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Original Article
Özgün Araştırma

Determination of natural adverse factors (aflatoxins, nitrate, nitrite, tannin, and sodium chloride) in some cage bird feeds

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Abstract: This study was carried out to determine the levels of aflatoxins, nitrate, nitrite, tannin and sodium chloride in some cage bird feeds sold in Ankara, Turkey. A total of 60 feed samples (20 each of budgerigar, canary and parrot feed) were analysed. Aflatoxin analyses by HPLC, nitrate, nitrite and tannin analyses spectrophotometrically and sodium chloride analyses by a method based on colour reaction were performed. Total aflatoxin was found to be 2.44-34.30 ppb in 11 budgerigar feeds, 0.91-16.75 ppb in 8 canary feeds and 1.43-19.29 ppb in 10 parrot feeds. Nitrite (0.00-6.19 ppm), nitrate (1.31-25.55 ppm), tannin (0.04-0.74%), and sodium chloride (0.05-0.44%) were found in all feeds. As a result, it was concluded that the level of AFB1 in only 1 budgerigar feed might pose a risk to the health of cage birds, while the levels of nitrate, nitrite, tannin and sodium chloride might not cause any health problems.

Keywords: Aflatoxin, cage bird feed, nitrate-nitrite, sodium chloride, tannin

Bazı kafes kuşu yemlerinde doğal olumsuzluk faktörlerinin (aflatoksin, nitrat, nitrit, tanen ve sodyum klorür) belirlenmesi

Özet: Bu çalışma, Ankara'da satılan bazı kafes kuşu yemlerinde aflatoksin, nitrat, nitrit, tanen ve sodyum klorür düzeylerini belirlemek amacıyla gerçekleştirildi. Toplam 60 yem örneği (muhabbet kuşu, kanarya ve papağan yeminin her birinden 20 adet) analiz edildi. HPLC ile aflatoksin analizleri, spektrofotometrik olarak nitrat, nitrit ve tanen analizleri ve renk reaksiyonuna dayalı bir yöntemle sodyum klorür analizleri yapıldı. Toplam aflatoksin 11 muhabbet kuşu yeminde 2.44-34.30 ppb, 8 kanarya yeminde 0.91-16.75 ppb ve 10 papağan yeminde 1.43-19.29 ppb olarak bulundu. Tüm yemlerde nitrit (0.00-6.19 ppm), nitrat (1.31-25.55 ppm), tanen (%0.04-0.74) ve sodyum klorür (%0.05-0.44) bulundu. Sonuç olarak, sadece 1 muhabbet kuşu yemindeki AFB1 düzeyinin kafes kuşlarının sağlığı için risk oluşturabileceği, nitrat, nitrit, tanen ve sodyum klorür düzeylerinin ise herhangi bir sağlık sorununa neden olmayabileceği sonucuna varıldı.

Anahtar kelimeler: Aflatoksin, kafes kuşu yemi, nitrat-nitrit, sodyum klorür, tanen

Introduction

With the widespread use of cage bird breeding, the frequency of veterinarians encountering care, nutrition and health problems of these animals is increasing (Salt et al. 2000). While veterinarian is evaluating the condition of sick animals, it is also very important to evaluate the mycotoxins, nitrate, nitrite, tannin and sodium chloride which are naturally present or may occur in feed or foodstuffs as well as infectious agents in terms of correct diagnosis and appropriate treatment (Barug et al. 2003; Basmacıoğlu and Ergül 2003; Blake 2008).

Aflatoxins constitute the most important group among mycotoxins. Aflatoxins are synthesised by *Aspergillus flavus*, *A. parasiticus*, *A. nomius* and various toxigenic *Aspergillus* and some *Penicillium* and *Rhizopus* moulds (Rustom 1997; Thompson and Henke 2000; Mishra and Das 2003; Nicholas 2003; O'keeffe 2003; Williams et al. 2004; Akande et al. 2006). It is reported that feed is contaminated with mycotoxins at a higher rate when compared to about 25 per cent of the agricultural products produced every year in the world (Akande et al. 2006; Dorina et al. 2008; Reddy and Waliyar 2009). Poisoning (mycotoxicosis) of acute or chronic character occurs in humans and animals eating food and feed contaminated with these toxins (Wood 1992; Nizamlıoğlu and Gözün 1996; Anon 2008a; Becer 2008).

Generally, birds are more sensitive than mammals. Young birds are more susceptible than adults. In poultry, the most susceptible species are ducklings and turkeys. Mortality due to aflatoxin

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exposure has been reported in migratory birds such as various duck species, Canada goose and crane (Robinson et al. 1982; Anon 2008b; Diaz et al. 1995).

Nitrate and nitrite are widespread in nature. Normally, fresh plants contain a few grams of nitrate per kilogramme. The nitrate and nitrite content of cereal grains ranges from 0.5 to 18 mg/kg depending on the species and growth condition (Diaz et al. 1995). Acute or chronic poisoning occurs when animals ingest excessive amounts of nitrate and nitrite accumulated in plants and groundwater for different reasons (Stoltenow and Lardy 1998; Casteel and Evans 2004; Nicholson 2007). The intensive and excessive use of nitrogen fertilisers in agriculture on the grounds that they increase yields leads to excessive nitrate accumulation in some plant species and groundwater. As a result of this accumulation, acute or chronic disorders occur in both humans and animals (Alçiçek and Başlar 1995).

Tannins are nitrogen-free, polyphenolic, amorphous compounds found naturally in plants. Tannins are degraded in the digestive system of animals and transformed into phenol structures such as gallic acid, pyrogallol and pyrocatechol which are more toxic than tannins (Schofield et al. 2001; Üstün and Aydın 2007). They form complex compounds with proteins, amino acids, polysaccharides, minerals and some vitamins and reduce their digestibility (Hagerman et al. 1992; Akar et al. 1994; Schofield et al. 2001).

If tannins are present in excessive amounts in feeds, they cause toxic effects in animals that consume these feeds or cause developmental retardation and decreased feed utilisation. In addition, tannins, which are known as a strong liver and kidney poison, can also cause carcinogenic effects. The toxicity of tannins is due to their astrengenic, irritant and haemolysing effects (Price et al. 1993; Reed 1995; Üstün and Aydın 2007).

Sodium chloride, which is a basic nutrient, is normally found in animal feeds at 0.5-1%. Due to its attractive and less irritating flavour, it can sometimes be taken in excessive amounts by animals and can cause poisoning. Poultry are very sensitive to sodium chloride poisoning (Kaya and Akar 2002).

There are not enough studies on the levels of aflatoxin, nitrate, nitrite, tannin and sodium chloride in cage bird feeds. In recent years in Turkey, the care, feeding and treatment of birds have been mainly included in the area of responsibility of veterinary medicine. Therefore, it is necessary to carry out toxicological analyses of bird feeds in terms of various residues and contaminants in terms of preventive medicine and diagnosis. When evaluated from this point of view, it is thought that the levels found as a result of this study will be a source for further research on the subject.

Materials and methods

Sampling

In this study, 20 feed samples of 3 different bird species, namely budgerigar, canary and parrot feed, and 60 feed samples in total were analysed. Feed samples were collected from pet shops, bird shops and markets in Ankara. Nitrate-nitrite, tannin and sodium chloride analyses of the feed samples were performed in the Application Laboratories of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, and aflatoxin analyses were performed in a laboratory accredited by the Turkish Accreditation Agency.

Aflatoxin analysis

Aflatoxin analyses were performed by HPLC according to the method reported by Stroka et al. (2003). The method was based on extraction of the analysed sample with acetone/water solvent mixture; clean-up with immuno-affinity column (IAK) containing monoclonal antibodies specific for aflatoxins B1, B2, G1, G2; post-column electrochemical bromine derivatisation and determination of aflatoxins B1, B2, G1, G2 by reverse phase liquid chromatography (RP-HPLC) with fluorescence detector.

For the determination of nitrate and nitrite levels in feeds, the method reported by Sen and Donaldson (1978), modified by Yavuz (1992) and Oruç and Ceylan (2001) and applied by Becer (2008) was used. The method is based on the extraction of nitrate and nitrite from homogenised or ground samples with distilled water. According to the method, the extracted nitrate was reduced to nitrite by passing through a cadmium column. The nitrite content at acidic pH was measured by a colorimetric method by adding N-(1-Naphthyl) ethylene diamine (NED) dihydrochloride mixed with sulfanilic acid as a colour reagent and converted into red azo dye.

Nitrite analysis

Five ml of the sample filtrate was measured and transferred to a 50 ml measuring cylinder. It was added 9 ml of ammonium chloride buffer, 5 ml of 60% acetic acid, 5 ml of sulfanilamide, 2 ml of NED. It was completed to 50 ml with distilled water. A blind sample was prepared in the same way using distilled water instead of filtrate. The prepared sample and

blind were kept in the dark for 25 min. Absorbance was read against the blind at a wavelength of 550 nm in a spectrophotometer.

Nitrate analysis

Two ml of the sample filtrate was measured and transferred to a 50 ml measuring cylinder. 5 ml of ammonium chloride buffer was added and mixed. Before nitrate determination, nitrate was reduced to nitrite by passing through the activated cadmium reduction column at a rate of 3-5 ml/min. After each sample filtrate passed through the column, the column was washed with 15 ml distilled water. To the sample passing through the column, 5 ml 60% acetic acid, 5 ml sulfanilamide, 2 ml NED were added respectively. It was completed to 50 ml with distilled water. A blind sample was prepared in the same way using distilled water instead of filtrate. The prepared sample and blind were kept in the dark for 25 min. Absorbance was read against the blind at a wavelength of 550 nm in a spectrophotometer.

Tannin analysis

Tannins in the feeds were determined according to the spectrophotometric method reported by Horwitz (1970) and Lepper (1950). Tannin, together with sodium carbonate, was coloured blue with Folin-Denis reagent. The amount of tannin was determined by measuring the blue coloured compound in a spectrophotometer at 760 nm. The results were given as percentage.

Sodium Chloride analysis

Sodium chloride level was determined according to USP-NF 1985. 5 g of feed sample was mixed with some distilled water in a mixer and the mixture was transferred into a 500 ml flask; the sample residues in the mixer and funnel were washed with distilled water. The mixture was heated at 45°C for 25-30 min to dissolve the salt and precipitate albumin, the volume was increased to 500 ml with distilled water, cooled and filtered. The filtrate was taken 50 ml, 1 ml of the indicator (potassium chromate 5% prepared in water) was added and titrated to brick red colour with 0.1 N AgNO3 solution. A blind (with 5 ml of water) was made in the same way. The volume of 0.1 N AgNO3 consumed was determined. The results were given as percentage.

Statistics

Statistical evaluation of the data obtained at the end of the study was performed with SPSS 17 statistical package programme. After determining whether total aflatoxin, nitrate-nitrite, tannin and sodium chloride levels were normally distributed or not by Kolmogorov-Smirnov test, the difference in the means between the normally distributed levels in terms of the specified variables was determined by one-way analysis of variance, and the groups showing differences were determined by Duncan least significant difference method.

Results

According to the results of the analyses, total aflatoxin levels (Table 1) were 2.44-34.30 ppb in 11 budgerigar feeds (Table 2), 0.91-16.75 ppb in 8 canary feeds (Table 3) and 1.43-19.29 ppb in 10 parrot feeds (Table 4). According to aflatoxin types, AFB1 was between 2.44-28.14 ppb in 11 budgerigar feeds, 0.91-15.5 ppb in 8 canary feeds and 1.43-18.5 ppb in 10 parrot feeds; AFB2 was between 0.29-1.66 ppb in 9 of the budgerigar feeds, 0.27-1.25 ppb in 3 of the canary feeds and 0.25-1.44 ppb in 5 of the parrot feeds; AFG1 was detected at levels between 3.9-8.13 ppb in 3 of the budgerigar feeds and 1.46 ppb in 1 of the parrot feeds and AFG2 was detected at levels of 0.6 ppb in only 1 budgerigar feed.

Table 1. Aflatoxin levels in all feeds (ppb).

Aflatoxins	Budgerigar X ± Sx	Canary X ± Sx	Parrot X ± Sx	General X ± Sx
AFB_1	5.41 ± 1.65	1.90 ± 0.82	3.48 ± 1.25	3.60 ± 0.75
min - max	0.00 – 28.14	0.00 – 15.50	0.00 – 18.50	0.00 – 28.14
AFB_2	0.34 ± 0.12	0.09 ± 0.06	0.21 ± 0.10	0.21 ± 0.06
min - max	0.00 – 1.66	0.00 – 1.25	0.00 – 1.44	0.00 – 1.66
AFG_1	0.80 ± 0.47	ND	0.07 ± 0.07	0.29 ± 0.16
min - max	0.00 – 8.13		0.00 – 1.44	0.00 – 8.13
AFG_2	0.03 ± 0.03	ND	ND	0.01 ± 0.01
min - max	0.00 – 0.60			0.00 – 0.60
Toplam AF	**6.58 ± 2.06**	**1.99 ± 0.88**	**3.76 ± 1.33**	**4.11 ± 0.88**
min - max	**0.00 – 34.30**	**0.00 – 16.75**	**0.00 – 19.29**	**0.00 – 34.30**

ND: No detectable levels were found.

Table 2. Aflatoxin levels in budgerigar feeds.

Sample Number	Result (ppb)				
	AFB_1	AFB_2	AFG_1	AFG_2	Total
1	14.76	1.66	3.9	-	20.32
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	13.79	0.6	-	-	14.39
8	2.53	0.29	-	-	2.82
9	2.95	0	-	-	2.95
10	6.48	0.38	-	-	6.86
11	-	-	-	-	-
12	7.14	0.42	-	-	7.56
13	9.41	0.47	-	-	9.88
14	-	-	-	-	-
15	-	-	-	-	-
16	28.14	1.66	3.9	0.6	34.3
17	-	-	-	-	-
18	12.7	0.8	-	-	13.5
19	7.95	0.55	8.13	-	16.63
20	2.44	-	-	-	2.44

- : No detectable levels were found.

Table 3. Aflatoxin levels in canary feeds.

Sample Number	Result (ppb)				
	AFB_1	AFB_2	AFG_1	AFG_2	Total
1	-	-	-	-	-
2	2.78	-	-	-	2.78
3	-	-	-	-	-
4	4.38	0.27	-	-	4.65
5	-	-	-	-	-
6	15.5	1.25	-	-	16.75
7	1.77	-	-	-	1.77
8	-	-	-	-	-
9	4.93	-	-	-	4.93
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-
15	0.91	-	-	-	0.91
16	2.65	-	-	-	2.65
17	-	-	-	-	-
18	-	-	-	-	-
19	5.05	0.3	-	-	5.35
20	-	-	-	-	-

- : No detectable levels were found.

Table 4. Aflatoxin levels in parrot feeds.

Sample Number	Result (ppb)				
	AFB_1	AFB_2	AFG_1	AFG_2	Total
1	-	-	-	-	-
2	-	-	-	-	-
3	11.69	1.23	-	-	12.92
4	10.21	0.51	-	-	10.72
5	-	-	-	-	-
6	-	-	-	-	-
7	2.18	-	-	-	2.18
8	3.29	-	-	-	3.29
9	-	-	-	-	-
10	1.43	-	-	-	1.43
11	-	-	-	-	-
12	-	-	-	-	-
13	18.5	0.79	-	-	19.29
14	-	-	-	-	-
15	1.58	-	1.46	-	3.04
16	-	-	-	-	-
17	14.6	1.44	-	-	16.04
18	-	-	-	-	-
19	3.68	0.25	-	-	3.93
20	2.52	-	-	-	2.52

- : No detectable levels were found.

In addition, an example of a negative (Figure 1) and positive chromatogram (Figure 2) of the analysed samples and an example of the chromatogram of the standard (Figure 3) are given.

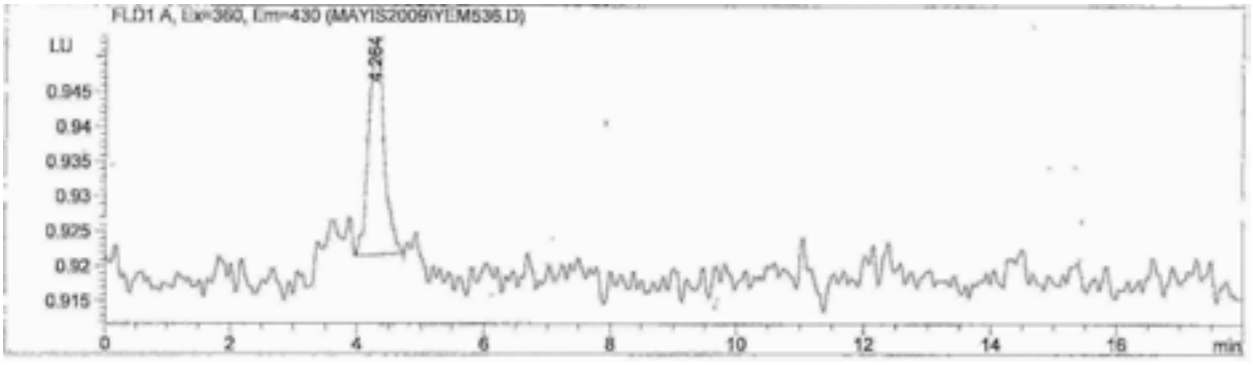

Figure 1. Negative feed sample chromatogram.

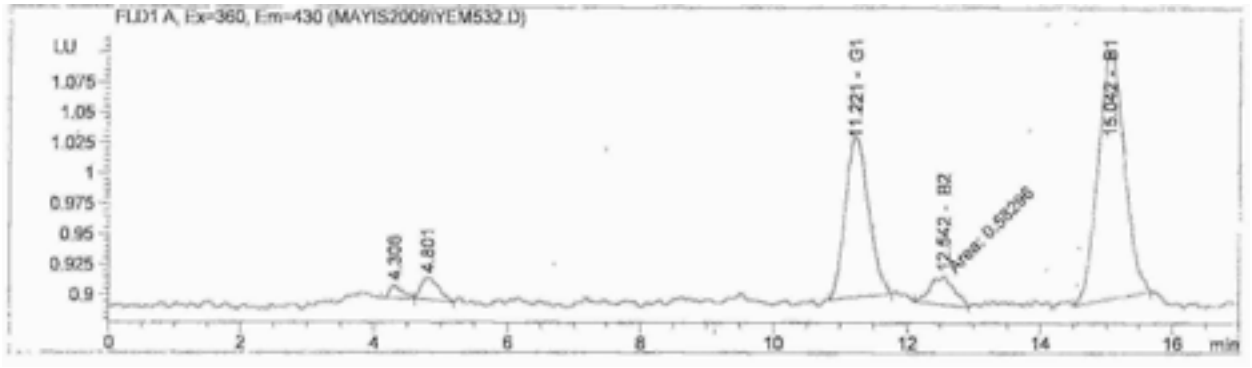

Figure 2. Positive feed sample chromatogram.

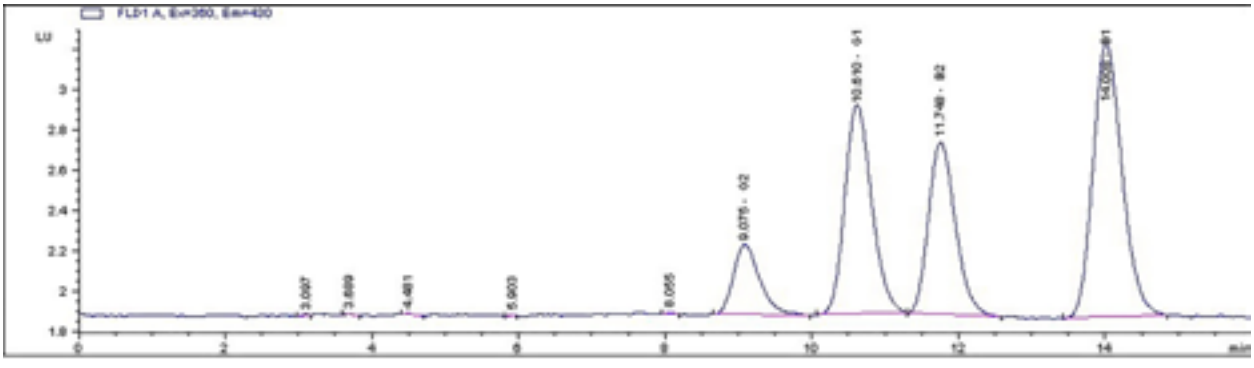

Figure 3. Chromatogram of the standard.

Feed varieties were similar in terms of total aflatoxin, AFB1, AFB2, AFG1 and AFG2 levels ($p>0.05$).

As a result of the analyses, nitrite levels were detected between 0.48-6.19 ppm in 32 samples and nitrate levels were detected between 1.31-25.55 ppm in all samples. According to the feed types, nitrate levels were 7.95±1.33 ppm (1.31-25.55 ppm) in budgerigar feeds, 7.47±0.60 ppm (2.62-13.76 ppm) in canary feeds, 6.95±0.60 ppm (1.97-12.45 ppm) in parrot feeds; nitrite levels were 1.48±0.41 ppm (0.00-6.19 ppm) in 10 samples in budgerigar feeds, 0.83±0.28 ppm (0.00-3.81 ppm) in 8 samples in canary feeds and 2.21±0.38 ppm (0.00-4.76 ppm) in 14 samples in parrot feeds (Table 5).

Table 5. Nitrate, nitrite, tannin and sodium chloride levels in feeds.

Substance	n	Budgerigar X ± Sx	Canary X ± Sx	Parrot X ± Sx	General* X ± Sx	P
Nitrite (ppm) min - max	20	1.48 ± 0.41[ab] 0.00 – 6.19	0.83 ± 0.28[b] 0.00 – 3.81	2.21 ± 0.38[a] 0.00 – 4.76	1.51 ± 0.22 0.00 – 6.19	x
Nitrate (ppm) min - max	20	7.95 ± 1.33[a] 1.31 – 25.55	7.47 ± 0.60[a] 2.62 – 13.76	6.95 ± 0.60[a] 1.97 – 12.45	7.46 ± 0.52 1.31 – 25.55	xxx
Tannin (percent) min - max	20	0.10 ± 0.007[a] 0.04 – 0.15	0.12 ± 0.005[a] 0.09 – 0.15	0.66 ± 0.013[b] 0.52 – 0.74	0.29 ± 0.034 0.04 – 0.74	xx
Sodium chloride (percent) min - max	20	0.25 ± 0.018[a] 0.11 – 0.44	0.11 ± 0.009[b] 0.05 – 0.18	0.23 ± 0.015[a] 0.09 – 0.35	0.19 ± 0.012 0.05 – 0.44	xxx

a, b: Differences between groups with different letters in the same row are significant.
x: $p < 0.05$ **xx:** $p < 0.001$ **xxx:** $p > 0.05$ ***:** n = 60

Tannin levels were found at ratios between 0.04-0.74% in all feeds. According to the feed types, 0.10±0.007% (0.04-0.15%) was found in budgerigar feeds, 0.12±0.005% (0.09-0.15%) in canary feeds and 0.66±0.013% (0.52-0.74%) in parrot feeds (Table 5).

Sodium chloride was found between 0.05-0.44% in all feeds. According to the feed types, 0.25±0.018% (0.11-0.44%) was found in budgerigar feeds, 0.11±0.009% (0.05-0.18%) in canary feeds and 0.23±0.015% (0.09-0.35%) in parrot feeds (Table 5).

In terms of nitrite, budgerigar feed was similar to canary and parrot feed. There was a significant difference between canary and parrot feeds ($p<0.05$). All groups were similar in terms of nitrate ($p>0.05$). In terms of tannins, budgerigar and canary feeds were similar; parrot feed was significantly different from these groups ($p<0.001$). In terms of sodium chloride, budgerigar and parrot feeds were similar ($p>0.05$), while there was a significant difference in canary feed ($p<0.05$).

Discussion and Conclusion

As a result of the scientific searches, a very limited number of studies with regard to the levels of aflatoxins, nitrate-nitrite, tannin and sodium chloride, which are natural adverse factors in cage bird feeds, were found.

In the study conducted by Oruç et al. (2001) by ELISA method in 22 bird feeds (12 budgerigars, 5 canary and 5 zoo bird feeds) in Bursa, total aflatoxin was found in 72.72% of the analysed feeds and it was determined to be in the levels of 0.0-9.2 ppb. In this study than the study of Oruç et al. (2001), total aflatoxin was detected at a lower rate (48.3%) but at higher levels (0.00-34.30 ppb).

In a study conducted by Nizamlıoğlu and Gözün (1996) by thin layer chromatography method in 12 different species and ages of poultry brought to the laboratory with suspicion of poisoning and in the feeds consumed, AFB1 has been detected in the feeds in levels of 2.5-25 ppb and it has been reported that pathological disorders due to aflatoxin are observed in the liver and kidneys of the animals as a result of necropsy. The levels of AFB1 found in this study (0.91-28.14 ppb) were similar to the levels (2.5-25 ppb) found according to the results of the analyses performed by the researchers.

Martins et al. (2003) have not detected aflatoxin in analysed 20 bird feeds (10 canary and 10 parrot feeds) for mycotoxins by HPLC method.

In a screening study conducted by Henke et al. (2001), aflatoxins ranging from 0 to 2780 ppb were detected in 142 wild bird feed samples consisting of corn, millet, sunflower seeds and cereal grains collected from different regions of Texas by Aflatest kits and fluorimetric method and it was reported that 17% of these feeds contained aflatoxins above 100 ppb. The aflatoxin levels found in this study (0.00-34.30 ppb) were much lower than the levels found by Henke et al.

In a study by Maia and Pereira Bastos de Siquiera (2002), AFB1 was detected in 26.7% of 30 bird feeds by thin layer chromatography method with a mean of 110 ppb in 26.7% of the bird feeds. It has been reported that all feeds in which aflatoxin is detected contain peanuts. When compared with the results of the studies here, the AFB1 levels found by the researchers (mean 110 ppb) were much higher than the mean AFB1 levels found in this study (3.60±0.75).

The contamination of feed and feed raw materials with aflatoxins varies significantly according to

feed content, countries, regions, seasons and time due to the preparatory factors causing toxin formation. For this reason, there were significant differences between the findings of the studies.

There is no data on aflatoxin tolerance levels in cage bird feeds. The levels obtained as a result of the research were evaluated according to the Communiqué on undesirable substances in feed numbered 2005/3 published in the Official Gazette dated 5 February 2005 and numbered 25718 and on the amendment of the Communiqué on undesirable substances in feed published in the Official Gazette dated 11 June 2008 and numbered 26903 within the framework of European Union harmonisation laws. According to this communiqué, the maximum amount of AFB1 that can be found in feedstuffs is reported as 0,02 mg/kg (20 ppb). Similarly, the Advisory Committee on Animal Feedingstuffs in the UK reported the maximum amount of AFB1 in feedstuffs for wild birds as 0.02 mg/kg. As a result of the study, it was determined that only 1 sample of budgerigar feed exceeded the specified levels.

In a study conducted by Oruç et al. (2001) on a total of 22 bird feeds including 12 budgerigars, 5 canaries and 5 zoo birds, 0.0-3.1 ppm nitrate and 0.0-1.3 ppm nitrite were found in the feeds. When compared with the results of this study (nitrate 1.35-25.5 ppm and nitrite 0.00-6.19 ppm), it was found that the levels found by the researchers were lower.

In a study conducted by Atef et al. (1991) in cockerels, it was determined that growth was slowed down, methaemoglobinaemia and changes in erythrocyte, glutamic pyruvic transaminase, creatinine and urea levels developed in both of the two different groups given 4.2 g/kg sodium nitrate and 1.7 g/kg sodium nitrite with feed. Therefore, it was stated that nitrate and nitrite might play a role in the aetiology of liver, kidney and immune system related diseases in poultry.

The levels found here were similar to the levels of nitrate and nitrite (0.5-18 ppm) normally found in cereal grains reported by Diaz et al. (1995).

Akar et al. (1994), in a study on tannin content in various feed materials, found that tannin content in feed materials ranged between 0 and 5.70 per cent and concluded that tannin content at these levels might cause health problems in poultry and adversely affect feed utilisation. The results obtained in this study (0.04-0.74%) were found to be lower than the results found by the researchers.

In a study conducted by Pour and Edriss (1997) in broiler chickens, it was reported that while no adverse effects were observed in animals fed with feed containing up to 0.26% tannin, performance decreased in animals fed with feed containing tannin above this level.

Poultry are more sensitive to sodium chloride poisoning than other species. For these animals, safe and toxic levels of sodium chloride are very close to each other (Balnave 2006).

Berger (2006) reported that the ratio of sodium chloride in the feed of chicken, turkey, duck, goose, pheasant and quail should be between 0.25-0.5%.

Although there is no data on the sensitivity limits of cage birds to sodium chloride, it was concluded that the levels determined as a result of the analyses (0.05-0.44%) would not cause any health problems in cage birds since they were below the levels specified in the literature.

The levels of nitrate-nitrite, tannin and sodium chloride were evaluated in the light of the literature on poultry and it was concluded that the levels found were at levels that would not cause any health problems in animals.

Conclusions

The levels of nitrate-nitrite, tannin and sodium chloride detected in feeds do not seem to pose any risk to the health of cage birds, but the level of AFB1 found in only 1 budgerigar feed may pose a risk to the health of cage birds. However, the lack of sufficient studies on the natural adverse factors found in bird feeds and their effects on the health of birds makes it necessary to carry out new studies in order to better evaluate the issue and to determine legal limits. In particular, the high levels and poisoning cases detected in some studies show that it would be useful to screen bird feeds in terms of natural adverse factors at certain intervals.

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Original Article
Özgün Araştırma

Retrospective evaluation of canine and feline mammary tumors diagnosed during the period from 2009 to 2022 in İzmir province

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Abstract: The aim of this retrospective study was to determine the incidence of canine and feline mammary tumors diagnosed in the Pathology Laboratory of Bornova Veterinary Control Institute in İzmir Province between January 2009 and December 2022, as well as to analyse the relationships between the age and breed characteristics of affected animals and the presence of mammary tumors. For this purpose, the available information regarding each mammary tumor case was collected from archive records. Dogs and cats with mammary tumors were divided into four age groups. All hematoxylin and eosin-stained slides of mammary tumors were histopathologically re-evaluated and classified. The study material consisted of 106 (35.81%) mammary tumor cases, of which 83 (37.55%) belonged to dogs and 23 (30.66%) to cats. The highest incidence of mammary tumors in both species was observed in the age group of 8 to 11 years. A statistically significant relationship was found between the incidences of mammary tumors according to the age groups and breeds in both species ($p<0.05$). Terrier (28 cases) in dogs ($p=0.000$) and mixed breed (14 cases) in cats ($p=0.0003$) were the most commonly affected breeds by mammary tumors. In dogs, 64 (77.10%) of 83 mammary tumors were malignant and 19 (22.90%) were benign; in cats, 18 (78.26%) of 23 mammary tumors were malignant and 5 (21.74%) were benign. Malignant mammary tumors were detected more frequently in Terrier dogs (34.37%) and mixed breed cats (72.22%). The most commonly diagnosed malignant mammary tumors were mixed type carcinoma (51.56%) in dogs ($p=0.000$) and tubular carcinoma (50%) in cats ($p=0.005$). Benign mixed tumor (47.37%) and complex adenoma (26.32%) were the most frequently observed benign mammary tumors in dogs. Simple adenoma constituted the majority (60%, 3 cases) of benign mammary tumors in cats.

Keywords: Cat, dog, mammary tumor, retrospective study, Türkiye

İzmir ilinde 2009-2022 yılları arasında teşhis edilen köpek ve kedi meme tümörlerinin retrospektif değerlendirmesi

Özet: Bu çalışmanın amacı, İzmir ilinde Ocak 2009-Aralık 2022 yılları arasında Bornova Veteriner Kontrol Enstitüsü Patoloji Bölümünde teşhis edilen köpek ve kedi meme tümörlerinin insidansını belirlemek ve meme tümörlü hayvanların ırk ve yaş özellikleri ile meme tümörlerinin varlığı arasındaki ilişkileri araştırmaktı. Bu amaçla her bir meme tümörü vakasına ilgili mevcut bilgiler arşiv kayıtlarından toplandı. Meme tümörlü köpek ve kediler 4 yaş grubuna ayrıldı. Meme tümörlerinin hematoksilen-eozin boyanmış slaydları histopatolojik olarak yeniden değerlendirildi ve sınıflandırıldı. Çalışma materyalini, 83 (%37,55) köpek ve 23 (%30,66) kediye ait 106 (%35,81) meme tümörü olgusu oluşturdu. Her iki türde en yüksek meme tümörü insidansı 8-11 yaş grubunda görüldü. Her iki türde yaş grupları ve ırklara göre meme tümörü insidansları arasında istatistiksel olarak önemli bir ilişki bulundu ($p<0,05$). Köpeklerde Terrier (28 olgu) ($p=0,000$) ve kedilerde melez ırk (14 olgu) ($p=0,0003$) meme tümörlerinin en sık görüldüğü ırklardı. Köpeklerde 83 meme tümörünün 64 (%77,10)'ü malign ve 19 (%22,90)'u bening; kedilerde ise 23 meme tümörünün 18 (%78,26)'i malign ve 5 (%21,74)'i bening karakterdeydi. Malign meme tümörleri, en çok Terrier ırkı köpeklerde (%34,37) ve melez ırk kedilerde (%72,22) belirlendi. Köpeklerde en sık teşhis edilen malign meme tümörleri, mikst tip karsinom (%51,56) ($p=0,000$) ve kedilerde tubuler karsinomdu (%50) ($p=0,005$). Köpeklerde bening mikst tümör (%47,37) ve kompleks adenom (%26,32) en sık gözlenen bening meme tümörleriydi. Kedilerdeki bening meme tümörlerinin (3 olgu, %60) çoğunluğunu basit adenom oluşturdu.

Anahtar kelimeler: kedi, köpek, meme tümörü, retrospektif çalışma, Türkiye

Introduction

Mammary tumors are one of the most common tumors of dogs, cats and humans (Goldschmidt et al., 2017). Female dogs and cats have a high incidence of mammary tumors, and mammary tumors are rarely seen in males (Munson and Moresco 2007). The

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annual incidence rate of mammary tumors in dogs has been determined as 198.8 cases in Alameda County, California (Dorn et al., 1968b) and 250 cases per 100,000 dogs in Northern Italy (Vascellari et al., 2016). In cats, the annual incidence rate of mammary tumors is lower compared to dogs and has been reported as 25.4 cases per 100,000 cats in Alameda County, California (Dorn et al., 1968b).

The incidence of mammary tumors and the impact of risk factors affecting the incidence in female dogs and cats varies according to countries and geographic regions (Dorn et al., 1968b; MacVean et al., 1978; Aydın et al., 2008; Dhami et al., 2010; Shida et al., 2010; Vascellari et al., 2016; Pastor et al., 2018; Viana et al., 2019). It is also directly related to ovariectomy and the age at which ovariectomy is performed (Schneider et al., 1969; Misdorp 1988; Sorenmo et al., 2000). Schneider et al. (1969) have reported that neutered bitches have 12% of the mammary cancer risk as compared to intact ones and that the risk of developing mammary tumors is significantly reduced (0.5% incidence) in female dogs neutered before the first estrus. Also, ovariectomy, even when performed at an advanced age, has been detected to be to some extent protective against mammary tumor development in dogs (Misdorp, 1988; Schneider et al., 1969). Similarly, it has been shown that intact female cats have a 7-fold higher risk of mammary cancer than neutered females (Dorn et al., 1968b) and that cats neutered before 1 year of age have an 86% reduction in the risk of feline mammary carcinoma development (Overley et al., 2005).

In this retrospective study, it was aimed to determine the incidence of canine and feline mammary tumors diagnosed in the Pathology Laboratory of Bornova Veterinary Control Institute in İzmir Province between January 2009 and December 2022 and also to investigate the relationships between the age and breed characteristics of affected animals and the presence of mammary tumors.

Materials and Methods

Animals and histopathological examination

In this retrospective study, a total of 106 mammary tumor cases from 221 dog and 75 cat tumor biopsy samples that were sent to the Pathology Laboratory of Bornova Veterinary Control Institute from various veterinary clinics in İzmir province during fourteen years between January 2009 and December 2022 were studied. Mammary tumors belonged to 83 dogs and 23 cats of different breeds and ages. The available information regarding each mammary tumor case was reviewed. The age, gender and breed characteristics of animals with mammary tumors were collected from their records. Dogs and cats with mammary tumors were divided into four age groups of 0-3, 4-7, 8-11 and 12-15 years according to their ages. The age information of 4 dogs and 1 cat, and the breed information of 5 dogs and 5 cats could not be recorded because such information wasn't available. Mammary tumors were defined as benign or malignant according to their histopathological examination protocols. All histological slides prepared from mammary tumor samples which had been previously fixed in 10% formalin and following routine tissue processing procedures, embedded in paraffin blocks, cut at 4-5 µm thickness and stained with hematoxylin and eosin were re-evaluated and classified under a light microscope, taking into account the histological classification of canine mammary tumors proposed by Goldschmidt et al. (2011) and the updated feline mammary tumor classification corresponding to this new classification in dogs (Goldschmidt et al. 2017).

Statistical analyses

Chi-square (χ^2) test was used to determine the strength of the relationships between the presence of mammary tumors and variables such as age group and breed. Similarly, the frequency of benign/malignant mammary tumors was also evaluated using the Chi-square test. T test or Mann Whitney U test was used to determine if there is a statistically significant difference between the mean ages of dogs and cats with mammary tumors and between the mean ages of animals with benign and malignant mammary tumors in both species. Statistical analyses were performed using the IBM SPSS Statistical Package Programme (version 24). Values of $p<0.05$ were considered significant.

Results

The distribution of canine and feline mammary tumors in total tumor samples by years (2009 to 2022) is presented in Figures 1 and 2, respectively.

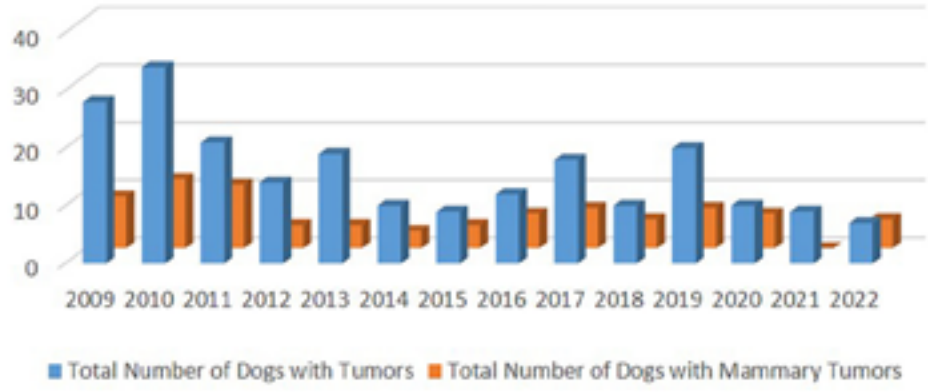

Figure 1. Distribution of canine mammary tumors in total tumor samples by years (2009-2022).

