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INVESTIGATION OF QUANTITY AND CHEMICAL CHARACTERISTICS OF BIGHEAD CARP *(Aristichthys nobilis)* BY-PRODUCTS

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Abstract

Bighead carp (*Aristichthys nobilis*) successfully grow in polyculture with carp, feeding on plankton. It has good quality of meat for both consumption and industrial processing. The quantity of by-products obtained during primary processing of bighead carp and chemical characteristics of internal organs were investigated in this paper. The total average weight of by-products was 760.45 g (42.31%) in relation to live body weight, which was cca 1797.5 g. The by-product contributing to the largest quantity to total live body weight was the head with 529.39 g (29.45% of live body weight), followed by complete internal organs and tail and fins, which weighed 137.67 g (7.66%) and 68.82 g (3.83%), respectively Chemical composition of internal organs of bighead carp was mostly water (60.99%), following by crude fats and crude proteins (21.20% and 10.61%, respectively). The low collagen content (15.25% of total crude protein) indicates the high nutritional quality of protein content from internal organs. Nitrogenous complexes from the internal organs were predominantly proteins. Digestible nitrogen was approximately equal to total nitrogen (92.04%), indicating that all proteins of the internal organs had high biological value. Based on the results obtained, it can be concluded that bighead carp internal organs could be important sources of proteins and fats, and thus, could be used in Serbia as a raw material for feed and technical fat production.

Key words: bighead carp by-products, quantity, chemical characteristics

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ISPITIVANJE KOLIČINA I HEMIJSKIH KARAKTERISTIKA SPOREDNIH PROIZVODA TOLSTOLOBIKA *(Aristichthys nobilis)*

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Kratak sadržaj

Sivi tolstolobik (*Aristichthis nigra*) uspešno raste u polikulturi sa šaranom, hrani se planktonima. Meso ove ribe je izvrsnog kvaliteta i veoma pogodno za industrijsku preradu. U ovom radu ispitivana je količina sporednih proizvoda dobijenih tokom klanja i primarne obrade sivog tolstolobika i hemijske karakteristike unutrašnjih organa. Prosečna ukupna masa sporednih proizvoda bila 760,45 g ili 42,31% u odnosu na masu žive ribe, 1797.5 g. Najveću masu imale su glave 529,39 g ili 29,45% u odnosu na masu žive ribe, slede unutrašnji organi (137,67 g or 7,66%) i masa repa i peraja (68,82 g or 3,83%). Hemijski sastav unutrašnjih organa sivog tolstolobika u osnovi sastoji se uglavnom od vode (60.99%), nakon čega sledi udeo sirovih masti (21,20%) i sirovih proteina 10,61%. Nizak sadržaj kolagena (15,25%) u ukupnim sirovim proteinima ukazuje na visoku hranljivu vrednost proteina unutrašnjih organa. Azotni kompleks ispitivane sirovine pretežno se sastoji od proteina. Svarljivost azota je približno jednaka sa ukupnim azotom (92,04%), što ukazuje da proteini unutrašnjih organa imaju visoku biološku vrednost. Na osnovu dobijenih rezultata može se zaključiti da unutrašnji organi sivog tolstolobika predstavljaju značajan izvor proteina i masnih kiselina, i tako se mogu koristiti kao sirovina za proizvodnju hrane za životinje i tehničke masti.

Ključne reči: sivi tolstolobik, sporedni proizvodi, hemijske karakteristike

INTRODUCTION

The aquaculture industry has grown rapidly over the last decade. Likewise, mariculture is expanding worldwide thereby increasing the demand for feed ingredients to support production (FAO, 2014). The rapidly growing sector directly depends on the aquafeed industry, which in turn largely depends on fish meal as its primary protein source.

Over the last decade, the global supply of fish meal has been limited, and meeting the demands of a growing industry has become challenging. In addition, fish meal proteins experience periodic fluctuations in pricing and availability (Tacon et al., 2008).

Freshwater fish contain high levels of PUFA, which makes them very important in human nutrition (Vladau et al., 2008). Since there are a number of biochemical interactions between the n-6 and n-3 series fatty acid, a balanced ratio between these fatty acids in the food is important for the normal functioning of the body in humans, as well as in animals (Đorđević et al., 2009). Consumption of fish meat is increasing, due to its high content of polyunsaturated fatty acids (PUFA), amino acids and lipid soluble vitamins which are important ingredients for human health. According to the latest data from FAO (Ćirković et al., 2012) the Republic of Serbia is a country where the average consumption of fish is 5-10 kg per capita per year, which is significantly below the European and global consumption (Ćirković et al., 2011).

Common carp is economically the most significant farmed fish species on the fish farms in Serbia (FAO, 2012), and the cyprinids are the most common species in the total world production of freshwater fishes (71.9%, 24.2 million tons in 2010) (Ćirković et al., 2012). Bighead carp (as well as grass carp) was imported in our country as a regulator of hydro vegetation. It feeds on higher underwater plants and its meat is of good quality (Vujković et al., 1993).

