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SEROEPIDEMIOLOGICAL INVESTIGATION OF *MYCOPLASMA BOVIS* IN CALVES

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Abstract

Within the multifactorial etiology of respiratory infections in cattle, Mycoplasmae play an important role. Most of the *Mycoplasma* microorganisms play a minor role in the development of the infections in cattle, contrary to *Mycoplasma bovis* (*M. bovis*) that is commonly the primary agent of the disease. Respiratory tract mucosa is the primary colonization site of *M. bovis* in cattle. Colonization of the upper respiratory tract of calves with *M.bovis* often occurs within the first few weeks of life. Animals with chronic infection and without clinical symptoms occasionally shed *M. bovis* and they are highly important for the epidemiology of the infection. Stress factors such as transportation, entrance into the feeding object, coldness, etc. are associated with the secretion of *M. bovis* from a nose. Diagnostic procedure relies on clinical symptoms and detection of causative agent, regardless of whether the infection is found in individual animals or in the entire herd. Serological detection of *M.bovis* antibodies is often a reliable diagnostic method. The most used indirect method is ELISA test. During a two-year period, blood serum samples from calves (beef cattle) were examined and analyzed. Calves originated from cattle farms (big and small) of Holstein- Friesian and Simmental breed. The total number of 3777 samples was examined applying ELISA (Biovet Inc. *Mycoplasma bovis* Antibody Test Kit Bovichek® M.bovis). Positive findings were obtained in 182

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animals, i.e. 4.81%. The obtained results confirmed the presence of *M.bovis* in cattle herds. Further research from epizootical aspect and the role of *M. bovis* in the occurrence of health problems in cattle population is necessary.

Key words: *Mycoplasma bovis,* calves, antibodies, ELISA

SEROEPIDEMIOLOŠKA ISPITIVANJA *MYCOPLASMA BOVIS* KOD TELADI

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Kratki sadržaj

U multifaktorijalnoj etiologiji respiratornih infekcija goveda, mikoplazme imaju značajno mesto. Većina vrsta mikoplazma imaju sekundarnu ulogu u nastajanju infekciji kod goveda, za razliku od *Mycoplasma bovis (M. bovis*) koja ima primarnu ulogu. Sluzokoža gornjih respiratornih puteva je primarno mesto za kolonizaciju *M. bovis* kod goveda. Naseljavanje M.bovis gornjeg respiratornog trakta kod telade nastaje već u prvim nedeljama života. Hronično inficirane jedinke bez kliničkih simptoma, koje povremeno izlučuju *M. bovis*, su značajna u epidemiologiji infekcije Stresni dogadjaji kao što su transport, ulazak u tovilište, hladnoća i drugo su povezani sa izlučivanjem *M. bovis* iz nosa. Na osnovu kliničke slike se postavlja sumnja na mikoplazmozu, a definitivna dijagnoza se postavlja dokazivanjem uzročnika, bilo da se radi o pojedinačnim grlima ili na nivou zapata. Dokazivanje infekcije *M.bovis* serološkim testovima se uspešno primenjuju. Danas se najčešće koristi indirektni metoda ELISA test. U toku dvogodišnjih ispitivanja pregledali smo krvne serume teladi koja su namanjena za tov. Telad su poticala sa farmi goveda (većih i manjih) i pripadali rasi Holštajn-Frizijskoj i Simentalskoj. U toku ispitivanja pregledali smo ukupno 3777 krvnih seruma teladi. Koristili smo metod ELISA (Biovet Inc. *Mycoplasma bovis* Antibody Test Kit Bovichek® M.bovis). Pozitivne rezultate utvrdili smo kod 182 životinja, što predstavlja 4,81%. Dobijeni rezultati ukazuju da je *M.bovis* prisutna u zapatima krava i da je potrebno vršiti dalja ispitivanja sa epizootiološkog aspekta i njene uloge u nastajanju zdravstvenih problema u populaciji krava.

Ključne reči: *Mycoplasma bovis,* telad , antitela, ELISA

INTRODUCTION

Mycoplasmas is recognized as the cause of some of the most severe and economically most costly diseases in cattle (Gonzalez et al, 1993; Gonzalez and Wilson 2003). More than 20 different *Mycoplasma* species have been isolated from cattle with different clinical symptoms of a disease (Henderson and Ball 1999). Most of the *Mycoplasma* microorganisms have a secondary role in cattle infection, contrary to *Mycoplasma bovis (M. bovis*), which has a primary role in the occurrence of the disease. *M.bovis* was first isolated back in 1961 in the US, as a disease with clinical symptoms of severe mastitis in cattle (Hale et al, 1962), and then during the next 40 years, it has spread to Europe, Asia and the rest of the world (Filioussis et al, 2007). So far, the presence of mycoplasmosis in cattle has been reported inmost European countries. The prevalence of *M.bovis* is underestimated and other bacteria are often isolated in calves with pneumonia or cattle with mastitis, where actually *M.bovis* is the primary cause. Only a few laboratories in the world routinely perform the monitoring of mycoplasmas. The occurrence of *M.bovis* in the herd is always associated with the cases of pneumonia, mastitis and arthritis (Pfutzner and Sachse, 1996). As compared to economic losses from respiratory diseases, the losses associated with mycoplasmosis in the cattle industry of US or UK are very high, tending to increase due to mycoplasmatic mastitis cases (Rosengarten and Citti, 1999).

M.bovis is widely spread among bovine population in enzootically infected areas. The infection is usually introduced into the new herds by clinically healthy calves or young cattle shedding the causative agents. Infected cattle shed mycoplasmas via the respiratory tract for many months or years representing the permanent reservoair of the infection. Respiratory tract mucosa is the primary site for the colonisation of *M. bovis* in cattle. Respiratory tract mucosa and mammary gland are the most important locations for the maintenance and secretion of *M. bovis*, which can persist even several months. Stress factors such as transportation, entrance into the feeding object, coldness, etc. are associated with the secretion of *M. bovis* from a nose. Animals with chronic infection and no clinical symptoms are occasionally shedding *M. bovis* and they are very important factor in the epidemiology of the infection. *M.bovis* has been proved a frequent causative agent of pneumonia, mastitis and arthritis in cattle (Nicholas et al, 2000). *M.bovis* can also be transmitted from an infected cow to a foetus or post partum to a newborn calf. The causative agent infects respiratory tract and stays there live and infectious until the pubescence or even the first calving (Bobos and Vidic, 2005)

Diagnostic procedure relies on clinical symptoms and detection of causative agent, regardless of whether the infection is found in individual animals or in the entire herd. ELISA is the mostly used indirect method. Serological detection of *M.bovis* antibodies is often a highly reliable diagnostic method. The level of antibodies detected by ELISA method persists for many months, especially in case of preceding month-long antibiotic therapy at herd level. In such cases, the isolation of the agent is very difficult. All other serological tests, such as possible indirect haemagglutination or film inhibition, are not as successful as indirect ELISA and thus not widely used. Commercial diagnostic tests are available in the market used worldwide.

MATERIALS AND METHOD

During a two-year period, blood serum samples from calves (beef cattle) were collected, examined and analyzed. The samples were taken from animals originating and living in different regions in the territory of Serbia. The calves of Holstein- Friesian and Simmental breed originated from several cattle farms (big and small. The total number of 3777 samples was collected and examined.

The diagnostic was performed using ELISA method. The diagnostic kit used in this research was a commercial Biovet Inc. *Mycoplasma bovis* Antibody Test Kit Bovichek® M.bovis, which is used in a routine laboratory work. The blood sera were analyzed using indirect ELISA test according to manufacturer's instructions. ELISA (Enzyme – linked immunosorbent assay) is a sensitive and specific method for detection of specific antibodies against certain infectious agent from blood sera. Antibodies from the serum bind with the antigen contained in a layer coating the wells of the test and an antigen-antibody complex is formed. Subsequently, the complex is stained to enable better visualisation.

RESULTS AND DISCUSSION

In total, 3777 blood serum samples of calves from different farms were analyzed for the presence of specific antibodies against *Mycoplasma bovis*. The analysis was performed using indirect ELISA method, a commercial kit. Positive findings were detected in 182 animals, i.e. 4.81% of the total population examined.

The finding of specific antibodies against *Mycoplasma bovis* is presented in Table 1.

Table 1. Findings of antibody against M.bovis in blood sera of calves

Farm	No. of examined calves	Positive	%
1	576	11	1,90%
2	234	18	7,69%
3	482	33	6,84%
4	675	27	4,00%
5	785	49	6,24%
6	311	8	2,57%
7	714	36	5,04%
Total	3777	182	4,81%

Similar results were obtained in another study performed in Serbia that included different regions and different diagnostic laboratory. In this research, 2.74% of calves proved positive to specific antibodies against *Mycoplasma bovis* in an indirect ELISA method (Vojinovic et al, 2012).

Mycoplasmae play an important role in the multifactorial etiology of respiratory infections in cattle. Several *Mycoplasma spp.* can cause severe mastitis in cattle, but *M.bovis* is the the predominant one. The disease spreads rapidly, that is, many cows manifest clinical signs of mastitis in one or more udder quarters in a very short period. In lactating cows, the infection mostly affects the entire udder. On farms with history of cattle mycoplasmosis, problems with joints, reproductive failures, pneumonia in calves and respiratory problems in adult cattle were recorded (Stokka et al, 2001). Dairy cows with mycoplasmatic mastitis show a drastic drop of milk production. Considering the infectious nature of the disease, clinical symptoms spread within the herd very fast, thus appropriate control measures have to be implemented (Vidic et al, 2012).

Unlike the majority of bacterial infections, the therapy of *Mycoplasma* infections is highly demanding, which is due to organism's resistance to mostly used antibiotics. Vaccination is a potential strategy to control *M.bovis* infection; however, the efforts to develop effective vaccine for use in young calves have been problematic so far. An effective program for the control of *M. bovis* infections includes a number of factors such as early detection of carriers and their removal from the heard, appropriate vaccination schedule, breeding conditions providing minimum environmental stress, housing with good air circulation, "all in all out" management practice to prevent infection transmission from older animals to younger ones or at least separating the calves from

adult animals as early as possible in case of the occurrence of endemic disease exists, etc (Nicholas and Ayling, 2003).

CONCLUSION

Despite the great number of clinical cases and significant economical losses, *M.bovis* is still considered an unimportant pathogen among veterinarians. Mycoplasmosis in cattle may have severe socio-economic impact from the aspect of export and international trade.

The obtained results demonstrated wide distribution of *M.bovis* in cattle herds, thus further research of epizootical features and role of *M. bovis* in the occurrence of health problems in cattle population is necessary. The research of *M.bovis* should be extended to a wider cattle population of different age, with a particular focus on older animals. Considering the possible economical losses, more comprehensive research should be taken into consideration.

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REFRENCES

1. Boboš S, Vidić B.: Mlečna žlezda preživara, morfologija-patologija-terapija, Novi Sad: Naučni institut za veterinarstvo, 2005
2. Filioussis G, Christodoulopoulos G., A. Thatcher , V. Petridou , E. Bourtzi-Chatzopoulou: Isolation of Mycoplasma bovis from bovine clinical mastitis cases in Northern Greece. *The Veterinary Journal* 173 , 215–218, 2007.
3. Gonzalez, R.N., B.M. Jayarao, S.P. Oliver SP, P.M. Sears: Pneumonia, arthritis and mastitis in dairy cows due to *Mycoplasma bovis*. In: Proc. 32nd Annual Meeting of the National Mastitis Council, pp 178-186, 1993.
4. Gonzalez, R.N., Wilson, D.J.: Mycoplasmal mastitis in dairy herds. *The Veterinary clinics of North America. Food animal practice* 19, 199–221, 2003.
5. Henderson J P , Ball H.J.: Polyarthritis due to *Mycoplasma bovis* infection in adult dairy cattle in Northern Ireland. *Vet Record* 145,13, 374-376, 1999.
6. Hale H.H., Helmboldt C.F., Plastridge W.N., Stula E.F.: Bovine mastitis caused by *Mycoplasma* species, *Cornell Veterinarien* 52, 582-591, 1962
7. Nicholas R., Baker R., Rayling R. et al: Mycoplasma infections in growing cattle. *Cattle Practice* 8, 2, 115-118, 2000.

8. Nicholas R.A.J., Ayling R.D.: *Mycoplasma bovis:*disease, diagnosis, and control. *Research in Veterinary Science* 74, 105/112, 2003
9. Pfutzner H., Sachse K.: *Mycoplasma bovis* as an agent of mastitis, pneumonia, artritis and genital disorders. *Scientific and Technical Review, Offices International Des Epizootes* 5, 1477-1494. 1996
10. Rosengarten R., Citti C.: The role of ruminant mycoplasmas in systemic infection. In: Stipkovits L, Rosengarten R, Frey J (Eds), Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics vol3, European Commission, Brussels 14-17, 1999
11. Stokka G.L., Lechtenberg K., Edwards T. et al: Lameness in Feedlot Cattle. *Vet Clin North Amer-Food Animal Practice* 17, 1, 196-202, 2001.
12. Vidić B., Boboš S., Savić S., Grgić Ž.: Mycoplasma as the cause of infections in cattle, *Arhiv veterinarske medicine,* 5, 2, 11-18, 2012
13. Vojinović D., Žutić J., Jovičić D., Đuričić B., Ilić Ž,. Samokovlija A., Elezović M.: Utvrđivanje prisustva antitela *Mycoplasma bovis*u krvnim serumima teladi u karantinu, tokom 2011. godine metodom indirektne ELISA. In: 2nd International Epizootical Days and XIV Serbian Epizootiology days, Belgrade, 142, 2012

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SEROPREVALENCE OF CANINE MONOCYTIC EHRLICHIOSIS IN HUNTING DOGS IN THE AUTONOMOUS PROVINCE OF VOJVODINA, SERBIA

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Abstract

Canine monocytic ehrlichiosis is a bacterial, vector-transmitted infection caused by *Ehrlichia canis*. The pathogen is mainly transmitted by ticks *Rhipicephalus sanguineus*. The disease highest distributionis most widely distributed in tropical and subtropical countries but it is also reported in Mediterranean countries of Europe (Spain, France, Italy, Turkey). Temperate continental climate and presence of these tick species are responsible for the maintenance and spreading of canine monocytic ehrlichiosis within the dog population in our region as well. Since hunting dogs are more exposed to tick bites then pet dogs, ed thisour study was conducted with the aims of determining the seroprevalence and basic epidemiological characteristics of monocytic ehrlichiosis infection in the population of hunting dogs, and comparing the obtained results with the results of other authors. This research involved 58 hunting dogs from the region of Autonomous Province of Vojvodina. All dogs were clinically examined and their basic epidemiological characteristics were recorded. Then, blood samples were collected in order to determine the presence of specific G class antibodies against *Ehrlichia canis* antigens. An indirect immunofluorescence test manufactured by VMRD, U.S.A., was used. In this study, the seroprevalence of monocytic ehrlichiosis in a population of examined hunting dogs from the region of Vojvodina was 13.79%. This rate is similar to the seroprevalence of monocytic ehrlichiosis in the general population of dogs in Vojvodina.

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Keywords: canine monocytic ehrlichiosis, *Ehrlichia canis*, hunting dogs, ticks, indirect immunofluorescence test, seroprevalence

SEROPREVALENCIJA MONOCITNE ERLIHIOZE KOD LOVAČKIH PASA U VOJVODINI

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Kratak sadržaj

Monocitna erlihioza pasa je bakterijska, vektorski prenosiva infekcija čiji je uzročnik *Ehrlichia canis*. Uzročnika oboljenja dominantno prenose krpelji *Rhipicephalus sanguineus*. Oboljenje ima najveću distribuciju u tropskim i suptropskim regionima, a registrovano je i u mediteranskim zemljama Evrope (Španija, Francuska, Italija, Turska). Umereno kontinentalna klima i prisustvo navedenih vrsta krpelja pogoduje održavanju i širenju monocitne erlihioze u populaciji pasa i u našem regionu. Obzirom da su lovački psi često izloženi ubodu krpelja, u poredjenju sa psima ljubimcima, cilj ovog istraživanja je da pruži uvid u osnovne epidemiološke karakteristike i seroprevalencu monocitne erlihioze lovačkih pasa, kao i da uporedi dobijene rezultate sa podacima o seroprevalenci monocitne erlihioze u opštoj populaciji pasa. U ovom istraživanju je pregledano 58 lovačkih pasa sa područja Vojvodine. Pre uzorkovanja pune venske krvi u cilju izdvajanja krvnih seruma, svi psi su klinički pregledani uz registrovanje osnovnih epidemioloških karakteristika. Za detekciju prisustva specifičnih antitela klase G na antigen *E. canis* korišćen je test indirektne imunofluorescencije proizvođača VMRD, USA. U ovom istraživanju utvrđeno je da seroprevalencija monocitne erlihioze u populaciji ispitivanih lovačkih pasa sa područja Vojvodine iznosi 13,79%, kao i da je ona slična sa prevalencijom ove infekcije kod opšte populacije pasa na području Vojvodine.

Ključne reči: monocitna erlihioza pasa, *Ehrlichia canis*, lovački psi, krpelji, test indirektne imunofluorescencije, seroprevalencija

INTRODUCTION

Monocytic ehrlichiosis of dogs is a bacterial, vector borne infection caused by *Ehrlichia canis*. Species of the genus *Ehrlichia* are obligatory intracytoplazmatic bacteria of the monocyte-macrophage system. Intracellular development of the bacteria is enabled by the reaction of numerous immunoreactive proteins, which to haveplay a key role in the infection, such as adhesives, regulators of the intake of nutrients and inhibitors of proinflammatory cytokines. The causative agent *E. canis* was first detected in 1935 in Algeria. Afterwards, in 1996, the causative agent of human monocytic ehrlichiosis, *E. chaffensis,* was found in dogs, broughtwhich gave more significance to this infection from the aspect of public health. The tick *Rhipicephalus sanguineus*, is the dominant vector for *Ehrlichia canis Rhipicephalus sanguineus*, but there is evidence on the transmission via *Dermacentor variabilis* (Johnson et al, 1998), *Dermacentor marginatus* (Satta et al, 2010), and *Ixodes* (Wielinga et al, 2006). The disease is most widely distributed in tropical and subtropical regions, but it has also been detected in Mediterranean countries of Europe (Spain, France, Italy, Turkey). Some parts of Spain and Italy are considered endemic regions for ehrlichiosis (Trotz-Williams et al, 2003). Temperate continental climate and presence of aforementioned tick species are predisposing factors for the maintenance and spread of monocytic ehrlichiosis in the population of dogs in our region. During the last few years, the disease has been detected in the neighboring countries including Hungary (Farkas et al, 2014), Romania (Mircean et al, 2012) and Bulgaria (Tsachev et al, 2006). Our previous seroepidemiological study of monocytic ehrlichiosis in the general population of dogs in the region of Vojvodina revealed the seroprevalence rates of 25%was found, and 16% obtained by iELISAand 16% was found in the same population of dogs, by and IIF tests, respectively. (Potkonjak et al, 2013).

