

**XIV. SIMPOZIJ
PERADARSKI DANI 2022.**
s međunarodnim sudjelovanjem

Hrvatska, Poreč, 11.-14. svibnja 2022.

**XIV SYMPOSIUM
POULTRY DAYS 2022**
With International Participation

Croatia, Poreč, May 11-14, 2022

**ZBORNIK
PROCEEDINGS**

Izdavač / Publisher
Hrvatski veterinarski institut / Croatian Veterinary Institute
Centar za peradarstvo / Poultry Centre

Urednica / Editor
Mirta Balenović

Lektorica i prevoditeljica / Language editor
Antonija Redovniković

Oblikovanje Zbornika i priprema za tisak / Proceedings design and layout
Berislav Jadro

Dizajn naslovnice / Cover design
Mirta Balenović
Eufrazijeva bazilika - mozaik - motiv preuzet od turističke zajednice grada Poreča
www.myporec.com

Tisak / Print
CRESCAT d.o.o., Zagreb

Naklada / Issue
300 primjeraka / 300 copies

SADRŽAJ / CONTENTS

Uvodna riječ / Opening Address

CENTAR ZA PERADARSTVO - OKOSNICA DIJAGNOSTIKE BOLESTI PERADI U REPUBLICI HRVATSKOJ POULTRY CENTRE - THE BACKBONE OF POULTRY DISEASE DIAGNOSTICS IN THE REPUBLIC OF CROATIA Tajana Amšel Zelenika	11
--	----

Usmena izlaganja / Oral presentations

PERADARSTVO TIJEKOM PANDEMIJE COVID-19, EPIDEMIJE HPAI TE IZAZOVI ODRŽIVOSTI I KONKURENTNOSTI SEKTORA U KONTEKSTU EU STRATEGIJE OD FARME DO STOLA I BIORAZNOLIKOSTI POULTRY INDUSTRY DURING COVID-19 PANDEMIC, HPAI, AND CHALLENGES TO SECTOR SUSTAINABILITY AND COMPETITIVENESS IN THE CONTEXT OF THE FARM TO FORK EU STRATEGY AND BIODIVERSITY Branko Bobetić	15
UTJECAJ NAČINA DRŽANJA NA OŠTEĆENJE PRSNE KOSTI KOKOŠI NESILICA EFFECT OF HOUSING ON KEEL BONE DAMAGES IN LAYING HENS Zlatko Janječić, Zlata Kralik, Zdravko Banovec, Dalibor Bedeković	18
PREVALENCE AND SEVERITY OF BREAST SKIN LESIONS IN COMMERCIAL TURKEYS UČESTALOST I TEŽINA OZLJEDA KOŽE NA PRSIMA U PURANA IZ KOMERCIJALNOG UZGOJA Olga Zorman Rojs, Jana Bergoč Martinjak, Hristo Hristov, Nina Mlakar Hrženjak, Alenka Dovč, Zoran Žlabravec, Jožko Račnik, Uroš Krapež, Brigita Slavec	25
KONTAKTNI DERMATITIS I BOLESTI KOŠTANO-MIŠIĆNOG SUSTAVA KAO POKAZATELJ DOBROBITI PILIĆA U TOVU FOOTPAD DERMATITIS AND MUSCULOSKELETAL DISORDERS AS WELFARE INDICATORS OF BROILER CHICKENS Kristina Matković, Danijel Marušić, Srećko Matković, Željko Pavičić, Ivana Sabolek, Marijan Benić, Mario Ostović	31
ZNAČAJNE PARAZITARNE BOLESTI U PERADI - PRIJETNJA PERADARSKOJ PROIZVODNJI SIGNIFICANT PARASITIC DISEASES OF POULTRY - A THREAT TO POULTRY INDUSTRY Tihomir Zglavnik, Tajana Amšel Zelenika, Marijana Sokolović	35
PROIZVODNI POKAZATELJI KOD <i>AD HOC</i> PRIMJENE AUTOGENOG CJEPIVA U PROIZVODNJI NAKON KLINIČKE POJAVE KOLIBACILOZE U JATIMA NESILICA LAKE PASMINE PRODUCTION PARAMETERS AFTER <i>AD HOC</i> APPLICATION OF AUTOGENOUS VACCINE DURING PRODUCTION FOLLOWING CLINICAL CONFIRMATION OF COLIBACILLOSIS IN EGG LAYER FLOCKS Željko Gottstein, Liča Lozica, Maja Lukač, Danijela Horvatek Tomić	45
HIDDEN INFECTIOUS BURSAL DISEASE VIRUS INFECTIONS IN CENTRAL EUROPE PRIKRIVENE INFEKCIJE VIRUSOM ZARAZNE BOLESTI BURZE U CENTRALNOJ EUROPI Tamás Mató, András Medveczki, István Kiss	51

SAFETY OF THE INTERMEDIATE PLUS VACCINE AGAINST INFECTIOUS BURSAL DISEASE BASED ON STRAIN G6 PROVJERA SIGURNOSTI CJEPIVA PROTIV ZARAZNE BOLESTI BURZE TEMELJENOG NA INTERMEDIJARNOM PLUS SOJU G6 Katarina Huić Babić, Lana Ljuma Skupnjak, Lovorka Špirić, Máté Halas	57
INFECTIOUS BRONCHITIS HATCHERY VACCINATION: COMPARISON BETWEEN TRADITIONAL SPRAY ADMINISTRATION AND A NEWLY DEVELOPED GEL DELIVERY SYSTEM IN FIELD CONDITIONS CIJEPLJENJE PROTIV ZARAZNOG BRONHITISA U VALIONICI: USPOREDBA TRADICIONALNE PRIMJENE RASPRŠIVANJEM I NOVO RAZVIJENOG SUSTAVA PRIMJENE GELOM U TERENSKIM UVJETIMA László Kőrösi, Mattia Cecchinato, Matteo Legnardi, Baranyay Henrik, András Gáspárdy	64
SAFETY OF THE LIVE ATTENUATED VACCINE AVISHIELD IB GI-13 AGAINST INFECTIOUS BRONCHITIS IN SPF CHICKENS DURING LAYING PERIOD NEŠKODLJIVOST PRIMJENE ŽIVOG ATENUIRANOG CJEPIVA AVISHIELD IB GI-13 PROTIV ZARAZNOG BRONHITISA KOD SPF KOKOŠI TIJEKOM RAZDOBLJA NESENJA Marina Gabrić Dragović, Leonida Kutle, Lovorka Špirić, Sunčana Abramović, Máté Halas, Ferenc Kelemen	71
USPOREDBA POSTUPAKA IZDVAJANJA DNA IZ PERA U KONTROLI CIJEPLJENJA PILENKI LAKE PASMINE KOKOŠI SOJEM CVI 988 VIRUSA MAREKOVE BOLESTI COMPARATIVE ANALYSES OF DIFFERENT DNA ISOLATION METHODS FROM FEATHER SHAFTS IN CONTROL OF VACCINATION OF LAYER PULLETS WITH MAREK'S DISEASE VIRUS STRAIN CVI 988 Sunčiča Sertić, Emanuel Budicin, Liča Lozica, Maja Lukač, Danijela Horvatek Tomić, Željko Gottstein	78
POJAVNOST SALMONELA IZDVOJENIH U OKVIRU PROVEDBE NACIONALNIH PROGRAMA I SLUŽBENOG UZORKOVANJA U REPUBLICI HRVATSKOJ U RAZDOBLJU OD 2017. DO 2021. GODINE PREVALENCE OF <i>SALMONELLA</i> SPP. RECORDED WITHIN THE SCOPE OF THE NATIONAL PROGRAMMES AND OFFICIAL SAMPLING IN THE REPUBLIC OF CROATIA 2017-2021 Borka Šimpraga, Luka Jurinović, Tajana Amšel Zelenika, Marijana Sokolović, Biljana Ječmenica, Ivana Lohman Janković, Fani Krstulović	84
UČESTALOST <i>SALMONELLA</i> SPP. U JATIMA TOVNIH PILIĆA NA PODRUČJU ISTOČNE HRVATSKE U RAZDOBLJU OD 2010. DO 2020. GODINE PREVALENCE OF <i>SALMONELLA</i> SPP. IN BROILER FLOCKS IN EASTERN CROATIA IN THE 2010 – 2020 PERIOD Marica Lolić, Mario Škrivanko, Marija Krajina, Davor Balić, Borka Šimpraga, Ivana Lohman Janković, Kristina Matković	91
THE PREVALENCE OF <i>CAMPYLOBACTER JEJUNI</i> AND <i>CAMPYLOBACTER COLI</i> IN CROATIAN BROILERS IN A FIVE-YEAR PERIOD (2017 – 2021) PREVALENCIJA <i>CAMPYLOBACTER JEJUNI</i> I <i>CAMPYLOBACTER COLI</i> KOD TOVNIH PILIĆA U HRVATSKOJ KROZ PETOGODIŠNJE RAZDOBLJE (2017.-2021.) Biljana Ječmenica, Borka Šimpraga, Fani Krstulović, Tajana Amšel Zelenika, Andrea Humski, Luka Jurinović	97
ANTIMICROBIAL SUSCEPTIBILITY, PHYLOGENETIC AND PROTEOMIC CHARACTERIZATION OF <i>ENTEROCOCCUS CECORUM</i> ANTIMIKROBNA OSJETLJIVOST, FILOGENETSKA I PROTEOMSKA KARAKTERIZACIJA BAKTERIJE <i>ENTEROCOCCUS CECORUM</i> Liča Lozica, Emanuel Budicin, Sunčiča Sertić, Snježana P. Kazazić, Maja Lukač, Danijela Horvatek Tomić, Željko Gottstein	102