Manufacturing and development of fish products could increase the offer and contribute to better sale of fish, not only in traditional fish markets, but also in retail stores and supermarkets. Technological processes, preservation and storage of fish meat differ from those characteristic for mammalian meat (Okanović et al., 2015). For proper manufacturing of fish products, knowledge about chemical composition and characteristics of raw fish meat is of highest importance in order to apply the most appropriate technology procedures adjusted to individual fish species.

Fish processing and development of new fish products can provide novel sale of fish, not only in traditional fish markets, but also in all other consumer goods stores (Baltić et al., 2009). The demands of modern markets are increas-

ingly directed towards processed fish, especially fillets. Larger quantities of edible and non-edible by-products are obtained in industrial conditions of primary fish processing (Ristić et al., 2002).

Fish yield, expressed as the ratio of the weight of the carcass without the head, scales, fins and internal organs and whole fish mass, are essential parameters for all technological operations related to fish processing, since the economy of production is directly dependent on it (Ljubojević et al., 2012).

On the basis of some previous research, it is well-established that by-products of bighead carp processing contain valuable nutrients, which may be sources for the food, pharmaceutical and feed industry (Ristić et al., 1992). In order to obtain more complete perception of the quality of animal by-product, it is necessary, in addition to knowledge of basic chemical composition, to obtain complete information on the quality of the most important nutritional components - proteins. However, the high crude protein content of some raw materials does not guarantee its high usability, ie. protein digestibility (Ristić et al., 2011).

Inedible by-products obtained during bighead carp slaughter belonging to the third category of by-products (Regulation EC, 2009) are important sources of proteins and fats that represent convenient raw materials for processing into proteinaceous feeds for swine and pets.

Due to the increasing industrial grass carp processing and need for complex utilization of obtained by-products, the aim of this research was to investigate the quantity of by-products and nutritive value of internal organs.

MATERIALS AND METHODS

The quantity of by-products and quality of internal organs were monitored during fish harvesting and processing of bighead carp from fish ponds in Vojvodina in industrial conditions.

Bighead carp from fish ponds Ečka were delivered live from fish farm to a manufacturing plant where they were immediately sacrificed. Mean values of bighead carp mass were approximately 1800 g. Scales, gills and viscera, heads (flat transverse incision just behind the gill arch) and the fins were removed with a knife (Photograph 1). The following values were measured: weight of fish before the cutting, meat head, mass of the tail and fins and mass of total internal organs. The total internal organs are not separated because in industrial conditions it is standard procedure.

Figure 1. Cuting of bighead carp

Investigation of chemical characteristics of internal organs were performed in the laboratory of the Institute of Food Technology in Novi Sad. Slaughtered bighead carp, according to the structure of the by-products, were used as one sample (was one set of internal organs from one fish) for further investigations. The samples composed in such a way were put in plastic bags, labeled and regrigerated at about 4°C. Four hours after the slaughter, samples were transferred into the chemical laboratory. All samples were ground with a homogenizer prior to examination and used for determining the chemical parameters. The samples were packed into aluminium foil bags and stored for 24 h at +4°C.

The basic chemical composition was assessed by determining moisture content (SRPS ISO 1442, 1997), total protein content (SRPS ISO 937, 1992), hydroxyproline content - the relative content of connective tissue proteins content (SRPS ISO 3496, 2002), free fat content (SRPS ISO 1444, 1997), and total ash content (SRPS ISO 936, 1998). Nitrogen fractions and digestible nitrogen were determined according the AOAC methods for free fat content (AOAC, 1998).

To the purpose of appropriate interpretation of the obtained data they were statistically evaluated (Stat Office RS, 2014) using calculations of arithmetic mean ($\bar{X}$), standard deviation (SD) and coefficient of variation (CV).

RESULTS AND DISCUSSION

The quantities of by-products obtained from bighead carp processing are presented in Table 1. After cutting-off the head, tail and fins and removal of complete internal organs, average carcass weight was 1013.55 g (56.39%) of total live body weight. Routine removal of skin, bones, spinal and rib carcass produced an average fillet weight of 817.29 g (45.47%) and the bones with the remains of the corresponding meat. Total average weight of by-products was 760.45 g (42.31%) in relation to livebody weight which was cca 1797.5 g. The by-product with the largest proportion of total live body weight was the head with 529.39 g (29.45%), followed by the whole internal organs and the tails and fins which had weight 137.67 g (7.66%) and 68.82 g (3.83%), respectively.

The head weight largely depends on the processing method (straight or round cut behind the gills). In the research of Tumbas and Petrović (1978), the head obtained with a circular cut was 11% of the live body weight. The weight of tail and fins 99.15 g (5.11%) was smaller than the weight of heads. Total internal organs weighed 143.77 g (12.22%). According to Ristić et al. (1992) bighead carp by-products percentages ranged from head 19.79%, tail and fins 3.09% and total internal organs 9.47%.

Table 1. Quantity of bighead carp by-product, g and %*

	$\overline{X}$	SD	CV	%
	1797.50	**98.23**	**5.46**	**100.00**
Scales	24.57	2.49	10.13	1.37
Head	529.39	31.65	5.98	29.45
Tail and fins	68.82	5.11	7.43	3.83
Total internal organs	137.67	12.22	8.88	7.66
Total by-products	**760.45**	**51.32**	**6.75**	**42.31**
Fillet	817.29	75.33	9.22	45.47
Bones and skin	196.26	19.56	9.97	10.92
Carcass	**1013.55**	**83.14**	**8.20**	**56.39**

* % according to bighead carp live weight

Results of the chemical composition of the internal organs are shown in Table 2.