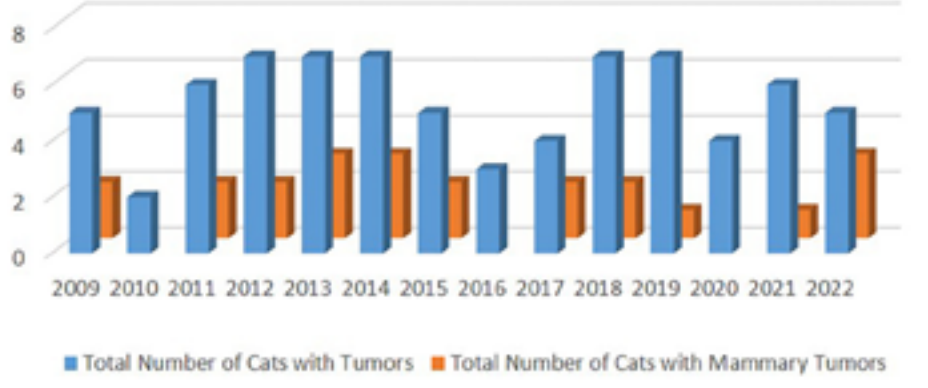

Figure 2. Distribution of feline mammary tumors in total tumor samples by years (2009-2022).

The incidence in 83 dogs and 23 cats with mammary tumors examined during the fourteen years covered by the study was determined as 37.55% and 30.66%, respectively. All mammary tumors belonged to female dogs and cats. The mean age of dogs with mammary tumors was 9.06±0.36, with a range from 3 to 15 years. The mean age of cats with mammary tumors was 10.68±0.56, with a range from 5 to 15 years. The difference between the mean ages of dogs and cats with mammary tumors was statistically significant (p= 0.0308). The highest incidence of mammary tumors in dogs and cats was observed in the age group of 8 to 11 years (Table 1). Statistically, when all age groups were taken into account, the differences between the incidences of mammary tumor according to the age groups in both species were significant (χ^2=20.19, p=0.0002 in dogs; χ^2=20.91, p=0.0001 in cats). However, when early age group (0-3 years) was excluded, this difference was insignificant in dogs (χ^2=0.97, p=0.62) but was significant in cats (χ^2=10.18, p=0.006).

Table 1. Distribution of dogs and cats with mammary tumors according to the age groups.

	Age Groups (years)						
DOGS (with tumors) n: 221	**0-3**	**4-7**	**8-11**	**12-15**	**UN**	**Total**	**Mean Age**
Number of dogs with mammary tumors	3	25	29	22	4	83	9.06±0.36
Percent (%)	3.61	30.12	34.94	26.50	4.82	37.55	-
CATS (with tumors) n: 75							
Number of cats with mammary tumors	-	2	14	6	1	23	10.68±056
Percent (%)	-	8.69	60.87	26.08	4.34	30.66	-

UN: Their age is unknown

The distribution of canine and feline malignant and benign mammary tumors according to the age groups and their breeds is presented in Table 2. In dogs, 64 (77.10%) of 83 mammary tumors were malignant and 19 (22.90%) were benign; in cats, 18 (78.26%) of 23 mammary tumors were malignant and 5 (21.74%) were benign. The malignant mammary tumors were observed much more frequently than benign tumors in both species and this difference was statistically significant ($\chi2=24.40$, $p<0.0001$ in dogs; $\chi2=7.35$, $p<0.0067$ in cats). The average ages of dogs with benign and malignant mammary tumors were 7.94±0.80 and 9.41±0.39 years, respectively. The average ages of cats with benign and malignant mammary tumors were 9.60±0.98 and 11.00±0.66 years, respectively. However, no statistically significant relationship between the average ages of dogs or cats with benign and malignant mammary tumors was found ($p>0.05$). Twenty-five (30.12%) dogs and 10 (43.48%) cats with malignant mammary tumors were in the age group of 8 to 11 years. Eight (9.64%) dogs and 4 (17.39%) cats with benign mammary tumors were in the age groups of 4 to 7 years and 8 to 11 years, respectively (Table 2). No statistically significant difference was also found between the incidences of benign and malignant mammary tumors according to the age groups in both species ($\chi2=5.54$, $p=0.14$ in dogs; $\chi2=2.88$, $p=0.24$ in cats).

As seen in Table 2, dog breeds such as Belgian Malinois, German shorthaired Pointer, Siberian Husky, English Pointer, Rottweiler, Shih Tzu, Chow Chow with one malignant or benign tumor were represented under Others. The difference between the incidences of mammary tumor according to the breeds in both species was statistically significant ($p<0.05$). Terrier (28 cases) in dogs ($\chi^2=103.33$, $p=0.000$) and mixed breed (14 cases) in cats ($\chi^2=16.33$, $p=0.0003$) were the most frequently affected breeds by mammary tumors. Malignant mammary tumors were more common in the Terriers (34.37%), followed by the Cocker Spaniels (9.37%) and German Shepherds (9.37%). The incidence of malignant mammary tumors in the Terriers showed an increase with age. The highest incidence of malignant mammary tumors in cats was found in the mixed breed (72.22%), followed by Siamese cats with 16.67% (Table 2). However, whereas no statistical association between the incidences of benign and malignant mammary tumors according to the breeds in dogs was found ($\chi^2=10.25$, $p=0.59$), the differences between these variables in cats were significant ($\chi^2=8.60$, $p=0.014$).

Table 2. Distribution of dogs and cats with malignant and bening mammary tumors according to the age groups and their breeds

	AGE GROUPS (YEARS)															
	MALIGNANT							BENIGN								
Breeds	**0-3**	**4-7**	**8-11**	**12-15**	**UN**	**Total**	**Percent %**	**0-3**	**4-7**	**8-11**	**12-15**	**UN**	**Total**	**Percent %**	**Overall Total**	**Overall Percent (%)**
DOGS																
German Shepherd	-	3	2	1	-	6	9.37	-	-	-	-	-	0	0	6	7.23
Cocker Spaniel		-	3	3	-	6	9.37	-	4	1	-	-	5	26.32	11	13.25
Golden Retriever	1	3	-	-	-	4	6.25	1	-	1	-	-	2	10.53	6	7.23
Labrador	-	-	2	-	-	2	3.13	-	-	-	-	-	0	0	2	2.41
Kangal dog	-	-	3	-	-	3	4.68	-	-	-	-	-	0	0	3	3.61
Terrier	-	4	8	8	2	22	34.37	-	2	2	2	-	6	31.58	28	33.74
Collie	-	-	-	1	-	1	1.56	-	-	-	1	-	1	5.26	2	2.41
Poodle	-	-	1	1	-	2	3.13	-	-	-	1	-	1	5.26	3	3.61
Doberman	-	1	1	-	-	2	3.13	-	-	-	-	-	0	0	2	2.41
English Setter	-	2	-	-	-	2	3.13	-	-	-	-	-	0	0	2	2.41
German Pinscher	-	1	1	-	-	2	3.13	-	-	-	-	-	0	0	2	2.41
Others	-	2	2	1	-	5	7.81	-	2	-	1	-	3	15.79	8	9.64
Mixed breed	-	-	1	1	-	2	3.13	1	-	-	-	-	1	5.26	3	3.61
UN*		1	1	1	2	5	7.81	-	-	-	-	-	0	0	5	6.03
Total	**1**	**17**	**25**	**17**	**4**	**64**	**100**	**2**	**8**	**4**	**5**	**-**	**19**	**100**	**83**	**100**
Percent (%)	**1.20**	**20.48**	**30.12**	**20.48**	**4.82**	**77.10**		**2.41**	**9.64**	**4.82**	**6.03**	**-**	**22.90**		**100**	
Mean Ages	**3**	**6± 0.30**	**9.5± 0.22**	**13.05± 0.26**	**-**	**9.41± 0.39**		**3**	**5.68± 0.21**	**8.75± 0.25**	**12.90± 0.40**	**-**	**7.94± 0.80**			

	AGE GROUPS (YEARS)															
	MALIGNANT							BENIGN								
Breeds	**0-3**	**4-7**	**8-11**	**12-15**	**UN**	**Total**	**Percent %**	**0-3**	**4-7**	**8-11**	**12-15**	**UN**	**Total**	**Percent %**	**Overall Total**	**Overall Percent (%)**
CATS																
Siamese	-	1	1	1	-	3	16.67	-	-	-	-	-	0	0	3	13.04
Persian	-	-	-	-	-	0	0	-	-	1	-	-	1	20	1	4.35
Mixed breed	-	-	8	5	-	13	72.22	-	-	1	-	-	1	20	14	60.87
UN*	-	-	1	-	1	2	11.11	-	1	2	-	-	3	60	5	21.74
Total	**-**	**1**	**10**	**6**	**1**	**18**	**100**	**-**	**1**	**4**	**-**	**-**	**5**	**100**	**23**	**100**
Percent (%)	**-**	**4.35**	**43.48**	**26.08**	**4.35**	**78.26**		**-**	**4.35**	**17.39**	**-**	**-**	**21.74**		**100**	
Mean Ages	**-**	**5**	**9.8± 0.36**	**14± 0.26**	**-**	**11± 0.66**		**-**	**6**	**10.5± 0.50**	**-**	**-**	**9.60± 0.98**			

UN*: Their breed is unknown **UN:** Their age is unknown

When the types of mammary tumors are histopathologically evaluated, 51.56% of malignant mammary tumors in dogs were mixed type carcinoma (Figure 3A), 10.94% were tubulopapillary carcinoma and 10.94% were complex carcinoma. The most frequently encountered benign mammary tumors were benign mixed tumor (47.37%) (Figure 3B) and complex adenoma (26.32%) (Table 3). Whereas no significant difference between the incidences of benign mammary tumor types according to the breeds in both species was found ($p>0.05$), the differences between the incidences of malignant mammary tumor types were statistically significant ($p<0.05$). Mixed type carcinoma in dogs ($\chi^2= 93$, $p=0.000$) and tubular carcinoma in cats ($\chi^2= 16.66$, $p=0.005$) were the most frequently observed malignant mammary tumors (Table 3, 4).

Table 3. Histological classification and distribution of canine mammary tumors according to the breeds

TUMOR TYPES	**DOG BREEDS**										
MALIGNANT MAMMARY TUMORS	**Terrier**	**Cocker Spaniel**	**German Shepherd**	**Turkish Kangal dog**	**Golden Retriever**	**Poodle**	**Mixed breed**	***Others**	**UN**	**Total**	**Percent %**
Tubular carcinoma	-	1	1	-	-	-	-	1	-	3	4.69
Tubulopapillary carcinoma	1	1	-	1	1	-	1	2	-	7	10.94
Cystic-papillary carcinoma	-	-	-	-	-	1	-	1	1	3	4.69
Solid carcinoma	1	-	-	2	1	-	-	-	1	5	7.81
Complex carcinoma	3	-	-	-	-	1	-	3	-	7	10.94
Mixed type carcinoma	15	4	4	-	2	-	1	4	3	33	51.56
Intraductal papillary carcinoma	-	-	-	-	-	-	-	1	-	1	1.56
Carcinosarcoma (Malignant mixed mammary tumor)	2	-	1	-	-	-	-	2	-	5	7.81
Total	**22**	**6**	**6**	**3**	**4**	**2**	**2**	**14**	**5**	**64**	**100**
Percent (%)	**34.37**	**9.38**	**9.38**	**4.69**	**6.25**	**3.12**	**3.12**	**21.88**	**7.81**	**100**	
BENING MAMMARY TUMORS											
Simple adenoma	1	-	-	-	-	-	-	1	-	2	10.52
Intraductal papillary adenoma	1	-	-	-	1	-	-	1	-	3	15.79
Complex adenoma	2	3	-	-	-	-	-	-	-	5	26.32
Benign mixed tumor	2	2	-	-	1	1	1	2	-	9	47.37
Total	**6**	**5**	**-**	**-**	**2**	**1**	**1**	**4**	**-**	**19**	**100**
Percent (%)	**31.58**	**26.32**	**-**	**-**	**10.52**	**5.26**	**5.26**	**21.06**	**-**	**100**	

UN: Their breed is unknown
*Others include the dog breeds such as Labrador, Collie, Doberman, English Setter, German Pinscher with one or two malignant and benign mammary tumors.

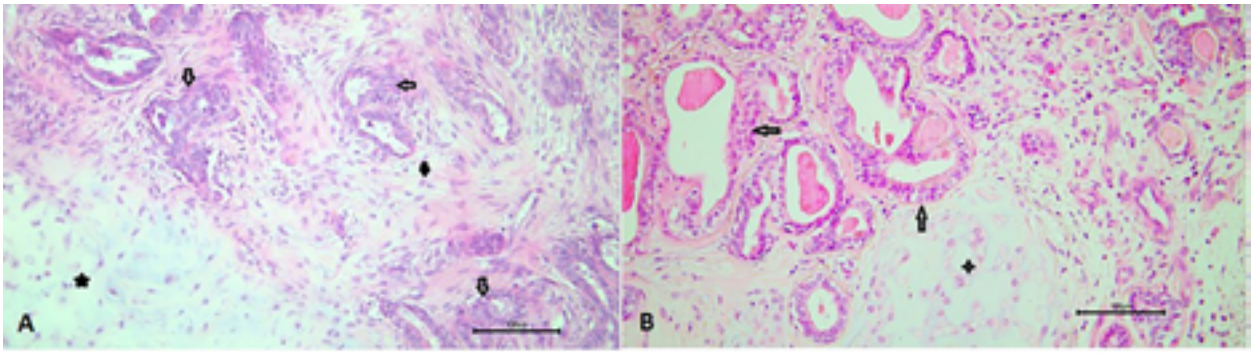

Figure 3. Hematoxylin and eosin (HE) staining of canine mammary tissues. Bars=100 µm. **A.** Mixed type carcinoma. Atypical epithelial cells arranged in irregular tubules (white arrows) with proliferating spindle-shaped myoepithelial cells (black arrow) and focus of chondroid differentiation which exhibit no atypia (asterisk). **B.** Bening mixed tumor. Mammary alveoli with hyperplastic epithelium and eosinophilic secretion (arrows) and chondroid focus (asterisk).

Mixed type carcinoma was frequently observed in the Terriers (15 cases) and to a lesser extent in the Cocker Spaniels (4 cases) and German Shepherds (4 cases). Complex adenoma was mostly seen in the Cocker Spaniels (3 cases) (Table 3). In cats, tubular carcinoma was mostly encountered in mixed breed cats (5 cases) (Figure 4A) and the less frequently in Siamese cats (3 cases). Simple adenoma constituted the majority (60%, 3 cases) of benign mammary tumors in cats (Table 4) (Figure 4B).

Table 4. Histological classification and distribution of feline mammary tumors according to the breeds.

TUMOR TYPES	**CAT BREEDS**					
MALIGNANT MAMMARY TUMORS	**Siamese**	**Persian**	**Mixed breed**	**UN**	**Total**	**Percent %**
Tubular carcinoma	3	-	5	1	9	50
Tubulopapillary carcinoma	-	-	1	-	1	5.56
Cystic -papillary carcinoma	-	-	-	1	1	5.56
Solid carcinoma	-	-	4	-	4	22.22
Comedocarcinoma	-	-	2	-	2	11.11
Mucinous carcinoma	-	-	1	-	1	5.56
Total	**3**	**-**	**13**	**2**	**18**	**100**
Percent (%)	**16.67**	**-**	**72.22**	**11.11**	**100**	
BENING MAMMARY TUMORS						
Simple adenoma		1	1	1	3	60
Intraductal papillary adenoma	-	-	-	1	1	20
Ductal adenoma	-	-	-	1	1	20
Total	**-**	**1**	**1**	**3**	**5**	**100**
Percent (%)	**-**	**20**	**20**	**60**	**100**	

UN: Their breed is unknown

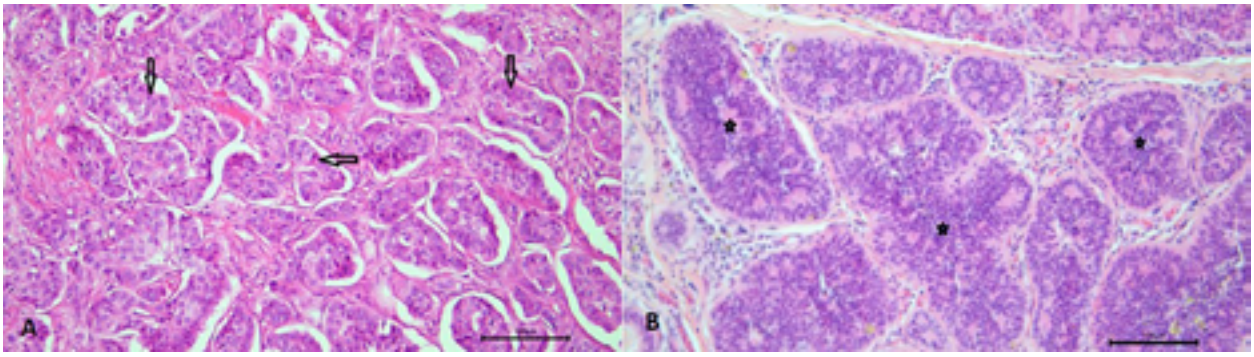

Figure 4. Hematoxylin and eosin (HE) staining of feline mammary tissues. Bars=100 µm. **A.** Tubular carcinoma. Atypical epithelial cells with tubular arrangement (arrows). **B.** Simple adenoma. The islands of uniform neoplastic cells consisting of cuboidal to columnar epithelial cells with basally located nuclei (asterisk).

Discussion and Conclusion

Retrospective epidemiological studies on neoplastic diseases represent both an important source of information by analyzing tumor behaviour over a particular time period and also a useful approach in determining the risk factors and prognostic criteria, based on clinical and histopathological features (Salas et al., 2015). In this retrospective study, data covering a 14-year period between January 2009 and December 2022 was evaluated by comparing it with data from many similar studies (Vural and Aydın 2001; Zatloukal et al., 2005; Sontas et al., 2009; Dhami et al., 2010; Shida et al., 2010; Salas et al., 2015; Andrade et al., 2017; Pastor et al., 2018; Kuruca et al., 2019; Viana et al., 2019; Tunç and Vural 2024).

Mammary tumors are one of the most common tumors of female dogs (Mitchell et al., 1974; Salas et al., 2015; Andrade et al., 2017; Pastor et al., 2018) and constitute 41.75% of all tumors seen in female dogs (Dorn et al., 1968a). It is much less common in males and has been reported at varying rates from 0.2 to 4.3 % (Dorn et al., 1968b; Moulton et al., 1970; Mitchell et al., 1974; MacVean et al., 1978; Vural and Aydın 2001; Vascellari et al., 2009; Nunes et al., 2018). In the present study, whereas the incidence (37.55%) of canine mammary tumors determined through the retrospective data analysis in İzmir province was below the incidence rates of 39.87%, 54 % and 60.37% reported from some countries such as Gujarat, Western India (Dhami et al., 2010), Italy (Vascellari et al., 2016) and Spain (Pastor et al., 2018), respectively, it was higher than the incidence rates reported from different provinces of Türkiye (Ertürk et al., 1971; Vural and Aydın 2001; Erer and Kıran 1993; Gülçubuk and Gürel 2003; Kuruca et al., 2019) and from some other countries (Mitchell et al., 1974; Bhaiyat et al., 2013; Salas et al., 2015; Viana et al., 2019). It has been suggested that the territorial/regional differences in the incidence of mammary tumors may be due to various factors such as the population distribution of certain breeds with a predisposition to mammary tumors, the prolonged time between tumor onset and clinical evaluation, the exposure of dogs to different environmental pollutants and failure to implement reproductive control measures such as neutering of female dogs at a young age (<2 years) (Pérez Alenza et al., 2000; Sorenmo et al., 2000; Sontas et al., 2009; Andrade et al., 2010; Dhami et al., 2010; Salas et al., 2015; Andrade et al., 2017; Nunes et al., 2018).

Mammary tumors in cats are reported as the third most frequently diagnosed tumors after hematopoietic system and skin tumors (Dorn et al., 1968a; Hayes and Mooney 1985) and account for 17.1% of all neoplasms in female cats (Dorn et al., 1968a). However, there are also some studies in which mammary tumors are seen the first (Gülçubuk et al., 2005) or second after skin tumors (Aydın et al., 2008; Vascellari et al., 2009; Shida et al., 2010). In Türkiye, retrospective studies evaluating different tumor types and mammary tumors in cats are much less compared to dogs (Ertürk et al., 1971; Köküuslu and Akkayan 1972; Gülçubuk et al., 2005; Aydın et al., 2008). In cats, mammary tumors are mostly malignant and show a lower incidence compared to dogs (Munson and Moresco 2007). In male cats, the incidence of mammary tumors has been reported to vary from 1.1% (Vascellari et al., 2009) to less than 1 % (Hayes et al., 1981). Contrary to some studies that the lower incidence rates of mammary tumors in cats are reported (Dorn et al., 1968a; MacVean et al., 1978; Aydın et al., 2008; Vascellari et al., 2009; Shida

et al., 2010), the frequency of mammary tumors in female cats in this study was 30.66% of all feline tumors. Consistent with the literature, the incidence (30.66%) of mammary tumors in cats was lower than that (37.55%) of dogs and the majority (78.26%) of them were malignant as in dogs.

The age-specific incidence for mammary tumors in dogs shows a marked increase between 6 and 10-11 years of age and decreases after 11 years of age with a shortening of life expectancy in dog. Mammary tumors are rarely seen in dogs younger than 2 years of age (Dorn et al., 1968b; Moulton et al., 1970). In the present study, the age of dogs with mammary tumors varied from 3 to 15 years, with an average of 9.06±0.36 years. The incidence of mammary tumors increased with age and the highest incidence was detected in the age group of 8 to 11 years (34.94%, n=29). Our finding is compatible with the findings of many studies that mammary tumors were the most often diagnosed in dogs between 8 and 12 years of age, with an average of 9 to 10 years (Moulton et al., 1970; MacVean et al., 1978; Egenvall et al., 2005; Zatloukal et al., 2005; Sontas et al., 2009; Dhami et al., 2010; Gupta et al., 2012; Bhaiyat et al., 2013; Salas et al., 2015; Andrade et al., 2017; Nunes et al., 2018; Tunç and Vural 2024). Also, the lowest incidence of mammary tumors in this study was found to be 3.61% in young dogs under 4 years of age. This finding is consistent with some studies that the incidence rates of 3 to 4.76% in similar age group were reported (Mitchell et al., 1974; Dhami et al., 2010; Kuruca et al., 2019; Tunç and Vural 2024). There were statistically significant differences between incidences of mammary tumor according to the age groups in the present study, compatible with observations of Vascellari et al. (2016). However, we did not detect a relationship between the incidences of benign and malignant mammary tumors according to the age groups. This finding is consistent with the finding of Salas et al. (2015).

Mammary tumors have been reported in cats between the ages of 2.5 and 19 years (mean age of 10.8 years), with an increased incidence rate from 7 to 12 years of age (Weijer et al., 1972). Also, Dorn et al. (1968b) have detected the highest tumor incidence in the age group of 10 to 11 years, with a marked increase after 9 years of age. Karabolovski et al. (2017) have reported that the mean age of detection of feline mammary tumors was 13.5 years. Unlike this finding, the mean age of 23 cats with mammary tumors in our study was 10.68±0.56 years. This is similar to those reported in some earlier studies (Weijer et al., 1972; Amorim et al., 2006; Cunha et al., 2017). In addition, as in dogs, the incidence of mammary tumors in cats also increased with age. Consistent with previous some studies (Dorn et al., 1968b; Weijer et al., 1972; Gülçubuk et al., 2005), the highest incidence rate in cats was determined in the age group of 8-11 years (60.87%, n=14) in this study.

Purebred dogs have been reported to have the higher breed predisposition to mammary tumors than mixed breed dogs (Dorn et al., 1968b; MacVean et al., 1978; Zatloukal et al., 2005; Salas et al., 2015; Vascellari et al., 2016; Pastor et al., 2018). Consistent with this evidence, the most frequently affected dog breeds by mammary tumors in the present study were Terrier with a statistically significant difference (33.74%, 28/83) and Cocker Spaniel (13.25%, 11/83), as indicated by similar findings of other some studies in Türkiye (Vural and Aydın 2001; Baştan and Zonturlu 2002; Kuruca et al., 2019; Baki Acar and Tunç 2022; Tunç and Vural 2024). This observation can be attributed to the breed popularity in and around İzmir Province under study, as reported by Perez Alenza et al. (2000) stating that breed predisposition can vary due to the different canine populations used in the studies. Additionally, the statistical relationship we found between the incidences of canine mammary tumors according to the breeds is also compatible with similar findings of Pastor et al. (2018) who detected that breed affects the development of the tumor, and of Salas et al. (2015) who showed a certain degree of association in some breeds.

Domestic shorthair, Persian and Siamese cat breeds have been suggested to have a high incidence of mammary tumors (Hayes et al., 1981; Amorim et al., 2006; Shida et al., 2010). In the pre-sent study, mammary tumors were mostly diagnosed in mixed breed cats (60.87%, 14/23) with a statistically significant difference and less frequently in Siamese cats (13.04%, 3/23). This finding is similar to observations of other some studies in which mammary tumors were detected more frequently in mixed breed and Siamese cats (Weijer et al., 1972; Cunha et al., 2017; Karabolovski et al., 2017). However, Shida et al. (2010) have reported that Persian and Siamese cats have high incidences of mammary tumors with statistically significant differences. In the present study, the high incidence of mammary tumors detected in mixed breed cats can be attributed to the fact that they constitute the majority of the domestic and stray cat population in and around İzmir Province.

Many studies in dogs have revealed that percentages of malignant mammary tumors were much higher than benign tumors, with a prevalence varying from 71.2 % to 96.15% (Vural and Aydın 2001; Oliveira et al., 2003; Zatloukal et al., 2005; Sontas et al., 2009; Pawar et al., 2015; Vascellari et al., 2016; Andrade et al., 2017; Nunes et al., 2018; Pastor et al., 2018; Kuruca et al., 2019; Tunç and Vural 2024). Similarly, we also detected more frequently canine malignant mammary tumors (77.10%, 64 cases) than benign tumors (22.90%, 19 cases). It has been reported that the high frequency of malignant mammary tumors in dogs may be associated with the criteria for histological evaluation of mammary tumors or the prolonged time between the onset of the tumor and clinical evaluation leading to the progression from benign to malignant tumors (Perez Alenza et al., 2000; Sorenmo et al., 2009; Andrade et al., 2017; Nunes et al., 2018). In addition, advanced age is accepted as an important risk factor in the formation of malignant tumors (Zatloukal et al., 2005; Sorenmo et al., 2009; Vascellari et al., 2016; Pastor et al., 2018). In the present study, the mean ages of dogs with malignant and benign mammary tumors were 9.41±0.39 and 7.94±0.80 years, respectively (Table 2). This finding is consistent with the finding of Sorenmo et al. (2009) who reported that the average ages of dogs with malignant and benign mammary tumors were 9.5 and 8.5 years, respectively and also supports many studies (Moulton et al., 1970; Sontas et al., 2009; Vascellari et al., 2016; Nunes et al., 2018) suggesting that benign mammary tumors occur 1 to 2 years earlier than malignant tumors. However, in contrast to some researchers (Sorenmo et al., 2009; Vascellari et al., 2016) who reported statistically significant differences, we did not find a significant difference between the average ages of dogs with benign and malignant mammary tumors, similar to the finding of Sontas et al. (2009).

Approximately 86-95% of mammary tumors in cats have been reported to be malignant (Hahn and Adams 1997; Gülçubuk et al., 2005; Amorim et al., 2006; Karabolovski et al., 2017). In the present study, the incidence of feline malignant mammary tumors was slightly lower (78.26%) than the reported percentages. The statistical difference we found between the incidences of benign and malignant mammary tumors according to the breeds in cats may be due to the absence of malignant mammary tumors in Persian cats.

Carcinomas and mixed tumors are the most commonly detected mammary tumors in dogs (Mitchell et al., 1974; Vural and Aydın 2001; Oliveira et al., 2003; Salas et al., 2015; Andrade et al., 2017; Nunes et al., 2018; Kuruca et al., 2019; Viana et al., 2019). Since benign mixed mammary tumors are composed of bening epithelial, myoepithelial and mesenchymal cells that can undergo malignant transformation, they often cause carcinomas and less frequently carcinosarcomas and sarcomas in mixed tumors (Cassali et al., 2012). Similarly, in the present study, mixed type carcinoma was the most frequently diagnosed malignant mammary tumor in dogs with a rate of 51.56% (33 cases). This was followed by tubulopapillar carcinoma (10.94%), complex carcinoma (10.94%) and to a lesser extent by other types of carcinomas. Benign mixed tumors and less often adenomas are the most common diagnosed benign mammary tumors in dogs (Mitchell et al., 1974; Vural and Aydın 2001; Salas et al., 2015; Nunes et al., 2018; Kuruca et al., 2019; Viana et al., 2019). Consistently, we also defined benign mixed tumors and various types of adenomas in dogs, with the percentages of 47.37% and totally 52.63%, res-pectively. However, in this study, the percentage of benign mixed tumors was lower than the percen-tages reported by some researchers in Türkiye (Vural and Aydın 2001; Kuruca et al., 2019). The tendency of Terrier dogs to malignant mammary tumors (Mitchell et al., 1974; Vascellari et al., 2016) and of Cocker Spanial dogs to adenomas (Mitchell et al., 1974) has been previously reported. Similarly, in the present study, malignant mammary tumors were mostly observed in Terriers, as reported by Kuruca et al. (2019) and Tunç and Vural (2024), and showed a higher incidence between 8 and 15 years of age. Mixed type carcinoma was detected in 68.18% of the Terrier dogs with malignant mammary tumors, unlike Kuruca et al. (2019) who reported that complex carcinoma and carcinosarcoma are seen more often in Terriers. Complex adenoma was also mostly found in Cocker Spaniels (3 cases).

In cats, the most frequently diagnosed malignant and benign mammary tumors have been reported as carcinomas/adenocarcinomas (Köküuslu and Akkayan 1972; Gülçubuk et al., 2005; Amorim et al., 2006; Shida et al., 2010; Karabolovski et al., 2017) and adenomas (Gülçubuk et al., 2005; Shida et al., 2010; Karabolovski et al., 2017), respectively. In compatible with these studies, tubular carcinoma (50%) and various types of adenomas (100%) constituted the majority of feline malignant and benign mammary tumors diagnosed in the present study, respectively. Carcinomas were observed mostly in mixed breed and less often in Siamese cats (Table

4), contrary to the findings of Hayes et al. (1981) who reported that carcinomas are seen 2 times more frequent in Siamese cats than other breeds. However, the Siamese breed was the only cat breed with malignant mammary tumor at 5 years of age in our study.

In conclusion, the present study has revealed that mammary tumors were one of the most frequently diagnosed tumors in female dogs and cats in İzmir province. The most commonly affected breeds by mammary tumors were Terrier in dogs and mixed breed in cats. Malignant mammary tumors were observed much more often than benign tumors. For this reason, it is of great importance to raise awareness about mammary health and the early diagnosis of mammary tumors among pet owners. It is thought that this study will contribute to the literature by providing data on canine and feline mammary tumors.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article
Özgün Araştırma

Eucalyptol (1.8-cineole) attenuates Gentamicin-induced liver injury

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Abstract: Gentamicin (GM), which is in the aminoglycoside antibiotic class, is frequently preferred today in the treatment of diseases caused by gram-negative bacteria. However, its significant side effects on liver and kidney functions limit its clinical usefulness. The antioxidant and anti-inflammatory medical activities of eucalyptol (EUC, 1.8-cineole) have been reported in different studies. This study aimed to evaluate the effects of EUC on GM-induced hepatotoxicity. The study groups are consisted of control (C), EUC, GM and GM + EUC, and there were 7 rats in each group. At the end of the study, the rats were euthanized under appropriate conditions and samples were collected and biochemical, histopathological and immunohistochemical analyzes were performed. It was determined that there was a important increase in serum alanine aminotransferase (ALT), aspartate transferase (AST) and gamma-glutamyl transferase (GGT) enzymes in the GM group relative to the C group ($p<0.001$). GM application is determined that it caused histopathological damage in livers. Additionally, immunohistochemically, it caused an important increase in the expressions of 4-hydroxynonenal (4-HNE), 8-hydroxy-2′-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) in rats in the GM group relative to the C group ($p<0.001$). Eucalyptol application (GM+EUC) regulated the increase in serum ALT, AST and GGT enzymes. It also attenuated 4-HNE, 8-OHdG and MDA expressions. It attenuated the histopathological damage caused by GM application. The results of this study revealed that EUC showed antioxidative, protective and curative efficacy in GM-induced liver damage.

Keywords: eucalyptol (1.8-cineole), gentamicin, hepatotoxicity, MDA, 8-OHdG

Okaliptol (1.8-sineol) Gentamisin kaynaklı karaciğer hasarını hafifletir

Özet: Aminoglikozid antibiyotik sınıfında yer alan gentamisin (GM), gram negatif bakterilere bağlı hastalıkların tedavisinde günümüzde sıklıkla tercih edilmektedir. Ancak karaciğer ve böbrek fonksiyonları üzerine belirgin yan etkilerinin olması klinik yararlılığını sınırlamaktadır. Okaliptolün (OKA, 1.8-sineol) antioksidan ve antiinflamatuar tıbbi aktiviteleri farklı çalışmalarda bildirilmiştir. Bu çalışmada GM kaynaklı hepatotoksisite üzerine OKA'nın etkilerinin değerlendirilmesi amaçlanmıştır. Çalışma grupları kontrol (K), OKA, GM ve GM + OKA'dan oluşmaktaydı ve her grupta 7 rat bulunmaktaydı. Çalışmanın sonunda ratlar uygun koşullar altında ötenazi edilerek örnekler toplandı ve biyokimyasal, histopatolojik ve immünohistokimyasal analizleri yapıldı. GM grubunda serum alanin aminotransferaz (ALT), aspartat aminotransferaz (AST) ve gama-glutamil transferaz (GGT) enzimlerinde K grubuna kıyasla anlamlı artış olduğu belirlendi ($p<0.001$). GM uygulamasının karaciğerlerde histopatolojik hasara neden olduğu belirlendi. Ayrıca immünohistokimyasal olarak GM grubundaki ratlarda K grubuna kıyasla 4-hidroksinonenal (4-HNE), 8-hidroksi-2′-deoksiguanozin (8-OHdG) ve malondialdehit (MDA) ekspresyonlarında anlamlı artışa neden oldu ($p<0.001$). Okaliptol uygulaması (GM+OKA) serum ALT, AST ve GGT enzimlerindeki artışı düzenledi. Ayrıca 4-HNE, 8-OHdG ve MDA ekspresyonlarını azalttı. GM uygulamasının neden olduğu histopatolojik hasarı azalttı. Bu çalışmanın sonuçları OKA'nın GM kaynaklı karaciğer hasarında antioksidan, koruyucu ve tedavi edici etkinlik gösterdiğini ortaya koydu.

Anahtar kelimeler: gentamisin, hepatotoksisite, MDA, okaliptol (1.8-sineol), 8-OHdG

Introduction

One of the drug classes frequently used in the treatment of diseases today is antibiotics. Antibiotics prevent many problems caused by infection. However, antibiotics can damage various organs such as skin, kidney, liver, brain, mouth, etc. Amino-

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glycoside antibiotics have been used as antibacterial treatment for many years. Aminoglycosides are defined as a class of antibiotics that consists of at least 2 amino sugars and are used to treat gram (-) bacteria. Following treatment with aminoglycoside antibiotics, approximately 5-10% of patients may experience ototoxicity, nephrotoxicity, and hepatotoxicity side effects (Mahi-Birjand et al., 2020; Thy et al.2023; Lang et al.2023)

Gentamicin (GM), which is in the aminoglycoside antibiotic class, is frequently preferred today in the treatment of diseases caused by gram-negative bacteria. However, its notable side effects on liver and kidney functions limit its clinical usefulness (Yarijani et al., 2019; Babaeenezhad et al., 2021; Akcakavak et al., 2024). The liver and kidneys are particularly sensitive to drug toxicity because they play important roles in normal homeostasis in detoxification and excretion of drugs and their metabolites. Currently, the exact mechanism of GM hepato and nephrotoxicity have not been fully clarified. Diffe-rent hypotheses have been proposed in many stu-dies and oxidative stress is the most emphasized in GM hepato and nephrotoxicity (Galaly et al., 2014; Laaroussi et al., 2021). Because reactive oxygen species (ROS) and other free radicals are suggested to be one of the important mediators of GM toxicity (Banday et al., 2008). Different studies have shown that GM stimulates excessive production of ROS metabolites, affecting different processes including lipid peroxidation, protein oxidation and DNA damage, leading to necrosis and cellular damage. In addition, excessive production of free radicals causes activation of nitrosative tissue stress, modulation of the caspase family and upregulation of the inflammatory process (Morales et al., 2002; Sanchez-Gonzalez et al., 2011; Ali et al., 2020; Bulboacă et al., 2022).

Eucalyptol (EUC, 1.8-cineole), a saturated monoterpene, is obtained from botanical sources such as eucalyptus, camphor and rosemary. EUC is known as the main active ingredient of eucalyptus essential oils. EUC has a long history of use in traditional medicine. It is also frequently used in food, fragrance and cosmetics due to its taste and aroma (Galan et al., 2020; Hoch et al., 2023). EUC has a number of medical effects including antimicrobial, anti-inflammatory, antioxidant, analgesic and bronchodilator (Galan et al., 2020; Cai et al., 2021; Kazak, 2022; Akcakavak, et al., 2024, Kazak et al., 2024a; Kazak et al., 2024b). It has been reported in various studies that EUC shows highly effective free radical scavenging activity and has the ability to defend cells from oxidative damage by neutralizing ROS (Rašković et al., 2014; Ryu et al., 2014). Ryu et al. 2014 stated that EUC significantly reduced ROS overproduction in patients with ischemic stroke. In an experimental arthritis study in rats, EUC administration was reported to reduce the levels of proinflammatory cytokines (Iqbal et al. 2024). In a lead acetate-induced liver injury study, it was stated that EUC could prevent damage by reducing inflammation and oxidative stress (Abdollahi et al.2024). It is also reported that EUC has an inhibitory effect against lipid peroxidation (Moon et al., 2014; Hsouna et al., 2019).

Medicinal plants have been widely used to treat many diseases since ancient times. Today, the use of herbal supplements has a special importance in the treatment of liver diseases due to their numerous properties and low side effects. EUC, a saturated monoterpene, is obtained from botanical sources such as eucalyptus, camphor and rosemary (Galan et al., 2020, Cai et al., 2021). Recently, many experimental researches are being conducted to prevent and/or treat GM-induced hepatoxicity using different agents (Babaeenezhad et al., 2021; Mirazi et al., 2021; Ogundipe et al., 2021). The main purpose of these studies is to find new, effective and safer antioxidant compounds that can prevent and/or treat GM-induced hepatotoxicity. EUC may be an important candidate due to a number of medicinal effects such as anti-inflammatory, antioxidant, antimicrobial, analgesic. Although there are studies in the literature evaluating the effects of EUC on hepatotoxicity induced by different agents, no study evaluating its effectiveness on GM-induced hepatotoxicity was found. The aim of the present study was to evaluate the effect of EUC on GM-induced hepatotoxicity by determining histopathological damage, hepatic functional enzymes, and lipid peroxidation markers in the tissue.