POJAVNOST PATOGENIH PLIJESNI U HRANI ZA PERAD OCCURRENCE OF PATHOGENIC FUNGI IN POULTRY FEEDSTUFFS Marijana Sokolović, Borka Šimpraga, Marija Berendika, Fani Krstulović	109
PRIMJENA EFEKTIVNIH MIKROORGANIZAMA U PERADARSKOJ PROIZVODNJI USE OF EFFECTIVE MICROORGANISMS IN POULTRY PRODUCTION Ivana Sabolek, Kristina Matković, Željko Pavičić, Mario Ostović	119
SYNERGY OF PHYTOGENICS AND BUTYRIC ACID TO IMPROVE INTESTINAL HEALTH IN BROILERS SINERGIJA FITOGENA I MASLAČNE KISELINE U POBOLJŠANJU ZDRAVLJA CRIJEVA KOD BROJLERA Jan Dirk van der Klis, Nick Slagman	123
THE EFFECT OF PROBIOTIC SUPPLEMENT ON THE CONTAMINATION OF BROILERS WITH <i>CAMPYLOBACTER</i> AND <i>SALMONELLA</i> UČINAK DODATKA PROBIOTIKA NA KONTAMINACIJU BROJLERA BAKTERIJAMA <i>CAMPYLOBACTER</i> I <i>SALMONELLA</i> Jana Avberšek, Bojan Papić, Darja Kušar, Maja Kavalič, Igor Gruntar, Jasna Mićunović, Matjaž Ocepek, Tomaž Knafelc, Neva Šemrov, Jasna Perc, Olga Zorman Rojs	127
ALUVIJALNI MINERALI U HRANI POBOLJŠAVAJU ZDRAVLJE I NESIVOST TEŠKIH HIBRIDNIH KOKOŠI ALLUVIAL MINERALS IN FEED IMPROVES HEALTH AND LAYING OF HEAVY HYBRID LAYERS Zlatko Janječić, Dalibor Bedeković, Mirta Balenović, Tajana Amšel Zelenika, Bratko Filipič, Hrvoje Mazija .	133
VLAKNA U HRANIDBI PERADI FIBRE IN POULTRY FEEDSTUFFS Marija Berendika, Marijana Sokolović, Gabrijela Krivec	141
PROMJENE U KRVNOJ SLICI KOKOŠI NESILICA LAKE PASMINE NAKON PRIMJENE FLURALANERA U TERAPIJI TEKUTI CHANGES OF HAEMATOLOGICAL PARAMETERS IN LAYER HENS AFTER FLURALANER TREATMENT OF MITES Emanuel Budicin, Liča Lozica, Danijela Horvatek Tomić, Gabriel Kontrec, Ivana Sabolek, Josip Miljković, Mario Ostović, Ana Shek-Vugrovečki, Jasna Aladrović, Željko Gottstein	148
ANTIMICROBIAL IMPACT OF NATURAL VOLATILE OILS ON MULTI-RESISTANT <i>ESCHERICHIA COLI</i> STRAINS ISOLATED FROM POULTRY ANTIMIKROBNI UČINAK PRIRODNIH HLAPLJIVIH ULJA NA VIŠESTRUKO REZISTENTNE SOJEVE BAKTERIJE <i>ESCHERICHIA COLI</i> IZOLIRANE IZ PERADI Camelia Tulcan, Maria Roberta Tripon, Oana Maria Boldura, Gaspar Cristina, Marijana Sokolović	155
PRIRODNI 1,25(OH) ₂ D ₃ -GLIKOZID UBLAŽAVA POSLJEDICE STRESA I POVEĆAVA KONCENTRACIJU KALCIJA I MAGNEZIJA U SERUMU KOKOŠI TEŠKE PASMINE U ZAVRŠNOJ FAZI PROIZVODNJE NATURAL 1,25(OH) ₂ D ₃ -GLYCOSIDE ALLEVIATE STRESS EFFECTS AND ENHANCE CALCIUM AND MAGNESIUM CONCENTRATION IN SERUM OF BROILER BREEDER HENS DURING FINAL STAGE OF PRODUCTION Josip Miljković, Lana Pađen, Ivan Lošić, Dalibor Bedeković, Kathrin Bühler, Liča Lozica, Danijela Horvatek Tomić, Željko Gottstein, Jasna Aladrović	162
EMISIJE STAKLENIČKIH PLINOVA IZ PERADARSKOJ PROIZVODNJE U REPUBLICI HRVATSKOJ OD 2010. DO 2020. GODINE GREENHOUSE GAS EMISSIONS FROM POULTRY PRODUCTION IN THE REPUBLIC OF CROATIA FROM 2010 TO 2020 Goran Kiš, Mihael Janječić, Dalibor Bedeković, Zlatko Janječić	169

UTJECAJ STABILIZIRANOG TEKUĆEG KLOR DIOKSIDA (ClO ₂) NA HIGIJENSKI STATUS VODOOPSKRBNOG SISTEMA NA BROJLERSKIM FARMAMA INFLUENCE OF STABILIZED LIQUID CHLORINE DIOXIDE (ClO ₂) ON HYGIENIC STATUS OF WATER SUPPLY SYSTEM IN BROILER FARMS Tijana Mujaković, Aida Kustura, Emina Rešidbegović, Damir Mujaković, Abdulah Gagić	174
--	-----