Basic chemical composition showed that this raw material, apart from

water (60.99%), contained mostly crude fat (21.20%) and then crude proteins (10.61%). The low proportion of collagen (15.25%) in the total crude protein indicates the high nutritional quality of the protein.

In the research of Ljubojević et al. (2013), the fat content in the bighead carp ranged from 2.3 to 16.8%, while the protein content was less variable and generally was in the range from 14 to 18%

Table 2. Chemical composition of bighead carp internal organs, %

Parameter	$\overline{X}$	SD	CV
Moisture	60.99	4.53	7.43
Crude protein	10.61	0.96	9.06
Relative content of connective tissue proteins	15.25	1.21	7.93
Crude fat	21.20	2.35	11.08
Ash	0.85	0.06	6.85
N-free extract	6.35	2.43	38.19
Non-protein N	0.79	0.11	24.70
Protein digestibility	92.04	0.92	1.00

As seen in Table 2, nitrogen complex of bighead carp internal organs was composed mostly of protein. The high digestibility of the protein (92.04%) indicates the high biological value of proteins of the internal organs. In the research of Ristić et al. (2002) it has been shown that a set of internal organs of bighead carp contained a higher share of fat (21.20%) as well as protein with good digestibility (92.04%).

CONCLUSIONS

Based on the results obtained in this study it is possible to conclude the following:

- After cutting-off the head, tail and fins and removal of complete internal organs the average carcass weight was 1013.55 g (56.39%) of the live weight.
- The total average weight of by-products was 760.45 g or 42.31% in relation to live body weight (cca 1797.5 g).
- The head had the largest proportion of weight in relation to live weight, being 529.39 g (29.45%) of live weight. Weights of tail and fins were much smaller,

being 68.82 g (3.83%). The total internal organs weighed 137.67 g (7.66%).
- Chemical analyses revealed that the internal organs, apart from water, contained significant amounts of crude fat (21.20%) and protein (10.61) making them suitable for feed processing.
- The amount of digestible nitrogen in the internal organs was approximately equal to total nitrogen (92.04%), indicating that all proteins from the internal organs have high biological value.
- Inedible internal organs obtained during bighead carp slaughtering could be an important source of nutritive components and could be used as raw material for processing into feeds for use in animal nutrition.

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EPIZOOTIOLOGICAL SITUATION OF AFRICAN SWINE FEVER IN EUROPE

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Abstract

African swine fever (ASF) is a viral disease of domestic pigs and wild boar. Due to the very serious socioeconomic consequences, the disease is one of the most important ones nowadays. African swine fever is an enzootic disease in many countries in Sub-Saharan Africa, in Sardinia, and Trans Caucasus countries. After its occurrence in Georgia in 2007, ASF spread to Armenia and Russian Federation, and in 2008. to Azerbaijan. Since then, its progressive moving toward the west has been recorded. Despite the number of undertaken preventive and control measures in the European Union (EU), ASF has been still spreading. During 2017, the disease has been reported in domestic pigs in Estonia, Italy-Sardinia, Latvia, Lithuania, Poland, Romania, and Ukraine. ASF cases in domestic pigs have also been reported in Moldova in 2017. The number of diagnosed cases in wild boar in 2017 is much higher than in domestic pigs. ASF outbreak in wild boar in the Czech Republic well describes the possible viral „jump" into a new region. The source of infection hasn't been confirmed yet, but it is common that such leaps are due to either swill feeding or improperly disposal of food rather than to the animal movements. Since the lack of effective vaccine makes eradication even more difficult, the prevention of viral entry into the new areas is of the most importance. With the same aim, since 2011.the surveillance of ASF has been implemented in Serbia.

Key words: African swine fever, domestic pigs, wild boar

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EPIZOOTIOLOŠKA SITUACIJA AFRIČKE KUGE SVINJA U EVROPI

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Kratak sadržaj

Africka kuga svinja (AKS) je virusna bolest domaćih i divljih svinja. Socioekonomske posledice ove bolesti svrstavaju je u najznačajnije bolesti današnjice. Africka kuga svinja je enzootska bolest u mnogim zemljama južno od Sahare, na Sardiniji i Kavkazu. Pošto se pojavila 2007. godine u Gruziji, AKS se iste godine proširila na Jermeniju i Rusiju, a 2008. na Azerbejdžan. Od tada se beleži progresivno kretanje virusa ka zapadu. Uprkos svim preventivnim i kontrolnim merama koje se sprovode u Evropskoj uniji (EU), africka kuga svinja se i dalje širi. Tokom 2017. godine kod domaćih svinja je dokazana u Estoniji, Italiji – Sardinija, Letoniji, Litvaniji, Poljskoj, Rumuniji i Ukrajini. Slučajevi AKS kod domaćih svinja u Moldaviji su takođe registrovani i u 2017. godini. Broj dijagnostikovanih slučajeva kod divljih svinja u 2017. je značajno veći u odnosu na broj slučajeva kod domaćih. Pojava AKS u Češkoj 2017. godine kod divljih svinja predstavlja veliki „skok" virusa u novo područje. Izvor infekcije još uvek nije potvrđen, ali je uobičajeno da se ovakve pojave dešavaju kao posledica hranjenja životinja ostacima hrane, a ne zbog kretanja životinja. Budući da je iskorenjivanje AKS veoma otežano u odsustvu efikasne vakcine, prevencija unosa virusa u nova područja je od najvećeg značaja. Sa tim ciljem, u Srbiji se od 2011. godine sprovodi nadzor kod divljih svinja na africku kugu.