The aims of this study was obtaining of basic epidemiological characteristics of monocytic ehrlichiosis, establishing of seroprevalence rate of this infection in hunting dogs and comparing the obtained data with the data from the literature on seroprevalence of monocytic ehrlichiosis in general population of dogs.

MATERIAL AND METHODS

The research encompassed 58 hunting dogs from the region of Vojvodina. All dogs were clinically examined and all relevant epidemiological data were recorded. Blood samples were taken for blood sera to be isolated. Samples of

full blood were taken by aseptic puncture of *v. cephalica antebrachii*. Blood sera were separated after centrifugation at 3000 rpm/min during 10 minutes. Detection of specific antibodies of G class against antigen of *E. canis was* performed using an indirect immunofluorescence test manufactured by VMRD, USA. Evident presence of the fluorescence of cytoplasmic bodies (morulas) was considered positive finding. The absence of fluorescence was considered negative finding. Serum titer values for G class immunoglobulins above 1:50 were considered positive finding.

RESULTS

The examination of 58 hunting dogs' blood sera using the indirect immunofluorescence test 58 blood sera samples from hunting dogs,revealed presence of antibodies of G class against *E. canis* antigen in 8 samples*E. canis* antigene was found, while the remaining 50 samples were seronegative to *E. canis*. The seroprevalence of monocytic ehrlichiosis in the population of examined hunting dogs from the region of Vojvodina was 13.79%. The positive finding of IF test for *E. canis* antigen is shown in Figure 1. The age of seropositive dogs was between 1.5 and 6 years, and of the investigated population included the following breeds: Posavic hound, English setter, German hunting terrier, German shorthaired pointer, German wirehaired pointer and brac dachshund. Antibodies of G class against *E. canis* antigen were found in three females and five males. For the prevention of ectoparasites, the following ectoparasiticides were used in positive dogs: Neostomosan sampoules, Frontline and Ectanon, Taktik and Baygon pulver. The basic epidemiological characteristics found in seropositive dogs to *E. canis* antigens are shown in Table 1.

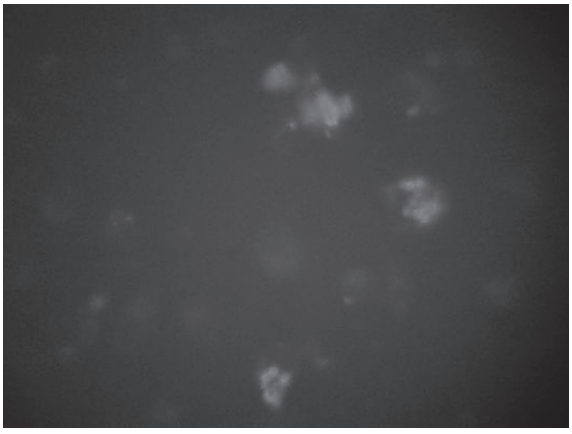

Figure 1. Positive finding of IIF test to antigen of *E. canis*

Table 1. Basic epidemiological characteristics found in dogs seropositive to *E. canis*

No of sample	Breed	Gender	Age	Ectoparasiticides used
4.	Posavic hound	Male	5 years	Neostomosan sampoules
21.	English setter	Female	1,5 years	Frontline and Ectanon
29.	German hunting terrier	Female	2,5 years	Ectanon and Taktik
30.	German shorthaired pointer	Male	2 years	Ectanon and Taktik
32.	German wirehaired pointer	Male	2,5 years	Frontline
47.	Brak dachshund	Female	6 years	Baygon pulver
53.	German hunting terrier	Male	4 years	Ectanon
54.	German hunting terrier	Male	3 years	Ectanon

DISCUSSION

Because of the climate changes and the geographical spread of the most important vector, *Rhipicephalus sanguineus* tick, proportional increase in prevalence of monocytic ehrlichiosis in dogs is found. Besides the tropical and subtropical regions, this tick species can also be found in the regions with temperate continental climate, including the region of our country (Christova et al, 2003; Parola et al, 2001).

Hornok et al performed molecular research on the presence of *E. canis* in ticks from the south region of Hungary and indicated the significance of wild animals as reservoirs of this infection. During the summer 2011, 348 ticks were collected from red foxes (*Vulpus vulpus),* golden jackals (*Canis aureus*) and German shepherd. The researchers established that *Dermacentor marginatus* and *Dermacentor reticulatus* prefer dogs as hosts, which was not the case before. *Dermacentor reticulatus* and *Ixodes canisuga* were most commonly found species in red foxes and golden jackals. In our research, in the nymphs of

D. marginatus and larvae of *I. canisuga* the presence of *E. canis* was established in the nymphs of *D. marginatus* and larvae of *I. canisuga*, which indicates potential significance of foxes as a reservoir of infection in the wilderness (Hornok et al, 2013). An experimental transmission of *E. canis* from grey foxes (*Urocyon cinereoargenteus*) to Beale dogs, with *Rhipicephalus sanguineus* as vector (Amyx et al, 1973)confirmed that foxes could be a potential reservoir in the wilderness. A research performed in Italy in 1995 revealed a seroprevalence rate of 72% in 154 examined stray dogs from the territory of Imola city in Emilia – Romagna region (Baldelli et al, 1995). During the following decade in Northern Italy, the researchers have found seroprevalence rates of 2.9% 8% in central region and 9.7% in southern Italy (Solano-Gallego et al, 2006). Seroepidemiological study of *E. canis* infection in 120 dogs with owners in northern Bulgaria in 2006, showed a total prevalence of 37.5% whilst the highest prevalence of 60% was established in a coastal city of Varna. In this research, IF test was used, also (Tsachev et al, 2006). In Former Yugoslav Republic of Macedonia in 2011, sporadic *E. canis* infection was found in a female of Samoyed breed, 11 years old, using ELISA test. In the same dog, *Leishmania infantum* was identified by IF test. In this case, the authors state that the co-infection has contributed to the deterioration of clinical findings (Atanaskova et al, 2012). Ina study done in 2012 in Romania, 1146 blood serum samples of dogs were analyzed and an *E. canis* seroprevalence of 2.1% was established (Mircean et al, 2012).

In previous seroepidemiological research on monocytic ehrlichiosis in general population of dogs in the region of Vojvodina, seroprevalence rates of 25% and 16% were established by iELISA and IF test, respectively (Potkonjak et al, 2013). In this research, the established seroprevalence of monocytic ehrlichiosis in the population of hunting dogs in the region of Vojvodina was 13.79%. This seroprevalence rate for *E. canis* is in accordance with the data reported by other authors. In spite of general opinion that hunting dogs are more exposed to tick bites, the results of this research demonstrated that the seroprevalence of monocytic ehrlichiosis in hunting dogs was similar to that in the general population of dogs in the same region of Vojvodina. To the purpose of a more efficient monitoring of monocytic ehrlichiosis in dogs in the region of Vojvodina, further and more detailed acarological and epidemiological research is needed in both dog population and the population of wild animals. In the context of the significance of wild animals as reservoirs of infection, we consider the investigation of wild animals and hunting dogs in hunting regions highly important.

CONCLUSION

The results obtained in this study demonstrated that seroprevalence of monocytic ehrlichiosis in the population of examined hunting dogs in the region of Vojvodina reached 13.79%. The significance of these results is reflected by the fact that the prevalence is very similar to prevalence of this infectionthat among the general population of dogs in the region of Vojvodina (Potkonjak et al, 2013).

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REFERENCES

1. Amyx H., Huxsoll D.: Red and gray foxes-potential reservoir hosts for Ehrlichia canis, *Jurnal of Wildlife Diseases*, 9, 1:47-50, 1973.
2. Atanaskova E., Kocevski Z., Nikolovski G., Stefanovska J.: Case report of canine co-infection with Leishmania infantum and Ehrlichia canis, *Mac. Vet. Rev*., 34, 1: 19 - 24, 2011.
3. Baldelli R., Di Francesco A., Fioravanti L., Borello B.: Ehrlichiosis canina. Indagine sieroepidemiologica in un canile dell'Emilia-Romanga, *Obiettivi e Documenti Veterinari*, 16, 61-63, 1995.
4. Christova I., Van De Pol J., Fioravanti L., Schouls L.: Identification of Borrelia burgdoferi sensu lato, Anaplasma and Ehrlichia species, and spotted fever group Rickettsiae in ticks from Southeastern Europe, *Europen Jurnal of Clinical Microbiology and Infectious Disease*, 22, 9, 535-542, 2003.
5. Farkas R., Gyurkovszky M., Lukács Z., Aladics B., Solzmosi N.: Seroprevalence of some vector-borne infections of dogs in Hungary, *Vector Borne Zoonotic Diseases*, 14, 4, 256-60, 2014.
6. Hornok S,. Fuente J., Horváth G., Fernández de Mera I., Wijnveld M., Farkas R., Jongejan F.: Molecular evidence of Ehrlichia canis and Rickettsia

massiliae in Ixodid ticks of carnivores from south Hungary, *Acta Veterinaria Hungarica*, 61, 1, 42-50, 2013.
7. Johnson E., Ewing S., Barker R., Fox J., Crow D., Kocan K.: Experimental transmission of Ehrlichia canis (Rickettsiales: Ehrlichieae) by Dermacentor variabilis (Acari: Ixodidae), *Vet Parasitol*, 74, 2-4, 277-88, 1998.
8. Mircean V., Dumitrache M., Györke A., Pantchev N., Jodies R., Mihalca A., Cozma V.: Seroprevalence and Geographic Distribution of Dirofilaria immitis and Tick-Borne Infections (Anaplasma phagocytophilum, Borrelia burgdorferi sensu lato, and Ehrlichia canis) in Dogs from Romania, *Vector-Borne and Zoonotic Diseases*, 12, 7, 595-604, 2012.
9. Parola P., Raoult D.: Ticks and tickborne bacterial diseases in humans: an emerging infectious threat, *Clinical Infectious Diseases*, 32, 6, 897-928, 2001.
10. Potkonjak A., Savić S., Jurišić A., Petrović A., Suvajdžić Lj., Lako B., Milošević N., Novaković Z.: Seroepidemiological research of canine monocytic ehrlichiosis in the Autonomous Province of Vojvodina, Serbia, *Acta Scientiae Veterinariae*, 41, 1106, 2013.
11. Satta G., Chisu V., Cabras P., Foisand F., Masala G. : Pathogens and symbionts in ticks: a survey on tick species distribution and presence of tick-transmitted micro-organisms in Sardinia, Italy, *Journal of Medical Microbiology*, 60, 1, 63-8, 2010.
12. Solano-Gallego L., Trotta M., Razia L., Furlanello T., Caldin M.: Molecular survey of Ehrlichia canis and Anaplasma phagocytophilum from blood of dogs in Italy, *Annals of the New York Academy of Sciences*, 1078, 515-518, 2006.
13. Trotz-Williams L.A., Trees A.J.: Systematic review of the distribution of the major vector-borne parasitic infections in dogs and cats in Europe, *Veterinary Record*, 152, 4, 97-105, 2003.
14. Tsachev I., Papadogiannakis I., Kontos V., Zarkov I., Petrov V., Pelagic V.: Seroprevalence of Ehrlichia canis infection among privately-owned dogs in northern Bulgaria, *Hellenic Veterinary Medical Society*, 57, 3, 206-216, 2006.
15. Wielinga P., Gaasenbeek C., Fonville M., de Boer A., de Vries A., Dimmers W., Akkerhuis Op Jagers G., Schouls L., Borgsteede F., van der Giessen J.: Longitudinal analysis of tick densities and Borrelia, Anaplasma, and Ehrlichia infections of Ixodes ricinus ticks in different habitat areas in The Netherlands, *Applied and Environmental Microbiology*, 72, 12, 7594-601, 2006.

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SEROPREVALENCE OF SOME INFECTIOUS DISEASES IN STRAY DOGS IN THE WIDER TERRITORY OF LESKOVAC CITY

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Abstract

Since the last decade, stray dogs have been significant ecological, economical and sociological problem in the world as well as in the Republic of Serbia. They occupy specific place in the ecology of big cities and their contact with human population represents danger since they are carriers of many zoonotic infectious diseases. The goal of this paper is to present epizootiological survey on some diseases of bacterial, viral and parasitic origin through serological investigation of blood sera and full blood. The prevalence of leptospirosis, Tularaemia, West Nile fever and dirofilariosis has been determined in this research. The material included full blood and blood sera of stray dogs in the wider territory of Leskovac city. The research methods encompassed standard serological tests: microscopic agglutination test *(MAT)* for determination of specific antibodies against *Leptospira spp*.; slow and fast agglutination test for determination of antibodies against Tularaemia causative agent (*Francisella tularensis*); agar gel immunodiffusion *(AGID)* for detection of specific antibodies against *West Nile virus –WNV*; modified Knot's test for the detection of microfilaria. The results revealed seropositivity for causative agents of Leptospirosis, Tularaemia

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and dirofilariosis, while there were no positive findings for WNV.

Key words: stray dogs, Leskovac, zoonoses

SEROPREVALENCE NEKIH ZARAZNIH BOLESTI U PASA LUTALICA U ŠIREM PODRUČJU LESKOVCA

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Kratki sadržaj

Tokom poslednjih deset godina, psi lutalice se smatraju značajnim ekološkim, ekonimskim i socijalnim problemom kako u celom svetu tako i u Republici Srbiji. Psi lutalice zauzimaju posebno mesto u ekologiji velikih gradova i njihov kontakt sa ljudima, predstavlja opasnost, jer oni mogu biti nosioci uzročnika mnogih zoonotskih infektivnih oboljenja. Cilj ovog rada je da se predstavi epizootiološka studija nekih bakterijskih, virusnih i parazitskih oboljenja, pomoću seroloških ispitivanja krvnih seruma i pune krvi pasa. Tokom ovog istrživanja, ispitivana je prevalenca na leptospirozu, tularemiju, groznicu zapadnog Nila i dirofilariozu. Materijal su predstavljali uzorci pune krvi i krvnog seruma pasa lutalica, iz šire okoline grada Leskovca. Metode ispitivanja su obuhvatale standardne serološke testove: test mikroskopske aglutinacije *(MAT)* za utvrđivanje specifičnih antitela protiv *Leptospira spp*.; spora i brza aglutinacija za utvrđivanje antitela protiv uzročnika tularaemije (*Francisella tularensis*); agar gel imunodifuzioni test *(AGID)* za detekciju specifičnih antitela protiv virusa *Zapadnog Nila –WNV*; modifikovani Knotov test za detekciju mikrofilarija. Rezultati ispitivanja pokazuju seropozitivnost na uzročnike leptospiroze, tularemije i dirofilarioze, dok nisu dobijeni pozitivni nalazi za WNV.

Ključne reči: psi litalice, Leskovac, zoonoze

INTRODUCTION

In past decades, the problem of stray dogs is widespread in the territory of the Republic of Serbia. The number of stray dogs in cities depends on the size of urban habitat and the number of pet owners, who produce major number

of future stray dogs. Considering the fact that stray dogs occupy important place in the ecology of cities because they are reservoirs and vectors of many infectious diseases and that they are commonly in close contact with humans, it is of a great importance to introduce and practice control measures with the aim of disease development prevention (Beck, 1975).

Leptospirosis is one of the most common infectious, zoonotic diseases of many animal species and humans caused by different serovars of spirochetes *Leptospira spp*. In nature, the organism is maintained in the circle between susceptible species, reservoirs and environment. The disease can be found in acute, subacute and chronic form. Chronic infections are difficult for clinical diagnostics. They are characterized by chronic hepatitis, renal failure and abortions, along with the presence of *Leptospira spp.* in kidneys and genital organs of animals (Genevieve, 2006). Many different sources of infection, numerous carriers, chronically infected animals and many serovars that cause the disease have significant role in spreading, diagnostics and suppression of Leptospirosis (Dmitrović et al., 2002; Hrnjaković Cvjetković et al., 2011).

Stray dogs are important reservoirs and transmitters of Leptospirosis to human population as well as to other animal species (Vojinović et al., 2012; Vojinović et al., 2013). The presence of Leptospira spp. in the population of stray dogs can be approached with several aspects: health care of humans, health care and welfare of animals as well as from an economical and political point of view.

Tularaemia is a zoonotic infectious disease caused by Gram negative, pleomorphic, immobile, non-sporulating bacteria *Francisella tularensis*. In nature, Tularaemia affects lagomorph species – rabbits, but the disease is confirmed in more than 100 different domestic and wild animal species, birds, amphibians and reptiles (Ellis et al., 2002). The causative agent is relatively resistant in nature and can survive for a long time in water. Natural infections of dogs are rare. The dogs can become infected during hunting, by eating infected rodents, through bites or scratches created by contaminated teeth or claws, or with infected ticks (Feldman K.A., 2003). In majority of cases, the disease in dogs is asymptomatic; however, in rare cases there short term anorexia, mild fever, depression, swelling of mandibular lymphatic nodes, myalgia, uveitis and conjunctivitis may occur (Green E.C., 2012; Spasojević Kosić et al., 2013). The literature data describe cases of dogs' death soon after contact with infected animals (William et al., 1979).

West Nile virus-*WNV* belongs to the genus *Flavivirus,* fam. *Flaviviridae*. It is a member of Japanese encephalitis serogroup. The virus is maintained in nature thanks to its circulation between vectors - mosquitoes (*Culex spp*. and *Ae-*

des spp.) and hosts - birds, horses, humans and other vertebrates (Campbell et al., 2002). West Nile fever can affect large number of bird species and mammals. In horses and humans, it causes significant clinical symptoms ranging from flu-like, mild respiratory syndrome to severe neurological symptoms and death. One of the first serological proofs of WNV circulation in the Republic of Serbia was published in 1972 when the seroprevalence was established in some regions in human population (Bordjoški et al., 1972). Currently, West Nile fever is spread worldwide. Vectors and migrating birds as reservoirs play an important role in further spreading. Epizootiological research on WNV seroprevalence in different animal populations in Serbia has started in 2008. The presence of specific antibodies was determined in horses, poultry, humans and for the first time in Serbia, in dogs as well (Samokovlija et al., 2012). Total of 1076 dogs were tested in period from 2008 to 2012 applying agar gel immunodiffusion method (*AGID*) and in 10 dogs (0.93%) the precipitation antibodies on WNV were found (Đuričić et al., 2013; Manić et al., 2013).

Dirofilariasis is a parasitic disease that has zoonotic character and is caused by *Dirofilaria immitis* and *Dirofilaria repens*. Mosquitoes from the genus *Aedes, Culex* and *Anopheles* are necessary vectors for spreading of this disease since one part of dirofilaria's living cycle takes place in them. The development form of parasite - microfilaria lives in the bloodstream of the host. Most common places for finding adult forms are heart cavity for *D. immitis*, or subcutaneous tissue for *D. repens*. Dirofilariosis was primarily disease characteristic for the Mediterranean basin, but, due to the climate changes, it spread out to the north, thus nowadays it can be found in middle Europe (Genchi et al., 2009). The first data suggesting the presence of dirofilariasis in Serbia (ex-Yugoslavia) were published in the 1990s (Dimitrijević S., 1999). Afterwards, the presence, seroprevalence, diagnostics, therapy and case occurrence were closely monitored in both animals and humans (Genchi et al., 2007; Rinaldi et al., 2011; Tasić et al., 2007; Savić et al., 2012; Pajković et al., 2010; Marić et al., 2013).

