

SERUM ZINC AND COPPER CONCENTRATIONS IN RAMS EXPERIMENTALLY INFECTED BY *MYCOPLASMA AGALACTIAE*

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Summary: In this study, 15 Kivircik rams aged 1.5 years, were used. After determining the spermatologic characteristics of all individuals, five of the rams were vaccinated to constitute the control group. *Mycoplasma agalactiae* 99 M (AIK 2) strain was inoculated in all of the rams 34 days after the vaccination. Before the inoculation and 15 days after, semen and blood samples were collected. The agent was isolated in blood on the 7th day after inoculation. Before and after the inoculation, serum zinc and copper levels were measured by using an Atomic Absorption Spectrometry. When copper and zinc level results were evaluated it was observed that, before and after inoculation copper level showed a non-significant decrease whilst zinc level had a significant decrease ($p < 0.05$). In post mortem examination, variable amounts of degeneration in the testes and an increase in the connective tissue of vesiculae seminales were observed. In conclusion it was suggested that, zinc deficiency played an important role in the formation of disease symptoms.

Key words: Mycoplasma infections; *Mycoplasma agalactiae*; semen-analysis; zinc-blood; copper-blood; spectrophotometry, atomic absorption; sheep-male

Introduction

Contagious agalactia in sheep and goats is a very contagious, acute, subacute or chronic disease (1). Aytuğ and others (2) reported that the disease could be seen in most European, Mediterranean, middle East and African countries. Mycoplasma infection is characterised by mastitis, polyarthritis and keratoconjunctivitis lesions (2, 3, 4, 5). In Spain (6), in a herd of 200 sheep, 20 % had uni- or bilateral keratoconjunctivitis and *Mycoplasma agalactiae* was isolated from conjunctival fluids collected from 20 ewes and 3 lambs.

This infection occurs in animals at every age but advanced pregnant and lactating animals are more susceptible (2, 4, 7, 8). It is reported that the incubation period of the disease is 1-2 weeks and recovered animals continue to be carriers for seven months (2, 4, 9)

Zavagli and others (10) reported that the

disease causes no clinical finding in rams and bucks but Philpott (11) claimed that it makes balanoposthitis. Foggie and others, (12) isolated the agent from the testes of experimentally infected rams.

The principal way of diagnosis is agent isolation, however, complement fixation and indirect hemagglutination tests are commonly used (2, 9, 13, 14). Some researchers (15, 16, 17, 18, 19) suggested, ELISA was more successful in the diagnosis of subclinic mycoplasma infections. Recently PCR has been used to search *M. agalactiae* in sheep milk samples (20).

While Nelson and others (21) reported serum zinc levels of 4.86-6.08 $\mu\text{mol/l}$ in a herd which suffered from cases of anorexia, wool eating, alopecia, hyperkeratosis and parakeratosis, Çamaş and others (22) have reported a level of 3.80-7.30 $\mu\text{mol/l}$ for similar symptoms.

In ruminants, normal serum zinc levels are between 11 and 18 $\mu\text{mol/l}$, and animals with levels below 10.5 $\mu\text{mol/l}$ are considered deficient (23). In Spain, values below 9.2 $\mu\text{mol/l}$ are considered low for ovine, whereas levels above 12.2 $\mu\text{mol/l}$ are

considered normal (24).

Normal serum copper levels for sheep as reported by various researchers (25, 26) are 9.26-15.86 $\mu\text{mol/l}$ and 12.56-18.84 $\mu\text{mol/l}$, respectively.

Deger and others (27) carried out a study to investigate changes in serum concentration of copper, zinc, and calcium in sheep naturally infested with lice. In their study, the concentrations of these elements in the infested animals were lower than in the healthy controls.

A study was carried out to determine the levels of trace elements (zinc, iron, copper), as well as of lead and cadmium, in both the soil and in organism in order to obtain more efficient economical results and healthier sheep breeds in Northwest Turkey by Or and others (28). In this study, while low zinc levels were determined in some districts, low copper levels were determined in full blood of 400 sheep from almost all districts of Northwest Turkey. The lowest values determined in copper in above study prove how serious the problem is in Northwest Turkey.

This study was performed to find out a possible relationship between zinc and copper trace element levels and clinical findings, specially genital defects (testis degeneration, spermatological differences, connective tissue increase in V.seminalis) in rams that experimentally infected by *M. agalactiae*.

Material and methods

The material of the study was 15 Kivırcık rams aged 1.5 years, which were free from *M. agalactiae* and were maintained under the same conditions in the Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Istanbul University. The rams were treated for parasites for one month and were infected by a *M. agalactiae* 99 M (AIK 2) strain which was obtained from Pendik Veterinary Control and Research Institute. Every ram received 3.7×10^6 /ml colony microorganism subcutaneously.