Ključne reči: africka kuga svinja, domaće svinje, divlje svinje

INTRODUCTION

African swine fever (ASF) is a viral disease of domestic pigs and wild boar. Due to the very serious socioeconomic consequences, the disease is one of the most important ones nowadays. Clinical symptoms vary depending on viral virulence and immune status of the host.

In European both domestic and wild pigs, ASF usually has the acute course with mortality 95-100% (Gallardo et al., 2015). Unlike in European species, in

African wild suids, the disease is subclinical and asymptomatic enabling them to be the reservoir of the virus.

However, soft ticks from the genus *Ornitodoros* are considered as the natural host and also vectors and reservoirs of the virus (Plowright et al., 1969). *Ornithodoros moubata* ticks are the vectors in the east and South Africa and Indian Ocean islands where the disease was confined until 2007. After 2007, despite the absence of competent vectors and reservoirs, the disease has been progressively spreading throughout Eurasia (Gallardo et al., 2015).

African swine fever was discovered in Kenya in 1909 after the European breeds of domestic pigs had been imported (Penrith et al., 2013). At that time, it was described as a haemorrhagic disease, causing almost total mortality of the domestic pigs. After its discovery, it has been confirmed that the causal agent had been circulating in the east and south Kenya since long ago in wild suids. Though later on the disease was reported in the other parts of Africa, geographically it was confined to Sub-Saharan region.

ASF occurred in Portugal in 1957, for the first time out of Africa, incurring from the west of Africa. After the two years of silence, it re-occurred in Portugal from where it has rapidly spread to the rest of Iberian Peninsula and other European countries – France (1964), Italy (1967, 1969, and 1983), Malta (1978), Belgium (1985) and the Netherlands (1986). During this period, ASF has been recorded also in central and south America – Cuba (1971, 1980), Brazil (1978), Dominican Republic (1978) and Haiti (1979). All those countries eradicated ASF, with the exception of Sardinia.

AFRICAN SWINE FEVER VIRUS

African swine fever virus is the only member of the family *Asfaviridae* and genus *Asfivirus*. It is a very complex DNA virus with 4 layers envelope (Dixon et al., 2005). The genome, 170-193 kbp long, possess 151-167 open reading frames (ORF) and codes 54 proteins and 100 polypeptides (Dixon et al., 2013). The capsid protein (p72) and two structural proteins, p30 and p54, are the most important ones. As the polyprotein pp62 is immunodominant, the antibodies generated after the natural infection are directed against it (Pastor et al., 1989). As of yet, 22 genotypes and 8 serogroups have been described (Gallardo et al., 2011). ASFV is very resistant to inactivation. However, many disinfectants such as lipid solvents, phenol, and iodide inactivate the virus at pH lower than 4 and higher than 11. The virus survives several weeks in the frozen or fresh meat as well as in the meat products. The temperature above 70 °C inactivates the virus (Sánchez-Vizcaínoa et al., 2009).

GEOGRAPHIC DISTRIBUTION

African swine fever is an enzootic disease in many countries in Sub-Saharan Africa, in Sardinia and Trans Caucasus countries. The virus in Africa circulates between wild suids and soft ticks. The disease is inapparent in the warthog, bush pig, and red river hog; the viremia in those species is at very low or even undetectable level but it does enable the infection of ticks (Jori and Bastos, 2009). In Europe, ASF is enzootic in Sardinia in wild boar population despite no soft ticks. Unlike in the past, today the viral circulation is being accomplished through 4 cycles – sylvatic cycle between ticks and wild boars, cycle between ticks and domestic pigs, viral circulation between domestic pigs and viral circulation between wild boars (Pietschmann et al., 2016). African swine fever occurred in 2007 in Georgia and afterward in other Trans Caucasus countries and Russian Federation as well, having destroyable effects on swine production (Rowlands et al., 2008). The incursion of ASF in this region has been linked to the international overseas transport between Africa and Georgia and swill feeding of pigs in the area surrounding the ports. After its occurrence in Georgia in 2007, ASF spread to Armenia and Russian Federation, and in 2008 to Azerbaijan. Since then the disease has progressively been moving toward the west: Ukraine (2012), Belorussia (2013), EU countries – Lithuania, Poland, Latvia and Estonia (2014), Moldova (2016), Czech Republic (2017), and Romania (2017). However, the competent vectors from the *Ornithodoros moubata* complex in this region have not been found. Ticks from the *O. erraticus* complex and *O. tholozani* have been found in some Mediterranean countries (Portugal, Spain, Italy, and Turkey) and Black sea countries (Moldova, Romania, and Georgia), Armenia, and Azerbaijan. Though those tick species have not been known as important for the disease spreading, they could be important for the maintenance of the infection in an area (Ravaomanana et al., 2010).

