Thirty eight microorganisms were isolated from 30 lung samples out of 56. While a single bacterium was isolated from 39.3% lung samples, more than one bacterium were isolated from 14.3% samples (Table 3). The most often isolated agents from lung samples were found to be *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* spp.

Table 2. Multiple viral infection rates for dairy cattle with respiratory problems

Viruses	No. of seropositive animals	
	n	%
1 virus ^a	3	5.4
2 viruses ^b	8	14.3
3 viruses ^c	17	30.4
4 viruses ^d	25	44.6
5 viruses ^e	3	5.4

^a BPIV-3 (n=2) and BRSV (n=1); ^b BRSV+BAV-3 (n=2) and BRSV+BPIV-3 (n=6);

^c BRSV+BPIV-3+BAV-3 (n=17); ^d BVDV+BRSV+BPIV-3+BAV-3 (n=25);

^e BoHV-1+ BVDV+BRSV+BPIV-3+BAV-3 (n=3)

Table 3. Microorganisms isolated from the lungs of cattle with respiratory problems

Isolated microorganisms	Number of isolated lungs	%
<i>E. coli</i>	10	17.9
<i>E. coli</i> + <i>S. aureus</i>	1	1.8
<i>E. coli</i> +KNS	1	1.8
<i>E. coli</i> + <i>M. haemolytica</i>	1	1.8
<i>S. aureus</i>	4	7.1
<i>S. aureus</i> +KNS	1	1.8
<i>Pseudomonas</i> spp.	1	1.8
<i>P. multocida</i> + <i>Proteus</i> spp.	1	1.8
<i>Streptococcus</i> spp.	3	5.4
<i>Klebsiella</i> spp.	1	1.8
<i>Klebsiella</i> spp.+KNS	1	1.8
<i>Klebsiella</i> spp.+ <i>Streptococcus</i> spp.	1	1.8
<i>Klebsiella</i> spp.+ <i>Candida</i> spp.	1	1.8
KNS	1	1.8
<i>Proteus</i> spp.	2	3.6
Total	30	53.8

Compared to other bacteria, the rates of viral infection accompanied by *Escherichia coli* (BRSV + BPIV-3 + BAV-3 + *Escherichia coli*; 8.9% and BRSV + BPIV-3 + *Escherichia coli*; 5.4%) were found to be higher (Table 4).

Table 4. Viral and bacterial multiple respiratory system infections in cattle

Viruses and Bacteria	No. of positive	%
BRSV+BPIV-3+BAV-3+ <i>E.coli</i>	5	8.9
BRSV+ BPIV-3+ <i>E.coli</i>	3	5.4
BRSV+BPIV-3+BAV-3+ <i>S. aureus</i>	2	3.6
BVDV+BRSV+BPIV-3+BAV-3+KNS	2	3.6
BoHV-1+ BRSV+BPIV-3+BAV-3+ <i>Pseudomonas</i> spp.	2	3.6
BVDV+BRSV+BPIV-3+BAV-3+ <i>Streptococcus</i> spp.	2	3.6
BRSV+ <i>S. aureus</i>	1	1.8
BPIV-3+ <i>E.coli</i>	1	1.8
BRSV+BPIV-3+ <i>S. aureus</i>	1	1.8
BRSV+BPIV-3+ <i>Klebsiella</i> spp.+ <i>Candida</i> spp.	1	1.8
BRSV+BPIV-3+BAV-3+ <i>Proteus</i> spp.	1	1.8
BRSV+BPIV-3+BAV-3+ <i>Klebsiella</i> spp.+ <i>Streptococcus</i> spp.	1	1.8
BoHV-1+ BVDV+ BPIV-3+ <i>P. multocida</i> + <i>Proteus</i> spp.	1	1.8
BVDV+BRSV+BPIV-3+BAV-3+ <i>Proteus</i> spp.	1	1.8
BVDV+BRSV+BPIV-3+BAV-3+ <i>E.coli</i>	1	1.8
BRSV+BPIV-3+BAV-3+ <i>Klebsiella</i> spp.+KNS	1	1.8
BVDV+BRSV+BPIV-3+BAV-3+ <i>M.haemolytica</i> + <i>E.coli</i>	1	1.8
BVDV+BRSV+BPIV-3+BAV-3+ <i>S.aureus</i> + <i>E.coli</i>	1	1.8
BoHV-1+BVDV+BRSV+BPIV-3+BAV-3+ <i>Streptococcus</i> spp.	1	1.8
BVDV+BRSV+BPIV-3+BAV-3+KNS+ <i>E.coli</i>	1	1.8
No bacteria and fungi	26	46.4
Total	56	100

DISCUSSION

It has been stated that various agents such as BoHV-1, BVDV, BRSV, BPIV-3 and BAV-3 generally cause respiratory problems in cattle that become a complex multifactorial infection with bacterial and mycoplasmal agents (Callan and Garry, 2002).