MATERIAL AND METHOD

The study included a total of 50 blood sera samples and 47 samples of full blood. All blood samples were taken from stray dogs that originated from the wider area of Leskovac city.

Blood sera were tested for the presence of antibodies against eight serovars of *Leptospira spp*.: *Icterohaemorrhagiae, Canicola, Pomona, Australis, Grippotyphosa, Bataviae, Sejroe* and *Bratislava*. To determine the presence of specific antibodies to the agents of leptospirosis, we used microscopic aggluti-

nation test (MAT), which is considered the gold standard.

The presence of specific antibodies for *F. tularensis* was determined using fast agglutination method on microscopic slide and slow agglutination method in tubes. Commercial antigen was used (*Francisella tularensis antigen, Bioveta, Czech Republic)* and method was performed according to manufacturer's instructions.

WNV specific antibodies were determined using agar gel immunodiffusion method (*AGID*) and antigen was prepared as described in the work of Đuričić et al. (Đuričić et al., 2013).

Total of 47 samples of full blood were tested for Dirofilariasis using modified Knot's test. This is fast and reliable method for detection of microfilaria in bloodstream.

RESULTS AND DISCUSSION

The results are shown on chart 1.

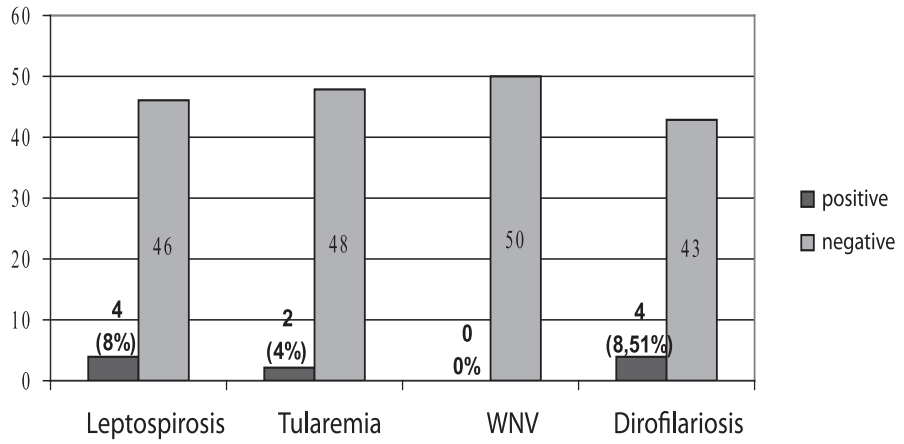

Chart 1. Seroprevalence of four infectious zoonotic diseases in stray dogs in wider territory of Leskovac city

From the total of 50 tested samples of blood sera, 4 (8%) were tested positive for the presence of specific antibodies for *Leptospira spp*. All samples were positive on *L. Canicola*- one sample presenting borderline titre 1:100, and three samples were positive in titre 1:300. *Leptospira canicola* is one of the most commonly diagnosed causative agents of Leptospirosis in dogs (Vojinović et al., 2013; Manić et al., 2013). Stray dogs are very significant as reservoirs and they actively, by constant bacterial shedding in case of chronic infection, con-

tribute to spreading and transmission of infection in human population and domestic animals.

By the use of fast agglutination method in the diagnostics of specific antibodies against *Francisella tularensis,* 2 positive dogs (4%) were found. Positive samples were examined by slow agglutination method and they did not present any positivity, meaning the low titre of antibodies. Tularaemia occurs sporadically in human population in the Republic of Serbia. During the period of ten years (2000-2011), 317 human cases were registered and only one case of disease in sheep in Mladenovac area (Marić et al., 2012). Beside findings in sheep, epizootiological data for Serbia are very scarce.

There were no positive results for the presence of specific antibodies against WNV by AGID method. Dogs rarely show symptoms of WNV infection, but they show seroconversion (Samokovlija et al., 2012; Đuričić et al., 2013).

rom the total of 47 full blood samples, using modified Knot's test, 4 stray dogs (8.51%) with microfilaria were found (figure 1). This disease has been becoming more common in dogs in Serbia in recent years (Savić et al., 2012; Pajković et al., 2010; Marić et al., 2013), impelling veterinarians and owners to perform checking for microfilaria during routine control of patients.

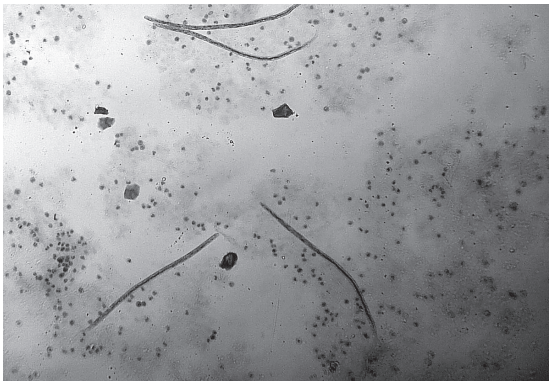

Figure 1. Modified Knot's test – microfilaria in full blood sample (Department of infectious diseases of animals and bee diseases, Faculty of Veterinary Medicine, University of Belgrade)

CONCLUSION

Considering the fact that dog shelters are one way of solving stray dog problem, the results presented in this paper are important indicator of the presence of infectious zoonotic diseases, which are underestimated in most instances. Leptospirosis is a global problem in veterinary medicine and human health care, and its presence in stray dog population poses danger for other

animals and humans (Adler and de la Pena, 2010). Dogs are considered relatively resistant animal species to causative agent of Tularaemia. Still, the presence of seropositive dogs can be good indicator that the causative agent is present in nature in the cycle between tick as vectors, rabbits and other wild animals (Green, 2012). WNV is present in the Republic of Serbia and it circulates in nature, which was identified in epidemics in 2012 and 2013 (Popović et al., 2013), but not in dog population. The occurrence and spreading of Dirofilariosis in the Republic of Serbia is serious problem, which has to be approached from all aspects. It is necessary to establish cooperation between physicians and veterinarians, as well as between sanitary and epidemiological service. The raise of awareness is of a great importance.

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REFERENCES

1. Adler B., de la Pena A.M,: Leptospira and leptospirosis. *Veterinary Microbiology*, 140, 287–296, 2010.
2. Beck A.M.: The Public Health Implications of Urban Dogs, *American Journal of Public Health*, 65, 12, 1315-1318,.
3. Bordjoški M., Gligić, A., Bošković, R.: Arbovirusne infekcije u RS Srbiji. *Vojnosanit. Pregl*., 29, 4,173-175, 1972.
4. Campbell L Grant, Marfin A Anthony, Lanciotti S Robert, Gubler J Duane: West Nile virus. *Infectious Diseases,* 2, 519-529, 2002.
5. Dimitrijević S.: Dirofilarioza ante portas. In: Clinica Veterinaria 1. Proceedings, pp 58, 1999.
6. Dmitrović R., Obradović M., Nedić LJ., Đerković V., Antonijević B., Pavlović N., Babić-Dunić V., Peklar P., Trbojević R., Gligić A., Jermolenko G., Vojinović D., Radosavljević M.: Zoonoze – zdravstveni problem na području Beograda. U: Dani Zavoda 2002 XIX Stručna konferencija, Beograd, str.57-72, 2002.
7. Đuričić Bosiljka, Vasić Ana, Rogožarski D., Vojinović Dragica, Elezović Radovanović Milica, Manić Marija, Marić J., Prokić Nataša, Ilić Živka, Novotny N., Gligić Ana: Seroepizootiological – epidemiological investigation and mapping of West Nile infection in the Republic of Serbia. *Acta veterinaria*, 63, 5-6, 569-579, 2013.

8. Ellis Jill, Oyston C. F. Petra, Green Michael, Titball W. Richard.: Tularaemia. *Clinical microbilogy reviews*, 631–646, Oct 2002.
9. Feldman Katherine Anne: Tularaemia . *JAVMA,* 222, 6, 725-730. March 15, 2003
10. Genchi Claudio, Rinaldi Laura, Cringoli Giuseppe: *Dirofilaria immitis* and *D. repens* in dog and cat and human infections. *Mappe parassitologiche,* 8, 2007.
11. Genchi C., Rinaldi L., Mortarino M., Genchi M., Cringoli G.: Climate and dirofilaria infection in Europe, *Veterinary Parasitology,* 163, 4, 286-92, 2009.
12. Genevieve Andre-Fontaine: Canine leptospirosis—Do we have a problem? *Veterinary Microbiology*, 117, 19–24. 2006.
13. Greene E.Craig: Infectious Diseases of the Dog and Cat - 4th Edition, Chapter 46 - Francisella and Coxiella Infections. 476-482, 2012.
14. Hrnjaković Cvjetković Ivana, Milošević Vesna, Jerant Patić Vera, Mikić Sandra Stefan, Cvjetković D., Radovanov Jelena, Kovačević Gordana: Najčešće bakterijske zoonoze u ljudi u Vojvodini u periodu 2005-2009. *Arhiv veterinarske medicine*, 4, 1, 11-18, 2011.
15. Manić Marija, Vojinović Dragica, Vasić Ana, Elezović Radovanović Milica, Prokić Nataša, Marić Jovan, Rogožarski D., Đuričić Bosiljka: Seroprevalence of some infectious diseases in stray dogs in the Republic of Serbia. Материалы международной научно-практической конференции "Актуальные вопросы постдипломного образования в ветеринарной медицине" Издательство: ФГОУ ВПО Волгоградский ГАУ "Нива", 3-9, 2013.
16. Marić J., Obrenović J., Milković M., Samokovlija A., Elezović M., Ljubić B., Stevanović G., Đuričić Đ. Đuričić B.: Tularemija u Republici Srbiji u periodu 2000–2011. godine. *Vet. glasnik*, 66, 5-6, 463 – 472, 2012.
17. Marić J., Simić V., Medić S., Nadaškić M., Manić Marija, Prokić Nataša, Pavlović I.: Presence of dogs microfilaria on wider territory of Belgrade. In: Proceedings of Third international epizootiology days and XV Serbian epizootiology days, Niška banja, 8-11 May 2013., urednik Tamaš Petrović, pp188-192, 2013.
18. Pajković D., Savić Sara, Veljković P., Grgić Ž.: Praćenje pojave dirofilarioze kod radnih pasa u službi vojske Srbije. *Arhiv veterinarske medicine*, vol.3, br.2, str.53-58, 2010.
19. Popović N, Milošević B, Urošević A, Poluga J, Lavadinović L, Nedelijković J, et al. Outbreak of West Nile virus infection among humans in Serbia, August to October 2012 . Euro Surveill. 2013;18(43):pii=20613. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20613
20. Rinaldi L., Genchi C., Musella V., Genchi M., Cringoli G.: Geographical

information systems as a tool in the control of heartworm infections in dogs and cats. *Veterinary Parasitology* 176, pp 286–290, 2011.
21. Samokovlija Ana, Vojinović Dragica, Rogožarski D., Elezović Milica, Marić J., Gligić Ana, Đuričić Bosiljka: Epizootiological and serological investigation of West Nile fever in central part of Serbia during 2011. In: Proceedings of II International epizootiology days, Beograd, 18-21 April 2012., urednik Tamaš Petrović, 55-59, 2012.
22. Savić S., Vidić B., Grgić Ž., Jurišić A., Ćurčić V., Ruzić M., Lolić Z.: Vektorske zoonoze pasa u Vojvodini. *Arhiv veterinarske medicine*, 5, 1, 77-87, 2012.
23. Spasojević Kosić Lj., Savić S.: Zdravstvena zastita lovačkih pasa. *Vet. Glasnik,* 67, 3-4, 259 – 268, 2013.
24. Tasić A., Tasić S., Miladinović-Tasić N., Zdravković D., Đorđević J.: Dirofilaria repens – potencijalna opasnost po zdravlje ljudi. *Acta Medica Medianae*, 46, 3, 52-55, 2007.
25. Vojinović D., Samokovlija A., Elezović M., Manić M., Prokić N., Maric J., Smiljanić J., Grgić Ž.: Results of representation *Leptospira spp*. with special reference to *Leptospira bratislava* in population street dogs in certain regions of the Republic Serbia. In: Proceedings of Third international epizootiology days and XV Serbian epizootiology days, Niška banja, 8-11 May 2013., urednik Tamaš Petrović. ISBN 978-86-81043-68-4, 72-76, 2013.
26. Vojinović D., Samokovlija A., Elezović M., Rogožarski D., Đuričić B.: Determination of specific antibodies against *Leptospira spp*. in population of stray dogs in the Republic of Serbia, Eurolepto 2012, Fakultet veterinarske medicine Sveučilišta u Zagrebu, 978-953-6062-87-4, http://www.vef.unizg.hr/eurolepto2012/prg.php, 2012.
27. William J. Martone, Lewis W. Marshall, Arnold F. Kaufmann, Jesse H. Hobbs, Martin E. Levy: Tularaemia Pneumonia in Washington. DC. A Report of Three Cases With Possible Common-Source Exposures. JAMA. 242:2315-2317, 2315-2317, 1979.

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FORENSIC INVESTIGATION OF SOW DEATHS IN THE INTENSIVE BREEDING SYSTEM

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Abstract

Sow mortality is a great challenge in intensive pig production worldwide. The aim of this study was to forensically examine the reason of sow death for a two-year period on two farms with intensive pig keeping, based on available data. In sows with a greater number of farrowings (over VII) greater number of deaths was found. Seasonality has an impact on sow mortality, in the summer period a larger number of sow death was found. Poor sow condition that usually occurs in the second half of lactation and after weaning, are the predisposition to the factors that lead to the sow death. On both examined farms in more than 60% of dead sows *Clostridium spp*. and *Escherichia coli* were isolated. In order to reduce the mortality of sows more attention should be paid to the older sows with a larger number of farrowings, provide better conditions in summer, cooling, and pay more attention to sows during the period from farrowing to the next insemination, respectively.

Key words: sows, death, forensics examination, risk factors

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FORENZIČKO ISPITIVANJE UGINUĆA KRMAČA U INTEZIVNOM SISTEMU DRŽANJA

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Kratak sadržaj

Uginuća krmača su veliki problem u intezivnoj proizvodnji svinja širom sveta. Cilj ovog istraživanja je bio da se na dve farme sa intezivnim načinom držanja svinja forenzički ispita razlog uginuća krmača u periodu od dve godine na osnovu dostupnih podataka. Kod krmača sa većim brojem prašenja (preko VII) ustanovljen je veći broj uginuća. Sezonalitet ima uticaj na uginuće krmača, pa je u letnjem periodu ustanovljen veći broj uginuća krmača. Loša kondicija krmača, do koje najčešće dolazi u drugoj polovini laktacije i nakon zalučenja, predstavlja predispozicju za faktore koji dovode do uginuća krmača. Na obe ispitivane farme kod preko 60% uginulih krmača izolovane su bakterije *Clostridium* spp. i *Escherichia coli*. U cilju smanjenja uginuća krmača potrebno je veću pažnju posvetiti starijim krmačama sa većim brojem prašenja, u letnjem periodu obezbediti bolje uslove, rashlađivanje, odnosno više se posvetiti krmačama u periodu od prašenja pa do narednog osemenjavanja.

Ključne reči: krmače, uginuće, forenzičko ispitivanje, rizični faktori

INTRODUCTION

Deaths of sows pose major challenges in pig production worldwide, causing direct economic losses in terms of saw loss, failure or drop of expected profit from piglets and the need of purchasing new gilts. A range of factors is responsible for sow deaths. In the European Union (EU), the implementation of new legislation dated 2013 implicates loose-housing of pregnant sows, i.e. group housing in shared boxes. Such housing system increases the risk of deaths because of potential injuries that are more likely than in the system, where sows are kept in individual boxes (Anil et al., 2003; Scott et al., 2009). Numerous authors reported age, poor body condition and stomach ulcers as factors associated with increased risk of death (Koketsu, 2000; Engblom et al., 2007; Sasaki and Koketsu, 2008). Moreover, the size of the herd and seasonality could affect the rate of death in sows (Christensen et al., 1995; Koketsu, 2000).

The pregnancy and farrowing itself also pose high risk of death (Chagnon et al., 1991). Averagely 84% of sows in intensive breeding systems are pregnant or in the stage of lactation (Koketsu, 2005), which increases the risk of death.

In the Republic of Serbia, sow-farms with intensive breeding system are often faced with the problem of sudden deaths of sows without any preceding symptoms of health disturbance (Stojanac et al., 2013). Sometimes, much greater number of sows' deaths than usual occur in a short time period of only few days, which poses a serious challenge from both aetiological and economical point of view. In that respect, the aim of this research was to perform forensic analysis and identify the possible reasons of sow deaths on two pig farms with intensive breeding system.

MATERIAL AND METHODS

The investigation encompassed two pig farms with the capacity of 2200 (Farm I) and 800 (Farm II) sows of Landrace, Yorkshire and F1 (Landrace x Yorkshire) breeds, and with a closed production system. At both farms, the farm management implicates weekly production system and strict application of the principle "all in/all out". The lactation period is 28 days, and after weaning, the sows were transferred into BUKARISTE. After artificial insemination, the sows remain in the bukariste for the following 30 days, when an ultrasound confirmation of the pregnancy is performed. Pregnant sows are then transferred into the cekaliste, until 110 days of gestation, and afterwards into the farrowing pens.

Forensic investigation extended over three-year period (2011-2013). In this period, 487 and 123 sows died at Farm I and Farm II, respectively. From dead animals, samples of internal organs (liver, kidney, spleen, small and large intestines, lungs, lymph nodes) were collected and subjected to standard bacteriological examination (aerobic and anaerobic isolation of the agent) (Quinn et al., 1998). Regular monitoring encompassed the control of chemical composition, microbial safety and presence of mycotoxins in sow feed by applying ELISA method.

The data sources used in this research involved the data obtained in the field, i.e. at the relevant farm, as well as official farm-records. The systematized data pertaining to all investigated parameters were evaluated using the measure of central tendency. The results were analyzed applying descriptive statistics and processed using Excel 2010.

RESULTS AND DISCUSSION

The investigation included two farms (I and II) and extended over two-year period (2011-2013). In this period, 487 sows died on Farm I whereas 123 sows died at Farm II. As related to the average number of sows (Table 1) the two-year rates of sow deaths were 23.21% and 16.33% on Farms I and II, respectively. The results obtained on Farm I are similar to those reported by Jensen et al., (2012), who reported death of 3% of sows in the period of three months and the results of Vestergaard et al. (2006) who established 14% saw deaths in the period of 12 months. The rate of sow deaths on Farm II was lower as compared to the reports of aforementioned authors.

Table 1. Average number of sows according to farrowing parity in the investigated period (2011-2013)

	Number of farrowings				**Total**
	I	II-VI	VII-X	≥XI	
Farm I	314	943	732	109	2098
Farm II	125	497	109	22	753

Chart 1 displays the rate of saw deaths by the number of farrowings in relation to average number of sows within given number of farrowings. High mortality rate in sows with higher number of farrowings is likely to be associated with the torsion of abdominal organs, vaginal and uterine prolapse, diseases of urinary tract and kidneys, which are often reported as characteristic for older sows (Christensen et al., 1995). According to our research, the increased mortality rate may be due to aforementioned factors, yet it was not confirmed by a patho-morphological examination. High mortality rates observed in saws that farrowed many times correspond with the results of some previous researches of other authors (Koketsu, 2000; Engblom et al., 2008).