PATHOGENESIS

ASFV infection of ticks in Africa is characterized by low infectious dose, lifelong infection and low mortality rate until the first laying of eggs. Unlike them, *Ornithodoros* ticks from the central and South America and Caribbean region show relatively high mortality of nymphs while the infection is not lifelong (Kleiboeker and Scoles, 2001). *Ornithodoros* ticks live up to 20 years enabling continuous viral maintenance in nature and in traditional pig keeping systems (Kleiboeker and Scoles, 2001). However, ticks have no role in long-

distance viral spreading but they do link the sylvatic ASF with the domestic pigs in Africa.

The most common infection routes in pigs are nasal and oral, with the exception of the dermal route in Africa via infected ticks. Once the disease has been established in a new area, the virus is usually transmitted through direct contact. Carriers, also, significantly contribute to the indirect routes of infection – via vehicles, rodents, equipment, people etc. Usually, incubation takes 4-19 days (Gallardo et al., 2015). Viral excretion in all excretes starts 2 days before the onset of the clinical symptoms and it can last up to 70 days. The extremely high viral titer is being found in the blood. The acute disease, clinically manifested, terminates within 4-5 days (Gallardo et al., 2015). Peracute and acute courses, with the high lethality, are common for the beginning of the epizootic. With the disease progression, more subacutely and chronically diseased animals are being found. At the same time, the viral virulence and mortality are decreasing. Infected pigs, usually, live several weeks but some of them can survive. Having the subclinical infection, they can live for a longer period of time. Survivals, persistently infected, with no clinical symptoms, play very important role in the disease maintenance in the enzootic regions and sporadic outbreaks in the new areas, as well.

CURRENT EPIZOOTIC SITUATION

Countries with the intensive pig production are the most vulnerable ones. Pigs, due to the fast growth, efficient feed conversion, and the fast turnover became the most important protein source for the human population. The majority of pig production is located in China, south-east Asia, west Europe, central and eastern USA, Americas, and south Brazil. However, there is still a deep gap between traditional and industrial pig production. African pig production is concentrated in Sub Saharan Africa, mostly in small family farms.

Though ASF had been existing in the majority of African countries, since 1995. a significant increase of outbreaks in Sub Saharan Africa and spreading into the free countries Madagascar and Mauritius were reported (Beltrán-Alcrudo et al, 2017). Those expansion of the disease along with the low awareness were the key factors for the viral incursion in the countries out of Africa in 2007. The transmission of ASF in Africa is very complex, depends on reservoirs, ticks, domestic pigs, the breeding system and human habits.

Since the first occurrence in 2007, ASF turned into large-scale epizootic in Europe and the part of Asia.

Beside of that, there are two distinguishable enzootic zones in Russia

Federation, in the central and in the southern part of the country (Gogin et al., 2013). There is, also, the evidence of survival wild boars. Such animals have no manifested clinical symptoms but could develop carrier status, enabling viral maintenance and spreading. In those regions, along with the appearance of sub clinically diseased wild boars, the mortality has been decreasing due to the acquired immunity, low infectious dose, viral adaptation to the new host and/or positive selection of lower virulent strains which normally appear after long time circulation in one population.

Non-EU countries combat with the ASF trying hard to stop it but the applied measures are apparently not efficient and do not prevent the viral spreading. The trade chains of cheap swine products, originating from the infected regions, are recognized as the major transmission routes of the ASF (Beltrán-Alcrudo et al., 2017). The leftovers of such products, usually improperly disposed or given to the pigs in swill, are the link between ASF and domestic pigs.

Almost since the first case of ASF in 2007, the preventive measures and surveillance have been implemented in EU (Gallardo et al., 2015). Along with the disease progression, the crisis plans have been updated in order to provide as earliest as possible disease detection. Currently applied preventive measures include regionalisation according to the World Trade Organisation, disinfection of vehicles at borders, strict controls at borders, ban of fairs, strict application of biosecurity measures, awareness campaigns, increase of number of tested animals, protective zones establishment, decrease of wild boar population, establishment of fruitful communication between the field and laboratories, promotion of necessary epizootic investigations etc. Nevertheless, it has been shown that the disease is possible to be put under control, like it has been since 2 decades in Sardinia, with no excursion out from the island.

Unlike in Russian Federation where ASF is mostly found in domestic pigs, wild boars are more affected in EU. Though it is assumed that ASF in EU has been spreading locally and independently in each, wild boar and domestic pigs, all cases in the domestic swine population were registered in the areas inhabited with both domestic and wild swine despite no contacts between them. Therefore, low biosecurity and swill feeding are considered as the most important routes of the viral introduction into the pig farms. ASF in domestic pigs occurred in EU in 2014 in Lithuania (Gallardo et al., 2015). It has been shown that for the efficient disease eradication, the early detection was a crucial factor. At the beginning of the epizootic in Lithuania, only sudden deaths were reported. Therefore, in high-risk areas, in the case of sudden deaths, ASF needs to be excluded. Viral transmission within the affected farm was further directed according to the type of the farm and applied biosecurity measures.