Poster prezentacije / Poster presentations

USPOREDBA STANDARDNE I MIKROVALNE METODE PRIPREME UZORAKA ZA ODREĐIVANJE PROFILA MASNIH KISELINA U ŽUMANJCIMA JAJA COMPARISON OF STANDARD AND MICROWAVE METHODS OF SAMPLE PREPARATION FOR DETERMINING THE FATTY ACID PROFILE IN EGG YOLKS Manuela Košević, Zlata Kralik, Olivera Galović	181
--	-----

ODREĐIVANJE REZIDUA FENBENDAZOLA U JAJIMA PERADI VEZANIM SUSTAVOM TEKUĆINSKA KROMATOGRAFIJA - TANDEMNA SPEKTROMetriJA MASA DETERMINATION OF FENBENDAZOLE RESIDUES IN POULTRY EGGS BY LIQUID CHROMATOGRAPHY - TANDEM MASS SPECTROMETRY Tiana Novosel, Damir Pavliček, Marija Denžić Lugomer	187
--	-----

ULOGA ŠKROBA U HRANIDBI PERADI ROLE OF STARCH IN POULTRY NUTRITION Marijana Sokolović, Marija Berendika, Gabrijela Krivec	197
---	-----

POJAVNOST I ANTIMIKROBNA OSJETLJIVOST BAKTERIJA RODA <i>ENTEROCOCCUS</i> IZDVOJENIH IZ PTICA KUĆNIH LJUBIMACA PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF <i>ENTEROCOCCUS</i> ISOLATED FROM PET BIRDS Danijela Horvatek Tomić, Petra Marcuš, Matea Mohenski, Liča Lozica, Maja Lukač, Željko Gottstein	206
---	-----

CO-INFEKCIJA VIRUSOM MAREKOVE BOLESTI I VIRUSOM PTIČJE LEUKOZE KOD KOKOŠI PASMINE BANATSKI GOLOŠIJAN Koinfekcija virusom Marekove bolesti i virusom ptičje leukoze kod kokoši pasmine Banatski gološijan Jelena Maletić, Branislav Kureljušić, Ljiljana Spalević, Ivana Vučićević, Ljubiša Veljović, Bojan Milovanović, Vesna Milićević	210
---	-----

UTJECAJ DOCJEPLJIVANJA ŽIVIM CJEPIVOM PROTIV ZARAZNE BOLESTI BURZE NA PROIZVODNE POKAZATELJE TOVNIH PILIĆA NAKON SLABE STOPE CIJEPLJENOSTI <i>IN OVO</i> EFFECT OF LIVE STRAIN BOOSTER VACCINATION AGAINST INFECTIOUS BURSAL DISEASE ON BROILER PRODUCTION PARAMETERS AFTER LOW <i>IN OVO</i> VACCINATION RATE Željko Gottstein, Liča Lozica, Sunčiča Sertić, Danijela Horvatek Tomić, Milivoj Dragošević, Mate Kraljević, Matija Ritoša, László Kőrösi	218
---	-----

ISPITIVANJE SVJEŽEG PORCIONIRANOG PILEĆEG MESA U MALOPRODAJI NA PRISUTNOST <i>LISTERIA MONOCYTOGENES</i> TESTING OF FRESH PORTIONED CHICKEN MEAT IN RETAIL FOR THE PRESENCE OF <i>LISTERIA MONOCYTOGENES</i> Zdenko Mlinar, Ivančica Kovaček, Vedran Prahin	223
---	-----

CO-INFECTION WITH MAREK'S DISEASE VIRUS AND AVIAN LEUCOSIS VIRUS IN THE BANAT NAKED NECK HENS

Jelena Maletić¹, Branislav Kureljušić¹, Ljiljana Spalević¹, Ivana Vučićević², Ljubiša Veljović¹, Bojan Milovanović¹, Vesna Milićević¹

¹ Serbian Institute of Veterinary Science, Belgrade, Serbia

² Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

Summary

Marek's disease virus and avian leucosis virus are causes of contagious, immunosuppressive, and oncogenic diseases. Many reports have described single virus infection, but detailed description of co-infection with Marek's disease virus and avian leucosis virus has been provided in a few previous reports. The aim of this study was to determine the cause of health disorders in a flock of the Banat Naked Neck hens at the age of 11 weeks. Clinical examination of the flock showed signs of cachexia, apathy, dropped wings, nervous symptoms in the form of bilateral leg paresis, sporadic diarrhoea, and the mortality reaching 20%. Post-mortem examinations showed the following changes: chronic pseudomembranous typhlitis, intestinal ascariasis, splenomegaly, granulomatous pneumonia, multiple yellowish caseous foci in the liver, and white tumour masses in the heart. Histopathological examination of the spleen, proventriculus and heart tissue revealed polymorphic infiltrates, while granulomatous inflammation was observed in the lung and liver. Gomori methenamine silver stain method revealed septate and arborized hyphae resembling the *Aspergillus* spp. fungi in the liver and lung granulomas. Bacteriological examination of faeces revealed the presence of *Escherichia coli*, and parasitological examination of the small intestine determined the presence of oocyst of *Eimeria acervulina* and *Eimeria tenella* in the caecum. The genomes of Marek's disease virus and avian leucosis virus were determined by polymerase chain reaction in tissue samples. In this case, specific pathological lesions, as well as molecular finding confirmed co-infection with Marek's disease virus and avian leucosis virus.

Key words: avian leucosis virus, Banat Naked Neck hens, Marek's disease virus

Introduction

There is an increase in the number of laying hens kept in alternative production systems such as organic, free-range and low-input production systems. These rearing systems are less standardisable than intensive ones, as biosecurity is poor in backyard flocks, and poultry have frequent access to the outdoors where they come in contact with wild birds and other animals such as rodents that can transmit different diseases (Gauly et al., 2002; Whitehead and Roberts, 2014; Carrisosa et al., 2021; Dal Bosco et al., 2021). The Banat Naked Neck hen is autochthonous poultry breed that is very tolerant to poor rearing conditions. It belongs to the breeds that are of national interest, as part of the strategy of preservation of total biodiversity in the Republic of Serbia and within the program of preservation of autochthonous genotypes, which is supported by the government of the

Republic of Serbia in the form of substantial annual subsidies (Milošević et al., 2013).

Oncogenic viral tumour diseases are one of the major threats to poultry industry as they are immunosuppressive and make poultry susceptible to various other illnesses. Under natural conditions, poultry can be infected by leucosis and Marek's disease virus (MDV). Avian leucosis virus (ALV) is a member of the genus *Alpharetrovirus*, family *Retroviridae*, associated with tumour formation, immunosuppression and decreased fertility that lead to enormous economic loss in the poultry industry, especially because there are no commercial vaccines to protect them from this infection. The virus has six subgroups, from A to E and J. It is known that subgroup A causes major disease and production problems in layers and that ALV-J (avian leucosis virus-J) has caused significant economic losses in meat-type chickens, but there are reports of field cases of leucosis

caused by ALV-J in commercial egg-type chickens (Simon et al., 2005; Payne and Nair, 2012). Marek's disease is a contagious, lymphoproliferative disease of poultry caused by the virus that belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Mardivirus* (Witter and Schat, 2003; Nair, 2005). The virus is transmitted only horizontally (Mete et al., 2016). The disease can be acute or chronic, and clinical signs depend on the strain virulence, poultry age and previous health condition (Baigent et al., 2006). Classical chronic form, caused by moderate and weak oncogenic strains of the virus, is characterized by changes in the nerves, i.e., paresis, then paralysis of the legs, torticollis, dropped wings, rarely changes in the iris and paralysis of the eyelids. Lymphomas occur in 5%-10% of infected birds (Rusov, 1997; Vučićević et al., 2014). This disease is the most commonly reported cause of mortality in backyard poultry, as many owners are not aware of the necessity of vaccination.