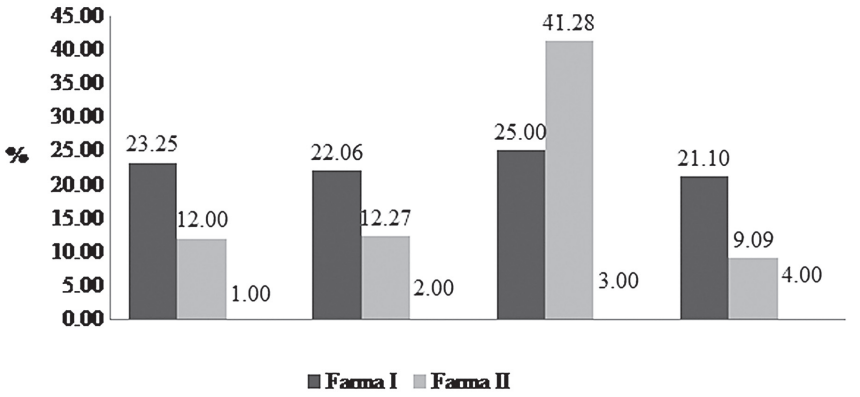


Chart 1. The percentage of dead sows according to number of farrowings

In Chart 2, the percentage of sow deaths according to seasonality is presented. The results indicated higher mortality rate in summer period (June-September) as compared to other seasons. Such result could be attributed to extremely high temperatures in Serbia during summer (over 35°C) and lack of air-conditioning systems in housing facilities, thus the inside temperature might sometimes reach even 45°C. Increased mortality rates during summer months were also reported by other authors. (Chagnon et al., 1991; Koketsu, 2000)

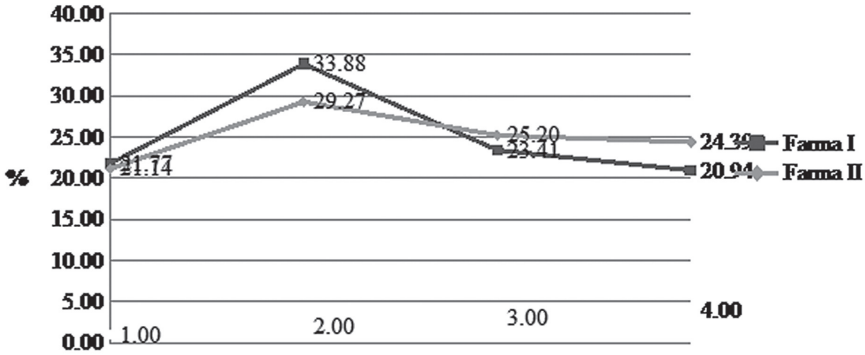
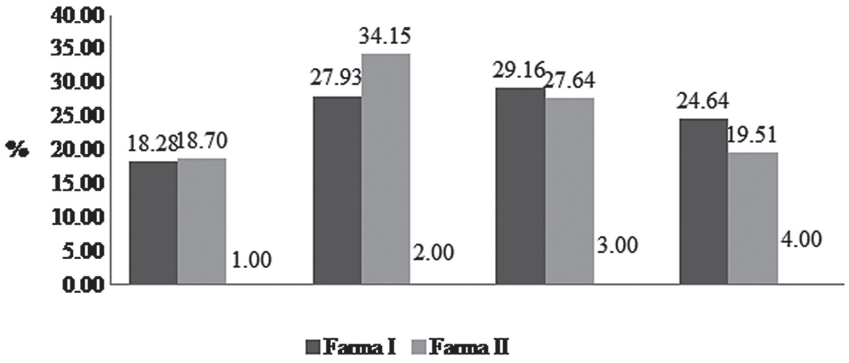


Chart 2. The percentage of dead sows according to the season of the year

Deaths of sows in relation to the stages of production cycle are presented in Chart 3. On both farms, the majority of sows died during lactation phase

and after weaning, before the next insemination. During lactation period, the body condition of sows decreases because of negative energy balance, which may induce metabolic and reproductive disorders (Kim and Suh, 2003). Moreover, numerous authors indicated that poor body condition increases the risk of stomach ulcer ant thus increased mortality rate in sows (Davies et al., 1996; Knauer et al., 2007).



IWOe-interval weaning oestrus
Chart 3. Number of dead sows according to cycle phases

The samples of internal organs (liver, kidney, spleen, small and large intestines, lungs, inguinal and mesenteric lymph nodes) were obtained from 97 dead sows from Farm I and 31 saws from Farm II, and submitted for bacteriological examination. In more than 60% of dead sows from both farms, *Clostridium* spp. and *Escherichia coli* were isolated. The finding of *Clostridium* spp. in dead sows was reported by other authors, and the organism is identified as the causative agent of sow death (Almond and Bilkei, 2005; Friendship and Bilkei, 2007; Jandowsky et al., 2013).

Table 2. The results of aerobic and anaerobic bacteriological examination of internal organ samples originating from dead sows

	Number of examined internal organ samples		**Number of positive samples (%)**	
	Farm I	Farm II	Farm I	Farm II
Streptococcus zooepidemicus	97	31	17 (17.52)	2 (6.45)
Clostridium spp.	97	31	59 (60.82)	24 (77.42)
Escherichia coli	97	31	65 (67.01)	21 (67.74)

CONCLUSION

The aforementioned forensic investigation of sow death in the intensive farming system revealed important role of different factors such as age, season, cycle stage and presence of infectious agents in increased mortality of sows. Farrowing associated stress, poor body condition in the second stage of lactation and after weaning are major predisposing factors for lethal outcomes. Limitations of our research such as effects of housing system, nutrition, genetic factors and farm management are important issues in intensive sow farming. However, in spite of aforementioned limitations, this research presents a useful source of information for both veterinarians and farmers, which can substantially contribute in decreasing the rate of deaths in sows in intensive farming systems.

REFERENCES

1. Almond P.H.D., Bilkei G.: Clostridium novyi caused outdoor sow mortality in Croatia. *Berliner Und Munchener Tierarztliche Wochenschrift*, 118, 7-8, 296-299, 2005.
2. Anil L., Bhend K.M.G., Baidoo S.K., Morrison R., Deen J.: Comparison of injuries in sows housed in gestation stalls versus group pens with electronic sow feeders. *J. Am. Vet. Med. Assoc*. 223, 1334–1338, 2003.
3. Chagnon M., D'Allaire S., Drolet R.: A prospective study of sow mortality in breeding herds. *Can. J. Vet. Res*. 55,180–184, 1991.
4. Christensen G., Vraa-Andersen L., Mousing J.: Causes of sow mortality among sows in Danish pig herds. *Vet. Rec.* 137, 395–399, 1995.

5. Davies P.R., Morrow W.E.M., Miller D.C., Deen J.: Epidemiologic study of decubital ulcers in sows. *J. Am. Vet. Med. Assoc*. 208, 1058–1062, 1996.
6. Engblom L., Lundeheim N., Dalin A-M., Andersson K.: Sow removal in Swedish commercial herds. *Livest. Sci*. 106, 76–86, 2007.
7. Engblom L., Lundeheim N., Strandberg E., Schneider M.P., Dalin A.M., Andersson K.: Factors affecting length of productive life in Swedish commercial sows. *J. Anim. Sci.* 86, 432–441, 2008.
8. Friendship C.R., Bilkei G.: Concurrent swine erysipelas and Clostridium novyi infections associated with sow mortality in outdoor sows in Kenya. *Veterinary Journal*, 173, 3, 694-696, 2007.
9. Jandowsky A., Bodenthin A., Seyboldt C., Frolich K.: Sudden death of outdoor housed pigs caused by Clostridium novyi. *Tieraerztliche Praxis Ausgabe Grosstiere Nutztiere*, 41, 6, 392-395, 2013.
10. Jensen T.B., Toft N., Bonde M.K., Kongsted A.G., Kristensen A.R., Sørensen J.T.: Herd and sow-related risk factors for mortality in sows in group-housed systems. *Preventive veterinary medicine*, 103, 1, 31-37, 2012.
11. Kim I-H., Suh G-H.: Effect of the amount of body condition loss from the dry to near calving periods on the subsequent body condition change, occurrence of portpartum diseases, metabolic parameters and reproductive performance in Holstein dairy cows. *Theriogenology,* 60, 1445–1456, 2003.
12. Knauer M., Stalder K.J., Karriker L., Baas T.J., Johnson C., Serenius T., Layman L., McKean J.D.: A descriptive survey of lesions from cull sows harvested at two Midwestern U.S. facilities. *Prev. Vet. Med*. 82, 198–212, 2007.
13. Koketsu Y.: Six component intervals of nonproductive days by breeding-female pigs on commercial farms. *J. Anim. Sci.* 83, 1406–1412, 2005.
14. Koketsu, Y.: Retrospective analysis of trends and production factors associated with sow mortality on swine-breeding farms in USA. *Prev. Vet. Med.* 46, 249–256, 2000.
15. Quinn P.J., Carter M.E., Markey B.K., Carter G.R.: Clinical Veterinary Microbiology. Mosby International, Linton House, London, 1998.
16. Sasaki Y., Koketsu Y.: Mortality, death interval, survivals, and herd factors for death in gilts and sows in commercial breeding herds. *J. Anim. Sci.* 86, 3159–3165, 2008.
17. Scott K., Binnendijk G.P., Edwards S.A., Guy J.H., Kiezebrink M.C., Vermeer H.M.: Preliminary evaluation of a prototype welfare monitoring system for sows and piglets (Welfare Quality® project). *Anim. Welf.* 18, 441–449, 2009.

18. Stojanac N., Stevančević O., Potkonjak A., Kovačević Z., Toholj B., Ljubojević D., Kragić S.: Seroprevalence of infectious causes of abortion in sows. *Contemporary Agriculture*, 62, 3-4, 206-211, 2013.
19. Vestergaard K., Bækbo P., Svensmark B.: Sow mortality and causes for culling of sows in Danish pig herds. In: Proceedings of the 19th International Pig Veterinary Society Congress, Copenhagen, Denmark, 2006, p. 255.

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EXAMINATION OF THE MOST COMMON ALLERGENS OF CANINE ATOPIC DERMATITIS - A RETROSPECTIVE STUDY

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Abstract

Canine atopic dermatitis (CAD) is one of the most common skin diseases of dogs. It is estimated that 10-15% of the dogs are showing clinical symptoms of this disease. Canine atopic dermatitis is defined as genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical symptoms. It is related to the production of IgE antibodies, mostly directed against external allergens. Allergens that can cause canine atopic dermatitis are quite numerous and depend on the geographical characteristics of research location. The aim of the study is to determine the most common allergens that can cause canine atopic dermatitis. The research was conducted according to medical records of 100 dogs during a period from the beginning of 2008 to the end of 2012 from the Department for skin diseases of small animals of the Clinic of equine, small animal, poultry and wild animal diseases at the Faculty of Veterinary Medicine, University of Belgrade. According to the clinical documentation, all of the tested dogs had clinically manifested symptoms of CAD. In order to confirm the diagnosis and detect the causative allergens, intradermal testing has been done. This testing has been performed with a standard set of 24 allergens specific to the geographical area of the research, produced in the Institute of Virology, Vaccines and Sera "Torlak". According to the results, the highest percentage of positive responses was established for the following allergens: house dust mites (*Dermatophagoides* sp.) 67%, ragweed (*Ambrosia artemisiifolia*) 61%, household dust 60%, cocks foot (*Dactylis* sp.) 59%, mix of weed pollen 57%.

Key words: canine atopic dermatitis, CAD, allergens, intradermal test

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ISPITIVANJE NAJČEŠĆIH ALERGENA ATOPIČNOG DERMATITISA PASA - RETROSPEKTIVA STUDIJA

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Kratak sadržaj

Atopični dermatitis pasa (ADP) je jedno od najčešćih oboljenja pasa. Procenjuje se da 10 do 15% pasa ispoljava kliničke simptome bolesti. Atopični dermatitis pasa se definiše kao genetički predisponirajuće oboljenje koje prati inflamatorni tok i alergijski svrab. Oboljenje nastaje zbog produkcije IgE antitela usmerenih na alergene iz spoljne sredine. Postoje mnogobrojni alergeni koji uzorkuju atopični dermatitis kod pasa zavisno od geografskih karakteristika lokacija koje se istražuju. Cilj studije je bio da se utvrdi koji su najčešći alergeni koji uzorkuju atopični dermatitis. Istraživanje je urađeno prema medicinskim protkolima za 100 pasa u period od početka 2008 do kraja 2012 godine iz ambulante za oboljenja kože Departmana za bolesti kopitara mesojeda, živine i divljači, na Faklutetu veterinarske medicine, Univerziteta u Beogradu. Prema kliničkoj dokumentaciji, svi testirani psi su imali kliničke manifestacije koje upoućuju na ADP. Kako bi potvrdili dijagnozu i detektovali uzročne alergene, potrebno je da se uradi intradermalni test. Navedeni test je urađen sa standardnim setom od 24 poznata alergena koji su specifični za geografsku oblast istraživanja, a proizvedeni su u Institutu za virusologiju, vakcine i serume "Torlak". Na osnovu dobijenih rezultata ustvrđeno je da je najčešći procenat pozitivnih odgovora dobijen kod sledećih alergena: grinja iz kućne prašine (*Dermatophagoides* sp.) 67%, ambrozije (*Ambrosia artemisiifolia*) 61%, kućne prašine 60%, mačije trave (*Dactylis* sp.) 59%, mešavine polena 57%.

Ključne reči: atopični dermatitis pasa, ADP, alergeni, intradermalni test

INTRODUCTION

Canine atopic dermatitis (CAD) is one of the most common skin diseases of dogs. It is estimated that 10-15% of dogs are showing clinical symptoms of this disease (Williams, 2001).

CAD is defined as genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical symptoms related to the production of IgE antibodies, mostly directed against external allergens (Halliwell, 2006). The pathogenesis of CAD is not yet entirely elucidated, but it is considered that a defect in the epidermal barrier allows breakthrough to the external and probably microbial allergens (Marsella 2006).

CAD usually occurs between 3rd and 6th month of age. Many studies from different periods and different geographical locations demonstrated that the following dog breeds are predisposed to CAD: West Highland White Terrier, Golden Retriever, Labrador Retriever, Bulldog (English and French), Chinese Sharr-Pei, Dalmatian and Poodle (Picco et al., 2008; Chanthick et al., 2008; Favrot et al., 2010).

Allergens that can cause CAD include house dust mites, pollen of trees, weeds and grasses, house and bed dust, fungal spores, animal and human dandruff, feathers, cigarette smoke, filling of the furniture, some bacterial allergens, insects (Chapman et al., 2007).

According to some authors, the primary lesion does not exist in CAD (Scott, 1981). Other authors indicated erythematous papula as a primary lesion (Chaberlain, 1974). Secondary lesions are the result of chronic pruritus and subsequent scratching, chronic inflammation of the skin and competitive secondary pyodermia and malassezia dermatitis. Partial or complete alopecia, reddish discoloration of hair because of the saliva, excoriation, papules, pustules, dry broken hair, hyper pigmentation, scaling and lichenification are noticed on the skin (Scott et al., 2001; Chanthick et al., 2008; Favrot et al., 2010).

The aim of this study was to determine the most common allergens that caused CAD in the Ambulance for skin desises of small animals of Department of equine, small animal, poultry and wild animal diseases at the Faculty of Veterinary Medicine, University of Belgrade in the period from the beginning of 2008 to the end of 2012.

MATERIAL AND METHODS

The research was conducted by using medical records of 100 dogs during a period from the beginning of 2008 to the end of 2012 in the Ambulance for

skin diseases of small animals of Department of equine, small animal, poultry and wild animal diseases at the Faculty of Veterinary Medicine University of Belgrade. According to the documentation, all tested dogs had clinically manifest symptoms of CAD. In all animals, general clinical examination was conducted, as well as specialist dermatological examination. Determination of the presence of fleas and skin scarification has been performed.

Before testing, the hair of dogs has been cropped on the lateral part of the thorax using electric reels with insert number 40. With a waterproof marker, spots for allergens applications , at the distance of 3cm from each other, have been marked on the cropped region. The testing has been conducted on non-sedated animals placed on the table in the latero-lateral position. Intradermal testing with seasonal and non-seasonal allergens was performed by standard aqueous solutions of the allergen manufactured by the Institute of Virology, Vaccines and Sera, "Torlak". Allergens for intradermal testing are supplied in a dissolved form - glass bottles of 10 ml. The allergen solutions were stored in refrigerator +4 C°. In addition to performing a positive (histamine phosphate 1:100,000 w / v) and the negative probe (0.9% physiological saline solution), intradermal testing was conducted using the following 24 allergens: house dust (250 PNU / ml), *Dermatophagoides* sp. (250 PNU / ml), a mixture of melds (500 PNU / ml), bacterial mixture of allergen (1000 PNU / ml), mixture of feathers (500 PNU / ml), bed linen dust (1000 PNU / ml), tobacco smoke (1000 PNU / ml), tree pollen mixture (1000 PNU / ml), the pollen of oak - *Quercus robur* (1000 PNU / ml), the pollen of poplar - *Populus* sp. (1000 PNU / ml), the pollen of birch - *Betula* sp (1000 PNU / ml), pollen of hazel - *Corylus* sp (1000 PNU / ml), pollen of lime - *Tilia* sp. (1000 PNU / ml), pollen of willow - *Salix* sp. (1000 PNU / ml), a mixture of grass pollen (1000 PNU / ml), cock's foot pollen - *Dactylis* sp. (1000 PNU / ml) Timothy grass pollen - *Phleum pratense* (1000 PNU / ml), perennial ryegrass pollen - *Lolium perene* (1000 PNU / ml), meadow fescue pollen - *Festuca pratensis* (1000 PNU / ml), a mixture of weed pollen (1000 PNU / ml), ragweed pollen - *Ambrosia artemisiifolia* (1000 PNU / ml), pollen of mugwort - *Artemisia vulgaris* (1000 PNU / ml), pollen of narrowleaf plantain - *Plantago lanceolata* (1000 PNU / ml), pollen sorrel - *Rumex acetosa* (1000 PNU / ml), 1:100 000, histamine phosphate, w / v physiological saline solution - 0.9% NaCl. In the tests, we used 18 individual allergens and 6 mixtures of allergens, including pollen of grass, trees, weeds, feathers, melds and bacteria.

For intradermal application of allergens extracts and the positive and negative controls, 1 ml syringes with a needle dimensions 26 G (0.5 mm) were used. On the previously marked area, 0.05 ml of prepared allergens extracts,

as well as positive and negative controls, was injected intradermally. Results of intradermal testing were interpreted after 15 minutes.

After this time, the reaction was measured (transparent ruler) and graduated. The reaction, which has been of the same size as the negative control has been marked as 0, as a reaction same with the histamine +4. Reactions marked as +2 and higher were considered positive.