Apart from the sudden deaths, other clinical symptoms must not be overlooked, in particular, fever even in a small number of animals. In EU, considering intensive and modern pig production, the special concern has been put on wild boar in regard to viral maintenance and the spread. However, their exact role has not been fully described so far. In Caucasus and Russian Federation, inhabited by the low dense wild boar populations, ASF was not persisting for a long time despite continuous viral introductions from the domestic pigs. With the disease progression toward the west and the high dense wild boar populations such as in Poland and Baltic countries, wild boars become more important particularly because of continuous disease occurrence (Beltrán-Alcrudo et al, 2017).

The majority of cases have been discovered during the summer time. Even in cold climates where the temperature during the winter stays below 0 °C, ASF is usually found in the warmer periods after the melting of infected cadavers. Still, there are two peaks of ASF in wild boars –winter and summer one. By using the spatiotemporal analytical methods, it has been determined that ASF spreads 2 km by month in Latvia and Estonia and 1 km monthly in Lithuania and Poland (Abrahantes et al., 2017).

By using the statistic models, it has been shown that both the virus and antibody prevalence have been increasing since 2014 in hunted wild boars in Estonia and Latvia, with the maximum in the winter time. The largest number of dead animals has been found during the summer due to the specific biology of wild boar. The virus prevalence in hunted wild boars is at low level – 0.5-3%. In found dead wild boars, it is 60-80% in Estonia, Latvia, and Lithuania but only 0.04-1.42% in Poland (Abrahantes et al., 2017). Since the start of the epizootic, the virus prevalence is higher than the seroprevalence in hunted animals. The linkage between ASF occurrence and the factors considered as risky, such as the number of populated places, human population size, number of domestic pigs and farms, roads net, forestation, wild boars habitats, has been investigated. It has been shown that the strongest link exists between ASF occurrence and the size of human population. The wild boar population density was considered as an important factor only in Estonia in the period 2014-2016 (Abrahantes et al., 2017). Despite many efforts and resources put into active surveillance and early detection of ASF, it has been shown that the passive surveillance was more efficient. All primary cases were discovered within passive surveillance.

Even though carriers in wild boar have not been found so far in EU, there are different ways which enable a long-term viral circulation. They are usually human-mediated: illegal trade of animals and animal products, low biosecurity level, and wild boars feeding (Abrahantes et al., 2017). Beside of those, cadavers

of infected animals serve also as a source of the infection (Probst et al., 2017).

The most probably, ASF spreads between subpopulations of wild boars by contact with the infectious materials (blood, cadaver, excretes). However, there is moderate to high likelihood that the direct contact between wild boars is crucial for the viral transmission, in particular where the feeders are in place. Moderate to high likelihood is applied also in case of finding improperly disposal of food leftovers (EFSA, 2015). Considering the consequences of ASF, different strategies to stop its spread have been implemented. One of them is the reduction of wild boar population. But, as every human intervention in nature, this one also produced the certain consequences. Intensive and often hunt during the depopulation campaigns lead to the wild boar dispersion and the disease spread. Reduction of a population for the more than 60% drives to the wild boar adaptation, the compensatory growth of the number of animals, and intensive movements out of the hunting area (EFSA, 2015).The feeding of wild boars prevents their dispersion but leads, due to their grouping, to the pathogens exchange. More effective measure for the control of wild boar population is the ban of feeding along with intensified hunting during the several consecutive years, aiming to reduce the number of females of all categories.

Therefore, there are two recommended types of managing wild boar populations: 1. Fast control measures meaning depopulation (killing over 70% of wild boars) or rapid disposal of carcasses, 2. Long-term measures meaning ban of feeding and hunting of females (EFSA, 2015).

However, despite all undertaken preventive and control measures, ASF has been still spreading. During 2017 it has been reported in domestic pigs in Estonia (3 cases), Italy - Sardinia (17 cases), Latvia (8 cases), Lithuania (30 cases), Poland (80 cases), Romania (2 cases) and Ukraine (110 cases). ASF in domestic pigs has also been registered in Moldova in 2017.Number of cases in wild boars is even higher – Czech Republic (115 cases), Estonia (542), Italy – Sardinia (28 cases), Latvia (749 cases), Lithuania (794 cases), Poland (398 cases), Ukraine (17 cases)[1]. AFS outbreak in the Czech Republic represents a huge „jump" of the virus into the new region. Though the source of the infection has not been documented yet, such events usually happen because of swill feeding or improper disposal of food leftovers. Two cases of ASF in domestic pigs in Romania, also, prove this.

Since the eradication of ASF is even more complex due to no vaccine availability, prevention of viral introduction is the most important. Having this as an aim, since 2011 there are implemented active and passive surveillance of ASF in Serbia (19). Considering both small wild boar population in Serbia

[1] https://ec.europa.eu/food/sites/food/files/animals/docs/ad_adns_outbreaks-per-disease.pdf

and traditional pig keeping despite the absence of competent tick vector species (20), ASF would produce destructive consequences for small producers. However, along with the surveillance and having learned lessons from the other countries, additional efforts are to be put into awareness and education of farmers, producers, and population in general.