Co-infection with ALV and MDV that cause contagious, immunosuppressive, and oncogenic diseases, has an important epidemiologic influence in poultry production. Some studies have described pathomorphological findings in co-infection with oncogenic viruses (Payne and Venugopal, 2000; Zhou et al., 2018; Liu et al., 2019).

In the conventional farming system, where poultry are kept in the outdoor systems, characterized by a high risk of acquiring infections, the prevalence of parasitic infection is high (Marcos-Atxutegi et al., 2009). In a situation where the organism has already been altered by oncogenic viral diseases, associated parasitic infestations can be fatal to the host. The most significant parasitic infections that influence poultry production are ascariasis and coccidiosis (Nnadi and George, 2010; Abebe et al., 2016). *Ascaridia galli* is the most frequent nematode disease of different avian species. In poultry that is extensively reared, the incidence of infection is >80%. It is accompanied by various clinical signs that include loss of appetite, anorexia, weight loss, ruffled feathers, dropped wings, retarded muscle and osteological development, altered hormone levels, depression and increased mortality, and in laying hens even lower egg production (Dahl et al., 2002; Thapa et al., 2017). Direct losses are caused by obstruction of the intestine and damage to the intestinal tract in hens, and indirect losses are due to reduced function of the immune system, which makes the birds more susceptible to secondary infections (Permin et al., 2006).

Coccidiosis is a disease caused by protozoan parasites from the genus *Eimeria*. It can cause increase of mortality, poor performance, lost productivity and great economic losses in poultry industry. This parasitic infection is followed by high morbidity, which manifests itself in the acute form, bloody enteritis with high mortality, or occurs in a subclinical form. The most important *Eimeria* spp. in poultry are *E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. mitis* and *E. praecox*. Differences among these species include invasion of certain part of the gut, pathogenicity, and the type of lesion. Clinical signs are ruffled feathers, listlessness, mucoid to bloody diarrhoea, reduced weight gain or weight loss, and in severe cases mortality (Lucas et al., 2019).

Aspergillosis is a non-contagious disease that primarily affects the lower respiratory system of birds and is caused by a fungal species of the genus *Aspergillus*. The disease occurs under the following conditions: immune compromised bird, overwhelming number of spores, stress, mismanagement problem in commercial and back yard poultry (Saif, 2008; Girma et al., 2016).

The aim of this study was to determine the cause of death in the Banat Naked Neck hens.

Materials and Methods

In September 2021, the backyard flock of the Banat Naked Neck hens, with 50 birds at the age of 11 weeks in extensive breeding was clinically examined. Food consumption was reduced. Wheat, barley, corn and feed concentrate were used in the diet, and water was provided *ad libitum*. The flock was not vaccinated against infectious diseases and was not in contact with other types of poultry.

In this study, during the farm visit we noticed leg paralysis, the birds were unable to move both legs, had weakened or dropped wings, and food consumption was reduced leading to severe weight loss, prominent sternum, and fat loss followed by death. Disorders of the digestive system in the form of bloody and black faeces were reported sporadically. The morbidity rate in the flock was 90%-100% and mortality was up to 20%. During clinical examination of the flock, samples of faeces were taken for bacteriological and parasitological analysis, which were further performed in the accredited laboratory of the Serbian Institute of Veterinary Science. *Ascaridia galli* and coccidiosis lesions were physically verified upon dissection of the whole intestine and caeca, as described by Sharma et al. (2015). Samples of caeca were taken for microscopic confirmation of coccidiosis, while the presence of adult *A. galli* was checked by visual examination. Collected samples were processed for microscopic examination to diagnose the presence of coccidian oocyst. Homogenized mixture of the faecal sample and distilled water was processed using sugar solution and distilled water for floatation and sedimentation methods, then placed on a glass slide and observed under the compound microscope. *Eimeria* oocysts were observed at 400× for confirmation of coccidiosis (Dryden et al., 2005; Fatima et al., 2015).

After necropsy of five birds, samples of the spleen, liver, lung, proventriculus and heart tissue were taken for histopathological examination and fixed in 10% buffered formalin, routinely processed and embedded in paraffin blocks. Paraffin sections about 5 µm thick were stained with haematoxylin-eosin (HE) method and Gomori methenamine silver (GMS) method.

During the pathomorphological examination, pooled samples of altered organs (spleen, liver, lung, proventriculus and heart tissue) were taken for molecular tests. Detection of Marek's disease virus DNA was done using real time polymerase chain reaction (PCR) (Hennig et al., 2003), and the genome of avian leucosis virus using gel-based PCR method (Mohammadi et al., 2008).

Results

In all five necropsied chickens, multiple yellowish-white caseous foci, 3 to 5 mm in diameter, were observed in the liver and white solid tumour masses in the heart. Consolidation of lung parenchyma with granulomatous pneumonic lesions was prominent in two birds (Figure 1).

During necropsy, all five corpses were found to have cachexia, and internal organ inspection revealed serous atrophy of epicardial adipose tissue, hypertrophic proventriculitis, presence of one specimen of *Ascaridia galli* in the gizzard of one bird, mild haemorrhagic enteritis sporadically, and marked splenomegaly (Figure 1) (2-3 times larger than the normal size). In three birds, heavy infection of the intestine with *Ascaridia galli* was observed, where 15-35 adult *Ascaridia galli* worms were noticed. Pathomorphological examination of the caecum revealed chronic pseudomembranous typhlitis, while microscopic examination of native smears of the caecal superficial mucosal layer indicated the presence of developmental forms of *Eimeria* species.

Polymorphic infiltrate was observed by histopathological examination of the spleen, heart, liver and proventriculus (Figures 2, 3 and 4). Consequent atrophy of the parenchyma was present in the liver, heterophilic and histiocytic infiltrates were observed in the spleen in addition to lymphoblastic proliferation, and lymphoblastic infiltration with capillary congestion and small haemorrhages

were observed in the proventriculus. Also, in the altered lung, well-organized granulomatous pneumonia with GMS staining positive hyphae was observed (Figure 5).

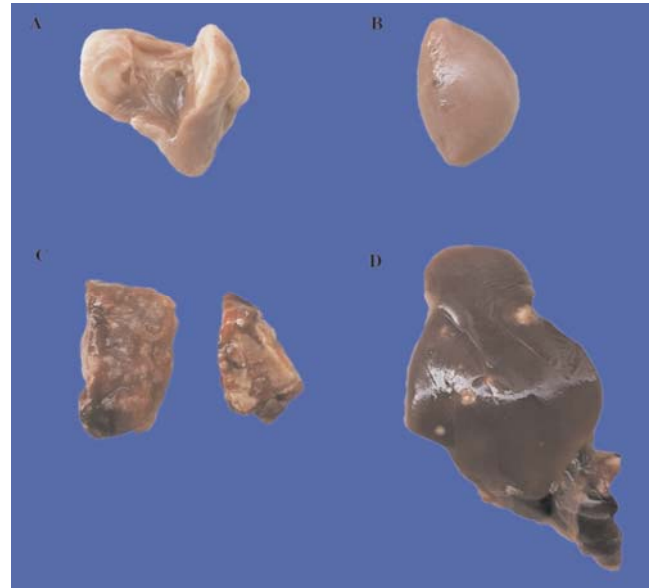


Figure 1. Macroscopic finding of the heart, spleen, lung and liver of Banat Naked Neck hens: (A) solid tumour of the heart; (B) splenomegaly; (C) granulomatous pneumonia; (D) granulomatous hepatitis