RESULTS

Table 1 displays the percentage of dogs, clinically suspected for CAD, which showed positive reactions to intradermal test on individual allergens. All tested dogs were multi-sensitive to intradermal testing. In the majority of dogs, between 6 and 11 positive reactions were recorded.

Table 1: Percentage of dogs, which showed positive reaction to intradermal testing on certain allergens

Allergens	% of dogs with positive reactions
Dermatophagoides sp. (250 PNU/ml)	
ragweed pollen - *Ambrosia artemisiifolia* (1000 PNU / ml)	61
house dust (250 PNU / ml)	60
cock's foot pollen (*Dactylis sp*) (1000 PNU/ml)	59
mixture of weed pollen (1000 PNU/ml)	57
mixture of grass pollen (1000 PNU/ml)	56
pollen of willow (*Salix sp*) (1000 PNU/ml)	55
pollen of poplar (*Populus sp*.) (1000 PNU/ml)	54
timothy grass pollen (*Phleum pratense*) (1000 PNU/ml)	47
perennial ryegrass pollen (*Lolium perene*) (1000 PNU/ml)	45
tree pollen mixture (1000 PNU/ml)	42
pollen of mugwort (*Artemisia vulgaris*) (1000 PNU/ml)	42
pollen of hazel (*Corylus sp*.) (1000 PNU/ml)	35
pollen of birch (*Betula sp*.) (1000 PNU/ml)	34
pollen of lime (*Tilia sp.*) (1000 PNU/ml)	32
mixture of feathers (500 PNU/ml)	20

Allergens	% of dogs with positive reactions
bed linen dust (1000 PNU/ml)	19
pollen of narrowleaf plantain (*Plantago lanceolata*) (1000 PNU/ml)	18
mixture of melds (500 PNU/ml)	17
bacterial mixture of allergen (1000 PNU/ml)	15
tobacco smoke (1000 PNU/ml)	14
pollen sorrel (*Rumex acetosa*) (1000 PNU/ml)	13
the pollen of oak (*Quercus robur*) (1000 PNU/ml)	12
meadow fescue pollen (*Festuca pratensis*) (1000 PNU/ml)	8

DISCUSSION

In the examined dogs, the highest number of positive reactions has been registered to the following allergens: house dust mites (*Dermatophagoides* sp.), ragweed (*A. artemisiifolia*), cock's foot (*Dactylis* sp.), weed pollen and house dust mixture (Table 1).

A total of 24 allergens were used, and all dogs were multi-sensitive. In the majority of dogs, between 6 and 11 positive reactions were recorded. One dog had 23 positive reactions. Also, the results in the literature confirm that the majority of the tested dogs had been multi-sensitive (Zur et al., 2002), which indicates the complexity of the CAD pathogenesis.

According to our study, house dust mites (*Dermatophagoides sp*.) were the most common allergen that caused positive reaction in dogs in intradermal testing (67%). However, literature data reported strong response in dogs (47-80%) to that allergen at the same concentrations of 250 PNU / ml that we used (Zur et al., 2002; Tarpataki et al., 2006; Chanthick et al., 2008). House dust mites belong to the family *Arachnidae* and they live freely in the epidermal debris of humans, animals, yeasts, and household food remains (Spieksma, 1990). House dust is a complex allergen, which consists of house dust mites, dandruff, mold spores, insect feces, bacteria, fibrous material from plants and animals, food debris and many other substances. There are different opinions about whether house dust mite allergen should be included in the intradermal testing because of its complex structure and strong irritating effect that may lead to false-positive reactions. Based on the results of intradermal testing, positive reaction to this allergen occurs in 39.4-75.5% of tested dogs (Willemse and Van den Brom 1983, Zur et al., 2002). In our study, reaction to house

dust has been identified in 60% of cases, and these data are consistent with the literature reports. In our study, we used concentrations of 250 PNU / ml, as recommended by other allergists (Reedy et al, 1997.; Sousa and Halliwell, 2001).

Weeds are annuals that grow wild and do not have any agricultural or decorative importance. The pollination of weeds takes place from the second half of July to November. According to data obtained by intradermal testing, weed pollen can cause a positive reaction in 2.7-45.5% of suspected atopic individuals (Willemse and Van den Brom 1983, Zur et al., 2002; Tarpataki et al., 2006; Chanthick et al., 2008). In our survey, the weeds were identified as an important allergen in our environment (57%), especially ragweed (61%). This information is not surprising considering the fact that the pollen of these plants manifests strong allergenic potential (Reedy et al , 1997.). This plant is most widespread in Eastern and Central Europe, Hungary, Serbia, Croatia, Slovakia and the Czech Republic (D'Ammato et al., 1991). Also, the data from our country indicate prevalence of these plants all over Serbia, especially in Vojvodina, Mačva, Podrinje, Šumadija and in the valleys of large rivers (Janjic et al., 2007). In veterinary medicine, there are no published data on the percentage of positive reactions to this allergen (Milicic Matic et al., 2010). All American authors agree that ragweed (group of weeds) is the most important allergen in dogs and point out its strong allergenic properties (Zur et al., 2002; Tarpataki et al., 2006; Chanthick et al., 2008).

Tree pollen is one of the important allergens and, according to the literature data, 12-35% of dogs showed a positive reaction to these allergens (Willemse antigens and Van den Brom 1983, Zur et al., 2002; Tarpataki et al., 2006; Chanthick et al., 2008). According to our results, the single most important pollen allergen were willow tree (55%) and poplar (54%). Grass pollen is considered responsible for 10-30% of all allergies in humans. In dogs, these data are different and mostly depend of the geographical location (Willemse and Van den Brom 1983, Zur et al., 2002; Tarpataki et al., 2006; Chanthick et al., 2008). In studies conducted so far, the intradermal testing was performed using the mixture of herb extracts, as well four of single allergens extracts: cock's foot (*Dactylis* sp), ryegrass (*Lolium perene*), timothy grass (*Phleum pratense*) and meadow fescue (*Festuca pratensis*). In our study, grass pollen was present in a slightly higher percentage (56%) as compared to the already published results.

CONCLUSION

The results of the conducted tests showed that most of the dogs with clinical signs of atopic dermatitis were multi-sensitive or demonstrated more than

2 positive reactions to the intradermal testing. The most common allergens that cause clinical symptoms of this disease were house dust mites, following ragweed, house dust, cock's foot, weed pollen mixture, mixed grass pollen, willow and poplar.

REFERENCES

1. Chaberlain K.W.: Atopic (allergic) dermatitis. *Vet Clin North Am* 4, 29-39, 1974.
2. Chanthick C., Anaman S., Buathet K.: The prevalence of positive intradermal allergy tests in 114 dogs with atopic dermatitis in the Bangkok metropolis, Thailand. *Veterinary Immunology and Immunopathology* 126, 256–262, 2008.
3. Chapman M.D., Pomes A., Breiteneder H., Ferreira F.: Nomenclature and structural biology of allergens. *J Allergy Clin Immunol* 119, 414-420, 2007.
4. D'Ammato G., Spieksma F.T.M., Bonini S.: Allergenic Pollen and Pollinisis in Europe. Science Publications, Oxford, 1991.
5. Halliwell R.: Revised nomenclature for veterinary allergy. *Veterinary Immunology and Immunopathology* 114, 207–208, 2006.
6. Janjić V., Vrbničanin S., Stanković-Kalezić R., Radivojević L., Marisavljević D.:Poreklo i rasprostranjenost ambrozije. U: Janjić V, Vrbničanin S: Ambrozija. Herbološko društvo,Srbije Beograd, 2007, str. 17–28.
7. Milčić Matić N., Popović N., Lazarević M., Medenica Lj.: The role of Ambrosia artemisiifolia allergen in canine atopic dermatitis. *Acta Veterinaria*, 60, 183-196, 2010.
8. Reedy L.M., Miller W.H., Williemse T.: Allergic Skin Disease of the Dog abd Cat, 2nd Edition, WB Saunders, London, UK, 1997.
9. Scott D.W.: Observations on canine atopy. *J Am Anim Hosp Assoc* 17, 91-100, 1981.
10. Sousa C.A., Halliwell R.E.: The ACVD task force on canine atopic dermatitis (XI): the relationship between arthropod hypersensitivity and atopic dermatitis in the dog. *Vet Immunol Immunopathol* 20, 233-237, 2001.
11. Spieksma F.T.M.: Mite biology. *Clinicals reviews in Allergy*, 8, 31-49, 1990.
12. Tarpataki N., Papa K., Reiczgel J., Vajdovich P., Voros K.: Prevalence and features of canine atopic dermatitis in Hungary. *Acta Veterinaria Hungarica* 54, 353–366, 2006.
13. Willemse T., Van den Brom W.E.: Investigations of the symptomatology and the significance of immediate skin test reactivity in canine atopic dermatitis. *Res Vet Sci* 34, 261-265,1983.

14. Williams H: Disease definition and measures of disease frequency. *J Am Acad Dermatol,* 45(suppl 1), 33–6, 2001.
15. Zur G., Ihrke P.J., White S.D., Kass P.H.: Canine atopic dermatitis: a retrospective study of 266 cases examined at the University of California, Davis, 1992-1998. *Vet Dermatology* 13, 89-102, 2002.
16. Marsella R.: Atopic Dermatitis: A New Paradigm**,** Proceedings from the Hill's 2006 Symposium on Dermatology held April 2-4 in Palm Springs, CA, 2006, 7-10**.**
17. Picco F., Zini E., Nett C., Naegeli C., Bigler B., Rüfenacht S., Roosje P., Gutzwiller M.E., Wilhelm S., Pfister J., Meng E., Favrot C.: A prospective study on canine atopic dermatitis and food induced allergic dermatitis in Switzerland, *Veterinary Dermatology* 19:150-155, 2008.
18. Favrot C, Steffan J, Seewald W, Picco F.: A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. *Vet Dermatology*, 21, 1, 23-31, 2010.
19. Scott D.W., Miller W.H., Griffin C.E.: Small Animal Dermatology, 6th Edition. W.B. Saunders, Philadelphia, 2001.

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NEONATAL DIARRHEA IN PIGS CAUSED BY *Clostridium perfringens*

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Abstract

The outbreaks of enteritic infections in piglets caused by *Clostridium perfringens* belongs to the disease group with marked age incidence i.e. it normally occurs in suckling piglets aged to 7 days, usually on 2nd or 3rd day. At necropsy, the predominant pathomorphological lesions are most frequently observed in small intestine, especially in jejunum. However, in some cases the pathomorfological lesions may macroscopically be absent. For that reason, diagnostic criteria should consider: the disease history data (mortality pattern), clinical signs of the disease (bloody diarrhea in suckling piglts), patomorhological lesions and bacteriologic findings. The material for research included samples from 5 swine farms, where health problems (diarrhea, increased mortality) in suckling piglets of different age were detected. In total 69 piglet carcases were submitted to necropsy. In typical cases the presence of bloody content in small intestine, snaky appereance of affected intestinal loops, the presence of emphysema in the intestinal wall were observed. Applying bacteriology testing (anaerobic cultivation) in the most examined cases *Clostridium perfringens* was detected in tissue samples.

Key words: suckling piglets, necrotic enteritis, *Clostridium perfringens*

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NEONATALNA DIJAREJA PRASADI UZROKOVANA SA *CLOSTRIDIUM PERFRINGENS*

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Kratak sadržaj

Crevne infekcije prasadi izazvane *Clostridium perfringens* pripadaju grupi oboljenja kod kojih je izražena zakonitost incidence u pogledu uzrasta obolelih jedinki tj. infekcija se ustanovljava u uzrastu do 7 dana, najćešće oko 2-3 dana života prasadi na sisi. Patomorfološkim pregledom lezije se najčešće ustanovljavaju u tankom crevu, naročito u jejunumu. Međutim, patomorfološki nalaz se ponekad može karakterisati i odsustvom makropatoloških lezija specifičnih za klostridijalnu infekciju. Zato se kao dijagnostički kriterijumi uzimaju u obzir i anamnestički podaci (uginuće u prvim danima života prasadi), klinička slika oboljenja (hemoragična dijareja prasadi na sisi), patomorfološke promene i bakteriološki nalaz. Materijal za ispitivanje je obuhvatao pet farmi svinja, na kojima su utvrđeni zdravstveni problemi (pojava proliva i uginuća) kod prasadi na sisi različitog uzrasta. Ukupno je patomorfološki pregledano 69 leševa prasadi na sisi. U tipičnim slučajevima, patomorfološkim pregledom ustanovljeno je prisustvo krvavog sadržaja u lumenu tankih creva, zmijoliki izgled crevnih uvojaka kao i prisustvo mehurića vazduha u zidu tankih creva. Bakteriološkim ispitivanjem (anaerobna kultivacija) iz tkiva uginule prasadi u najvećem broju slučajeva izolovan je *Clostridium perfringens*.

Ključne reči: prasad na sisi, nekrotični enteritis, *Clostridium perfringens*

INTRODUCTION

Clostridium perfringens is a Gram-positive, spore forming bacterium that can cause a variety of toxic-specific lesions and gastrointestinal diseases in domestic and wild animals as well as in humans (*van Asten et al., 2010*). Owing to its ability to produce spores under adverse environmental conditions, it is one of the most widespread potential bacterial pathogens in nature as well as in the gastrointestinal tract of most animal species (Backer et al., 2010). Based on the production of 4 major toxins, alpha (CPA), beta (CPB), epsilon (ETX) and iota

(ITX), *C.perfringens* isolates are classified into 5 toxino-types (A-E) (Miclard et al., 2009; Songer and Taylor, 2006). Two other toxins, enterotoxin (CPE) and beta2 (CPB2) can be produced by all types of *C. perfringens*, although they are not used in typing *(Songer and Uzal, 2005*).

C. perfringens type C infection occurs in all swine-producing areas of the world (Backer et al., 2010; Jäggi et al., 2006). Type C causes frequently hemorrhagic, often fatal, necrotic enteritis in young piglets (Songer and Taylor, 2006). It is mostly seen in the first 7 days of life (Jackson and Cockcroft, 2007; Prodanov-Radulović et al., 2013). Enteric disease, necrotic enteritis, caused by these organisms impacts producers, veterinary practitioners, and diagnosticians, despite long-term availably of immunoprophylactic products (toxoid vaccines) for swine protection (Songer and Uzal, 2005). In view of high morbidity and mortality rates, necrotic enteritis is a cause of serious financial losses in pig rearing (Springer and Selbitz, 1999).

MATERIAL AND METHODS

The material for this research originated from five swine farms, where certain disorders and health problems in suckling piglets were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: anamnestical evaluation and clinical investigation, pathomorphological examination, standard bacteriological examination for detection the precence of aerobic and anaerobic bacteria in the organs and tissue samples derived from diseased and/or died suckling piglets.

RESULTS AND DISCUSSION

On the first two examined farms, the evaluation of anamnestic data indicated apparent health problems and increased mortality in suckling piglets. Clinical manifestations occurring in suckling piglets (first 5 and 10 days after farrowing) included severe diarrhea and signs of body dehydratation. Despite the fact that the piglets were therapeutically treated, there was no evident respond to applied medication. Decrease in growth rate was evident in some survived nursed piglets, which did not die but they remain stunted. On the second evaluated farm, the sows are vaccinated during pregnancy but recently the vaccine has been changed (i.e. vaccine from another producer was introduced). During data control, the difference in vaccine composition was noticed: the old one had 3 types of toxoid C. perfringens (type B and purified toxoid type C and D) while the newly applied contains only one type of C beta-toxoid.

The control of farm anamnestic data, it was discovered that severe diarrhea in piglets was most frequently in the litters deriving from first litter and older sows. By clinical examination of piglets aged 11-15 days yellow-brownish colored diarrhea accompanied by staining of peritoneum was detected. In the 4-days old litters, traces of the reddish-brown diarrhea on the piggery floor were discovered. In the piglets from the first litter sow, bloody diarrhea in the first day of life was evident. The pathomorphological examination of the dead suckling piglets revealed lesions dominantly on the mucosal surface of the digestive tract: hemorrhagic and necrotic enteritis, catarrhal and hemorrhagic gastritis, diphteroid-necrotic gastritis and enteritis (*Enteritis necroticans porcellorum*). The stomach was often full of milk and mucosa of the small intestine, dark red and necrotic. Bacteriological examination (anaerobic cultivation) of tissue samples (spleen, liver, kidneys and mesenterial lymph nodes) from dead suckling piglets, revealed presence of *Clostridium perfringens*.

The feces of saws contained small numbers of type-C organisms and these multiply rapidly in the small intestine of piglets, out-competing other bacteria and becoming the dominant organisms in the population (Songer and Uzal, 2005; Songer and Taylor, 2006). Oral infection of piglets, acquired in most cases through teats contaminated with feces, leads to replication of *C. perfringens* type C in the intestines resulting in the production of protein toxins (exotoxins), including β-toxin. The β-toxin is not degraded because of poor synthesis of digestive enzymes by piglets and high anti-trypsin content in sows` milk. It consequently has a decisive influence on the pathogenesis of necrotic enteritis (Springer and Selbitz, 1999). Lethal and necrotizing effects of CPB play key roles in tissue damage. Death is likely due to the effects of intestinal damage and toxemia (Miclarad et al., 2009). Hypoglycemia and secondary bacteremia due to *C. perfringens* or *Escherichia coli* may raise the fatality rate (Songer and Uzal, 2005).

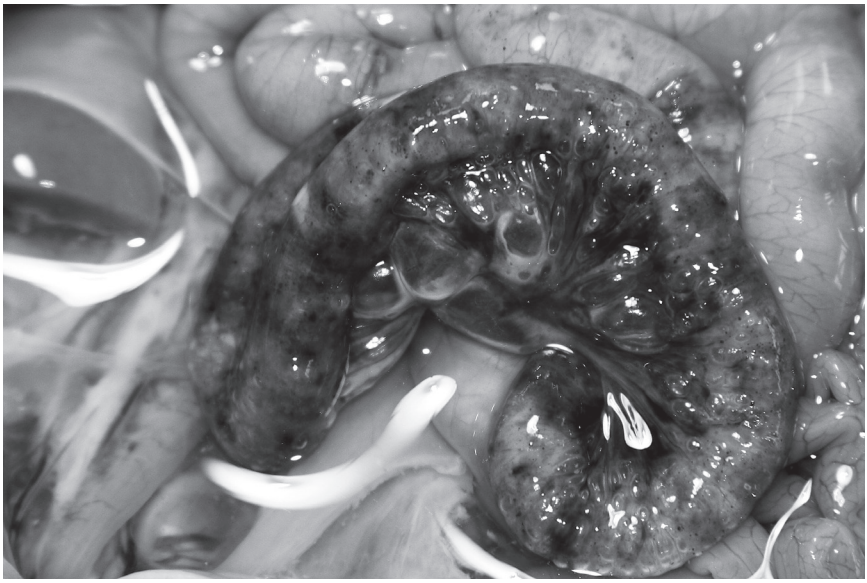
Clinical signs vary according to immune status and age of affected piglets (Songer, Uzal 2005). Clinical disease can take the per acute, acute or chronic course with signs of intense abdominal pain, depression, and bloody diarrhea, which begins 8 to 22 hours after exposure to *C. perfringens* type C. The course of the disease is usually 24 or fewer hours in 1- to 2-day old piglets (Jackson and Cockcroft, 2007), but chronic disease (usually in older animals) can persist for 1 or 2 weeks, and is characterized by persistent diarrhea without blood and dehydration (Songer, Uzal 2005). Disease is most common in 3-dayold piglets, but may occur as early as 12 hours after birth (Jackson and Cockcroft, 2007; Prodanov-Radulović et al., 2013). Piglets become weak, move with reluctance and rapidly become moribund, risking crushing by the sow. Many of them are found dead within 12-36 hours after birth. However, death also occurs in some

animals without diarrhea being seen (Songer and Taylor, 2006).