There are studies – Pernthaner *et al.* (1990) 23%, Durham and Hassard (1990) 37.8%, Bulut *et al.* (2006) 15.8%, Gurses (2008) 25.6%, Duman *et al.* (2009) 35.3%, and Sakhæe *et al.* (2009) 30.4% on BoHV-1 presence in which low prevalence was obtained in dairy cattle. In this study, BoHV-1 seroprevalance (7.1%) was detected to be lower than in other studies. In the study, although BVDV antigen presence was not found, BVDV antibody positivity rate was detected as

50%. This result showed to be in a agreement with the results of Bulut *et al.* (2006), Okur-Gumusova *et al.* (2007) and Yildirim *et al.* (2009), but were lower compared to findings by Suzan *et al.* (1983), Ghirotti *et al.* (1991) and higher than recorded by Durham and Hassard (1990), Yavru *et al.* (2005). In the study, seropositivity rates against BRSV and BPIV-3 viruses were found to be 94.6%. These results were similar to those of Duman *et al.* (2009) BRSV 94.4%, BPIV-3 92.8%, Gurses (2008) BRSV 95.6%, BPIV-3 91.1%, and Sakhaee *et al.* (2009) BRSV 100%, BPIV-3 100%. Besides, the finding of BAV-3 was highly in accordance with the results of other researchers – Burgu and Toker (1985) (89%), Ghirotti *et al.* (1991) (87.4%), Okur-Gumusova *et al.* (2007) (81.4%), Gurses (2008) (83.3%), Duman *et al.* (2009) (86%). In the middle and southwest France, from serological scannings of respiratory viruses in cows in 20 cattle herds, while high seroprevalance at rates of BRSV (93.6%), BPIV-3 (100%), BAV-3 (66.5%) and BVDV (66.7%) were detected, low seroprevalance for BoHV-1 (16.6%) was reported (Valarcher and Hagglund, 2006). In our study, high seroprevalance for BRSV, BPIV-3, BAV-3 and BVDV, but low for BoHV-1 was detected.

The main source of respiratory problems in cattle are said to be viral agents combined with single, multi (combined) or other pathogens (Valarcher and Hagglund, 2006). That is why many researchers have worked on the presence of multiple viral infections in cattle with respiratory problems. Duman *et al.* (2009) reported that they detected antibody presence against minimum one virus (1.4%) and maximum 4 viruses (49.6%), Okur-Gumusova *et al.* (2007) minimum 1 virus (6.9%) and maximum 3 viruses (58.5%), Gurses (2008) minimum 1 virus (1.7%) and maximum 4 viruses (55.4%), Yavru *et al.* (2005) minimum 7 viruses (0.4%) and maximum 2 viruses (36.2%), Yildirim *et al.* (2009) minimum 5 viruses (7.2%) and maximum 3 viruses (32.1%), Sakhaee *et al.* (2009) minimum 4 viruses (5.5%) and maximum 4 viruses (53%). In this study, antibody presence was detected against minimum 1 and 5 viruses (5.4%). Most often (44.6%) it was the case of a multiple infection caused by 4 viruses.

BRSV is believed to be responsible for most respiratory infections (Ames, 1997). BPIV-3 is common amongst cattle all over the world and has a high serum antibody prevalence amongst mature animals (Bryson, 1990). BPIV-3 is thought to be the predisposing factor in shipping fever and enzootic pneumonia. That is why it can be detected concurrent with bacteria and *Mycoplasma* spp. in many events (Storz *et al.*, 2000). In other viruses such as BVDV, adenovirus, rhinovirus, reovirus, and influenza virus are stated to be detected in cattle with respiratory problems and are generally coexisting with other pathogens (Valarcher and Hagglund, 2006).