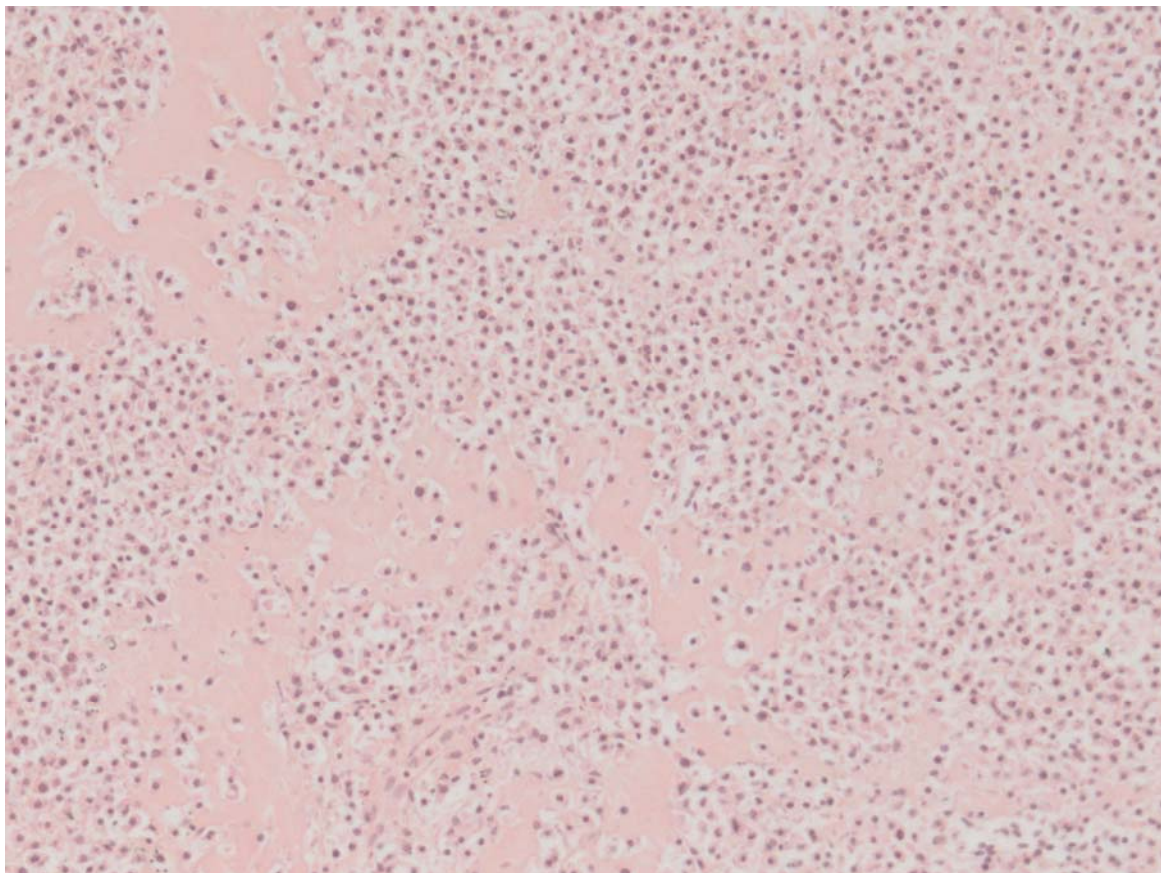


Figure 2. Microscopic finding of the spleen of Banat Naked Neck hens: polymorphic infiltrate. (HE, magnification $\times 40$)

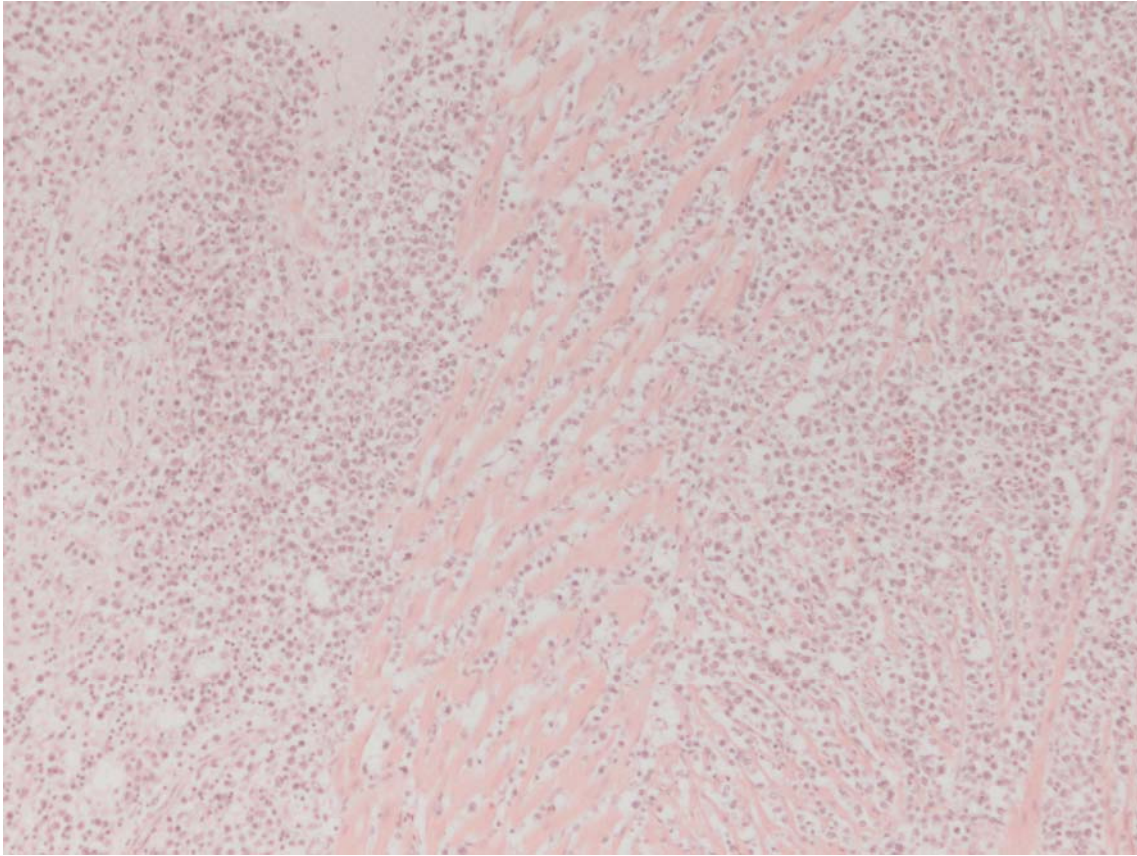


Figure 3. Microscopic finding of the heart of Banat Naked Neck hens: polymorphic infiltrate. (HE, magnification $\times 20$)