Lesions are typically noticed in the jejunum and ileum; they may extend anterior to within a few centimeters of the pylorus and posterior to the proximal colon. Gross mucosal lesions are reddish or black in color, with intense hemorrhage and gas bubbles in the intestinal wall. Contents of the affected area contain blood, and may be found as far distal as the rectum. Jejunum portion of intestine may be loosely adherent to adjacent segments by acute fibrinous peritonitis. Intestinal wall is usually thickened and yellow or grayish, and its contents may be bloodstained and contain necrotic debris. Hallmark lesions include profound mucosal necrosis and emphysema in small intestine, sometimes extending into cecum and proximal colon. Type C is a primary pathogen but can apparently colonize lesions associated with other diseases such as transmissible gastroenteritis (TGE) (Songer and Taylor, 2006). Characteristically, dead piglets are in good condition (Jackson and Cockcroft, 2007). At necropsy, the predominant lesions are most frequently observed in small intestine, especially jejunum; occasionally lesions may be confined to large intestine. Lesions are similar in all segments of the intestines and in acute cases consisting of diffuse or segmental extensive fibrinonecrotic (pseudomembranous) enteritis with emphysema and bloody gut contents. There may be fibrin strands on the intestinal serosa, and adhesions may develop between intestinal loops (Songer, Uzal 2005). One may see localized peritonitis, but the piglet usually dies before this can develop (Jackson and Cockcroft, 2007).

Figure 1. Necrohemorrhagic enteritis in 3-day old piglet

Figure 2. Acute *C. perfringens* type C enteritis in 3-day-old piglet: an emphysematous segment of the intestine (jejunum)



On the third examined swine farm, the health problems in suckling piglets were connected with the purchasing of breeding animals (gilts). Despite the fact that all gilts originated from one farm, after the farrowing, all litters died in the first 2 days of life. Clinically, severe dehydration, depression, piglets' cohorting, the yellow or light brown colored diarrhea was observed. In some animals the purple-red-colored watery feces was evident already on the first days of life. Eventually, all farrowed litters died. The pathomorphological examination of the dead suckling piglets aged 2-3 days revealed catarrhal gastritis, angry purplish-red color of jejunum (i.e. color like rot-cherry). In some cases, the small intestines manifesting snaky appearance of affected intestinal loops, the presence of emphysema in the intestinal wall or with extensive whitish sediment (gypsum-like content) were observed. Applying anaerobic cultivation, *C. perfringens* was isolated from the organs and tissue samples (liver, spleen, kidney, mesenterial lymph nodes) of died suckling piglets.

Outbreaks of *C. perfringens* infection often follow the introduction of infected breeding stock, and the disease persists in herds for up to 2 months; however, in herds where new stock is constantly introduced, the outbreaks may continue for up to 15 months (Prodanov-Radulović et al., 2013; Songer and Taylor, 2006). Farrowing houses or areas may become heavily contamina-

ted. Typically, three or four litters or part of a litter in a herd may be affected by severe disease but up to 50 litters have been reported to be affected in some outbreaks. Herds may be infected with the organism but typical disease may be absent (Songer and Uzal, 2005). In some cases, this is attributed to early administration of antimicrobials as either treatment or prevention, but most commonly, it is the results from increasing practice of including *C. perfringens* type C toxoid in vaccines given to sows to prevent piglet diarrhea (Springer and Selbitz, 1999). Where protective antibody is present in the colostrum at adequate levels, no disease will be seen. Where levels are inadequate or the intake is insufficient, clinical signs may develop somewhat later and be mild and difficult to recognize (Songer and Taylor, 2006). However, Prodanov-Radulović et al. (2011) suggested that enteric disease of suckling piglets could be provoked with the feed quality, i. e. the presence of mycotoxins in the feed for lactating sows and in the piglets' first feed. The authors reported the occurrence of hemorrhagic enterotoxaemia in piglets despite the fact that dams were vaccinated twice during gestation.

Clostridium perfringens is characterized by a very short generation time, and type C organisms can multiply to numbers approaching 10^8-10^9 per gram of contents in only a few hours (Songer and Taylor, 2006).

Attachment to jejunal epithelial cells at villous apices is followed by desquamation of these cells and proliferation of the organism along the basement membrane. Necrosis of the villous lamina propria is extensive, and hemorrhage accompanies necrosis (Gurtner et al., 2010). The necrotic zone advances to involve crypts, muscular mucosa and sub mucosa, and occasionally the muscular layers. Perforation of intestinal wall leads to emphysema in muscle layers, beneath the peritoneum, and in mesenteric lymph nodes (Songer and Taylor, 2006*).*

On the last two examined swine farms, by controlling the epizootical and anamnestic data, certain irregularities in the implementation of immunoprophylactic measures were found. The sows were vaccinated, but not according to manufacturer's recommendation (i.e. only once before farrowing). In suckling piglets aged 5 days, the signs of diarrhea are observed but not in all litters. After controlling the piggeries, it was established that there was a full-floor facility with straw bedding, while the boxes were separated by the wooden wall. The health problem has been apparent for about one year, but diarrhea was not recorded in each litter. A large number of piglets got sick and died in 12 to 24 hours after farrowing. After farrowing, the piglets had good birth weight, but pinky-colored diarrhea occurred as soon as on the second day of life. Treatment with antimicrobials was of little use in diseased piglets

and all diseased animals eventually die. Also, the problem was a high mortality rate in nursing piglets. Clinical examination revealed that diseased piglets were dehydrated, with loss of body condition. Nursing was minimal, and these piglets rapidly lost condition and became gaunt and weak. They had reddish-brown to yellow-brown colored diarrhea accompanied by staining of perineum as well as redness and swelling of the anus.

By gross pathological examination in dead suckling piglets, distinct lesions on small intestine were evident: snaky appearance of affected intestinal loops and the presence of emphysema (i.e. gas bubbles) in the intestinal wall. The jejunum of affected piglets was also swollen with an angry purplish-red color, with bloodstained fluid content. In some cases, the mucous surface of small intestine was covered by a grayish-yellow deposits and the intestinal wall was thickened and friable. Also, hemorrhagic gastritis, diffuse hemorrhage of the kidneys, enlarged and reddened mesenteric lymph nodes could be found. In a laboratory testing (anaerobic cultivation), *C. perfringens* was isolated from the examined tissue samples.

The disease occurs epizootically in non-vaccinated populations, and prevalence of affected litters can reach 100%. The case fatality rate varies, but 100% mortality in litters from non-immune sows is not unusual, and herd mortality may be > 50% (Jäggi et al., 2009). The study of Springer and Selbitz (1999) clearly demonstrate that vaccination of sows with the toxoid vaccine and concomitant administration of a penicillin preparation in the piglets leads to a drastic reduction in piglet losses. With increased herd immunity, the disease may become enzootic, with mild cases developing over a period of months. Continued appearance of acute disease suggests herd immune deficiency (such as by frequent introduction of immune-naïve gilts) or failure of piglets to receive adequate amounts of colostrum (Songer and Uzal 2005).

Diagnostic criteria - mortality pattern, clinical signs of the disease, and pathological gross lesions are sufficient basis for a presumptive diagnosis of *C. perfringens* type-C enteritis in piglets. More detailed herd infection history, exclusion of other causes of necrotic enteritis and bacteriological culture may be needed to establish a presumptive diagnosis in chronic cases (Songer and Taylor, 2006). Final diagnosis, however, should be based on bacteriological cultivation of intestinal contents (isolation of large numbers of *C. perfringens* followed by genotyping of isolates and/or CPB detection from intestinal contents) (Songer and Uzal, 2005**;** van Asten et al., 2010). Although *C. perfringens* type C can be found in the intestines of clinically healthy pigs, this is not frequently the case, which usually advocates the diagnostic relevance of isolation of this microorganism from the intestines of diseased pigs. Also, small amounts

of *C. perfringens* type C are usually isolated from the intestines of clinically normal pigs, whereas large numbers of the organism are usually isolated from pigs with necrotic enteritis (Songer and Uzal, 2005). The clostridial enteritis infections have a complex pathogenesis, thus the diagnosis cannot be based on mere isolation of the clostridia involved, and other factors should be taken into consideration to establish the final diagnosis (Songer and Taylor, 2006; Prodanov-Radulović et al., 2011; Prodanov-Radulović et al., 2013).

CONCLUSION

The early age at which this disease occurs, the rapid course, compatible clinical signs, mortality pattern and typical necropsy findings suggest the diagnosis, which can be readily confirmed by bacteriological examination. However, in some cases, diagnosis should be based upon findings in the herd as a whole rather than on the examination of individual diseased animals. The treatment is of little use in animals with clinical signs, and prophylaxis is the preferred approach. The disease can be effectively prevented by vaccination of pregnant sows, by re-evaluation and correction of the environmental conditions and management system since these factors may have considerable influence on the occurrence of the disease.

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REFERENCES

1. Baker A. A., Davis E., Rehberger T., and Rosener D.: Prevalence and Diversity of Toxigenic *Clostridium perfringens* and *Clostridium difficile* among Swine Herds in the Midwest. *Applied and Environmental Microbiology*, 76, 9, 2961–2967, 2010.
2. Gurtner **C.,** Popescu F., Wyder M., Sutter E.,Zeeh F., Frey J.,von Schubert C., Posthaus H.: Rapid Cytopathic Effects of *Clostridium perfringens* Beta-Toxin on Porcine Endothelial Cells. *Infection and Immunity*, 78, 7, 2966–2973, 2010.
3. Jackson P., Cockcroft P. D.: Clostridium perfringens type C infection (haemorrhagic enterotoxaemia). In: Handbook of Pig Medicine, Saunders El-

sevier Publishing, 2007, p. 89-91.
4. Jäggi M., Wollschläger N., Abril C., Albini S., Brachelente C., Wyder M., Posthaus H.: Retrospective study on necrotizing enteritis in piglets in Switzerland. *Schweiz Arch Tierheilkd.*, 151, 8, 369-375, 2009.
5. Hendriksen S.W., van Leengoed L.A., Roest H.I., van Nes A.: Neonatal diarrhea in pigs: alpha- and beta2-toxin produced by Clostridium perfringens. *Tijdschr Diergeneeskd.*,131, 24, 910-913, 2006.
6. Miclard J., Jäggi M., Sutter E., Wyder M., Grabscheid B., Posthaus H.: Clostridium perfringens beta-toxin targets endothelial cells in necrotizing enteritis in piglets, *Vet Microbiol*, 137:320-325, 2009.
7. Prodanov-Radulović J., Došen R., Stojanov I., Pušić I., Živkov-Baloš M., Ratajac R: Interaction between the mycotoxins and causative agents of swine infective diseases. Proc. Nat. Sci. Matica Srpska 120, 251-259, 2011.
8. Prodanov-Radulović J., Došen R., Stojanov I., Pušić I., Ratajac R.: The necrotic enteritis by *Clostridium perfringens* in suckling piglets: practical observations, control and diagnostics. In: editor in chief Milan Popović, Proceedings, The 23rd International Symposium 'New Technologies In Contemporary Animal Production', June 19-21, Novi Sad, Faculty of Agriculture, 2013, 180-182.
9. Songer J.G., Uzal F.A.: Clostridial enteric infections in pigs. *J Vet Diagn Invest*, 17, 528-536, 2005.
10. Songer J.G., and Taylor D.J.: Clostridial infections. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, Diseases of Swine, Iowa: Blackwell Publishing, 2006, p.613-628.
11. Springer S., Selbitz H.J.: The control of necrotic enteritis in sucking piglets by means of a *Clostridium perfringens* toxoid vaccine. *FEMS Immunology and Medical Microbiology* 24, 333-336, 1999.
12. van Asten J.A.M., Nikolaou G.N., Gröne A.: The occurrence of cpb2-toxigenic *Clostridium perfringens* and the possible role of the b2-toxin in enteric disease of domestic animals, wild animals and humans. *The Veterinary Journal*, 183,135-140, 2010.

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HEALTH CONTROL OF PIG HERDS ON COMMERCIAL FARMS

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Abstract

The concept of modern industrial production of pigs on commercial farms is based, among other things, on the implementation of biosecurity measures as well as solving problems of environmental protection, which greatly burden the production. It is well known that good health is a prerequisite for good pig reproduction, that is, a successful and profitable production. The health status of the herd depends on many factors such as the maintenance technology, nursing, nutrition, organization, level of staff training and systematic implementation of good health care policies. Today, we are witnessing high incidence of bacterial diseases, viral etiology and certain parasites that seriously affect the pig production in intensive farming conditions. Keeping such diseases under control is possible only by applying appropriate prophylactic and therapeutic measures, as well as by increased monitoring by professional services.

Keywords: swine, breeding diseases, reproduction, biosecurity,

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KONTROLA ZDRAVLJA STADA SVINJA NA KOMERCIJALNIM FARMAMA

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Kratak sadržaj

Koncept savremene industrijske proizvodnje svinja na komercijalnim farmama zasnovan je, između ostalog i na sprovođenju biosigurnosnih mera, kao i na rešavanju problema ekološke zaštite koji u velikoj meri opterećuju proizvodnju. Poznato je da dobro zdravlje svinja predstavlja preduslov dobre reprodukcije, odnosno uspešne i profitabilne proizvodnje u svinjarstvu. Zdravstveni status stada zavisi od velikog broja činilaca , kao što su su tehnologija držanja, nege, ishrane, organizacija, stepen obučenosti kadrova kao i sistematsko sprovođenje mera zdravstvene zaštite . Danas smo svedoci da veliki broj oboljenja bakterijske, virusne etiologije kao i pojedine parazitoze mogu ozbiljno ugroziti proizvodnju svinja u intenzivnom uzgoju. Ove bolesti moguće je primenom profilaktičkih i terapeutskih mera, kao i pojačanim nadzorom stručnih službi držati pod kontrolom.

Ključne reči: svinje, uzgojne bolesti, reporodukcija biosigurnost

INTRODUCTION

In intensive pig farming, is more valid parameters that can be used to show the success and profitability of production, such as the number of live or weaned piglets, length of fattening period, the number of non-reproductive days annual etc. Today it is common pig production on commercial farms would present a number of breeding piglets and fattening pigs per sow delivered during calendar year. The production parameters vary considerably between countries with more or less developed production pigs (Radojičić at al. 2002). To be able to work on improving the production of pigs on the farm it is important to assure good health of breeding sows and piglets in the first days after farrowing (Bojkovski et.al. 2005, 2011, 2013b).

In this review paper presents a summary of our research that are related to solving reproductive health and biosecurity issues on commercial swine farms as well as an overview of environmental contaminants that were present on farms with some of the possible solutions.

Flexible cooperation of farm breeding with professional services, with respect to the implementation of technical knowledge and application of range of biotechnical measures and putting the emphasis on prevention of disease in order to promote the good health of pigs. It is possible to improve the welfare of pigs an actual production (Hristov et. al. 2008)

COMMON HEALTH – REPRODUCTIVE PROBLEMS ON COMMERCIAL PIG FARMS

In intensive pig production, the control of the reproduction herd is the primary task. It is well known that in comparison to other breed of domestic animals, swine are characterized by a very high reproductive potential, given that the early sexually mature, have a high value of ovulation, the period of gestation and the lactation period is relatively short and can quickly establish a pregnancy after weaning the previous litter. From an economic point of view, proper, regular reproductive activity of pigs is of great importanceThe reproductive efficiency of a herd is usually estimated on the basis of: age of **female** animals at first farrowing, the length of their reproductive exploitation, the duration of the interval between individual farrowing, and the size of the litter at weaning. Reproductive activity of pigs is influenced by the number of factors including hereditary, endogenous (hormones , immunoglobulins , enzymes) a dn environmental ones, the presence of pathogens as well as management and production technology (Uzelac , Vasiljević , 2011.). Reproductive efficiency is further determined by the system of keeping, diet , season , farm location, microclimate, implementation of biosecurity measures , herd size , herd health status (presence of breeding , parasitic and infectious diseases), body score condition , and methods of artificial insemination (Lončarević et al . , 1997 Petrujkić et al . 2011).

Infertility is a common problem on commercial farms. The causes of infertility are diverse and numerous. Current problem at most of our farms is the emergence of seasonal infertility that is present during summer months and is a serious impediment to producers who want to maximize reproductive efficiency of the herd (Petrujkić et al., 2009, 2010 , 2011) . In this sense, in intensive pig production today much attention is paid to optimizing the microclimate conditions in housing facilities by using computerized ventilation systems and

automated equipment for air conditioning , lighting, feeding, manure disposal , etc. Programming of desired parameters provides favorable conditions for the animals and maximize expressing of their genetic potential and increase their reproductive productivity while greatly reducing the stress.

Adequate health care for farm animals , high level of hygiene of animals, vehicles and staff , as well as the strict application of all desired methods in the technology of artificial insemination are the primary requirements to accomplish high reproductive efficiency of breeding animals (Stančić et al . 2012).

Conventional assessment of semen quality in boars as an important segment in the technology of artificial insemination is widely practiced on our commercial farms. Classical procedure for evaluation of semen quality in condition of commercial breeding can identify the ejaculates with low fertilization potential; however , it did not prove effective in predicting fertility parameters in the field conditions (Tsakmakidis , 2011) . Therefore, in order to overcome the problem of infertility and to control the reproductive efficiency of pigs, a cooperation with the veterinary institute offers a range of novel laboratory methods such as motion estimation using computer analyzer (CASA) , automated sperm morphology analysis (ASMA) , flow cytometry for determination of chromatin integrity , HOS test etc. Thus, the fertility of boars can be continuously monitored enabling a prompt response to the immediate production. Technology of the preparation of heterosperm insemination doses involving sperm of two or more terminal boars has been applied in the process of artificial insemination at our commercial farms to produce a large number of piglets per sow (Vasiljević , 2012)

Use of deep- frozen semen is common on industrial swine farms . The advantage of frozen semen is its ability to preserve the genetic material for a longer period, and significantly reduce the risk of introduction of the disease in the herd (Stanković et al ., 2007). However, the deep freezing is not the part of common practice because of certain technological drawbacks of deep-freezing procedure and low rates of pregnancy and litter size (Vidović et al . , 2011).

