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PROSPECTIVES AND NECESSITY OF ERADICATION OF INFECTIOUS BOVINE RHINOTRACHEITIS / INFECTIOUS PUSTULAR VULVOVAGINITIS IN THE REPUBLIC OF SERBIA

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Abstract

Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) is a disease affecting cattle population of all breeds, categories and age. The disease can take different clinical courses, infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) being the most common ones. Both diseases, an especially infectious bovine rhinotracheitis (IBR), pose severe health threat and cause major economic losses and are considered one of the most costly diseases in cattle industry. The causal agent is a virus categorized into the family *Hepesviridae* and designated as bovine herpesvirus type 1 (BHV-1) or IBR/IPV virus. Any positive result to IBR/IPV specific antibodies in non-vaccinated cattle, in either blood or milk, indicates the infection, and the animal is considered a source of infection. In this article, we described the needs and prospects for the eradication of IBR/IPV in the Republic of Serbia. The eradication of IBR/IPV is a complex process implying strictly defined program of measures. The implementation of such measures requires systematic strategy involving different phases and activities that can continue over several years. The program requires substantial efforts as well as financial resources, which should be justified and paid off through a successful eradication of IBR/IPV

Key words: IBR/IPV, eradication, prospectives, necessity

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MOGUĆNOSTI I POTREBE ISKORENJIVANJA INFEKTIVNOG GOVEĐEG RINOTRAHEITISA/INFEKTIVNOG PUSTULOZNI VULVOVAGINITISA U REPUBLICI SRBIJI

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Kratak sadržaj

Infektivni goveđi rinotraheitis/infektivni pustulozni vulvovaginitis (IBR/IPV) su bolesti od koje oboljevaju goveda svih rasa, kategorija i uzrasta. Bolest se klinički pojavljuje u više formi, ali najčešće pojave bolesti su infektivni goveđi rinotraheitis (IBR) i infektivni pustulozni vulvovaginitis (IPV). Obe forme bolesti, a posebno infektivni goveđi rinotraheitis (IBR), nanose velike zdravstvene i ekonomske gubitke, pa se IBR smatra jednom od „najskupljih bolesti" u govedarstvu. Uzročnik bolesti je virus. Sistematizovan je u familiju *Hepesviridae* i označen je kao goveđi herpesvirus tip 1 (BHV-1) ili kao IBR/IPV virus. Svaki nalaz antitela protiv IBR/IPV virusa kod nevakcinisanih goveda, bilo u krvi ili mleku, ukazuje da je grlo inficirano i da predstavlja izvor infekcije. U radu su predstavljene mogućnosti i potrebe iskorenjivanja IBR/IPV u Republici Srbiji. Iskorenjivanje IBR/IPV je proces i sprovodi se prema jasno definisanom programu, koji može da traje više godina, a čine ga više faza sa različitim aktivnostima. Sprovođenje programa zahteva veliki rad i značajna materijalna i finansijska sredstva, koja treba da budu opravdana i nadoknađena uspešnim iskorenjivanjem IBR/IPV.

Ključne reči: IBR/IPV, iskorenjivanje, perspektive, potreba

INTRODUCTION

Infectious bovine rhinotracheitis / infectious pustular vulvovaginitis (IBR/IPV) is a disease affecting cattle population of all breeds, categories and age. The disease can take different clinical courses, infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) being the most common ones. Both clinical forms, an especially infectious bovine rhinotracheitis (IBR) brings on severe health threat and substantial economic losses and is considered one of the most costly diseases in cattle industry. The causal agent is a virus from the family of *Herpesviridae*, sub-family *Alphaherpesviridae*, named according to the clinical diseases it caused - bovine herpesvirus type 1 (BHV-1) or IBR/IPV virus.

IBR/IPV is an issue of major importance from both health and economic point of view. Acute form of IBR is associated with severe health risk and considerable economic damage. The initial disease manifestations involve rhinotracheitis and *keratoconjunctivitis*, which in turn progress to severe bronchopneumonia. Most severely affected animal populations are young cattle categories, i.e., calves and bull calves. Some animals that survive the acute form of the disease manifest apparent developmental defects as a consequence of severe injury of respiratory organs, especially lungs. In adult animals, that is, cows and in-calf heifers, in addition to respiratory syndrome, the infection can result in abortion, fetal mortality, prolonged service period and decreased milk yield (*Von Krage E et al., 1989; Hagee JJ et al., 1996; Hagee JJ et al., 1998; Graham A.David 2013*). Establishment of latent infection in all animals that survived the acute form is highly characteristic for the disease. Latently infected animals do not manifest clinical signs of the disease, while presence of virus-specific antibodies is the only indicator that the animal is potential infection reservoir. Moreover, a loss in milk yield of some 0.9 kg/day during nine weeks has been established in latently infected cows (*Straub Ch.O., 2001*). In addition to these and many other direct losses, latent IBR/IPV infection can cause a range of rather non-quantifiable indirect losses. Some typical examples include bans on trading breeding cattle, semen and embryos originating from infected herds or even regions. Such bans may bring even greater losses than the IBR/IPC epidemics itself.

With an aim of preventing the occurrence of the disease and consequential economic losses the problem of IBR/IPV has been addressed by a range of researchers worldwide. Extensive researches have been conducted to determine disease prevalence mainly because of its latent form and its importance for cattle trading. By the end of 20th century, numerous countries developed and implemented relevant programs for eradication of IBR/IPV to enable an unhindered cattle trading. Low incidence of IBR/IPV among cattle herds in Switzerland, Austria, Denmark and all Scandinavian countries resulted in complete eradication of IBR/IPV and obtaining of IBR/IPV-free status. Such status granted the aforementioned countries possibility of free international trading with all cattle categories as well as with semen for artificial insemination (*Ackermann M., Engels M., 2006*). Some other EU Member States have put substantial effort to eradicate the disease and obtain IBR/IPV free status in clearly delineated territories. Thus, the region of Bayern in Germany and Bolzano in Italy are nowadays certified as regions free from IBR/IPV. Moreover, all EU Member States developed and implemented their own national programs for eradication and control of IBR/IPV (*Ackermann M., Engels M., 2006*). Some of

them, such as Germany, Poland, Slovakia, Slovenia, Czech Republic, Hungary etc., eradicated IBR/IPV in almost all regions and are close to declaring the IBR/IPV-free status in either entire territory or at least some regions. According to OIE records, an outbreak of IB/IPV was established by the beginning 2015 in Austria, which had been considered IBR/IPV-free country since 2004. IBR/IPV was detected in 27 herds from four Austrian regions - Vorarlberg (3), Tyrol (18), Upper Austria (1) and Lower Austria (5).

The investigation of IBR/IPV in the Republic of Serbia was initiated in the 60ties of the last century. In 1967, Bratanović U et al. isolated the virus, which was the first isolation of IBR/IPV in our country. Later on, a number of researchers reported on isolation and seroprevalence of IBR/IPV in different cattle herds and regions in Serbia (*Jermolenko G et al., 1978*, *Pavlović R et al., 1981*, *Lazić S et al., 1995*, *Lazić S et al., 2008.*). It should be emphasized that many cattle herds in the Republic of Serbia have preserved an IBR/IPV-free status. Moreover, current farm practices among many farmers imply introducing only IBR/IPV-free animals into the newly-formed cattle herds. In that respect, we can state that certain number of cattle herds in the Republic of Serbia is considered IBR/IPV-free. Such herds can be certified as IBR/IPV free herds after relevant examination procedure.

PROGRAMS FOR ERADICATION OF IBR/IPV

Huge health-economic losses associated with IBR/IPV prompted a number of West-European countries to develop legal regulations and provisions for getting this infection under control. Eradication of IBR/IPV in many EU countries was carried out in line with the provisions of the Directives 64/432/EEC, 2004/558/EEC and other relevant legislation. All countries interested in participation in international cattle trading were obligated to ensure development and implementation of national programs for control, suppression and eradication of IBR/IPV. Even if not the member of EU/EEC, Switzerland was one of the first countries in the world, which developed and implemented the program for control, suppression and eradication of IBR/IPV. (*Bommeli W., Kihm U., 1982., Ackermann M., et al. 1990*).

The program of eradication of IBR/IPV in Switzerland has continued throughout 10 years and was based on the principle "test and remove", without using vaccines. The eradication process in Switzerland prolonged over some 10 years including four simultaneous phases. With an aim of preventing disease transmission restrictions were imposed on trade of breeding animals with IBR/IPV positive finding. Such animals were sent to slaughter. All breed-

ing and fattening animals were examined and animals with positive finding of IBR/IPV specific antibodies were slaughtered. Continuous monitoring has been conducted in order to maintain the status of IBR/IPV-free country.

Austria is also one of the first European countries that introduced the program for eradication of IBR/IPV. Since 1990, eradication has been mandatory for all cattle producers. Because of low seroprevalence, the eradication strategy of IBR/IPV included slaughtering of all seropositive animals without vaccination. Continuous monitoring is practiced in line with relevant legislation (*Ackermann M., Engels M., 2006*).

By the 1990s, Denmark, Finland, Norway and Sweden implemented successful programs for eradication of IBR/IPV and are now considered disease-free states at their entire territory. Since the seroprevalence was relatively low, eradication strategy included slaughtering of seropositive animals without vaccination and with continuous surveillance by serological testing (*Ackermann M., Engels M., 2006*). The "test and slaughter"approach (*Nuotio L. et al.,* 2007) was applied in all aforementioned countries.

Some other EU Member States such as Germany (except Bavaria), Italy (except region of Bolzano), Poland, Hungary, Slovenia, Ireland, Belgium, France, Netherlands, Portugal, Lithuania, Spain and Great Britain developed national programs for eradication of IBR/IPV based on vaccination (mostly using marker vaccines against IBR/IPV) and continuous monitoring of the disease. Many of those countries such as Germany (all regions), several provinces in Italy, Poland as well as Czech Republic, Slovakia and Slovenia have already completed or are close to complete eradication of IBR/IPV in all regions.

As emphasized by numerous authors, appropriate monitoring is the crucial element of an IBR/IPV eradication program. The monitoring defines the methods and manner of disease control. Serological testing is the key segment, thus, type and number of samples per one herd as well as the testing-intervals needs to be precisely defined (*Graat E.A.M. et al., 2001, Knopf L. et al., 2007*). Herd size, seroprevalence and the level of applied biosecurity measures are individual characteristics of each herd, which significantly affect the effectiveness of an eradication program disregarding the applied methods and model of eradication.

ERADICATION OF IBR/IPV IN THE REPUBLIC OF SERBIA

According to the research results from previous period as well as from current research, the presence of the disease is likely in many herds, and some 50% of cattle are suspected to be latently infected with IBR/IPV virus.

The eradication of IBR/IPV in our country relies on voluntary participation of cattle owners, same as in majority of other countries that have already eradicated or are close to complete eradication of the disease, yet with the support and participation of governmental institutions in view of appropriate monitoring and certification. Herd certification is performed by Veterinary Directorate through relevant veterinary institutes, field veterinary service and under supervision of veterinary inspection board. Veterinary Directorate issues the certificate and keeps the Records on issued certificates. The certificates need to be available on-line from the web-site of the Veterinary Directorate and all relevant data on the herd, IBR/IPV eradication and validity date of the certificate should be visible for all interested parties.

Eradication of IBR/IPV is highly complex and comprehensive procedure encompassing several phases. Depending on disease prevalence within the herd the process can prolong over several years. Once the disease has been eradicated, continuous yearly monitoring is crucial for the maintenance of IBR/IPV free status of the herd as well as for preventing introduction and spreading of IBR/IPV virus within the herd. The surveillance can prevent a range of health problems while continuous monitoring confirms the IBR/IPV free status of the herd resulting in renewal of relevant certificates.

Initial phase, or phase I, encompasses the confirmation of the presence of the disease in the herd. The detection relies on examination of milk samples (bulk samples or individual samples) and/or blood serum testing. Bulk milk sample can contain milk samples collected from up to 20 cows. The size of the herd, that is, number of cattle in the herd, determines the number of samples/animals to be examined in order to confirm the presence of the disease. If the presence of the disease has not been confirmed in milk samples, blood samples are collected from non-milking cows (dry cows and all other animals older than 2 months) in order to determine herd disease status. Negative findings for IBR/IPV virus in milk and blood samples are argumentative enough to confirm absence of the infection, i.e., to consider the herd free from IBR/IPV. The herd can be certified as IBR/IPV-free.

Positive finding of antibodies against IBR/IPV virus in milk samples requires precise determination of disease prevalence within the herd in order to define the program, i.e., the method and procedures for eradication of the disease. Precise determination of seroprevalence encompasses testing of individual milk samples and blood samples from strictly defined number of animals. The crucial factor for the determination of prevalence is the number of examined animals in the herd calculated using Cannon's formula (*Cannon R.M. 2001)* which is applicable for a reliable determination of seroprevalence

at herd level. According to the prevalence value, either physical removal of infected animals or vaccination is applied. Removal (culling) of infected animals from the herd is justified with prevalence rates below 5%. Removal of latently infected animals is considered the most effective method for eradication of IBR/IPV. After removing the last infected bovine from the herd, dual control is performed after 28-day period (two incubation periods) and should include all animals in the herd. Obtaining of two successive negative results at both testings qualifies the herd for certification as IBR-free.

In conditions of high disease prevalence, vaccination is considered the most suitable instrument for eradication of IBR/IPV. Vaccination needs to be well planned and comprehensive, and must include all cattle categories. The vaccination schedule is determined according to the type of selected vaccine. Vaccination should be conducted throughout the period of several years, that is, until removing all latently infected animals from the herd. Monitoring of vaccination effects should be performed throughout the entire vaccination period to determine the IBR/IPV status of the herd. In the eradication of IBR/IPV, very good results were obtained with the vaccine containing *glycoprotein E-deleted* (*gE*) gene, so-called IBR/IPV marker vaccine, which was developed for usage in IBR/IPV eradication programs. Application of this marker vaccine enables differentiation between vaccinated and infected animals (DIVA principle) during the monitoring process – testing of seroconversion and vaccination effects using appropriate diagnostic tools. This provides useful information on disease status within the herd, i.e., stagnation or progress. Successful eradication of IBR/IPV using marker vaccine has been accomplished in many bovine herds in EU.

In the process of eradication of IBR/IPV, vaccination period and schedule are determined by herd overhaul plan. It usually lasts 4-5 years, as it is likely that some 20-25% of all latently infected animals will be eliminated from the herd. Monitoring is performed one year post vaccination encompassing examination of bulk milk samples and blood samples from non-milking animals. Negative finding qualify the herd for certification as IBR/IPV free.

The maintenance of certified IBR/IPV-free status implicates continuous adherence to relevant biosecurity measures, monitoring of health status and periodic control of blood samples and bulk milk samples. Implementation and adhering to relevant biosecurity measures is crucial to the prevention of numerous diseases including IBR/IPV. Newly purchased animals are potential entry portal for many diseases, thus, purchase of new animals should be performed only from herds certified as free from IBR/IPV. Such animals must be quarantined prior to be introduced into the herd. Isolation period can extend

even up to one month, that is, until obtaining laboratory confirmation on IBR/IPV virus-free status for all purchased bovines. Monitoring of animals' health status implies exclusion of IBR/IPV virus as a causal agent in cases of abortion, fetal mortality and manifested respiratory syndrome in the herd. Control examination is one of the most important factors for maintenance of IBR/IPV free status. Such examination encompasses detection of IBR/IPV specific antibodies in blood and/or milk samples as reliable indicators of the disease and is performed at 6-month intervals. The number of examined animals to be examined is determined according to herd size applying Cannon's formula, and must be representative at herd level. These activities ensure the certification of the herd, which needs to be documented by relevant record of issued by authorized institutions, describing all activities performed in a certified IBR/IPV-free cattle herd.

CONCLUSIVE REMARKS

Eradication of IBR/IPV in the Republic of Serbia is absolutely indispensable and economically justified. It offers multiple benefits, above all for producers of breeding material but also for other economic branches such as dairy and meat industry as well as other business entities involved in cattle industry either directly or indirectly. Having in mind the complex nature of program implementation, it is essential to identify the participating subjects and their responsibilities. Cattle owners (farmers, cooperative societies, associations) play the major role in the process. Eradication of IBR/IPV should be voluntary for farmers and not the subject of coercive measures.

Aside from farmers, successful realization of the program requires participation of other subjects such as field veterinary service, veterinary inspection board, relevant departments of scientific or specialized veterinary institutes and Veterinary Directorate. Farmers and field veterinarians assisted by professionals from veterinary institutes and under supervision of veterinary inspection board launch the initiative and submit the request for eradication of IBR/IPV to the Veterinary Directorate. After obtaining positive response, professionals from veterinary institutes develop detailed program of activities in cooperation with field veterinary service and farmers and under supervision of veterinary inspection board. Field veterinary service in cooperation with farmers performs the sampling and submission of samples to the relevant veterinary institute under supervision of veterinary inspection board. If the eradication of infection requires vaccination, field veterinary service is responsible to supply the vaccines and carry out the vaccination according to vaccination

schedule designed by professionals from veterinary institutes. Testing results, opinions and recommendation on IBR/IPV eradication of the relevant veterinary institute are submitted to farmers, field veterinary service and veterinary inspection board. At the moment of termination of the program, relevant veterinary institute and veterinary inspection board submits to the Veterinary Directorate relevant documentation and the proposal for certification of the herd as IBR/IPV-free. Veterinary Directorate issues the certificate and keeps the Records on issued certificates. The certificates need to be available from the web-site of the Veterinary Directorate („*on-line*").

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BLUETONGUE DISEASE - EPIZOOTIOLOGY SITUATION IN SERBIA IN 2015, DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

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Abstract

Bluetongue disease is non-contagious, vector borne, viral disease mainly of sheep but also of other domestic and wild ruminants. Bluetongue virus (BTV) belongs to the family *Reoviridae*, genus *Orbivirus* and is characterized by segmented double-stranded RNA. Virus is transmitted from one to another susceptible animal by hematophagous insects of the genus *Culicoides*. According to official data, between 2002 and 2014, Serbia has belonged to BTV free countries. After that, the first outbreak occurred in August 2014. The last case was reported in December of the same year. During 2015, 74 samples were examined for exclusion of bluetongue disease: 8 in cattle, 65 in sheep and one in goat. In order to detect viral genome, 73 blood samples and one tissue sample were examined by reverse transcription - polymerase chain reaction (RT-PCR). None of tested samples was confirmed to be BTV positive. Following the Instruction of the Ministry of Agriculture and Environmental Protection - Veterinary Directorate, monitoring program for Bluetongue disease in Serbia started from October 2015. The program consists of insect identification and detection of viral genome in *Culicoides spp.* by RT-PCR assay. Of the 80 samples that were received during the program realization in 2015, only four, which were collected during late autumn, have contained insects of *Culicoides spp*. In none of them, BTV was detected. For differential diagnosis, 65 ovine blood samples were examined for the presence of viruses of contagious ecthyma, sheep and goat pox as well as eight bovine blood samples were tested for viruses of bovine viral diarrhea, infectious bovine rhinotracheitis / pustular vulvovaginitis and malignant catarrhal fever. The samples were analyzed using

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molecular methods (PCR and RT-PCR). Only two bovine blood samples gave positive reaction for the presence of bovine viral diarrhea virus.

Key words: bluetongue disease, differential diagnosis

BOLEST PLAVOG JEZIKA – EPIZOOTIOLOŠKA SITUACIJA U SRBIJI U 2015. GODINI, DIJAGNOSTIKA I DIFERENCIJALNA DIJAGNOSTIKA

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Kratki sadržaj

Bolest plavog jezika je nekontagiozno, vektorski uslovljeno virusno oboljenje koje se javlja uglavnom kod ovaca, ali i kod ostalih domaćih i divljih preživara. Virus plavog jezika (Bluetongue Virus – BTV) pripada familiji *Reoviridae*, rod *Orbivirus* a karakteriše ga segmentirana dvolančana RNK. Virus među prijemčivim životinjskim vrstama prenosi hematofagni insekt iz roda *Culicoides*. Prema zvaničnim podacima, Srbija se u periodu između 2002. i 2014.godine smatrala zemljom slobodnom of BTV. Nakon toga, bolest se pojavila prvi put u avgustu 2014. Poslednji slučaj prijavljen je u decembru iste godine. Tokom 2015. Godine ispitana su 74 uzorka sa ciljem isključivanja bolesti plavog jezika, i to 8 uzoraka poreklom od goveda, 65 od ovaca i jedan od koza. U cilju detekcije virusnog genoma 73 uzorka krvi i jedan uzorak tkiva su ispitani metodom reverzibilne lančane reakcije polimeraze (RT-PCR). Ni jedan od testiranih uzoraka nije bio pozitivan na BTV. U skladu sa Instrukcijom Ministarstvo poljoprivrede i zaštite životne sredine – Uprave za veterinu, u oktobru 2015. godine započet je program monitoringa bolesti plavog jezika u Srbiji. Program podrazumeva identifikaciju insekata i detektovanje genoma virusa kod *Culicoides spp.* primenom metode RT-PCR. Od ukupno 80 uzoraka koji su primljeni na ispitivanje tokom realizacije programa u 2015. godini, samo četiri uzorka koji su prikupljeni tokom kasne jeseni sadržali su insekte iz roda *Culicoides spp*. Ni u

jednom od uzoraka nije detektovan BTV. U svrhu diferencijalne dijagnostike 65 uzoraka ovčije krvi ispitano je na prirustvo virusa kontagioznog ektima, boginja koza i ovaca, a osam uzoraka krvi goveda testirano je na viruse goveđe virusne dijareje, infektivnog goveđeg rihotraheitisa / pustularnog vulvovaginitisa i maligne kataralne groznice. Uzorci su analizirani primenom molekularnih metoda (PCR i RT-PCR). Kod samo dva uzorka krvi goveda ustanovljena je pozitivna reakcija na prisustvo virusa goveđe virusne dijareje.

Ključne reči: bolest plavog jezika, diferencijalna dijagnoza

INTRODUCTION

Bluetongue disease is non-contagious, vector borne, viral disease that infects mainly sheep but also other domestic and wild ruminants. Bluetongue virus (BTV) belongs to the family *Reoviridae*, genus *Orbivirus*. The genome of bluetongue virus is segmented, double-stranded RNA. Up to date, 27 serotypes of bluetongue virus have been discovered (Maan et al., 2012, Jenckel et al., 2015). The virus is transmitted between susceptible animals by hematophagous insects of the genus *Culicoides*. Only females can transmit the virus. They live around 70 days, and suck blood every 3-4 days (Radojičić et al., 2011). Being the vector borne disease, the presence of vectors is crucial for occurrence of the infection in animals. Seasons with low temperatures, which are free of vectors, influence the epizooty of disease in such way that the disease incidence decreases to zero.