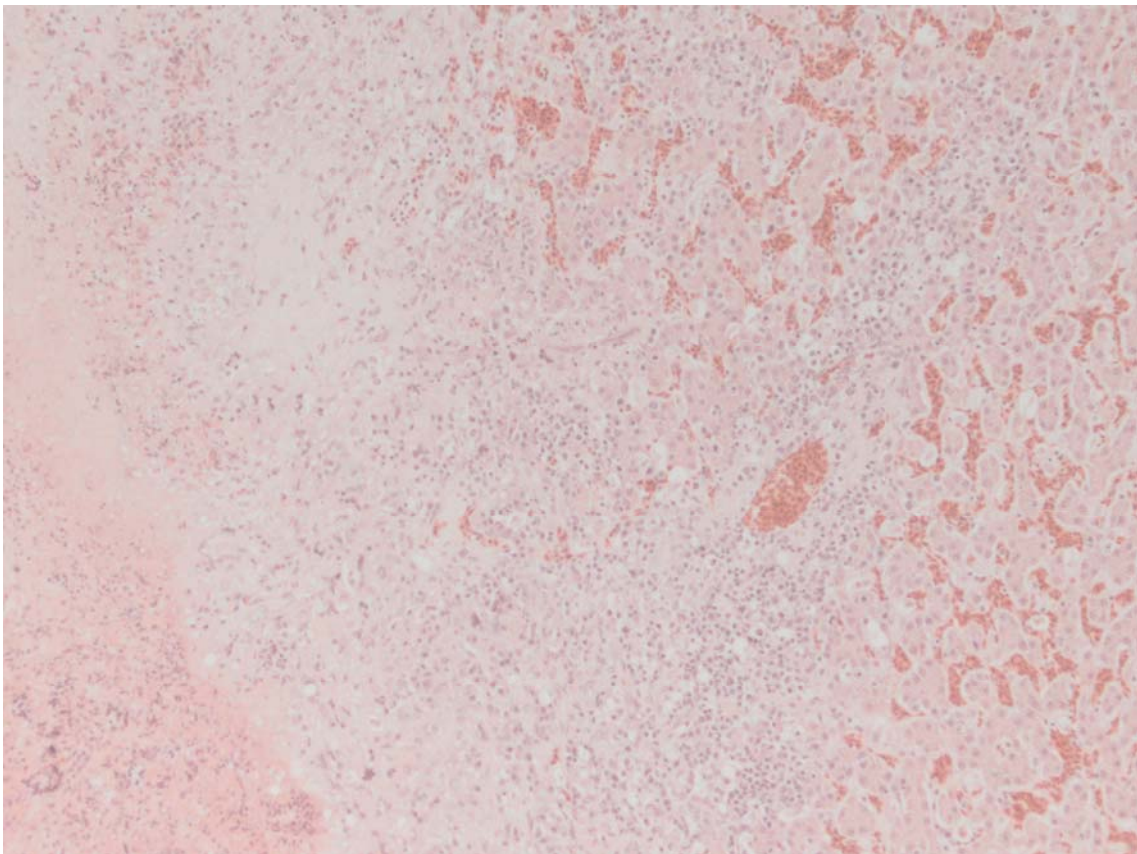


Figure 4. Microscopic finding of the liver of Banat Naked Neck hens: polymorphic infiltrate. (HE, magnification $\times 20$)



Figure 5. Microscopic finding of the lung of Banat Naked Neck hens: arborized and septate hyphae. (GMS, magnification $\times 10$)

Bacteriological analysis of faeces revealed the presence of *E. coli*, and parasitological analysis of faeces confirmed the presence of *Eimeria acervulina* and *Eimeria tenella* oocysts. The genome of Marek's disease virus serotype 1 and genome of avian leucosis virus were confirmed by PCR analysis.

Discussion

Concurrent infections with oncogenic viruses and parasites are economically very important to the poultry industry, especially in the conventional farming. There is no infrastructure for biosecurity and poultry is exposed to environmental stresses, which lead to huge economic losses for farmers (Abbas et al., 2015).

Many reports have described histological changes associated with a single virus infection, but detailed description of histological findings from naturally occurring cases of co-infection has been provided in a few reports (Wen et al., 2018; Lui et al., 2019). In the present study, we describe clinical signs and pathological findings associated with MDV and ALV co-infection in a naturally infected flock of the Banat Naked Neck hens. In this case, co-infection with two oncogenic viral diseases induced immune suppression and increased susceptibility to secondary infections including coccidiosis, ascaridiasis and aspergillosis.

Marek's disease (MD) is a major cause of mortality in backyard chickens worldwide (Metz et al., 2013) because

backyard poultry are primarily kept for a hobby with poor biosecurity measures and vaccination strategies (Elkhoraibi et al., 2014). Cell-associated alphaherpesvirus that causes Marek's disease is highly contagious, environmentally resistant, and continues to be a significant problem in the alternative production systems due to the lack of vaccination strategies for backyard poultry flocks. The findings by Spalević et al. (2016) proved the genome of Marek's disease virus serotype 1 as a cause of impaired health in the flock of Holland white crested chickens. Clinical signs in this case manifested as cachexia, feed intake decrease, and sporadic signs of diarrhoea. Pathomorphological changes in certain internal organs are in correlation with our findings. White nodules in the heart, also hypertrophic proventriculitis, are consistent with previous reports. Histopathological examination revealed that the proventriculus wall was thickened, while congestion of capillaries with small haemorrhages and signs of glandular cystic dilatation were observed in the mucosa. Multifocal lymphoid infiltrations and heterophilic and histiocytic infiltrates in different organs are similar to the histopathological changes described by other authors (Pejović et al., 2007; Spalević et al., 2016; Metz et al., 2016).

Our findings are also in correlation with the findings reported by Wen et al. (2018), who describe clinical signs and pathological findings associated with MDV and ALV-J co-infection in a naturally infected flock of egg-laying hens. Using immunohistochemical method and sequences of the

viral oncogenes isolated from the diseased egg-laying hens, they found that concurrent infection with MDV and ALV-J contributed to tumorigenicity observed in the flock.

In the present study, sporadic occurrence of bloody and black faeces could have been a result of haemorrhages and erosions in caecal mucosa due to the infection with *Eimeria*, but mild haemorrhagic enteritis in the small intestine could have occurred due to the *A. galli* larvae emerging from the mucosa. *E. acervulina* and *E. tenella* have been established as the major causes of bloody diarrhoea in poultry coccidiosis (Makai et al., 2007; Ngongeh et al., 2019). Coccidiosis is a challenge for backyard flocks as they are floor-raised and have continual exposure to *Eimeria* spp. oocysts from contaminated environmental areas (Godwin and Morgan, 2015).

Atrophy of epicardial adipose tissue may have occurred as a consequence of fat mobilization, which is in correlation with the findings reported by Sharma et al. (2018), who demonstrated that hens with high *A. galli* burden had consistently lower lipid reserves compared to uninfected hens. *A. galli* alters nutrient utilization and absorption from feed, which leads to reduction in the final growth. Due to the increased use of energy reserves from the body to elevate the immune response against parasites such as *A. galli* infection may affect stored energy reserves such as liver lipids in laying hens (Das et al., 2010; Sharma et al., 2019). The presence of *A. galli* infection also leads to cannibalism, which endangers the welfare and behaviour of chickens (Gauly et al., 2007). Direct losses are caused by obstruction of the intestine and damage to the mucous membrane of the intestinal tract in hens, and indirect losses are a consequence of reduced function of the immune system, hence increased susceptibility to secondary infections. Subclinical *A. galli* infections may have an immunosuppressive effect, allowing *E. coli* to establish itself (Permin et al., 2006). According to the research by Dahl et al. (2002), even subclinical *A. galli* infection seems to enhance the establishment of *P. multocida*.

Pathological features induced by aspergillosis indicate permanent organ damage (Nururrozi et al., 2020). Our findings are consistent with the previous research, which reports that granulomas caused by *Aspergillus* usually occur in the lungs and air sacs (Ivetić et al., 2003; Saif 2008; Kureljušić et al., 2011; Kureljušić et al., 2012). In this case, co-infection with ALV, MDV and ascariasis were found, where all agents induced immune suppression and increased susceptibility to secondary infections, so it is not surprising that the flock was infected by aspergillosis as well.