The phenomenon of stress is also one of the serious problems on commercial farms. The farms that are still developing their management strategies have a mor pronounced problem with stress than farms with well-organized production system. The requirements of modern pig production today have reduced the stress to a minimum and provided maximum comfort for the animals (*welfare)* . In this regard, it is important to understand the mechanisms of adaptation syndrome and stress reactions. Providing adequate living conditions for the animals positively affects their productivity that reaches the expected and desired levels. The high level of corticosteroids in the blood of animals exposed

to stress results in the reduction in their resistance thus making them highly susceptible to various infections. Therefore, it is very important to improve the welfare of animals at farms through the development and promotion of human consciousness in view ofof the respect, care and responsibility towards animals. The application of technical and technological solutions in animal production can provide maximum comfort and convenience to animals.

Technology of feeding farm animals takes an important place in the prevention of stress and is a very important factor in maintaining good health and reproductive status . Fattened sows , which carry a large number of fetuses and consume large amounts of feed in facilities with increased humidity and temperature , are more susceptible to stress and often manifest signs of respiratory distress . It is one of the reasons for the introduction of new dietary guidelines according to specific production stages and animal categories. The dietary curve for breeding sows is precisely defined for each particular stage of production in order to enable early estrus after weaning of piglets , to maximize the number of ovulated and implanted embryos , increase the number of delivering live and vital piglets , and to increase the milk yield during lactation while preserving good condition and health status of female animals. All this would prolong the life and productivity of animals and decrease the administration of drugs. Thanks to this approach, modern commercial farms are characterized by 35 or more weaned piglets per sow per year.

Pig production on commercial farms is heavily burdened by a range of diseases affecting piglets . Piglet pathology is highly dynamic within the herd as a whole, as substantial agglomeration of animals in a limited space enhances both horizontal and vertical transmission of the infection. Intensive production conditions may result in the occurrence of so called production, i.e. technological diseases caused by certain microorganisms. Variations of pathogenic organisms that commonly affect piglets are of great importance , not only in view of their resistance to drugs , but because of their potential genetic recombination affecting the clinical picture and course of the disease that may complicate the diagnosis as well as the therapeutic and prophylactic management (Blackburn , 1995 Bojkovski et al . 1997, 2005) . The following diseases were observed on our pig farms: neonatal colibacillosis , edema disease , necrotic enteritis , dysentery, circovirus infection , and respiratory disease complex (PRDC) .

In recent years, massive outbreaks ofrespiratory disease complex (PRDC) were recorded on pig farms in both our country and worldwide, which is becoming a serious health problem at all technological stages of production . PRDC of pigs is a simultaneous infection of lung tissue with a number of respiratory

pathogens and is a common term for pig pneumonia characterized by multifactorial etiology. Isolated pathogens may vary between and within production herds (Honnold 1999 , Ivetić et al . 2005 Golinar et al . 2006) . The control of PRDC is difficult and complicated . The importance of respiratory disease complex is based on the interaction between multiple respiratory pathogens . Knowledge on such interactions must be taken into consideration in order to accomplish implementation of effective control measures . Respiratory disease of pigs develops as a consequence of the presence of living infective agents in the immediate surroundings of the animal or is due to a sudden drop of immunoprotective mechanism of the respiratory system (Ivetić et al. 2005). Contrary to the control of common diffuse infectious diseases of pigs that persist in our country and are encompassed in national legislation on mandatory suppression of infectious diseases, the detection and suppression of technopathies rather represents an economic need of the producers themselves.

CYTOGENETIC METHODS AS A PART OF BIOSECURITY PLANNING ON COMMERCIAL PIG FARMS

In modern pig production, thegenetics is applied with an aim of improving productive capacity of existing breeds on commercial farms by creating breed varieties with higher genetic potential, animal growing in pure breed or cross-breeding for commercial purposes. A part of our research was focused on the investigation of karyotype changes in intensive breeding. We established that karyotype changes may occur under the influence of chemical substances, which can originate from feed, water or the environment of the investigated animals (Bojkovski et.al.2010). Our recommendation to commercial-type farms and reperoduction and artificial insermination centers implicate application of the results of cytogenetic methods that enable detection of carriers of hereditary anomalies. Implementation of these methods into the biosecurity plans on the farms will positively affect the health status of the herd and thus improve the production results.

ECOLOGICAL ISSUES ON COMMERCIAL PIG FARMS

The presence of chemical pollutants (heavy metals) and their impact on animal health on commercial farms has been monitored for a prolonged period of time. Heavy metals, which react with organic molecules and alter their structure and function, are particularly hazardous for all living systems. Heavy metals are absorbed into the body via respiratory and digestive systems, and skin. The results of a several-year long research have pointed out the risk

of feed contamination with heavy metals and its deposition in the body of animals, with consequences on the health and reproductive capacity of domestic animals.

The toxicity of heavy metals results in the formation of free radicals by inhibiting the activity of antioxidant enzymes and glutathione oxidation, and resulting in the creation of malonyl - dialdehyde (MDA) as a marker of oxidative stress . Their toxicity is derived from the tendency to form covalent links with sulfhydryl groups of biomacromolecules, or displace certain cofactors thus inhibiting the activity of particular enzymes (Bojkovski et al . 2008a, b ,2010a).

Our recommendation for commercial farms is to apply measures for reducing the risk of toxic heavy metals , to implement multiple monitoring of the quality of raw materials and finished products as well as adequate protector of the toxic effects of these agents (Bojkovski et al , 2010b , c)

BIOSECURITY ON COMMERCIAL PIG FARMS

Biosecurity plans are critical in the prevention of disease and unwanted situations as well as in business improvement (Uhlehoop , 2007) . The global objective of contemporary swine production in developed countries is to prevent the entry of disease in the herd, that is, maximally protect pigs from the contact with infectious agents from the environment and prevent or minimize the transfer of pathogenic organisms between certain categories of animals within the herd.. Therefore, special attention is paid to technical solutions that enable protection of pig herds from harmful external influences. Such measures include the construction of a quarantine for newly purchased animals, establishment of a separate department for the delivery of animals and special entrance for the personnel organized in line with the prescribed hygienic measures and strictly defined behavior protocol. All measures aimed at protecting the herd from infection are known as biosecurity measures and include measures of external and internal biosecurity defined in a biosecurity protocol. Outside biosecurity measures include the multi-side housing system , access control (personnel , feed , equipment , materials, semen for artificial insemination, control of vehicles accessing the farm , control of rodents , insects and birds , entry protocols for the staff , control of animal delivery, disposal of dead animals , quarantine for newly purchased animals)and are aimed at preventing the transmission of infectious agents from the environment and other herds in the region. . Internal biosecurity pertains to procedures regulating personnel access and behavior on the farm (shower , farm clothing, circulation of people and animals through the farm, the use of tools and equipment for the work,

etc.) , applyingthe principle "all in-allout" protocols for cleaning , washing and disinfection as well as infection control applying a program of preventive and curative health care of animals (Uzelac , Vasiljević , 2011). Assessment of biosecurity based on relevant indicators should be a routinemechanism for the evaluation of biosecurity on farms, indicating the direction of future operations and, possibly, their improvement (Lončarević et al, 1997, Stankovićet al., 2008). For example, based on consideration of the failures in providing biosecurity Stanković and Hristov (2009) reported the level of biosecurity on one of the investigated pig farms to be 3.96 (very good) . This result indicates the current biosecurity status of the farm , but one should always keep in mind the mutual interaction and overall activity of all biosecurity parameters (Stanković and Hristov , 2009).

The farmers bear the primary responsibility to protect their herds in terms of introduction of the disease by applying movementcontrol, by following proper procedures for housing of particular animal groups and appropriate sanitation. The personnell on the farm and visitors must be aware of their role in maintaining safe health status on the farms (Stanković and Hristov , 2009) .

CONCLUSION

The aim of intensive pig farming on commercial farms is to produce a large number of weaned piglets and fatteners per sow per year . To achieve this goal, it is necessary to establish high reproductive efficiency of breeding animals . This can be achieved by adequate health care, modern technology and good organization of the production applying appropriate procedures in the technology of artificial insemination.

The main goal of modern production on commercial farms is to reduce the phenomenon of stress to the lowest possible level .

High-health status and health control applying appropriate healthcare programs along with preventive and curative measures and protocols for external and internal biosecurity are an imperative of modern pig farming.

Our recommendation for commercial farms and centers for the reproduction and artificial insemination is to apply cytogenetic methods that enable detection of carriers of hereditary anomalies.

In order to reduce the risk from the chemical pollutants, implementation of multiple monitoring of the quality of raw materials and finished products is highly required, as well as the application of adequate protective agents that minimize toxic effects of these pollutants .

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REFERENCES

1. Blackburn P.W.: Curent problems on a new approaches to pig health Chapter 7 In: The health of pigs By Jon Hill and David Sainsbury, Logman Veterinary Health Sourcies, 221-22,1995
2. Bojkovski J., Radojičić Biljana, Petrujkić B.: Savremeni aspekti u dijagnostici i terapiji uzgojnih bolesti svinja. Proceedings of workshop: "Clinica Veterinaria", Ohrid 3-7.09., 251-257. ,2005
3. Bojkovski J., Dobrić Đ., Erski-Biljić Milanka, Zakarija Dolores.: Rezistencija domaćih životinja na antibiotike i njena genetska osnova. U: I Simpozijum mutageneze, genotoksikologije, Zlatibor, 15-18 septembar. Zbornik kratkih sadržaja radova,C37,1997
4. Bojkovski J., Relić Renata, Hristov S., Stanković B., Savić B., Petrujkić T.: Contribution to knowledge of health, reproduction, biosecurity and ecological problems in intensive pig production. *Bulletin UASVM, Veterinary Medicine*, 67, 2, 37-39..
5. Bojkovski J., Radojičić B., Petrujkić T., Borozan S.: A contribution to insight of the most important etiological factors with influence of farm animal health in Serbia. In: Proceedings of the International Symposium on new research in biotehnology, Bucharest, Romania, Biotehnology, series F, Special volume, 101- 114, 2008a
6. Bojkovski J., Stanković B., Petrujkić T., Petrujkić B., Savić B., Đoković R., Pantić I. Turajačanin D.: Review of investigations on influence of environmental chemical contaminants on hereditary base and reproductive capacities of landras breed boars from pig farm, center for reproduction and artificial insemination and biosecurity measures in Serbia. *Lucrări stiinifice medicină veterinară Timisoara* , 18, 2, 25-33, 2010b
7. Bojkovski J., Petrujkić T., Stanković B., Petrujkić B.: Menadžment zdravlja svinja U: Zbornik referata i kratkih sadržaja 21. Savetovanje veterinara Srbije, sa međunarodnim učešćem, Zlatibor, Zbornik kratkih sadržaja, 83, 2010c
8. Bojkovski J., Radojičić B.,Petrujkić T., Borozan S.: Prilog poznavanju etioloških činiliaca koji utiču na zdravlje farmskih životinja. U: 20. saveto-

vanje veterinara Republike Srbije sa međunarodnim učešćem, Zlatibor , Zbornik referata i kratkih sadržaja , 405-412, 2008
9. Bojkovski J., Savić B., Rogožarski D.: Pregled uzročnika oboljenja svinja na farmama industrijskog tipa. U: Deveti simpozijum zdravstvene zaštite selekcija i reprodujkcije svinja, Srebrno jezero, Zbornik radova, 62-75, 2011
10. Bojkovski J.: Efekti faktora sredine na kariotipske varijacije sisara, doktorska diseertcija, Univerzitet u Beogradu, Fakultet veterinaraske medicine, 1994
11. Bojkovski J., Savić B., Rogožarski D., Stojanović D., Vasiljević T., Apić I., Pavlović I.: An outline of clinical cases of disease in pigs at commercial farms. In: Proceedings of 23th International symposium " New Technologies in Conteproary Animal Production", Novi Sad (Serbia) 19-21 jun, 163-166.,2013a
12. Bojkovski J**,** Rogožarski D, Vasiljević T,Stojanović D, Savić B, Pavlović I**,** Relić R, Janjušević J.: Morphological changes in the kidneys of pigs caused by ochratoxin-feeding on the slaughter house (case report) . *Bulltein USAVM, Veterinarinary Medicine*, 70, 2 195-197, 2013b
13. Golinar O.I., Valenčak Z.: Porcine respiratory disease complex(PRDC) in Slovenia.Proceedings In: The 19th Ineternational Pig Veterinary Society. Congres, Copenhagen, Denmark, 291, 2006
14. Hristov S., Stanković B., Relić Renata, Joksimović-Todorović Mirjana, Davidović, Vesna, Milojković D.: Urogenitalne infekcije priplodnih krmača. *Biotechnology in animal husbandry*, 22, 761 - 772.,2006
15. Hristov S., Stanković B., Relić Renata, Todorović-Joksimović Mirjana: Dobrobit i biosigurnost na farmama. *Biotechnology in animal husbandry*, 24 (spec.issue), 39-49., 2008
16. Honnold C.: Porcine respiratory disease complex , http//www. ces.purdue. edu/ pork health/caryhonnold.html (8 july 2008date last accessed) 1999
17. Lončarević A., Maričić Z., Tosevski J., Pavlović I.: Osnove sistematskog zdravstvenog nadzora i programiranje zdravstvene zaštite svinja u intenzivnom odgoju. U: A. Lončarević, Zdravstvena zaštita svinja u intenzivnom odgoju, Naučni institut za veterinarstvo Srbije, Beograd, 517-523,1997
18. Ivetić V., Žutić M., Savić B., Milošević B.: Kompleks respiratornih bolesti kod svinja dijagnostika i mere kontrole U: Zbornik radova i kratkih sadržaja17. Savetovanja veterinara Srbije sa medjunarodnim učešćem 7-10 septembar, 190-198, 2005
19. Petrujkić T., Bojkovski J., Petrujkić B.: Reprodukcija svinja, monografija, Naučni institut za veterinarastvo Srbije, Beograd, 2011
20. Radojičić, B., Đuričić B., Gagrčin M.: Epizootiološko-dijagnostički značaj

kontrole reperoduktivnog i respiratornog sindroma svinja, *Veterinarski glasnik*, 56,1-2,231-31, 2002
21. Stančić I., Radović I., Dragin S., Erdeljan M., Apić I.: Veterinarska i zootehnološka situacija u veštačkom osemenjavanju svinja na vojvođanskim farmama, *Savremena poljoprivreda*, 61, 1-2, 2012
22. Stanković B., Hristov S., Petrujkić T., Relić Renata, Petrović Milica., Todorović-Joksimović Mirjana, Davidović Vesna: Polno prenosive bolesti svinja. *Savremena Poljoprivreda*, 56, 1-2, 99-105. 2007
23. Stanković B., Hristov S., Petrujkić T., Todorović-Joksimović Mirjana, Davidović Vesna, Bojkovski J.: Biosigurnost na farmama svinja u svakodnevnoj praksi. *Biotechnology in animal husbandry*, 24, 601-608, 2008
24. Stanković B., Hristov S.: Najčešći propusti u obezbeđenju biosigurnosti na farmama goveda i svinja. *Zbornik naučnih radova Instituta PKB Agroekonomik*, 15, 3-4,103-110, 2009
25. Tsakmakidis I.: Komparacija predviđanja fertiliteta veprova in vitro probama i klasičnih metoda evaluacije semena. U: Zbornik radova IX Simpozijuma – Zdravstvena zaštita, selekcija i reprodukcija svinja, sa međunarodnim učešćem, Srebrno jezero,2011, 116. ,2011
26. Uhlepnhoop E.: Biosecurity planning for livestock farms. Dobrobit životinja i biosigurnost na farmama.U: 1. Međunarodna konfrencija o doborbiti i biosigurnosti na farmama u Srbiji, Zemun, 14 i 15 novembar, Poljoprivredni fakultet, Zemun, 227-237, 2007
27. Uzelac Z., Vasiljević T.: Osnove modernog svinjarstva. Petrovaradin, Futura,, 2011
28. Vasiljević, T.: Tehnologija pripreme heterospermnih doza semena nerastova i ostvareni rezultati na farmama. U: Zbornik radova X Simpozijuma – Zdravstvena zaštita, selekcija i reprodukcija svinja, sa međunarodnim učešćem, Srebrno jezero 2012, 96-110, 2012
29. Vidović V., Šubara V., Višnjić V, Punoš D.: Savremeno gajenje svinja, Novi Sad, Poljoprivredni fakultet, Departman za veterinarsku medicinu., 2011

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THE ROLE OF EFFLUX PUMP AND OTHER MECHANISMS OF ANTIMICROBIAL RESISTANCE TO (FLUORO)QUINOLONES IN EPIDEMIC ISOLATES OF *SALMONELLA* TYPHIMURIUM, *SALMONELLA* KENTUCKY AND *SALMONELLA* INFANTIS

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Abstract

This paper briefly describes mechanisms of resistance to fluoroquinolones in several worldwide distributed Salmonella clones. The isolates have acquired multiple resistance traits over the years due to extensive antibiotic treatment both in human and veterinary medicine. Except for the *Salmonella* Typhimuirum DT104 where efflux pump appears to play a major role in resistance to (fluoro)quinolones, in other serovars mutations on topoisomerase genes seem to firstly occur and have been primary mechanisms of resistance. Plasmid borne resistance is rarely detected but because of horizontal gene transfer needs to be recorded. Understanding the genetic events at the molecular level is crucial in epidemiology work and provides insight in spreading of resistance clones of *Salmonella*.

Key words: *Salmonella* Typhimuirum, *Salmonella* Kentucky, *Salmonella* Infantis, efflux pump, resistance, topoisomerase

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ULOGA EFLUKS PUMPE I DRUGIH MEHANIZAMA REZISTENCIJE NA FLUORO(HINOLONE) KOD EPIDEMIOLOŠKIH IZOLATA SALMONELLA TYPHIMURIUM, SALMONELLA KENTUCKY I SALMONELLA INFANTIS.

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Kratki sadržaj

U ovom radu kratko su opisani mehanizmi antimikrobne rezistencije na fluorohinolone kod nekoliko široko rasprostranjenih klonova Salmonela. Izolati su stekli multiplu rezistenciju tokom vremena usled prekomerne upotrebe antibiotika u humanoj i veterinarskoj medicini. Sa izuzetkom klona STDT104 kod kog efluks pumpa igra važnu ulogu u rezistenciji na FQ, drugi serotipovi rezistenciju primarno stiču mutacijama na topoizomeraznim genima. Plazmid posredovana rezistencija je retka kod Salmonela ali je izuzetno značajna zbog mogućnosti horizontalnog prenošenja gena. Razumevanje mehanizama rezistencije na molekularnom nivou je od vitalnog značaja u epidemiologiji zato što omogućava uvid u načine širenja klonova kod Salmonela.

Ključne reči: *Salmonella* Typhimuirum, *Salmonella* Kentucky, *Salmonella* Infantis, efluks pumpa, rezistencija, topoizomeraze

INTRODUCTION

Quinolone resistance and decreased susceptibility to fluoroquinolones in *Salmonella* arise as a consequence of treatment with antibiotics. Some of the most prominent and most frequent mechanisms of resistance of Salmonella are point mutations in quinolone resistance determining region (QRDR) of the *gyrA* gene. In Salmonella highly resistant to fluoroquinolones, mutations on *gyrB*, *parC* and *parE* genes have also been detected. The *gyrA* and *gyrB* genes encode GyraseA subunit, while *parC* and *parE* encode the topoisomerase

IV. These enzymes are of vital importance for bacterial DNA transcriptional process. Screening of mutations is performed by PCR along with sequencing of the QRDR region to detect non-synonymous substitutions.