Materials and methods

Animals

The material for the study consisted of 28 female Wistar Albino rats weighing 300-400 g. The rats were housed in standard plastic cages, at room temperature of 20-22°C, in 12 hours of light and 12 hours of darkness, and were fed *ad libitum*.

Experimental procedure

Rats were randomly divided into 4 groups, 7 in each group; control (C), EUC, GM, GM+EUC. GM was implemented as a single dose of 100 mg/kg intra-

peritoneal (i.p.) for 10 consecutive days of the study. EUC solution was implemented by oral gavage at 100 mg/kg for 10 consecutive days. EUC solution was prepared relative to the previously stated study (Akcakavak et al., 2024). In addition, the vehicle solution was administered to C and GM groups by oral gavage for 10 consecutive days throughout the experimental period. On the 11th day of the study, intracardiac blood was taken from the experimental animals under xylazine and ketamine (10-90 mg/kg) anesthesia, and the animals were sacrificed. Afterwards, the liver tissues of the necropsied rats were placed in 10% neutral formaldehyde solution for histopathological and immunohistochemical examinations.

Determination of serum biochemical parameters

Blood samples taken under anesthesia were centrifuged at 5000 rpm for 15 minutes at 4°C. The obtained sera were transferred to cryotubes and stored at -80°C. Liver function was evaluated via measurement of ALT, AST and GGT activities in serum using autoanalyzer (AU5800, Beckman Coulter, Japan) and commercial kits.

Histopathological examination

Liver tissues taken following necropsy were fixed in 10% neutral formaldehyde solution. Then, formaldehyde was removed by washing and passed through graded alcohols (70º, 80º, 90º, 100º) and xylene steps, respectively. Afterwards, the relevant liver tissues were embedded in paraffin. Sections were taken from paraffin blocks onto ground slides and stained with Hematoxylin-Eosin (H-E). Histopathological evaluation was performed semi-quantitatively in 10 different fields at x20 magnification (0; none, 1; mild, 2; moderate, 3; severe).

Immunohistochemical examination

4-5 µm sections were taken from paraffin blocks onto adhesive slides. The sections were subjected to paraffin extraction and rehydration processes. Immunohistochemical staining was performed using a commercial kit according to the previously stated method (Akcakavak et al., 2023). 8-OHdG (8-hydroxy-2′-deoxyguanosine, Santa Cruz Biotechnology, sc-393871, 1/200 dilution, 1 hour incubation), 4-HNE (4-Hydroxynonenal, Abcam, ab46545, 1/200 dilution, 1 hour incubation), MDA (Malondialdehyde, Abcam, ab6463 1/1000 dilution, 1 hour incubation) antibodies were used as primers. 3. 3′-diaminobenzidine (DAB) was used as a chromogen and was examined under light microscopy after counterstaining with Mayers hematoxylin. Immunohistochemical scoring was performed semi-quantitatively (0; none, 1; mild, 2; moderate, 3; severe)(Akcakavak et al., 2024).

Statistical analysis

The statistical program SPSS (Inc., Chicago, USA 25.0) was used to analyse the obtained data. The biochemical, histopathological and immunohistochemical findings obtained in the study were evaluated using One-way ANOVA and Duncan's test as post-hoc test. $p<0.05$ was used as the significance limit.

Results

Serum biochemical findings

Serum biochemical findings of the groups regarding liver function tests are presented in Table 1. The activities of the ALT, AST and GGT enzymes in the GM group were 2.1, 1.7 and 2.2. fold respectively higher than those of the C group ($p<0.001$). The activities of the ALT, AST and GGT enzymes in the GM+EUC group significantly decreased (0.7, 0.8 and 0.7 fold, respectively) compared with the GM group, although the activities of the ALT, AST and GGT enzymes in the GM+EUC group significantly increased (1.4, 1.3 and 1.6 fold, respectively) relative to C group ($p<0.001$).

Table 1. The impacts of EUC on the activities of ALT, AST, and GGT in rat liver, intact and with GM-induced liver injury (Mean±SE, n;7)

Liver function tests	C	EUC	GM	GM+EUC
ALT (ul/l)	35.83±1.67^{c}	39.62±2.94^{c}	75.83±1.83^{a}	52.34±2.09^{b}
AST (ul/l)	90.33±3.57^{c}	93.50±2.86^{c}	152.17±5.49^{a}	121.00±2.90^{b}
GGT (ul/l)	4.50±0.56^{c}	4.67±0.42^{c}	9.83±1.37^{a}	7.10±0.60^{b}

$^{a-c}$ Indicates statistical significance between groups in the same line ($p<0.001$). (**ALT**; alanine aminotransferase, **AST**; aspartate transferase, **GGT**; gamma-glutamyl transferase, **C**; Control, **EUC**; eucalyptol, **GM**; gentamicin, **GM+EUC**: gentamicin+eucalyptol)

Histopathological results

Histopathological statistical scores of the groups are give in Table 2. C and EUC groups showed normal histology (Figure 1.A-B). Degeneration and necrosis were detected in hepatocytes in GM and GM+EUC groups. Especially degenerative and necrotic changes were localized in the centrilobular region (Figure 1.C-E). Degeneration and necrosis scores were significantly reduced in the GM+EUC group relative to the GM group ($p<0.001$). In addition, bile duct proliferation, inflammatory cell infiltration and sinusoidal dilatation were seen in the GM and GM+EUC groups (Figure 1.C-E). The GM+EUC group significantly reduced the relevant disorders relative to the GM group ($p<0.001$). Moreover, hemorrhage foci were detected in places in the GM and GM+EUC groups.

Table 2. Histopathological scores between groups (Mean+SE, n;7)

Histopathological lesion	C	EUC	GM	GM+EUC
Degeneration of hepatocytes	$0.50+0.22^{c}$	$0.67+0.21^{c}$	$3.17+0.17^{a}$	$2.17+0.17^{b}$
Necrosis of hepatocytes	$0.33+0.21^{c}$	$0.50+0.22^{c}$	$2.83+0.17^{a}$	$1.83+0.30^{b}$
Inflammatory cell infiltration	$0.33+0.21^{c}$	$0.33+0.21^{c}$	$2.67+0.21^{a}$	$1.67+0.33^{b}$
Bile duct proliferation	$0.50+0.22^{c}$	$0.50+0.22^{c}$	$2.33+0.33^{a}$	$1.33+0.21^{b}$
Sinusoidal dilatation	$0.67+0.21^{c}$	$0.83+0.17^{c}$	$2.5+0.22^{a}$	$1.67+0.21^{b}$

a-c Indicates statistical significance between groups in the same line ($p<0.001$). (**C**; Control, **EUC**; eucalyptol, **GM**; gentamicin, **GM+EUC**: gentamicin+eucalyptol)

Immunohistochemical results

The immunohistochemical scores of the groups are given in Table 3. In the C and EUC groups, immunopositivity was mild or absent for the relevant primers (8-OHdG, 4-HNE and MDA). 8-OHdG, 4-HNE and MDA immunopositivity was significantly increased in the GM group compared to the C group ($p<0.001$), and immunopositivity was prevalent in the centrilobular regions (Figure 2). The GM+EUC group was found to have significantly reduced immunopositivity (8-OHdG, 4-HNE and MDA) relative to the GM group ($p<0.001$).

Table 3. Immunohistochemical scores between groups (Mean+SE, n;7)

Primers	C	EUC	GM	GM+EUC
4-HNE	$0.17+0.17^{c}$	$0.33+0.21^{c}$	$2.67+0.21^{a}$	$1.83+0.17^{b}$
8-OHdG	$0.00+0.00^{c}$	$0.17+0.17^{c}$	$2.83+0.17^{a}$	$2.00+0.25^{b}$
MDA	$0.17+0.17^{c}$	$0.33+0.21^{c}$	$2.67+0.21^{a}$	$1.83+0.30^{b}$

a-c Indicates statistical significance between groups in the same line ($p<0.001$). (**MDA**; malondialdehyde, **8-OHdG**; 8-hydroxy-2′-deoxyguanosine, **4-HNE**; 4-Hydroxynonenal, **C**; Control, **EUC**; eucalyptol, **GM**; gentamicin, **GM+EUC**: gentamicin+eucalyptol)

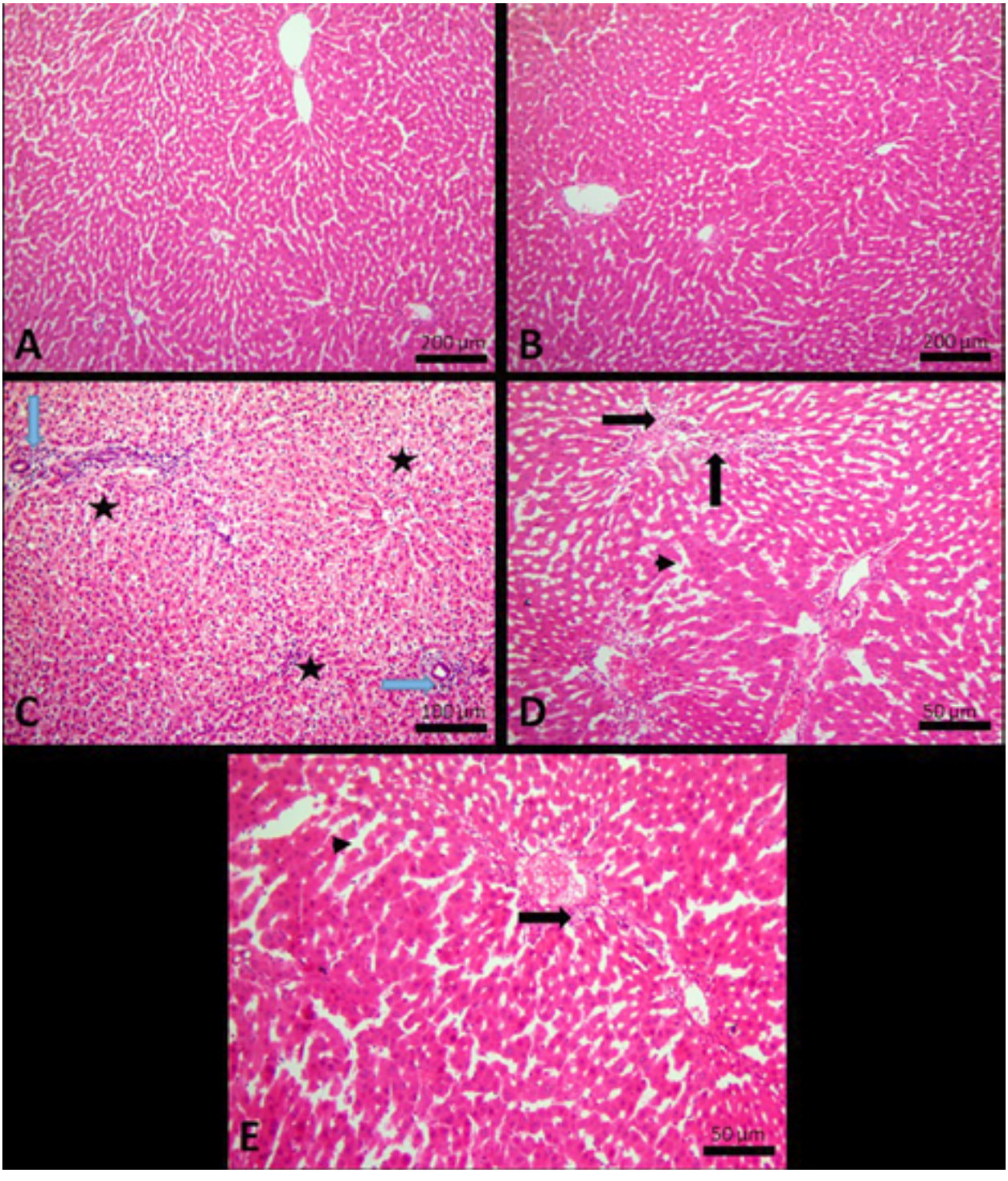

Figure 1. Histopathological examination between groups, Hematoxylin-Eosin, **A**; C group, **B**; EUC group, **C-D**; GM group, **E**; GM+EUC group. (Degeneration in hepatocytes (stars), necrosis (black arrows), inflammatory cell infiltration (blue arrows), sinuosidal dilatation (arrowheads), C; Control, EUC; eucalyptol, GM; gentamicin, GM+EUC; gentamicin+eucalyptol)

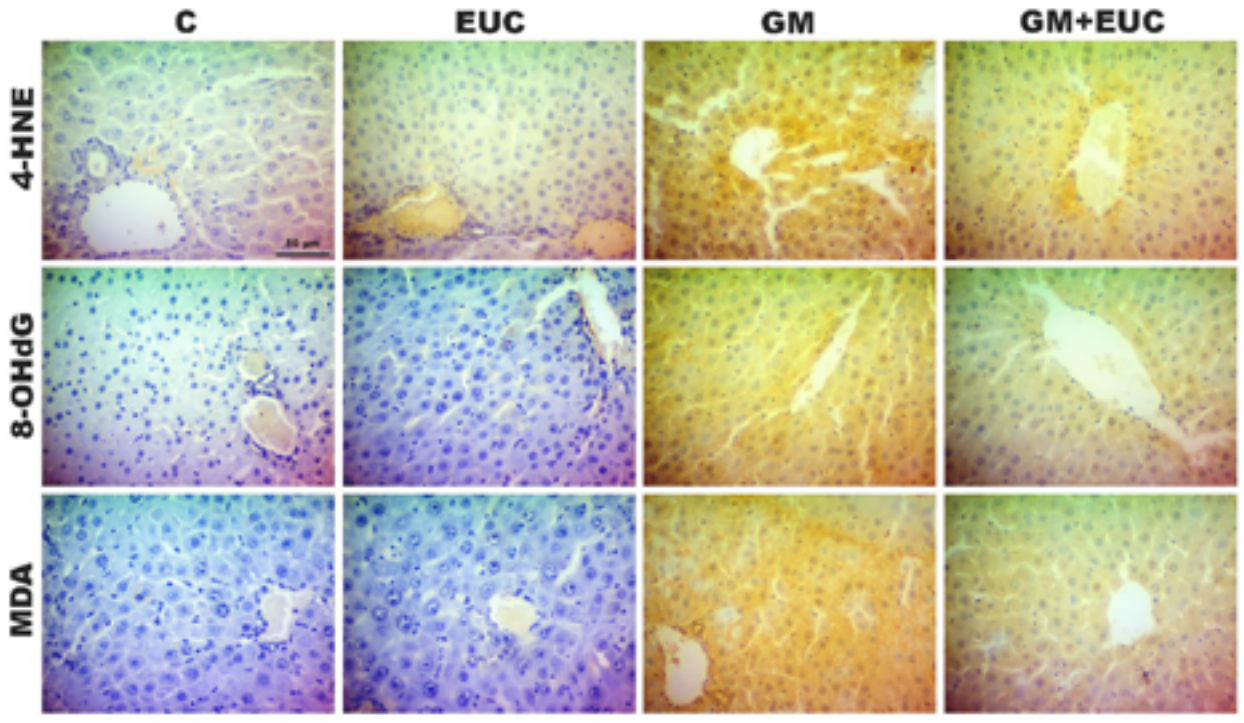

Figure 2. Immunohistochemical examination between groups (DAB), (**4-HNE**; 4-Hydroxynonenal, **8-OHdG**; 8-hydroxy-2'-deoxyguanosine, **MDA**; malondialdehyde, **C**; Control, **EUC**; eucalyptol, **GM**; gentamicin, **GM+EUC**; gentamicin+eucalyptol)

Discussion

Although the effects of many phytochemical components on GM-induced liver toxicity have been evaluated, no study evaluating the effect of EUC on hepatoxicity has been found. Present study, we aimed to evaluate the effects of EUC (100 mg/kg, 10 consecutive days) on liver damage induced by GM (100 mg/kg, 10 consecutive days) in rats. The current biochemical, histopathological and immunohistochemical findings showed that GM administration caused liver hepatoxicity and that the changes related to hepatoxicity could be prevented with simultaneous administration of EUC with GM.

Oxidative stress is a condition that occurs when the balance between free radicals and antioxidants in normal homeostasis is disrupted. Antioxidants are defined as protective systems that protect cells against the damaging effects of free radicals. It is known that suppression of the antioxidant system for various reasons can lead to a wide variety of pathological conditions, including liver damage. Gentamicin reduces the activity of antioxidant enzymes such as CAT, GPx and SOD in cells, causing the redox system to deteriorate and the formation of free radicals (Ďuračková, 2010; Randjelovic et al.2017; Laaroussi et al., 2021; Bulboacă et al., 2022).

AST is present in both hepatocyte cytosol and hepatocyte mitochondria while ALT exists prevalently in the hepatocyte cytosol. The release of these enzymes from hepatocytes is largely due to hepatocellular injury (Zoppini et al., 2016). The enzyme GGT is also associated with hepatitis and biliary tract obstruction (Ibraheem et al., 2021). Literature revealed that GM may directly lead to hepatotoxicity in rats through oxidative stress and apoptosis and finally cause the elevation of the serum activities of ALT, AST and GGT. It has been found that GM increased ALT, AST and GGT activities in rats (Yarijani et al., 2019; Babaeenezhad et al., 2021; Ibraheem et al., 2021; Bulboacă et al., 2022). In keeping with such findings, the present study demonstrated that GM caused liver damage as shown by important increases in serum ALT, AST and GGT activities. Thus, the elevation of ALT, AST and GGT in GM administered animals can imply the injury of liver cytoarchitecture and hepatic cell integrity, linked to microsomal membrane fluidity, mitochondrial dysfunction, and free radical generation. In addition, the activities of the ALT, AST and GGT enzymes in serum of the

groups that received GM and EUC importantly reduced, which indicates the protective effect of EUC against hepatotoxicity induced by GM.

GM is often prescribed to treat bacterial conjunctivitis, sepsis, endocarditis, and infections caused by gram-negative bacteria (Chen et al., 2014). Although it has many beneficial effects, it increases ROS levels by inhibiting enzymatic and non-enzymatic antioxidants in the liver. Thus, in addition to increasing oxidative stress, it causes liver damage by causing damage to lipids, nucleic acids and cellular proteins in the membrane (Laaroussi et al., 2021; Ogundipe et al., 2021). Galan et al. (2014) detected histopathologically degeneration, steatosis, congestion, inflammatory cell infiltration and bile duct proliferation in liver injury induced by GM in rats. In another study examining liver damage due to GM, histopathological findings included steatosis, congestion, inflammatory cell infiltration, sinusoid dilatation, and an increase in the number of Kupffer cells (Al-Khamas et al. 2020). In another study, degeneration, necrosis and sinusoid dilatation were detected histopathologically in GM-induced liver damage (Wijayanti et al.,2023). When the histopathological findings of the current study were evaluated, it was seen that the histopathological changes observed in the GM group were consistent with the findings of previous studies. It is thought that the finding of degeneration and necrosis in the centrilobular region (Figure 1), especially in the GM group, may be because of the increase in ROS levels for GM application and the subsequent oxidative stress.

It was determined that GM-induced histopathological damage was reduced when EUC was administered together with GM. It has been reported in various studies that EUC shows highly effective free radical scavenging activity and has the ability to protect cells from oxidative damage by neutralizing ROS (Rašković et al., 2014; Galan et al., 2020; Akcakavak et al., 2024). EUC is suggested that it exhibits its antioxidant and anti-inflammatory medical activities through manipulations on Nrf2 and NF-kB pathways. Research in the literature reports that EUC causes upregulation of the Nrf2 transcription factor and downregulation of the NF-kB pathway, thus exhibiting strong antioxidant and anti-inflammatory bioactivities (Cai et al., 2021; Venkataraman et al., 2023; Akcakavak et al., 2024; Iqbal et al., 2024). The current study shows that EUC administration can prevent the upregulation of oxidative stress and inflammatory processes that occur with GM administration and thus reduce liver damage. In addition, the fact that EUC down-regulated pro-inflamatory cytokine levels in previous studies further strengthened our idea (Akcakavak et al., 2023; Akcakavak et al., 2024; Iqbal et al., 2024).

Excessive formation of ROS due to chemical toxicity causes oxidative damage to DNA. This situation contributes to the formation of 8-OHdG, which is the most crucial effect of DNA damage. 8-OHdG has been commonly assesed as a biomarker of oxidative DNA damage in recent years (Graille et al., 2020; Akcakavak et al., 2023). Another oxidative reaction induced by excessive ROS levels is lipid peroxidation. 4-HNE and MDA are known as the cytotoxic end products of lipid peroxidation (Yang et al., 2003; Ayala et al., 2014). In the literature, it is reported that causes upregulation of 4-HNE, 8-OHdG and MDA in different GM-induced toxicity studies (Aycan-Ustyol et al., 2017; Cui et al., 2019; Mohammed et al., 2019; Ince et al., 2020). In the present study, higher expressions of 4-HNE, 8-OHdG and MDA were detected in the GM group relative to the control groups and were consistent with the literature (Figure 2). Present findings showed that GM (100 mg/kg, 10 consecutive days) administration caused oxidative DNA damage and lipid peroxidation. GM and EUC administration provided protection against GM-induced hepatotoxicity by reducing the expressions of 4-HNE, 8-OHdG and MDA relative to the GM only group.

In conclusion, it has been determined that GM application causes complications characterized by degeneration and necrosis in hepatocytes, inflammatory cell infiltration, bile duct hyperplasia and sinusoidal dilatation, and this structural and cellular damage in the liver causes an increase in serum liver enzymes. The current study shows that EUC administered simultaneously with GM plays a protective/curative role in reducing GM-induced liver damage by suppressing liver function tests (AST, ALP, GGT), histopathological changes and 8-OHdG, 4-HNE and MDA expressions. These results suggest that EUC may be a promising candidate for clinical drug development in GM-induced liver damage.

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Original Article
Özgün Araştırma

Determination of *Neospora caninum* in cattle fetuses from the Central Black Sea region using PCR

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Abstract: Neosporosis is an infectious disease caused by the *Neospora caninum*, which leads to abortions in cattle. It causes significant economic losses in both global livestock farming and has recently become one of the leading causes of abortions. This study aims to determine the prevalence of *Neospora* infection in aborted fetuses from 136 cattle in enterprises where large-scale cattle farming is intensively practiced in the Central Black Sea Region (Amasya, Samsun, and Tokat provinces). For the diagnosis of *N. caninum*, DNA isolation was performed aborted fetuses, and these samples were subsequently analyzed using the PCR test method. According to the results obtained, *N. caninum* was found in 3 out of 136 aborted fetuses. In conclusion, this study conducted on cattle in the Central Black Sea Region detected the presence of *Neospora caninum* in aborted fetal samples at a rate of 2.2%. These findings indicate that *N. caninum* should not be overlooked in future studies involving aborted fetal samples. Conducting comprehensive research on the definitive hosts of the parasite will play a crucial role in controlling neosporosis and contribute to the development of effective strategies to prevent the spread of the disease.

Keywords: Cattle, Central Black Sea, Fetus, Neosporosis, PCR

Orta Karadeniz Bölgesindeki sığır fetüslerinde *Neospora caninum'un* PCR ile belirlenmesi

Özet: Neosporozis, *Neospora caninum'un* neden olduğu, sığırlarda aborta neden olan enfeksiyöz bir hastalıktır. Hastalık, büyükbaş hayvan yetiştiriciliğinde önemli ekonomik kayıplara neden olmakta ve son yıllarda abortların başlıca nedenleri arasında yer almaktadır. Bu çalışma, Orta Karadeniz Bölgesinde (Amasya, Samsun ve Tokat illeri) büyükbaş hayvancılığın yoğun olarak yapıldığı işletmelerde abort yapan 136 sığıra ait atık fetüslerde *Neospora sp. varlığını ve* prevalansını belirlemeyi hedeflemektedir. *Neospora caninum*'un teşhisi için atık fetüslerden DNA izolasyonu yapılmış ve ardından bu örnekler PCR test yöntemi ile analiz edilmiştir. Elde edilen sonuçlar doğrultusunda, 136 sığıra ait atık fetüslerden 3'ünde *N. caninum*'a rastlanmıştır. Sonuç olarak, Orta Karadeniz Bölgesindeki sığırlarda gerçekleştirilen bu çalışmada aborte fetüs numunelerinde *N. caninum*'un varlığı %2.2 oranında tespit edilmiştir. Bu bulgular, aborte fetüs numuneleri ile yapılacak gelecekteki çalışmalarda *N. caninum*'un göz ardı edilmemesi gerektiğini göstermektedir. Parazitin son konaklarıyla ilgili kapsamlı araştırmaların yürütülmesi, neosporosisin kontrolünde önemli bir rol oynayacak ve hastalığın yayılmasını engellemeye yönelik etkili stratejiler geliştirilmesine katkı sağlayacaktır.

Anahtar kelimeler: Fetüs, Neosporozis, Orta Karadeniz, PCR, Sığır

Introduction

Neosporosis is a disease caused by the protozoan *Neospora caninum*, which has a two-host heteroxenous life cycle. This disease can lead to clinical signs in various animal species, especially cattle and dogs. Neosporosis is recognized as a parasitic factor causing abortions in both wild and domestic animal species worldwide, particularly in cattle (Dubey et al. 2007; Kaltungo and Musa 2013). Transplacental infection in cattle is an important source of transmission for the parasite; however, the primary route of infection occurs through the oral intake of oocysts shed in dog feces (McAllister et al. 1998). The prevalence of *N. caninum* is attributed to the consumption of placental, aborted fetal, or uterine debris by dogs, which serves as a source of postnatal infection (Davison et al. 2001; Schares et al. 2002; Dubey 2003; Toolan 2003; Salehi et al. 2009; Goodswen et al. 2013). The presence of definitive host dogs in areas where cattle are kept and the contamination of feed and water with their feces are believed to contribute to the spread of infection (Dijkstra et al. 2002; Dubey et al. 2007).

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In the diagnosis of *N. caninum* infection, various serological and molecular tests, along with histopathological and immunopathological examinations using light and electron microscopy, can be employed. These methods are important tools for accurately diagnosing the infection and gaining more insight into the pathogenesis of the disease (Barber et al. 1995; Lally et al. 1996; Ortega-Mora et al. 2006; Lindsay and Dubey 2020). The determination of *N. caninum*-specific antibodies in cattle is typically preferred through serological methods (Dubey and Schares 2006). An observed increase in antibody titers during mid-pregnancy in seropositive animals is considered an indication of reactivation of latent infection (Lindsay and Dubey 2020). Neosporosis has been associated with abortion in seropositive animals (Anderson et al. 2000; Dubey 2003; Açıcı et al. 2019). However, it has been observed that approximately 95% of calves born to seropositive cows are clinically normal despite being congenitally infected (Dubey 1999a; Quintanilla et al. 2000; Dubey 2003). The most effective method for controlling the disease is the culling of infected animals once a definitive diagnosis has been made (Reichel et al. 2013). Nevertheless, the absence of clinical signs in infected cattle other than abortion complicates the diagnosis of the disease (Barber et al. 1995).

Neosporosis represents a significant parasitic threat to cattle populations, with a high prevalence reported globally. This disease is responsible for considerable economic losses (McAllister et al. 1998; Trees et al. 1999; McAllister et al. 2000). The prevalence of *N. caninum* varies by region: 3.4% to 36.2% in Africa Ayinmode et al. (2017), Abdeltif et al. (2022); 37.5% to 70% in northeast Thailand Kashiwazaki et al. (2001); 0.5% to 3.9% in the Czech Republic Václavek et al. (2007), Bártová et al. (2015); 4.1% in Germany and France Dubey et al. (2007); 2.7% to 44.4% in Australia Dubey et al. (2007), Nasir et al. (2012); 23.6% to 91.2% in Brazilian Ragozo et al. (2003), Guedes et al. (2008) and 5.2% in North Dakotato Khaitsa et al. (2006). In Türkiye, serological studies have reported the seroprevalence of neosporosis in cattle to be between 2% and 37.2% (Bıyıkoğlu et al. 2001; Aktaş et al. 2005; Pişkin and Ütük 2009; Kasap et al. 2020; Bulut et al. 2021; Köse et al. 2021; Kula and Gökpınar 2021). While some studies exist on the prevalence of the disease in Türkiye, they seem insufficient considering the cattle population in the country. Regularly conducted parasitological studies at specific intervals would particularly help in determining the prevalence of the disease. The objective of this study is to ascertain the prevalence of neosporosis in abortion cases in the provinces of Amasya, Samsun, and Tokat, where extensive cattle farming is the predominant practice.

Materials and Methods

Collection of fetal samples

The samples used in this study were obtained from the abortions of cattle sent for routine diagnosis to the Samsun Veterinary Control Institute from Amasya (n=16), Samsun (n=80), and Tokat (n=40) provinces. The organ samples taken from these calves were delivered to the Parasitology Laboratory in sterile containers, numbered and stored at -20°C until analysis. The provinces from where the fetal samples were sent are shown in Figure 1.

Figure 1. The provinces from where the fetal samples were taken for analysis regarding *Neospora caninum*

This study aimed to determine the prevalence of *Neospora* infection in aborted fetal tissues (lung, heart, liver, spleen, and stomach contents) from 136 cattle that were aborted in the provinces of Amasya, Samsun, and Tokat, located in the Central Black Sea Region. Approximately 25 mg tissue pieces (lung, heart, liver, spleen, and stomach contents) were taken from each fetal sample and transferred to 7 mL cryo-tubes, followed by the addition of 3 mL of PBS. The samples were homogenized in an automatic homogenization device (Bead Ruptor Elite, Bead Mill Homogenizer, SKU 19-042E, OMNI International, USA) at 7000 rpm for 1 minute. Following homogenization, the samples were centrifuged at +4°C at 4000 rpm for 10 minutes, and 100 µL of the supernatant was taken for DNA extraction according to the manufacturer's protocol (Genomic DNA Mini Kit/Invitrogen). Specific primers for *Neospora*

caninum, Np6/Np21 primers (5′-GGG TGT GCG TCC AAT CCT GTA AC-3′, 5′-CTC GCC AGT CAA CCT ACG TCT TCT-3′) were used for PCR (Kamali et al. 2014). The PCR mixture was prepared in a total volume of 25 µL, consisting of 2.5 µL Dream Taq buffer, 0.5 µL dNTP Mix (10 mM), 0.8 µL of each primer (10 pmol), 0.4 µL Dream Taq DNA polymerase (5 U/µL), and 15 µL sterile distilled water, with 5 µL of template DNA added to reach a total volume of 20 µL. The PCR mixture was placed in a thermal cycler with the following amplification conditions: an initial denaturation at 94°C for 7 minutes, followed by 35 cycles consisting of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute. The process concluded with a final extension step at 72°C for 7 minutes. Subsequently, the amplified PCR products were subjected to electrophoresis in a 1% agarose gel stained with 0.05% ethidium bromide (5 mg/mL) at 90 V and 100 mA for 50 minutes, and the presence of DNA bands was visualized under UV light using a gel imaging device. A PCR product showing a band of 337 bp was considered positive.

Statistical analysis

The frequencies of aborted fetal samples from 136 cattle between 2018 and 2020 were analyzed and summarized in a frequency table 1.

Results

As a result of this study, 136 aborted fetal samples from cattle were analyzed using Polymerase Chain Reaction (PCR), and *Neospora caninum* was detected in 3 samples (2.2%). Of the 80 samples from Samsun, 2 (2.5%) were positive, and of the 40 samples from Tokat, 1 (2.5%) was positive. No *N. caninum* was found in any of the 16 samples from Amasya. The number of samples collected by province and the positivity status are presented in Table 1.

Table 1. Distribution of cattle abortion materials in this study according to provinces and results.

Provinces	Taken samples	Positive	Negative
Amasya	16	0	16
Samsun	80	2	78
Tokat	40	1	39
Total	**136**	**3**	**133**

The PCR image of the *N. caninum* abortion samples is shown in Figure 2.

Figure 2: For *Neospora caninum*, PCR analysis of the samples showed specific banding at 337 bp. M: Molecular weight marker (100 bp ladder), PC: Positive control, NC: Negative control, 1,8: Positive samples, 2-7: Negative samples

Discussion and Conclusion

Neosporosis is one of the most important parasitic causes of cattle abortions, widely observed both globally and in Türkiye, leading to significant economic losses. *N. caninum* is a parasite with a broad host spectrum and can cause infections in many domestic and wild animals, particularly cattle. This situation increases the impact of *N. caninum* on both livestock farming and natural ecosystems, resulting in serious economic losses. The diversity of hosts facilitates the spread of the parasite and complicates its control. Therefore, the prevention and management of *N. caninum* infections are crucial for animal health and productivity (McAllister et al. 1998; Dubey 1999b; Dubey et al. 2007; Şentürk et al. 2020). Congenital infections associated with *N. caninum* can lead to abortions, stillbirths, and the birth of clinically or subclinically infected calves at different stages of pregnancy. This situation poses a significant problem for cattle breeding and causes economic losses. Since the timing of these infections has a decisive impact on animal health and productivity, careful monitoring and management are required (Innes et al. 2007). One of the main reasons for abortions caused by *N. caninum* is the presence of definitive host dogs on farms. The presence of these dogs in the same environment as cattle facilitates the contamination of feed and water sources with feces from canids, thereby promoting the spread of infection (Dubey et al. 2007; Kaltungo and Musa 2013).

Abortions related to neosporosis can occur in any season of the year (Anderson et al. 1991; Moen and Wouda 1995; Thurmond et al. 1995). Numerous studies using various serological methods have been conducted on cattle in different countries around the world. The prevalence of *N. caninum* has varied, with rates of 56.9% in Argentina Campero et al. (1998), 0.5% to 3.9% in the Czech Republic Václavek et al. (2007), Bártová et al. (2015); 12.5% in Wales and England Davison et al. (1999); 36.8% in Spain Quintanilla-Gozala et al. (1999), 15.6% in Poland Cabaj et al. (2000), and 10.7% in Sudan (Ibrahim et al. 2012). In Türkiye, a study by Eşki and Ütük covering *N. caninum* seroprevalence research up to 2018 reported an average prevalence of 13.06% (1023/7830) in cattle. Similarly, a study conducted by Demir et al. in 2020 reviewed all serological studies on cattle in Türkiye and reported an average seroprevalence of 14.7% (1672/11,373) for *N. caninum*.

Globally, PCR diagnostic studies for *Neospora caninum* in aborted cattle fetuses have been conducted. Sager et al. (2001) reported 21% positivity in 58 out of 242 samples in Sweden, while Sadrebazzaz et al. (2004) found *N. caninum* in 33% of 12 aborted fetuses in Iran. In Brazil, Cabral et al. (2009) detected *N. caninum* in 6.7% of 105 aborted fetuses, and Şuteu et al. (2012) found 38.9% positivity in 21 aborted fetal samples. Macedo et al. (2017) detected *N. caninum* DNA in 38.8% of 14 tissue samples from 35 aborted fetuses. In Türkiye, PCR studies on aborted calf fetuses have reported varying results; Özkaraca et al. (2017) found *N. caninum* in 25.49% of 102 aborted fetuses in Elazığ, while Açıcı et al. (2019) reported a 49.4% positivity rate in 44 aborted fetuses from 89 farms using Real-time PCR. In Şenel (2022) doctoral thesis, which investigated *N. caninum* in the Marmara Region, DNA from brain, heart, liver, lung, spleen, and kidney tissues of 84 aborted samples revealed *N. caninum* DNA in 26.19% of cases. Additionally, a study by İrehan et al. (2022) detected *N. caninum* in 8 out of 30 aborted fetuses using Real-time PCR, with only two of these also testing positive by conventional PCR. The lower positivity rate found in our study (2.2%) compared to the 49.4% reported by Açıcı et al. (2019) could be attributed to the lower detection rate of conventional PCR. Furthermore, the lower prevalence of *N. caninum* in the Central Black Sea Region compared to previous studies may be explained by the high level of integrated farming practices in the provinces where this study was conducted and the minimization of contact between cattle and uncontrolled dogs.

The type of cattle farming systems and management strategies are significant risk factors influencing the prevalence of *N. caninum*. Studies have shown that the seroprevalence of *N. caninum* is lower in integrated farms compared to rural family farms. It has been noted that cattle in rural family farms are more exposed to uncontrolled dogs, and those cattle that come into contact with these dogs carry a higher risk of infection (Öcal et al. 2014; Noori et al. 2019). The variability in results from PCR studies for *N. caninum* diagnosis conducted globally and in our country may stem from differences in regions, cattle breeds and rearing conditions, sample sizes, types and quantities of examined tissues, parasitic load, the presence of risk factors associated with *N. caninum*, and the different tests used. Therefore, the presence of definitive host dogs in areas where cattle are located, which can contaminate feed and water with their feces, is thought to play a significant role in the spread of infection (Dijkstra et al. 2002; Dubey et al. 2007).

In conclusion, understanding the relationship between intermediate and definitive hosts of *Neospora caninum* and implementing preventive measures is of great importance. Additionally, since calves born from *N. caninum* infections can transmit the infection from generation to generation, and due to the lack of effective treatment or vaccines, it is considered essential to conduct herd-wide screenings and remove infected animals from the herd. Informing veterinarians and farmers about infections that cause abortion in cattle is crucial for combating these infections and, consequently, for the national economy. Furthermore, the use of double or triple test combinations in diagnosing *N. caninum* has been signficantly evaluated for accurate diagnosis.

Ethical Statement: This study was conducted with permission from the Local Ethics Committee for Animal Experiments of the Samsun Veterinary Control Institute, under the letter dated 07.07.2022 with reference number 19572899/031-65 (Decision no: 2022/5).

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Forestry. Neither the the Republic of Türkiye Ministry of Agriculture and Forestry can be held responsible for". This text is included in accordance with the recommendation of our Ministry.

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Original Article
Özgün Araştırma

The effect of dietary *Sanguinaria canadensis* extract and/or Mannan-Oligosaccharide supplementation on body weight and serum total antioxidant activity in broilers under heat stress

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Abstract: *Sanguinaria canadensis* L. is an herbalceous perennial that contains benzophenanthridine alkaloids, including sanguinarine and dihydrosanguinarine. Mannan-oligosaccharide (MOS) is derived from the cell wall of the yeast *Saccharomyces cerevisiae*. The aim of the study was to investigate the effects of the supplementation of *Sanguinaria canadensis* extract (SCE) and/or MOS on body weight and serum total antioxidant activity in broilers under heat stress (HS[+]) and normal (HS[-]) conditions. A total of 72 one-day-old Ross 308 broiler were randomly assigned to 8 pens in two environmentally controlled rooms (4 pens per room). The dietary treatments were: (1) basal diet (control), (2) basal diet plus 1 g/kg of SCE, (3) basal diet plus 1 g/kg of MOS, (4) basal diet plus 1 g/kg of SCE and 1 g/kg of MOS. At 15 days of age, the chickens in one of the two rooms were exposed to HS (34±2°C) for 6 h, while the chickens in another room were continuously kept under normal conditions, serving as control treatment (22±2°C). During the study, body weights were significantly different and these differences were depended on diet and heat. HS[+] groups had lower body weights, however, the supplementation of SCE and MOS improved this situation positively. During the study, it was also determined that there was an interaction between diet and heat. Differences for serum antioxidant activity between HS[-] and HS[+] groups were significant for CUPRAC analysis results and unsignificant for ABTS analysis results.