Conclusion

Comprehensive understanding of contagious diseases of backyard poultry flocks is important to provide poultry health information to flock owners, veterinarians, and animal health officials, as they may spread unchecked. Concurrent diseases are common in free-range flocks. Helminth infections and poultry coccidiosis are usually conjoint and

they occur as a result of impaired management, due to the lack of the basic biosecurity knowledge. We recommend that the infections be diagnosed regularly with focus on the determination of subclinical occurrence, and controlled promptly. Cases of Marek's disease are not uncommon in our country due to the lack of vaccination strategies for backyard poultry flocks, but this was for the first time that we detected MDV and ALV-J as a concurrent infection. Birds with aspergillosis must be culled because of irreversible pathological damage. Farmers should be aware of the importance of management, biosecurity measures, transmission of pathogens among flocks, regular deworming and vaccination. In this way, we would reduce the impact of infections on the poultry production, and it will improve the overall health and welfare of backyard flocks and farm profitability.

Acknowledgement

The study was funded by the Serbian Ministry of Education, Science and Technological Development (Contract No. 451-03-9/2021-14/200030).

References

- ABBAS, G., S.H. KHAN, M. HASSAN, S. MAHMOOD, S. NAZ, S.S. GILANI (2015): Incidence of poultry diseases in different seasons in Khushab district. Pakistan. J. Adv. Vet. Anim. Res. 2:141-145.
- ABEBE, B., A. MEKONNEN, A. MIHRETU (2016) Review on major gastrointestinal parasites that affect chickens. Journal of Biology, Agriculture and Healthcare Vol.6, No.11, 2016
- BAIGENT, S. J., L. P. SMITH, V. K. Nair, R. J. W. Currie (2006): Vaccinal control of Marek's disease: Current challenges and future strategies to maximize protection. Vet. Immunol. and Immunopathol. 112 (1-2), 78-86.
- CARRISOSA, M., S. JIN., B. A. McCrea, K. S. MACKLIN, T. DORMITORIO, R. HAUCK (2021): Prevalence of Select Intestinal Parasites in Alabama Backyard Poultry Flocks. Animals, 11, 939.
- DAL BOSCO, A., S. MATTIOLI, A. C. MANCINELLI, E. COTOZZOLO, C. CASTELLINI (2021): Extensive rearing systems in poultry production: the right chicken for the right farming system. a review of twenty years of scientific research in Perugia University, Italy. Animals, 11(5):1281.
- DAHL, C., A. PERMIN, J. P. CHRISTENSEN, M. BISGAARD, A. P. MUHAIRWA, K. M. D. PETERSEN, J. S. D. POULSEN, A. L. JENSEN (2002): The effect of concurrent infections with *Pasteurella multocida* and *Ascaridia galli* on free range chickens. Vet. Microbiol. 86, 313-324 .
- DAS, G., F. KAUFMANN, H. J. ABEL, M. GAULY (2010): Effect of extra dietary lysine in *Ascaridia galli*-infected grower layer. Vet. Parasitol. 170, 238-243.
- DRYDEN, M. W., P. A. PAYNE, R. RIDLEY, V. SMITH (2005): Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet. Ther. 6 (1), 15-28.
- ELKHORAIBI, C., R. A. BLATCHFORD, M. E. PITESKY, J. A. MENCH (2014): Backyard chickens in the United States: a survey of flock owners. Poult. Sci. 93, 2920-2931.

- FATIMA, T., M. S. SAJID, M. K. SALEEMI, Z. IQBAL, R. M. SIDDIQUE (2015): Descriptive epidemiology of endoparasitic fauna in layer birds (*Gallus domesticus*) of central Punjab. Pak. J. Agri. Sci. 52, 815-820.
- GAULY, M., C. BAUER, R. PREISINGER, G. ERHARDT (2002): Genetic differences of *Ascaridia galli* egg output in laying hens following a single dose infection. Vet. Parasitol. 103, 99-107.
- GAULY, M., C. DUSS, G. ERHARDT (2007): Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (*Gallus gallus domesticus*). Vet. Parasitol. 146, 271-280.
- GIRMA, G., M. ABEBAW, M. ZEMENE, Y. MAMUYE, G. GETANEH (2016): A Review on Aspergillosis in Poultry. J Vet Sci Technol 7: 382.
- GODWIN, R. M., J. A. MORGAN (2015): A molecular survey of *Eimeria* in chickens across Australia. Vet. Parasitol. 214, 16-21.
- HENNIG, H., N. OSTERRIEDER, M. MULLER-STEINHARDT, H. M. TEICHERT, H. KIRCHNER, K. P. WANDINGER (2003): Detection of Marek's disease virus DNA in chicken but not in human plasma. Journal of Clinical Microbiology, 2428-2432.
- IVETIĆ, V., D. VALTER, I. PAVLOVIĆ, B. MILJKOVIĆ, D. MASLIĆ-STRIŽAK, Ž. ILIĆ, B. SAVIĆ, S. STANO-JEVIĆ, LJ. SPALEVIĆ (2003): Atlas bolesti živine. Institute of Veterinary medicine of Serbia, Belgrade, 77-80.
- KURELJUŠIĆ, B., B. SAVIĆ, R. PRODANOVIĆ, J. ĐEKIĆ, V. ADAMOV, D. JAKIĆ-DIMIĆ, B. MILJKOVIĆ, O. RADANOVIĆ, V. IVETIĆ (2011): Primena različitih histohemijskih metoda u dijagnostici aspergiloze mozga kod ćurica. Veterinarski glasnik 65, 1-2, 43-50.
- KURELJUŠIĆ, B., O. RADANOVIĆ, J. KURELJUŠIĆ, N. JEZDIMIROVIĆ, D. MASLIĆ-STRIŽAK, R. PRODA-NOVIĆ, V. IVETIĆ (2012): The occurrence of aspergillosis in flock of turkey poults. Biotechnology in Animal Husbandry 28 (1), 129-136.
- LUI, H., M. KUI, M. LIU, C. YANG, H. XUETING, Z. YU, Q. KEZONG (2019): Histologic findings and viral antigen distribution in natural coinfection of layer hens with subgroup J avian leukosis virus, Marek's disease virus, and reticuloendotheliosis virus. Journal of Veterinary Diagnostic Investigation, Vol. 31(5), 761-765.
- LUCAS, A. N., G. U. ESTHER, B. F. BARINEME (2019): Consequences of Concurrent Infections with *Ascaridia Gallii* and *Eimeria* in Broiler Chickens. Asian Journal of Applied Sciences Vol. 07, Issue 01.
- NAIR, V. (2005): Evolution of Marek's disease – a paradigm for incessant race between the pathogen and the host. Vet. Journal 170, 175-183.
- NGONGEH, L. A., E. G. UGWUZOR, B. B. FAKAE (2019): Consequences of Concurrent Infections with *Ascaridia Gallii* and *Eimeria* in Broiler Chickens. Asian Journal of Applied Sciences, Volume 07, Issue 01.
- NNADI, P. A., S. O. GEORGE (2010): A Cross-Sectional Survey on Parasites of Chickens in Selected Villages in the Sub humid Zones of South-Eastern Nigeria. Journal of Parasitology Research, 14: 6, 18-24.
- NURURROZI, A., Y. YANUARTONO, S. WIDYARINI, D. RAMANDANI, S. INDARJULIANTO (2020): Clinical and pathological features of aspergillosis due to *Aspergillus fumigatus* in broilers. Veterinary World, 13(12), 2787-2792.
- MARCOS-ATXUTEGI, C., B. GANDOLFI, T. ARANGUENA, R. SEPULVEDA, M. AREVALO, F. SIMON (2009): Antibody and inflammatory responses in laying hens with experimental primary infections of *Ascaridia galli*. Vet. Parasitol., 161(1-2):69-75.
- MAKAI, V. A., H. K. MAKERI, A. A. ADEIZA, B. V. O. MAKAI (2007): Preliminary studies of anticoccidial effect of Mahogany (*Khaya senegalensis*) and African Locust Bean Tree (*Parkia biglobosa*) aqueous bark extracts on chicken infected with coccidia. Savannah Journal of Agriculture, 2: 43-45.
- METE, A., F. GIANNITTI, B. BARR, L. WOODS, M. ANDERSON (2013): Causes of mortality in backyard chickens in Northern California: 2007– 2011. Avian Dis. 57:311-315.
- METE, A., R. GHARPURE, M. E. PITESKY, D. FAMINI, K. SVERLOW, J. DUNN (2016): Marek's Disease in Backyard Chickens, A Study of Pathologic Findings and Viral Loads in Tumorous and Nontumorous Birds. Avian Dis., 60(4), 826-836.
- MILOŠEVIĆ, N., L. PERIĆ, M. ĐUKIĆ-STOJŠIĆ, S. TRIVUNOVIĆ, V. RODIĆ, S. BJEDOV (2013): Autochthonous hen breeds in the Republic of Serbia – Banat Naked Neck and Sombor Crested. World's Poult. Sci. J., 69, 153-162.
- MOHAMMADI, A., K. ASASI, M. MASOUDIAN, B. BOZORGHAMI (2008): Detection of avian leukosis virus (ALV) in albumen of Shiraz commercial and local layer flocks using ELISA and RT-PCR. Iranian Journal of Veterinary Research, Shiraz University, Vol. 9, No. 3, Ser. No. 24.
- PAYNE, L. N., K. VENUGOPAL (2000): Neoplastic diseases: Marek's disease, avian leukosis and reticuloendotheliosis. Rev Sci Tech, 19, 544-564.
- PAYNE, L. N., V. NAIR (2012): The long view: 40 years of avian leukosis research. Avian Pathol. 41, 11–9.
- PEJOVIĆ, N., M. VELHNER, V. POLAČEK, S. ALEKSIĆ-KOVAČEVIĆ, D. MARINKOVIĆ, M. KNEŽEVIĆ (2007): Morphological and immunohistochemical examination of tumor cells in Marek's disease. Acta Veterinaria 57 (1), 27-35.
- PERMIN, A., J. P. CHRISTENSEN, M. BISGAARD (2006): Consequences of concurrent *Ascaridia galli* and *Escherichia coli* infections in chickens. Acta Vet Scand., 47 (1), 43-54.
- RUSOV ČEDOMIR (1997): Marekova bolest. izd. Jugoslovensko naučno udruženje živinara, Beograd
- SAIF, Y. M. (2008): Diseases of poultry. Twelfth edition, Blackwell Publishing, Ames, Iowa, USA
- SPALEVIĆ, LJ., D. MASLIĆ-STRIŽAK, B. KURELJUŠIĆ, V. MILIĆEVIĆ, O. RADANOVIĆ, N. JEZDIMIROVIĆ (2016): Marek's disease in the Holland White Crested Chicken. Veterinarski glasnik, 70 (5-6), 225-232.
- SHARMA, S., S. AZMI, A. IQBAL, N. NASIRUDULLAH, I. MUSHTAQ (2015): Pathomorphological alterations associated with chicken coccidiosis in Jammu division of India. J. Parasit. Dis. 39, 147-151.
- SHARMA, N., P. W. HUNT, B. C. HINE, N. K. SHARMA, A. CHUNG, I. RUHNKE (2018): Performance, egg quality and liver lipid reserves of free-range laying hens naturally infected with *Ascaridia galli*. Poult. Sci. 97, 1914-1921.
- SHARMA, N., P. W. HUNT, B. C. HINE, I. RUHNKE (2019): The impacts of *Ascaridia galli* on performance, health and