After determining the spermatologic characteristics of all individuals, five of the rams were vaccinated to constitute the control group. *Mycoplasma agalactiae* 99 M (AIK 2) strain was inoculated in all of the rams 34 days after the vaccination.

Before vaccination and inoculation, six ejaculate samples were collected from each ram. Two ejaculates were discarded to minimise the effect of sexual rest and the remaining four ejaculates were evaluated to determine the spermatological characteristics. The rams were

clinically observed for a 15 day incubation period. They were ejaculated by means of an electro ejaculator for nine times during this period. Seminal volume, mass activity, motility, concentration and morphological evaluations were carried out.

M. agalactiae isolation was carried out according to the literature (29) on semen, blood and organs after slaughter.

Blood samples were collected from each ram prior to inoculation and 15 days after inoculation. 10 cc blood was taken from V.jugularis into tubes without anticoagulant, kept at room temperature for 30 minutes and serum was separated by centrifuging at 5000 rpm for 3-5 minutes. These sera were stored at -20°C until analysis.

Serum copper and zinc measurements were carried out with a Shimadzu Atomic Absorbtion Spectrometry model AA-680 in accordance with the technique described in the references (30, 31). In order to prevent contamination from glassware, plastic materials were used during the measurements of trace elements.

For copper measurements, 0.5 $\mu\text{g/ml}$ and 1 $\mu\text{g/ml}$ copper standard solutions were prepared from 9987 Titrisol 1000+0.002 gram Copper ($\text{CuCl}_2 + \text{H}_2\text{O}$) Merck (E.Merck, D-6100 Darmstadt, Germany) standard stock solution. Bidistilled water was used as a blank solution. The device was operated to a 324.8 nm wave length, an air-acetilen gas mixture (8/1.8), a slit space 0.5 nm and B.G.C. mode were chosen. Blank and standard solutions were given to the device under these conditions. Then samples to be measured were given to the device and Copper concentrations were measured.

For zinc measurements, 0.5 $\mu\text{g/ml}$ and 1 $\mu\text{g/ml}$ zinc standard solutions were prepared from 9953 Titrisol 1000+0.002 gram zinc ($\text{ZnCl}_2 + \% 0.06 \text{ HCl}$) Merck (E.Merck, D-6100 Darmstadt, Germany) standard stock solution. Bidistilled water was used as a blank solution. The device was operated to a 213.9 nm wave length, an air-acetilen gas mixture (8/2.0), a slit space 0.5 nm and B.G.C. mode were chosen. Blank and standard solutions were given to the device under these conditions. The samples were given to the device like copper measurements and zinc concentrations were measured.

All rams were killed 79 days after inoculation and bacteriological and histopathological examination, of the samples taken from the testes, cauda epididymis, vesiculae seminalis and synovial fluids were carried out according to the literature (29).

Statistical evaluations of serum zinc and

Table 1: Mean values and standard deviations of serum Cu and Zn levels of control and treatment group rams

Parameter/Unit	Control (n=5)				Treatment (n=10)			
	Before		After		Before		After	
	Inoculation		Inoculation		Inoculation		Inoculation	
	X ± SD		X ± SD		X ± SD		X ± SD	
Copper (µmol/l)	13.22	0.43	13.14	0.39	13.46	0.48	12.85	0.45
Zinc (µmol/l)	9.91	0.24	9.90	0.27	9.44	0.25	8.39*	0.28

* $p < 0.05$

copper element levels were done by student t test and of spermatological characteristics by Duncan test (32).

Results

Clinical results

No clinical effects except a slight temperature rise were observed in rams infected by *M. agalactiae*.

Blood serum results

Serum zinc and copper levels of infected rams before and after inoculation and their statistical evaluations are presented in Table 1.

When copper and zinc level results were evaluated it was observed that, before and after inoculation copper level showed a non-significant decrease whilst zinc level had a significant decrease ($p < 0.05$).

Spermatological results

Characteristics of the control and treatment groups before and after inoculation are presented in Table 2.

At the comparison of average results, volume, mass activity, motility and concentration values of the treatment group decreased and the morphological defect ratio, specially head defects were observed to be increasing ($p < 0.05$).

Microbiological results

The agent was not isolated from blood and

semen samples collected before inoculation. The agent was isolated from heparin containing blood and semen samples collected from the 7th day post inoculation and cultured on PPLO media. No agent was isolated from the blood and semen of the control group. The agent was isolated from the testes, epididymis, V.seminalis and synovial fluids of the slaughtered rams. Tests for identification confirmed that the agent was *M. agalactiae*.