According to official data, Serbia was considered BTV free country during the period 2002 – 2014[2]. This period ended with an outbreak of the disease in August 2014, in the south of the country. Later on, the virus has extended over almost complete territory of Serbia. It was confirmed that the virus that caused the disease belonged to serotype 4. The last case was reported in December of the same year. During this period, 644 outbreaks have been reported, all of them caused by BTV serotype 4 (BTV4) (Anonymous, 2015, Veljović et al., 2015). Laboratory diagnostic and confirmation were carried out in National Reference Laboratory (NRL) for Bluetongue Disease - Institute of Veterinary Medicine of Serbia (IVMS).

Following the Instruction of the Ministry of Agriculture and Environmental Protection - Veterinary Directorate (Anonymous, 2015), Monitoring program of bluetongue disease has been implemented in October 2015. The program included insect identification and detection of viral genome in *Culicoi-*

[2] http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Immsummary

des spp. by Reverse Transcription – Polymerase Chain Reaction (RT-PCR) and was aimed to determine the vector free periods.

Although clinical signs in sheep are quite typical, there are many other viral diseases with similar manifestation. Clinical signs in cattle are rather rare but can easily be misinterpreted. Therefore, all ill domestic ruminants with facial oedema, erosions of the nasolabial plate, congestion and haemorrhage of mucous membranes, inflammation and necrosis of the skin should be differentially tested for contagious ectyme of sheep, bovine viral diarrhoea, poxvirus infections, viral stomatitis etc.

The aim of this study was to present epizootiological situation of bluetongue disease during 2015 and establishment of differential diagnosis after BTV has been excluded in samples originating from clinically ill ruminants.

MATERIAL AND METHODS

Laboratory diagnostics of the bluetongue disease was performed at virology department of The Institute of Veterinary Medicine of Serbia, which is a NRL for bluetongue. During 2015, 74 samples originating from sheep, cattle and goat with symptoms of bluetongue were tested by RT-PCR. The majority of tested samples included unclotted blood from ill animals (73 samples). Only one sample originated from a dead goat and was composed of parts of morphologically altered organ (spleen).

Monitoring program for bluetongue disease, issued by the Ministry, prescribes entomology and virology examination of insects. During twelve months (since 1st October 2015 until 30th September 2016), each of twelve Serbian veterinary institutes had to submit to the Institute of Veterinary Medicine of Serbia insects collected on two locations of their respective counties, two times per month (Anonymous, 2015). In the period from 1 October to 31 December 2015, 80 pooled insect samples were received. After insect detection by morphological examination, the samples containing *Culicoides spp*. were examined for the presence of bluetongue virus by RT-PCR.

Detection of bluetongue virus in blood, tissue samples and midges was performed using molecular technique - Reverse Transcription - Polymerase Chain Reaction (RT-PCR), which detects viral nucleic acid. Extraction of the nucleic acid was performed using RNA extraction kit Bioline Isolate RNA Mini Kit (Bioline, UK) in accordance with the manufacturer's instruction. For Reverse Transcription - Polymerase Chain Reaction (RT-PCR) commercial kit Verso 1-Step RT-PCR Kit ReddyMix (Thermo Fisher Scientific Inc., USA) was used. Conventional, nested RT-PCR assay targeting the NS1 gene of BTV was

performed using primers and thermal protocol referred in the OIE (*fr. Office International des Epizooties*) Terrestrial Manual 2014, Chapter 2.1.3.[3]

Beside examination on BTV, differential diagnostic assays were performed on all blood samples. Samples originated from sheep (65 blood samples) were analyzed for the presence of contagious ectyme and sheep and goat poxviruses, while eight bovine blood samples were analyzed for the detection of bovine viral diarrhea virus, infectious bovine rhinotracheitis / pustular vulvovaginitis and malignant catarrhal fever virus. Polymerase Chain Reaction (PCR) and Reverse Transcription - Polymerase Chain Reaction (RT-PCR) were used for detection of viral genome. Viral DNA was extracted with QIAampDNA Mini Kit (QIAGEN, Germany), and Hot Star Taq PLUS Master Mix Kit (QIAGEN, Germany) was used for PCR. Diagnostic of the contagious ectyme was done following the protocol of Hosamani et al. (2006) where used primers amplify complete sequence for envelope protein (B2L). For the detection of sheeppox and goatpox viruses, PCR protocol for highly conserved region which encodes protein called *viral growth factor,* was used (Jônatas et al., 2009).

According to recommended differential diagnostic tests,[4] all bovine blood samples were tested for viruses of bovine viral diarrhea (BVD), infectious bovine rhinotracheitis / pustular vulvovaginitis (IBR/IPV) and malignant catarrhal fever (MCF). For the detection of nucleic acid of those viruses, we used primers and protocols according to Weinstock et al. (2001) for BVD, Deka et al. (2005) for IBR/IPV and Teankumet al. (2006) for MCF.

RESULTS AND DISCUSSION

Analysis of 73 samples of unclotted blood as well as 1 tissue sample for the presence of bluetongue virus gave negative result, what leads to a conclusion that during 2015 there were no laboratory confirmed outbreak of bluetongue disease. Those results represent combination of many biotic and abiotic factors, as well as climatic changes that led to the completion of outbreak of bluetongue disease during the winter 2014/2015.

The process of spreading of BTV and development of epizooty highly depends on vectors. For the transmission of the virus, competent vectors[5], viremic and susceptible animals are necessary. Thus, efficient transmission on the location with viremic animals requires presence of specific species of

[3] http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.03_BLUETONGUE.pdf
[4] http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/BLUETONGUE.pdf
[5] Vector competence defines ability of a vector to become infected and to have a possibility to replicate and disseminate a virus

Culicoides, which can transmit the virus. Until now, four species of Culicoides in which BTV can propagate have been identified in the territory of Serbia: *Culicoides pulicaris, C. nubeculosus, C. obsoletus* and *C. parroti* (Pavlović Ivan, 2015). Besides the necessity of the presence of competent vector species, the vectors during their lifetime have to be fed on viremic animal and survive extrinsic incubation period (which depends on outside temperature, humidity, etc), whilst virus multiplies in the gut of the insect. To spread the infection, infected midges have to bite susceptible, naïve animal and to excrete sufficient amount of the virus into its blood. Many physiological barriers may act to limit or constrain dissemination of the virus throughout the insect's organs and thus prevent transmission. It is important to emphasize that only *Culicoides* females suckle blood, and that they live probably 10-20 days, exceptionally longer (between 44 and 90days) (Mellor et al., 2000).

With the arrival of wintertime, conditions for reproduction of insects become unfavorable. Among other weather conditions that can influence vectorial capacity of *Culicoides spp.*, the temperature is the most important one. It affects the number of generations that can arise during one season and the population size. Temperature influences the virus infection/replication in the bodies of adult midges. For few vector species, it was proved that the complete inhibition of virus replication occurs when ambient temperature decreases below 15^{o}C (Mullens et al., 1995). Contrary to that, infection rate and speed of virus replication grows at higher temperatures, but the life of midges shortens (Mullens et al., 1995). Increase of precipitation during summer season favors their reproduction.

Beside the competent vector, viremic animal is another essential factor in this equation with many variables. The duration of viremia in bluetongue disease is variable. It lasts from 3 to 300 days (Radojičić et al., 2011). Longer duration of viremia implies higher possibility for the occurrence of new disease outbreaks after winter. It is well known that among susceptible species the longest duration of viremia is recorded in cattle – maximum 300 days (Radojičić et al., 2011). Therefore, this species is considered most responsible for overwintering of the virus and new outbreaks after long period with unfavorable climatic conditions.

Having in mind the aforementioned facts, it is clear that the process of transmission and overwintering of the virus can fail in many points.

Although the predictions from the beginning of 2015 suggested that new outbreaks of bluetongue will appear in our country again (Veljović et al., 2015), it became obvious that climatic factors were not favorable for the development of *Culicoides spp*. Most probably, cold wave with temperatures below -10°C

during December 2014 and January 2015[6] caused disappearance of adult competent vectors and termination of reproductive cycle. On the other hand, long and very hot summer in 2015, with six hot waves and average precipitation[6], reduced the number of newly borne *Culicoides spp.* Moreover, animal population that is partially seroconverted makes unfavorable circumstances for new epizooty of BTV serotype 4.

Many surrounding countries encountered outbreaks of bluetongue during 2015 (Hungary, Romania, Macedonia, Albania, Bosnia and Herzegovina and Croatia)[7]. Only Hungary and Croatia faced the outbreaks that happened close to the border with Serbia, but transmission across the border has not been confirmed.

Monitoring program that has begun in October 2015 is aimed at examining and establishing local presence of *Culicoides spp*. and defining seasons throughout the year that are free of vectors. To the end of 2015, 80 pooled samples of insects have been collected and delivered to the IVMS. Among them, only four, which were collected during November, contained insects of *Culicoides spp*. (*C.obsoletus*). As RT-PCR and nested PCR for detection of NS1 gene[8] gave negative results it could be concluded that midges had not been infected with bluetongue virus.

Differential diagnostic assays were performed on all 73 animal blood samples. Sixty-five ovine samples have been examined for viruses of contagious ectyme, and sheep and goat pox. All samples gave negative results for the presence of genome segment that encodes synthesis of *viral growth factor* of sheep and goat poxviruses. According to the official data, the virus circulated in neighboring countries between 2013 and 2015 (Bulgaria - 2013 and Greece 2013-2015)[9], but not in close proximity to the Serbian border. As this is highly contagious disease that cause high morbidity (around 75%) and mortality (around 50%) in naive populations (Radojičić et al., 2011), it was expected that these analyses would give negative result.

Contagious ectyme is widespread across our territory. During 2014, six positive samples (animals) were detected at virology department of The Institute of Veterinary Medicine of Serbia. Out of 65 blood samples tested during 2015, not even one gave positive reaction. Genome of parapoxvirus has not been detected in blood, though it was expected because of its presence in ovine population during 2014. For a more accurate diagnosis, the sampling and sam-

[6] http://www.hidmet.gov.rs/ciril/meteorologija/klimatologija_produkti.php
[7] http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Immsummary
[8] http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.03_BLUETONGUE.pdf
[9] http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Immsummary

ple submission for laboratory diagnostics should be done during the clinical phase of the disease. As soon as the first symptoms with skin changes appear, the sample of choice is a fluid from the vesicles, and later on a scab. Although unclotted blood is the best sample for BTV detection (because of quite long viremia), in case of poxvirus infections, virus is present in blood for short period of time and therefore this type of sample is not reliable particularly if taken after viremic stage of the disease.

After primary and secondary viremia, the highest amount of the virus is present in skin pustules and in scabs that arise after eruption of pustules (Baxby D., 1996). Thus, these are the preferable samples for laboratory testing when speaking of animals in this phase of the disease. Negative results in our testing were probably due to the fact that blood is not enough suitable sample for poxvirus PCR analysis.

Bovine samples collected for the detection of bluetongue virus were differentially examined for viruses of bovine viral diarrhea, infective bovine rinotracheitis / pustular vulvovaginitis and malignant catarrhal fever. Among these viruses, only viral genome of bovine viral diarrhea virus was detected. Two samples positive for bovine viral diarrhea virus originated from cattle from the settlements of Gaj, municipality Kovin, and Dobrica, municipality of Alibunar. Such result was expected having in mind unfavorable epizootiological situation in whole country.

CONCLUSION

Opposite to predictions, bluetongue disease was not confirmed in NRL for bluetongue during 2015. Which combination of factors drive up to the preferable epizootiology situation is still an open question. Thank to it, epizootiological situation is better now than in the last year (2014). The monitoring program has been carried out until the autumn 2016, and its results should help us to deal with new potential epizooties. In addition, a program of vaccination of ruminants in regions where outbreaks occurred and in regions where combination of abiotic, biotic and climatic factors is favorable for bluetongue disease was started at the end of 2015. Killed vaccine should prevent infection of ruminants with BTV serotype 4 and decrease the probability of new outbreaks. Differential diagnostic assays served for exclusion and confirmation of other viral diseases with similar symptoms. In order to obtain better and more reliable results, appropriate samples (beside blood) from ill animals should be collected and used for laboratory examination.

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SEROPREVALENCE OF MOSQUITO-BORN AND TICK-BORN MICROORGANISMS IN HUMAN POPULATION OF SOUTH BACKA DISTRICT

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Abstract

Chikungunya virus is an Arbo virus belonging to the family *Togaviridae*. In urban areas, antropophilic *Aedes aegypti* and *Aedes albopictus* mosquitoes are vectors for virus transmission to human population. Chikungunya virus has attracted the professional and scientific public attention in 2013 causing a massive outbreak on the American continent. In Europe, autochthonous transmissions of Chikungunya virus infections have been recorded in Italy in 2007 as well as in France in 2010 and 2014. Usutu virus is a RNA virus from the family *Flaviviridae*. The virus circulates in a transmission cycle between wild birds and Culex mosquitoes. The virus has been detected in numerous bird species across Europe. Manifestations recorded in humans include meningoencephalitis and skin rash. First human cases in Europe were recorded in immunocompromised individuals in Italy in 2009. Spirochete *Borrelia burgdorferi sensu lato* is transmitted to humans by ticks and causes Lyme disease, a multisystemic disease with dermatological, neurological, cardiological or articular manifestations. Ninety three persons interviewed about risk factors for vector-borne infections were examined. The examination was performed using commercial ELISA IgG for Chikun-

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gunya and Usutu virus and ELISA IgM and IgG test for *Borrelia burgdorferi* in line with manufacturer's instructions (Euroimmun, Germany). Out of 93 examined individuals Usutu virus specific IgG antibodies were identified in 7.5% (7/93) persons. The results of ELISA IgG test for Chikungunya virus were negative in the majority of tested samples, whereas 7.5% (7/93) of samples revealed borderline result. In 9.7% (9/93) participants, antibodies against *Borrelia burgdorferi* were detected only by ELISA IgM test. Recent infection with *Borrelia burgdorferi* was confirmed in 2.15% (2/93) individuals, whereas IgG antibodies against *Borrelia burgdorferi* were detected in only one participant. The obtained results indicated that Usutu virus is active in the territory of South Bačka District contrary to Chikungunya virus as well as that *Borrelia burgdorferi* is an important pathogen in the investigated region.

Key words: Usutu virus, Chikungunya virus, Borrelia burgdorferi, ELISA IgG

SEROPREVALENCIJA MIKROORGANIZAMA KOJE PRENOSE KOMARCI I KRPELJI KOD HUMANE POPULACIJE U REGIONU JUŽNA BAČKA

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Kratak sadržaj

Chikungunya virus je Arbo virus iz porodice Togaviridae. U urbanim zonama antropofilne vrste komaraca Aedes aegypti i Aedes albopictus su vektori koji infekciju prenose na ljude. Chikungunya virus je privukao pa-

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žnju 2013. godine izazvavši veliku epidemiju na američkom kontinentu. U Evropi je registrovana autohtona transmisija Chikungunya virusa 2007. god. u Italiji i 2010. i 2014.god. u Francuskoj. Usutu virus je RNK virus iz porodice Flaviviridae. Održava se u transmisivnom ciklusu između divljih ptica i komaraca iz genusa Culex. Virus je dokazan u različitim vrstama evropskih ptica. Virus u čoveka izaziva meningoencefalitis i osip na koži. Prvi humani slučajevi u Evropi su registrovani u Italiji 2009. god. u imunodeficitarnih osoba.

Spiroheta Borrelia burgdorferi sensu lato se prenosi na ljude krpeljima i izaziva lajmsku bolest, multisistemsko oboljenje koje se ispoljava dermatološkim, neurološkim, kardiološkim manifestacijama i promenama na zglobovima. Pregledano je 93 ispitanika od kojih je anketiranjem dobijen podatak da su izloženi riziku inficiranja mikroorganizmima koje prenose vektori. Testiranje je izvršeno komercijalnim ELISA IgG na Chikungunya i Usutu virus te ELISA IgM i IgG testom na Borrelia burgdorferi, striktno prema uputstvima proizvođača (Euroimmun, Nemačka). Od 93 ispitanih osoba IgG antitela protiv usutu virusa nađena su u 7,5% (7/93). Rezultat ELISA IgG testa na Chikungunya virus je bio negativan u većini testiranih uzoraka dok je u 7,5% (7/93) uzorka rezultat bio graničan. U 9,7% (9/93) ispitanika dokazana su antitela samo ELISA IgM testom na bakteriju Borrelia burgdorferi. U 2,15% (2/93) ispitanika dokazana je nedavna infekcija bakterijom Borrelia burgdorferi. U jednog ispitanika dokazana su samo IgG antitela na Borrelia burgdorferi. Dobijeni rezultati ukazuju da je za razliku od Chikungunya virusa usutu virus aktivan na teritoriji Južnobačkog okruga kao i da je Borrelia burgdorferi značajan patogen na ispitivanom okrugu.

Ključne reči: Usutu virus, Chikungunya virus, Borrelia burgdorferi, ELISA IgG

INTRODUCTION

Chikungunya virus is an Arbo virus belonging to the family *Togaviridae*, genus *Alphavirus*. In African jungles, various nonhuman primates serve as the reservoir of Chikungunya virus after being infected by its vectors – various types of mosquitoes. Infected mosquitoes may transmit the virus to humans during the blood meal. In urban areas, antropophilic *Aedes aegypti* and *Aedes albopictus* mosquitoes are responsible for transmission of the disease (Weaver et al., 2012). Chikungunya virus causes a sudden onset of clinical symptoms, fever over 38,5^{0}C, polyarthralgia and skin rash. The prognosis is most commonly favorable, although hepatitis, Guillain-Barre syndrome, cardiologic and

neurologic disorders like encephalitis, myelopathy and polyneuropathy may occur. The ability of *Aedes aegypti* and *Aedes albopictus* mosquitoes to colonize wide new geographic areas, facilitated by rapid development of international traffic and global climate changes, significantly contributes to Chikungunya virus expansion. Chikungunya virus has attracted the professional and scientific public attention in 2013 causing a massive outbreak on the American continent. Chikungunya virus infections have been recorded in Africa, Asia, Europe and on the islands in the Indian and Pacific Oceans before 2013. At the end of 2013, the first local transmission of Chikungunya virus on the American continent was recorded in the Carribean. Since then, almost 1.7 million chikungunya cases have been identified in 45 countries on the American continent (CDC, 2015). Local transmission of Chikungunya virus was reviewed in 198 cases in Puerto Rico and in 4 cases at the U.S. Virgin Islands (CDC, 2016).

Usutu virus is another arbovirus, belonging to the family *Flaviviridae*, serogroup of Japanese encephalitis. Usutu virus infection is endemically present in Africa, where the birds serve as the reservoir of infection, while the Culex mosquitoes play the role of the vector. African birds have become well adapted to Usutu virus during their evolution, so the infection goes mostly asymptomatically in these birds. In contrast, Usutu virus is highly virulent for European birds and the infection may result in necrotizing focal encephalitis, degenerative myocarditis and fatal encephalitis (Baconyi et al., 2007). The virus is presumed to be introduced into Europe by migratory birds that became infected during either living in or overflying of endemic areas in Africa. The first human cases have been recorded in immunocompomised persons in Italy (Pecorari et al., 2009). The virus causes meningoencephalitis and skin rash in humans (Cavrini et al, 2011).

Infection with spirochete *Borrelia burgdorferi sensu lato* is transmitted to humans by ticks and causes Lyme disease, a multisystemic disease with dermatological, neurological, cardiological or articular manifestations. Although even 13 different species of Borrelia belong to *Borrelia burgdorferi sensu lato*, one of those, *Borrelia burgdorferi sensu stricto,* is the most significant in the United States of America, whereas *Borrelia afzelii* and *Borrelia garinii* are the most important members of *Borrelia* species in Europe. Incidence of Lyme disease in the United States ranged from 7.0/100 000 (in 2012) to 9.8/100 000 (in 2009) during the period of 2004 - 2015, so as Lyme disease is the most common vector-borne disease in the United States. It is the most common tick-borne disease in Europe as well. In endemic areas of the Euroasia, various species of vertebrates, small mammals and birds infested by larvae and nymphs of *Ixodes ricinus* ticks serve as reservoirs of *Borrelia burgdorferi.*

MATERIAL AND METHODS

In 2015, 93 otherwise healthy persons referred to the Public Health Institute of Vojvodina for serological testing within regular pregnancy check-ups or pre-operative preparation were examined. Persons were interviewed about risk factors for mosquito-borne or tick-borne infections such as outdoor recreational activities, professional stay in nature, exposure to the mosquitoes or use of the repellents. In total 53 (56.9%) subjects were females, while 40 (43.1%) were males. Sera were tested for the presence of Usutu and Chikungunya viruses by enzyme immunoassay (ELISA) for specific IgG antybodies and IgM and IgG for *Borrelia burgdorferi* (manufacturer Euroimmune, Lübeck, Gemany) on Analyzer I, Euroimmune.

RESULTS

IgG ELISA – positive results for Usutu virus were obtained in 7 (7.5%) samples (four from males, three from females) with average age of 53.9 years. Among subjects seropositive on Usutu virus, 71.4% reported either outdoor or indoor exposure to the mosquitoes. 6/7 (85.7%) subjects seopositive for Usutu virus live in rural areas, whereas 5/7 of them (71.4%) live close to the water. None of the tested subjects either has travelled abroad during the last year or has been vaccinated against yellow fever. In addition, none of tested subjects was positive on Chikungunya virus. Indeterminate results for Chikungunya virus were obtained in 7 subjects of average age 52.6 years.

In two subjects (2.15%), acute *Borrelia burgdorferi* infection (both IgM ELISA and IgG ELISA positive) has been found; one of them was a 49 year old male and the other was a 22 year old female. In the case of one 21 year old female (1.07%) there were only IgG antibodies against *Borrelia burgdorferi* found. Isolated IgM ELISA positive finding was identified in 9 (9.7%) subjects, indicating probable infection, but only follow-up of the subjects with serological testing performed both in acute and convalescent sera was able to provide the final diagnosis.

DISCUSSION

Usutu virus has been found in the birds and/or mosquitoes in 12 European countries: Austria, Croatia, Czech Republic, Germany, Greece, Hungary, Italy, Spain, Serbia, United Kingdom, Switzerland and Belgium (Nikolay et al., 2015). Among neighboring countries, the presence of Usutu virus has

been confirmed by detection of the virus in birds using reverse transcription – polymerase chain reaction, immunohistochemistry and in situ hybridization (Bakonyi T., 2007). Usutu virus has been proven serologically in horses in Serbia during investigation on West-Nile virus seroprevalence (Lupulović et al., 2011). Symptomatic Usutu virus infections in humans have been described in two European countries: Italy and Croatia, which is our neighboring country (Vilibic - Čavlek et al., 2015, Nikolay et al., 2015). These findings encouraged the authors of this paper to investigate the Usutu virus seroprevalence in a minor sample of human population of Vojvodina. Antibodies against Usutu virus were confirmed in 7/93 (7.5%) subjects by IgG ELISA. Taking into account that the West-Nile virus is active too in Serbia and cross-reactions with ELISA test among flaviviruses may occur, the results should be confirmed by more sensitive and more specific test, such as plaque reduction neutralization test (Petrić et al., 2012, Hrnjakovic et al., 2015).