- immune responses of laying hens: new insights into an old problem. *Poultry Science* 0, 1-10.
- THAPA, S., S. M. THAMSBORG, N. V. MEYLING, S. DHKSL, H. MEJER (2017): Survival and development of chicken ascarid eggs in temperate pastures. *Parasitology*, 144(9):1243-1252
- VUČIĆEVIĆ, I., S. NEŠIĆ, V. KUKOLJ, S. ALEKSIĆ KOVAČEVIĆ (2014): Expression of CD3, CD79 and PCNA on tumor cells in Marek's disease. *Macedonian Veterinary Review* 5-7, 56.
- WEN, Y., Q. HUANG, C. YANG, L. PAN, G. WANG, K. QI, H. LIU (2018): Characterizing the histopathology of natural co-infection with Marek's disease virus and subgroup J avian leucosis virus in egg-laying hens. *Avian Pathology*, Vol. 47, No. 1, 83-89.
- WHITEHEAD, M. L., V. ROBERTS (2014): Backyard poultry: Legislation, zoonoses and disease prevention. *J. Small Anim. Pract.* 55, 487-496.
- WITTER, R. L., K. A. SCHAT (2003): Marek's disease. In: Saif YM editor in chief, *Disease of Poultry 11th Edition*, Blackwell Publishing Co, Iowa State Press, 407-65, 2003.
- ZHOU, J., G. L. ZHAO, X. M. WANG, X. S. DU, S. SU, C. G. LI, V. NAIR, Y. X. YAO, Z. Q. CHENG (2018): Synergistic viral replication of Marek's disease virus and avian leukosis virus subgroup J is responsible for the enhanced pathogenicity in the superinfection of chickens. *Viruses* 18:10.

KOINFEKCIJA VIRUSOM MAREKOVE BOLESTI I VIRUSOM PTIČJE LEUKOZE KOD KOKOŠI PASMINE BANATSKI GOLOŠIJAN

Sažetak

Virus Marekove bolesti i virus ptičje leukoze uzrokuju zarazne, imunosupresivne i onkogene bolesti. Mnoga izvješća opisuju infekciju pojedinim virusom, ali tek mali broj izvješća podrobnije opisuje koinfekciju virusom Marekove bolesti i virusom ptičje leukoze. Cilj ovog istraživanja bio je utvrditi uzrok zdravstvenih poremećaja u jatu kokoši pasmine Banatski gološijan u dobi od 11 tjedana. Klinički pregled jata pokazao je znakove kaheksije, apatije, spuštenu krila, živčane simptome u obliku pareze nogu, sporadični proljev te 20%-tnu smrtnost. Postmortem pregledi pokazali su sljedeće promjene: kronični pseudomembranski tiflitis, crijevnu askaridijazu, splenomegaliju, granulomatoznu pneumoniju, višestruka žučkasta sirasta žarišta u jetri i bijele tumorske tvorbe u srcu. Patohistološki pregled tkivnih uzoraka slezene, proventrikla i srca otkrio je polimorfne infiltrate, dok je u plućima i jetri zabilježena granulomatozna upala. Metenamin srebrno bojenje po Gomoriju otkrilo je septirane i arborizirane hife nalik gljivama *Aspergillus* spp. u jetrenim i plućnim granulomima. Bakteriološke pretrage fecesa pokazale su prisutnost *Escherichia coli*, dok je parazitološka pretraga tankog crijeva utvrdila prisutnost oocita *Eimeria acervulina* te *Eimeria tenella* u cekumu. PCR-om su genomi virusa Marekove bolesti i virusa ptičje leukoze utvrđeni u tkivnim uzorcima. U ovom istraživanju su specifične patološke promjene i molekularni nalazi potvrdili koinfekciju virusom Marekove bolesti i virusom ptičje leukoze.

Ključne riječi: virus ptičje leukoze, kokoš pasmine Banatski gološijan, virus Marekove bolest