Another relevant mechanism of resistance to quinolones in *Salmonella* spp. is plasmid mediated, that arises by production of a small protein termed QNR. The QNR is characterized by pentapeptide repeats, composed of 218 amino acids and it preserves Gyrase from quinolone action, without the influence on the function of the enzyme. Plasmid borne QNR proteins are rarely found in isolates highly resistant to quinolones (Hopkins et al., 2005, Velhner et al., 2012) although Kehrenberg et al. (2006) have found *qnrS* gene in plasmid pINF5 of the *S*. Infantis obtaining MIC to NAL 512 mg/L indicating its strain specific occurrence.

Significant semi-specific mechanism of antimicrobial resistance in Salmonella is tripartite efflux pump AcrAB-TolC, classified as a member of Resistance Nodulation Division Family (RND) of transporters, and is responsible for extruding antimicrobial agents and other toxic substances out of the cell. It is usually involved in mediating multidrug resistance in bacteria as a nonspecific response since its primary evolutionary role has been to excrete bile salts from bacterial cell (Piddock 2006).

The AcrAB efflux pump is constituted of three proteins that form the channel in the cytoplasmic membrane. This system is energy dependent and subsequently regulated by active transport. Efflux pump consists of: periplasmic accessory protein (AcrA), transporter protein (AcrB) and the outer membrane protein (TolC), encoded by genes termed *acrA*, *acrB* and *tolC*. The expression of the AcrAB is fine tuned by several global and local activators and repressors, encoded by *acrR, ramAR*, *soxRS* and *marRAB* regulatory genes (Piddock LJV, 2006, Abouzeed et al. 2008, Giraud et al., 2013).

The overexpression of efflux genes occurs if local or global repressor genes are mutated. Two experiments are conducted to reveal these processes at molecular level. Firstly, the overexperssion experiments were aimed at showing increased level of the mRNA of activator efflux genes, applying qRT-PCR or dot blot technique with anti polyclonal antibodies. Further, the sequencing of local and global repressor genes is performed to determine sequence alterations compared to wild type, which are responsible for deactivation of repressor genes and subsequent overexpression of major efflux pump regulatory genes.

This paper briefly describes the role of efflux mechanism as well as target gene mutations and plasmid borne resistance to (fluoro)quinolones in *Salmonella* spp., which is one of the most important food borne pathogens worldwide. Several different fluoroquinolone resistant strains of *Salmonella* spp. have been detected by now and it is vital to determine their most im-

portant antibiotic resistance mechanisms, since certain resistotypes are highly competitive in the conditions of extensive drug use.

Development of FQ resistance in Salmonella enterica serovar Typhimurium

Antibiotic susceptible *Salmonella* Typhimurium definite phage type 104 (STDT104) has been isolated from humans in England in 1960's. Multi-drug resistant STDT104 was firstly found in samples obtained from cattle in the UK in 1980 and in humans nine years later. Since then, STDT104 has spread worldwide and is considered an important pathogen in both human and veterinary medicine and it has been extensively studied over the years. This strain is multiple resistant to ampicillin (AMP), chloramphenicol (CHL), florfenicol (FLO), streptomycin (STR), spectinomycin (SPT), sulphonamides (SSS) and tetracyclines (TET). Resistance genes are clustered in salmonella genomic island 1 (SGI1) close to its 3'end. The SGI1 is located between *thdF* and *int2* genes bounded by 18 kb inverted repeats and it has been determined that insertion has been driven by site specific recombination. (Cloeckaert and Schwarz, 2001, Carattoli et al., 2002, Doublet et al., 2005).

Since 1993, STDT104 in farm animals has also acquired additional mechanisms of resistance to quinolones due to the therapy with enrofloxacin. Resistance to quinolones and decreased fluoroquinolone susceptibility is primarily attributed to active efflux as well as to mutations on topoisomerase genes, mainly the *gyrA* gene (Giraud et al., 2000, Threlfall, 2000). Experiments on mutant cells produced by disruption of *acrB* gene or homologous recombination to obtain transformants with deleted *tolC* gene have shown that efflux mechanism plays an important role in resistance to quinolones but also to CHO, FLO and TET. Baucheron et al. (2004) demonstrated that multiple drug resistance to TET, CHO, FLO is driven not only by overexpression of *floR* and *tet(G)* pump genes but is also highly dependent on AcrAB-TolC efflux system. Briefly, specific transporters Flo(R) and Tet(G) deliver antibiotics from cytoplasmic membrane to the periplasm while AcrB recognize the substrate (antibiotic) and with TolC extrude the compounds out of the cell. Simultaneous action of both types of transporters induce higher levels of resistance to FLO, CHL and TET through cumulative effect of multicomponent AcrAB efflux pump and major facilitator superfamily (MFS) transporters.

During the period 1991-1995, highly ciprofloxacinresistant STDT204 var Copenhagen has been isolated from animals and humans in several countries and it was discovered that multiple point mutations on topoisomerase genes are associated with resistance to fluoroquinolones. The mutations were found on *gyrA* Ser83®Ala, Asp87®Asn, on *gyrB* Ser464®Phe and on *parC* gene, Ser80®Ile (Heisig et al, 1995, Guerra et al., 2003).

However, different isolates have shown different levels of MIC values of quinolones even in cases where isolates have carried identical mutations on topoisomerase genes. Decreased accumulation may in part account for decreased susceptibility and is mediated by overexpression of AcrABTolC efflux pump system. Additionally, in *acrB* inactivated mutants of the STDT204, the role of another efflux pump termed AcrEF has been discovered. AcrAB and AcrEF are characterized by high sequence homology and it appears that if *acrB* gene is deleted the expression of other efflux pump genes (*acrD* and *acrF*) could potentially be increased. Also, the insertion sequences (IS*1* or IS*10*) were detected upstream of *acrEF*. These insertion sequences contain putative promoters and increase the expression of the *acrEF* genes while the expression of the putative local repressor *acrS* is not affected.

Subsequently it is believed that high resistance to fluoroquinolones in STDT 204 is developed by multiple target mutations on topoismerase genes as well as on global regulatory genes of efflux pump systems (Olliver et al., 2005, Giraud et al., 2006).

FQ resistance of *Salmonella enterica* serovar Kentucky is strain-specific.

During the period of five years (2000 - 2005) in France, *Salmonella* Kentucky ST198, CIP^r (SKST198) was isolated from travelers who have returned from northeast and east Africa (Weill et al., 2006). In the year 2003, it caused nosocomial outbreaks in two hospitals in Slovakia and in 2005 it was isolated from a patient who returned to Belgium after visiting Libya. Since then, SKST198 has been sampled in Europe mostly from the travelers. In animals, *S.* Kentucky commonly infects poultry and turkey and these isolates usually harbor the resistance to (fluoro)quinolones (Le Hello et al., 2011, LeHello et al., 2013). Some strains feature salmonella genomic island 1 (SGI1), carrying class 1 integrons containing several antibiotic resistant gene cassettes. SGI1 is considered a non-replicative element, which integrates into a chromosome by recombination and it has been experimentally demonstrated that SGI1 could be conjugally transferred to recipient strain from chromosome to chromosome by a helper plasmid (Doublet et al., 2005). Several SGI1variants have been detected in S. Kentucky and subsequently labeled SGI1-A to - O. SGI1-K, P and Q variants are characterized by the presence of IS26 elements, which are often associated with antibiotic resistance genes and transposon structures which facilitate horizontal gene transfer among bacteria (Doublet et al. 2008). These particular mechanisms of genetic transfer and different integration patterns could possibly induce spread of one of the most important epidemic clones - SKST198.

The efflux mechanism in *S.* Kentucky has been studied by Baucheron et al., (2013). Out of 27 isolates collected from all over the world, the mutations on

repressor *ramR* gene were found in only three of them. Function of RamR has been affected by the presence of frame shift GATC duplication, frame shift G insertion and 91 bp deletions in the *ramR* gene. These genetic events induce 2 to 4 fold increase of resistance to fluoroquinolones due to increased expression of the AcrAB-TolC efflux system.

Monoclonal spread of *Salmonella enterica* serovar Infantis

Salmonella Infantis (SI) is most frequently found in broiler chickens and may present serious food contaminant. The presence of SI is usually recorded in farm animals (mainly poultry) and humans and it has become a very important pathogen worldwide. Moreover, similar or identical pulsotype and MLST (multi locus sequence type) has been described in studies from Japan (Shahada et, al., 2006), Israel (Gal More et al., 2010), Hungary (Nógrády et al., 2007), Germany (Hauser et al., 2012) and Serbia (Velhner et al., 2014). On the other hand, multidrug resistant SI subclone was identified in Germany and Hungary. It is believed that a clonal spread of a single clonal lineage has arisen as a result of a mechanisms, which protect the serovar from genetic rearrangements or horizontal gene transfer (Hauser et al., 2012).

Another mechanism of drug resistance of SI is plasmid mediated, obtained by the presence of *qnrS* gene (Kehrenberg et al., 2009). However, resistance to (fluoro)quinolones is mainly attributed to mutations on topoisomerase genes. Antimicrobial effect of fluoroquinolones arises as a result of interference with topoismerase enzymes thus preventing replication of bacterial genome. Mutation on topoismerase genes as well as the small protein QNR prevents binding of fluoroquinolone molecules to topoisomerase enzymes.

The role of active efflux was also documented in some strains of SI described by Velhner et al. (2014). Namely, eight SI isolates out of 64 harboring resistance to quinolones has shown 5-6 fold decrease in MIC concentration to NAL in the presence of Phe-Arg-β-Naphthylamide (PAβN), efflux pump inhibitor (EPI). There is also evidence which implicate that overexpression of AcrABTolC efflux pump system may increase resistance to fluoroquinolones in SI.

The role of global regulators in expression of efflux pump in Salmonella was studied by Kehrenberg et al., (2009) in detail. The authors have developed *in vitro* mutants after exposing S. Infantis and several other serotypes to ciprofloxacin 1-128x of the MIC. MIC of the bacteria that have already shown resistance did not increase and no additional mutations compared to parent strain were found on topoisomerase genes. However, previously susceptible strains have developed mutations on *gyrA* and elevated MICs to NAL and CIP. In mutants obtained *in vitro*, mutational changes in regulatory genes of the efflux

pump were detected on *ramRA* and *soxRS*. Increased expression of *ramA* (the activator gene) in *S*. Infantis (mutant 2) has arisen due to 10 bp deletion that created early stop codon in *ramR* gene. Several other mutational types have been also detected in *ramR* gene and its flanking regions, causing the up-regulation of *ramA* gene and consequently overexpression of the efflux pump, in mutants of *Salmonella* serovars other than Typhimuirum.

Involvement of *soxRS* system in efflux genes expression in salmonella may not be very frequent but is very contributive fluoroquinolone resistance mechanism. It has been described in different *Salmonella* serovars, but the 49 bp insertion in *soxR* in *S.* Virchow 2 mutant (strain 1) generated an early stop codon at *soxR* gene, resulting in significantly higher upregulation of *soxS* than previously detected *soxR* mutations. Conclusively, upregulation of the AcrAB efflux system is highly dependent on slight alterations of expression of *soxRS* genes and may play important role in FQ resistance in *Salmonella* spp.

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REFERENCES

1. Abouzeed Y.M., Baucheron S., Cloeckaert A.: *ramR* mutations involved in efflux-mediated multidrug resistance in *Salmonella enterica* serovar Typhimurium. *Antimicrob Agents Chemother,* 52, 2428-2434, 2008.
2. Baucheron S., Tyler S., Boyd D., Mulvey M.R., Chaslus-Dancla E., Cloeckaert A.: AcrAB-TolC directs efflux-mediated multidrug resistance in *Salmonella enterica* serovar Typhimurium DT104, *Antimicrob Agents Chemother*, 48, 3729-3735, 2004.
3. Baucheron S., Le Hello S., Doublet B., Giraud E., Weill F.X., Cloeckaert A.: ramR mutations affecting fluoroquinolone susceptibility in epidemic multidrug-resistant *Salmonella enterica* serovar Kentucky ST198. *Front Microbiol*, 4, 1-6, 2013.
4. Caratolli A., Fietici E., Villa L., Dionisi M., Ricci A., Luzzi I.: Antibiotic resistance genes and Salmonella genomic island 1 in *Salmonella enterica* serovar Typhimuirum isolated in Italy. *Antimicorb Agents Chemother*, 46, 2821-2828, 2002.
5. Cloeckaert A., Schwarz S.: Molecular characterization, spread and evolu-

tion of multidrug resistance in *Salmonella enterica* Typhimuirum DT104. *Vet Res*, 32, 301-310, 2001.
6. Doublet B., Boyd D., Mulvey M.R., Cloeckaert A.: The *Salmonella* genomic island 1 is an integrative mobilizable element. *Mol Microbiol*, 55, 1911-1924, 2005.
7. Doublet B., Praud K., Bertrand S., Collard J.M., Weill F.X., Cloeckaert A.: Novel insertion sequence and transposon-mediated genetic rearrangements in genomic island SGI1 of *Salmonella enterica* serovar Kentucky. *Antimicorb Agents and Chemother*, 52, 3745-3754, 2008.
8. Gal-Mor O., Valinsky L., Weinberger M., Guy S., Jaffe J., Schorr Y.I., Raisfeld A., Agmon V., Nissan I.: Multidrug-resistant *S. enterica* seorvar Infantis, Israel. *Emerg Infecti Dis*, 16, 1754- 1757, 2010.
9. Giraud E., Cloeckaert A., Kerboeuf D., Chaslus-Dancla E.: Evidence for active efflux as the primary mechanism of resistance to ciprofloxacin in *Salmonella enterica* serovar Typhimurium. *Antimicrob Agents and Chemother*, 44, 1223-1228, 2000.
10. Giraud E., Baucheron S., Cloeckaert A.: Resistance to fluoroquinolones in *Salmonella*: emerging mechanism and resistance prevention strategies. *Microb Infect* 8, 1937-1944, 2006.
11. Giraud E., Baucheron S., Virlogeux-Payant I., Nishino K., Cloeckaert A.: Effects of natural mutations in the *ramRA* locus on invasiveness of epidemic fluoroquinolone-resistant *Salmonella enterica* serovar Typhimuirum isolates. *J Infect Dis* 207, 794-802, 2013.
12. Guerra B., Malorny B., Schroeter A., Helmuth R.: Multiple resistance mechanisms in fluoroquinolone-resistant *Salmonella* isolates from Germany. *Antimicrob Agents Chemother*, 47-2059, 2003.
13. Hauser E., Tietze E., Helmuth R., Junker E., Prager R., Schroeter A., Rabsch W., Fruth A., Toboldt A., Malorny B.: Clonal dissemination of *Salmonella enterica* serovar Infantis in Germany. *Foodborne Pathog Dis*, 9, 352-360, 2012.
14. Heisig P., Kratz B., Halle E., Gräser Y., Altwegg M., Rabsch W., Faber JP:Identification of DNA Gyrase A mutations in ciprofloxacin-resistant isolates of *Salmonella* typhimuirum from men and cattle in Germany. *Microb Drug Res*, 1, 211-218, 1995.
15. Hopkins K.L., Davies R.H., Threlfall E.J.: Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: Recent developments. *Int JAntimicrob Agents*, 25, 358-373, 2005.
16. Kehrenberg C., Friederichs S., de Jong A., Brenner Michael G., Schwarz S.: Identification of the plasmid-borne quinolone resistance gene *qnrS* in *Sal-*

monella enterica serovar Infantis. *J Antimicrob Chemother*, 58, 18-22, 2006.
17. Kehrenberg C., Cloeckaert A., Klein G., Schwarz S.: Decreased fluoroquinolone susceptibility in mutants of *Salmonella* serovars other than Typhimuirum: detection of novel mutations involved in modulated expression of *ramA* and *soxS*. *J Antimicorb Chemother*, 64, 1175-1180, 2009.
18. Le Hello, Hendriksen R.S., Doublet B., Fisher I., Møller Nielsen E., Whichard J.M., Bouchrif B., Fashae K., Granier S.A., Jourdan-Da Silva N., Cloeckaert A., Threlfall E.J., Angulo F.J., Aarestrup F.M., Wain J., Weill F.X.: International spread of an epidemic population of *Salmonella enetrica* serotype Kentucky ST198 resistant to ciprofloxacin. *J Infect Dis*, 204, 675-684, 2011.
19. Le Hello S., Bekhit A., Granier A., Barua H., Beutlich J., Zajac M., Münch S., Sintchenko V., Bouchrif B., Fashae K., Pinsard J.L., Sontag L., Fabre L., Garnier M., Guibert V., Howard P., Hendriksen R.S., Christensen J.P., Biswas P.K., Cloeckaert A., Rabsch W., Wasyl D., Doublet B., Weill F.X.: The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. *Front Microbiol*, 4, 1-10, Article 395, 2013.
20. Nógrády N., Tóth A., Kostyák A., Pászti J., Nagy B.: Emergence of multidrug-resistant clones of *Salmonella* Infantis in broiler chickens and humans in Hungary. *J Antimicrob Chemother*, 60, 645-648,2007.
21. Olliver A., Vallé M., Chaslus-Dancla, Cloeckaert A: Overepression of the multidrug efflux operon *acrEF* by insertional activation with *IS1* or *IS10* elements in *Salmonella enterica* serovar Typhimurium DT204 *acrB* mutant selected with fluoroquinolones. Antimicorb Agents Chemother, 49, 289-301, 2005.
22. Piddock L.J.V.: Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev*, 19, 382-402, 2006.
23. Shahada F., Chuma T., Tobata K., Okamoto K., Sueyoshi M., Takase K.: Molecular epidemiology of antimicrobial resistance among *S. enterica* serovar Infantis from poultry in Kagoshima Japan, Int J Antimicrob Agents, 28, 302-307, 2006.
24. Threlfall E.J.: Epidemic *Salmonella typhimuirum* DT104 - a truly international multiresistant clone. *J Antimicrob Chemother*, 46, 7-10, 2000.
25. Velhner M., Kozoderović G., Jelesić Y., Stojanov I., Dubravka Potkonjak, Jelena Petrović: Plasmid mediated resistance to quinolones in *Salmonella*. *Arhiv Vet Med*, 5, 19-29, 2012
26. Velhner M., Kozoderović G., Grego E., Galić N., Stojanov I., Jelesić Z., Kehrenberg C.: clonal spread of *Salmonella enterica* serovar Infantis in Serbia:

Acquisition of mutations on topoisomerase genes *gyrA* and *parC* leads to increased resistance to fluoroquinolones. *Zoonoses Public Hlth*, 6, 364-370, 2014

27. Weill F.X., Bertrand S., Guesnier F., Baucheron S., Grimont P.A.D., Cloeckaert A.: Ciprofloxacin resistant *Salmonella* Kentucky in travelers. Emerg Infect Dis, 12, 1611-1612, 2006.

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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.

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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.

Address of the journal

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