As for European countries, autochthonous transmission of Chikungunya virus was registered in northeast Italy, where there were 205 cases during the period from July to September 2007. In addition, two autochthonous cases were registered in 2010 and 12 cases more in 2014 in France (Rezza G, 2007), (Gould, 2010). During the period 2008 – 2012, 51 Chikungunya cases were registered in European Union (ECDC, 2014). There have been no registered (even imported) Chikungunya cases in Serbia so far. Moreover, not a single case positive on Chikungunya virus has been identified in Serbia so far by serological examination.

According to reviewed data of the Institute of Public Health of Vojvodina, the incidence of Lyme disease was 8.3 in Serbia, respectively 5.7/100.000 residents in Vojvodina (Institute for Public Health of Serbia, 2014). Only 2/93 subjects had serological markers of acute infection (both positive IgM and IgG antibodies against *Borrelia burgdorferi*).

CONCLUSION

Obtained results showed that Usutu virus (unlike Chikungunya virus) is active in South Bačka District as well as that *Borrelia burgdorferi* is a significant pathogen in the same area.

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ELECTROLYTES – SODIUM, POTASSIUM AND CHLORIDES IN POULTRY NUTRITION

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Abstract

Sodium, potassium and chlorides play a crucial role in maintaining body acid-base balance as well as osmotic pressure in body fluids. These processes are the result of synergetic action of all three elements, and the role of each individual component is difficult to define without knowing and taking into consideration the other two elements. The maintenance of this value is determined by three major factors – balance and ratio of electrolytes in feed, endogenous acid production and level of renal activity. Electrolyte imbalance is quite rare, since body's buffering system provides maintenance of normal physiological pH value. This article will give an overview of the role, importance and needs of poultry for sodium, potassium and chlorides, as well as occurrences related to deficit and the imbalance of those elements in feed.

Key words: sodium, potassium, chlorides, poultry

ELEKTROLITI – NATRIJUM, KALIJUM I HLORIDI U ISHRANI ŽIVINE

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Kratak sadržaj:

Natrijum, kalijum i hloridi imaju ključnu ulogu u održavanju acido-bazne ravnoteže u organizmu i osmotskog pritiska u telesnim tečnostima. Ovi elementi deluju zajednički i vrlo je teško definisati ulogu svakog

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pojedinačno, bez poznavanja uloge ostala dva. Održavanje acido-bazne ravnoteže je pod uticajem tri najvažnija činioca: balansa i odnosa elektrolita u hrani, endogene produkcije kiselina i nivoa renalne aktivnosti. Debalans elektrolita je retka pojava, pošto puferni sistem organizma obezbeđuje održavanje normalne, fiziološke pH vrednosti, međutim kada do njega dođe mogu nastati značajne ekonomske štete u živinarskoj proizvodnji. U ovom radu biće opisani uloga, značaj i potrebe živine za natrijumom, kalijumom i hloridima, kao i pojave vezano za deficit i debalans navedenih elemenata u hrani.

Ključne reči: natrijum, kalijum, hloridi, živina

INTRODUCTION

While requirements for sodium, potassium and chlorides have been clearly definied, there is currently an understanding of the need to achieve a balance between cation and anion supply (*Leeson and Summers, 2001*). Most commonly, the electrolyte balance is described by a simple formula Na+K-Cl and expressed as mEq/kg meal. The amount 250 mEq/kg is considered optimal for normal physiological function. Electrolyte imbalance is quite rare, since body's buffering system provides maintenance of normal physiological pH value. The maintenance of this value is determined by three major factors – balance and ratio of electrolytes in feed, endogenous acid production and level of renal activity. Electrolyte balance formula designated by *Mongin (1981)* is as following:

Na+K-Cl in feed = feed cations – anions + endogenous acids + excess base

Feed anion balance value other than 250 mEq/kg feed results in acidosis or alkalosis, which causes both production and health problems. Supplementation of Na (without Cl^-) in feed leads to an increase in concentration of HCO3- ions and elevated blood pH, whereas supplementation of Cl^- (without Na) decreases concentration of HCO3- ions and pH value. Endogenous acid production affects the electrolyte balance. It changes also with the change of protein source in feed, that is, replacement of soybean meal with fish meal results in modification of electrolyte balance from17.4 to 12.1 mEq/kg feed (*Mongin, 1981).* Anion imbalance can be solved by bicarbonate supplementation in the diet. The same author recommends that fishmeal-based poultry diets should be supplemented with sulfates to ensure electrolyte balance. The use of some divalent ions can interfere with electrolyte balance. Thus, supplementation of calcium chloride (CaCl2) in the diet can induce acidosis in poultry, contrary to chloride sources such as NaCl and KCl, which are associated with acidosis to a much lesser extent. This is due to the fact that calcium absorption from CaCl2

is lower than that of sodium from NaCl. Calcium bounds to carbonates from CaCO3 using up bicarbonates from blood, and excessive unabsorbed chlorine causes acidosis.

Some exams of electrolyte imbalance include tibial dyschondroplasia and respiratory alkalosis. Tibial dyschondroplasia in chicks can occur as the consequence of electrolytic misbalance. The condition is associated with a range of factors including administration of NH4Cl in feed production (*Leeson and Summers, 2001*).

Respiratory alkalosis occurs at high temperatures consequent to excessive loss of carbon-dioxide induced by panting. The condition can result in poor growth rate in meat industry and poor quality of eggshell that quite often affects highly-productive laying hens. Acid-base balance substantially affects the process of eggshell formation. The acid-base status of the intrauterine extracellular fluid strongly affects calcium solubility (precipitation); however, its influence on bicarbonate flows is still unclear. In normal conditions, the process of eggshell formation induces renal acidosis as a result of total reabsorption of filtrated bicarbonates. Concurrently, metabolic acidosis due to formation of insoluble CaCO3 occurs, since calcium ion (Ca^{++}) pushes out the hydrogen ion (H^{+}) from $HCO3^{-}$. Hydrogen ions induce elevation of pH value, and the balance is established in intrauterine extracellular fluid through the influence of bicarbonate from body's buffer system. Such acidosis is considered normal. Metabolic competition for $HCO3^{-}$ ion that plays a role in both maintenance of acid-base balance and the process of eggshell formation induces severe acidosis, which results in reduced production of the eggshell. Moreover, feed supplementation with NH4Cl can produce severe acidosis. In normal production conditions, substitution of NaCl portion with NaHCO3 can positively affect the eggshell formation process. In conditions of commercial egg production, maintenance of electrolytic balance by adding electrolytes in the feed should be avoided *(Kapetanov et al., 2015)*.

Broilers and layers differ in their requirements and reactions to electrolyte therapy in conditions of heat stress. Thus, treatment with aqueous electrolyte solutions positively affects the growth rate and decreases the mortality in broilers. *Whiting et al. (1991)* reported that positive effects of electrolyte supplementation in drinking water for broilers during heat stress are attributed to the increased water intake rather than to anion/cation status in the electrolyte supplement itself.

It should be emphasized that exposure of poultry to high temperatures should be prevented because the blood bicarbonate levels decrease in such conditions, while extreme conditions may induce metabolic acidosis. The poul-

try tolerates stable high temperatures much better than sudden temperature changes (increase or cyclic temperature challenge) (*Kohne and Jones, 1975*).

Electrolyte balance can affect the metabolism of numerous amino acids, especially lysine and methionine. It is well established that deficit of potassium in feed induces increased lysine accumulation in tissues. The accumulation rate correlates with potassium level. Such conditions of potassium deficiency result in decreased growth rate in chicks. High levels of sodium chloride, regardless of amino-acid balance, negatively affect the growth rate in poultry. It is likely that negative effects of electrolyte disbalance are more pronounced when using low-protein diet, since nitrogen turnover imbalance represents more serious problem (*Leeson and Summers, 2001*).

Electrolyte imbalance can be prevented by balancing anion and cation contents in poultry feed formulations. Thus, wheat has better electrolyte balance than maize, whereas soybean and other protein feeds have very low electrolyte balance due to high potassium content. Electrolyte balance should be taken into consideration when changing the protein source in the diet. Feed formulations with low electrolyte balance strongly require electrolyte supplementation (NaHCO3). Also, the problem of electrolyte imbalance is of importance in conditions when feed contains excessive sulfur or chloride. Balancing the electrolytes in feed by adjusting the cation concentration is very difficult if the feed contains high level of chlorides. Decrease of chloride level is feasible, as signs of chloride deficiency occur only when its level drops below 0.10%. A portion of sodium chloride can be substituted with sodium-carbonate, bearing in mind the minimum requirements for chlorides that have to be satisfied.

SODIUM (Na)

Sodium is the major cation in extracellular fluid and essential element for normal life cycle and metabolism of both plants and animals. Sodium makes some 93% of the total cation content in blood plasma (*Leeson and Summers, 2001*). Its crucial role has first been established as early as in 1881. Sodium is the basic constituent of salt, and all animals manifest strong need for salt.

Physiological role of sodium. Basic physiological functions of sodium are the following:

- participation in the maintenance of acid-base balance and optimal osmotic relationships;
- participation in the regulation of body fluid volume;
- participation in muscle cell contractions;
- close relation with adrenal gland functions;

- playing a role in carbohydrate absorption, that is, energy turnover in the body

Sodium content in poultry ranges averagely from 0.1 to 0.14% of body mass. Some 30-40% of sodium is found in the skeleton, tightly bound to the inorganic part of the bone, thus being hardly available for satisfying animal's needs. Since contained mainly in blood plasma, the element can hardly be found in blood cells. This is the reason why it is considered the most important cation responsible for maintenance of pH in blood plasma. Sodium content in blood plasma of chickens is 8.4 mg/ml (*Leeson and Summers, 2001*), that is, 122–160 mmol/l in chickens and 145–147 mmol/l in turkey poults *(Puls, 1990)*.

Sodium absorption and homeostasis. Sodium salts manifest ability to accumulate in the body by decreasing the excretion in conditions of low salt intake. Moderate increase in sodium intake is not considered big problem in poultry having in mind that concentration of this element in the water is not particularly high. With high sodium levels in the diet, the poultry will increase the water intake and remove excess sodium from the body via body excreta. The symptoms of toxicities will not manifest as long as birds have enough drinking water. However, in case of the intake of excess water, the symptoms of water toxicity will occur. On the other hand, substantial negative loss of water is associated with sodium deficit, where spending of accumulated extracellular fluid leads to severe dehydration of the birds.

Poultry diet is hypotonic in view of sodium content, yet it provides maintenance of stable concentration of the element in the body regardless of variable intake. Since homeostatic processes in blood and entire body are crucial for good health, all living organisms have developed a range of mechanisms for their maintenance.

Sodium is readily absorbed from small intestines, while certain amount is absorbed in the stomach. Some 85–90% of dietary sodium is excreted in the urine in the form of phosphates or chlorides (*Jovanović et al., 2001)*.

Sodium metabolism. Activation of the majority of mitochondrial enzymes is mediated by intracellular ions of potassium and magnesium, whereas extracellular sodium ions (Na^+) inhibit the activity of mitochondrial enzymes. A range of anions such as proteins, tricarboxylic acid, organic phosphates, glycerophosphates and keratin-phosphates, bicarbonates and some chloride ions are present in muscle and tissue cells. Some anions manifest ability to *diffuse* across cell *membranes, while some are fixed to cytoplasmic structures or otherwise prevented to move and leave the cell. Such ions electrostatically attract*

cations including sodium. Together with potassium and anions, sodium forms buffer that is crucial for maintaining optimal pH value of the cytoplasm. Upon the entry of sodium and potassium into the cell, the accumulation of potassium ions is much higher than that of sodium ions.

Active transport of sodium and potassium ions is of vital physiological importance. More than one third of adenosine triphosphate (ATP) consumed by a resting animal is used for active transport of sodium and potassium (*Leeson and Summers, 2001*). Adenosine triphosphates are enzymes hydrolyzing ATR in the presence of sodium and potassium together with magnesium ions. By breaking ATR, the enzymes provide energy for active ion transport and *pump* three *sodium ions out of the cell*.

Sodium deficiency. Sodium deficiency results in decrease in osmotic pressure and consequent disturbance of acid-base balance. Symptoms of pronounced sodium deficiency include heart failure, decreased blood pressure, increased hematocrit and decreased elasticity of subcutaneous tissue as well as low adrenal gland function leading to elevated levels of uric acid in the blood and subsequent shock and death.

Less pronounced sodium deficiency in chickens causes poor growth, soft bones, corneal keratinization and decreased blood plasma volume. In laying hens, the symptoms mainly include decreased egg production, impaired growth and sometimes even cannibalism. A range of diseases are associated with vast excretion of sodium from the body, diarrhea and other gastrointestinal disorders or urinary losses consequent to renal failure or adrenal gland insufficiency. Poultry diet with sodium content below 0.012–0.050% is considered sodium-deficient *(Puls, 1990).*

Excess sodium. Sodium content in poultry feed at levels above 0.5% is considered toxic. Even dietary sodium contents of some 0.35% stimulate increased water intake in poultry, thus causing electrolyte imbalance, and elevated sodium levels result in water toxicity. Excess of dietary sodium in laying hens (above 0.19% of feed mix) causes decreased egg fertility *(Puls, 1990*) and poor quality of the eggshell (*Leeson and Summers, 2001*). Ducks are particularly sensitive to increased sodium content in the diet.

Sodium requirements for poultry. Sodium requirements for young birds are 0.15% of feed, provided that the level of chlorides is the same. For laying hens, the recommended levels range from 0.17-0.19%. The ratio of sodium and chloride levels in poultry diet should be 1 : 1.

Introduction of nipple drinkers to poultry farms revealed that poultry consume inadequate amount of water because of anatomic incapability/limitation or lethargy, thus, the requirements for sodium are increased. In that respect,

Murakami et al. (1997) recommend 0.25% sodium content in formulations for 21-day broilers. Linear relationship between growth rate and dietary sodium level for young poultry has been established, and the limit is commonly determined by manure consistency.

Sodium sources. Feeds of animal origin are particularly rich in sodium, as it is present in almost all tissues. Feeds of plant origin contain significantly lower sodium levels, and its content varies depending on numerous factors. Sodium content in grains ranges from 80 mg/kg in maize to over 500 mg/kg in oat grain, the contents in oilseed range 150-170 mg/kg and in wheat bran around 250 mg/kg *(Živkov-Baloš et al., 1999)*. Kitchen salt/animal salt (NaCl) is the major sodium source containing some 38% sodium. As high levels of salt result in increased water consumption, a portion of dietary salt (30%) can be replaced with sodium bicarbonate without negative effects to production performance of poultry (*Jovanović et al.,2001)*. In such cases, somewhat dry droppings can be observed.

POTASSIUM (K)

Potassium is the third most abundant element found in the body of most animals (*NRC, 2005)*. Contrary to sodium, potassium is found in the body, that is, inside the cell itself. Blood cell level of potassium is somewhat 25 times higher than that of plasma cells. High potassium contents are characteristic for muscle and nerve cells, being some 4 mg/kg in muscles as compared to 0.1 mg/ml in blood plasma (*Leeson and Summers, 2001*).

Physiological role of potassium. The functional role of potassium is similar to that of sodium; however, its activity is taking place inside the cell. The most important roles of potassium include:

- participation in the maintenance of acid-base balance and optimal osmotic relationships;
- activation of the range of intracellular enzymes;
- participation in protein and carbohydrate metabolism;
- playing essential role in preserving normal heart function as it decreases the contractility of cardiac musculature and favors heart muscle relaxation;
- increasing the permeability of cell membrane;
- promoting the absorption of free neutral amino acids such as glycine

Potassium metabolism and deficiency. The major manifestation of potassium deficiency (hypokalemia) includes generalized muscle weakness with subsequent limb weakness, decreased intestinal tonus associated with disten-

sion, cardiac insufficiency as well as respiratory insufficiency and failure. Hypokalemia can occur consequent to severe stress. Stress conditions are associated with an increase of plasma proteins, which triggers adrenalin-mediated renal excretion of potassium into the urine. Upon reestablishment of glycogen stores, potassium returns to the liver. This can temporarily postpone hypokalemia. In the period of adaptation to stress, blood flow to the muscle gradually improves and lost potassium content is reestablished.

Low levels of dietary protein combined with low potassium levels or during fasting period result in poor growth of animals, yet without apparent manifestation of potassium deficiency. This is due to the fact that potassium produced by decomposition of tissue proteins compensates the deficit of dietary potassium, i.e., potassium excreted in the urine. In such instances, the potassium contents in feed can be reduced.

Potassium to nitrogen ratio in muscles and urine is relatively stable, thus, these two elements are released simultaneously during the process of body-tissue decomposition. Supplementation of proteins to poultry diet lacking nitrogen will cause increase in blood nitrogen level, but the birds will most probably manifest symptoms of hypokalemia. The lack of potassium results in decrease of tissue levels of neutral amino acids, whereas the content of basic amino acids increases in order to compensate potassium deficit. Thus, the consequences of excess lysine in feed can successfully be prevented through potassium supplementation in feed. Potassium is absorbed in the small intestines and eliminated in the urine.

Excess potassium. Potassium toxicities in healthy animals is rare. This is due to the body's ability to readily excrete potassium as well as regulate absorption. The major causes of hyperkalemia are excessive potassium intake, reduced renal losses, and redistribution of potassium (*NRC, 2005*). *Teeter and Smith (1986)* conducted a set of experiments and found no adverse effects when potassium (as KCl) was supplied in water with corn-soybean base diet containing 7,300 mg K/kg when fed to week-old chicken pullets for two weeks under near optimal environmental conditions. Under heat stress conditions, offering water with 1,000 to 1,500 mg K/L resulted in improved average daily gain in broilers, but blood pH and feed efficiency were not improved.

Smith et al. (2000) reported that the increase in the dietary concentration of potassium from 2,300 to 20,000 mg/kg caused a linear increase in water intake, water to feed ratio, and excreta moisture of layers.

Potassium requirements for poultry. Potassium deficiency is rarely observed in practice since standard poultry feed mixes contain more than 1% K *(Puls, 1990*) and feed with 0.1% K is considered potassium-deficient.

Potassium contents in feed should range between 0.4 and 0.6% of feed mix. Nutritional recommendations depend on the age of poultry (Table1). Puls (1990) indicated that potassium requirements increase for 63% in conditions of an increase of ambient temperature from 24 °C to 35°C.

Potassium level in the body correlates with muscle mass, so it is used to calculate the body mass *in vivo* applying radioactive isotope of potassium – K40, since ratio of K40 and total K is constant (*Jovanović et al., 2001).*

Table 1. Potassium requirements for poultry *(Puls, 1990*)

Age (weeks)	Requirements (% K in dry matter)
0–2	0.40
2–4	0.30
4–8	0.23
8–18	0.10
Layers	0.10
Turkeys – all ages	0.40

Potassium sources. Feeds of plant origin are rich in potassium, thus, supplementation is not necessary. *Živkov-Baloš et al. (1999)* reported potassium levels in maize, wheat and sunflower meal being 2,500-2,800 mg/Kg, 3,500 mg/Kg and 13,850 mg/Kg, respectively.

CHLORIDES (Cl^-)

Major part of chlorine, that is, chloride is found in extracellular fluids but also in red blood cells and other tissues. The elements are absorbed in small intestines; excess chloride is eliminated in the urine and is usually associated with excess sodium and potassium.

Physiological role of chlorides. Though closely associated with sodium, chlorides have some distinct independent functions such as:

- Chlorine is major anion of gastric juice and builds up gastric acid together with hydrogen ion;
- It participates in carbon-dioxide transport in the blood thus increasing plasma bicarbonate content

Chloride Metabolism and deficiency. Chloride ions have weak affinity of binding to protein ions, and enter the cell together with potassium. Chlorides are actively transported particularly via the cells of gastric mucosa. Chloride

deficiency in chickens is associated with extremely poor growth, limb weakness, poor bone mineralization, high mortality rate, dehydration and high blood levels of chlorides as well as tetany-like neural symptoms. Feeds containing less than 0.05% sodium chloride (0.03% chlorine) are considered chloride-deficient. Deficiency occurs more commonly in herbivores, since forage and grains are relatively poor in salt.

Chloride requirements for poultry. Chloride contents for poultry have to be balanced according to the requirements and/or potassium and sodium levels in the diet. Basically, the concentration of chlorides in the feed should be for some 10-15% higher than that of sodium.

Minimum chloride requirements for maximal growth rate of chickens are 0.13% and for turkey poults 0.10%. Optimal levels for chickens and turkey poults are 0.30% (0.20-0.40%) and 0.25%, respectively, whereas optimal level for turkeys is 0.27% *(Puls, 1990*).

Adequate dietary chlorides are of utmost importance for laying hens, as it prevents feather picking and cannibalism. Elimination of chlorides from layers' feed results in laying pause for 14-21 days. Returning of salt into diet results in accomplishment of 80% production peak within 16 days.

Excess chloride. Excess salt in drinking water manifests more severe toxic effects as compared with feed. Water with total soluble matter content higher than 3,000 mg/l is not appropriate for use in poultry industry. Tolerable chloride levels in water range from 0.015 to 0.018% (0.25 to 0.30% salt), whereas levels higher than 0.033% chloride (more than 0.54% salt) are considered toxic *(Puls, 1990*). In young growing birds, 2.7% salt in the diet, lead to rapid mortality *(Morrison et al., 1975).*

Tolerable salt levels in chicken feed are 0.9% chlorine (1.2% salt), and for layer hens 1.2% chlorine (2% salt) *(Puls, 1990*). Turkey poults are less tolerable to high salt contents in feed. *Scrivner (1946)* found that 1% sodium chloride in the feed for turkey poults was without effect, whereas 1% salt in the drinking water resulted in 100% mortality (within 48 hours) characterized by edema and ascites. Ducks tolerate excess chloride better than other domestic poultry. Dietary salt levels above 1% result in increased water content in the feces.

Excess chlorides in poultry feed leads to wet feces, extreme water consumption, ascites, edema, reduced thickness and hardness of the eggshell. The study of *Smith et al. (2000)* demonstrates that for every 0.25 % increase in sodium chloride content of the diet there will be an additional 9 g water excreted per g of feces.

Chloride sources. Feed formulations based on maize and soybeans are very poor sources of natural sodium chloride. Supplementation of fishmeal

into the feed reduces the requirements for salt. Major source of chlorides in animal feed is animal salt or kitchen salt.