Keywords: Broiler; Heat stress; Mannan oligosaccharide; *Saccharomyces cerevisiae*; *Sanguinaria canadensis*

Broyler diyetlerine *Sanguinaria canadensis* ekstraktı ve/veya Mannan-Oligosakkarit ilavesinin sıcaklık stresi altında canlı ağırlık ve serum total antioksidan aktivitesi üzerine etkisi

Özet: *Sanguinaria canadensis* L., sanguinarine ve dihydrosanguinarine dahil olmak üzere benzophenanthridine alkaloidleri içeren çok yıllık bir bitkidir. Mannan-oligosakkaritler (MOS) *Saccharomyces cerevisiae* mayasının hücre duvarından elde edilmektedir. Bu çalışma, *Sanguinaria canadensis* ekstraktı (SCE) ve/veya MOS ilavesinin sıcaklık stresi (HS[+]) ve normal (HS[-]) koşullar altındaki broylerlerde canlı ağırlık ve serum total antioksidan aktivitesi üzerindeki etkilerini araştırmak amacıyla yapılmıştır. Toplam 72 adet bir günlük broyler civcivleri (Ross 308), çevre kontrollü iki odada 8 kümese (oda başına 4 kümes) rastgele ayrılmıştır. Gruplara (1) bazal diyet (kontrol), (2) bazal diyet + 1 g/kg SCE, (3) bazal diyet + 1 g/kg MOS, (4) bazal diyet + 1 g/kg SCE + 1 g/kg MOS şeklinde 42 gün boyunca besleme uygulanmıştır. Hayvanlar 15 günlük olunca, iki odadan birindeki gruplar 6 saat boyunca sıcaklık stresine (34±2°C) maruz bırakılırken, diğer odadaki gruplar kontrol muamelesi olarak sürekli normal (22±2°C) koşullarda tutulmuştur. Çalışma boyunca, vücut ağırlıkları önemli ölçüde farklılık göstermiş ve bu farklılıklar diyet ve sıcaklığa bağlı olmuştur. Sıcaklık stresine maruz kalan gruplar daha düşük vücut ağırlığına sahipken, SCE ve MOS takviyesi bu durumu olumlu yönde iyileştirmiştir. Çalışma sırasında diyet ve sıcaklık arasında bir etkileşim olduğu da tespit edilmiştir. HS[-] ve HS[+] gruplar arasındaki serum antioksidan aktivite farklılıkları CUPRAC analiz sonuçları için önemli, ABTS analiz sonuçları için ise önemsiz bulunmuştur.

Anahtar kelimeler: Broiler; Sıcaklık stresi; Mannan oligosaccharide; *Saccharomyces cerevisiae*; *Sanguinaria canadensis*

Introduction

Stress is considered as a reflex response that inevitably occurs in organisms exposed to adverse environmental conditions and can affect many systems, leading to negative effects such as decreased immunity, live weight gain, feed consumption, and even death (Puvadolpirod and Thaxton 2000; Etim et al. 2013). Heat stress is the most important stressor in poultry and one of the most important causes of economic losses in poultry production. The re-

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commended ambient temperature for broiler chickens from the 4th week of age is between 20-24ºC and when this temperature exceeds 35ºC, it leads to an increase in mortality and morbidity of broilers (Arjona et al. 1988). Although it is thought that the decrease in performance parameters such as body weight, feed conversion, meat and egg yield due to heat stress in poultry is directly related to the decrease in feed intake, many findings from studies on this subject have shown that heat stress can also cause oxidative stress leading to the formation of reactive oxygen species (ROS) that disrupt cell structure in poultry and antioxidant system disorders that negatively affect nutrient absorption and metabolism (Sur et al. 2023). Indeed, various stress factors on living organisms, including heat and cold stress, lead to the overproduction of ROS and the formation of free radicals that exceed antioxidant capacity causes damage to proteins, lipids and DNA (Babior 2000; Belhadj Slimen et al. 2014). Although free radicals are normally neutralized by the body's antioxidant defense system, it is not successful in eliminating the excessive free radical load caused by high environmental stressors and abnormal conditions, and therefore the necessity for the addition of natural or synthetic antioxidants to poultry diets arises (Hosseini-Vashan et al. 2015).

Different feeding strategies to reduce the negative effects of heat stress on livestock include reducing heat production (e.g., increasing dietary fat), compensating for low nutrient content in the diet (e.g., increasing the amount of concentrate), and reducing metabolic changes due to heat stress (e.g., using different feed additives) (Babinszky et al. 2011). Within the scope of different feed additives; especially after the banning of antibiotics by the European Union in 2006, the interest in alternative feed additives to antibiotics such as plant and plant extracts, botanical mixtures, organic acids, probiotics, prebiotics, symbiotics, essential oils, enzymes as growth promoters has increased (Huyghebaert et al. 2011).

Sanguinaria canadensis, also known as bloodroot, is a member of the *Papaveraceae* family, a family of lactiferous latex-producing plants (Croaker et al. 2016). *S. canadensis* biologically contains eight isoquinoline alkaloids, including six quaternary benzophenanthridine alkaloids as sanguinarine, chelerythrine, sanguilutine, chelilutine, sanguirubine, chelirubine and two protopine alkaloids as protopine and allocryptopine (Bambagiotti-Alberti et al. 1991). Among phytobiotics, isoquinoline alkaloids constitute the most interesting group due to their antimicrobial, anti-inflammatory and immunomodulatory effects (Kishore et al. 2009) and within this group, benzophenathridine alkaloids are believed to be the main bioactive components of *Sanguinaria spp.* (Harkrader et al. 1990) and almost all studies report that sanguinarine is the most bioactive component of this alkaloid group (Senchina et al. 2009). It has also been reported that sanguinarine has antimicrobial, anti-inflammatory and antioxidant properties (Adhami et al. 2004).

Mannanoligosaccharide (MOS) is known as a new active antigen substance derived from the cell wall of Saccharomyces cerevisiae, a yeast cell (Xue et al. 2022). The main reasons for the addition of MOS to diets as an alternative to antibiotics are that it prevents the adhesion of pathogenic bacteria to intestinal cells and increases the immunological effect by stimulating the immune system (Genç et al. 2011). In addition, it has also been reported that MOS improves body antioxidant capacity in various animal species such as laying hens (Bozkurt et al. 2012), sheep (Zheng et al. 2018), rabbits (Attia et al. 2015) by acting as a free radical scavenger.

The aim of this study was to investigate the effects of single and combined supplementation of *S. canadensis* extract (SCE) and MOS on body weight and total antioxidant activity in broilers exposed to heat stress, which is an important stressor for poultry.

Materials and Methods

Animals, diets and experimental design

A total of 72 one-day old broiler chicks (Ross 308) were used in this study. The animals were randomly allocated equally into two temperature-controlled rooms with 4 pens each (8 birds per pen). The basal diets used for starter (d 1-21) and grower (d 22-42) phases in the experiment were formulated as isocaloric (ME: 12.34 and 12.74 MJ/kg for starter and grower diets, respectively.) and isonitrogenous (CP: 21% and 18.2% for starter and grower diets, respectively.) to contain all nutrients at appropriate levels according to NRC (1994). During the experiment, the control group in both rooms was fed only with the basal diet, while the experimental groups were fed with basal diets supplemented (1 g/kg diet) with MOS, a prebiotic obtained from the cell wall of the *Saccharomyces cerevisiae* yeast cell, and/or *Sanguinarina canadensis* extract (SCE). After a 15-day adaptation period, the groups in the room without heat stress (HS[-]) were kept under normal thermal conditions of 22±2°C during the experiment, while the groups in the room under heat stress (HS[+]) were exposed to 34±2°C for 6 hours a day. Chicks

were provided *ad libitum* access to feed and water during the experimental period (42 days).

Collection of data and analytical profiles

On day 21 and 42, the animals were fasted for 12 hours and weighed individually, and their body weights (BW) were recorded. Samples collected from the diets given to each group were analyzed for ME and CP according to the standard procedures reported by the Association of Official Analytical Chemists (AOAC 2000).

Blood samples were collected from the brachial vein via vacuum tubes on day 42 from all animals in the experiment before the morning feeding. Serums were separated by centrifugation for 15 min at 3000 g at 4°C and stored (-86°C) for further analysis. CUPRAC (cupric reducing antioxidant capacity) assay according to the procedures reported by Apak et al. (2005) and ABTS (2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay according to the method reported by Re et al. (1999) were applied to determine total antioxidant activity in serum samples. Analyte concentrations were measured by ICP-OES (Agilent 700 ICP-OES). Assays were calibrated with trolox and results are expressed in trolox equivalents (mM).

Statistical analysis

All data were subjected to ANOVA using the general linear models (GLM) procedure of SPSS (1999). Statistical differences among means ($p<0.05$) were identified using Tukey's multiple range test.

Results

The effects of the experimental period on BWs of broilers on d 21 and 42 are presented in Table 1. In HS[-] groups, SCE supplementation to the diet caused a significant increase in BW on d 21 compared to the control and MOS groups ($P<0.05$), while MOS supplementation did not cause a significant difference compared to the control group. The addition of both additives together caused a significant increase in live weight compared to the control and MOS group ($P<0.05$). When the groups in the same room were compared in terms of body weight on day 42, no significant difference was found between the SCE, MOS and control groups, but the combination of SCE and MOS resulted in significantly higher final BW than both the control and MOS groups ($P<0.05$).

Table 1. Body weights of broilers (n=9)

Groups	HS Exposure	Supplementation (g/kg diet)		Body weight (g)	
		SCE	MOS	d 21	d 42
1	[-]	-	-	889.78^{cd}	2650.23^{b}
2	[-]	1	-	1009.34^{ab}	2780.89^{ab}
3	[-]	-	1	898.89^{cd}	2658.45^{b}
4	[-]	1	1	1036.67^{a}	2994.20^{a}
5	[+]	-	-	772.00^{e}	2158.88^{c}
6	[+]	1	-	959.32^{bc}	2821.34^{ab}
7	[+]	-	1	870.89^{d}	2618.89^{b}
8	[+]	1	1	906.40^{cd}	2762.67^{ab}
SEM				6.85	23.12
Diet					
Control				830.89	2404.56
SCE				984.33	2801.11
MOS				884.89	2638.67
SCE + MOS				971.56	2878.44
Heat					
[-]				958.67	2770.94
[+]				877.17	2590.40
Variation source (p<)					
Diet				0.0001	0.0001
Heat				0.0001	0.0001
Diet × Heat				0.024	0.001

HS: Heat stress; **SCE:** *Sanguinaria canadensis* extract; **MOS:** Manan-oligosaccharide; **SEM:** Standard error mean; $^{a, b, c, d, e}$ Means within a column with different superscript letters indicate significant differences ($p<0.05$).

In HS[+] groups, BWs on d 21 were significantly higher in the experimental groups than those of control (P<0.05). It was also recorded that SCE supplementation had a higher effect than MOS in terms of BWs on the same day (P<0.05). On d 42, when BWs of the groups in the same room were compared, the addition of SCE and MOS to the diet individually and combined did not cause a significant difference bet-ween these experimental groups, but all experimental groups had significantly higher BW compared to the control group (P<0.05).

When the groups in HS[+] and HS[-] rooms were compared, temperature stress caused a significant decrease in body weight in the unsupplemented group (P<0.05). The addition of SCE and MOS to the diets individually and combined significantly improved the decreased BW in the HS[+] groups (P<0.05) and brought it closer to BW value of the control group in the HS[-] room. In addition, when the experimental groups in the HS[+] and HS[-] rooms were compared, except for the group fed diet supplemented with the combination of the relevant additives in both rooms, it was noted that there was no significant difference in BW between the counterparts of the experimental groups in the HS[-] room on d 21. On day 42, it was also observed that there was no significant difference in final BW between all experimental groups in both rooms. As a result, it was determined that significant differences in BWs during the experiment were related to diet and heat (P<0.001). Heat stress led to lower BW, but the addition of SCE and/or MOS to the diets improved this situation positively. It was also found that there was an interaction between diet and heat during the experiment (P=0.024 and P=0.001, respectively).

The results of CUPRAC and ABTS analysis performed to determine antioxidant activity in serum samples obtained from the control and experimental groups at the end of the trial (d 42) are presented in Table 2. According to the CUPRAC analysis results, no statistically significant difference was found in serum antioxidant activity at the end of the trial among all groups in HS[-] room. The same situation was also found among the groups in HS[+] room. When HS[+] and HS[-] rooms were compared, HS treatment had a negative effect on serum antioxidant activity and it was significantly lower than the groups housed at normal temperature (P<0.05).

Table 2. Serum antioxidant activities on d 42 according to CUPRAC and ABTS assay (n=9)

Groups	HS Exposure	Supplementation (g/kg diet)		Assay (mM TR-equivalent)	
		SCE	MOS	CUPRAC	ABTS
1	[-]	-	-	0.59[a]	1.83
2	[-]	1	-	0.53[a]	1.92
3	[-]	-	1	0.54[a]	1.80
4	[-]	1	1	0.59[a]	1.82
5	[+]	-	-	0.28[b]	1.94
6	[+]	1	-	0.33[b]	1.88
7	[+]	-	1	0.32[b]	1.93
8	[+]	1	1	0.31[b]	2.05
SEM				0.013	0.027
Diet					
Control				0.44	1.88
SCE				0.42	1.90
MOS				0.43	1.86
SCE + MOS				0.46	1.93
Heat					
[-]				0.56	1.84
[+]				0.32	1.95
Variation source (p<)					
Diet				0.793	0.827
Heat				0.0001	0.046
Diet × Heat				0.388	0.377

HS: Heat stress; **SCE:** *Sanguinaria canadensis* extract; **MOS:** Manan-oligosaccharide; **SEM:** Standard error mean; [a, b] Means within a column with different superscript letters indicate significant differences (p<0.05).

According to the ABTS analysis results, whether or not the groups were subjected to HS and whether or not the related feed additives were used in the diets did not have a significant effect on serum antioxidant activities and no significant differences were found between all groups in terms of the related parameter. In addition, there was no significant interaction between diet and heat in terms of serum antioxidant activity according to both analysis methods (P=0.39 and P=0.38, respectively).

Discussion and Conclusion

Due to increasing concerns about the transfer of residues to consumers through the food chain and the growth of resistant bacteria that threaten both public and animal health, studies on the use of herbal extracts and purified ingredients obtained from plants as alternative additives have increased, especially following the ban on the use of antibiotic feed additives as growth promoters by the European Union in 2006 (Kostandinović and Lević 2018). In addition to herbal extracts, various neutraceuticals such as botanical mixtures, organic acids, prebiotics, probiotics, symbiotics, exogenous enzymes, essential oils are also considered as alternative feed additives to antibiotics (Huyghebaert et al. 2011; Yadav and Jha 2019). In this context, this study was conducted to evaluate individual and combined dietary supplementation of *Sanguinaria canadensis* plant extract, which is rich in quaternary benzo phenanthridine alkaloids (Wu et al. 2020), and MOS derived from the cell wall of *Saccharomyces cerevisiae*, a yeast cell, in terms of body weight and serum antioxidant activities in broilers housed under normal thermal and heat stress conditions as two different environmental temperatures. In this study, the addition of SCE alone to the diet of broilers housed at normal temperature caused a significant increase in BW on d 21 compared to the control group, but did not cause a significant difference on d 42. This positive result obtained in terms of BW on d 21 was comparable with the results of the same period in the studies conducted on broilers by Vieira et al. (2008), Liu et al. (2020), Hasan et al. (2020) and Abudabos et al. (2020). In contrast, a 28-day trial in broilers with a phytobiotic containing 1.5% sanguinarine (sangrovit WS ®) showed no significant difference in final BW between groups (Khatun et al. 2023). In the present study, the addition of the relevant extract to the diet created a statistically insignificant difference in terms of BW on d 42 compared to the control group, which was similar to the results of no significant difference between the control and experimental groups in terms of BW values on d 42 reported by Vieira et al. (2008), on d 35 reported by Zdunczyk et al. (2010) and Aljumaah et al. (2020), and on d 56 reported by Liu et al. (2020). MOS is a natural substance derived from the cell wall of the yeast cell S. cerevisiae and contains a carbohydrate-like mannan component (Zbeda 2021). In this study, the addition of MOS alone to the diet at a rate of 1 g/kg in broilers housed in a normal temperature environment did not cause a significant difference in BWs on d 21 and 42 compared to the control group. Contrary to this finding, in a study in which MOS was added to the diet at a rate of 50 g/100 kg in broilers, Taye et al. (2021) reported that there was an improvement in weekly BW values compared to the control group, and this result was attributed to the view that the carbohydrate in the structure of MOS can reduce pathogenic bacterial colonization in the gastrointestinal tract by binding to bacterial fimbriae, thus improving broiler performance. However, in the broiler diets, there was no significant difference compared to the control group in the BW values on d 21 and 50 in the study in which 0.15%, 0.1% and 0.05% MOS was added by Eseceli et al. (2010) for the starter, grower and finisher periods, respectively, and in the BW values on d 28 and 42 in the study in which 0.5, 1.0 and 1.5 g/kg MOS was added by Zhou et al. (2021), which are parallel to the results of the present study. The combined addition of SCE and MOS to the diets of broilers housed in the HS[-] room resulted in a significant increase in BWs on d 21 and 42 compared to the control group. The fact that the combined use of the relevant additives was more effective than adding them separately to the diet, especially on day 42, strengthens the possibility of a possible synergistic effect. There are also evidences that the combination of phytobiotics with probiotics or prebiotics has a synergistic effect on performance. Indeed, the combination of rosmarinic acid as a phytobiotic and multi-strain Lactobacillus as a probiotic in dairy calves (Stefańska et al. 2021), the mixture of carob pulp, chicory and fenugreek as a phytobiotic-prebiotic mixture in fattening pigs (Juhász et al. 2023), the combination of hop extract, oregano essential oil and MOS in broilers (Bozkurt et al. 2009) resulted in a significant improvement in BW compared to the control group, which is remarkable in terms of synergistic effect. In other studies where phytobiotics were used together with other additives such as probiotics and prebiotics, including the study conducted by Ren et al. (2019) in broilers to determine the effects of the combination of a commercial phytobiotic, containing carvacrol, cinnamaldehyde and eugenol as active ingredients,

together with host-specific *Lactobacillus* strains as probiotics, the common view is that these combinations have an improving effect on the intestinal microbiota and animal health by reducing the survival of potential pathogenic microorganisms in the intestine. With this context, it would not be unreasonable to consider the possibility that such a mechanism of action may play a role behind the improving effect of the combined dietary supplementation of SCE and MOS on BW in broilers in the present study.

Heat stress is the most important environmental stressor for the poultry industry worldwide. Factors such as thick feather layer, inadequate sweat glands and high metabolic characteristics, make poultry more sensitive and vulnerable to heat stress (Song et al. 2018). In this study, it was observed that the BW values of the HS[+] control group on d 21 and 42 were significantly lower than the HS[-] control group, as expected. It is known that HS has negative effects on parameters such as feed intake, body weight gain, carcass weight and immunity in poultry (Sahin and Kucuk 2003; Niu et al. 2009; Ghazi et al. 2012). It is also believed that the decrease in appetite and feed consumption leading to less body weight gain in animals exposed to HS is a defense mechanism to reduce heat production in the animal (Sohail et al. 2012). It has also been reported that exposure to high ambient temperature increases the production and release of corticosteroids in poultry, leads to lower levels of important growth hormones such as triiodothyronine and thyroxine, and causes the release of various cytokines and glucocorticoids that induce protein catabolism (Sahin and Kucuk 2003; Zhou et al. 2016; Yıldırım 2016). In the HS[+] room, although HS caused a significant decrease in BW in the control group, dietary SCE supplementation resulted in a significant increase in BW on d 21 and 42, resulting in an improvement of 24.3% and 30.7%, respectively. Even though it seems to be no significant difference between the control group in the HS[-] room and SCE supplemented group in HS[+] room, it is also noteworthy that the addition of SCE caused an increase in BW values of approximately 8% and 6.5% for the same days, respectively. In a trial conducted by Wang et al. (2022) on broilers with the extract of *Macleaya cordata* plant, which has similar alkaloids to *S. canadensis*, the addition of 1000 mg/kg of the relevant extract to the diet of the HS group significantly increased the final BW value compared to the HS control group, and also, the addition of this extract reduced the deterioration in the intestinal flora caused by HS by reducing the relative density of *Bacteroidota* and *Bacteroides* in the intestinal flora, and thus reducing the decrease in growth performance caused by HS. In studies with 100 ppm by Kikusato et al. (2021) and 60 and 100 mg/kg isoquinoline alkaloids by Khongthong et al. (2023) in broilers under HS, it was reported that the addition of these extracts to the diet led to a significant increase in final BW. In these studies, which are consistent with the positive effects of SCE on BW in broilers under HS in the present study, the ameliorative effects of the relevant extracts on BW were attributed to the modulating effect on caecal flora composition (Wang et al. 2022), the reducing effect on oxidative damage, protein catabolism, intestinal barrier function, intestinal inflammation and the correcting effect on cortisone release (Kikusato et al. 2021), and the improving intestinal integrity by reducing inflammation in the intestines and the suppressing effect of anorexigenic regulation by modulating the gut-brain axis (Khongthong et al. 2023). This situation also raises the possibility that there are similar mechanisms underlying the improving effect of SCE on BW in broilers under HS in the present study.

According to Gibson and Roberfroid (1995), prebiotics are nondigestible food substances that exert beneficial effects by selectively stimulating the growth and activity of one or a limited number of beneficial bacteria in the host intestine. In this study, the addition of 1 g/kg MOS to the diet of broilers under HS significantly improved the decrease in BW values caused by HS on d 21 and 42 and even succeeded in approaching the BW values of the control group housed under normal temperature. When compared to the group supplemented with SCE under HS, it was observed that although MOS supplementation fell behind the group supplemented with SCE on d 21 in terms of BW, it closed this gap on d 42. In this study, the result, which showed that the addition of MOS to broiler diets under HS had a significantly higher BW value compared to the control group in the same environment, was in accordance with the results obtained in studies in which 0.2 g/L MOS was added to drinking water by Hasan et al. (2014), 0.5 ml/L commercial prebiotic was added to drinking water by Sayed et al. (2023), and 0.5% MOS was added to the diet by Sohail et al. (2012) in broilers under HS. The positive effects of MOS on BW under HS may be a result of its ability to reduce the proliferation of potentially pathogenic bacteria in the intestinal environment due to stress, to increase the population of beneficial bacteria, to support intestinal functions, and in addition to these, to reduce the release of cortisone, which increases under

stress, to normal levels. As a matter of fact, it has been reported that dietary MOS supplementation significantly decreased serum corticosterone levels in broilers under HS (Sohail et al. 2012; Hosseini et al. 2016; Cheng et al. 2018; Cheng et al. 2019). In the present study, it was found that the group supplemented with the combination of SCE and MOS housed in the HS[+] room had significantly higher BW than the control group, as in the same group housed in the HS[-] room. Considering the previously mentioned positive effects of adding SCE and MOS individually to the diet on BW under HS, the fact that using them together produced similar results shows that there is no negative interaction between themselves in terms of BW, and it is also possible that they created a cumulative effect in terms of the possibilities leading to the positive result on BW.

Free radicals and antioxidants, which still attract the attention of researchers as a subject of study, are in balance under normal conditions in living organisms, and when this balance changes in favor of free radicals, it can lead negative effects caused by oxidative stress (Gheisar and Kim 2018). The antioxidant activity of additives such as plant extracts, essential oils, prebiotics and probiotics is a subject of great interest due to their free radical scavenging abilities that may play a role in the prevention of free radical-induced diseases (Miguel 2010; Gheisar and Kim 2018; Chaves et al. 2020; Musazadeh et al. 2023). Total antioxidant status is an important criterion used to evaluate the entire antioxidative status in the body (Erel 2004). In this context, measurement of total antioxidant capacity is an application that provides important data in determining antioxidant effects. In particular, measuring total antioxidant capacity or activity in biological samples such as plasma, which contain various antioxidant compounds, provides valuable clues about the antioxidant status (Ghiselli et al. 2000). There are various analysis methods such as ORAC (oxygen radical absorbance capacity), FRAP (ferric reducing antioxidant power), CUPRAC (cupric reducing antioxidant capacity), ABTS (2,20 - azinobis - (3 - ethylbenzothiazoline - 6 - sulfonic acid)), DPPH (2,20 - diphenyl - 1 - picrylhydrazyl), Folin for analyzing antioxidant capacity (Apak et al. 2005). In this study, CUPRAC and ABTS analysis methods were used to determine serum antioxidant activity. When the serum antioxidant activity values obtained according to the CUPRAC method were examined, the first striking finding was that the antioxidant capacity in the HS[+] groups was significantly lower than in the HS[-] groups. On a room basis, the statistically insignificant differences in serum antioxidant activities between the control and experimental groups of each room showed that the differences were entirely due to heat stress, regardless of dietary additives. This may be due to the change in the antioxidant defense system due to increasing environmental temperature. Indeed, it has been reported that broilers exposed to heat stress produce excessive levels of reactive oxygen species (ROS) leading to oxidative stress due to decreased mitochondrial respiratory chain activity and that heat stress disrupts the balance between synthesis and catabolism in this production (Lin et al. 2006; Yang et al. 2010). It was observed that there was a decrease in the antioxidant defense system due to the decrease in SOD, catalase and glutathione peroxidase concentrations in broilers housed under heat stress (Sahin et al. 2010), and there was a significant decrease in the activity of antioxidant enzymes paraoxonase and arylesterase (Sohail et al. 2011). In the present study, when CUPRAC and ABTS analysis methods were compared in terms of HS, it was observed that the CUPRAC method provided more effective results than the ABTS method, while the ABTS method did not exhibit effective sensitivity results against the changes in serum antioxidant activity due to HS. Cecchini and Fazio (2020), who determined that there was no significant difference in antioxidant capacity analyzed by ABTS method in blood samples collected from hens artificially stressed with dexamethasone compared to the healthy group, while this difference was significantly higher in the FRAP test, reported that the lower sensitivity of the ABTS test in the detection of imbalance in antioxidant status compared to the FRAP test may be due to the different technology on which the two tests are based. In the present study, the lower sensitivity of the ABTS method compared to the CUPRAC method in measuring the change in antioxidant activity due to HS may be due to a similar reason. In addition, it is also thought that some unique advantages of the CUPRAC method may play a role in its higher sensitivity than the ABTS method. For example, better reagent stability than radical reagents such as ABTS, a neutral pH of 7.0, which is close to biological systems, and compatibility with hydrophilic and lipophilic solvents are some of the advantages of the CUPRAC assay (George et al. 2022).

In the present study, the addition of SCE to the diet did not cause a significant difference in serum total antioxidant activity between the control and experimental groups housed in each of the HS[-] and HS[+] rooms. Although there are no data on total antioxidant capacity directly related to SCE

supplementation in broilers, similar results were obtained from some studies in which partially similar active ingredients were used on different animal species. The use of dihydrosanguinarine in rats by Vrublova et al. (2008), Sangrovit, a mixture of quaternary benzo[c]phenanthridine alkaloids obtained from *M. cordata* in rats by Zdarilova et al. (2008), and Sangrovit® Extra, a mixture of isoquinoline alkaloids obtained from *M. cordata* in pigs by Le et al. (2020) did not cause any significant difference in terms of total antioxidant capacity. Additionally, Bavarsadi et al. (2017) reported that sanguinarine had no directly remarkable antioxidant properties in laying hens, and in a study by Karakçı et al. (2022) on laying quails, it was reported that a mixture of magnolia and sanguinarine as aromatic plant extracts did not create a significant difference in plasma total antioxidant status.

In this study, the addition of MOS to the diet did not cause significant differences in serum antioxidant activity between the control and experimental groups housed in each of the HS[-] and HS[+] rooms. This result was in agreement with the recent study conducted by Abd El Latif (2023) in broilers, in which no significant difference was detected in serum total antioxidant capacity with the addition of MOS to the diet compared to the control group. Also, in a trial conducted by Yang et al. (2022), the addition of xylo-oligosaccharides, fructo-oligosaccharides and iso-maltooligosaccharides to broiler diets had no significant difference in total antioxidant capacity in liver, breast muscle and thigh muscle samples. In an experiment conducted by Zbeda (2021) in rabbits as a different animal species, it is also a similar finding that the addition of MOS to the diet did not cause any difference in terms of serum total antioxidant capacity values.

In conclusion, in this study, it was determined that body weights were significantly different and these differences were depended on diet and heat stress. Heat stress caused lower body weights in broilers, however, the supplementation of *Sanguinaria canadensis* extract and mannan-oligosaccharide improved this situation positively. In terms of serum antioxidant activity, differences between thermoneutral and heat stressed room were significant for CUPRAC and insignificant for ABTS analysis results. The supplementation of *Sanguinaria canadensis* extract and/or mannan-oligosaccharide had no significant effect on serum antioxidant activity in both analysis method.

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Original Article
Özgün Araştırma

Detection of rotavirus in raw and ready-to-eat food samples

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Abstract: Rotaviruses are the common cause of acute gastroenteritis. Since the transmission is generally occurs person-to-person by fecal-oral route, the importance of foodborne transmissions can be underestimated. Food can be contaminated with rotavirus at any stage of food process, from farm to fork. In this study, 105 samples were collected from seafood (mussels, fish, etc.), red meat (sausage, meatballs, etc.) and poultry meat products (turkey ham, wings, etc.). The samples were tested for the presence of rotavirus by RT-PCR. Cell culture based virus isolation methods carried out for the positive samples. DNA sequencing and phylogenetic analysis were performed for the genotyping of positive samples. Rotavirus was detected in 2 mussel sample groups by RT-PCR. DNA sequence analysis was carried out of both VP4 and VP7 gene regions of the rotavirus for one sample and determined that it was a group A human rotavirus G1P[8]. The rotavirus isolation did not occur after the inoculation the samples to cell culture. This study demonstrates the presence of rotavirus in raw mussel samples on market.

Keywords: Foodborne diseases, Genotype, MA-104, Rotavirus, RT-PCR,

Çiğ ve tüketime hazır gıda örneklerinde rotavirusun tespiti

Özet: Rotaviruslar, akut gastroenterit olgularının yaygın bir nedenidir. Rotavirus infeksiyonlarında bulaşmanın genellikle insandan insana fekal-oral yolla gerçekleşmesi sebebiyle gıda kaynaklı bulaşmaların önemi gözardı edilebilmektedir. Gıdalar, çiftlikten sofraya kadar geçen sürecin herhangi bir aşamasında rotavirus ile kontamine olabilmektedir. Bu çalışmada deniz ürünleri (midye, balık vb.), kırmızı et (sosis, köfte vb.) ve kanatlı et ürünlerinden (jambon, kanat vb.) 105 örnek toplanmıştır. Örnekler, RT-PCR ile rotavirus varlığı açısından test edilmiştir. Pozitif örneklerden virus izolasyonu için hücre kültürüne ekim gerçekleştirilmiş ve örneklerin genotiplendirilmesi için DNA dizilimi ve filogenetik analiz yapılmıştır. RT-PCR ile 2 midye örnek grubunda rotavirus tespit edilmiştir. Bir örnek için rotavirusun hem VP4 hem de VP7 gen bölgelerinden DNA dizi analizi yapılmış ve tespit edilen virusun grup A insan rotavirus G1P[8] olduğu belirlenmiştir. Örneklerin hücre kültürüne inokulasyonundan sonra rotavirus izolasyonu gerçekleşmemiştir. Bu çalışma, çiğ midye örneklerinde rotavirus varlığını ortaya koymaktadır.

Anahtar kelimeler: Gıda kaynaklı hastalıklar, Genotip, MA-104, Rotavirus, RT-PCR

Introduction

Acute gastroenteritis is an illness that affects people worldwide and rotavirus is one of the most common agent that causes this condition. Rotavirus infections affect people of all ages with mild symptoms in adults, but young children are affected severely. According to estimates, rotavirus infections caused diarrhea that led to the deaths of 215.000 children under the age of 5 worldwide in 2013 and 37% of diarrhea-related deaths in children under the age of 5 in 2008 (Tate et al., 2012). Children aged 4-23 months are in the risk group for severe rotavirus infections, which may include hospitalization or death. Repeated infections often occur due to insufficient immunity at this age. Immunity against rotavirus gradually increases with each subsequent infection, and therefore symptoms get milder in reinfections (Velazquez et al., 1996; Gladstone et al., 2011). There is no significant difference reported in incidence of rotavirus infections between developed and developing countries. However, mortality rates in developing countries are much higher than those in developed countries. It is reported that more than 80% of fatal rotavirus infections occur in developing countries with poor hygiene, sanitation and malnutrition (Parashar et al., 2003; 2006).

Person-to-person transmission is a common way of rotavirus infections via the fecal-oral route. Additionally, foodborne transmission plays an important role in outbreaks. Foods can be contaminated with rotaviruses by the use of sewage polluted water for irrigation or washing, results in contamination of vegetables in the field. Harvest of seafood from contaminated areas is also one of major risk. Meat can be contaminated with rotavirus in slaughterhouse if precautions are not taken. In addition,

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food can be contaminated with rotavirus in restaurants, canteens, and food courts by foodhandlers; it has been reported that food handlers are the most important source of such contamination (Koopmans and Duizer, 2004).

Foods act as a transport vehicle until they deliver the viruses to the target host (Jaykus, 2000). Putrefaction, deterioration or color changes are not observed in foods contaminated with viruses, as in bacterial contaminations. Therefore, it is not possible to perform a sensorial pre-examination of virus-contaminated food before consumption (Richards, 2001; Hasoksuz, 2008). Thus, when even foods with a high viral load consumed, no particular difference can be observed from safe food.

The aim of this study was to investigate the presence of rotavirus in ready-to-eat or raw food samples of animal origin by RT-PCR. Positive samples were subjected to cell culture-based virus isolation study and sequenced to determine the genotype of the circulating virus.

Materials and Methods

Samples and positive control

A total of 105 raw and ready-to-eat food samples were collected (Table 1) in Istanbul, exceeding the minimum required sample size for the study parameters of a 95% confidence level, 10% margin of error. The samples were brought to the laboratory with their original packages or in a sterile plastic bag in cold chain.

Bovine rotavirus B223 strain was used as positive control in molecular and cell culture based virus isolation studies.

Table 1: Raw and ready-to-eat food samples tested in the study.

Red Meat	Qty.	Seafood	Qty.	Poultry Meat	Qty.
Ground meat from butcers	5	Raw fish (internal organs)	5	Spicy raw chicken wings	5
Packaged ground meat from supermarkets	5	Raw mussels	10	Raw chicken	5
Packaged meatballs from supermarkets	5	Internal organs of frozen fish from supermarkets	5	Raw chicken wings	5
Sudjuk	5	Packaged mussel from supermarkets	5	Schnitzel	5
Salami	5	Raw squid from restaurants	5	Turkey Ham	5
Sausage	5			Sausage	5
Raw kebab from restaurant	5			Salami	5
				Turkey sausage	5
Total	**35**		**30**		**40**

Homogenization

The samples were dissected with sterile disposable scalpels, diluted with PBS in a sterile plastic bag and homogenized with stomacher (Seaward Stomacher 400C) at 260 RPM for 20 min. The supernatant was transferred to 50 ml tubes and centrifuged with 4000 rpm at 4°C for 35 minutes, then supernatant transferred to 15 ml tubes in duplicate, one was stored at -20°C for molecular studies and the other at -80°C for cell culture-based isolation studies.

Molecular diagnose

RNA isolation was performed by using Roche High Pure Viral Nucleic Acid Kit (Cat. no:11858874001) according to the manufacturer's instructions. RT-PCR used to detect the VP4 and VP7 gene regions of rotavirus by using 'Con3/Con2' (Gentsch et al., 1992) and 'sBeg9/End9' (Gouvea et al., 1994) primers (Table 2). For each sample, 5µl of the isolated RNA product including 0.8 µl DMSO, 0.6 µl forward and reverse primers was incubated in a dry block heater at 94°C for 5 minutes, and then kept on ice for 2 minutes. After that cDNA synthesis carried out by using Promega Reverse Transcription System (Cat. No: A3500) according to manufacturer instructions. Promega, GoTaq® G2 Flexi DNA Polymerase (catalog no: M7805) kit used for PCR applications. The PCR conditions are shown in Table 3 and 4 for amplification of VP4 and VP7 genes. Agarose gel electrophoresis carried out to visualise the DNA fragments.

Table 2. Primers used for molecular diagnosis.

Gene Region	Primer	Sequence	Lenght (bp)
VP4	Con3	TGG CTT CGC CAT TTT ATA GAC A	877
	Con2	ATT TCG GAC CAT TTA TAA CC	
VP7	sBeg9	GGC TTT AAA AGA GAG AAT TTC	1062
	End-9	GGT CAC ATC ATA CAA TTC TAA TCT AAG	

Sequencing

PCR amplicons were visualized by agarose gel electrophoresis and purified by Roche High Pure PCR Product Purification Kit (Catalog No: 11732668001) according to the manufacturer's instructions. Sanger sequencing method performed in ABI 3130XL Genetic Analyzer device using the same primers as for RT-PCR amplification. Mega X software used for nucleotides alignment with CLUSTRAL W algorithm. For the construction of the phylogenetic tree, sequences obtained from GenBank, and the full names of the sequences were shortened in G1 and P[8] groups. Phylogenetic tree was evaluated by Neighbor-Joining method using 1000 replicates bootstrap analysis.

Table 3. PCR conditions used for detection of VP4.

Master Mix (per sample)		PCR programme			
5X Green GoTaq® Flexi Buffer	5µl	Initial denaturation	94°C	3 min	1 cycle
MgCl2 (25mM)	3µl	Denaturation	95°C	1 min	35 cycles
dNTP (10mM each)	1µl				
Primer (F) (20 pmol/ µl)	1µl	Annealing	52°C	2 min	
Primer (R) (20 pmol/ µl)	1µl				
GoTaq® G2 Flexi DNA Polymerase (5u/µl)	0.5µl	Extension	72°C	1 min	
Template DNA	5µl				
Water	34.5µl	Final extension	72°C	7 min	1 cycle
Total	50 µl				

Cell culture based virus isolation:

For the RT-PCR positive samples, homogenates which were prepared before and kept at -80°C in 15ml tubes, were thawed at room temperature, centrifuged at 4000 rpm at 4°C for 30 minutes. After the supernatant was passed through membrane filters with a pore diameter of 0.2 µm, inoculated onto MA-104 cell monolayers (ATCC, CRL-2378.1) according to previous protocol with minor modifications (Arnold et al., 2009). Briefly the homogenates incubated at 37°C for 1 hour with the concentration of 10µg/ml trypsin (Sigma Aldrich, T4799). Following the incubation, the homogenate diluted with EMEM to reach a final concentration of 2 µg/ml trypsin and inoculated on the MA-104 cell monolayer. Five subsequent blind passages were performed**.**

Table 4. PCR conditions used for detection of VP7.