Histopathological results

At post-mortal macroscopic examinations, although the testes of the rams in treatment and control groups were normal in size, some of the treatment rams' testes were observed to be softer.

When the preparations from different testes regions of all rams were examined, dystrophic changes of various degree were observed in all testes.

Discussion

M. agalactiae infection is an economic disease of sheep and goats characterised by mastitis, polyarthritis and ceratoconjunctivitis (2, 3, 4, 8, 9). Zavagli and others (10) reported that the disease showed no symptoms in rams and bucks, also showed that rams are more resistant to experimental infections (33). In our study, we agreed with the above researches because no animal showed any symptom other than a slight temperature rise. In accordance with previous studies (2, 4, 9, 33, 34) we can isolate the agent from blood, testes, epididymis, V.seminalis and synovial fluids.

Burnet (35), in his early studies reported delay in growth, testicular atrophy, hyperkeratosis and

Table 2: Spermatological-morphological characteristics of control and treatment rams

Parameter/Unit	Control (n=5)				Treatment (n=10)			
	Before		After		Before		After	
	Inoculation		Inoculation		Inoculation		Inoculation	
	X	± SD	X	± SD	X	± SD	X	± SD
Volume (ml)	0.89 ^a	0.61	0.97 ^a	0.12	0.94 ^a	0.34	0.74 ^b	0.39
Mass activity (0-4)	3.45 ^a	0.51	3.06 ^a	0.62	3.64 ^a	0.41	2.32 ^b	0.91
Motility	87.25 ^b	4.99	75.44 ^a	10.96	90.54 ^a	4.23	54.63 ^b	21.12
Concentration (x10 ⁹ /m)	1.87 ^a	0.56	2.01 ^a	0.86	2.02 ^a	0.33	1.39 ^b	0.68
Head (%)	8.90 ^a	3.51	8.67 ^b	3.75	8.27 ^a	2.99	12.34 ^a	6.24
Mid Piece (%)	1.90 ^a	1.92	2.18 ^a	2.53	1.36 ^a	1.42	1.68 ^a	1.79
Tail (%)	5.75 ^a	2.86	10.27 ^a	5.20	4.21 ^b	2.00	10.75 ^a	5.88

a,b: The differences between the means of characters in each row that contain different letters are significant ($p < 0.05$)

anorexia with zinc deficiency, he also claimed that immune response is affected seriously by zinc deficiency. In our study, there was a considerable decrease (8.39 $\mu\text{mol/l}$) in the experimental incubation period in the treatment group and we supposed it was due to the degeneration in testicular tissue and defects in spermatogenesis.

Researchers (36) reported anemia, neutropenia, collagen and elastin formation defects in connective tissue, skeletal and cardiovascular lesions, neural demyelination degeneration and neonatal ataxia at a copper deficiency. In our study we investigated copper in relation to arthritis which is one of the main symptoms of the disease, but there was no arthritis in the treatment group and in accordance with this situation no significant decrease in copper levels were observed.

At the end of the study, it is concluded that Zn deficiency was important in the formation of symptoms.

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KONCENTRACIJE CINKA IN BAKRA V SERUMU OVNOV, EKSPERIMENTALNO OKUŽENIH Z *MYCOPLASMA AGALACTIAE*

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Povzetek: V študiji smo uporabili 15 leto in pol starih ovnov pasme kivircik. Vsem smo določili spermatološke lastnosti, nato pa 5 ovnov zaščitno cepili, da so predstavljali kontrolno skupino. 34 dni po cepljenju smo vse živali okužili z mikoplazmo *Mycoplasma agalactiae* sev 99 M (AIK 2). Pred okužbo in 15 dni po njej smo živalim vsak dan jemali vzorce sperme in krvi. Mikoplazme smo izolirali iz krvi sedmi dan po okužbi. V krvnem serumu smo z atomsko absorpcijsko spektrometrijo določali raven bakra in cinka. Ko smo primerjali koncentracijo obeh elementov pred okužbo in po njej, smo ugotovili neznačilen padec vsebnosti bakra in velik padec vsebnosti cinka ($p < 0.05$). Pri posmrtnem pregledu smo ugotovili različne stopnje degeneracije v modih in zvečanje količine vezivnega tkiva v mehurnici. Ugotavljamo, da ima pomanjkanje cinka pomembno vlogo pri oblikovanju simptomov bolezni.

Ključne besede: mikoplazma infekcije; *Mycoplasma agalactiae*; sperma-analize; cink-kri; baker-kri; spektrofotometrija, atomska absorpcija; ovce; samci