CONCLUSION

Sodium, potassium and chlorides play a crucial role in maintaining body acid-base balance as well as osmotic pressure in body fluids. These processes are the result of synergetic action of all three elements, and the role of each individual component is difficult to define without knowing and taking into consideration the other two elements. Based on data presented in this paper the biological role of these three elements in normal metabolism during production of poultry is essential. Sodium, potassium and chlorides are a relatively nontoxic elements that is required in relatively large amounts to sustain life. Disturbances in metabolism can result in they being toxic. However, a combination of relevant quality control programs in the animal feed industry, as well as the implementation of good manufacturing practices and adequate education nutritionists can significantly reduce the risks associated with the appearance of electrolytic imbalance and toxicities.

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COMPARATIVE ANALYSIS OF DIFFERENT STRATEGIES FOR THE CONTROL OF CLASSICAL SWINE FEVER IN THE REPUBLIC OF SERBIA USING MONTE CARLO SIMULATION

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Abstract

Several different strategies for control and eradication of Classical Swine Fever (CSF) were compared using a Monte Carlo method-based simulation model. The control strategy analyzed in this paper, in addition to other CSF control measures, includes application of biosecurity measures on pig farms and rural backyard holdings. Elements of the control strategy are based on applicable regulations and include the simulation of detection of the disease, setting up the protected and surveillance zones, standstill of pig movements and restricted movement of animals, vehicles, equipment, and people with strong control measures in protection and surveillance zones, euthanasia of susceptible pigs, protective vaccination of pigs, compensation etc. During the simulation, different output parameters were compared such as: duration of epizootic of a disease, number of affected holdings and animals, direct costs such as those for dead or culled animals, costs of surveillance, disposal of infectious materials, cleaning and disinfection. Depopulation of affected animals with early diagnostics and vaccination in protection and surveillance zone proved to be the most effective measures to stop spreading and eradication of the disease. However, during the simulation, systematic implementation of biosecurity measures in all pig production clusters demonstrated to be appropriate strategy for sustainable

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control of CSF and setting up a stable epizootiological situation.

Key words: classical swine fever, Monte Carlo, biosecurity measures, control strategy

KOMPARATIVNA ANALIZA RAZLIČITIH STRATEGIJA ZA KONTROLU KLASIČNE SVINJSKE KUGE UPOTREBOM MONTE CARLO SIMULACIJE

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Kratak sadržaj

Nekoliko različitih strategija za kontrolu i iskorenjivanje klasične kuge svinja je upoređivano primenom modela simulacije zasnovanog na Monte Karlo metodi. Kontrolne strategije koje su obrađivane u ovom radu, pored opštih i posebnih mera suzbijanja KKS, uključuju i različite nivoe primenjenih biosigurnosnih mera na komercijalnim, porodičnim farmama i seoskim gazdinstvima na kojima se ga je svinje. Mere neškodljivog uništavanja obolelih životinja i životinja koje su bile u kontaktu sa obolelim životinjama, uspostavljanje zaštitnih zona pod nadzorom, kontrola i zaustavljanje prometa životinja i mehaničkih vektora (vozila i ljudi) unutar zona, rano otkrivanje bolesti i mera vakcinacije su bile uključene u simulaciju. Izlazni parametri kao što su: vreme trajanja epizootije, broj zaraženih gazdinstava i životinja, broj uništenih životinja, troškovi nadzora i direktne štete nastale zbog pojavljivanja bolesti s upoređivani tokom simulacije. Vakcinacija, neškodljivo uklanjanje obolelih životinja i rana dijagnostika su se pokazale kao najefektivnije mere zaustavljanja širenja i iskorenjivanja bolesti. Međutim, kao mera dugoročne strategije kontrole KKS i uspostavljanja stabilne epizootiološke situacije, tokom simulacije pokazala se mera planskog i sistematskog podizanja biosigurnosnih mera u svim klasterima proizvodnje

svinja (komercijalne farme, porodične farme tipa A, porodične farme tipa B i seoska gazdinstva).

Ključne reči: klasična kuga svinja, Monte Karlo, biosigurnosne mere, kontrolna strategija

INTRODUCTION

Classical swine fever (CSF) is highly contagious disease of viral etiology affecting domestic and wild pigs. From economic aspect, CSF is the most severe threat to national pig industry in all countries. The disease is spread worldwide and is reported at all continents. Models and simulation of CSF epizootics enables assessment of disease dynamics as well as economic effects of implemented control measures. The objective of this article is to analyse potential control strategies for eradication of CSF in the Republic of Serbia based predominantly on improvement of biosecurity measures on a typical rural holdings and gradual upgrading of rural holdings to higher farm categories.

MATERIAL AND METHODS

Description of the simulation model

The simulation has been conducted in the territory of the municipalities of Sremska Mitrovica and Šid. The area is characterized by high density of pigs as well as substantially heterogeneous pig-breeding technology, production habits and policies as well as production scale. The simulation encompassed all pig-breeding holdings including commercial farms, family farms type A, family farms type B and backyard holdings. The data on the number of pigs, production categories, and geographical locations were collected during the field investigation or obtained from the Central Database of Ministry of the Agriculture. Data processing was performed using ARC GIS 10.0. software package (Gatrell, 2004; Stanojevic, 2014). In cases where data about geographical location of rural holdings were not accessible, these were obtained from the Central Database of Ministry of Agriculture and the geographic coordinates were determined in ARC GIS 10.0, by randomly selecting (Gatrell, 2004). The simulation was performed applying the North American Animal Disease Spread Model based on the Monte Carlo method. NAADSM is a computer program based on the Monte Carlo method and is developed for the simulation of contagious animal diseases. The software was developed by a team of experts of the Center for Epidemiology and Animal Health US Department of Agriculture from Fort Collins, Colorado (Jalvingh et al., 1999; Harvey et al., 2007; Reeves et al., 2012).

The basic idea underlying the Monte Carlo method is the approximation of the expected value E(*X*) by the arithmetic mean of the results of a large number of independent tests all with the same distribution as *X*. The stochastic simulations use random variables and are based on the law of random numbers (Jalvingh et al., 1999).The model simulates daily disease transmission between farms and rural holdings for pig production. The simulation includes both direct and indirect contacts. The events such as "contact between the various epizootical units – adequate contact" and "contact between the various units that caused the transmission of diseases – effective contact" are generated stochastically. The variability of the obtained results after 1,000 replications provide the information about the potential pattern of disease spreading (Gatrell, 2004; Engel et al., 2005; Harvey et al., 2007).At the beginning of the simulation, all backyard holdings and farms are considered "susceptible", except in cases where a number of pigs are vaccinated. Once acquiring the status "infected", a holding/farm has to pass through all other statuses predefined in the model. Table 1. describes the definition of the disease transition states.

Table 1. Definition of statuses through which animals pass in the model

Status	Definition of status
Susceptible	All animals in the herd not infected and can be infected in case of contact with a diseased animal.
Latent	Period between exposure and infection. Some animals in the herd are infected, but still do not shed the virus.
Subclinical infection	Some animals in the herd are infected and shed the virus. No clinical symptoms.
Clinical infection	Some animals in the herd are infected, shed the virus, and show a clinical image of the disease.
Vaccinated	Animals in the herd are vaccinated and are not susceptible to CSF.
Dead from disease	Animals died due CSF
Culled	All animals in the herd are culled during implementation of CSF eradication measures.

After a short period of latency, all infected pigs disseminate the virus

among susceptible population. However, there are certain differences in probability of an outbreak of the disease after adequate contact. Such differences are determined by intensity of direct and indirect contact between animals, the type of the holding and farming system itself as well as the level of implemented biosecurity measures. Some potential scenarios entail that certain number of pigs is vaccinated, thus possessing artificially induced immunity, which makes them non-susceptible. Defining parameters for disease spread sets down the modelling of control measures, laid down by relevant regulations. Upon completing the simulation, the following data are analysed: total number of infected farms and holdings, total number of diseased and culled animals, number of farms and holding where euthanasia was performed, duration of the outbreak, financial data such as costs of euthanasia, disinfection and cleaning, expenses of safe disposal of carcasses, costs of laboratory examination etc. Depending on the scenario, several hypothetic situations were simulated including preventive vaccination of animals and no-vaccination scenario. The initial scenario describes actual status of CSF control in Serbia. Other scenarios simulated spread of the disease in conditions with no vaccination or emergency vaccination aimed at preventing virus transmission outside of infested area (Table 2).

Table 2. Scenario set up

No.	Preventive vaccination policy	Herd immunity	Protective vaccination (radius)	Depopulation: r= 500 m around infected farm	Depopulation: r= 100 m around infected farm	Depopulation: r= 50 m around infected farm	Biosecurity measures
1.	yes	49%	-	CF, A, B, RH1, SG2	-	-	-
2.	yes	49%	-	CF	A	B, RH1, RH2	-
3.	yes	49%	-	CF	-	A, B, RH1, RH2	-
4.	yes	49%	-	-	CF	A, B, RH1, RH2	-
5.	no	0%	ne	CF, A, B, RH1, SG2	-	-	-

6.	no	0%	10km	CF	A	B, RH1, RH2	-
7.	no	0%	3km	CF	A	B, RH1, RH2	-
8.	no	0%	10km	CF	-	A, B, RH1, RH2	
9.	no	0%	3km	CF	-	A, B, RH1, RH2	
10.	no	0%	10km	-	CF, A	B, RH 1, RH 2	-
11.	no	0%	3km	-	CF , A	B, RH 1, RH 2	-
12.	no	0%	3km	-	CF, A	B, RH 1, RH 2	no natural mating (NNM)
13.	no	0%	3km	-	CF , A	B, RH 1, RH 2	NNM & 25% indirect contacts
14.	no	0%	3km	-	CF, A	B, RH 1, RH 2	NNM & 50% indirect contacts

CF–commercial farm; A- family farm type A; B- family farm type B; RH1- rural holding category 1; RH2- rural holding category 2

Disease parameters

The diseases characteristics and input parameters, used for analysis with NAADSM are based on data from literature and results of retrospective analyses of CSF cases in the Republic of Serbia in 2005, 2006 and 2007 (Table 3) (Backer et al., 2011).

Table 3. Disease transmission parameters

Parameters	**Probability distribution**	**The mean value / standard deviation in days**
The latent period (Laddomada, 2000)	Poisson distribution	7(1); 8(1)
Subclinical period (Martinez-Lopez et al., 2011)	Poisson distribution	21
Infectious period (Baker et al., 2011)	Gamma distribution	Alfa: 13.5, beta: 1
Immune period after vaccination (Qui et al., 2006)	The normal Gaussian distribution	300/60
The number of direct contacts between animals daily- direct sales to owners *	Poisson distribution	*Type of farm* — *Intensity* Industrial farms — 0.07 Type A — 0.009 Type B — 0.0074 Back yard farm — 0.0036
Number of contacts direct natural mating (mating animals) *	Poisson distribution	*Type of farm* — *Intensity* Type B — 0.016 Rural farm — 0.0057
The probability of transmission of the virus through direct contact if the farm / farm source of infection (Karsten et al., 2005a)	Bernoulli distribution	*Type of farm* — *Probability* Industrial farms — 0.7 Type A — 0.7 Type B — 0.8 Rural farm — 0.8
Number of indirect contacts per day *	Poisson distribution	*Type of farm* — *Intensity* Industrial farms — 0.1428 Type A — 0.1428 Type B — 0.0330 Rural farm — 0.2850
Local spread of the virus (Karsten et al., 2005a)	Bernoulli distribution	*Distance from the farm* — *Mean value* 150m — 0.020 150-250m — 0.010 250-500m — 0.004 500-1000m — 0.002

Parameters	**Probability distribution**	**The mean value / standard deviation in days**	
The probability of detecting the first appearance of clinical symptoms of CSF from the moment when the farm became infectious (Engel et al., 2005)	Fixed value	*Number of days*	*Probability*
		8	0%
		10	3%
		20	7%
		25	10%
		37	50%
		47	90%
		50	100%
The probability of detecting cases of death due to infection by the CSFV from the time of occurrence of the first cases of death (placing suspected on the basis of the findings pathoanatomic) (Klinkenberg et al., 2005)	Fixed value	*Number of days*	*Probability*
		1	20%
		2	30%
		3	40%
		4	50%
		5	60%
		6	70%
		7	80%
		8	100%
The probability of successful tracking of shipments of animals that have left an infected farm (**)	Bernoulli distribution	*Type of farm*	*Mean value*
		Commercial Farm	0.80
		Family farm type A	0.80
		Family farm type B	0.80
		Rural farm	0.40
The probability of successful detection and monitoring of indirect contact with infected farm (Martinez-Lopez et al., 2011)	Bernoulli distribution	*Type of farm*	*Mean value*
		Commercial Farm	0.95
		Family farm type A	0.90
		Rural farm	0.20
Sensitivity and specificity of diagnostic tests	Fixed value	Sensitivity	99%
		Specificity	99%

* Data collected in the field

**Data based on CSF epidemic in Serbia in 2010

Figure 1. Latent period

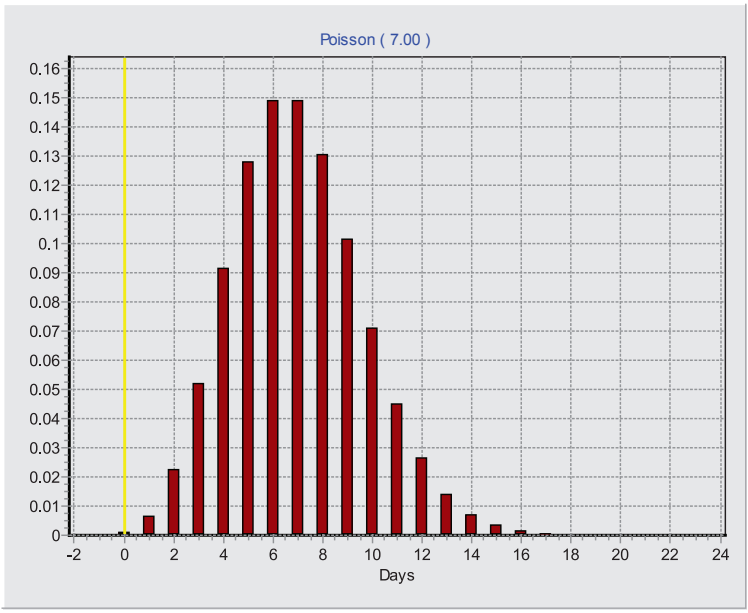


Figure 1. Clinical infectious period

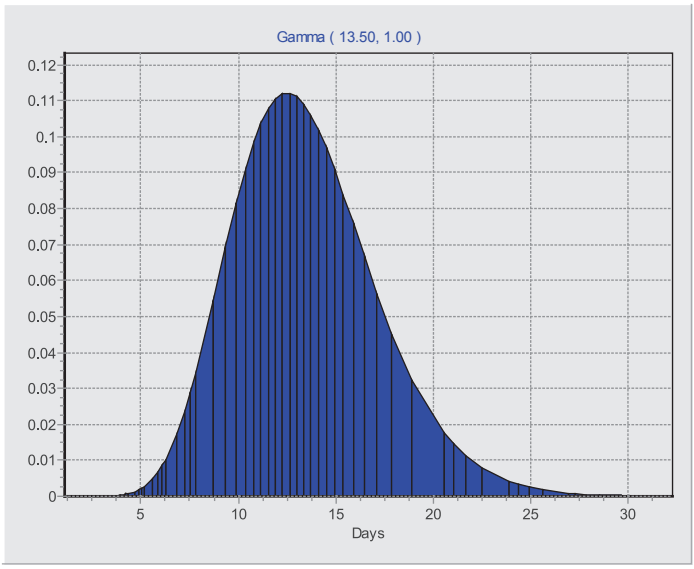
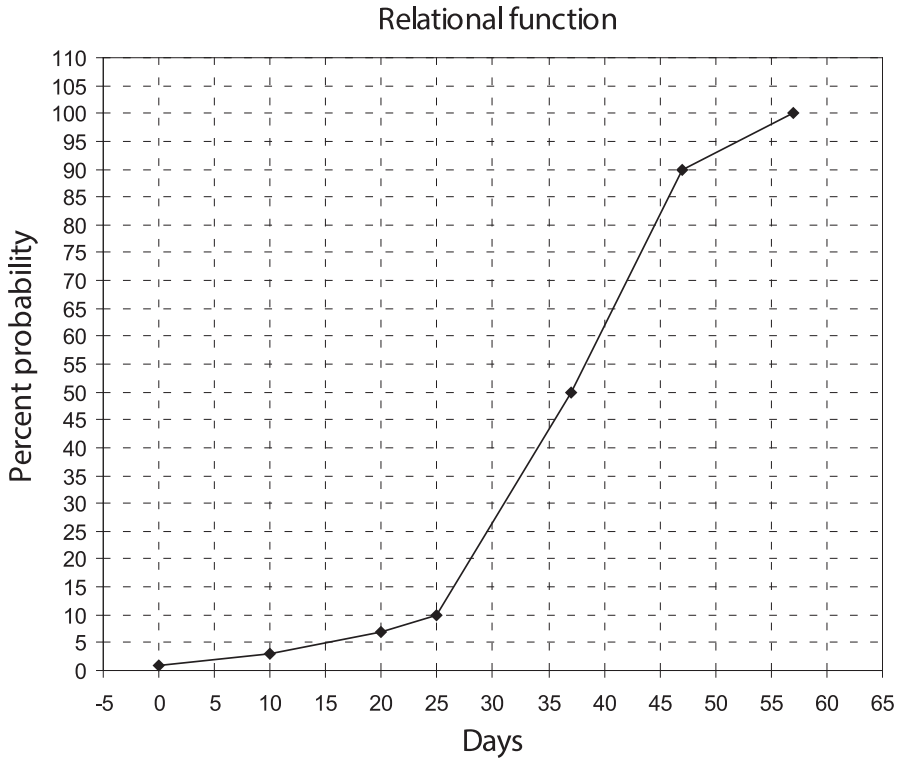


Figure 3. The probability of detection of first cases or observing clinical signs of CSF on industrial farm, type A, B and backyard farm. The detection probability was modeled as based on the results from Klinkenberg et al. (2005) and Engel et al. (2005) and available data from epidemics of CSF in Serbia between 2005 and 2010.



RESULTS

Simulation of the scenario No.1 entailed analysis of the effects of implementation of currently relevant measures for CSF control in case of disease outbreak, taking into consideration factors such as actual production conditions and capacities of field veterinary service. Scenarios 2-4 dealt with potential modifications of current control strategies and the assessment of their effects when applied in conditions of CSF control using preventive vaccination policy. In scenarios No. 5-14, hypothetical situations of no-vaccination CSF control were tested. The testing included also the hypothesis on CSF control using protective vaccination as well as the improvement of biosecurity meas-

ures on rural holdings and family farms type B such as restriction of natural mating and intensity of indirect contacts. Fourteen different hypothetical scenarios have been analysed (Table 2).

The analysis of obtained results revealed that CSF control using preventive vaccination strategy results in less direct economic losses, less number of diseased and culled animals as well as significantly shorter duration of the epizootic. The simulation indicated that the area for preventive euthanasia of pigs should be set to a radius of max 50m around extensive rural holdings and family farms type A and B, and 500m around commercial pig farms. The simulation also revealed no statistically significant differences regarding duration of epidemics, number of infected holding or animals even if the depopulation radius was limited to 100m around the infected commercial farm.

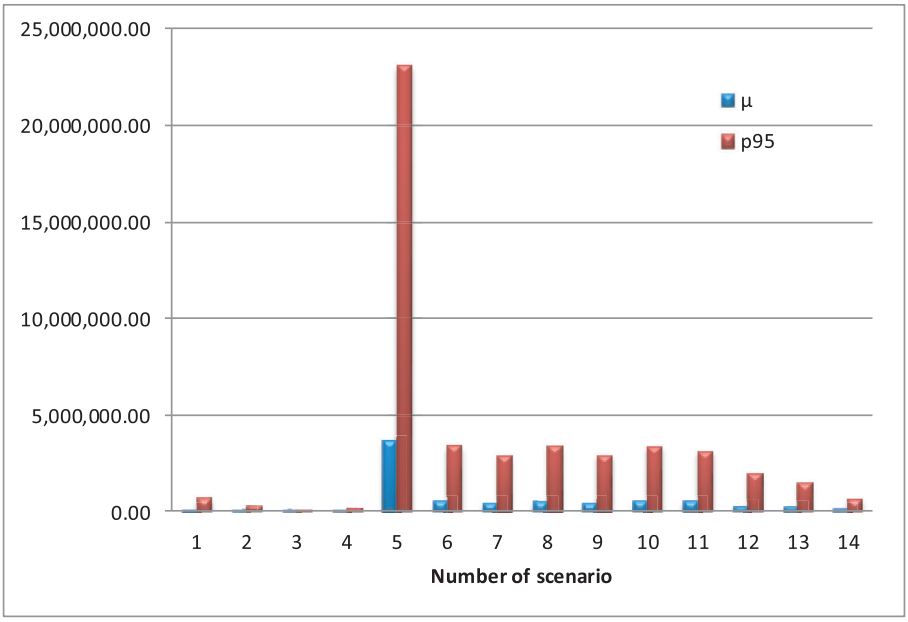
The most severe losses were observed in a scenario that was identical with the current field conditions, yet presuming cessation of vaccination program and absence of protective vaccination in case of CSF outbreak. In scenarios No. 12, 13 and 14 characterized by absence of preventive vaccination but with restricted natural mating and improved biosecurity measures on rural holdings and family farms type B, the results revealed statistically significant decrease in number of diseased animals as well as lower economic damage.

In conditions of termination of vaccination, the scenario No. 9 proved most appropriate, that is, the following measures are most effective: depopulation radiuses set to 500m and 50 m around commercial farms and rural holdings/family farms type A and B, respectively; implementation of protective vaccination policy and other measures laid down in relevant legislation. In all simulation models, there were no statistically significant differences between the effects of protective vaccination applied in the radius of 10km or 3 km around the commercial farms.

Table 4 depicts the results obtained in simulation models for 14 different scenarios. In scenarios No.1-4, potential modification of current CSF control strategy relying on preventive vaccination are analysed. The results obtained in simulation scenario No. 5 indicated that veterinary service is unable to control CSF without protective vaccination. As obvious from Table 4, implementation of controlled natural mating in case of CSF outbreak results in statistically significant decrease in number of diseased animals as compared with scenarios lacking this measure (scenario No. 12 in Table 4).

Furthermore, if controlled natural mating in case of an outbreak of CSF would be associated with a decrease in intensity of indirect contacts for 25% and 50%, the number of diseased animals would be even more decreased (scenarios No. 13-14, Table 4).

Graph 1. Comparative graph of total economic damage expressed in EUR



Legend:
- CFS control applying preventive vaccination (scenarios 1-4);
- No-vaccination CFS control (scenario5);
- CFS control applying protective vaccination as the alternative to mass pig (scenarios 6-11);
- CFS control applying protective vaccination and improvement of biosecurity measures at rural holding and family farms type B (scenarios 12-14).