Master Mix (per sample)		PCR programme			
5X Green GoTaq® Flexi Buffer	10µl	Initial denaturation	94°C	3 min	1 cycle
MgCl2 (25mM)	5µl	Denaturation	95°C	1 min	35 cycles
dNTP (10mM each)	1µl				
Primer (F) (20 pmol/ µl)	1µl	Annealing	55°C	2 min	
Primer (R) (20 pmol/ µl)	1µl				
GoTaq® G2 Flexi DNA Polymerase (5u/µl)	0.5µl	Extension	72°C	1 min	
Template DNA	5µl				
Water	26.5µl	Final extension	72°C	10 min	1 cycle
Total	50 µl				

Results

Two raw mussel sample groups were positive for rotavirus. Sequence data were obtained from one of the positive sample for both VP4 and VP7 regions using the same primers for detection. The sequence data submitted to GenBank (Accession number OP598880 and OP598881) and compared with the sequences available on sequence database to determine the G/P genotype. The phylogenetic analysis showed that VP7 sequence was belong to G1 and VP4 was P[8] (Figure 1, 2). Both G1 and P[8] sequences presented high nucleotide identity (>99%) with Human Rotavirus A Wa (G1P[8]) backbone strain.

Virus isolation studies carried out for the two positive samples on MA-104 cell monolayers but no CPE were observed after 5 blind passages.

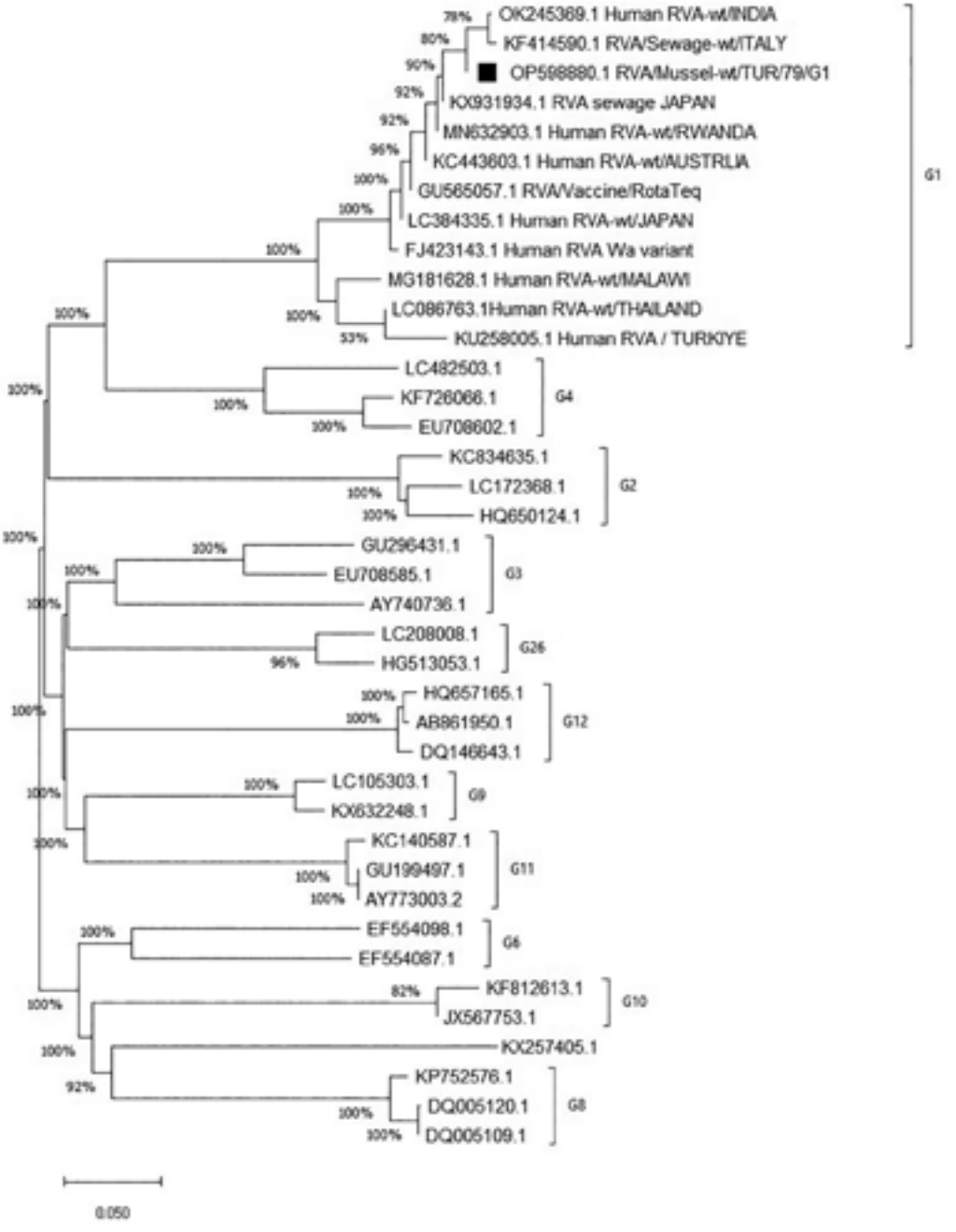

Figure 1. Phylogenetic tree of the nucleotide sequences of positive sample based on VP7.

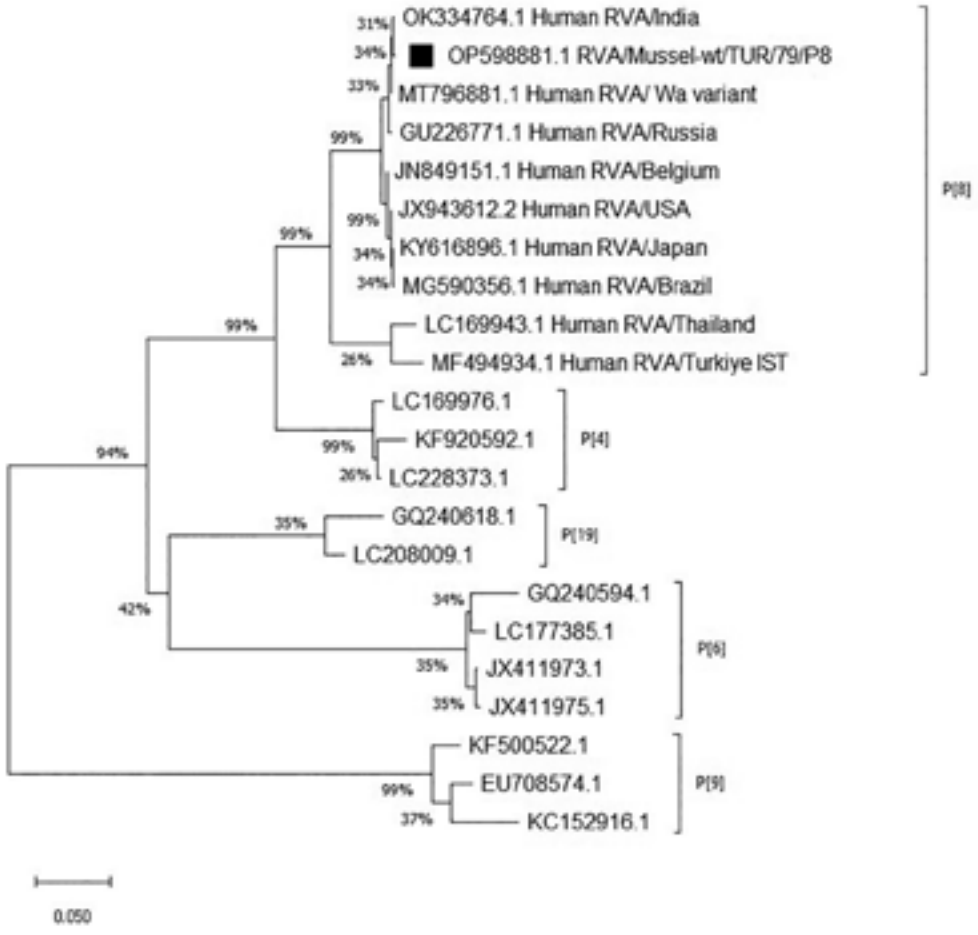

Figure 2. Phylogenetic tree of the nucleotide sequences of positive sample based on VP4.

Discussion and Conclusion

The most common cause of acute gastroenteritis in children under the age of five is rotavirus, and everyone experiences rotavirus infection at least once in their lifetime (Parashar et al., 2009). Studies conducted in Türkiye have also reported that rotavirus is one of the most common agents causing gastroenteritis (Bicer et al., 2014, Balkan et al., 2016; Tapisiz et al., 2019).

The growing industrial food production sector, changing food consumption habits as a result of crowded cities worldwide, and spread of international trade in food have increased the risk of foodborne diseases. Poor hygiene conditions in the food industry can cause serious gastroenteritis cases in humans. Since foodborne viruses are highly contagious, the source of infection is not attributed to food until the first cases are detected, and can be defined as an epidemic transmitted person-to-person (Newell et al., 2010). Food can be contaminated with rotavirus in various ways, such as using waste water for irrigation on the field, or zoonotic transmission may occur through consumption of meat, contaminated by sick animals at the slaughterhouse (Machnowska et al., 2014; Jones and Muehlhauser, 2017). Food-handlers are also one of the main source of contamination. A case of acute gastroenteritis occured in schools in some parts of Japan, and studies identified it as a rotavirus-related case that may result from lunch (Hara et al., 1978). In a similar gastroenteritis case in Fukui, Japan, where 3000 people were affected in 7 primary schools, rotavirus was identified as the cause. Although the virus was not detected in any food or water samples, it was interpreted as foodborne due to the fact that lunch was distributed to all 7 schools from a single source (Matsumoto ve ark., 1989). In a rotavirus outbreak

in a mother and child sanatorium in Germany, rotavirus was detected in a sample of potato stew and sequence comparison with a stool sample showed that two viruses were identical (Mayr et al., 2009).

In this study, while rotavirus was not detected in red and poultry meat products, 2/15 (13%) raw mussel samples were positive for both the VP4 and VP7 gene regions. Although our sample size of raw mussels was limited, the results are in agreement with the studies conducted in Italy that found 9% and 12% positivity of rotavirus in raw mussels collected from production sites on the coast yards (Fusco et al.,2017; 2019). In a study conducted in Brazil, rotavirus was detected in all 11 mussels collected from the sea coast (Keller et al., 2013), and 5.4% of bivalve shellfish were positive in Thailand (Kittigul et al., 2014). Our results contrast with a study from Istanbul, in which the presence of rotavirus investigated with multiplex real-time PCR in 52 groups (1350 pieces) of mussels collected in April, and none of the samples was positive (Ghalyoun and Unver Alcay, 2018). This disagreement might be due to the sample collection period of two studies. It has been reported that the incidence of rotavirus infections increases in winter (Patel et al., 2013; Gundeslioglu et al., 2018). In this study, the samples were collected in December and January, this factor can explain the dissimilarity of results.

The sequence analyses showed that the virus detected from the mussels in this study was a human rotavirus A G1P[8] type. It has been reported that rotavirus G1P[8] is the most common genotype in human rotavirus infections globally (Banyai et al., 2012; Doro et al., 2014). In Türkiye, studies showed that G1P[8] is also the predominant genotype causing gastroenteritis in humans. (Ceyhan et al.,2009; Bozdayi et al., 2008; Altindis et al., 2016). The sequence of the sample presented high nucleotide identity with Wa backbone strain. It has been reported that G1P[8] genotypes are usually associated with the Wa-like backbone (Rasebotsa et al., 2020), which is also genetically close to Rotarix and Rotateq vaccine strains. Evidence of vaccine virus shedding has been reported previously (Yoshikawa et al., 2019; Simsek et al., 2022), but the genetic data in the current study are insufficient to make such an interpretation.

The fact that the rotavirus detected in the mussel samples was of human origin and the samples were raw, suggests that this contamination most likely did not originate from a food-handler. It is more likely that, mussels harvested from a coast of city where probably sewage-contaminated stream meets the sea. As mussels are bivalves and they are filter-feeders, environmental viruses from the contaminated water concentrate in their body during the filtering.

Isolation studies were carried out in cell culture from food samples that were positive for rotavirus and no CPE were observed. With this result, it was thought that the rotavirus detected in mussel samples was inactive. This inactivation may have occurred due to the long period of time between harvest in the coastyard and collection from the market.

Our study has some limitations regarding the method used for molecular diagnosis of food products. It is widely agreed that various chemical ingredients in food matrices can affect the results of molecular detection of targeted nucleic acid region (Piskata et al., 2019). Although, ISO/TS 15216-1:2013 standard was defined for detection of hepatitis A virus and norovirus in food, there has no standard diagnose method defined for detection of rotavirus. Still, the method in this standard could have been adapted by using an internal positive control for the efficiency of molecular diagnose which could have enhanced the accuracy of the negative results obtained in this study.

In conclusion, the results of this study demonstrate that mussels can be contaminated with rotaviruses, posing a threat to public health if consumed undercooked. Although there are designated restricted areas for harvesting mussels that are free from sewage contamination, the mussels investigated in this study were likely not collected from these safe zones. Our findings reinforce the importance of strictly adhering to harvesting mussels only from these restricted areas to ensure they are free from contamination.

Conflict of interest: The authors declared that there is no conflict of interest.

Acknowledgments: This study is produced from the first author's PhD thesis. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the Republic of Türkiye Ministry of Agriculture and Forestry. Neither the the Republic of Türkiye Ministry of Agriculture and Forestry can be held responsible for them.

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Özgün Araştırma
Original Article

Solunum yolu hastalığı belirtileri gözlenen keçilerde bazı *Mycoplasma* türlerinin izolasyon ve identifikasyonu

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Özet: Keçi yetiştiriciliğinde karşılaşılan pnömoni olguları önemli verim kayıplarına yol açmaktadır. Bu nedenle solunum sistemi hastalıklarında rol oynayan bakteriyel etkenlerin izolasyon ve identifikasyonu, hastalıkla mücadelede önem arz etmektedir. Bu çalışmada makroskobik olarak pnömoni lezyonları tespit edilen keçi akciğer örneklerinde bazı *Mycoplasma* türlerinin izolasyon ve identifikasyonu amaçlandı. Bu doğrultuda Siirt ili Belediye mezbahasında kesimi yapılan 270 keçiden alınan akciğer örnekleri incelendi. Örneklerde *Mycoplasma capricolum* subsp. *capricolum*, *Mycoplasma capricolum* subsp. *capripneumoniae*, *Mycoplasma agalactiae* ve *Mycoplasma putrefaciens* varlığı bakteriyolojik konvansiyonel ve moleküler yöntemler kullanılarak araştırıldı. Çalışmada incelenen 270 örneğin selektif besiyerine ekim sonucunda 4 (%1,48)'ünde *Mycoplasma* spp. şüpheli koloniler elde edildi. Tür spesifik primerlerin kullanıldığı PCR ile örneklerin 3 (%7,0)'ünde *M. capricolum* subsp. *capricolum*, 1 (%25,0)'inde ise *M. capricolum* subsp. *capripneumoniae* identifiye edildi. Örneklerde *M. agalactiae* ve *M. putrefaciens* identifiye edilmedi. Sonuç olarak Siirt yöresinde yetiştiriciliği yapılan ve bölge halkı için önemli bir geçim kaynağı olan keçilerde meydana gelen pnömoni olgularında *M. capricolum* subsp. *capricolum* ve *M. capricolum* subsp. *capripneumoniae* etkenlerinin rol oynayabileceği belirlendi.

Anahtar kelimeler: *Mycoplasma*, Keçi, Pnömoni, PCR.

Isolation and identification of some *Mycoplasma* species in goats with respiratory disease symptoms

Abstract: Pneumonia cases encountered in goat breeding cause significant yield losses. Therefore, isolation and identification of bacterial agents that play a role in respiratory system diseases are important in combating the disease. In this study, it was aimed to isolate and identify some *Mycoplasma* species in goat lung samples with macroscopic pneumonia lesions. In this context, lung samples taken from 270 goats slaughtered in the Siirt Municipality slaughterhouse were examined. In the samples, the presence of *Mycoplasma capricolum* subsp. *capricolum*, *Mycoplasma capricolum* subsp. *capripneumoniae*, *Mycoplasma agalactiae* and *Mycoplasma putrefaciens* were investigated using bacteriological conventional and molecular methods. *Mycoplasma* spp. suspected colonies were obtained in 4 (1.48%) of the 270 samples examined in the study as a result of inoculation on selective medium. Using species-specific primers, PCR identified *M. capricolum* subsp. *capricolum* in 3 (75.0%) of the samples and *M. capricolum* subsp. *capripneumoniae* in 1 (25.0%) of the samples. *M. agalactiae* and *M. putrefaciens* were not identified in the samples. In conclusion, it was determined that *M. capricolum* subsp. *capricolum* and *M. capricolum* subsp. *capripneumoniae* may play a role in pneumonia cases occurring in goats, which are raised in Siirt region and are an important source of livelihood for the people of the region.

Keywords: Goat, *Mycoplasma*, Penumoniae, PCR

Giriş

Tüm dünyada olduğu gibi ülkemizde de önemli bir geçim kaynağı olan keçi yetiştiriciliği insan beslenmesinde kaliteli protein kaynağı sağlaması açısından da önemli bir yere sahiptir (Kaymakçı ve ark. 2005). Bakım maliyetlerinin düşük olması, tarımsal arazi olarak değerlendirilemeyen alanlara kısa sürede uyum göstermesi ve keçilerin daha dayanıklı türler olması nedeniyle keçi yetiştiriciliğinin ekonomik faaliyetlere katkısı yüksek olmakla birlikte, Siirt bölgesi için önemli bir ekonomi sektörü haline gelmiştir (Sen ve ark. 2018; Bakır ve Mikail 2019; Aziz ve Laf-

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ta 2022). Türkiye İstatistik Kurumu (TÜİK) verilerine göre 2023 yılında Türkiye'de 10.302.940 baş keçi bulunurken bunun 1.197.307'si Siirt bölgesinde bulunmaktadır (TÜİK 2023).

Keçilerde tespit edilen solunum sistemi hastalıklarının etiyolojisinde çeşitli bakteriyel etkenler rol oynamaktadır. Keçilerde meydana gelen pnömoni olgularından *Mycoplasma* spp., *Mannheimia haemolytica*, *Pasteurella multocida*, *Trueperella pyogenes*, *Histophilus somni*, *Corynebacterium pseudotuberculosis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae* gibi bakteriyel etkenler sıklıkla izole ve identifiye edilmektedir (Lacasta ve ark. 2008; Tijjani ve ark. 2012; Aher ve ark. 2012; Shah ve ark. 2016; Doley ve Ahmed 2017; Thakur ve ark. 2019). Ayrıca kötü bakım ve olumsuz hava koşulları, aşırı kalabalık ağıllar ve stres faktörleri de keçilerde pnömoni vakalarının görülme sıklığını arttırmaktadır (Momin ve ark. 2011; Shah ve ark. 2016; Doley ve Ahmed 2017).

Mollicutes sınıfında yer alan ve hücre duvarından yoksun olan *Mycoplasma* türleri, evcil geviş getiren hayvanların solunum sistemi hastalıklarında önemli rol oynamaktadır. Bu sınıfta yer alan *Mycoplasma (M.) mycoides* subsp. *mycoides*, *M. agalactiae* ve *M. capricolum* subsp. *capripneumoniae* Dünya Sağlık Örgütü (OIE) tarafından da önemli patojenler listesine eklenen türler arasında yer almaktadır. *M. capricolum* subsp. *capripneumoniae* %100 morbidite ve %60-80 oranında mortalite ile seyreden keçi ciğer ağrısı hastalığının etkeni olarak bilinmektedir (OIE 2020; Solangi ve ark. 2023). Dünya'da hastalıktan kaynaklı yılda 500 milyon ABD dolarından daha fazla ekonomik kayıp yaşanmaktadır (Yatoo ve ark. 2019).

Ayrıca *Mycoplasma* türleri arasında yer alan *M. capricolum* subsp. *capricolum* ve *M. putrefaciens*'de özellikle keçilerde solunum sistemi hastalıklarına neden olan önemli türlerdir (Pavone ve ark. 2023). *Mycoplasma capricolum* subsp. *capripneumoniae* ve *Mycoplasma capricolum* subsp. *capricolum*, *Mycoplasma putrefaciens* ve *Mycoplasma agalactiae* ile birlikte keçilerde MAKePS sendromu olarak bilinen mastitis, artritis, keratokonjuktivit, pnömoni ve septisemi olgularına da neden olmaktadırlar (Thiaucourt ve Bölske 1996; Chakraborty ve ark. 2014; Ejaz ve ark. 2015).

Konu ile ilgili ulusal ve uluslararası alanda yapılan çalışmalar değerlendirildiğinde keçilerde görülen solunum yolu hastalıklarında *Mycoplasma* spp. izolasyon oranının %7.16 ile %46.2 arasında değiştiği bildirilmektedir (Yener ve ark. 2001; Abdou 2002; Mostafa 2003; Rania 2006; Abdel Halium ve ark. 2019; Mousa ve ark. 2021).

Bu çalışmada da Siirt ili Belediye mezbahasında kesimi yapılan keçilerden alınan akciğer örneklerinden bazı mikoplazma türlerinin bakteriyolojik konvansiyonel ve moleküler yöntemlerle izolasyon ve identifikasyonu amaçlandı.

Materyal ve Yöntemler

Materyal

Çalışmada Eylül 2023-Haziran 2024 tarihleri arasında Siirt ili mezbahasında kesimi yapılan keçilerden alınan ve makroskobik olarak pnömoni lezyonları belirlenen 270 akciğer örneği kullanıldı.

Akciğer örneklerinin alınması

Siirt ili Belediye mezbahasında kesimi yapılan keçilerin akciğerleri incelendi. Yapılan incelemeler sonucunda makroskobik olarak pnömoni lezyonları tespit edilen akciğerlere enine kesitler atılarak örnekler alındı. Alınan örnekler %20 gliserinli PBS içeren örnek toplama kaplarına aktarıldı ve soğuk zincir koşulları sağlanarak Siirt Üniversitesi, Veteriner Fakültesi, Mikrobiyoloji ABD Laboratuvarı'na getirildi.

Yöntem

İzolasyon

Alınan örnekler bakteriyolojik konvansiyonel yöntemlerle *Mycoplasma* spp. izolasyonu için Mycoplasma Broth Base (CM0403, Oxoid, England)'e ekimler yapılarak 37°C'de %5-10 CO_2 içeren ortamda 7 gün boyunca inkübe edildi. İnkübasyon periyodu sonucunda üreme görülen sıvı besiyerlerinden Mycoplasma Agar Base (CM0401, Oxoid, England)'e ekimler yapılarak 37°C'de %5-10 CO_2 içeren ortamda 7-10 gün boyunca inkübe edildi. İnkübasyon sonucunda besiyerinde üreme olup olmadığı 40x'lik büyütmede stereomikroskop altında incelendi ve sahanda yumurta görüntüsüne sahip olan koloniler *Mycoplasma* spp. şüpheli olarak kabul edildi (Samiullah 2013; Farooq 2018; Abd-Elrahman ve ark. 2020). Şüpheli bulunan izolatlar PCR ile tür düzeyinde identifikasyonları yapılana kadar %50 at serumu eklenmiş Mycoplasma Broth Base'de -20°C'de saklandı.

PCR ile identifikasyon

Mycoplasma spp. şüpheli izolatların PCR ile tür düzeyinde identifikasyonlarının gerçekleştirilmesi amacıyla kullanılan tür spesifik primerler ile ilgili bilgiler Tablo 1'de gösterildi.

DNA İzolasyonu

Mycoplasma spp. şüpheli izolatlardan genomik DNA eldesi, ticari genomik DNA izolasyon kiti (GeneAll, ExgeneTM Clinic SV Mini, 108.101, Korea) kullanılarak elde edildi. Elde edilen genomik DNA, izolatların PCR ile identifikasyonunda kullanılana kadar -20°C'de saklandı. Bununla birlikte akciğer örneklerinden de DNA izolasyonu yapılarak etkenlerin varlığı PCR ile araştırıldı. Bu amaçla küçük parçalara ayrılan örneklerden ticari DNA izolasyon kiti (Hydra, HY-DGDA-100, Türkiye) kullanılarak genomik DNA elde edildi.

Amplifikasyon

Hem şüpheli izolatlardan hem de doku örneklerinden mikoplazma türlerinin varlığının belirlenmesi amacıyla kullanılan PCR karışımının hazırlanmasında ticari mastermix (BioLabs, Taq 2X Master Mix, M0270S, İngiltere) kullanıldı ve üretici firmanın önerileri dikkate alındı. Karışımın optimizasyonu için 12.5 µl mastermixe, 2 µl genomik DNA, 1.5 µl primerlerden ilave edilerek toplam hacim PCR suyu ile 25 µl'ye tamamlandı. Amplifikasyon işleminde primerlerin sentez ettirildiği firmanın önerileri doğrultusunda bağlanma sıcaklığı optimize edildi. Karışımın ön denatürasyonu için 94°C'de 10 dk bekletildi. Amplifikasyon aşaması toplam 35 siklus olarak uygulandı. Denatürasyon için 94°C'de 1 dk, uzama aşaması için de 72°C'de 1 dk bekletildi. Final uzaması 72°C'de 10 dk olarak ayarlanırken, kullanılan primerlerin bağlanma sıcaklıkları tablo 1'de belirtildi. PCR sonucu elde edilen amplikonlar, agaroz jelde elektroforeze tabi tutularak, DNA marker ile kıyaslandı ve jel görüntüleme cihazında (Gen-Box ImagER, Ankara, Türkiye) incelendi. PCR işlemlerinde negatif kontrol olarak DNA içermeyen PCR suyu, pozitif kontrol olarak *Mycoplasma agalactiae* ATCC® 35890 kullanıldı.

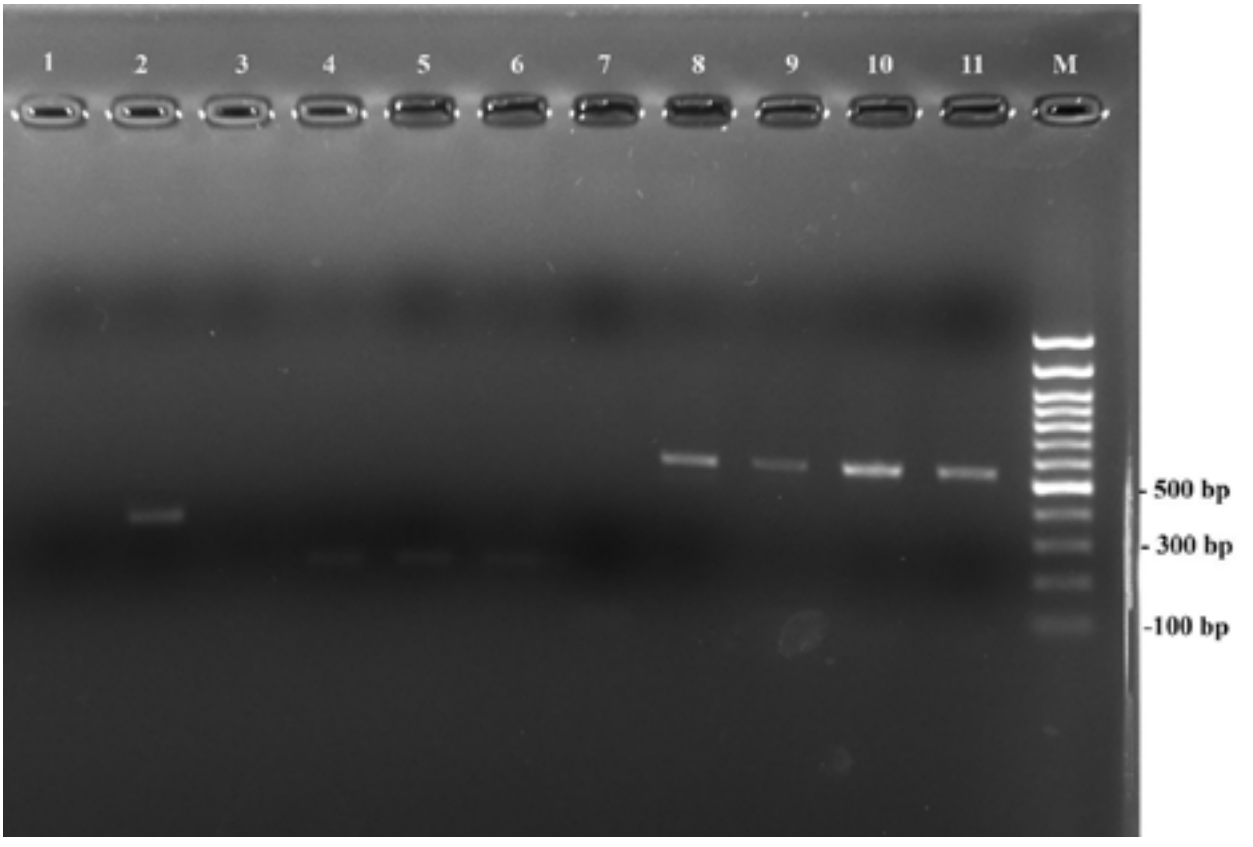
Tablo 1. Şüpheli izolatların PCR ile identifikasyonunda kullanılan primer dizilimleri.

Etken	Oligonukleotid (5'-3')	Amplikon Büyüklüğü (bp)	Bağlanma Sıcaklığı	Referans
Mycoplasma mycoides cluster	F: CGAAAGCGGCTTACTGGCTTGTT R: TTGAGATTAGCTCCCCTTCACAG	548	62°C	Bascunana ve ark. 1994
Mycoplasma capricolum subsp. *capripneumoniae*	F: ATCATTTTTAATCCCTTCAAG R: TACTATGAGTAATTATAATATATGCAA	316	51°C	Woubit ve ark. 2004
Mycoplasma capricolum subsp. *capricolum*	F: ACTGAGCAATTCCTCTT R: GTAAACCGTGTATATCAAAT	192	48°C	Hernandez ve ark. 2006
Mycoplasma agalactiae	F: CCTTTTAGATTGGGATAGCGGATG R: CCGTCAAGGTAGCGTCATTTCCTAC	360	60°C	Gonzalez ve ark. 1995
Mycoplasma putrefaciens	F: GCGGCATGCCTAATACATGC R: AGCTGCGGCGCTGAGTTCA	540	60°C	Shankster ve ark. 2002

Bulgular

Çalışmada incelenen 270 örneğin 4 (%1,48)'ünde *Mycoplasma* spp. şüpheli koloniler tespit edildi. Yapılan PCR analizlerinde izolatların *Mycoplasma mycoides* cluster spesifik primer ile pozitif sonuç verdiği belirlendi. Tür spesifik primerlerin kullanıldığı PCR ile izolatların 3 (%75,0)'ü *M. capricolum* subsp. *capricolum*, 1 (%25,0)'i *M. capricolum* subsp. *capripneumoniae* olarak identifiye edildi (Şekil 1).

Doku örneklerinden elde edilen genomik DNA'larda gerçekleştirilen PCR işlemlerinin de bakteriyolojik yöntemlerle elde edilen sonuçlar ile paralellik gösterdiği belirlendi. Çalışmada incelenen örneklerde *M. agalactiae* ve *M. putrefaciens* türleri tespit edilmedi.



Şekil 1. PCR ile *Mycoplasma* spp. tespit edilen örneklerden elde edilen amplikonların agaroz jel görüntüsü. 1: *M. capricolum* subsp. *capripneumoniae* negatif kontrol, 2: *M. capricolum* subsp. *capripneumoniae* pozitif örnek (316 bp); 3: *M. capricolum* subsp. *capricolum* negatif kontrol, 4-6: *M. capricolum* subsp. *capricolum* pozitif örnek (192 bp); 7: *M. mycoides* cluster negatif kontrol, 8-11: *M. mycoides* cluster pozitif örnek (548 bp); M: 100 bp DNA marker.

Tartışma ve Sonuç

Et ve süt için yetiştiriciliği yapılan keçiler, özellikle düşük kaliteli meraları, çalılık ve fundalık alanlarını değerlendirmeleri nedeniyle diğer hayvanlara oranla daha fazla tercih edilmektedirler. Ayrıca Dünya nüfusunun giderek artış göstermesi ve bu doğrultuda da hayvansal kökenli gıdalara talebin artması keçi yetiştiriciliğini daha da önemli kılmaktadır (Tüfekci, 2023). Ancak keçilerde meydana gelen solunum sistemi hastalıkları nedeniyle hem ekonomi hem de verim olumsuz yönde etkilenmektedir (Farooq 2018; Ahmad ve ark. 2021).

Bu çalışmada keçilerde solunum yolu enfeksiyonlarına neden olan *M. capricolum* subsp. *capricolum*, *M. capricolum* subsp. *capripneumoniae*, *M. agalactiae* ve *M. putrefaciens* etkenlerinin varlığının tespit edilmesi amaçlandı.

Keçilerde meydana gelen solunum sistemi hastalıklarından *Mycoplasma mycoides* sınıfında yer alan *Mycoplasma capricolum* subsp. *capripneumoniae*, *Mycoplasma capricolum* subsp. *capricolum* ile *Mycoplasma agalactiae ve Mycoplasma putrefaciens* izolasyonu ve identifikasyonu amacıyla uluslararası alanda yapılan bazı çalışmalar bulunmaktadır.

Bu amaçla İran'da yapılan bir çalışmada; Khodakaram-Tafti ve ark. (2023), 50 pnömonik keçi akciğer örneğinin %22 (n=11)'sinde *Mycoplasma* spp. ve %6 (n=3)'sında *M. capricolum* subsp. *capripneumoniae* identifiye ettiklerini rapor ederken, İran'da yapılan başka bir çalışmada ise araştırmacılar inceledikleri 4 keçi akciğer örneğinin tamamında *M. capricolum* subsp. *capripneumoniae* identifiye ettiklerini rapor etmişlerdir (Abdollahi ve ark. 2023). Mousa ve ark. (2021), Mısır'da yapmış oldukları bir çalışmada sağlıklı (n=40) görünen ve hasta olduğu belirlenen (n=60) keçilerden aldıkları 100 akciğer örneğini ince-

lemişlerdir. Araştırmada sağlıklı ve hasta keçilerden sırasıyla %17 ve %56,6 oranında *Mycoplasma* spp. tespit edilmiştir. Mısır'da yapılan başka bir çalışmada ise keçilerden alınan 620 kan serumu örneğinin %20 (n=24)'sinde *M. capricolum* subsp. *capripneumoniae* yönünden pozitiflik bildirilmiştir (Selim ve ark. 2021). Thakur ve ark. (2019), Hindistan'da koyun ve keçilerden 44 burun svabı ve 6 akciğer olmak üzere toplam 50 adet örnek topladıklarını ve keçilerden alınan burun svap örneklerinin 11 (%22)'inde *Mycoplasma* spp. tespit ettiklerini ancak koyunlardan alınan örneklerde söz konusu etkene rastlamadıklarını rapor etmişlerdir. El-Deeb ve arkadaşlarının 2017 yılında Suudi Arabistan'da yapmış oldukları çalışmada keçilerden topladıkları 700 örneğin 67 (%9,57)'sinde *Mycoplasma mycoides* sınıfında yer alan *Mycoplasma* türlerini ve bu doğrultuda örneklerin 55 (%7,85)'inde de *Mycoplasma capricolum* subsp. *capripneumoniae* identifiye ettiklerini bildirilmişlerdir. Ejaz ve ark. (2015), Pakistan'da yaptıkları bir çalışma kapsamında koyun (n=240) ve keçilerden (n=200) almış oldukları toplam 440 burun svabı örneğinin %7.5'inde *Mycoplasma mycoides* cluster, %5'inde *Mycoplasma putrefaciens*, %1,25'inde *Mycoplasma capricolum* subsp. *capricolum* identifiye ettiklerini rapor etmişlerdir. Aher ve ark. (2013), Hindistan'da yaptıkları çalışmalarında hasta ve sağlıklı keçilerden alınan 198 örneğin 1 (%0,7)'inden *Mycoplasma* spp. izole ettiklerini; Pakistan'da yapılan başka bir çalışmada ise 1920 keçi burun svabı örneğinin 177 (%9,6)'sinde *Mycoplasma* spp., 123 (%6,40)'ünde *Mycoplasma mycoides* cluster, 34 (%1,77)'ünde *Mycoplasma capricolum* subsp. *capricolum* ve 20 (%1,04)'sinde de *Mycoplasma putrefaciens* bulduklarını bildirmişlerdir (Awan ve ark. 2012). Kumar ve arkadaşları 2011 yılında Hindistan'da yapmış oldukları çalışmada 358 akciğer örneğinin 30 (%8,35)'unda *Mycoplasma* spp., 11(%3,07)'inde *Mycolasma capricolum* subsp. *capricolum* ve 19 (%5,30)'unda *Mycolasma mycoides* subsp. *capri* identifiye ettiklerini beyan etmişlerdir. Ürdün'de yapılan bir çalışmada ise keçilerden alınan 310 burun svabı örneğinin 12 (%3,9)'sinde *Mycoplasma* spp., 6 (%1,93)'sında *M. capricolum* subsp. *capricolum*, 5 (%1,61)'inde *M. putrefaciens*, 1 (%0,32)'inde de *Mycoplasma mycoides* subsp. *mycoides* large colony tipi tespit ettiklerini ancak *M. agalactiae* yönünden herhangi bir pozitiflik bulamadıklarını rapor etmişlerdir (Al-Momani ve ark. 2006).

Ülkemizde ise bu kapsamda yapılan sınırlı sayıda çalışma bulunmaktadır. Bu doğrultuda; Öztürk ve Yaman'ın 2024 yılında Isparta'da yapmış oldukları bir prevalans çalışmasında 813 kan serumu örneğinin ELİSA testi ile 83 (10,2%)'ünü *M. agalactiae* yönünden pozitif bulduklarını beyan etmişlerdir. Çetinkaya ve ark. (2009), Malatya, Elazığ, Bingol, Bitlis, Muş ve Siirt bölgelerinden keçilerden aldıkları 31 akciğer ve 1 burun svabı örneğinde PCR ile %38,7 oranında *M. capricolum* subsp. *capripneumoniae* tespit ettiklerini rapor etmişlerdir. Yener ve ark. (2001), Bitlis'deki bir mezbahadan aldıkları 42 pnömonik keçi akciğer örneğinin %28,57'sinden *Mycoplasma* spp. identifiye ettiklerini bildirmişlerdir.