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INVESTIGATION OF *MYCOPLASMA SYNOVIAE* SEROPREVALENCE IN BROILER BREEDER FARMS IN SOUTH BAČKA REGION

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Abstract

Mycoplasma synoviae is known to cause respiratory disorders, synovitis, subclinical infections, air sacculitis and eggshell apex abnormalities in domestic poultry worldwide. The aim of this study was to determine *M. synoviae* seroprevalence in 5 different broiler breeder farms in South Bačka from 2014 to 2017. A total of 1511 samples were tested using commercial indirect enzyme linked immunosorbent assay (ELISA) for detection of antibodies in the blood sera. In this study, the seroprevalence of 25.21% was found and 47 (40.87%) flocks out of 115 tested were positive to *M.synoviae*. Seroprevalence varied between 31.02% in 2015 and 16.78% in 2016. Flock prevalence ranged from 31.03% in 2014 to 55.88% in 2015. These results suggest that *M. synoviae* infection is present in broiler breeder farms in South Bačka, and that is necessary to conduct further research, systematic monitoring and to improve biosecurity measures on broiler breeder farms.

Key words: Mycoplasma synoviae, seroprevalence, broiler breeder

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SEROPREVALENCA *MYCOPLASMA SYNOVIE* NA FARMAMA RODITELJSKIH JATA TEŠKIH LINIJA U JUŽNOBAČKOM OKRUGU

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Kratak sadržaj

Poznato je da *Mycoplasma synoviae* uzrokuje respiratorna oboljenja, sinovitis, subkliničke infekcije, zapaljenje vazdušnih vreća i deformitete ljuske jaja kod domaće živine širom sveta. Cilj ovog istraživanja bio je da se utvrdi seroprevalenca *M. synoviae* na 5 različitih farmi roditeljskih jata teških linija od 2014. do 2017. godine. Indirektnom ELISA tehnikom ukupno je testirano 1511 krvnih seruma. Utvrđena je seroprevalenca od 25.21%, a od 115 testiranih jata 47 (40.87%) je bilo pozitivno na antitela protiv *M. synoviae*. Seroprevalenca se kretala od 16.78% u 2016. godini do 31.02% u 2015. godini. Prevalenca jata je iznosila od 31.03% u 2014. godini do 55.88% u 2015. godini. Rezultati dobijeni u ovom istraživanju ukazuju da je infekcija *M. synoviae* prisutna na farmama roditeljskih jata teških linija u Južnobačkom okrugu i da je neophodno sprovesti dalja istraživanja, sistemski monitoring i poboljšati mere biosigurnosti na farmama.

Ključne reči: *Mycoplasma synoviae,* seroprevalenca, roditeljska jata teških linija

INTRODUCTION

Mycoplasma synoviae (Ms) is one of the most important pathogens in domestic poultry worldwide, including breeders, broilers and layers (Vardaman et al., 1973; Kleven and Ferguson – Noeln, 2008). Ms infections in poultry have always been regarded as less important than infections with *Mycoplasma gallisepticum*, but during the last decade, the importance of infections with Ms have been highlighted in several researches and there is an increased consciousness to produce Ms free poultry (Feberwee et al., 2008; Landman, 2014). In chickens, Ms infections have been associated with the respiratory disorders, synovitis, subclinical infections, air sacculitis (Vardaman et al., 1973; Macowan

et al., 1984), as well as eggshell apex abnormalities and egg production losses (Landman and Feberwee, 2001; Van Beek et al., 2002; Landman and Feberwee, 2004; Feberwee et al., 2007).

Mycoplasma synoviae can cause significant economic losses due to decrease of egg production rate, growth and hatchability rates, poor eggshell quality, and condemnation of carcasses at slaughter because of air sacculitis and arthritis (Fiorentin et al., 2003; Kleven 2003a; Peebles et al., 2011). An additional contribution to the importance of Ms infection in domestic poultry is brought by the high seroprevalence of Ms in countries with developed poultry industry (Feberwee et al., 2008; Feberwee and Landman, 2012) and its ability to interact with other pathogens such as Newcastle disease virus and infectious bronchitis virus (Kleven et al., 1972; Hopkins and Yoder, 1982; Feberwee et al., 2009). Reduction in egg production of 5 to 10 %, a reduction in hatchability of 5 to 7 % and more than 5 % increased mortality in the young chickens were reported in infected breeder flocks (Stipkovits and Kempf, 1996). Broiler flocks from the seropositive broiler breeders showed higher rate of mortality from air sacculitis (King et al., 1973; Macowan et al., 1984). The economic impact of Ms infection is mainly focused on increased condemnation of carcasses due to air sacculitis (Sentíes-Cué et al., 2005). Reduced weight gain and poorer feed conversion are also the result of Ms infection in broilers (King et al., 1973). *Mycoplasma synoviae* can be transmitted horizontally and vertically (Jordan, 1975). Vertical transmission is considered to be a major way of spreading of Ms in domestic chickens, therefore the most efficient method of control would be regular monitoring and elimination of positive breeder flocks (Kleven and Ferguson-Noeln, 2008; Lockaby et al., 1998). Although various live and attenuated Ms vaccines have been developed and used worldwide (Morrow et al., 1998), the Ms vaccine is not yet available in Serbia.