Table 4. Results of the simulations applying various control strategies. Average values, standard deviation and 95.percentile for the total number of infected animals, total number of vaccinated animals, total number of slaughtered animals and duration of epizootics.

Scenario No.	Herd immunity	Total number of infected animals			Total number of vaccinated animals			Total number of slaughtered animals			Total number of examined blood samples			Duration of epizootics		
		μ	SD	p95	μ	SD	p95	μ	SD	p95	μ	SD	p95	μ	SD	p95
1.	49%	116	325	519	23,406	37,217	104,780	1,165	2,571	5,640	7,333	6,855	17,552	57	23	104
2.	49%	140	472	562	25,897	42,293	117,274	357	1,595	1,017	7,774	7,397	18,712	58	24	104
3.	49%	116	320	548	24,353	38,251	111,029	222	691	782	7,480	6,790	17,961	58	23	106
4.	49%	129	364	575	26,120	39,493	112,772	311	1,093	933	7,429	6,887	17,594	58	23	106
5.	0%	9,781	21,768	65,782	0	0	0	37,478	78,826	238,756	20,186	25,706	83,131	137	158	499
6.	0%	3,771	9,909	29,191	82,463	94,177	286,775	4,389	10,941	31,133	14,478	15,261	51,915	81	42	162
7.	0%	3,397	8,696	23,148	80,942	92,927	286,980	3,897	9,441	25,664	14,289	14,467	50,078	81	43	169
8.	0%	3,808	9,847	27,432	81,354	95,082	286,020	4,330	10,852	31,180	14,420	15,276	51,242	82	44	166
9.	0%	3,363	9,103	23,803	76,856	89,722	276,794	3,841	14,638	26,482	13,457	13,809	47,890	81	67	168
10.	0%	3,971	9,873	29,799	87,534	97,035	292,412	4,374	10,510	30,455	15,108	15,307	51,955	84	45	168
11.	0%	3,748	9,597	27,729	80,417	93,138	287,650	4,265	10,582	27,981	14,702	15,601	52,424	82	44	168
12.	0%	1,935	6,890	12,718	57,442	78,604	250,911	2,348	7,867	17,056	10,309	12,256	28,333	67	34	134
13.	0%	1,506	5,478	9,938	56,272	75,391	228,351	1,841	611	12,422	8,919	10,785	26,865	66	34	135
14.	0%	921	4,291	2,501	47,728	67,302	195,894	1,094	4,379	4,508	6,496	8,512	19,429	64	32	132

μ- average value within population; SD- standard deviation; p95- 95.percentil;

DISCUSSION

Simulation and mathematic modelling enable the pre-estimation of optimal control strategies, quantification of potential epizootic outcomes, adjustment of relevant control plans, assessment of veterinary service and necessary resources (Jalvingh et al., 1999; Karsten et al., 2005a). This research offered a review of potential outcomes of CSF outbreak in a limited area characterized by high pig density and highly heterogeneous pig production system. Potential dynamics of CSF epizootic as well as the level of consequent damage were described relating to different approaches to disease control and eradication. The simulation revealed that rural holdings are highly susceptible to CSF; however, in such holdings, the potential for virus spread over large distance is lower, which corresponds with simulation results reported in Bulgaria (Backer et al., 2011). Spread of the disease over large distances is mainly associated with family farms type A and B and commercial farms. Rural holdings producing pigs for their own needs are not considered to be of high potential risk for disease transmission. The simulation model also suggested that, when speaking of rural holdings, local transmission is the most common route of infection with CSF virus. As regards the farms type B, most common infection routes include both local spreading and indirect contacts. The obtained results correspond with the results of the study analysing the potential of local spread of CSF virus conducted in Holland during a CSF epidemics in 1997-1998 (Karsten et al., 2005b; Klinkenberg et al., 2005). The simulation emphasized the role of uncontrolled natural mating in disease spreading at settlement level. Occurrence of CSF on family farms type A is mostly associated with the purchase of animals for fattening from producers from family farms type B and rural holdings. When speaking of commercial farms, two potential risk factors are most commonly associated with CSF outbreaks – introduction of infected animals into the herd and contacts with rural holdings through the personnel employed at the farm, who have their own pigs at home. Safe elimination of animals and protective vaccination proved most effective. On the other hand, the least economic losses are observed in conditions of continuous maintenance of appropriate immunity status of animals. Improvement of biosecurity measures proved highly important for substantial reduction of both disease transmission and economic damage in case of epidemic outbreak. Combined with other biosecurity measures such as good on-farm production practices, controlled access of visitors and vehicles, elimination of unnecessary contacts with other pig owners and preventing contacts with wild boars contributes to substantial reduction of the risk for CSF outbreaks.

CONCLUSIONS

Based on the results obtained in this research, we may conclude as following:

1. In endemic areas with predominate extensive pig production, relying on CSF control, strategy based on preventive vaccination proved most cost effective.
2. Preventive slaughtering of pigs should be carried out in the radius of max 50 m around the infected rural holdings, whereas destruction radius for commercial farms is set to 100 m.
3. If selecting the no-vaccination CSF control policy, modified EU strategy based on protective vaccination and limited pig depopulation in a radius of 500m around infected commercial farms, i.e., 50 m around infected family farms type A and B and rural holdings, has proved most cost effective.
4. For all simulation models, where protective vaccination was used as control measures in combination with limited depopulation, there were no statistically significant differences between the effects of protective vaccination if applied within the radius of 10 km around infected farm as compared to the radius of 3 km.
5. Restriction of natural mating and its limitation to one's own herd significantly reduces the risk of virus transmission and CSF outbreak.
6. Under the present conditions in the Republic of Serbia, it is not reasonable to implement a SCF control strategy without vaccination, particularly on rural holdings and family farms with lower levels of implementation of biosecurity measures.

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TETRACYCLINE RESISTANCE IN ESCHERICHIA COLI ISOLATES FROM POULTRY

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Abstract

The objectives of the present paper are the analysis and comparison of the results from available literature regarding the prevalence of tetracycline resistance in *Escherichia coli* strains isolated from poultry in different countries. Tetracycline is still one of the most commonly used antibiotic in many developing countries both in human and in veterinary medicine. The main reasons are its relatively low cost and availability. Besides that, this class of antibiotics is still used in developed countries for prophylactic and therapeutic purposes. The widespread use of tetracycline in poultry farming could result in horizontal transfer of resistance determinates from poultry to humans as well as to the environment. *Escherichia coli,* is a commensal bacteria from human and poultry digestive systems, and present one of the most important reservoirs of antibiotic resistance and has a significant role in the transfer of various resistance determinants. Some strains of *Escherichia coli* are highly pathogenic and can cause several diseases in poultry which require antibiotic therapy. Positive correlation between the usage of antibiotics both in human and in veterinary medicine and the corresponding antibiotic resistance were reported by many authours. Furthermore, there is also some evidence that the positive correlations were also found between the usage of antibiotics in veterinary medicine and the appereance of antibiotic resistance in bacteria isolated from humans. The prudent use of tetracycline antibiotic in poultry production is essential as well as permanet monitoring of the presence of the tetracycline resistance.

Key words: *Escherichia coli*, poultry, resistance, tetracycline

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REZISTENCIJA NA TETRACIKLIN KOD IZOLATA ESCHERICHIA COLI ŽIVINE

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Kratki sadržaj

Predmet ovog rada je analiza i poređenje rezultata iz raspoložive literature u pogledu prevalentnosti rezistencije na tetracikline sojeva *Escherichia coli* izolovanih kod živine u različitim zemljama. Tetraciklin je još uvek jedan od najčešće primenjivanih antibiotika u većini zemalja u razvoju, kako u humanoj tako i u veterinarskoj medicine. Glavni razlozi za to su njegova široka dostupnost kao i relativno niska cena. Osim toga, ova klasa antibiotika se još uvek koristi u profilaktičke i terapeutske svrhe i u razvijenim zemljama. Široka primena teteraciklina u živinarskoj industriji može da izazove horizontalni transfer determinanata rezistencije sa živine na ljude in a okolinu. *Escherichia coli* je komensalna bakterija prisutna u digestivnom traktu ljudi i živine pa tako i jedan od najvažnijih izvora rezistencije na antibiotike. Ovaj mikroorganizam takodje igra važnu ulogu u prenosu različitih determinanata rezistencije. Neki sojevi *Escherichia coli* imaju izražen patogeni potencijal i izazivaju niz oboljenja kod živine koja zahtevaju antibiotsku terapiju. Pozitivnu korelacija između primene antibiotika u humanoj i veterinarskoj medicine i posledičnu rezistenciju na antibiotike opisali su brojni autori. Osim toga, postoje dokazi o pozitivnoj korelaciji između primene antibiotika u veterinarskoj medicine i pojave rezistencije na antibiotike kod bakterija izolovanih kod ljudi. Pažljiva primena tetraciklina u živinarstvu je od velikog značaja kao i kontinuirani monitoring pojave rezistencije na tetracikline.

Ključne reči: *Escherichia coli*, živina, rezistencija, tetraciklin

INTRODUCTION

The antibiotics used in poultry farming may sometimes be the same, or belong to the same class, as those used in human medicine. The main consequences of the use of antibiotics, especially the same or from the same class, both in human and poultry can cause dissemination of antibiotic resistance as result of the continuous positive selection of resistant bacterial clones, whether

these are pathogenic, commensal or environmental bacteria (Sengeløv et al., 2003). Antibiotic resistance occurs often in poultry farms and surrounding environment and can be spread to humans via food or water chain, and also by routes such as environmental contamination by poultry waste and direct contact with animals (Velhner et al., 2010). The resistant zoonotic bacteria are of particular public health concern. Zoonotic infection can be transmitted to humans directly from animals or through the contaminated food. During the last two decades, the use of antibiotic in poultry farming has changed, with use of antibiotics as growth promoters banned or severely restricted in some countries. This has lead to distinctive differences between countries in regard to prevalence of antibiotic resistance (Van den Bogaard et al., 2001; Adelowo et al., 2009; EFSA, 2016). Furthermore, because antibiotic use practices are very different among countries across the world we have taken the opportunity to find out more about the general effects of antibiotics, by comparing the patterns of antibiotic resistance, among poultry from different parts of the world.

This review presents the prevalence and some of the latest problems regarding tetracycline resistant *Escherichia coli* strains, isolated from poultry in different countries worldwide, with a focus on broiler chickens. Several reviews of antibiotic resistance in *E. coli* have been published recently in Serbia (Velhner et al., 2010; Todorović et al., 2015) but the problems with tetacycline resistance in *E. coli* in relation to poultry farming have not been particularly addressed.

TETRACYCLINE –MODE, USE, PRICE, RESISTANCE

The mode of action of tetracycline has been reviewed by Velhner and Milanov (2015). Briefly, it consists of reversible inhibition of bacterial protein synthesis by binding to the ribosomal complex, preventing the association of aminoacyl-tRNA with the bacterial ribosome. The consequence is weakening the ribosome-tRNA interaction and stopping the synthesis of proteins.

Tetracycline are a family of broad-spectrum antibiotics that are frequently used in poultry production. First-generation, such as tetracycline, chlortetracycline, and oxytetracycline, have been widely used as growth promoters in poultry production for decades and the second-generation, such asminocycline and doxycycline, are commonly used both in the prophylactic and therapeutic purpose in human and veterinary medicine (Eliopoulos and Roberts, 2003; Nsofor et al., 2013). Tetracyclines are used for all food production animal species, including poultry. The main reasons are their broad spectrum of activities, the relatively low price and availability. On the other hand, the

extensive usage of tetracycline may often lead to the emergence of resistant bacteria (Chopra and Roberts, 2001). The extended use of tetracycline leads to the selection pressure and consequently the fact that tetracycline resistance is very frequent in zoonotic, pathogenic, intestinal, commensal bacteria such as *E. coli*.

The study conducted by Bryan et al. (2004) indicated that the environmental exposure of humans and animals to tetracycline and also the other antibiotics leads to the development and dissemination of antibiotic resistance by horizontal gene transfer. The continuous search for new antibiotics in the time of resistance is response to reduced utility of tetracycline (Chopra and Roberts, 2001). The continued transfer of antibiotic resistance determinants among various environmental and clinical bacterial populations is a permanent problem.

THE USE OF TETRACYCLINE IN HUMAN CLINICAL PRACTICE

Tetracycline as a broad-spectrum antibiotics are still used in clinical practice in human medicine although with some limitation (Chopra and Roberts, 2001). As one of the cheapest classes of antibiotics available today, they are very suitable for use in developing countries. Tetracycline's are effective for the therapy of human parasitic diseases, so they are the drug of choice in the therapy of mefloquine–resistant *Plasmodium falciparum* infection (Eliopoulos and Roberts, 2003). Tetracyclines are used for treatment of diseases caused by Gram-negative, gram-positive bacteria, chlamydia, mycoplasmas, rickettsia and protozoan parasites and also as growth promoters in livestock production worldwide (Velhner and Milanov, 2015).

Mechanisms of resistance (Acquired tetracycline resistance genes)

Four mechanisms such as efflux, ribosomal protection, enzymatic inactivation and target modification are recognized as mediators of tetracycline resistance in bacteria (Velhner and Milanov, 2015). The first report of transferable tetracycline resistance was in *Shigella dysenteriae* in 1960 (Eliopoulos and Roberts, 2003). After that 23 genes encoding efflux pump and 11 genes encoding ribosomal protection proteins in bacteria have been reported (Chopra and Roberts, 2001). The genes associated with an efflux mechanism, namely tet(A), tet(B), tet(C), tet(D), and tet(E) are important part of tetracycline resistance in *E. coli* (Chopra and Roberts, 2001). Bryan et al. (2004) found that the gene tet(M) is a mediator of ribosomal protection mechanism of resistance, in isolates from pig and chickens and it was the first report of tet(M) in *E. coli,* while

Jones et al. (2006) first reported the identification of the tet(M) tetracycline resistant determinant in a human clinical isolate of *E. coli*.

The prevalence of tetracycline resistance in *E. coli* can be a useful tool to monitor resistance in poultry production and furthermore it can provide a good model for epidemiological studies of antimicrobial resistance. Tetracycline-resistant *E. coli* can be used as a useful indicator for antibiotic resistant bacteria in poultry farming due to its high occurrence. Numerous studies have demonstrated the prevalence and characterization of tetracycline resistant *E. coli* from broiler chickens (Sengeløv et al., 2003), laying hens (Van den Bogaard et al., 2001), humans (Miles et al., 2006), and poultry litter, facilities, manure (Adelowo et al., 2009).

E. coli - commensal and pathogenic, the role in resistance

Commensal *E. coli* are commmonly present in the digestive tract in humans and in animals and in feces and it is well known that *E. coli* could be a source of contamination of food during evisceration after slaughter, during contact with contaminated water or during food handling. Besides that, *E. coli* are also very important pathogen and can be involved in intestinal but also in extra-intestinal infectious diseases. Pathogenic strains of *E. coli* can cause respiratory disease in poultry and in such cases antibiotics are very important for the treatment of these conditions and these compounds are commonly administered as feed supplements. On the other hand, their frequent use can lead to development of multi-resistant *E. coli*. Moreover, some strains of *E. coli* are zoonotic pathogens and can be causative agents of food-borne diseases in humans. Furthermore, infections with antibiotic resistant strains of *E. coli* may lead to failures in clinical therapy or the use of second-line antibiotics for therapy can be required. The importance of commensal bacteria, particularly *E. coli* is the fact that they also can be a reservoir for resistance genes, which further may be transferred between bacterial species, including zoonotic microorganisms (Todorović et al., 2015). Apart from being one of the most important reservoirs of antibiotic resistance genes, *E. coli* could be one of the primary causes of hospital-acquired infections. It was established that the occurrence of antibiotic resistance in commensal *E. coli* could be used as excellent indicator of the selection pressures due to the use of antibiotics and the resistance patterns which could be expected in pathogenic bacteria. *E. coli* may be susceptible to many antibiotics but isolates from poultry may be frequently resistant to one or more antibiotics, and particularly to those that have been widely used in poultry farming for a long time such as tetracycline's (Zahraei Salehi and Farashi Bonab, 2006). The number of factors affects the occurrence of antibiotic resistance in commensal *E. coli*. For instance the

selective pressure from the use of antibiotics in poultry, clonal spread of resistance, the dissemination of genetic determinants of resistance such as plasmids and the co-selection of multi-resistant organisms are involved in establishing the population of resistant microorganisms in animals.

CORRELATION BETWEEN THE USE OF ANTIBIOTICS AND OCCURRENCE OF TETRACYCLINE RESISTANCE

The use of antibiotics is often necessary for the therapy of bacterial infections in intensive poultry production and tetracycline is a frequently used first line antibiotic for poultry and other domestic animals. The inappropriate use of antibiotics, such as exposure of bacteria to subtherapeutic doses of antibiotic, the use of antibiotics in food producing animals, especially as growth promoters or in prophylactic purpose are the main factors which strongly contribute to the development of resistance. Also, the therapy is frequently conducted without the previously bacteriological examination and without determination of the antibiotic resistance profile of a pathogen which could lead to appearance of the antibiotic resistance (Velhner et al., 2010). Besides that, bacteria can be exposed to tetracycline in environment since tetracyclines are naturally derived compounds (Miles et al., 2006). Also there is a lacks of information regarding the amount of antibiotics administered in sub-therapeutically doses in poultry in many countries. Schroeder et al. (2004) stated that selective pressure promote the emergence of antibiotic resistance bacteria each time when antibiotics are used but it should be emphasized that sub-therapeutic use of antibiotics can be an additional factor which contribute to the emergence of antibiotic resistant bacteria.

The widespread and long use of tetracycline in the poultry industry is undoubtedly one of the reasons for the high prevalence of resistance to tetracycline's in broilers (Van den Bogaard et al., 2001) and it should be mentioned that about 50-90% of antibiotics administered to animals on farms are excreted into the environment as unmetabolized or as metabolic intermediates which are inactive but may be transformed and become active in the environment (Diarra et al., 2007). On the other hand, Álvarez-Fernández et al. (2013) found that the relatively high resistance to tetracycline in *E. coli* isolated from poultry from organic system of rearing is unexpected and surprising. Thibodeau et al. (2008) found resistance to tetracycline in *E. coli* isolated from broilers in Canada even if these flocks were not treated with antibiotics (of this class). Knežević and Petrović (2008) reported tetracycline resistance in *E. coli* isolates from broiler farms even if no antibiotics were used. Such finding could be explained

by the fact that tetracycline's have been used on poultry farms for treatment of infectious diseases for a long time, so there is possibility that *E. coli* may evolve and become resistant to tetracycline's, which further lead to widespread distribution in animals as reservoirs regardless the type of production and the practice of using antibiotics. Moreover, Diarra et al. (2007) suggested that antibiotic resistant clones are stable and they could persist in poultry flocks during several production cycles-rotations, even in the cases when selective pressure on the farm is not present for a long period of time. So, sometimes the use of antibiotics may not be directly correlated with the antibiotic resistance rate.

The possibility of transfer of tetracycline resistant bacteria from poultry to humans is still controversial (Miles et al., 2006). Schroeder et al. (2004) stated that there is no documented case of antibiotic treatment failure of infections which is directly related to ingestion of foodborne *E. coli* and consequence of that is the fact that because of a lack of a definitive link between antibiotic resistant *E. coli* in food and failure of clinical treatment, the real degree of treat to human health due to antibiotic resistant *E. coli* in poultry remains putative and controversial.

Cross resistance

Velhner and Milanov (2015) highlighted the fact that tetracycline are provoking resistance to other classes of antibiotics and vice versa. Resistance to tetracycline often leads to a cross resistance to other antibiotics, such as fluoroquinolones (Todorović et al., 2015). Cross resistance of tetracyclines to fluoroquinolones and third generation of cephalosporins in *E. coli* from broiler chickens and fattening turkeys in 2013 and 2014 was observed in Austria, Germany, Hungary, Italy, Poland, Portugal, Spain (EFSA, 2015; 2016). Velhner and Milanov (2015) suggested that this is probably the consequence of the fact that the resistance determinants are often found on mobile genetic elements. The main consequence is threatened to humans and animals health especially in the cases when the antibiotics are necessary for the therapy of certain diseases.

THE OCCURENCE OF TETRACYCLINE RESISTANCE IN POULTRY IN EUROPE

The European Commission banned the use of antibiotics as growth promoters in animal nutrition by 1 January 2006 due to the emergence of bacteria resistant to antibiotics, which are used for the therapy of human and animal infections. Sweden banned use of antibiotics as growth promoters in all food producing animals in 1986 and after that the European Union banned use of

avoparcin in 1997 and use of bacitracin, spiramycin, tylosin and virginiamycin in 1999 as growth promoters. Also, consumer pressure is pushing the poultry industry to produce chickens without antibiotics as growth promoters, but removal of antibiotics as growth promoters has led to reduced growth performance and feed efficiency as well as increased incidence of diseases such as clostridial necrotic enteritis in broilers (Casewell et al., 2003). Moreover, Casewell et al. (2003) noted the increase in the sales of tetracycline (by 36 tonnes) for therapeutic purposes form 1999 to 2000 after the EU ban of the above mentioned antibiotics as growth promoters.

Van den Bogaard et al. (2001) examined the percentage of faecal samples containing resistant strains of *E. coli* in three poultry population; broilers and turkeys commonly given antibiotics and laying hens treated with antibiotics relatively rarely in the Netherlands. They reported that the prevalence of resistant faecal *E. coli* from poultry, poultry farmers and poultry slaughters to oxytetracycline in turkeys was 87%, in broilers 78% in laying hens 76%, in turkey farmers 79% in broiler farmers 61% and in laying hens farmers 36% in turkey slaughters 55% and in broiler slaughters 43%. They noted that for oxytetracycline the percentage of samples with a high degree of resistance in chickens and turkeys was significantly higher compared to laying hens. And the same tendency was observed in human population and also the resistance to oxytetracycline was significantly higher in slaughters than in laying-hen farmers.

Sengeløv et al. (2003) examined *E. coli* isolates from diseased and healthy broilers in Denmark for the presence of tetracycline resistance genes tet(A), (B), (C), (D) or (E) and found that the tet(A) and tet(B) were the most prevalent. These results is probably consequence of the fact that the tet(A) and the tet(B) genes are the predominant in the intestinal environment of food producing animals and/or the presence of specific conjugative plasmids. They noted that pathogenic *E. coli* isolates from diseased broilers were of serogroup O2 or O78. They examined 18 tetracycline resistant pathogenic isolates of *E. coli* and 17 tetracycline isolates from healthy broilers and found that one isolate contained the tet(D) gene, while they did not detected tet(C) and tet(E) genes in any of the isolates. The authors did not observed significant difference between the prevalence of tet(A) and tet(B) genes among *E. coli* isolates originated from healthy and diseased broilers. In isolates form healthy broilers tet(A) was present in 41.2% and tet(B) in 52.9% and tet(D) in 5.9% and in isolates originated from diseased broilers tet(A) was present in 72.2% and tet(B) in 27.8% samples.