Yapılan çalışmalar değerlendirildiğinde; keçilerden alınan örneklerden *M. mycoides* cluster teşhis oranı %5,30-%9,57 değişirken, *Mycoplasma* spp. oranının %0.7-%56.5; *M. capricolum* subsp. *capricolum* oranının %1,25-%3,07; *M. capricolum* subsp. *capripneumoniae* oranının %6-%100, *M. agalactiae* oranının %0-%10, *M. putrefaciens* oranının ise %1,04-%5 arasında değiştiği görülmektedir. Sunulan bu çalışmada ise *M. mycoides* cluster izolasyon oranı %1,48 iken, *M. capricolum* subsp. *capricolum* izolasyon oranı %1,11, *M. capricolum* subsp. *capripneumoniae* izolasyon oranı %0,37 olarak bulunmuştur. Bu duruma coğrafi farklılıkların, bölgesel iklim özelliklerinin farklı olması ile hem örneklerin alınma şekli hem de örneklem büyüklüğünün ve kullanılan teşhis yöntemlerinin farklı olmasının neden olabileceği düşünüldü. Ayrıca bölgede konar-göçer tarzda hayvancılık faaliyetlerinin sürdürülmesi, bakteriyel pnömonilerde predispoze faktör olan ağıl kalabalıklığının da önüne geçerek, izolasyon oranlarının daha düşük olmasına neden olmuş olabileceği düşünüldü.

Sonuç olarak bu çalışmada Siirt bölgesinde yetiştirilen keçilerde pnömoni olgularına neden olarak önemli verim kayıplarına sebebiyet veren *M. capricolum* subsp. *capricolum* ile *M. capricolum subsp. capripneumoniae* etkenlerinin varlığı bakteriyolojik konvansiyonel ve moleküler yöntemlerle ortaya koyuldu. Elde edilen verilerin bölgede yetiştiriciliği yapılan keçilerde pnömoni vakalarına karşı oluşturulacak koruma-kontrol stratejilerine katkı sağlayacağı düşünüldü. Konuyla ilgili ileride yapılacak olan çalışmalarda daha fazla sayıda örnek ile çalışılmasının yanı sıra pnömoni olgularına neden olan diğer etkenlerin varlığının da araştırılmasının bölge hayvancılığına katkı sağlayacağı kanaatine varıldı.

Etik Onay: Çalışma Siirt Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 167/02/2023 tarih ve 2023/01/09 sayılı kararı ile onaylandı.

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Özgün Araştırma
Original Article

Bovine Ephemeral Fever virus enfeksiyonunun retrospektif olarak 2012-2023 yılları arasında seroprevalansının araştırılması

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Özet: Bovine Ephemeral Fever (BEF) ülkemizin özellikle Güneydoğu Anadolu ve Akdeniz Bölgesi'nde şiddetli salgınlara neden olan vektör kaynaklı viral bir hastalıktır. Bu çalışmada 2012-2023 yılları arasında BEFV antikorlarının varlığının belirlenmesi ve sonuçların konu ile ilgilenen araştırmacılara toplu olarak sunulması amaçlanmıştır. Bu bağlamda 2012-2023 yılları arasında Adana, Adıyaman, Gaziantep, Hatay, Kahramanmaraş, Mersin, Osmaniye, Şanlıurfa ve Kilis illerinden toplanan 2840 adet sığır serum örneği "Blocking ELISA" ile incelendi. On yıllık süre içerisinde ortalama pozitifliğin %38,09 (1082/2840) olduğu, pozitifliğin en yüksek olduğu ilin Adana %53,81 (247/459), en düşük olduğu ilin ise %26,53 (65/245) ile Kilis olduğu tespit edildi. Yıllara göre bir değerlendirme yapıldığında ise en yüksek pozitiflik %96,69 (117/121) ile 2015, en düşük pozitiflik ise %10,12 (82/810) ile 2017 yılında tespit edildi. Sonuç olarak BEFV bu bölgelerde varlığını devam ettirmektedir ve periyodik aralıklarla salgınlara yol açarak bölge hayvancılığı açısından risk oluşturmaktadır. Bu nedenle hastalığın takibi, korunma ve kontrolüne yönelik çalışmaların sürdürülmesi önem arz etmektedir.

Anahtar kelimeler: Bovine Ephemeral Fever, ELISA, Seroloji, Virus.

Restrospective investigation of seroprevalence of Bovine Ephemeral Fever virus infection between 2012-2023

Abstract: Bovine Ephemeral Fever (BEF) is a vector-borne viral disease that causes severe epidemics especially in the Southern Anatolia and Mediterranean region of our country. In this study, it was aimed to determine the presence of BEFV antibodies between the years 2012-2023 and to present the results to the researchers who are interested in the subject. For this aim, 2840 sera samples collected from Adana, Adıyaman, Gaziantep, Hatay, Kahramanmaraş, Mersin, Osmaniye, Şanlıurfa and Kilis provinces between 2012-2023 years were analyzed by ''Blocking ELISA". It was seen that the average positivity was 38.09% (1082/2840) in a ten year period, the provinces with the highest positivity was Adana 53.81% (247/459), the province with the lowest positivity was Kilis 26.53% (65/245). When we make an evaluation by years, it was determined the highest positivity was 96.69% (117/121) in 2015, the lowest positivity was 10.12% (82/810) in 2017. As a result, BEFV continues to exist in these regions and pose a risk for the livestock breeding in the region by causing outbreaks at periodic intervals. For this reason, it is important to continue the studies for the follow-up, prevention and control of the disease.

Keywords: Bovine Ephemeral Fever, ELISA, Serology, Virus.

Giriş

Üç gün hastalığı olarak da adlandırılan Bovine Ephemeral Fever (BEF), sığır ve mandalarda et ve süt veriminde azalma sebebiyle yüksek ekonomik kayıplara neden olan akut seyirli viral bir hastalıktır (Zheng ve ark. 2009; OIE 2016; Pyasi ve ark. 2020). Neden olduğu ekonomik kayıplar sebebiyle BEF vakası bildirilmeyen ülkelerde dahi salgınların yakından takip edilerek sıkı önleyici tedbirlerin alınmasının önemli olduğu düşünülmektedir (Tonbak ve ark. 2013).

Bovine Ephemeral Fever Virus (BEFV), *Rhabdoviridae* familyasından *Ephemerovirus* genusunda yer almaktadır (Abayli ve ark. 2017; Dorey-Robinson ve ark. 2019; Omar ve ark. 2020; ICTV 2024). Viron 14,9 kb boyutunda negatif iplikçikli ve tek sarmallı RNA (ssRNA) genomuna sahiptir ve önemli beş yapısal proteini (N, P, M, G ve L), bir (GNS) yapısal olmayan proteini ile birkaç küçük yardımcı proteini kodlayan gen bölgeleri bulunmaktadır (Gleser ve ark. 2023; Benevenia ve ark. 2024). G proteini yüzeyinde dört antijenik bölge (G1, G2, G3 ve G4) tanımlanmıştır. Sadece anti-BEFV antikorları ile G1 parçacığı reaksiyona girmektedir. Blocking ELISA ve Indirekt ELISA G1 parçacığını tespit etmek için kullanılmaktadır (Zheng ve Qiu 2012).

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BEFV'nin dünya çapında tek bir serotipi bulunmaktadır (Walker ve ark. 1991; Trinidad ve ark. 2014). Sağlıklı sığır ve insektlerden serolojik çapraz reaksiyon gözlenen birkaç virus izole edilmiştir. Ancak bunların hiçbiri hastalığa neden olmamıştır. Bunlar arasında Avustralya'dan Berrimah virus (BRMV) ve Kimberley virus (KIMV), Afrika'dan Malakal virus ve Asya'dan Puchong virus sayılmaktadır (Walker ve ark. 1991).

Hastalığın mortalite oranı düşük, morbidite oranı ise yüksektir (Walker ve Klement 2015; OIE 2016). BEFV, Avustralya, Afrika, Orta Doğu ve Asya'nın tropik ve subtropik bölgelerinde görülür (Lavon ve ark. 2023; Benevenia ve ark. 2024). Afrika'da çeşitli yabani ruminantlarda anti-BEFV antikorları tespit edilmesine rağmen, hastalığın meydana geldiği bölgelerde sığırlarla birlikte bulunan koyunlarda doğal enfeksiyonlar bildirilmemiştir (Kirkland 2002).

Dünya çapında 38 ° K ve 36 ° G enlemleri arasında BEFV'nin endemik görüldüğü kabul edilmektedir (Pyasi ve ark. 2020). Kıtalar ve ülkeler arası ana bulaşma yolları tamamen açıklanamamıştır (Boaron ve ark. 2012). Vektörlerin hakim rüzgarlar tarafından taşınması ile epizootiklerin meydana geldiği rapor edilmiştir. (Walker 2005; Chaisiririat ve ark. 2018). Kan emen sineklerden (Culicoides spp.) izole edilen BEFV'nin bulaştırılmasında rol alan vektörler ile ilgili çalışmalar devam etmektedir (Kirkland 2002; Trinidad ve ark. 2014). Virusun *Culicoides imicola, Culicoides coarctus, Culicoides kingi, Culicoides nivosis, Culicoides bedfordi, Culicoides pallidipennis, Culicoides puncticollis, Culicoides brevitarsis, Anopheles bancroftii, Culex, Uranotaenia ve Aedes* gibi çeşitli kan emen sineklerden izole edildiği rapor edilmiştir (Walker ve Klement 2015).

Hastalık sıcak mevsimlerde belirgin bir artış sergilemektedir (Tonbak ve ark. 2013). Hastalık Orta Doğu'da Suudi Arabistan'da 1930 yılında, İsrail'de 1931 yılında ve Türkiye'de de1985 yılında ilk olarak rapor edilmiştir (Omar ve ark. 2020). Türkiye'de salgınlar her 4-5 yılda bir ve genellikle aynı bölgelerde ortaya çıkmaktadır (Erol ve Ark. 2015, Oğuzoğlu ve ark. 2015). Bu bölgeler subtropikal iklim özelliği gösterdiğinden virusun vektörleri için uygun yaşam alanları sağlamaktadır. Akdeniz kıyı bölgesinde BEFV salgınları genellikle yaz sezonu sonunda ortaya çıkmaktadır (Oğuzoğlu ve ark. 2015).

Hastalığın seyri esnasında bifazik ateş, kaslarda sertlik, salivasyon, gözyaşı ve burun akıntısı, iştahsızlık, ruminasyonun durması, topallık ve yatma gibi klinik semptomlar gözlenebilmektedir (Walker 2005). Epidemiyolojik gözlemler ve klinik semptomlar hastalıktan şüphenilmesine sebep olsa da kesin teşhis enfekte sığırlardan elde edilen örneklerden virusun izolasyonunun yapılması ile gerçekleştirilebilmektedir. Serolojik teşhis amacıyla iki hafta ara ile çift serum örnekleme yapılarak BEF antikorları ortaya konulmaktadır (Tonbak ve ark. 2013).

Blocking Enzyme-Linked Immunosorbent Assay (B-ELISA) ve indirekt Enzyme-Linked Immunosorbent Assay BEFV'nin G1 proteinine karşı oluşan antikorları tespit etmek için geliştirilmiştir (Zheng ve Qiu 2012). Hastalığın teşhis ve izlenmesinde B-ELISA testinin, Virus Nötralizasyon testine göre uygulamasının daha kolay ve hassasiyetinin ise daha yüksek olduğu (Zakrzewski ve ark. 1992), bu nedenle geniş çaplı sürü taramalarında tercih edilerek kullanılabildiği vurgulanmıştır (Zheng ve ark. 2010). Doğal BEFV enfeksiyonu geçiren hayvanlarda oluşan nötralizan antikorlar konakları virusa karşı uzun bir süre koruyabilmektedir (Walker 2005).

Bu çalışmada 2012-2023 yılları arasında Adana, Adıyaman, Gaziantep, Hatay, Kahramanmaraş, Mersin, Osmaniye, Şanlıurfa ve Kilis illerinden toplanan kan serum örneklerinde tespit edilen anti-BEFV antikorlarının yaygınlığı, illere ve yıllara göre tasnif edilerek konu ile ilgilenen araştırmacılara toplu bir şekilde sunulması hedeflenmiştir.

Gereç ve Yöntem

Gereç: Bu çalışmanın materyalini, 2012-2023 yılları arasında saha taraması için Haziran-Ekim ayları arasında Adana Veteriner Kontrol Enstitü Müdürlüğü Viroloji Laboratuvarına Adana, Adıyaman, Gaziantep, Hatay, Kahramanmaraş, Mersin, Osmaniye, Şanlıurfa ve Kilis illerinden eşkal bilgileri olmadan gönderilen veya enstitümüz uzmanları tarafından sahadan toplanan kan serum örnekleri oluşturmuştur. Laboratuvarımıza 2015 yılında Şanlıurfa (n:50) ve Adana (n:71)'dan gönderilen serum örnekleri aşılı hayvanlardan elde edilirken geri kalan örneklerin tamamı aşısız hayvanlardan elde edilmiştir. Toplanan serum örnekleri 56°C'de 30 dk inaktive edilmiş ve serolojik testlerde kullanılıncaya kadar -20°C'de saklanmıştır. Toplanan örnek sayıları, örneklerin illere ve yıllara göre dağılımları Tablo 1'de özetlenmiştir.

Yöntem: Toplanan kan serum örnekleri ticari B-ELISA (Virology Laboratory, EMAI, Camden NSW Avustralya) ile kit prosedürüne uygun olarak incelendi. Test pleytleri 450 nm absorbans değerinde ELISA okuyucu'da (Biochrom Ezread400, İngiltere) okutuldu. Elde edilen absorbans sonuçları <%40 negatif, %40-59 şüpheli ve >%60 pozitif olarak değerlendirildi.

Bulgular

On yıllık süre içerisinde ortalama pozitifliğin %38,09 (1082/2840) olduğu, pozitifliğin en yüksek olduğu ilin Adana %53,81(247/459), en düşük olduğu ilin ise %26,53 (65/245) ile Kilis olduğu tespit edildi. Yıllara göre bir değerlendirme yapıldığında ise en yüksek pozitiflik %96,69 (117/121) ile 2015, en düşük pozitiflik ise %10,12 (82/810) ile 2017 yılında tespit edildi. Adana ve Şanlıurfa illerine ait aşılı hayvanlardan örnek toplanan 2015 yılı haricinde dokuz yıllık süre içerisinde değerlendirme yapıldığında aşısız hayvanlarda ortalama pozitiflik %35,49 (965/2719) olarak belirlendi.

İncelenen numunelere ait detaylı test sonuçları Tablo 1'de özetlenmiştir.

Tablo 1. Hayvan örneklerinde anti-BEF antikorlarının tespiti için yapılan B-ELISA test sonuçlarının yıllara ve illere göre dağılımı

İL	2012 % (+/n)	2013 % (+/n)	2014 % (+/n)	2015 % (+/n)	2016 % (+/n)	2017 % (+/n)	2018 % (+/n)	2021 % (+/n)	2022 % (+/n)	2023 % (+/n)	TOPLAM % (+/n)
ADANA	%41,17 (14/34)	%33,84 (22/65)	%75 (3/4)	%98,59 (70/71)	%10 (4/40)	%12,22 (11/90)	%20 (6/30)	%92 (23/25)	%96 (48/50)	%92 (46/50)	%53,81 (247/459)
ADIYAMAN	%68,75 (11/16)	---	---	---	%27,5 (11/40)	%10 (9/90)	%13,33 (4/30)	%68 (17/25)	%52 (26/50)	%34 (17/50)	%31,56 (95/301)
GAZİANTEP	---	---	---	---	%7,5 (3/40)	%5,55 (5/90)	%6,66 (2/30)	%48,14 (13/27)	%96 (48/50)	%44 (22/50)	%33,40 (93/287)
HATAY	---	---	---	---	%12,5 (5/40)	%10 (9/90)	%8 (4/50)	%76 (19/25)	%72 (36/50)	%32 (16/50)	%29,18 (89/305)
KAHRAMAN-MARAŞ	---	---	---	---	%15 (6/40)	%8,88 (8/90)	%20 (6/30)	%80 (20/25)	%90 (45/50)	%28 (14/50)	%35,73 (99/285)
MERSİN	%14,28 (1/7)	---	---	---	%20 (8/40)	%11,11 (10/90)	%6,66 (2/30)	%48 (12/25)	%74 (37/50)	%34 (17/50)	%29,79 (87/292)
OSMANİYE	%43,75 (7/16)	---	---	---	%15 (6/40)	%10 (9/90)	%16,66 (5/30)	%68 (17/25)	%78 (39/50)	%64 (32/50)	%38,20 (115/301)
ŞANLIURFA	%86,66 (26/30)	---	---	%94 (47/50)	%10 (4/40)	%12,22 (11/90)	%13,33 (4/30)	%92 (23/25)	%84 (42/50)	%70 (35/50)	% 52,60 (192/365)
KİLİS	---	---	---	---	---	%11,11 (10/90)	%6,66 (2/30)	%60 (15/25)	%50 (25/50)	%26 (13/50)	% 26,53 (65/245)
TOPLAM	%57,28 (59/103)	%33,84 (22/65)	%75 (3/4)	%96,69 (117/121)	%14,68 (47/320)	%10,12 (82/810)	%12,06 (35/290)	%70,04 (159/227)	%76,89 (346/450)	%47,11 (212/450)	%38,09 (1082/2840)

Tartışma ve Sonuç

Dünyada ve ülkemizde yüksek ekonomik kayıplara neden olmasından dolayı BEF üzerine yapılmış çeşitli araştırmalar bulunmaktadır (Erol ve ark. 2015; Zaghawa ve ark. 2016, 2017).

Tibette 2012-2015 yılları arasında ELISA testi ile incelenen 1123 adet Tibet Sığırı (Yak)'nın, 454 (%40,4)'ünde pozitiflik tespit edilmiş ve seroprevalansların 2012, 2013, 2014, 2015 yıllarında sırasıyla %49,3, %36,0, %44,1 ve %34,0 olduğu bildirilmiştir (Liu ve ark. 2017).

Suudi Arabistan'da ortaya çıkan salgınlarda seroprevalans oranlarını 2007 yılındaki salgında Jizan bölgesinde %66,7; Doğu bölgesinde %69,1; Qasim bölgesinde %69,7; Riyadh bölgesinde %70; 2009 yılındaki salgında Jizan bölgesinde %31,7; Doğu bölgesinde %32,2; Qasim bölgesinde %30,0; Riyadh bölgesinde %40,0; 2011 salgınında ise Jizan bölgesinde %30,4; Doğu bölgesinde %28,7; Qasim bölgesinde %33,1; Riyadh bölgesinde ise %32,4 olarak tespit edilmiştir (Zaghawa ve ark. 2017). Yine Suudi Arabistan'da 2010 yılının yaz aylarında 1480 adet sığıra ait seropozitiflik oranları Doğu'da %18, Jizan'da %18, Qasim'de %26 ve Riyad'da ise %12 olarak bildirilmiştir (Zaghawa ve ark. 2016).

İsrail'de 1990, 1999, 2004 yıllarında meydana gelen salgınlarda serum nötralizasyon testi ile BEF'in sürü düzeyindeki insidensini belirlemek için 192 sığır sürüsü incelenmiş ve insidensler 1990, 1999, 2004 yıllarında sırasıyla %78,4, %97,7 ve %100 olarak belirlenmiştir (Yeruham ve ark. 2010). Yine

İsrail'de yapılan diğer bir çalışmada nötralizasyon testi ile seroprevalans oranları mandalarda %13,79 (4/29), ceylanlarda %4,44 (3/68) ve alageyiklerde %0,68 (2/296) olarak tespit edilmiştir (Aziz-Boaron ve ark. 2015).

Mısırın El Dakhla vahasında 40 hayvan melezinden (Friesian x Balady) viremi ve viremiden iki hafta sonraki dönemde alınan serum örnekleri anti BEFV antikorları yönünden ELISA testi ile incelemiş ve prevalans viremi döneminde %20 (8/40), sonraki dönemde ise %60 (24/40) olarak belirlenmiştir (Elnaby ve Rateb 2019).

Türkiye'de Ege bölgesinde bulunan Aydın (n=125) ve Muğla (n=100) illerinden toplam 225 adet serum örneği B-ELISA ile incelemiş ve pozitiflik tespit edilmemiştir (%0) (Erol ve ark. 2015). Orta Karadeniz bölgesinde bulunan beş ilden toplanan örneklerde (Ordu, Samsun, Tokat, Sinop ve Amasya) ortalama seroprevalans %13,5 olarak tespit edilmiştir. İller düzeyinde seroprevalans oranları ise Samsun'da %2,5, Amasya'da %27,5 ve Sinop'ta %37,5, olarak belirlenirken Ordu ve Tokat'ta ise seropozitifliğe rastlanmamıştır (%0) (Albayrak ve Özan 2010). Türkiye'nin Trakya bölgesinden toplanan 557 sığıra ait serum örneği incelenmiş ve ortalama seroprevalans %8,04, iller bazında prevalans ise İstanbul'da %2,8, Edirne'de %15,3, Çanakkale'de %2,5, Kırklareli'nde %13 ve Tekirdağ'da %6,6, olarak belirlenmiştir (Karaoğlu ve ark. 2007). Adana, Adıyaman ve Sakarya illerinden toplam 55 hayvana ait örneklerin incelendiği bir çalışmada anti-BEFV antikorları tespit edilememiştir (Tonbak ve ark. 2013).

Bu çalışmada 10 yıllık süre içerisinde Adana, Adıyaman, Gaziantep, Hatay, Kahramanmaraş, Mersin, Osmaniye, Şanlıurfa ve Kilis olmak üzere toplam dokuz ili kapsayan geniş bir coğrafi alandan 2840 serum örneği incelenmiş ve 1082 (%38,09) örnekte anti-BEFV antikorları tespit edilmiştir (Tablo 1).

Yapılan incelemede pozitifliğin en yüksek olduğu ilin Adana %53,81 (247/459), en düşük olduğu ilin ise %26,53 (65/245) ile Kilis olduğu tespit edilmiştir. Hastalığın endemik olduğu ve bu iki ildeki prevalans oranlarındaki farkın yapılan örneklem farklılığından kaynaklanabileceği kanaatine varılmıştır. Yıllara göre bir değerlendirme yapıldığında ise en yüksek pozitiflik %96,69 (117/121) ile 2015, en düşük pozitiflik ise %10,12 (82/810) ile 2017 yılında tespit edilmiştir. 2015 yılındaki yüksek seropozitifliğin Şanlıurfa ve Adana illerinde aşı uygulanan çiftliklerden örneklem yapılmasından ve çalışmada kullanılan tanı kitinin enfeksiyona bağlı pozitiflik ile aşı kaynaklı pozitifliği ayırt edememesinden kaynaklandığını düşündürmüştür. Ülkemizde BEF enfeksiyonuna karsı periyodik düzenli bir aşı uygulaması yapılmamaktadır. Ancak hastalık çıkan yerlerde aşı uygulaması yapan işletmeler bulunmaktadır 2015 yılı haricinde aşısız sürülerde 9 yıllık süre içerisinde değerlendirme yapıldığında ortalama pozitiflik %35,49 (965/2719) olarak belirlenmiştir.

Ayrıca Tonbak ve ark (2013), Oğuzoğlu ve ark (2015) ve Abaylı ve ark (2017) 2012 salgınında yaptıkları filogenetik analiz sonuçlarına ek olarak 2012 yılında belirlenen %57,28'lik seropozitiflik oranı ile 2020 yılında Tokgoz ve ark (2023) yaptıkları çalışmada bildirdikleri %69,04'lik seropozitiflik oranı ve belirtilen yıllara ait saha gözlemleri birer salgına işaret etmektedir. Nitekim 2020 yılında Karayel-Hacıoğlu ve ark (2021) Şanlıurfa'dan, Tokgöz ve ark (2023) Adana ile Şanlıurfa'dan elde ettikleri izolatlar ile yaptıkları filogenetik analizler sonucunda Orta Doğu soy hattında yer aldıklarını bildirmişlerdir.

Seroprevalans sonuçları göstermektedir ki subtropikal bölgede yer alan bu illerde BEFV enfeksiyonu enzootik olarak görülmekle beraber antikor varlığı yıllara bağlı olarak değişiklik göstermektedir. Salgından sonra gelişen antikor varlığının zamanla azalma eğilimi gösterdiği için enfeksiyonla mücadelede riskli bölgelerde koruma ve kontrol kapsamında bölgesel mücadele önlemlerinin alınması gerekliliğini ortaya koymaktadır. Bu sebepten dolayı ortalama 3-5 yılda bir salgınların ortaya çıktığı düşünüldüğünde salgın beklenen bölgelerde düzenli antikor varlığına bakılması ve antikor varlığının azaldığı dönemlerde koruyucu amaçla aşı uygulamalarının yapılması önerilmektedir.

Etik Kurul Kararı: Bu çalışma Adana Veteriner Kontrol Enstitü Müdürlüğü Yerel Etik Kurulunun 27.09.2024 tarih ve 2024-3/426 sayılı kararı gereği çalışmanın Etik Kurul İznine tabi olmadığına karar verilmiştir. Tarım ve Orman Bakanlığı Gıda ve Kontrol Genel Müdürlüğü tarafından 30.09.2024 tarih ve E-71037622-325.99-16028049 sayılı yazı ile bu çalışma için gerekli izin alınmıştır. İfade edilen görüş ve düşünceler yalnızca yazara aittir ve T.C. Tarım ve Orman Bakanlığı'nın görüşlerini yansıtmak zorunda değildir. Türkiye Cumhuriyeti Tarım ve Orman Bakanlığı bunlardan sorumlu tutulamaz.

Teşekkürler: Laboratuvar çalışmalarındaki yardımlarından dolayı Veteriner Sağlık Teknikeri Ali ÖZ'e ve rahmetli Veteriner Hekim Mehmet MİRİOĞLU'na teşekkür ederim.

Maddi destek ve çıkar ilişkisi: Çalışmayı maddi olarak destekleyen kişi ve kuruluş yoktur ve yazarların arasında herhangi bir çıkara dayalı ilişki yoktur.

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Bibliometric analysis of articles produced from animal health research projects supported by TAGEM according to scientific fields

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Derleme
Review Article

Sığırlarda BVDV enfeksiyonları

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Özet: Bovine Viral Diyare Virus (BVDV) olarak da bilinen Pestivirus enfeksiyonları, günümüzde özellikle süt sığırcılığı endüstrisinde yatırımcı ve yetiştiriciler için ağır ekonomik kayıplara sebep olan viral bir hastalıktır. İlk defa ilan edildiği 1946 yılından bugüne dek gerek Türkiye'de gerekse Dünya'da birçok farklı araştırmacı tarafından farklı genotip ve subgenotipleri olduğu keşfedilen BVDV hala sığır sağlığını etkilemektedir. Ülkemizde koruma ve kontrol yöntemlerine dair çok fazla çalışma olmamasına karşın, farklı ülkelerde çeşitli eradikasyon programları uygulanmaktadır. Fakat buna rağmen BVDV kontrolünde sorunlar devam etmektedir. Bu nedenle bu derlemede BVDV enfeksiyonlarındaki son durum ve gelişmeler hakkında bilgiler sunuldu.

Anahtar kelimeler: Bovine Viral Diarrhea Virus, BVD, MD, Pestivirus, Sığır

BVDV infectıons in cattle

Abstract: Bovine Viral Diarrhea Virus (BVDV), also known as Pestivirus infections, is a viral disease that causes significant economic losses to investors and farmers, particularly in the dairy cattle industry. Since its first identification in 1946, BVDV has been found to have various genotypes and subgenotypes, discovered by many researchers both in Türkiye and globally, and continues to affect cattle health. Although there have been few studies on protection and control methods in Türkiye, and despite the implementation of various eradication programs in other countries, challenges in controlling BVDV persist. Therefore, this review provides information on the current status and developments regarding BVDV infections.

Keywords: Bovine Viral Diarrhea Virus, BVD, Cattle, MD, Pestivirus

Giriş

Sığırlarda Bovine Viral Diyare Virus (BVDV), akut ya da persiste enfeksiyonlara sebep olan; solunum, sindirim ve reproduktif sistem bozukluklarına yol açabilen endemik bir enfeksiyondur. *Flaviviridae* familyasına mensup *Pestivirus* genusunda bulunan BVDV, Dünya'da ilk kez 1946 yılında yapılan bir çalışmada keşfedilmiştir. Sonraki yıllarda Dünya genelinde yapılan çalışmalarda hastalığın endemik bir enfeksiyon olduğu anlaşılmıştır. Küresel anlamda birçok ülkede Bovine Viral Diyare (BVD) hastalığına sebep olan varyant genotip ve subgenotip Pestivirus suşları mevcuttur. Bu nedenle kontrol ve eradikasyon çalışmaları ile ilgili hem ülkeler arası hem de bölgesel farklılıklar yaşanmaktadır. Türkiye'de 1990'lı yıllardan beri çeşitli bölgelerde BVD ile ilgili çalışmalar yapılmaktadır. Hastalığın akut formunda enfekte hayvanda şiddetli verim kayıpları görülmektedir. Morbiditesi yüksek bir enfeksiyon olduğundan dolayı, hastalık kısa süre içinde sürüde yayılmakta ve bireysel kayıpların yanı sıra sürü bazında da bu verim kayıpları etkili olmaktadır. Hastalığın persiste formunda (Pİ) virusun sürü içinde sirkülasyonu ve devamlılığı söz konusu olduğundan, koruma ve kontrol noktasında her çiftliğin kendi kontrol programını oluşturması gerekmektedir. Kontrol programlarının eksik ya da yanlış uygulandığı durumlarda sürü içerisinde buzağı ölümleri, kongenital anomaliler, reproduktif bozukluklar, süt veriminin azalması gibi önemli ekonomik kayıplar görülmektedir.

Bu derlemede, BVD ile ilgili gelişmeler ve koruma, kontrol ve eradikasyon stratejilerinin etkin bir biçimde ortaya konulmasıyla ilgili bilgilerin sunulması ve sığır sağlığı ile sığırcılık endüstrisine fayda sağlamak hedeflendi.

Etiyoloji

Hastalığa ilişkin yapılan ilk çalışmalar 1946 yılında akut gelişen bir hastalığın görülmesi sonucu başlamış olup başlarda hastalığın toksikasyon sebepli olabileceği düşünülse de yapılan analizler sonucunda hastalığın zehirlenme kaynaklı olmadığı anlaşılmış ve hastalığın bir etken kaynaklı olabileceği

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belirtilmiştir (Olafson ve ark., 1946). Sonraki yıllarda hastalığın sürü içi yayılımı daha düşük fakat daha şiddetli seyreden bir formu ortaya konulmuş ve hastalığın bu formu Mukozal hastalık (Mucosal disease-MD) olarak adlandırılmıştır (Ramsey ve Chivers, 1957). BVD ve MD hastalıklarının aynı etkenlerden dolayı olduğu ise 1961 yılında yapılan bir çalışmada tespit edilmiştir (Kniazeff ve ark., 1961). BVD ile MD arasındaki bu farklılık hastalık etkenlerinin farklı biyotiplere sahip olması ile açıklanmıştır.

BVD, deneysel olarak sitopatik ve nonsitopatik olarak 2 biyotipe ayrılmıştır. Nonsitopatik viruslar, hücrelerde dejeneratif etkilere sebep olmazlar. Buna karşın sitopatik viruslar hücre morfolojilerinde çeşitli değişikliklere ve nihayetinde apoptozise neden olurlar (Lee ve Gillespie, 1957). Nonsitopatik tipte bir virus ile enfekte bir fetüsün, doğumu takiben sitopatik tipte bir virus ile süperenfeksiyonu neticesinde MD şekillenmektedir (Brownlie, 1990).

Tablo 1. Hafif ve Şiddetli Seyirli BVD/MD İnfeksiyonları ile Persiste İnfeksiyonlarda Sitopatik ve Nonsitopatik Virusların Etkileri (Deregt ve Loewen, 1995)

Hastalığın Seyri	NCP[a]	CP[b]	Olası Sonuçlar
Hafif seyirli BVD[c]	+	+	Ateş, ishal, iyileşme
Pİ[d]	+	-	MD, Pİ enfekte hayvanlar
MD[e]	+	+	Ölümcül akut/kronik sonuçlar
Trombositopeni[f]	+	-	Hemoroji, ölüm/iyileşme
Şiddetli seyirli BVD[f]	+	-	MD benzeri semptomlar, ölüm ya da iyileşme

[a]Nonsitopatik

[b] Sitopatik

[c] NCP ya da CP viruslar hafif seyirli olabilir

[d] Persiste İnfeksiyon, Fetüsün gebelik sürecinde olan enfeksiyonu

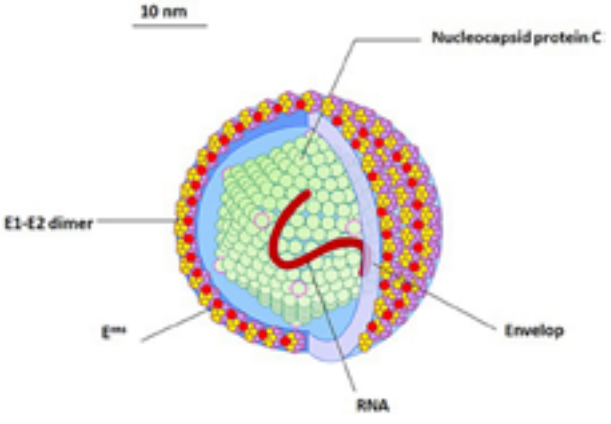
[e] Hem NCP hem de CP biyotipteki viruslar MD ile ilişkilidir. Sadece Pİ hayvanlar MD olabilir.

[f] Virulansı yüksek NCP viruslar şiddetli seyreden BVD enfeksiyonlarına ve trombositopeniye sebep olabilir.

Etken

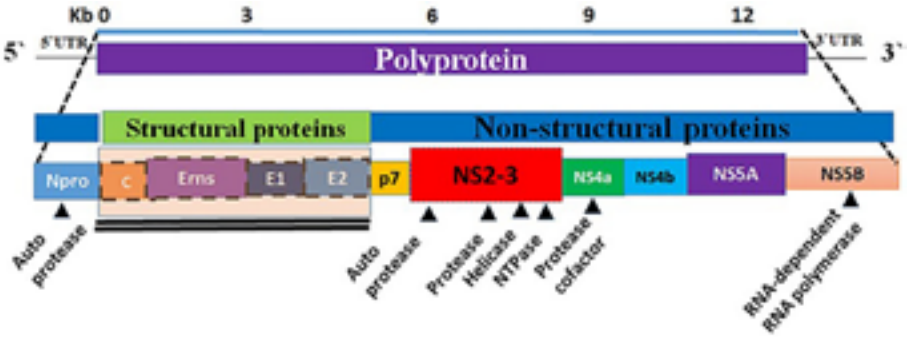
BVDV, 12.3–16.5 kilobaytlık (kb) genom büyüklüğünde, zarflı ve tek iplikli, (+) polariteye sahip bir RNA virusudur (Şekil 1) (Chi ve ark., 2022). BVD/MD'ye sebep olan etkenler *Flaviviridae* ailesine mensup *Pestivirus* genusunda yer almaktadır. *Pestivirus* genusunda yer alan ve BVDV olarak adlandırılan virus, BVDV-1, BVDV-2 ve BVDV-3 olarak üç genotipe ayrılmıştır. Güncel taksonomiye göre BVDV-1 *Pestivirus bovis* olarak, BVDV-2 ise *Pestivirus tauri* olarak yeniden adlandırılmıştır. Fakat güncel yayınlarda araştırmacılar sıklıkla, söz konusu genotiplerden BVDV-1 ve BVDV-2 olarak bahsetmektedir. BVDV-3 ise günümüzde *Pestivirus Brazilense* ya da Hobi-Like virus olarak tanımlanmaktadır. Bu genotipler ile ilgili yapılan çalışmalar neticesinde, virusun farklı subgenotiplere ayrıldığı tespit edilmiştir. Bu çalışmalar sonucu günümüzde, 24 adet BVDV-1 (1a–1x), beş adet BVDV-2 (2a–2e) ve beş adet BVDV-3 (a–e) olmak üzere toplam 34 subgenotip ortaya çıkmıştır. (Anonim, 2023 ; Baumbach ve ark., 2023 ; Yeşilbağ ve ark., 2024). Türkiye'de 3 genotipin de varlığı bilinmektedir. Bunlar arasında en yaygın olarak BVDV-1 genotipi görülmektedir. BVDV-1 subgenotiplerinden BVDV-1l ve BVDV-1r subgenotipleri diğerlerine göre daha yaygındır. Bunların yanında BVDV-1a, 1b, 1c, 1d, 1f ve 1h subgenotipleri de Türkiye'de sirkülasyonda olan subgenotiplerdendir (Cagirgan ve ark., 2022 ; Yilmaz ve ark., 2022). BVDV-3'ün varlığı ilk kez Erzurum ve Elazığ'da yapılan bir çalışmada, 2019 yılında bildirilmiştir (Timurkan ve Aydın, 2019).

Virusun yapısal proteinleri E^{rns}, E1, E2 ve C proteinidir (Şekil 2). Yapısal olmayan proteinleri ise N^{pro}, p7, NS2/3, NS4A, NS4B, NS5A, NS5B'dir (Neill, 2013).



Şekil 1. Pestivirus Şematik Yapısı (Al-Kubati ve ark., 2021)

BVDV genomu 5've 3' bölgelerinde bulunan bilgi kodlamayan alanlar (Untranslated regions-UTRs) ile çevrili tek bir açık okuma alanından (open reading frame-ORF) oluşur (Kokkonos ve ark., 2020). RNA, yaklaşık 3900 amino asitlik bir poliproteini kodlayan uzun bir protein sentezleme yeteneği olan bölgeyi (ORF) içerir (Goga ve ark., 2014). Virusun genomlarını oluşturan yapısal ve yapısal olmayan proteinler 5'UTR ile 3'UTR bölgesi arasında dizili halde bulunmaktadır (Al-Kubati ve ark., 2021).