In general, viral respiratory system infections among cattle are seen mostly in autumn and winter (Valarcher and Hagglund, 2006). Van der Poel *et al.* (1993) stated that infections arising from BRSV infected cattle were seen at low levels or were never diagnosed. However, as a result of ongoing reinfections or presence of persistently infected animals, an important increase in antibody titer among seropositive cattle was detected. Similarly, in their study on viral respiratory tract infections (BRSV, BPIV-3, BoCV, BVDV) of cattle in the spring time (March-May), Hagglund *et al.* (2006) stated that the control mechanisms prepared for herd

biosafety could not be successful during this period because of the latent period, reactivation and ongoing virus circulation in closed herds. In this study, the presence of antibodies to viral agents detected in the respiratory system of dairy cattle in the summer time could be because of ongoing reinfections and persistent presence of infection.

Many studies were carried out on the isolation of bacterial agents from the lungs (Erdag *et al.*, 1993; Girgin *et al.*, 1989; Gunduz and Erganis, 1998; Kilic, 2003). In these studies, bacterium isolations as single or mixed infections from cattle lungs with pneumonia were described. The fact that single or more bacteria were isolated from the lungs with pneumonia and viral infection was seen in the same animals showed that various bacteria played a primary or secondary role in the etiology of pneumonia.

Although *Pasteurella* spp. agents were generally isolated from cattle pneumonia (Erdag *et al.*, 1993; Gunduz and Erganis, 1998; Yates, 1982), in this study the isolation rate was low (1.79%). *Mycoplasma* spp. and *Haemophilus* spp., which were often isolated together with viral infections in cattle pneumonia, could not be isolated was associated to the fact that the study was carried out in the summer time. These results support the researchers who stated that pneumonia events in cattle are seen at low levels in the summer (Maity and Deb, 1991). In this study, the rate of *Escherichia coli* (23.2%) isolated from lungs with pneumonia was found to be extremely high. Although *Escherichia coli* was an opportunistic pathogen, in Turkey, there were studies stating that *Escherichia coli* isolation in cattle pneumonia was at high levels (Erdag *et al.*, 1993; Girgin *et al.*, 1989; Gunduz and Erganis, 1998). However, there are also studies stating that *Escherichia coli* isolation rate (2.6%) was at low levels (Kilic, 2003). It was believed that this difference between the bacteria and their rates could be due to climatic conditions, feeding, housing conditions, and the age of animals. In the studies (Kilic, 2003; Yates, 1982), it was stated that bacteria and isolation rates isolated from pneumonia cases could change according to the age and gender of animals, hygiene, climatic conditions and their feeding.

Finally, we found that in dairy cattle with respiratory problems, viruses (BRSV+BPIV-3+BAV-3, BRSV+BPIV-3 and others) could be the primary responsible agents and bacteria could be secondary accompanying agents.

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NEKE VIRUSNE I BAKTERIJSKE INFEKCIJE RESPIRATORNOG TRAKTA MLEČNIH GOVEDA U LETNJEM PERIODU

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SADRŽAJ

U ovom radu su izneti rezultati ispitivanja zastupljenosti nekih virusnih i bakterijskih infekcija respiratornog trakta mlečnih goveda tokom leta u regiji Burdur, Turska. Uzorci krvi su uzeti od 56 životinja neposredno pre klanja i ispitivani na prisustvo antitela protiv govedeg herpes virusa 1 (BoHV-1), virusa govede dijareje (BVDV), sincicijalnog govedeg virusa (BRSV), govedeg parainfluenca virusa 3 (BPIV-3) i govedeg adenovirusa (BAV-3) ELISA metodom. Uzorci pluća za bakterijsku kultivaciju uzimani su neposredno posle klanja. Procenat seropozitivnih

jedinki za pet ispitivanih virusa (BoHV-1, BVDV, BRSV, BPIV-3 i BAV-3 je iznosio: 7,14%, 50%, 94,64%, 94,64% i 82,14% respektivno. Kod 5,36% životinja je dokazano prisustvo antitela na samo jedan virus, kod 14,29% na dva, kod 30,36% na tri, kod 44,64% na četiri i kod 5,36% na pet virusa. Ukupno je izolovano 36 bakterija iz 30 od ukupno 56 uzoraka pluća. U 39,3% uzoraka (22/56) izolovana je samo jedna bakterijska vrsta, a više od jedne u 14,3% (8/56). U većini izolata su se nalazile *E. coli*, *Staphylococcus aureus* i *Streptococcus spp.* Utvrđena je veća povezanost virusnih infekcija sa prisustvom *E. coli* u odnosu na druge bakterije (BRSV, BPIV-3 i BAV-3 + *E. coli* - 8,92% i BRSV, BPIV-3 + *E. coli* 5,35%). Virusi su uzrokovali primarne infekcije pluća goveda dok se bakterije pojavljuju kao sekundarni agensi.