Lanz et al. (2003) examined occurrence of antibiotic resistance on clinical *E. coli* isolates from laying hens with septicemia collected in Switzerland between 1999 and 2001 and reported that resistance to tetracycline in laying hens

was 26%. They found that the frequency of resistance determinants among tetracycline –resistant and tetracycline intermediate isolates in laying hens was 64% for tet(A), 30% for tet(B), 0% for tet(C) and in 6% of isolates they did not find any of the resistance determinants, while tet(D) and tet(E) genes were not detected in any of the *E. coli* isolates.

Marchant et al. (2013) examined isolates of the *E. coli* from healthy chickens belonging to the oldest (1998/99) and the latest (2006) surveillance programs in Spain. They noted correlation between the presence of integrons and resistance to tetracycline in chicken *E. coli* isolates from the Veterinary Antimicrobial Resistance Surveillance (VAV) Network and according to their data integrons were detected in 49% of the isolates from 1999 while resistance to tetracycline was determined in 94% of isolates. Integrons were present in 49% of isolates from 2006 while resistance to tetracycline was determined in 80% of *E. coli* isolates.

Resistance to tetracycline in broilers was very high or extremely high in most countries of the EU in 2014, with the exception of the Nordic countries (Finland, Denmark, Norway and Sweden) which reported low to moderate resistance to tetracycline, and the overall tetracycline resistance in EU was 50.1% (EFSA, 2016). The highest levels of resistance from fattening turkeys was reported for tetracycline and it was 70.9% in *E. coli* isolates in 2014 (EFSA, 2016). The occurrence of tetracycline resistance in *E. coli* isolates also varied significantly between European countries in 2013 and the resistance level to tetracycline in EU in broilers to tetracycline was 45.6 % (EFSA, 2015).

The occurrence of resistance to tetracycline in indicator *E. coli* from *Gallus gallus* in countries reporting MIC data in 2013, using harmonized ECOFFs was as follows: 22.6% in Austria, 60.3% in Belgium, 56.0% in Croatia, 15.2% in Denmark, 65.8% in France, 35.2% in Germany, 37.5% in Hungary, 35.8% in Netherlands, 46.1% in Poland, 64.1% in Spain, 7% in Norway and 23.8% in Switzerland (EFSA, 2015).

Furthermore, the occurrence of resistance to tetracycline in indicator *E. coli* from *Gallus gallus* in countries reporting MIC data in 2014 was as follows: 29% in Austria, 45.6% in Belgium, 43.5% in Croatia, 5.8% in Denmark, 63.3% in France, 33.9% in Germany, 39.4% in Hungary, 42.4% in Netherlands, 62% in Poland, 60.6% in Spain, 1.5% in Norway, 96.5% in Bulgaria, 78.2% in Cyprus, 24% in Czech Republic, 18.3% in Estonia, 10.9% in Finland, 68% in Greece, 52.7% in Ireland, 73.5% in Italy, 53.1% in Latvia, 56.1% in Lithuania, 45% in Malta, 62.6% in Portugal, 73.8% in Romania, 43.5% in Slovakia, 31.8% in Slovenia, 9.6% in Sweden and 60.4% in United Kingdom (EFSA, 2016). Regarding the trends in resistance among commensal *E. coli* isolates from broil-

ers over the seven year period from 2008 to 2014, the Netherlands registered significant declines in tetracycline resistance over the last 5 years and statistically significant decreasing trends over years was observed for tetracycline in France (EFSA, 2016).

Resistance to tetracycline in *E. coli* from fattening turkeys was generally very high or extremely high and amounted from 23.7% to 87.6% in EU countries in 2014 (EFSA, 2016). Extremely high levels (>70%) were reported in France (75.2%), Italy (77.6%), Poland (73.5%), Portugal (85.9%), Romania (84.2%), Spain (87.6%), UK (79.2%). Very high (50-70%) resistance levels to tetracycline were observed in Germany (56.5%), Hungary (64.1%); while high (20-50%) resistance levels were reported in Austria (40.8%) and in Sweden (23.7%) (EFSA, 2016).

THE OCCURENCE OF TETRACYCLINE RESISTANCE IN POULTRY IN AFRICA

Generally, in Africa the use of antibiotic in food producing animals, including poultry is unrestricted and the policies regarding antibiotics are not often enforced, so antibiotics, especially tetracycline are widely used in poultry production (Nsofor et al., 2013; Olonitola et al., 2015). According to study conducted by Nsofor et al. (2013) more than 83% of poultry farmers use tetracycline, and they are the most widely used in poultry feeds in Africa and as a consequence 100% of the bacterial isolates from tested feeds were resistant to tetracycline. Furthermore, in the majority of countries a prescription for antibiotics is not need even for human use (Olonitola et al., 2015) and the antibiotics that are used both human and in veterinary medicine is important, especially having in mind that even the use of distinct antibiotics can be a risk for developing of resistance. Olonitola et al. (2015) mentioned the fact that there is a great shortage in data related to the emergence of antibiotic resistant pathogens in Africa, but undoubtedly African communities suffer from infections with antibiotic resistant bacteria.

Adelowo et al. (2009) examined the prevalence of multi-resistant bacteria in the waste dumpsite on ten poultry farms in Nigeria. The resistance to tetracycline was 100% among the isolated *E. coli* strains. They noted that the antibiotics which are used frequently in the farms have caused the higher rate of resistance in bacteria. Such was the case with the tetracycline, oxytetracycline and chlortetracycline which were used regularly on ten farms, which they have studied.

Olonitola et al (2015) also stated that tetracycline's are one of the most

often used antibiotics in Nigeria not only in veterinary medicine, but also in human clinical practice. The main reason is the fact that tetracycline is highly available and its price is relatively low. The use of tetracycline in poultry production could lead to horizontal gene transfer via bacteria which colonized intestinal tract, such as *E. coli*.

The authors from Nigeria showed that tetracycline resistance is present in the examined poultry and poultry farms as well as in the facillities surrounding the farms and they all highlighted the need for strict regulation and control of tetracycline use in poultry farming in Nigeria as well as in other African countries. They further stated that the use of antibiotics, including tetracycline which is without control in poultry industry, present a serious problem from the public health point of view, having in mind the possibility of dissemination of resistant strains from poultry to environment, and spread of resistance determinants to pathogenic strains of bacteria in Nigeria.

THE OCCURENCE OF TETRACYCLINE RESISTANCE IN POULTRY IN NORTH, CENTRAL AND SOUTH AMERICA

In the United States some antibiotics are used for prophylactic purposes in livestock or as growth promoters. Tetracycline have been used as a useful growth promoters in farm animals in North America since the 1950s (Eliopoulos and Roberts, 2003). For the first time in experimental purposes, in the USA Levy et al. (1976) fed chickens with tetracycline supplemented feed and as they expected the intestinal microbiota of the chickens contained almost entirely tetracycline resistant bacteria. Furthermore, they also showed that feeding chickens with tetracycline-supplemented feed lead to the emergence of tetracycline-resistant *E. coli* in people from that farm, but not their neighbors.

Diarra et al. (2007) examined the effect of addition of approved antibiotics such as bambermycin, penicillin, salinomycin, and bacitracin or a combination of salinomycin plus bacitracin in British Columbia (Canada) on the prevalence and distribution of antibiotic resistance in 197 commensal *E. coli* isolates from broiler chickens over 35 days and reported that all isolates showed some degree of multiple antibiotic resistance. They observed that the resistance to tetracycline was most prevalent and amounted 68.5%. Furthermore, they noted that the overall resistance levels decreased from day 7 to day 35. They further characterized 104 tetracycline resistant *E. coli* isolates from 7 to 28 days old chickens fed different growth promoters and detected a decrease in the incidence of isolates carrying tet(B) gene from days 7 to 35, so their results demonstrated that multi-resistant strains of *E. coli* may be isolated from broil-

ers regardless of the antibiotics used as growth promoters. On the other side, the phenotype and the distribution of resistance determinants in *E. coli* can be changed by feed with the addition of some of the antibiotics used in broilers farming.

Miles et al. (2006) investigated the prevalence of tetracycline resistance in fecal *E. coli* isolates from healthy broiler chickens and compared obtained results with isolates obtained from hospitalized patients in Jamaica. They examined eighty-two *E. coli* strains isolated from faecal samples of broiler chickens and urine and wound specimens of hospitalized patients. According to their results tetracycline resistance occurred at a frequency of 82.4% in isolates from broilers compared to 43.8% in human isolates. Moreover, they detected multi-resistant strains in isolates from both chickens and humans and noted that it was usually associated with tetracycline resistance. Isolates resistant to tetracycline from both sources contained one or several plasmids which were transmissible by transformation of chemically-competent *E. coli*. They also observed that tetracycline resistance was mediated by efflux genes tet(B) and/or tet(D). The results obtained by Miles et al (2006) highlighted the prevalence of multi-resistant strains of *E. coli* among healthy broilers in Jamaica and possibility that it is associated with expression of tetracycline resistance. They noted that the genes which encode resistance were similar in the strains from chickens and human origin and suggested that genes are disseminated in the environment but they did not find any tetracycline resistant *E. coli* isolates from feed and water samples from any of the five tested poultry farms so the further investigations are required to examine the possibility of chicken sources as potential reservoirs for tetracycline resistance in humans. Further, they noted that most multi-resistant strains isolated from chickens were resistant to kanamycin, tetracycline and nalidixic acid and the most isolates from humans were resistant to kanamycin and tetracycline or kanamycin and gentamicin and that fact suggested that isolates from chickens that were resistant to tetracycline are more likely to become resistant to other antibiotics. On the other hand, they found that strains which were resistant to tetracycline isolated from chickens were susceptible to ampicillin, amoxicillin/clavulanate, chloramphenicol, ciprofloxacin and gentamicin and these findings indicated limited cross-resistance which enables the existence of therapeutic options for poultry colonized by tetracycline resistant *E. coli*. They also provided the evidence of existing significant tetracycline resistance in *E. coli* isolates from chickens originated from farms without recorded use of antibiotics and hospitalized patients in Jamaica. Further, they reported that tetracycline resistance was associated with plasmids and they discovered that 69% of the tetracycline resistant isolates had

resistance plasmids. Based on their results Miles et al. (2016) further suggested that many human and poultry commensal bacteria carry the same *tet* genes, plasmids, transposons and integrons as pathogenic bacteria and that all these resistance determinates could be transferred to pathogenic bacteria which can cause disease.

Ferreira et al. (2016) examined cloacal swabs which were harvested from 2011 to 2012 from 40-day old commercial broilers in two poultry farms from São Paulo State, Brazil for the presence of Extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae*. They detected 13 ESBL-producing *E. coli* and observed that among the isolates, 92.3% were also resistant to tetracycline's.

THE OCCURENCE OF TETRACYCLINE RESISTANCE IN POULTRY IN ASIA AND MIDDLE EAST

The majority of antibiotics is used for prophylactic purposes in livestock or as growth promoters in China and the same situation is also recorded in many developing countries in Asia and Middle East. The results of research from various authors (Dai et al., 2008; Lei et al., 2010) showed that expansion of poultry production in China and the accompanying widespread use of antibiotics have resulted in widespread resistance to antibiotics, especially to tetracycline.

Dai et al (2008) investigated antibiotic resistance of *E. coli* isolated from chickens from 49 farms located in southern, central and northern China between 2001 and 2006 and found that the most common pattern of resistance was ampicillin–enrofloxacin–doxycycline resistance, which was observed in 125 (23.3%) of the *E. coli* isolates. The resistance to doxycycline was observed in 75.0% of isolates and it was consistently high (over 70%) from 2001 to 2006. According to their results resistance to doxycycline was found in 70.9% in 2001, 74.4% in 2002, 72.2% in 2003, 72.4% in 2004, 77.8% in 2005 and 82.4% in 2006, so in total during six years number of investigated isolates was 536 and resistance to doxycicline was 75% (402 isolates).

Lei et al. (2010) provided evidence of the relationship of the use of antibiotics in poultry farming and its selection of antibiotic resistance in *E. coli* isolates from broilers and their results showed very high resistance to tetracycline in China. They examined 187 *E. coli* isolates from chicken and found that the level of resistance to tetracycline was 95.2%. Based on the obtained data, they noted that China faced with serious problem, and that the practice of abundant use of antibiotics, including tetracycline in poultry, especially in poultry feed as additives, consequently lead to high resistance in *E. coli* isolates.

Salehi and Bonab (2006) investigated 50 avian pathogenic *E. coli* (APEC) strains isolated from broilers with colisepticemia and found that the rate of resistance to oxytetracycline was 95%, to chlortetracycline was 95%, to tetracycline was 94% and to doxycycline was 88%. Their results showed a very high incidence of resistance to tetracycline in *E. coli* strains from poultry in Iran and the obtained results is probably due to the increased use of antibiotics as feed additives for growth promotion and prevention of diseases, as well as the use of inappropriate antibiotics for treatment of the disease. They also reported that antibiotics are used intensively in order to decreasing the huge losses caused by colibacillosis caused by *E. coli* in poultry farming in Iran. The resistance transfers among different bacteria and possible cross resistance between antibiotics used in poultry farming are also significant factors that contribute to high level of tetracycline resistance in *E. coli* isolates from poultry in Iran.

Van et al. (2008) examined a current resistance profile of *E. coli* from poultry in Vietnam. They isolated *E. coli* from 43 samples of broilers feces and noted that the rates of multiresistance were up to 95 in chicken feces isolates and resistance was most frequently observed to tetracycline (95%). They collected faeces from two poultry farms where chickens were less than 1 month old. They further reported that the tet(A) gene was the most prevalent of the tetracycline resistance genes detected (71.1% of the isolates), followed by tet(B) (18.4%) in Vietnam.

Ozaki et al. (2011) investigated the effects of rearing practices of commercial broilers on the incidence of antibiotic resistance in commensal *E. coli* isolates in Japan so they collected fecal *E. coli* isolates from 4 farms wherein in 2 out of 4 farms no antibiotics were used during the rearing period and antibiotics had not been used in farming practice for more than 1 year, whereas on the other two farms, following collection of the fecal samples at 14 and 15 days of age, oxytetracycline, sulfadimethoxine, and tylosin were given to chickens on one farm and sulfadimethoxine was given to chickens on the other farm. They reported resistance to oxytetracycline in 46 to 62% of isolates obtained from farms 1, 2 and 4 while 100% of the isolates from farm 3 were resistant to oxytetracycline. They found that the level of resistance to oxytetracycline on farm 1 at 2, 17 and 50 days of age was 38%, 57% and 45%, respectively; on farm 2 at 2, 17 and 48 days of age resistance to oxytetracycline was 53%, 29% and 78%, respectively; on farm 3 at age 2, 14 and 47 days the resistance rate amounted 100%; on farm 4 at age 2, 15 and 48 days the resistance rate amounted 67%, 49% and 70%, respectively. They suggested that isolates resistant to oxytetracycline from the farm where antibiotic were not used were shed from the intestinal tract and remained in the environment. It has been well documented in the

study conducted by Diarra et al. (2007) that some resistant strains can persist in the farm environment and colonize new flocks. The authors also stated that it was not known whether antibiotics had been used in broiler parent flocks. It is well known that such practices can affect prevalence of resistant strains in commercial broiler farms without using antibiotics in feed. The author also strongly suggested that the acquired resistance to antibiotics including tetracycline occurred due to horizontal transmission of resistant plasmids regardless of the use of antibiotics during the rearing period.

THE OCCURENCE OF TETRACYCLINE RESISTANCE IN POULTRY IN SERBIA

The few studies have investigated antibiotic resistance, including tetracycline resistance, in *E. coli* from the poultry farms in Republic of Serbia (Krnjajić et al., 2005; Knežević and Petrović, 2008). Krnjajić et al. (2005) took samples from 42 cattle, pig and poultry farms. The samples were feces of young healthy, young unhealthy and adult animals or internal organs, cloacal swabs and eggs in case of dead animals. Prevalence of resistance to tetracycline of the isolated *E. coli* strains isolated from healthy chicken was a 100%, in case of dead chicken, the isolated *E. coli* strains showed a 85% resistance to tetracycline, while the prevalence of tetracycline resistance of the *E. coli* strains from hens was 75%. Knežević and Petrović (2008) examined the resistance in commensal non-pathogenic *E. coli* from three farms from Vojvodina. They chose commensal strains because they represent a good indicator of commensal resistance and as opportunistic pathogens may spread resistance genes by horizontal gene transfer to other bacteria, including pathogens. They sampled sixty rectal or cloacal swabs from randomly selected animals settled in different objects without direct contact including 20 broilers which were 6 week old from three different farms. They reported that the broilers were not treated with antibiotics. They grouped intermediate strains with the sensitive isolates. Among *E. coli* isolates from broilers the 94.7% isolates were resistant to tetracycline. The authors claimed that the high resistance rate to tetracycline of broiler isolates is not associated with its usage. Gavrović et al. (2011) examined samples of internal organs, feces and swabs from ill animals, including poultry and they found that the incidence of resistance to tetracycline in *E. coli* isolated from poultry was 56%.

THE OCCURENCE OF TETRACYCLINE RESISTANCE IN POULTRY IN AUSTRALIA

The use of antibiotics in Australia in animal production is regulated and enforced. Obeng et al. (2012) examined 251 *E. coli* isolates from intensive meat and free range egg layer chickens collected between December 2008 and June 2009 in South Australia. For that purpose they collected a total of 311 faecal samples including 155 from free range meat chickens, 69 from free range egg layer chickens and 87 from indoor commercial chickens and found that phenotypic resistance to tetracycline varied between different classes of poultry. Tetracycline resistance was identified in 53 (39.5%) of free range meat chicken isolates, 39 (67.2%) of indoor commercial meat chickens and in 10 (16.9%) of the free range layers. In total of 251 *E. coli* isolates the abundant resistance was detected to tetracycline (40.6%). Furthermore, they found multiple resistances to three or more antibiotics in 26 (10.6%) of the 251 isolates and co-resistance involving tetracycline was the most prevalent detected. They found the tet(A) gene in 48 isolates, the tet(B) in 7 isolates, the both tet(A) and tet(B) genes in one isolate, tet(A) and tet(C) genes in 11 isolates and the combination of tet(B) and tet(C) genes in 3 isolates.

The prevalence of tetracycline resistance and the occurrence of resistance genes detected in the *E. coli* isolates in relation to distribution in the various groups of chickens were as follows: in free range meat chickens tet(A) was found in 20, tet(B) in 1; tet(A) and tet(C) in 4; tet(B) and tet(C) in 2 and Integron 1 in 20 and Integron 2 in 6 isolates; in free range egg layers tet(A) was detected in 3, tet(B) in 3 isolates, while no integrons were found; in indoor commercial meat chickens tet(A) was found in 25, tet(B) in 3, tet(A) and tet(B) in 1, tet(A) and tet(C) in 7, tet(B) and tet(C) in 1, Integron 1 in 13 and Integron 2 in 1 isolate. They found association between tetracycline resistance and integrons, and in total of 251 isolates, tetracycline resistance was detected in 102 isolates (40.6%) and Integron 1 was found in 33 (13.1%) of the isolates while Integron 2 was found in 7 (2.8%) of the isolates. The authors highlighted the fact that their results showed a considerable reduction in resistance to tetracycline in comparison with previous study of intensively reared chicken conducted in their laboratory which has shown resistance to tetracycline in 85% of isolates and they concluded that tetracycline resistance are determined by the antibiotic used. In Australia only chlortetracycline is registered for therapy of egg-producing birds, which explains in part the low level of tetracycline resistance in free range birds. A wider range antibiotic such as oxitetracycline is registered for use in meat producing birds. Furthermore, authors included

the possibility of residual resistance to tetracycline due to co-selection. The authors highlighted the fact that there were no significant differences between intensive and free-range chickens in occurrence of resistance but they explained that by the fact that they supplied the free-range chickens from the same hatcheries as intensive producer chicken so there is a possibility that resistance genes are passed vertically from breeder flock and that antibiotics are probably used in breeder flock rather than in the meat type chickens.

Management practices and preventive measures on poultry farms

The microbiota of poultry intestines is influenced by management practices including feeding and antibiotic use on the poultry farms. The monitoring of antibiotic resistance in commensal *E. coli* isolated from randomly selected healthy poultry, provides valuable information on the levels of the resistance in that population. In addition, determining the occurrence of resistance to antibiotics in commensal *E. coli* provides information useful for the examination of the relationship of the selective pressure exerted by the use of antibiotics on the intestinal microbiota in poultry.

It should be mentioned that epidemiological data implicate that urinary tract infections in humans may be associated with poultry consumption (Manges et al., 2007). Such data endorses the need for more rigorous surveillance and improved practises in poultry production in order to reduce the carriage of genes of antibiotic resistance and thereby minimize the likelihood of horizontal gene transfers of these resistance determinats via the food chain.

Presented data emphasize the need for further improvements of poultry farming practice, mainly the strict regulation of antibiotic usage. The proper measures can reduce the likelihood of horizontal gene transfer of mobile antibiotic resistance genes to other bacteria via the food chain. The presented data also suggested that *E. coli* strains resistant to tetracyclines are stable and able to transmit and persist in poultry even when there are no selective pressures of antibiotics or long time after the withdrawal of antibiotics.

CONCLUSIONS

The relationship between the use of tetracycline as growth promoter in poultry industry and development of resistance has been established by many authors during the past decades. Such long-term subtherapeutic use of tetracycline antibiotics in feed is of great concern because it leads to selective pressure on the *E. coli* carried by the poultry and also in the farms environment, so the beneficial effects of tetracycline will become limited as bacterial

resistance increases. The necessity to reduce nontherapeutic use of tetracycline in poultry industry is obvious. Moreover, the education of the farmers, veterinarians, public and health is very important in order to ensure the proper use of tetracycline and to ensure that they will not be used without need, such as treatment of infections caused by viruses, prophylactic use and the use as growth promoter.

All in all, resistance to tetracycline in *E. coli* from poultry has been reported worldwide. Generally, it is often associated with the use of antibiotics, but the tetracycline resistance was noted also in several cases when antibiotics are not being used.

Monitoring of tetracycline resistance among commensal bacteria such as *E. coli* is of great importance not only in humans but also in poultry in order to detect the possible route of transfer of resistant bacteria or resistant determinants from poultry to humans.