Şekil 2. Bovine Viral Diyare Virusu Genomu: Yapısal Ve Yapısal Olmayan Proteinleri Kodlayan Genlerin Organizasyonu (Al-Kubati ve ark., 2021)

Viral replikasyon, lipid metabolizması ve birçok faktörle ilişkili karmaşık bir süreçtir (Stapleford ve Miller, 2010). Replikasyonun başlaması için virus, konak hücrenin yüzey lipidleri ile etkileşime girer (Martín-Acebes ve ark., 2011). Sığırlardaki hücresel reseptörlerden CD46, tip-1 transmembran glikoproteinidir (Maurer ve ark., 2004 ; Krey ve ark., 2006 ; Riedel ve ark., 2020). BVDV replikasyonu, viral membran proteininin CD46 reseptörüne bağlanması yoluyla endositoz ile başlar (Iqbal ve ark., 2000 ; Rodenhuis-Zybert ve ark., 2010). BVDV zarf proteinleri E1, E2 ve E^{rns} bu süreçte önemli rol oynarlar. BVDV E2 proteini konak hücre yüzeyindeki lipoproteinlere bağlanır ve böylece BVDV'nin hedef hücrelere girişi tamamlanır. Daha sonra BVDV RNA'sı üretim süreci başlar. Viral genomik RNA paketleme ve virus fragmentlerinin salınması sürecinde, C protein, endoplazmik retikulum boşluğundaki viral genomik RNA'ya bağlanır ve BVDV virionları hücre içi organellere ait veziküllerde olgunlaşır. Daha sonra replikasyon tamamlanarak virionlar hücreden dışarı salınır (Ma ve ark., 2022). BVDV yapısında bulunan poliproteinlerde, CP ve NCP biyotipleri arasında farklılıklar vardır. NCP biyotipte NS2/3 şeklindeki protein, CP biyotipte NS2 ve NS3 olmak üzere 2 farklı protein olarak bulunmaktadır (Behrens ve ark., 1998) Hücre kültüründe NS2/3'ün, enfeksiyonun erken evresinde parçalandığı görülür. İleri evrelerde ise parçalanamayan NS2/3 proteini birikmeye başlar. BVDV enfeksiyonunda, parçalanmamış NS2/3, virion oluşumu için önemlidir (Klemens ve ark., 2015) Hücre kültüründe, NS2/3 parçalanması, enfeksiyonun erken evresinde gözlenir (Lackner ve ark., 2004) Bu birikmenin persiste enfeksiyonlara sebep olabileceği bildirilmiştir (Klemens ve ark., 2015)

Fiziksel ve kimyasal özellikler

Pestiviruslar, yapılarında bulunan lipid özellikli zarf sebebiyle alkol ve deterjan gibi çeşitli maddelerle hızlıca inaktive olurlar (Depner ve ark., 1992). Buna karşın stabilitelerini geniş bir pH aralığında (5.7 ile 9.3 pH) koruyabilirler. Aynı zamanda pestiviruslar, 40 °C ve daha fazla sıcaklığa maruz kaldığında etkinlikleri büyük ölçüde azalır. Dondurma işlemine tabi tutulmaları ise enfektivitelerinde değişiklik meydana getirmez (Ridpath, 2005).

Konak duyarlılığı

Pestiviruslar pek çok hayvan türünü etkilemektedir (Riedel ve ark., 2020). Virusun ilk tanımlanmasından bu zamana kadar keçi, koyun, deve ve lama gibi pek çok hayvan türünde virus tespit edilmiştir (Evans ve Reichel, 2021).

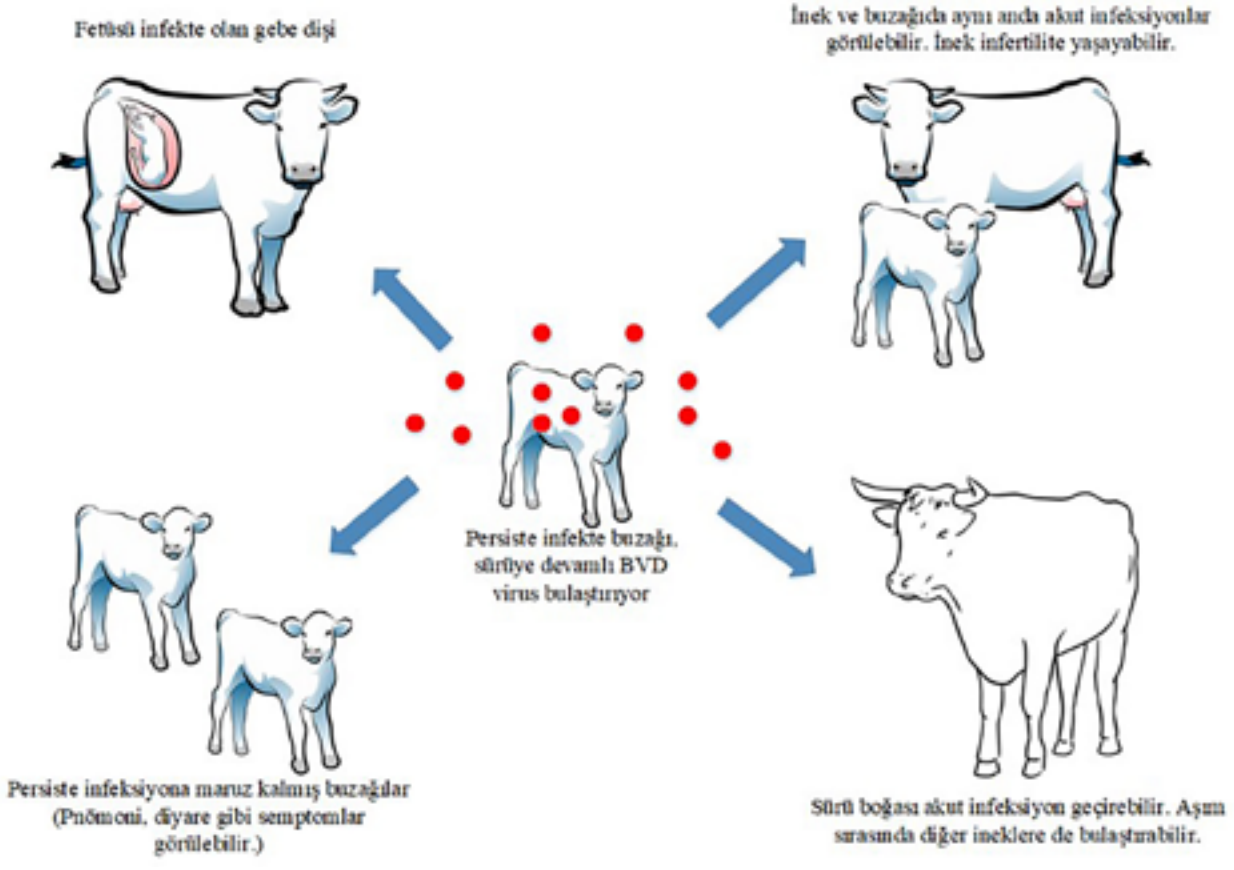
Koyunlarda BVDV enfeksiyonlarının etkilerinin, BVDV ile enfekte sığırlarda görülen etkilere benzediği rapor edilmiştir (Scherer ve ark., 2001). Gebe olmayan koyunlarda önemli klinik semptomlar görülmezken (Evans ve ark., 2015), gebe koyunlarda akut enfeksiyonlar ciddi klinik belirtilere yol açabilir (Evans ve Reichel, 2021). Gebe keçilerde ise BVDV enfeksiyonunun sığır ve koyunlarda olduğu gibi, abortus ve reproduktif sistem semptomlarına sebep olduğu bilinmektedir (Broaddus ve ark., 2009).

Bulaşma

BVDV hem vertikal hem de horizontal olarak bulaşabilir. Virusun vetrikal olarak anneden fetüse geçmesi ile hastalık için çok önemli olan persistentlik mekanizması gelişir (Şekil 3) (Schweizer ve Peterhans, 2014). Gebe hayvanlarda henüz fetüsün immun sistemi gelişmediği bir dönemde (gebeliğin ilk 100 günü), nonsitopatik biyotipteki virusa maruz kalınması durumunda, fetusta immun tolerans gelişir ve doğum sonrası da mevcut antijene karşı bağışıklık tepkisi görülmez (Walz ve ark., 2020). Bu hayvanlar Pİ olduklarından dolayı yaşamları boyunca antijen yönünden pozitif olmalarına karşın antikor yönünden negatiftirler. Dolayısıyla ömür boyu virus saçarak sürülerde virusun sürekli olarak sirküle olmasına sebep olurlar. Bu durum hastalığın yayılımının ve dolayısıyla kontrol edilmesinin zorluğunun en büyük sebeplerindendir. Ayrıca Pİ hayvanların sadece sürüdeki yayılımların sebebi olmakla kalmayıp, virusun varyasyo-

nuna katkı sağladığı yönünde önemli veriler mevcuttur (Neill ve ark., 2012).

Akut enfeksiyonlu hayvanlar ise virusu devamlı saçmazlar. Virusun virulansı ve konağın direncine bağlı olarak bu süre yaklaşık 2-3 hafta olmasına karşın, bu hayvanların daha uzun sürelerde virus saçabildiği de bildirilmiştir (Brownlie ve ark., 1987; Goto ve ark., 2021). Genel olarak BVDV'nin sürü içinde yayılmasına sebep olan etkenler Pİ hayvanlar, akut enfekte hayvanlar, vektörler, aeresol olarak bulaşma ve kontamine ekipmanlardır. Ayrıca semen, embriyo ve iatrojenik yolla bulaşma da hastalığın önemli bulaşma yollarındandır (Gunn, 1993; Niskanen ve ark., 2002 ; Niskanen ve Lindberg, 2003).



Şekil 3. BVDV'nin Vertikal Yolla Bulaşma Ve Yayılması (Khodakaram-Tafti ve Farjanikish, 2017)

Patogenez

BVDV enfeksiyonları birçok farklı sistemde patolojik değişimlere sebep olabilir. Sindirim sistemi, solunum sistemi, reproduktif sistem, immun sistem ve sinir sisteminde oluşabilecek tüm patolojik etkiler birçok faktöre bağlı olarak değişkenlik gösterebilir. Hayvanın gebe olup olmadığı, gebelik dönemi, hayvanın bağışıklık durumu, virusun biyotipi ve enfeksiyonun şiddeti gibi etkenler patogenezde farklılıklara sebep olur (Grooms, 2004 ; Al-Kubati ve ark., 2021).

BVDV ilk olarak sindirim sistemi veya solunum sistemi vasıtası ile orofarengeal mukozaya ulaşır. Daha sonra, epitel hücrelerde ilk replikasyon aşaması meydana gelir. Bu replikasyonu takiben viruslar ekzositoz ile serbest bırakılır. Serbest kalan viruslar, direkt kan serumu veya enfekte lökositler ile sistemik olarak yayılabilir. Ek olarak virus, erkek sığırlarda seminal veziküller ve prostat bezinde de çoğalabilir. BVDV, hayvanlarda akut bir enfeksiyonla seyredebileceği gibi, persiste enfeksiyonlara, fetal enfeksiyonlara ve MD'ye de sebep olabilir. Akut enfeksiyonlarda virusa maruz kalındıktan yaklaşık 3 gün sonra iştahsızlık, ateş, burun akıntısı ve ishal gibi semptomlar görülebilir ve yaklaşık 2 hafta sonra iyileşme gerçekleşir. İnfeksiyonun şiddetine ve hayvanın bağışıklık durumuna göre immunsupresyon oluşursa, sekonder enfeksiyonlar gelişebilir (Meyling ve ark., 1990 ; Potgieter, 1997 ; Khodakaram-Tafti

ve Farjanikish, 2017). Hastalığın epidemiyolojisinde çok önemli olan persiste enfeksiyonlar ise, gebe hayvanların gebeliğin ilk trimesterinde virusa maruz kalması durumunda oluşur (Peterhans ve ark., 2003). Fetüs, virusu kendi yapısının bir parçası olarak tanımladığından dolayı, doğan buzağılarda yaşamları boyunca antijen bulunmasına karşın antikor bulunmaz. Bu hayvanlar, virus rezervuarı olarak sürülerde BVDV'nin sürdürülmesi konusunda çok önemli role sahiptir (Smirnova ve ark., 2008). Bununla birlikte virus sebebiyle intrauterin enfeksiyonların şekillenmesi de mümkündür (Khodakaram-Tafti ve Farjanikish, 2017). Gebeliğin ilk 45 gününde virusa maruz kalınması durumunda genellikle embriyonik ölüm gerçekleşir. Gebe hayvanın 45-145. günler arasında enfekte olması durumunda ise BVDV'ye veya farklı etkenlere bağlı abort şekillenmezse fetüs Pİ olarak doğar. Ayrıca gebeliğin 100-150. günleri arasındaki fetal enfeksiyonlarda, serebellar hipoplazi, ensefalopati, retinal dejenerasyonlar, büyüme geriliği ve iskelette çeşitli malformasyonlar gibi kongenital anomaliler şekillenebilir. Fetal enfeksiyon, gebeliğin 150. gününden sonra meydana geldiğinde ise, fetüs genellikle antikor geliştirme mekanizmasına sahiptir. Doğan yavrular klinik olarak sağlıklı ve antikor yönünden de pozitiftir (de la Concha-Bermejillo ve Romano, 2021).

MD ise Pİ hayvanların BVDV'nin CP biyotipi ile süperenfeksiyonu sonucunda oluşur; yüksek ateş, şiddetli ishal ve dehidrasyon gibi semptomlarla birlikte çoğunlukla ölümle sonuçlanır (Wilhelmsen ve ark., 1991 ; Kelling, 2004).

Kuzey Amerika'da araştırmacılar tarafından BVDV'nin klinik semptomlarının çok şiddetli olduğu hastalık salgınları ortaya konuldu. Bu salgınlar; solunum sisteminde bozukluklar, ishal, abort, multisistemik kanamalarla birlikte seyreden trombositopeni ve ani ölümle karakterize edildi. Etkilenen sığırlarda MD'yi düşündüren klinik semptomlar mevcut olmasına rağmen, MD oluşumu için gerekli olduğu bilinen, aynı anda her iki BVDV biyotipi ile (CP-NCP) enfeksiyon oluşmadığı görüldü. Yapılan tanı testleri ile enfeksiyonun BVDV-2 genotipi tarafından oluştuğu tespit edildi. Daha sonra yapılan deneysel çalışmalar BVDV-2 genotipinin benzer şekilde şiddetli bir hastalığa sebep olduğunu doğruladı. Farklı bölgelerde de farklı araştırmacılar tarafından şiddetli klinik tablo ve hemorajik sendrom semptomları görülen sığırlarda BVDV-2 genotipinin tanımlanması, araştırmacıların BVDV-2 genotipinin BVDV-1 genotipine kıyasla virulansının daha yüksek olduğunu düşünmesine sebep olmuştur (Walz ve ark., 2020)

Tanı

BVD, hayvan sağlığı bakımından oldukça önemli bir hastalıktır ve erken tanı, kontrol programları hastalıkla mücadelede oldukça etkilidir (Qi ve ark., 2022). Hastalığın tanısı, dışkı svabı, burun svabı, doku parçaları ve iç organlar gibi örnekler kullanılarak moleküler, serolojik ve virusun saptanmasına yönelik çalışmalar ile ortaya konulabilmektedir (Wang ve Pang, 2024). Ayrıca hastalığın tanısında kan ve süt de dahil olmak üzere çok çeşitli örnekler kullanılmaktadır. BVDV'ye özgü antijen ve antikorların tespit edilmesi hastalığın tanısında çok önemlidir (Lanyon ve ark., 2014). Virusun saptanmasına yönelik olarak virus izolasyonu, immunohistokimya testi ve immunofloresan testlerinden faydalanılmaktadır (Sandvik, 2005 ; Piña ve ark., 2022 ; Harms ve ark., 2023). Tanı için serolojik yöntemlerden ELISA Testi (Enzyme-Linked Immunosorbent Assay) oldukça sık kullanılmaktadır. BVDV antijeni veya antikorunun tespiti için hızlı ve spesifik bir yöntemdir. Virus nötralizasyon testi ise daha çok aşı çalışmalarında tercih edilmektedir (Wang ve Pang, 2024). Moleküler yöntemlerden Ters transkripsiyon-polimeraz zincir reaksiyonu (RT-PCR) ve Real-time RT-PCR (Quantitative RT-PCR-RT-qPCR), BVDV teşhisi için kullanılan yöntemlerdendir. Yapılan değerlendirmeler sonucunda klinik vakaların tanısında RT-qPCR'ın kullanılması önerilmiştir (Wernike ve Beer, 2024). RT-PCR testi sonucunda elde edilen ürünlerin sekans analizleri ve filogenetik çalışmaları yapılarak BVDV genotiplerinin tespit edilmesi ve virusların sınıflandırılması oldukça önemlidir (Flores ve ark., 2002). Aynı zamanda filogenetik ağaç oluşturulması ve genotipler arasındaki benzerlikler ve farklılıkların ortaya konulması hem moleküler epidemiyoloji hem de aşılama stratejilerinde koruma ve kontrol açısından önemlidir (Xia ve ark., 2007 ; Kapli ve ark., 2020). Sürü ve sürüler arasındaki bulaşmanın ana kaynağı olan Pİ hayvan varlığının tespit edilebilmesi çok önemlidir. Pİ hayvanlardaki virus yükünün fazla olması, çeşitli tanı yöntemleriyle antijen tespitini kolaylaştırmaktadır. Bununla birlikte şiddetli bulgularla seyreden MD'nin varlığının ortaya konulabilmesi için de etkilenen hayvandan BVDV'nin hem CP hem de NCP biyotipi izole edilebilmelidir. Ancak etkilenen hayvanda Pİ teşhisiyle birlikte MD semptomlarının görülmesi tanıyı doğrulamaya yardımcı olur (Lanyon ve ark., 2014).

Koruma, kontrol ve eradikasyon

BVDV, sürü bazında genel olarak verimin azalması gibi doğrudan veya koruma ve kontrol programları için yapılan harcamalar gibi dolaylı yollarla sığır endüstrisinde büyük ekonomik kayıplara neden

olur (Pinior ve ark., 2017). Hastalık için risk faktörleri değerlendirildiğinde vertikal, direkt ve indirekt bulaşmaların tümü ele alınmalıdır. Pİ hayvanlar, klinik semptom göstermediğinden dolayı hastalığın yayılması açısından en büyük risk teşkil eden faktördür. Dolayısıyla Pİ hayvanların sürüden uzaklaştırılması çok önemlidir. Bununla birlikte hayvan hareketlerinin izlenmesi, çiftlik çalışanlarının, ziyaretçilerin ve taşıma araçlarının kontrol çalışmaları da oldukça önemlidir (Rossi ve ark., 2017). Tüm risk faktörleri ele alındığında hastalığın yayılımının önlenmesi için 6 kategori üzerinde çalışmaların yapılması önerilmiştir. Bunlar hayvan hareketlerinin kontrolü, insan kaynaklı bulaşmaların önlenmesi, işletme araçları ve ekipmanların kontrolü, karantina ve izolasyon uygulamaları, sıhhi önlemler alınması ve çiftlik yönetiminin önleyici tedbirlerini kapsar (Ferreira ve ark., 2024).

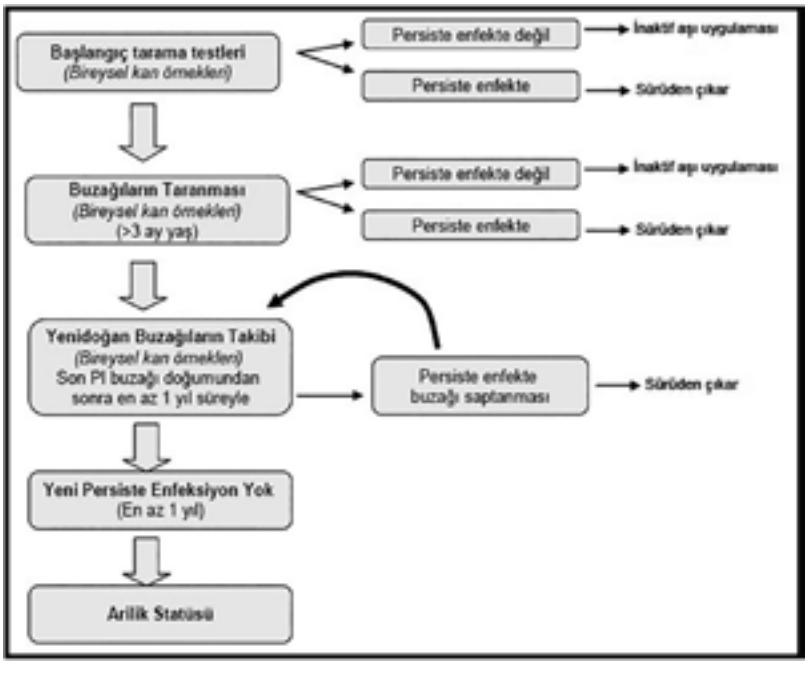
Pİ hayvanlar, BVDV'nin sürü içindeki yayılımında en önemli sebep olduğundan, hastalığın eradikasyonu açısından birincil hedef bu hayvanların teşhisi olmalıdır (Lanyon ve ark., 2014). Bir sürüye yapılacak olan gebe düve alımlarında ve işletmede sürüye katılacak tüm hayvanlar için bir veteriner hekim otoritesinin gözetiminde izolasyon uygulanması ve karantina uygulamasının yapılması yerinde olacaktır. Bu uygulama en az üç hafta sürmeli, süreç boyunca diğer hayvanlarla doğrudan ya da dolaylı olarak temas engellenmeli ve tanı çalışmaları yapılmalıdır (Walz ve ark., 2010 ; Ferreira ve ark., 2024).

Şekil 4. BVDV Koruma Kontrol Ve Eradikasyon Çalışmalarında Almanya'da Bir Çiftlikte Yapılan Uygulamalar (Moennig ve ark., 2005)

Hayvan nakilleri esnasında taşıma araçları da hastalığın yayılması açısından risk oluşturabilmektedir (Şekil 4) (Benavides ve ark., 2021). Yapılan çalışmalarda virusun 1-2 saat süreyle metal yüzeylerde canlı kalabildiği belirtilmiştir (Stevens ve ark., 2011). Hayvan taşıma araçları dışında işletmede kullanılan diğer araçlar ve yem taşıma araçları ile ilgili de riskler mevcuttur. Özellikle küçük ölçekli işletmelerde aynı araçlar farklı işletmeler tarafından kullanılabilmektedir. Bu paylaşım direkt olarak bulaşmada risk teşkil edebileceği gibi araç şoförlerinin de, enfeksiyonu duyarlı sürülere taşıyabileceği unutulmamalıdır (Benavides ve ark., 2021). Bununla birlikte hastalığın bulaşmasında özellikle meslek grupları olarak veteriner hekimlerin, teknisyenlerin, teknikerlerin ve çiftlikte çalışan işçilerin çok fazla etkiye sahip olduğu bilinmektedir. Çiftliklerde kullanılan çizmelerin ahırlara yapılan ziyaretlerden sonra dezenfekte edilmesi, dışarıdan ziyaretçi kabul edilmemesi, dışarıdan çiftliğe sağlık personeli gelmesi durumunda tek kullanımlık galoş, önlük, bone kullandırılması enfeksiyonun duyarlı sürülere bulaşmasının önlenmesi açısından önem arz etmektedir (Hoe ve Ruegg, 2006). Diğer bir risk faktörü ise hayvancılık işletmelerinde hayvan besleme sırasında ve atık bertarafında aynı makine, alet, ekipman kullanılmasıdır. Dolayısıyla alet, ekipman ve araçların düzenli olarak dezenfeksiyonu, hastalık bulaşmasını önemli ölçüde engeller. Hastalığın bulaşma yöntemleri göz önünde bulundurulduğunda gübre ve atık yönetimi de hastalığın bulaşmasının engellenmesi açısından alınacak tedbirler arasında yer almaktadır.

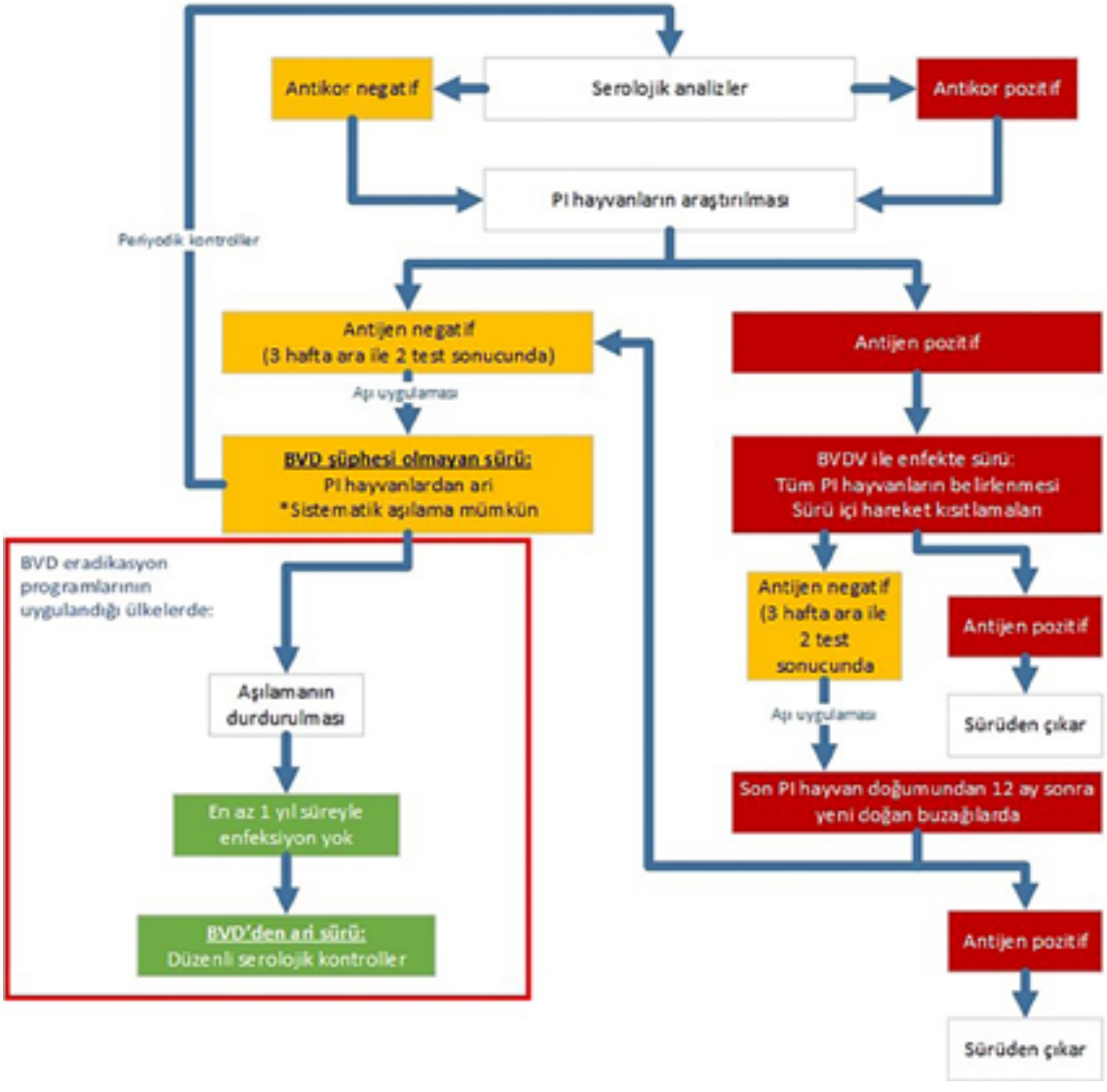
Tüm bu programlar devlet ve çiftlik yönetimi tarafından belirlenen ve önerilen yöntemler doğrultusunda yapılmalıdır. Eradikasyon ve kontrol çalışmaları, izolasyon ve biyogüvenlik uygulamalarının yanında aşı uygulamalarını da kapsamalıdır (Şekil 4) (Ferreira ve ark., 2024). Nitekim ülkelerin BVD ile mücadelede farklı yaklaşımları mevcuttur. Bazı ülkelerde kontrol ve eradikasyon programları uygulanmakta ve başarılı sonuçlar alınmaktadır. Bazı Avrupa ülkelerinde persistent enfeksiyon oranlarında ciddi oranlarda düşüş görülmekte iken, İsveç, Norveç ve Finlandiya'da hastalığın eradike edildiğine yönelik raporlar bulunmaktadır (Scharnböck ve ark., 2018).

Ülkemizde direkt olarak BVDV ile ilgili kontrol ve eradikasyon programı olmamasına karşın, bulaşıcı hastalıkların yayılmasının önlenmesi amacıyla taşıma araçlarının nakil öncesi dezenfeksiyonu gibi kanuni yükümlülükler şart koşulmuştur (Anonim, 2022). Mevzuat gereği yapılan uygulamaların yanında çiftlik bazlı kontrol ve eradikasyon programları uygulanmaktadır. Ulusal ve bölgesel bazda bir kontrol ve eradikasyon programı bulunmaması dolayısıyla tanı ve aşılama yöntemlerinin ciddiyetle uygulanması önemlidir. Tanıda tespit edilen BVDV pozitif hayvanların yanında mutlaka Pİ hayvanlara karşı tarama yapılmalı ve Pİ olduğu tespit edilen hayvanlar sürüden çıkarılmalıdır. Şekil 5'te 2012 yılında Bursa'da kurulan bir çiftlikte uygulanan kontrol programı neticesinde uygulamadan olumlu sonuçlar alınabileceği bildirilmiştir.



Şekil 5. Bursa'da Bir Çiftlikte Uygulanan Kontrol ve Eradikasyon Şeması (Yeşilbağ ve ark., 2012).

BVDV ile mücadelede, sürüdeki Pİ hayvanların teşhisi ve sürüden çıkarılmalarını takiben yapılacak aşı uygulamaları hastalığın sürü içindeki varlığını önemli ölçüde azaltacaktır. Bunun yanında yıllık yapılacak kontrollerde yine Pİ hayvanlara yönelik tanı çalışmalarının sürdürülmesi ile düzenli yıllık kontrol ve aşı çalışmaları yapılmasıyla birlikte, hastalığın sürüden eradikasyonu mümkün olabilmektedir. Şekil 6'da yapılabilecek örnek kontrol şeması verilmiştir.



Şekil 6. Ülkemizde uygulanabilir bir kontrol ve eradikasyon şeması

Aşılama çalışmaları

BVDV aşıları inaktif aşılar ve modifiye canlı aşılar olmak üzere 2 şekilde bulunmaktadır. Son yıllarda attenüe delesyon aşıları üretilmiş ve ülkemizde de kullanıma başlamıştır. İnaktif aşılar sıcaklık ve kimyasallardan dolayı oluşabilecek deaktivasyona karşı dirençli ve bağışıklık tepkisi oluşturmak için güvenilir bir yöntem olsa da aktif bağışıklığı sağlamak için tek uygulama yapılması yeterli olmaz. Modifiye canlı aşılar ise uzun süreli bir bağışıklık tepkisini tetikler ve tek bir doz kullanımından sonra immunite sağlama avantajına sahiptir. Fakat uygulama öncesi nakliye esnasında soğuk zincirin sağlanamaması riskine karşı stratejik önlemler gerektirir. Bunun yanında canlı aşıların yapımında kullanılan fetal sığır serumunun kontamine olma olasılığı göz ardı edilmemelidir. Yapılan çalışmalarda canlı aşıların kontaminasyonunun çoğunlukla NCP bir suş sebebiyle olabileceği bildi-

rilmiştir (van Oirschot ve ark., 1999). Günümüzde, Türkiye'de inaktif kombine aşı uygulaması yaygındır (Woolums ve ark., 2013 ; Newcomer ve ark., 2017 ; Yilmaz ve ark., 2022).

Virus iki biyotipe ve birden fazla genotip ve subgenotipe ayrıldığı için, değişik monovalan ve multivalan aşı formülasyonları mevcuttur. NCP biyotip sığır popülasyonlarında daha yaygın görülse de BVDV'nin CP suşlarının persiste enfeksiyona yol açmadığı düşünüldüğünden dolayı güvenlik tedbirleri sebebiyle aşı formülasyonlarının büyük kısmında CP biyotip kullanılır. NCP biyotip içeren aşıların kullanılması, iyi bir bağışıklık tepkisi oluşturabilir. Ancak, NCP biyotipin Pİ oluşturma etkisinin dikkate alınması ve sürü sağlığının etkin olarak korunabilmesi amacıyla aşı seçimini belirlemek için her aşı tipinin avantajları ve dezavantajları ile önerilen aşılama zamanlamasının değerlendirilmesi gerekir. Bunların dışında BVDV'nin E proteininin virustan çıkartılmasıyla elde edilen attenue bir aşı olan delesyon aşılarının da immuniteyi yeterli miktarda uyardığı ve canlıda aktif bir enfeksiyon geliştirmediği bildirilmiştir. Maternal antikorların azalmaya başladığı dönem itibariyle aşının bir kez uygulanmasını takiben yıllık olarak aşılamaya devam edilmesi uygun görülmüştür (Woolums ve ark., 2013 ; Newcomer ve ark., 2017 ; Rajput ve ark., 2020 ; Yilmaz ve ark., 2022).

Sonuç

Sığırların uluslararası ve yurt içinde nakliyesinin sık olması birçok ülkede BVDV'nin endemik hale gelmesinde kritik bir rol oynamaktadır. Hastalık sığır endüstrisinde çok büyük ekonomik kayıplara sebep olduğundan, enfeksiyona sebep olan etkenin bilinmesi ve hastalığın risk faktörlerinin anlaşılması, çiftlik bazında ve ulusal bazda koruma, kontrol ve eradikasyon programları yaklaşımlarını yönlendirecektir. Özellikle persiste enfeksiyonların hastalığın yayılmasındaki öneminin bilinmesi ve persiste enfekte hayvanların sürülerden uzaklaştırılması çok önemli olmakla birlikte kontrol programlarındaki nihai başarı için biyogüvenlik stratejilerin doğru şekilde belirlenerek uygulanması gerekmektedir. Ayrıca virusun fazla sayıda genotip ve subgenotipe sahip olması aşılama stratejilerinin bölgesel olarak değerlendirilmesi gerektiği sonucunu doğurmaktadır. Bölgelerde sirküle olan genotip ve subgenotipler kullanılarak aşılama programları yapılmasında yarar vardır. Özetle BVDV enfeksiyonlu tek bir hayvanın dahi duyarlı bir sürüye girmesi sonucunda virus sürüye ve bölgeye yayılıp yıllarca kalabilir. Dolayısıyla enfeksiyonun özelliklerinin bilinmesine ek olarak ekonomik kayıpların en aza indirilebilmesi ve hayvan sağlığı stratejilerinde önemli bir adım atılabilmesi için, Devlet tarafından yetiştiricilere ve işletme çalışanlarına hastalıkla ilgili eğitim-yayım faaliyetleri yapılmasında, eradikasyon programları geliştirilmesinde ve aşı programları oluşturulmasında yarar vardır.

Deney Hayvanları Kullanımı, Etik Kurulu ve Diğer Etik Kurul Kararları: Çalışmada canlı örnek kullanılmamasından dolayı etik kurul kararı bulunmamaktadır.

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Derleme
Review Article

Tahtakuruları (Hemiptera: Cimicidae)'nın insan sağlığı açısından önemleri ve mücadelesi

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Özet: İnsan sağlığı açısından önemli olan tahtakuruları Cimicidae ve Reduviidae ailelerinde yer alırlar. Bu ailelerde bulunan bazı türler nimf ve ergin dönemlerinde insanlardan ve hayvanlardan kan emerek beslenirler. Cimicidae ailesinde yer alan türlerden *Cimex lectularius* ve *Cimex hemipterus* insan sağlığını yakından ilgilendirirler. Türkiye'de, özellikle Konya başta olmak üzere bazı illerde, öğrenci evlerinde ve yurtlarda *Cimex lectularius*'tan kaynaklanan tahtakurusu salgınlarına son yıllarda sıklıkla rastlanmaktadır. Bu makale meslektaşlarımı, öğrencilerimi ve halkı tahtakuruları hakkında aydınlatmak üzere hazırlanmıştır.

Anahtar kelimeler: *Cimex lectularius*, Konya, Tahtakurusu, Türkiye

Bedbugs (Hemiptera: Cimicidae), their importance for human health and control

Abstract: Bedbugs, which are important for human health, belong to the families Cimicidae and Reduviidae. Some species in these families feed by sucking blood from humans and animals during their nymphal and adult stages. Among the species in the family Cimicidae, *Cimex lectularius* and *Cimex hemipterus* are closely related to human health. In Türkiye, bedbug outbreaks caused by *C. lectularius* have been frequently observed in some student houses and dormitories especially in Konya and some provinces. This article has been prepared to enlighten my colleagues, students and the public about bedbugs.

Keywords: Bedbug, *Cimex lectularius*, Konya, Türkiye

Giriş

Tahtakuruları Hemiptera Linnaeus takımında yer alan, hemimetabol (yarı başkalaşıma sahip) ve geceleri aktivite gösteren (nokturnal) artropodlardır. Bu takımda şimdiye kadar 302 ailede yer alan yaklaşık 104 bin tür bulunduğu bildirilmiştir (Kavasima ve ark. 2022). Bu takımda, daha önce Hemiptera'nın sinonimi olarak kabul edilen, fakat şu andaki taksonomide alt takım olarak değerlendirilen Heteroptera alt takımındaki türlerden 1519'unun Türkiye'de de tespit edildiği bildirilmiştir (Çerçi ve ark. 2024). Bu türlerin çoğunluğu doğada serbest olarak yaşar ve genellikle bitkilerde parazitlenirler. Bununla birlikte, Cimicidae (Şekil 1) ve Reduviidae (Şekil 2) ailelerindeki tahtakuruları insan ve hayvanlardan kan emerek beslendikleri ve hatta bazı patojenlere vektörlük yaptıkları ya da olası vektör oldukları için veteriner ve tıp hekimliği açısından önemlidir.

Cimicidae ailesinde altı alt aile, 25 cins ve 110'dan fazla tür olduğu ve bu türlerin çoğunun yarasalarda parazitlendiği bildirilmiştir (Kavasima ve ark. 2022; Dursun ve Fent 2024). Cimicinae alt ailesinde yer alan, kozmopolit bir tür olan, insanlardan, yarasalardan ve kuşlardan kan emerek beslenen ***Cimex lectularius*** Linnaeus ve daha çok tropik ülkelerde görülen, insan ve kuşlardan kan emerek beslenen ***Cimex hemipterus*** (Fabricius) ile Cacodminae alt ailesinde yer alan, insanlardan ve yarasalardan kan emen ve Batı Afrika'da görülen ***Leptocimex boueti*** (Brumpt) (Krinsky 2002; Giorda ve ark. 2013) insan sağlığı açısından önemli türlerdir. *Cimex* Linnaeus cinsinde yer alan *Cimex lectularius* (Şekil 1A, Şekil 3) ve *C. hemipterus* insan sağlığı açısından en önemli türlerdir. Bu türler nimf ve ergin dönemlerinde insanlardan, kuşlardan ve yarasalardan kan emerek beslenirler (Goddard 2014; Dik 2015).