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EVALUATION OF THE MANAGEMENT OF BROWN HARE POPULATION IN VOJVODINA REGION FOR THE PERIOD 1967-2011

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Abstract

Every year during the past 45 years (1967–2011), hunting organizations have been sending eye lenses (*lens cristallina*) to the Laboratory for hunting of the hunting association of Vojvodina for testing and analysis of the percentage of young hares in a micro population to establish the real growth rate. These two parameters combined with the number of hares in spring and cull rate from last year were sufficient to provide hunting organizations with precise information about hunting dynamics and rate of hunting in current hunting season. At the beginning, (in 1967), only 20% of hunting organizations submitted the samples, but ten years after the first sample had been collected, the percentage has gradually increased to 30% in 1977 Since then, the percentage of hunting organizations that were sending samples increased to 45% in 1987, 60% in 1997, and 70 % in 2007 with a maximum of 77% percentage in 2008. Throughout the research period of 45 years, the total number of examined and processed eye lenses was 363,380. Out of 8,727 samples approximately 8,075 eye lenses were processed yearly. A small number of hunting organizations which didn't send any samples haven't been hunting brown hare in their hunting grounds during these years. During this research, several conclusions were made: the percentage of young hares in populations varies from 38% in 2010 to 70.3% in 1994. The average percentage for period of 45 years was 58.4% of young hares in a population. According to the research, the coefficient of real growth was 1.58 young hare per female hare. The minimum was 1.13 in 2010, and the maximum was 2.33 in 1994. These analyses provided the actual information

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about stability and health of hare populations and enable a sustainable long-term planning of these game species.

Key words: brown hare, *Lepus europaeus Pallas*, game management, real growth rate

GAZDOVANJE POPULACIJOM ZECA U VOJVODINI ZA PERIOD OD 1967. DO 2011. GODINE

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Kratak sadržaj

U proteklom periodu od 45 godina (1967-2011), u Laboratoriju za lovstvo Lovačkog saveza Vojvodine lovačke organizacije su neprekidno svake godine slale očna sočiva (lens cristallina) radi utvrđivanja, učešća mladih u mikropopulaciji, i na osnovu toga realnog prirasta. Ova dva parametra su, zajedno sa prolećnim brojnim stanjem i odstrelom u prethodnoj godini, bila dovoljna da im Stručna služba Saveza da preporuku kako i koliko treba da love u tekućoj lovnoj godini. U početku (1967. godine) je oko 20% lovačkih udruženja slalo uzorke, da bi nakon 10 godina (1977.) to iznosilo preko 30%, 1987. godine preko 45%, 1997. godine preko 60% i 2007. godine oko 70%, sa maksimumom zabeleženim u 2008. godini od skoro 77%. Za period od 45 godina pregledano je ukupno 363.380 očiju ulovljenih zečeva, što prosečno godišnje iznosi 8.075 očnih sočiva iz 8.727 uzoraka. Manji broj lovišta koji nije slao uzorke nije ni lovio zeca tih godina. Istraživanjima je utvrđeno da je za ovako dugi period prosečan procenat mladih je bio 58,4% (sa minimumom od 38,0% zabeleženim 2010. godine i maksimumom od 70,3% zabeleženim 1994. godine), odnosno da je prosečan koeficijenat realnog prirasta bio 1,58 zečića po zečici (sa minimumom od 1,13 zabeleženim 2010. godine i maksimumom od 2,33 zabeleženim 1994. godine). Ovakve analize omogućuju najbolji način za praćenje populacije, kao i plansko i dugoročno održivo gazdovanje.

Ključne reči: zec, Lepus europaeus Pallas, gazdovanje, realni prirast

INTRODUCTION

The actual age of hare *(Lepus europeus Pallas, 1778)* can be determined in several ways. One method is currently used for scientific purposes in Vojvodina and hunting organizations plan their hunting quotas according to the annual growth rate for micro populations of brown hare.

With more than 45 years of experience, since 1967, the annual growth rate is determined in October, after the first few hare hunting days. At the beginning, from 1967 to 1987, the analyses were conducted at the Faculty of Agriculture of Novi Sad. Since 1987, the research were conducted in the laboratory for hunting at the Hunting Association of Vojvodina. The minimum number of eye samples required to be submitted for examination is 30. After submission, in the controlled laboratory conditions, the research is conducted and eye lenses are processed. Subsequently, the hunting laboratory issues a recommendation for further hunting in the specific hunting ground. After collecting information about annual grow rate, spring counting of hares in hunting grounds, and number of hares hunted in the first few hunts it is easy to give advice and plan the dynamics of the cull in each hunting organization.

If the analyses reveal the share of young is less than 50% it is very important to stop further hunting. Although this method is in use in different countries (Hungary, Check Republic), its highest application is recorded in Vojvodina region.

The research of Valentinčić (1956) on the ideal annual grow rate was performed at one hunting ground in Vojvodina (study area – island "Biserno") in 1954 and 1955. The results of this research showed that brown hares have 5 litters per year in this region of Vojvodina.

The *first litter* appears in March (8 – 25), with a peak from 18-20 March. That means, the main mating season starts on 25th of January and lasts till 11th of February, with a peak around 5th of February. This data pertained to year 1954, but in 1955, the litters were found from the 1-20 March with a peak around 10-15 of March. Thus, we can conclude that the prime mating season started on 17 January and lasted up to 5 February, with highest mating activity from 27 January until 1 February. In the first litter, during these two years of research, the number of young hares per female was 1.23. The *second litter* in 1954 appeared in the period 25 April – 5 May. That means that the second mating season for hares was in March, i.e., from 15 to 25 March. For a two-year research period, the average number of small hares per litter was 2.9 per year. The *third litter* was born from 5 to 23 June with a peak on 5 June. Thus, the third mating season was between 23 April and 15 May with a peak on 5 May.

In the third litter, the number of young hares was 2.75 per female. In the *fourth litter*, small hares were born in the period from the end of July until 10 August, peaking on 5 August. Hares were mating in June (20 – 30), and the number of the young per litter was 1.2. The *fifth litter* of hares was born between 5 and 20 September with a peak on 12 September. The mating season started on 25 July and ended by 10 August with a peak on the 1 August.

According to the research results we can conclude that of the majority of females gave birth five times a year, and the first litter can be found from 10 – 20 March, second from 25-30 April, third from 1-15 June, fourth litter is born from 1 to 15 August, and the fifth litter can be found between 12 – 25 September.

The second and third litters are major litters in the year; if they are successful, it is very likely that hare population will be plentiful in this year. These hares are born between end of April and middle of June, thus, the hunters must be highly concerned and put maximum efforts to preserve this precious game. It is very interesting that during this research, there wasn't a single female that gave birth in the same year when she was born.

Analyzing data for three years (1974-1976) Jovanović and Aleksić (1976) have concluded the following: 1) In the first litter, 57% females were fecundated and had 0.6 fetus after the cull in autumn; 2) In the second litter, 88% females were fecundated and had 2.5 fetus in uterus; 3) In the third litter, 92% females were fecundated and had 3.9 fetus in uterus; 4) In the fourth litter, 100 % females were fecundated after the cull and had 3.4 fetuses in uterus; and 5) In the fifth litter, 46% females were fecundated after the cull and they had averagely 1.2 fetuses.

At the conclusion of this research, it was apparent that 79% of hunted female hares were fecundated. Valentinčić (Valentinčić, 1955) established that the percentage of fecundated females was 68% in 1954 and 1955, while the number of fetuses in uterus was 1.5. However, the research from 1976 revealed that percentage of fetus was higher, being 2.4.

Valentincić established that the most numerous litters were second and third (3.3 and 2.5), and Aleksić and Jovanović concluded that the most numerous litters were third and fourth (3.9 and 3.4).

There is a range of methods for determination of the age of hares. Some can be performed in the field, do not require any specific equipment and are easy to learn and to perform. The methods that do not require particular scientific knowledge and laboratory equipment include measuring the body mass of hare, assessment how easily ears are torn, forehead color, and tear bone These methods are not always accurate but they are easy and fast, so we use them to make some rough estimation of the age of hunted hares.

Lord (Lord, 1959) found a new and scientifically based method to determine the age of hares. He did his experiment on a hare from Florida *(Sylvilagus floridanus).* The experiment was based on the data reported by English ophthalmologist Smith (Smith, 1883), who found that eye pupil and its elements is the only part of hare's body that never stops growing. Lord experimented with 92 hares with known age. His experiment was totally functional and was a great scientific discovery. Nowadays, thanks to him, the age of hares and hares can be estimated with a month-level precision. In European terminology, the method is known as Rieck method (Rieck, 1962) as he was the first one who applied it on brown hare (*Lepus europaeus Pallas*).

The most accurate measurements and experiments on brown hare were conducted by Cabon-Raczynski and Raczynski (Cabon-Raczynski, Raczynski, 1972) on a sample of 2,277 hares. They were measuring the mass of dried eye lenses and established the borderline values for young hare and older ones. The borderline value is 275 mg, that is, the mass below this value indicates that the hare is young, while lenses of bigger weight belong to older hares.

Another method for determination of hare age is that of Strahov (Stroch, 1931), which is based on capture re-capture method; however, is not that accurate and it differs for some 20 % from the method of dried eye lenses. The authors who applied this method were Andersen and Jensen (Andersen, Jensen, 1972). The method is still in use, but it is far more unreliable than the Lord method (Lord, 1956).

Several other researchers used the method of *dry eye* lenticular weight for determining of age not only of hares but also some other game species. Some authors examined the influence of different methods of fixation and drying of eye lenses on the precision of results. Andersen and Jensen (Andersen, Jensen, 1972) recommend the drying at 60°C to obtain the best results, whereas Černe (1976) recommends the drying temperature of 110°C.

MATERIAL AND METHODS

Brown hare age determination based on the weight of eye lenses

In the Laboratory for Hunting of the Faculty of Agriculture in Novi Sad, the method of determining the age of hares has been used since 1967. The process of fixing and processing of the material is done by carefully pulling the eyeball of the harvested hares, which is then placed in a 5-10% solution of formalin (formaldehyde), and left there for 3-4 days. Then, the lens is removed and dried for 72 hours at 55-60°C in the thermostat. Once completely dry, they lenses are weighed on Mettler scale with an accuracy of 5 mg. Uniformity of

the preparation, drying and measurement of the lenses from the beginning of this method is guaranteed by the laboratory and offers the possibility of comparing the results from different years. The resulting data have already been used to determine the real hunting quota of hares, as well as active measures to protect the hare population in Vojvodina (Šelmić, 1977).

Based on the weight when dry, the population was categorized into six age classes.

I age class, of up to 100 mg - hares up to 3 months
II age class, of 100 to 200 mg - hares aged 3- 6 months
III age class, of 200 to 280 mg - hares aged 6 -12 months
IV age class, from 280 to 310 mg - hares aged 1 to 2 years
V age class, of 310 to 370 mg - hares aged 3-4 years
VI age class, over 370 mg - hares over 4 years of age

Values between age classes were determined on the basis of research by Andersen and Jensen (Andersen, Jensen, 1972) and the criteria of Miller (Möller, 1975) and Šelmić (Šelmić, 1977). This type of research in Vojvodina has continuously been done for 45 years (1967-2011), and 363,380 lenses from 7,873 samples have been processed and measured so far.

Calculating growth rate based on the share of young

As aforementioned, this type of research has been performed in Vojvodina for over 40 years, and up to now, the method has given excellent results. Thanks to this research annual hunting bag is carefully and sustainably planned.

Based on the established share of young hares in the population (up to one year old) at the beginning of hunting season and criteria for evaluation of real growth (Šelmić, 1977) as well as data obtained from hunting associations on the numerical strength of the spring population and the hunting bag from that year, expert service is able to make a recommendation on hunting possibilities. The proposal to reduce hunting quotas refers mostly to those hunting organizations, in which the participation of young hares in a total population is less than 50%.

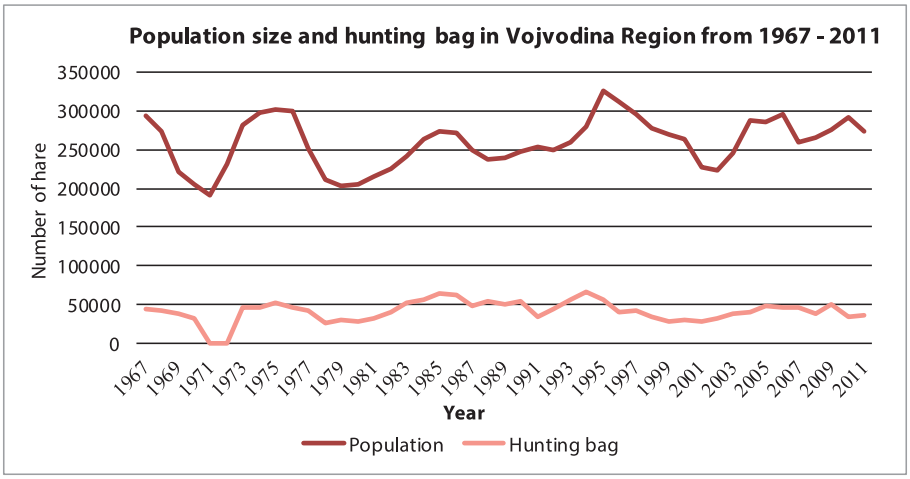
Table 1. Growth assessment based on the number of young hares in the population

Growth assessment	Share of young hares in the population	Periodic real growth rate in relation to the spring population
Very low	Up to 40%	Up to 20%
Low	from 41% - 50%	21% - 40%
Good	from 51% - 57%	41% - 62%
Very good	from 58% - 63%	63% - 90%
Great	over 64%	over 90%

The growth of the population of hares depends on a number of environmental factors, modern machinery, the use of pesticides in the hunting ground, increased number of predators and other; therefore, these studies have so far proved valuable because they preserved the population stable.

RESULTS AND DISCUSSION

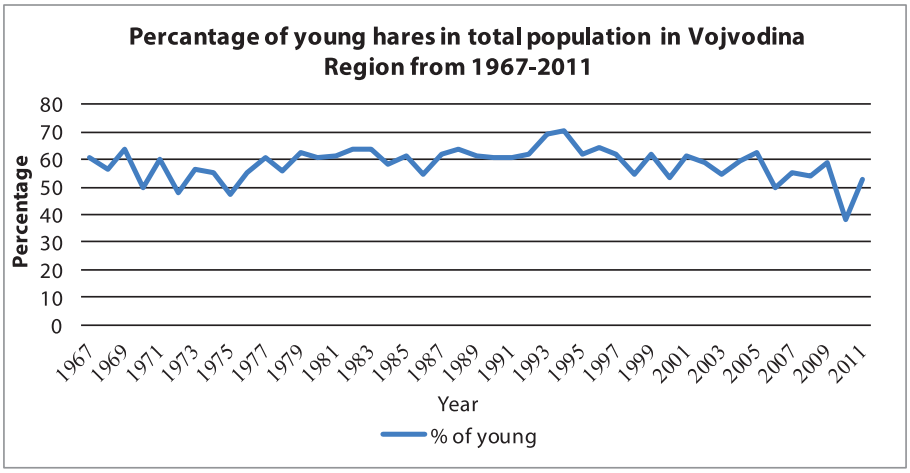
The coefficient of real growth until the hunting season, calculated on the basis of the share of young hares in the population at the beginning of the hunt, shows a balanced flow for the entire observed period. Two extremes were recorded in 1993 (2.26) and 1994 (2.33), thus ending up with highest real growth rate in 42 years since this method is applied in practice (Graph 2).



Graph 1. Population and annual hunting bag of hare (*Lepus europaeus* Pall.) for 1967-2011 period

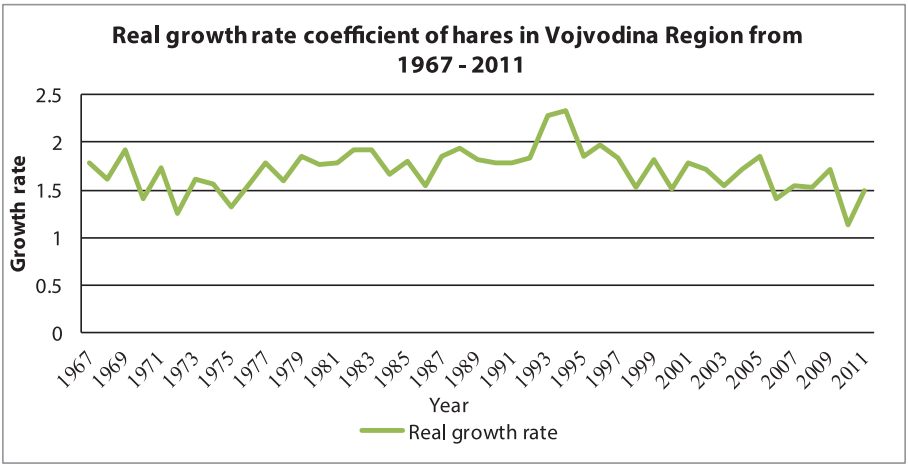
Quantity of hare population in Vojvodina (Graph 1) in the observed period was balanced except in 1971 when the least number of hares was recorded. Consequently, two-year ban on hare hunting was imposed (1971 and 1972), except for experimental purposes (Vapa et al, 2007).

Hunting bag in the same period ranged mostly between 35,000 and 45,000 (Graph 1), with the overall average being 43,033. The highest annual hunting bag was recorded in 1985 (63,591) and 1994 (65,848). The highest percentage of use was recorded in 1985 (23.3%) and 1986 (22.7%), with the average value for the entire period being 16.63%. The percentage of use was the lowest in 1999 remaining at 10.1%. In the 1990s, throughout entire Vojvodina region, partial ban on hunting was imposed because of war. Share of young hares in the population (Graph 2) at the beginning of the hunting season and calculated real growth rate (Graph 3) show balanced flow in the observed period. Lowest share of young hares was recorded in 1975 (47.3%) and 2010 (38.0%), with the average for the entire observed period being 58.4%.



Graph 2. Share of young hares in the population for the period 1967-2011

Real growth coefficient (Graph 3) was the lowest in 1972 (1.25), when hunting was banned and in 2010 being only 1.13. The maximum coefficient of real growth was recorded in 1993 (2.28) and 1994 (2.33), with an average for the entire observed period being 1.58.



Graph 3. Real growth coefficient of hares for the period 1967-2011

CONCLUSION

Determining the real annual growth of hares is of an invaluable importance for an effective evaluation of hare population. Using data on the spring population number and winter losses, an overall assessment can be made in order to preserving the hare population in our country.

When preparing relevant documents and planning hare population dynamics, it is recommended to apply the average real growth coefficient of 1.58 calculated on the basis of the average values for several decades. Using a higher coefficient than calculated could quickly result in the reduction in the number of hares in the hunting area, and consequent unrealistic harvest.

The method for determining the share of young hares in the population in Vojvodina, and subsequently calculated real growth was fully accepted by hunting organizations and hunting grounds. Most of them regularly submit the eye lenses from first hunts, and then, upon completion of laboratory processing of specimens, they decide whether or not the hunting will continue, and for how long. Due to this fact, we can conclude that stable hare populations exist in over 70% of hunting grounds in Vojvodina. This strongly suggests that a sustainable management is accomplished and the population will most likely remain stable for years to come if such management practices continue. .

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MALIGNANT TUMOURS IN LABRADOR RETRIEVERS USED FOR NARCOTIC DETECTION

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Abstract

The two corpses of police dogs, of Labrador retriever breed used for finding narcotics, were sent to the Scientific Veterinary Institute "Novi Sad", and examined post-mortem, in order to determine the cause of death. Post-mortem examination revealed the presence of neoplastic changes in the mandibular region, pulmonary parenchyma and axillary region. The samples of neoplastic changes were sent to the Laboratory of Patohistology within the Faculty of Veterinary Medicine, University of Belgrade, in order to specify the diagnosis. Histopathology examination revealed an adenocarcinoma in lungs and *haemangioendothelioma* in axillary region. Is there any role of drug sniffing on the occurrence of neoplasia in Labrador retriever breed is still unknown, but it could be an important factor in the development of neoplasia in these dogs.

Key words: lung adenocarcinoma, *Haemangioendothelioma,* Histopathology, Labrador retriever.

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MALIGNI TUMORI KOD LABRADOR RETRIVERA KOJI SE KORISTE ZA PRONALAŽENJE NARKOTIKA

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Kratak sadržaj

Dva leša policijskih službenih pasa, rase Labrador retriver koji se koriste za pronalaženje narkotika, dostavljeni su u Naučni institut za veterinarstvo "Novi Sad", kako bi se na osnovu patomorfološkog pregleda utvrdio uzrok smrti. Tokom obdukcije utvrđeno je prisustvo neoplastičnih promena u mandibularnoj regiji, plućnom parenhimu i aksilarnoj regiji. Uzorci promenjenog tkiva dostavljeni su na Fakultet veterinarske medicine, Univerziteta u Beogradu, u laboratoriju za patohistologiju kako bi se odredila tačna dijagnoza. Patohistološkim nalazom utvrđeno je prisustvo adenokarcinoma u plućima i hemangioendotelioma u predelu aksilarne regije. Kakvu ulogu može imati udisanje narkotika na pojavu neoplazmi kod Labrador retrivera još uvek nije poznato, ali se pretpostavlja da to može biti jedan od faktora u nastanku neoplastičnih promena kod ovih službenih pasa.

Ključne reči: adenokarcinom pluća, hemangioendoteliom, patohistologija, Labrador retriver.

INTRODUCTION

Labrador retriever dogs are often used as sniffer dogs – they are trained by a large number of law enforcement agencies and rescue teams worldwide. These dogs are able to detect the scents of illegal drugs, explosives, flammable materials, and other contraband items such as illegal imports of ivory (Oesterhelweg et al., 2008). Drug-sniffing dogs may be exposed to illicit drugs and other toxic agents in the line of duty, most commonly through inhalation and ingestion. Ingestion is the most common route of exposure. They may ingest whole bags of drugs which must be removed surgically or via endoscopy to prevent rupture and massive exposure. Police dogs may be at increased risk

for malicious poisonings (Gupta R., 2012). Inhalation exposures are generally of lesser magnitude but may result in a more rapid onset of action of the agent (Llera and Volmer, 2006). When compared to the pet population, police dogs had a higher risk of developing neoplasms (Kippens and Grondalen, 1999). This case presentation describes the presence of malignant tumours in two police dogs, Labrador retriever breed trained for finding drugs.

CASE PRESENTATION

During 2015 and 2016, two corpses of official police dogs used for drug detection were brought to the Scientific Veterinary Institute ``Novi Sad`` to determine the cause of death. The necropsy was performed at the autopsy hall of the Scientific Veterinary Institute ``Novi Sad``. The first dog was a 12-years-old male Labrador retriever weighing around 40 kg. The second dog was a 15-years-old male Labrador retriever weighing approximately 50 kg. Both dogs were non-neutered dogs, older than 10 years and in very poor body condition. They were short hair yellow dogs. Both dogs were found dead in police rooms intended for the accommodation of service dogs. There were no data regarding a previous treatment of dogs.

During external post-mortem examination of the first dog, in the area of mandibular region globular formation of hard consistency was observed. This neoplastic change was 2x2cm in diameter, while the surrounding tissue was oedematous, haemorrhagic, jelly-like consistency. The left lung was enlarged, and during palpation of the lung parenchyma, neoplastic change 5x3cm in diameter was detected (Figure 1-A). This neoplastic change was hard consistency. On cross-section of the left lung, sticky dark red liquid was observed (Figure 1-B). By separating synsarcosis connection of front left limb of second dog, in the area of the axillary region tumour mass was detected (Figure 2-B). This neoplastic change was round shaped, about 10 cm in diameter, hard consistency, and protruded above the surface (Figure 2-A). On cut section, the structure of this tumour was liver-like, dark red coloured (Figure 2-C). The structure of the affected ribs had been completely destroyed, and this neoplastic change penetrated in the area of the chest cavity (Figure 2-D).

After necropsy and tissue sampling, the samples were sent to the Laboratory for Patohistology, at the Faculty of Veterinary Medicine, University of Belgrade in order to specify the diagnosis. For patohistological analysis, the sample was fixed in 10% neutral-buffered formalin, embedded in paraffin, and sections of 4 μm thickness were cut. These sections were stained with haematoxylin and eosin (H&E). Histopathologically, a well-differentiated adenocarcinoma

with squamous metaplasia was diagnosed at the left lung of 12-years-old dog. Microscopically, the neoplastic tissue of lungs showed mix of small basaloid cells and larger differentiated cells that were polygonal, with abundant glassy eosinophilic cytoplasm and prominent atypia of neoplastic cells. Acinar differentiation with formation of lumens was clearly evident, along with squamous differentiation of cells. Histopathological examination of neoplastic change of 15-years-old dog confirmed *Haemangioendothelioma* of axillary region. Microscopically, neoplastic tissue of axillary region had numerous irregular vascular channels and large areas of haemorrhage. Tumour cells were consisted of pleomorphic endothelial cells, ranging from spindle-shaped to polygonal to ovoid.

DISCUSSION

Malignant tumours are a common health problem in dogs worldwide. All dog breeds, as well as crossbred dogs may be affected, and it is notable that some purebred dogs appear to be at increased risk of certain types of malignant tumours, suggesting underlying genetic predisposition (Dobson, 2013).

Adenocarcinoma is a malignant tumour originating in the glandular and epithelial tissue (the lining of the internal organs). This type of malignant tumour growth can take place in many parts of the body. It commonly affects older dogs, usually more than six years of age. No particular breed is known to be predisposed and it is more common in male dogs than females. This type of tumours usually has a poor prognosis. The exact cause is still unknown. According to Scanziani et al. (1991) genetic cause is suspected in Belgian shepherds.

Reported to Ogilive et al. (1989) incidence of lung cancers in dogs is markedly lower than in humans, and accounts for approximately 1.2% of all tumours (Brodey and Craig, 1965; Sato et al, 2005). Pulmonary carcinoma with components of both adenomatous and squamous cells is classified as adenocarcinomas with squamous metaplasia. This type of tumours have been reported in large-scale surveys in dogs, accounting for approximately 13% of primary lung tumours (Hahn et al., 1996; Griffey et al., 1998; Sato et al., 2005). Metastatic lung cancers are much more common in dogs than primary lung cancers. Most of primary lung tumours are diagnosed in dogs average age of 10 to 12 years.

Haemangiosarcoma (HSA, *malignant hemangioendothelioma*), a malignant tumour of endothelial cells, occurs most frequently in old dogs, but is less common than haemangioma. Hemangiosarcoma is an aggressive and common cancer in dogs and it has been estimated to represent 7% of canine malignant tumours (Dobson, 2013). German shepherd dogs are most commonly affected,

and some other breeds are over-represented. Hemangiosarcoma is a highly malignant tumour arising from blood vessels, probably less common than some of the other mesenchymal malignancies. The most common primary sites for hemangiosarcoma in dogs are visceral organs, notably the spleen and liver; it may also arise in the right atrial appendage. (Dobson, 2013). Metastasis most often occurs to the liver, omentum, mesentery, lungs either hematogenously or through transabdominal transplantation via seeding after tumour rupture. Most affected dogs die from acute internal haemorrhage secondary to rupture of the tumour. Despite surgical and chemotherapeutic management, the median survival time for dogs diagnosed with HSA is little more than 6 months (Hammer et al., 1991; Clifford et al., 2000; Sorenmo et al., 2000; Sabattini and Bettini, 2009). The breed that appears to be predisposed is the German shepherd dog, and other commonly reported breeds include the Golden retriever, Pointer, Boxer, Labrador retriever, English setter, Great dane, Poodle, and Siberian husky (Smith, 2003). German shepherd dog has been reported to has an increased risk with an odds ratio of 4.7, compared to other purebred dogs (Prymac et al., 1985). More recently hemangiosarcoma appears to has become a significant problem in Golden retrievers in North America with an estimated life-time risk of 1 in 5 reported by the Golden Retriever Club of America (Tamburini et al., 2009; Dobson, 2013). The tumour occurs predominantly in older dogs between 8 and 10 years of age; mean age at time of diagnosis is 9 to 12 years (Sharma, 2012). Labrador retriever and Golden retriever dog breeds count for a high ratio of visceral hemangiosarcomas and skin hemangiomas and a slightly lower ratio of nonvisceral hemangiosarcoma. Dogs with hemangiomas are younger than those with hemangiosarcoma. Any type of hemangiosarcoma is rare in young dogs (Schultheiss, 2004).

The epidemiological study performed by Mialot and Lagadic (1990) remarked positive correlation between age of dogs and cats with tumour incidence. The increase in tumour incidence is correlated until the age of 10 years with animal age, in both dogs and cats. Tumour frequency remains high until the age of 12 years in dogs and 13 years in cats, then it gradually reduce, because of the life duration of these species.

There is no evidence which support addiction of dogs trained to detect hard narcotics, or even run the risk of becoming addicted, to the narcotics which they are trained to detect. A sniffer dog during the training for narcotic detection does not come in physical contact with narcotic agents. The only contact the dog has with the narcotic sample is related to sniffing the sample package. Two cases of malignant tumour in dogs that are used for the detection of narcotics are insufficient to examine the relationship between tumour

occurrences in these working dogs. In the literature there is no enough information about what kind of impact drugs can have on the health of sniffer dogs and the occurrence of tumours in this breed, although many drug-induced diseases are known in human population (Irey, 1976; Challita et al., 2014). It has been confirmed that neoplastic changes were the second leading cause of death or euthanasia in working dogs in the USA (Moore et al., 2001). This findings may suggest a possible role of chronic drug exposing to occurrence of neoplastic changes in sniffing dogs, so the further investigations may determinate the possible correlation.

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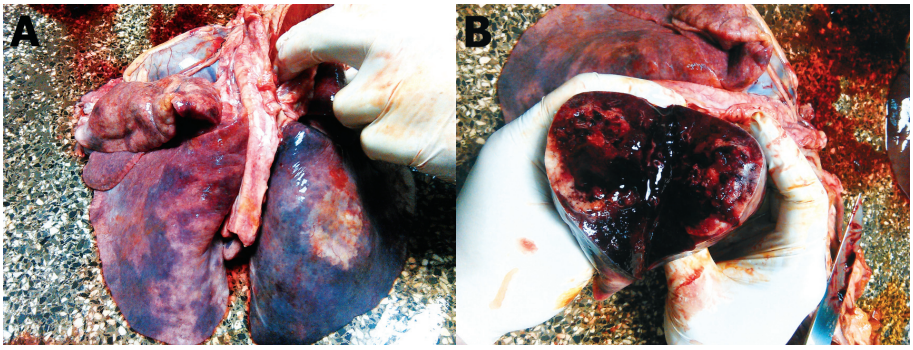

Figure 1. Post-mortem examination: Gross appearance (A) and cut of surface of the tumour (B).

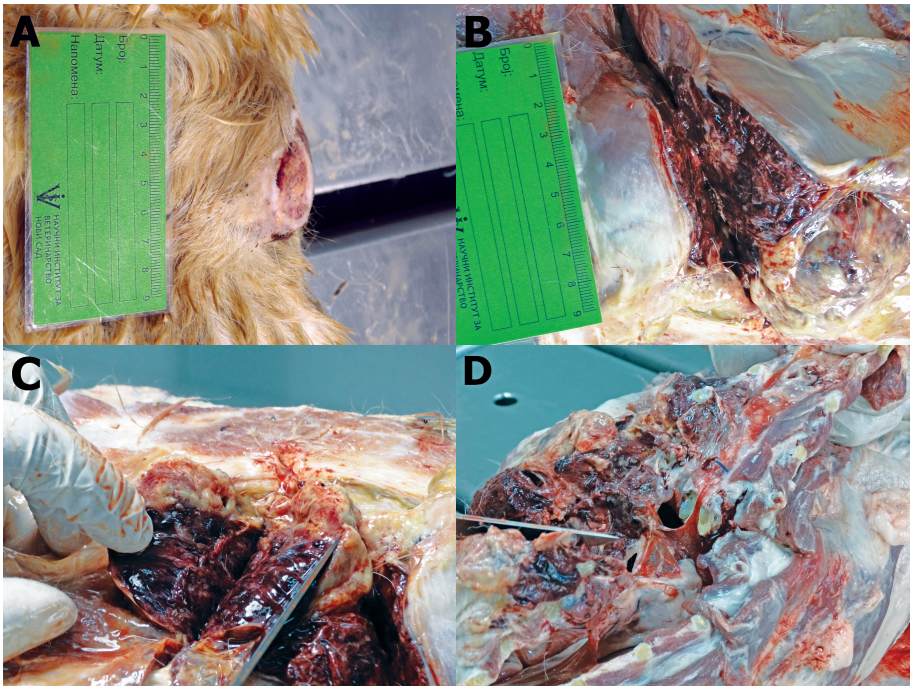

Figure 2. Post-mortem examination: Clinical aspect (A), gross appearance (B), cut of surface of the tumour (C) and structure of the affected ribs (D).

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UPUTSTVO AUTORIMA ZA PRIPREMANJE RUKOPISA

ARHIV VETERINARSKE MEDICINE je časopis Naučnog instituta za veterinarstvo"Novi Sad" u Novom Sadu. Časopis objavljuje originalne, stručne i pregledne radove, priloge iz prakse, izveštaje sa kongresa i stručnih sastanaka, prikaze knjiga, radove iz istorije veterinarske medicine

Sve primljene rukopise Uređivački odbor šalje recenzentima radi stručne procene. Ukoliko recenzenti predlože izmene i dopune, tada se kopija recenziranog rukopisa dostavlja prvom autoru s molbom da tražene izmene unesu u tekst ili pak u protivnom da argumentovano izraze svoje neslaganje sa datim primedbama recenzenta. Konačnu odluku o prihvatanju rada za štampu donosi glavni i odgovorni urednik zajedno sa uređivačkim odborom.

Časopis se štampa na srpskom jeziku, a kratak sadržaj se prevodi na engleski. Radovi stranih autora se štampaju na engleskom jeziku sa kratkim sadržajem na srpskom.

Molimo saradnike da svoje radove pišu u skladu sa sledećim uputstvima.

Opšta uputstva

Tekst rada se kuca u programu za obradu teksta Word, latinicom, fontom Times New Roman, veličina slova 12 tačaka (12 pt), dupli proredom. Levu i desnu marginu podesiti na 20 mm, a gornju i donju na 30 mm, na A4 strani. Ukoliko se u tekstu koriste specijalni znaci (simboli), koristiti font Symbol. Rukopis rada dostaviti odštampan jednostrano papiru, ali i u elektronskoj formi. Paginacija na desnoj strani lista, počevši od naslovne strane. Reference u tekstu treba da navedu ime autora, iza kojeg se stavlja zarez i godina. Ukoliko ima više od dva autora, tada se u zagradi piše samo prezime prvog autora uz dodatak «i sar.,» pa godina (Vidić i sar., 2004).

Ukoliko je rad iz programa nekog projekta na kraju rada navesti finansijera projekta i evidencioni broj.

Naslovna strana

Na prvoj stranici treba napisati sledeće:

- naziv članka, odnosno rada treba pisati velikim slovima bez podvlačenja i bez skraćenica
- imena autora pisati ispod naslova punim imenom i prezimenom, razdvojena samo zarezom.

Iznad prezimena se brojem označava ustanova u kojoj radi autor (autori):

- navesti punu adresu ustanova u kojima autori rade; navoditi onim redosledom koji odgovara redosledu autora u radu;
- na dnu stranice treba navesti ime e-mail jednog od autora, radi korespondencije.

Kratak sadržaj

Na posebnoj stranici uz rad treba priložiti i kratak sadržaj rada, obima 300 reči. Pored naslova i imena autora i ustanova, kratak sadržaj treba da sadrži najvažnije činjenice iz rada. Takođe, ispod kratkog sadržaja treba navesti 3-8 ključnih reči.

Pisanje teksta

Svi podnaslovi se pišu velikim boldiranim slovima. U radu koristiti kratke i jasne rečenice. Tekst treba da bude u duhu srpskog jezika, a sve strane izraze za koje postoje odgovarajuće reči u našem jeziku ne treba koristiti. Za nazive lekova koristiti isključivo njihova internacionalna nezaštićena imena (tj. generička imena) i pisati ih onako kako se izgovaraju (ne na latinskom ili engleskom jeziku). Ukoliko se, pak, želi ipak istaći ime nekog preparata, onda se njegovo ime (zajedno sa imenom proizvođača) stavlja u zagradu iza naziva aktivne supstancije. Uređaji ili aparati se takođe označavaju njihovim trgovačkim nazivima, s tim što se i ovde u zagradi mora navesti ime i mesto proizvođača. Za svaku skraćenicu, koja se prvi put javlja u tekstu treba navesti i pun naziv. Skraćenice nikako ne koristiti u naslovu, a u kratkom sadržaju ih takođe treba izbegavati. Decimalne brojeve pisati sa zarezom i bar još jednom nulom. Obim rukopisa bez priloga, ne treba da bude veći od 8 stranica kucanog teksta. Izbegavati veliki broj priloga.

Tabele se označavaju arapskim brojevima (iznad tabela) po redosledu navođenja u tekstu, sa nazivom na srpskom jeziku. Koristiti font Times New Roman, veličina slova 12 pt, sa jednostrukim proredom i bez uvlačenja. Ukoliko se u tabeli koriste skraćenice treba ih objasniti u legendi ispod tabele.

Grafikoni se takođe označavaju arapskim brojevima (ispod grafikona) po redosledu navođenja u tekstu, sa nazivom na srpskom jeziku. Koristiti font Times New Roman i veličinu slova 12 pt, sa jednostrukim proredom i bez uvlačenja. Ukoliko se koriste skraćenice, treba ih objasniti u legendi ispod grafikona.

Sheme (crteži) se označavaju arapskim brojevima (ispod shema) po redosledu navođenja u tekstu, sa nazivom na srpskom jeziku. Koristiti font Times New Roman i veličinu slova 10 pt, sa jednostrukim proredom i bez uvlačenja. Ukoliko se koriste skraćenice, treba ih objasniti u legendi ispod sheme.

Fotografije se označavaju arapskim brojevima (ispod fotografije) po redosledu navođenja u tekstu, sa nazivom na srpskom jeziku. Primaju se isključivo originalne fotografije (crno-bele ili u boji) na sjajnom (glatkom, a ne mat) papiru. Na poleđini svake fotografije treba napisati redni broj i strelicom označiti gornji deo slike. Za svaki primerak rukopisa dostaviti po jednu sliku.

Poglavlja rada

Poglavlja rada su: **Uvod, Materijal i metode rada, Rezultati, Diskusija (ili Rezultati i diskusija zajedno), Zaključak i Literatura.**

U uvodu treba ukazati na najvažnije, odnosno najnovije činjenice i poglede vezane za temu rada, sa kratkim obrazloženjem cilja sopstvenih ispitivanja.

Materijal i metode rada. U ovom poglavlju treba opisati uslove pod kojima su ogledi izvedeni, navesti pun naziv metoda koje su korišćene u ispitivanjima, materijal i životinje na kojima su izvedena ispitivanja.

Rezultati. Rezultate prikazati pregledno uz pomoć tabela ili grafikona. Svuda treba da stoji redni broj i tekst, koji opisuje šta određena slika, tabela, grafikon prikazuje. Redni broj sa tekstom se stavlja iznad tabela, a kod svih ostalih prezentacija ispod.

Diskusija. U ovom poglavlju se prikazuju uporedna analiza dobijenih rezultata sa rezultatima i mišljenjima drugih autora sa isticanjem značaja ispitivanja ali bez donošenja zaključaka.

Zaključak. U ovom poglavlju autor iznosi svoja zaključna razmatranja.

Literatura. U ovom poglavlju autor treba da iznese literaturne podatke, odnosno radove, koje je koristio u toku izrade svog rada. Poželjno je da korišćena literatura bude što novija. Reference treba pisati jednu ispod druge (numerisati ih arapskim brojevima) i abecednim redom prema prvom slovu prezimena prvog autora. Broj referenci nije u principu ograničen ali se preporučuje da ne bude veći od 15. Reference članaka koji su prihvaćeni za štampu treba označiti kao «u štampi» i priložiti dokaz o prihvatanju rada.

Primeri navođenja referenci:

1. **Članak u časopisu:**

Stojanović D., Maličević Ž., Ašanin R.: The use a new model for the investigation of sepsis. Acta Veterinaria, 52, 2/3, 125-131, 2002

2. **Knjige i druge monografije:**

Qinn P.: Clinical Veterinary Microbiology. London, Mosby, 1998

3. Poglavlje u knjizi:

Vidić B., Boboš S., Lako B., Lončarević A.: Dijagnostika bruceloze. U: Aleksandar Lončarević, Bruceloza svinja, Beograd: Poljoprivredni fakultet, 2000, str. 47-49.

4. **Članak u zborniku radova sa naučno-stručnog skupa:**

Valčić M., Lazić S., Rašić Z.: Mesto i uloga terenskog veterinara u epizootiološkom radu.

U: Dragiša R.Trailović, urednik, Zbornik radova, X regionalno savetovanje iz kliničke patologije i terapije životinja, 1-5. septembar, Kragujevac, Beograd: Fakultet veterinarske medicine, 2008, 75-82.

Napomena

Rad koji ne ispunjava sve gore navedene uslove neće biti poslat na recenziju i biće vraćen autorima da ga dopune i isprave.

Adresa časopisa

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NOTE FOR CONTRIBUTORS

ARHIVE OF VETERINARY MEDICINE is a journal of the Scientific Veterinary Institute "Novi Sad" in Novi Sad. The journal publishes original, expert and review papers, case reports, reports from symposia and other meetings, book reviews, cases from history of veterinary medicine.

All manuscripts are sent for a review and evaluation. In the case the reviewer suggests additional changes, the manuscript will be sent to the first author with a kind request to change the manuscript. In the case the author does not want to change, appropriate argumentation should be given. Final decision on accepting the manuscript is given by the editor in chief, together with editorial committee.

The journal is published in the Serbian language, followed by an abstract in English. The papers of foreign authors are published in English followed by an abstract in Serbian.

The manuscript should be written according to the following instructions:

General notes

The paper should be in Word program, Latin characters, size 12 pt, Times New Roman, double spaced. Left and right margins 20 mm, top and foot margins 30 mm, paper size A4. If special symbols are used, use font Symbol. The manuscript should be submitted on paper size A4, but also in electronic form. Pagination on the right side, starting from the title page. References and notes are cited in the text by authors' names, followed by the year of publication. If there are more than two authors, only the name of the first author is written followed by the abbreviation "i sar." (Vidić i sar., 2004).

If the paper is part of a project, name the financier and the project number at the end.

Title page

On the title page the following should be written:

- the title of the paper in capital letters, without underlining and abbreviations
- the names of the authors (first and second name, followed by a comma).

Above the second name place a number that denotes the institution where the author works:

- full name of the institutions should be given.

- at the bottom of the page write E-mail address of one author, for correspondence.

Summary

Every paper should be followed by a summary (300 words). Beside the title, name of the authors and institutions, it should contain the most important facts from the paper and three to eight key words.

Text

All the subtitles write in bold capital letters. Use short and concise sentences. Name the drugs as their International Nonproprietary Names (so called generic names). If the name of a specific drug is to be stressed, name it together with the producer (in brackets). The names of devices write as used in trade (name of the producer in brackets). When using an abbreviation for the first time, write the words that stand for. Abbreviations cannot be used in the title and summary. Text should not be longer than 8 pages. Avoid long enclosures.

Tables number with the Arabic numerals (above the table). Use Times New Roman, 12 pt, single space, without indentation. If abbreviations are used, give an explanation bellow the table.

Graphs number with the Arabic numerals (below the graph). Use Times New Roman, 12 pt, single space, without indentation. If abbreviations are used, give an explanation bellow the graph..

Scheme number with the Arabic numerals (bellow the scheme). Use Times New Roman, 10 pt, single space, without indentation. If abbreviations are used, give an explanation bellow the graph.

Photographs number with the Arabic numerals (bellow the photo). Only original photographs can be used (black and white). On the back side write ordinal number of the photo and mark the top of the photo.

Headings

Headings in the paper are**: Introduction, Material and Methods, Results, Discussion (or Results and Discussion), Conclusion and Literature.**

Introduction points on the most important, i.e. most recent data regarding the topic with a short presentation of the aims of this research.

Material and Methods. Here describe the conditions in the experiment, name the used methods, material and animals.

Results. The results are displayed through tables or graphs, numbered with ordinal numbers and with an explanation what the photo, table or graph shows.

Discussion. Here give analyses of the obtained results comparing to the results and opinions of other authors, pointing the importance of this research, without giving a conclusion.

Conclusion. Here the authors gives his final conclusions.

Literature. The author should list the references, preferably the most recent one. References should be numbered with Arabic numerals, one under the other, written in alphabetical order according to the surname of the first author. In general, the number of references is not limited, but it is advisable to write 15 references.

Examples of references:

1. **Articles in journals:**

Stojanović D., Maličević Ž., Ašanin R.: The use a new model for the investigation of sepsis. Acta Veterinaria, 52, 2/3, 125-131, 2002

2. **Books:**

Qinn P.: Clinical Veterinary Microbiology. London, Mosby, 1998

3. **Chapters in books:**

Vidić B., Boboš S., Lako B., Lončarević A.: Dijagnostika bruceloze. U: Aleksandar Lončarević, Bruceloza svinja, Beograd: Poljoprivredni fakultet, 2000, str.47-49

4. **Articles in proceedings:**

Valčić M., Lazić S., Rašić Z.: Mesto i uloga terenskog veterinara u epizootiološkom radu.

U: Dragiša R.Trailović, urednik, Zbornik radova, X regionalno savetovanje iz kliničke patologije i terapije životinja, 1-5. septembar, Kragujevac, Beograd: Fakultet veterinarske medicine, 2008, 75-82

Note

A paper that is not in accordance to the aforementioned instructions will not be sent for a review and will be returned to the authors for corrections.

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