Şekil 1. Cimicidae; A: *Cimex lectularius*; dişi, B: *Cimex hirundinis*; erkek, orijinal

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Şekil 2. Reduviidae; ergin (solda) ve nimf (sağda), orijinal

Merdivenci (1967) Türkiye'de, *Cimex* cinsindeki türlerden ***C. hemipterus*** [(= ***C. rotundatus*** (Signoret)]'u kobay ve tavşanlarda, ***C. columbarius*** Jenyns'u güvercinlerde, ***C. pipistrelli*** Jenyns'yi ise küçük yarasalarda saptadığını bildirmiştir. The Global Biodiversity Information Facility (GBIF) tarafından *C. rotundatus C. hemipterus*'un sinonimi olarak kabul edilmektedir (GBIF 2024). Köse ve ark. (2017) ise Ev kırlangıcı (*Delichon urbica*) yuvalarında ***Cimex hirundinis*** Lamarck **(= *Oeciacus hirundinis*)** (Şekil 1B)'i tespit etmişlerdir. Çerçi ve ark. (2024) Türkiye'de Cimicidae ailesi, Cimicinae alt ailesinde dört türün; ***C. columbarius*** (Güvercin tahtakurusu), ***C. lectularius*** (Tahtakurusu), ***C. pipistrelli*** (Yarasa tahtakurusu) ve ***C. hirundinis*** (Kırlangıç tahtakurusu) bulunduğunu belirtmişlerdir. C*imex hemipterus*'un coğrafi yayılışı dikkate alındığında, bu türün Türkiye'deki varlığı şüpheli görünmektedir. Bununla birlikte, *C. hemipterus*'un tropikal ve subtropikal bölgelerde yaygın olduğu, fakat Orta Doğu, Rusya, Fransa, İtalya ve Avustralya gibi ülkelerde de görüldüğü ifade edilmiştir (Dang ve ark. 2021; Topluoğlu ve ark. 2023). Türkiye'de yayınlanan klasik entomoloji ve artropodoloji kitaplarında (Mimioğlu 1973; Unat ve ark. 1995; Nalbantoğlu 2015; Dik 2015) genellikle *C. lectularius* ve *C. hemipterus* hakkında bilgi verilmiştir.

Cimex hemipterus'un kökeni belirsiz olmakla birlikte *C. lectularius*'un Ortadoğu'da, mağaralarda yaşayan insan ve yarasalardan köken aldığı kaydedilmiştir (Krinsky 2002; Giorda ve ark. 2013). Mısır'da, Tell el-Amarna'da yapılan kazılardan elde edilen sonuçlara göre ***Cimex lectularius***'un en azından 3500 yıldan fazla bir süredir insanları rahatsız ettikleri bildirilmiştir (Panagiotakopulu ve Buckland 1999; Giorda ve ark. 2013). Bu türün milattan önce (MÖ) 400'de Yunanistan'da, milattan sonra (MS) 77 yılında İtalya'da, 11. yüzyılda Almanya'da, 13. yüzyılda Fransa'da ve 1583 yılında da İngiltere'den kaydedildiği bildirilmiştir (Krinsky 2002). İkinci Dünya savaşı öncesinde endemik olan tahtakurusu salgınları, 1945 yılından sonra, sosyal ve ekonomik gelişmelerin ve özellikle de DDT gibi yeni ve etkili insektisitlerin bulunmasıyla birlikte azalmıştır (Giorda ve ark. 2013). *Cimex lectularius* ve *C. hemipterus* kaynaklı tahtakurusu istilası 1990'ların sonunda Avrupa, ABD ve Avustralya'da neredeyse eşzamanlı olarak başlamıştır (Davies ve ark. 2012). ABD (Sabou ve ark. 2013; Singh ve ark. 2013; Goddard 2014; Singh ve ark. 2017; Abbar ve ark. 2020; Dang ve ark. 2021), Fransa (Sabou ve ark. 2013; Chebbah ve ark. 2021), Almanya, İspanya ve İtalya (Giorda ve ark. 2013; Sabou ve ark. 2013), İngiltere (Naylor ve Boase 2010) ve Slovakya (Totkova ve ark. 2019) gibi Avrupa ülkelerinde, İran'da (Berenji ve ark. 2019), Avustralya (Sabou ve ark. 2013; Dang ve ark. 2014; 2015), Afrika, Endonezya, Malezya, Singapur, Tayland, Orta Avrupa, Rusya (Dang ve ark. 2021), Çin (Wang ve ark. 2013; Dang ve ark. 2021) ve Kore (Sabou ve ark. 2013)'de de tahtakurusu istilası bildirilmiştir. Avustralya'da, 1999-2006 yılları arasındaki tahtakurusu salgınlarının 1999 öncesine göre %4500, ABD'de 2002 ve 2003'te %500 artış gösterdiği ve 1998'den sonra 50'den fazla ülkede tahtakurusu enfestasyonlarının arttığı bildirilmiştir (Topluoğlu ve ark. 2023). Slovakya'da 1970 ve 1980'li yıllarda sporadik olarak görülen tahtakurusu istilasının daha sonra büyük bir halk sağlığı sorunu haline dönüştüğü belirtilmiştir (Totkova ve ark. 2019).

Türkiye'de tahtakurusu istilasıyla ilgili sorunların olduğu, sporadik kanıtlar olmasına rağmen, bu konuda yeterli bilginin bulunmadığı ifade edilmiştir (Topluoğlu ve ark. 2023). Ülkemizin birçok ilinde sık görülmeye başlanan tahtakurusu (*C. lectularius*) salgınları son yıllarda Konya'da ve diğer bazı illerimizde de yaygın olarak görülmeye başlanmıştır.

Bu makalede *C. lectularius* ve kısmen *C. hemipterus* hakkında genel bilgi verilecek ve özellikle tahtakurusu mücadelesi üzerinde durulacaktır.

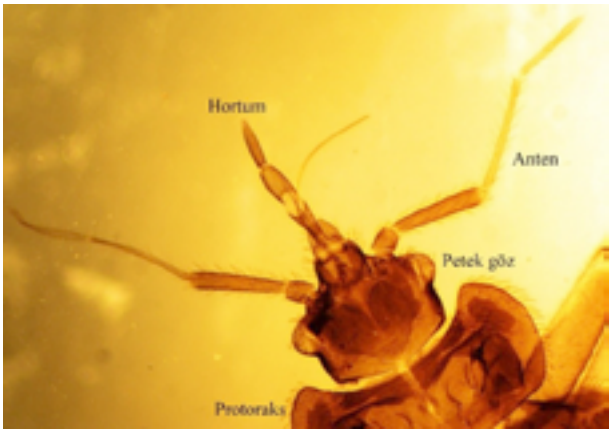
Cimex lectularius **Linnaeus**

Cimex lectularius kanatsız olup, vücut yukarıdan-aşağıya basıktır. Ortalama uzunlukları 4–5 mm olmakla birlikte, 7 mm'ye kadar çıkabilir. Vücutları çok geniştir. Kan emmemiş örnekler soluk sarı-kahverengi iken, kan emen örnekler kırmızımsı-kahverengi veya koyu kahverengi renktedirler (Kettle 1993; Krinsky 2002; Dik 2015; Beugnet ve ark. 2021; Hamlili ve ark. 2023) (Şekil 1A). Erkek ve dişi morfolojik olarak birbirine benzer. Bununla birlikte; dişide, 5. abdominal segmentteki paragenital sinüs, erkekte

ise son abdomen segmentinde yer alan paramerler erkek ve dişinin ayrılmasını kolaylaştırır (Şekil 3).

Baş beşgenimsidir ve öne doğru daralarak uzamıştır. Vücuda oranla oldukça küçüktür. Ağız organları delici ve emici tipte olup, hortum (rostrum) şeklinde uzamıştır. Hortum üç segmentlidir ve dinlenme sırasında, ventralde geriye doğru bükülmüştür. Petek gözler az sayıda fasetten meydana gelmiştir ve başın iki yanında belirgin olarak yer alırlar. Ocelli yoktur. Antenler dörder segmentlidir. İlk segment çok kısa olup, diğerlerine oranla daha kalındır. Son üç segment ince ve uzundur (Şekil 4).

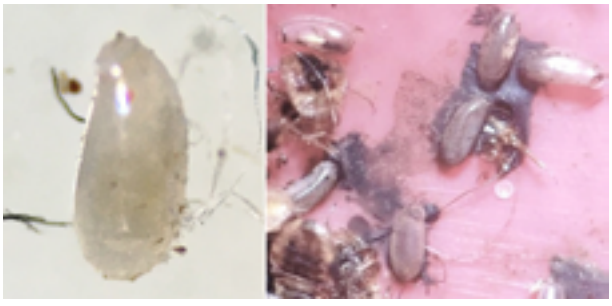
Şekil 3. *Cimex lectularius*; erkek (solda), dişi (sağda), (antenlerin 3. ve 4. segmentleri kopmuş), orijinal



Şekil 4. *Cimex lectularius*; baş ve protoraks, orijinal

Toraks kısadır ve başa oranla daha geniştir. Protoraks anteriorda iç bükey, lateralde ise dış bükeydir (Şekil 4). Antero-lateralde öne doğru uzayarak kısmen başı kuşatmıştır. Üç çift bacaktan kısa olan birinci çift öne doğru uzamıştır. İkinci ve üçüncü çift bacaklar daha uzundur ve arkaya doğru uzamıştır. Ayak uçlarında bir çift ince tırnak bulunur. Kanatları yoktur (Kettle 1993; Krinsky 2002; Dik 2015).

Abdomen geniştir ve 11 segmentten meydana gelmiştir. Bununla birlikte ilk iki ve 9-11. segmentlerdeki kaynaşma nedeniyle sekiz segment (2-9) görülebilir. Erkekte, abdomenin posteriorunda, koyu renkte, içe doğru bükülmüş paramerler bulunur (Kettle 1993; Krinsky 2002; Dik 2015).



Şekil 5. *Cimex lectularius* yumurtaları, orijinal

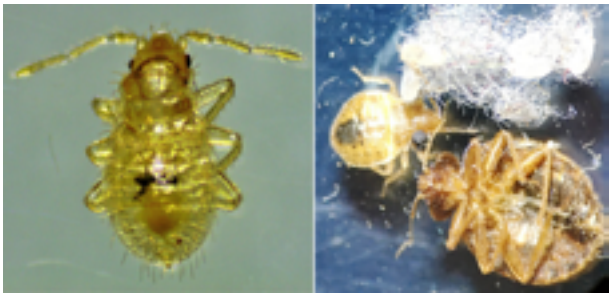
Cimex hemipterus genel olarak *C. lectularius*'a benzemekle birlikte, pronotumun daha dar olmasıyla *C. lectularius*'dan ayrılır (Krinsky 2002).

Cimex lectularius ovipardır. Dişiler çiftleştikten sonra kan emerler. 3-6 gün içinde yumurtlamaya başlarlar (Krinsky 2002) ve kuytu yerlere yaklaşık olarak 150–200 tane yumurta bırakırlar (Şekil 5). Yumurtlama işi birkaç gün sürer. Ovipozisyon ve bırakılan yumurta sayısı kan emme sıklığına göre değişir. Sıcaklık ve nem oranına göre değişmekle birlikte, dişiler her 3-4 günde bir kan emerler ve her hafta ortalama 3-8 yumurta bırakırlar. Bazı dişilerin günde 12, ömürleri boyunca 540 kadar yumurtladıkları gözlenmiştir (Krinsky 2002). Yumurtalar yapışkan bir sıvı ile yapıştırılırlar. Yumurtaların gelişimleri ve nimflerin çıkış süresi sıcaklıkla yakından ilgilidir. Yumurtalardan 23°C'de 3–14 gün, 30–35°C'de ise 4–5 günde nimfler çıkarken, 13°C'nin altındaki ve 37 °C'nin üstündeki sıcaklıklarda yumurtalar açılmazlar. Yumurtalar 13°C'nin altındaki sıcaklıklarda üç ay canlı kalabilirler. Bu durum soğuk kış aylarında tahtakurularının nesillerini devam ettirebilmeleri açısından önemli bir noktadır (Kettle 1993; Dik 2015; Beugnet ve ark. 2021).

Cimex lectularius yarı başkalaşıma sahip bir böcek olduğu için yumurtadan birinci dönem nimf çıkar (Şekil 6). Yumurtalardan çıkan nimfler erginlere benzerler ve beş kez gömlek değiştirdikten sonra olgun hale geçerler (Şekil 6 ve 7). Nimfler de kan emerek beslenirler. Biyolojik çember 1–3 ayda tamamlanır (Dik 2015). Gelişmeleri için en uygun (Optimal) sıcaklık 30°C'dir ve bu sıcaklıkta yumurtadan ergin hale geçinceye kadar ki süre 24 gündür. Bu süre 18°C'de 128 güne kadar çıkar. Nemin gelişme

üzerine etkisi ya çok azdır ya da hiç yoktur (Krinsky 2002).

Cimex lectularius uzun ömürlüdür. Nimfler düşük sıcaklıkta, beslenmeden 5-6 ay canlı kalabilirler. Erginler ise daha uzun süre canlı kalabilirler (Krinsky 2002). Açlığa uzun zaman dayanabilirler. Genellikle gece kan emmelerine karşılık, gündüzleri de konaklarına hücum edebilirler (Dik 2015). Kan emmeden bir yıl canlı kalabilirler (Beugnet ve ark. 2021).



Şekil 6. *Cimex lectularius*; 1. dönem nimf (solda), yumurta, nimf ve ergin (sağda), orijinal

Şekil 7. *Cimex lectularius*; kan emmiş ergin (solda) ve değişik nimf dönemleri (1.dönem nimf en sağda), orijinal

Epidemiyoloji

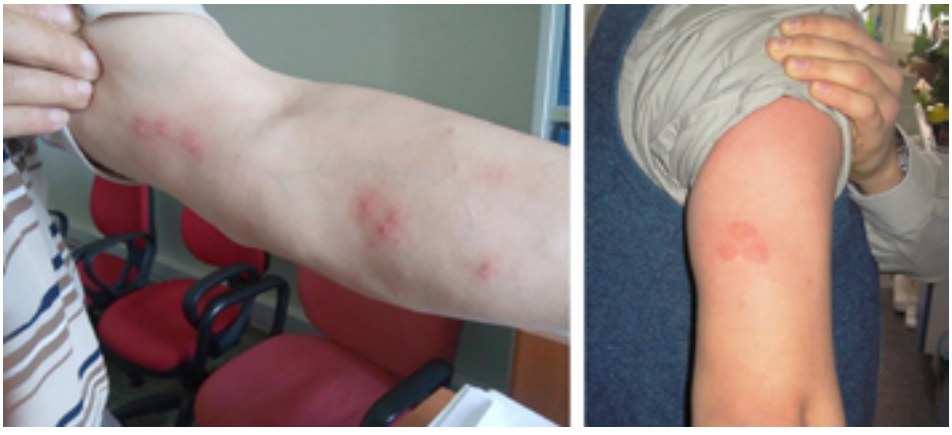
Cimex lectularius ve *C. hemipterus* kozmopolit türlerdir. *Cimex lectularius* Antarktika dışında, tropikal bölgeler ağırlıklı olmak üzere, dünyanın her yerinde görülmektedir (Hamlili ve ark. 2023). Tahtakuruları geceleri konaklara saldırarak kan emerler. Gündüzleri kuytu yerlerde, duvarların yarık ve çatlaklarında, tahta kanepelerin, koltukların veya buna benzer eşyaların altlarında, dikiş yerlerinde, şiltelerde vb yerlerde saklanırlar. Tiyatrolarda, ofislerin bekleme salonlarında ve otobüs koltuklarında da görülürler (Krinsky 2002, Naylor ve Boase 2010). Tahtakuruları bulundukları ortamlarda karakteristik tatlımsı bir koku salgılarlar. Bu koku, o ortamda tahtakurusu olduğunu gösterir (Krinsky 2002). Tahtakuruları ergin ve gelişme dönemleriyle bulaşmış eşyaların taşınmalarıyla veya yukarıda belirtilen ortak kullanım alanlarının kullanılmasıyla bir yerden bir başka yere taşınırlar. Tahtakurularının konak seçicilikleri yoktur. Bu nedenle hem insanlardan hem de memeli ve kanatlı hayvanlardan kan emebilirler. Hijyen kurallarının yeterli olmadığı ortamlarda tahtakurularına sık olarak rastlanmaktadır (Dik 2015). İnsanların gelir seviyelerinin artması veya Erasmus öğrenci değişim programları gibi imkanların ortaya çıkması ile ülke içi veya uluslararası seyahatler artmaktadır. Turizm veya eğitim amaçlı seyahatlerin artması tahtakurularının yayılmasına neden olmaktadır (Haberkorn ve ark. 2023).

Son yıllarda, gerek Konya'da, Selçuklu başta olmak üzere, merkez ilçelerden ve gerekse diğer illerden tarafıma gelen şikayetler ve gönderilen örnekler, ülkemizde de *C. lectularius* kaynaklı tahtakurusu salgınlarında büyük bir artış olduğunu göstermektedir. Öğrencilerin yoğun olarak yaşadıkları Bosna-Hersek Mahallesi'ndeki öğrenci evlerinde ve bazı yurtlarda tahtakurusu sorunu olduğu, bana iletilen bildirimlerden, getirilen veya gönderilen böceklerin *C. lectularius* olarak teşhis edilmesinden anlaşılmaktadır. Bu olgularda; tahtakurularının istila ettiği evlerin öğrenciler tarafından farkında olmadan kiralanmasının veya bu evlerde genellikle ikinci el eşyaların kullanılmasının önemli rol oynadığı gözlenmiştir. Yurtlarda ise, bazen sığınmacıların, bazen depremzedelerin kalmalarının, kaldıkları süreler içinde hijyen kural-

larına tam olarak uyulamamasının veya yurtların boşaltılmasından sonra, insektisitlerin yeterince ve istenilen şekilde uygulanamamasının veya bunun için yeterli sürenin olmamasının tahtakurularının yayılmasında etkili olduğu gözlenmiştir.

Patogenez

Cimex lectularius geceleri konaklarına saldırarak insanlardan, sıcakkanlı hayvanlardan, kuşlardan ve yarasalardan kan emer. Kan emdikleri yerlerde bir hafta kadar süren kızarıklık, kaşıntı ve şişlikle karakterize (Şekil 8), seyrek olarak da sistemik alerjik lezyonlar şekillenir (Goddard 2014; Dik 2015; Berenji ve ark. 2019). *Cimex lectularius* ve *C. hemipterus'*da Hepatit-B, HIV, *Borrelia recurrentis, B. duttoni, Coxiella burnetii, Bartonella quintana* ve *Rickettsia rickettsii, Trypanosoma cruzi, Aspergillus* spp. gibi viral, bakteriyel, paraziter ve fungal 40'ın (Berenji ve ark. 2019; Chebbah ve ark. 2021; Sabet ve ark. 2023), hatta 50'nin üzerinde (Hamlili ve ark. 2023) mikroorganizma izole edilmiştir. Dışkılarında da bu virüsün tespit edilmiş olması, virüsün temas yoluyla duyarlı kişilere bulaşabileceği ihtimalini akla getirmektedir. Bununla birlikte bugüne kadar herhangi bir hastalığa vektörlük yaptıkları kanıtlanamamıştır (Krinsky 2002; Dang ve ark. 2021; Hamlili ve ark. 2023).



Şekil 8. *Cimex lectularius*'un kan emmesi sonrası oluşan alerjik lezyonlar, orijinal

Mücadele

Tahtakuruları ile mücadelede oldukça zordur. Özellikle öğrencilerin yurtta kaldığı dönemlerde, insektisit uygulamasının zor ve riskli olması nedeniyle yeterli mücadele bilimsel yaklaşımla yapılamamakta ve bu yüzden de sorun devam etmektedir. Çoğu evlerde ise ya bilinçsiz mücadele yapılmakta ya da bilimsel mücadele yapılmamaktadır. Kullanılan insektisitlerin bir kısmı, tahtakurularının saklandıkları yerlere ulaşamadıklarından veya bazı *C. lectularius* suşlarının dirençli olmalarından dolayı yeterince etkili olmamaktadır. Apartmanlarda veya benzer şekilde çok fazla odaları ve kullanıcıları olan yerleşim yerlerinde, bina sakinlerinin tahtakuruları hakkında yeterince eğitilmemeleri, bilinçlendirilmemeleri, düşük gelirli insanların ekonomik durumları, bazı insanların insektisit uygulamalarına karşı isteksiz olmaları veya evlerinde bulunan tahtakurularını bildirmemeleri, değişik yaşam tarzları insektisit kullanımında aksaklıklara yol açmakta ve bu da mücadelenin başarısını azaltmaktadır (Romero ve ark. 2017). Diğer taraftan, sıklıkla ve yüksek yoğunlukta kullanılan insektisitler insan ve çevre sağlığı açısından risk oluşturmakta, diğer taraftan ekonomik anlamda da yük oluşturmaktadır. Gazete ve televizyon haberlerinden edindiğimiz bilgilere göre insektisit uygulaması sonrasında bazı ölümler de görülmektedir. Konya'nın Karatay ilçesinde, 2024 yılı başlarında, tahtakurusuna karşı, depo zararlılarına karşı kullanılan alüminyum fosfit kullanılması ve sonucunda ölümlerin ortaya çıkması ve başka bir ilimizde ortaya çıkan benzer bir olguda insektisit uygulanan evdeki bir karı kocanın ölmesi buna örnek olarak gösterilebilir.

Tahtakurularıyla mücadelede; fiziksel, kimyasal ve biyolojik mücadele yöntemleri ayrı ayrı veya birlikte kullanılabilir.

Fiziksel mücadele

Fiziksel mücadelede; tahtakurusundan şüphelenildiği zaman eşyaların ve saklanma yerlerinin sık sık kontrol edilmesi, uygun denetim, sık yıkama, dağınıklığı giderme, vakumlama gibi kimyasal olmayan yöntemler, yatakların ve bazaların kaplanması, buharla işleme, yoğun şekilde istila edilmiş eşyaların atılması ve yapısal ısıl işlemlerin hepsi etkilidir; ancak bunlar kalıcı koruma sağlamazlar (Kells 2006; Wang ve ark. 2012; 2015; Singh ve ark. 2017; Abbar ve ark. 2020, Ranabhat ve Wang 2020). Değişik tipte aspi-

ratörlerin, pilli veya elektrikli süpürgelerin kullanılmasının tahtakurularıyla mücadelede yararlı olacağı da bildirilmiştir (Bérenger ve ark. 2015). Çamaşır makinesinde, 60°C'de yıkamanın veya -17°C'de iki saat bekletmenin çamaşırlardaki tahtakurularının bütün dönemlerine karşı etkili olduğu kaydedilmiş, 40°C'de yıkanan veya deterjansız suda 24 saat bekletilen çamaşırlarda ise, yumurtalar hariç diğer gelişme dönemlerinin hepsine karşı etkili olduğu bildirilmiştir (Naylor ve Boase 2010). Tahtakurusu istilasına karşı yüksek sıcaklık kullanımından da yararlanılmaktadır. Bunun için gerekli olan ekipmanların geliştirilmesi bu yöntemin yaygınlaşmasını sağlamıştır. Isıl işlemler, yalıtımlı kutular ve ısıtmalı nakliye römorkları gibi konteynerler içinde uygulanabilir veya ısı doğrudan bir yapı içindeki odalara ve içeriklere verilebilir (Kells ve Goblirsch 2011). Sıcaklığın genel olarak 45-52°C arasında olması tercih edilmekle birlikte, bazen 55-60°C'ye kadar çıkarılabilir. Tahtakurusu mücadelesinde erginler için öldürücü sıcaklığın (LTemp99) 48,3°C, yumurtalar içinse 54,8°C olduğu, 45°C'ye maruz kalan yetişkin tahtakurularının LTime99 süresi 94,8 dakika, yumurtalar için 45°C'de 7 saat, 48°C'de ise 71,5 dakika olduğu saptanmıştır (Kells ve Goblirsch 2011).

Kimyasal mücadele

Kimyasal mücadelede, ilk önceleri arsenik, civa, pretrum, sülfür ve siyanid gazının ve 1940'lı yıllarda DDT'nin kullanıldığı bildirilmiştir (Bayram Delibaş 2017). Daha sonraları Klorlu hidrokarbonlar (DDT, Dieldrin vb), karbamatlı (Carbaryl, Propoxur) organik fosforlu (Diazinon, Dichlorvos, Fenitrothion, Malathion, Pyrimiphos-methyl, Phoxim vb) kullanılmaya başlanmıştır. Yakın yıllarda ise, tahtakurusu mücadelesinde bu insektisitlere ek olarak; Acetamiprid, Thiamethoxam, Imidacloprid ve Dinetofuran gibi neonikotinoidler, Sipermetrin (cypemethrin), Permetrin, Deltametrin ve Cyfluthrin gibi sentetik pretroidler, Chlorfenapyr, Juvenil Hormon Analogları gibi kimyasal insektisitler ve diyatomlu toprak, sedir yağı (Ceddar oil) gibi bazı doğal ürünler de kullanılmıştır (Goddard 2014, Wang ve ark. 2015). Sentetik pretroitli ve neonikotinodli insektisitler halen yoğun olarak kullanılmaya devam etmektedir. Çevre sağlığı ve insektisit çeşitliliği dikkate alındığında, tahtakurusu mücadelesinde, klorlu hidrokarbonlara, karbamatlara ve organik fosforlu insektisitlere karşı direnç gelişmesi, bu insektisitlerin kullanılmalarının yasaklanması veya kalıcı etkilerinin çevre sağlığına zarar vermesi nedeniyle, tahtakurusu mücadelesinde uzun zamandır genellikle sentetik pretroidler veya neonikotinoidli insektisitler, ya ayrı ayrı ya da birlikte kullanılmaktadır (Davies ve ark. 2012; Dik 2015; Wang ve ark. 2015; Haberkorn ve ark. 2023; Yu ve ark. 2023). Diğer ülkelerde de uzun yıllardır tahtakurusu mücadelesinde sentetik pretroidlerden (Cyfluthrine, Deltamethrin, Lambda-Cyhalothrine, Cypermethrin, Alpha-cypermethrin vb) yararlanılmaktadır (Davies ve ark. 2012; Haberkorn ve ark. 2023; Yu ve ark. 2023). Sentetik pretroitli insektisitlere direnç gelişmesi veya mücadelede farklı seçeneklerin denenmesi amacıyla tahtakurusu mücadelesinde neonikotinoidli (Acetamiprid, Thiamethoxam, Imidacloprid, Dinetofuran) insektisitler de kullanılmaya başlanmıştır. Sentetik pretroitlerin neonikotinoidlerle birlikte kullanılmasının, sentetik pretroitli insektisitlerin yalnız kullanılmasından daha etkili olduğu ifade edilmiştir (Yu ve ark. 2023). Bu insektisitler tahtakurularının saklandıkları yerlere serpme, püskürtme, reçineli şerit veya tütsü (fumigasyon) tarzında uygulanırlar (Wang ve ark. 2015). Toz şeklinde kullanılan insektisitlerin püskürtme tarzında kullanılan insektisitlere oranla daha etkili olduğu, toz insektisitlerin kalıcı etkilerinin daha fazla olduğu kaydedilmiştir (Abbar ve ark. 2020; Singh ve ark. 2016; Singh ve ark. 2017; Ranabhat ve Wang 2020). Toz halinde 4.9 g/m2 dozda birçok kez kullanılan %1'lik Cyfluthrin'in (Tempo 1% Dust, Bayer Environmental Science) tahtakurularına karşı püskürtme tarzında kullanılan insektisitlere ve sentetik pretroidlere dirençli tahtakurularına karşı daha etkili olduğu belirlenmiştir (Singh ve ark. 2016).

Avustralya'da, *C. hemipterus*'a karşı 110 mg/m^2 Pesquard FG161 (% 4.4 d-tetramethrin & (%13.2) cyphenothrin) ve Sumithrin 10SEC (%10 d-phenothrin)'e karşı yüksek direnç, Tandem (thiamethoxam [11.6%], lambda-cyhalothrin [3.5%]) 183.96 mg/m^2; Temprid SC (imidacloprid [21%], beta-cyfluthrin [10.5%]) 106.13 mg/m^2 ve Sumithion 20CS (fenitrothion [20%]) 250 mg/m^2'a ise düşük ve yüksek direnç saptanmıştır (Leong ve ark. 2020). Yine Avustralya'da *C. hemipterus* ve *C. lectularius*'un değişik suşlarına karşı deltametrin ve imidaclopridin etkinlikleri araştırılmış, *C. lectularius*'ta deltametrine karşı yüksek bir direncin gözlendiği ve etkisinin %15-100 arasında değiştiği, imidaclopride karşı direncin değişiklik göstermekle birlikte, etkisinin %95'in üstünde olduğu belirtilmiştir (Lilly 2017). Dinetofuran (%0.25) ve toz halinde diyatomlu toprağın (%95) (Alpine aerosol ve Alpine dust) uygulandığı düşük gelirli evlerde ergin tahtakurularına karşı etkisinin altı aylık kullanım sonunda %96.8 olduğu kaydedilmiştir. Laboratuvar çalışmalarında, doğrudan uygulanan Alpine aeorosolün nimflere %100, yumurtalara %91.3 oranlarında etkili olduğu kaydedilmiştir (Singh ve ark. 2013). ABD, New Jersey'de, tahtakurularına karşı

Tandem (3.5% lambda-cyhalothrin, 11.6% thiamethoxam, Syngenta Crop Protection, Greensboro, NC, USA), Temprid SC (10.5% beta-cyfluthrin, 21% imidacloprid, Bayer Environmental Science, Research Triangle Park, NC, USA), Transport Mikron (6% bifenthrin, 5% acetamiprid, FMC Corporation, Philadelphia, PA, USA)'un etkileri araştırılmış, sekiz hafta sonunda bifenthrin+acetamiprid karışımının %98'in üzerinde etkili olduğu, onu %89 ile lambda-cyhalothrin ve %87 ile beta-cyfluthrin, 21% imidacloprid'in izlediği bildirilmiştir (Wang ve ark. 2013).

Böcek mücadelesinde CO_2 de kullanılmaktadır (Wang ve ark 2012). *Cimex lectularius* mücadelesinde karbondioksitin (CO_2) etkisinin araştırıldığı bir çalışmada; tahtakurusunun bütün gelişim dönemleri üzerine öldürücü etki gösterebilmesi için CO_2'in 25°C'de, en az %30 yoğunlukta ve 24 saat kullanılması, yumurtaların %100 öldürülmesi için CO_2'in %100 yoğunlukta 20, 25 ve 30°C'de, sırasıyla 3, 7 ve 8 saat, erkek ve nimfleri öldürmek içinse, aynı sıcaklıklarda sırasıyla 8, 13 ve 14 saat fumigasyona tabi tutulması gerektiği bildirilmiştir (Wang ve ark 2012). Aynı araştırmada, 23-24°C'de, fumigasyon veya kuru buz şeklinde kullanılan CO_2'in kumaş vb malzemelerle dolu kutu veya sızdırmaz torbalarda saklanan tahtakurularının bütün gelişme dönemlerine karşı %90 etkili olduğu belirtilmiştir (Wang ve ark 2012).

Tahtakurusu mücadelesinde sulfuryl fluoride'in de fumigasyon tarzında kullanılabileceği ifade edilmiş, 25°C'de, 103,7 g-h/m3'lük dozda ergin ve ileri dönem nimflere %100 etkili bulunmuştur (Phillips ve ark. 2014). Bununla birlikte, bu insektisitin kullanılması için eğitimli personele ve özel ekipmana gereksinim duyulmasının dezavantaj olduğu ifade edilmiştir (Bayram Delibaş 2017).

Methopren ve Hydropren gibi kitin sentezi inhibitörlerinin (Insect Growth Regulator) tahtakurularının nimf dönemlerine karşı etkili olabileceği, fakat erginlerine karşı etkisiz olduğu ve etkilerinin yavaş olduğu kaydedilmiştir (Bayram Delibaş 2017).

İnsanlarda, tedavi dozunda kullanılan moksidektin ve ivermektinin tahtakurularında morbidite ve mortaliteye sebep oldukları, ivermektinin tahtakurularını tamamen ortadan kaldırmadığı, daha yüksek ve daha çok uygulamaya gerek duyulabileceği, moksidektinin ise ABD'de, FDA (Food and Drug Administration) tarafından insan kullanımında ruhsatlandırılmadığı bildirilmiştir (Sheele ve ark. 2017).

Doğal ürünlerle mücadele

Tahtakurusu mücadelesinde, kimyasal insektisitlerin yanı sıra, çevre sağlığı açısından daha güvenli olan silikon dioksid (silisyum dioksid) ve diyatomlu toprak gibi doğal ürünler (yeşil ürünler) de kullanılmaya başlanmıştır (Kells 2006). Bunlar sınıflarda, gündüz bakım merkezlerinde, huzurevlerinde, hastanelerin belirli alanları gibi hassas yerlerde kullanılabilirler (Goddard 2014). Diyatomlu toprak suda yaşayan organizmaların, alglerin fosilleşmiş kalıntılarından oluşan doğal bir üründür (Goddard 2014, Kerdsawang ve ark. 2023). Silikon dioksid (Silicone dioxide) bazlı (SiO_2) insektisit tozlar memeliler için düşük toksisiteye sahip olup, kuru ortamlarda kalıcı etkileri uzun sürmekte ve sentetik pretroidli insektisitlere karşı dirençli tahtakurularına etkili olmalarından dolayı tahtakurusu mücadelesinde kullanılmaktadır (Singh ve ark. 2016; Abbar ve ark. 2020; Kerdsawang ve ark. 2023). Toz halinde kullanılan Silica jelin, tahtakurularına etkisinin bir gün sonra %95, beş gün sonra %96.7, 10 gün sonra ise %100 olduğu bildirilmiştir (Singh ve ark. 2016). ABD'de yapılan bir çalışmada, silikon dioksitli tozların *C. lectularius*'a karşı etkisinin 72 saat sonra %90'nın üzerinde olduğu belirtilmiştir (Yu ve ark. 2023). Başka bir araştırmada; birisi duyarlı diğeri dirençli iki *C. lectularius* suşuna karşı laboratuvar şartlarında kullanılan 0.1 g/L ve 0.05 g/L silica dioksidin (ChinChex®: Silicon dioxide %55, amorphous silica %45) tam etkili olduğu ve 21 gün içinde tahtakurularını tamamen ortadan kaldırdığı kaydedilmiştir (Kerdsawang ve ark. 2023). Silikon dioksit, sentetik pretroid ve piperonyl butoxide veya karbondioksit ile karıştırılarak kullanıldığında da tahtakurularına karşı yüksek etkili bulunmuştur (Abbar ve ark. 2020).

Biyolojik mücadele

Biyolojik mücadelede mantarlardan yararlanılmaktadır. Bunlardan en önemlisi *Beauveria bassiana*'dır. Bu mantarın özellikle piretroidlere dirençli tahtakurusu suşlarına karşı kullanılabileceği belirtilmiştir (Bayram Delibaş 2017). *Beauveria bassiana*'nın duyarlı ve pretroidiere dirençli suşlarına karşı %94 oranında etkili olduğu ve bu etkinin ortalama dört gün sonra ortaya çıktığı belirtilmiştir (Barbarin ve ark.2017). *Wolbachia*'nın da tahtakurularının büyümelerini yavaşlattığı belirtilmiştir (Bayram Delibaş 2017).

Direnç

Tahtakuruları insektisitlere karşı oldukça dirençlidirler ve hâlihazırda mücadelede kullanılan insektisitlere karşı direnç geliştiği bildirilmiştir (Romero ve ark. 2010; Dang 2014; 2015; Akhtar ve Isman 2016; Lilly ve ark. 2016; Barbarin ve ark. 2017; Vassena ve ark. 2019; Abbar ve ark. 2020; Haberkorn ve ark. 2023, Kerdsawang ve ark. 2023; Yu ve ark. 2023). Son yıl-

larda, *Cimex lectularius* ve *C. hemipterus*'ta karbamatlılara, organik fosforlulara, neonikotinoidlere, fenilpirazollara (phenylpyrazol) ve sentetik pretroidlere karşı sıklıkla direnç görüldüğü rapor edilmiştir (Dang 2014; Goddard 2014; Dang 2015; 2017; Lilly ve ark. 2017; Berenji ve ark. 2019; Abbar ve ark. 2020; Dang 2021, Haberkorn ve ark. 2023; Wang ve ark. 2012; Yu ve ark. 2023). Daha önceleri tahtakurusu mücadelesinde yaygın olarak kullanılan DDT'ye karşı oluşan direncin sentetik pretroitli insektisitlere karşı çapraz dirence yol açmasından dolayı sentetik pretroitli insektisitlere karşı da yüksek oranda direnç gözlenmektedir (Yu ve ark. 2023). Pretroidlere karşı dirençte tek bir genin değil, birden fazla genin baskılamasının rol oynadığı ve sodyum kanal genlerinde iki mutasyon saptandığı ifade edilmiştir (Bayram Delibaş 2017). ABD'nde özellikle deltametrine karşı yaygın bir direnç gözlendiği, sentetik pretroidli ve neonikotinoidli insektisitlere ve bunların kombinasyonuna karşı da tahtakurularında direnç gözlendiği kaydedilmiştir (Yu ve ark. 2023). İnsektisitlere karşı şekillenen direncin davranışsal direnç ve fizyolojik direnç olmak üzere iki şekilde ortaya çıktığı ifade edilmiş ve *C. lectularius*'a karşı oluşan direncin fizyolojik direnç olduğu, davranışa bağlı direncin tespit edilemediği bildirilmiştir (Dang ve ark. 2017, Haberkorn ve ark. 2023). Diğer bir makalede (Dang ve ark. 2021) ise tahtakurularında direnç gelişmesinden hedef bölgenin duyarsızlığı, artan metabolik detoksifikasyon ve penetrasyon direnci olmak üzere üç ana direnç mekanizmasının sorumlu olduğu belirtilmiştir. Sentetik pretroidlere karşı davranışsal direnç, kütiküler direnç, metabolik direnç ve DNA düzeyinde ikame mutasyonlar nedeniyle hedef bölge duyarsızlığı direnci şeklinde direnç şekilleri görüldüğü bildirilmiş (Dang ve ark. 2014; Lilly ve ark. 2016), DDT ve pretroidlere karşı *C. hemipterus*'ta şekillenen yüksek direncin multiple *kdr* mutasyonlarından kaynaklanmış olabileceği belirtilmiştir (Dang ve ark. 2021).

Korunma

Korunmada, özellikle seyahatlerde, otel odalarında tahtakurularının saklanabilecekleri yerlerin gözden geçirilmesi, valiz ve çantaların yatak üzerine veya dolaplara konulmaması, yataklardan, zeminden uzak tutulması, naylon poşetlerle sarılarak olası tahtakurularının valiz ve çantalara girmelerinin önlenmesi, valiz ve çantaların seyahat dönüşü doğrudan banyoda açılmaları, yüksek sıcaklıkta buhara tutulması ve içindeki yıkanabilir eşyaların çamaşır makinesinde, yüksek sıcaklıkta yıkanması önerilmektedir (Valtchev ve Chalakov 2020).

Deney hayvanları kullanımı etik kurulu ve diğer etik kurul kararları ve izinler: Derleme niteliğinde olduğu için herhangi bir izne gerek bulunmamaktadır.

Maddi destek ve çıkar ilişkisi: Çalışmayı maddi olarak destekleyen kişi/kuruluş ve yazarın herhangi bir çıkara dayalı ilişkisi yoktur.

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