In Serbia, serological monitoring of Ms is still on voluntary basis and data about seroprevalence of Ms in poultry farms are lacking. It is necessary to identify Ms seropositive farms, to assess the epidemiological risks in different poultry categories and to improve biosecurity measures on farms. The aim of this study was to investigate Ms seroprevalence on different broiler breeder farms and to perceive the epidemiological situation of Ms infection in South Bačka Region.

MATERIAL AND METHODS

A total of 1511 blood samples were taken from 115 broiler breeder flocks from 5 different farms in South Bačka region. The samples were taken from

2014 to 2017, following the voluntary *M. synoviae* control program established by Scientific Veterinary Institute "Novi Sad". Blood samples were aseptically taken from non-vaccinated broiler breeders that had no clinical symptoms.

Sera were tested for the presence of antibodies to *M. synoviae* by a commercial ELISA test kit (IDEXX 99-06728, IDEXX Laboratories, Westbrook, ME) according to manufacturer's instructions. Briefly, 100 µl of each diluted serum sample (1:500) were added to the wells previously coated with Ms antigen. Undiluted positive and negative controls were tested in duplicate wells. Samples were incubated at 18-26 °C for 30 minutes. After washing the plates with distilled water 3 to 5 times, 100 µl of conjugate (Goat antichicken antibodies: Horseradish peroxidase conjugate) were added to each well and incubated at 18-26 °C for 30 minutes. Plates were washed again 3 to 5 times with distilled water and 100 µl of TMB (tetramethyl benzidine) substrate were dispensed into each well of the plate. The substrate solution was incubated at 18-26 °C for 15 minutes. The reaction was quenched with 100 µl of stopping solution. The absorbance was measured at 650 nm. Serum samples with S/P values greater than 0.5 (titer greater than 1076) were considered positive.

RESULTS

The obtained results are presented in Table 1 and Table 2. In general, 381 samples were found positive for the presence of anti-Ms antibodies, with the overall prevalence of 25.21%. The highest – 31.02% and the lowest – 16.78% seroprevalence was found in 2015 and 2016, respectively (Table 1). It was found that 47 (40.87%) out of the 115 tested flocks were positive. Positive flock rates in different years ranged between 31.03% and 55.88% (Table 2).

Table 1. Seroprevalence of Ms from 2014 to 2017

Year	Number of tested samples	Number of positive samples	Prevalence (%)
2014	361	100	27.70
2015	432	134	31.02
2016	453	76	16.78
2017	265	71	26.79
Total:	**1511**	**381**	**25.21**

Table 2. Results of the presence of Ms in poultry flocks from 2014 to 2017

Year	Number of tested flocks	Number of positive flocks	Positive rates (%)
2014	29	9	31.03
2015	34	19	55.88
2016	33	12	36.36
2017	19	7	36.84
Total	**115**	**47**	**40.87**

DISCUSSION

Mycoplasma synoviae is one of the most important mycoplasma species that affect domestic poultry during laying period. Our results revealed Ms infection seroprevalence of 25.21% in broiler breeders. Another study conducted in Serbia showed seroprevalence of 36.66% in 2000 and 22.60% in 2009. The decrease in seropositivity in 2009 compared to 2000 was due to specific measures that were applied in layer and broiler breeder flocks (Kapetanov et al., 2010). Similar serological survey in China demonstrated an overall seroprevalence of 41.19 %, which is twice as high in comparison to our results. Seroprevalence in China varied in different provinces from 5.10% to 100%. Commercial vaccine is not yet available in China, so the authors concluded that detected antibodies originated from the natural infection (Xue et al., 2017). In Serbia, the situation in view of the availability of vaccine against Ms is the same as the one in China, so our results indicated seroconversion following natural infection as well. A study in Portugal revealed Ms seroprevalence of 40.3% (Moreira et al., 2015), which is similar to the one found in China.

Survey in Portuguese broiler breeder flocks revealed high prevalence of Ms infection, with 483 positive samples and 24 (66.7%) positive flocks out of 36 tested (Moreira et al., 2015). In the Netherlands, flock prevalence was 35% (Feberwee et al., 2008), in South America 15% (Buim et al., 2009) and in Middle East 27% (Amer et al., 2012). In our study, flock prevalence was 41%, which is lower in comparison to those from Portuguese flocks, and higher in comparison to other above-mentioned results. These results suggest worldwide distribution of Ms infection in breeder flocks. Another study addressing the Ms flock prevalence was carried out in Serbia by Kapetanov et al. (2010), and the authors reported flock prevalence of 40.48 in 2000 and 20.74% in 2009. Flock prevalence in our study varied from 31.03% in 2014 to 55.88% in 2015. It can

be concluded that Ms flock prevalence is different from year to year, because the occurrence of infection is influenced by numerous factors.

Since Ms can be transmitted vertically and horizontally (Stipkovits and Kempf, 1996), it can cause infection in broilers with increased mortality, feed conversion and condemnation. (Xue et al., 2017; Moreira et al., 2015). Feberwee et al. (2008) reported lower seroprevalence in meat rearing breeder stock (6%) than in meat-type grandparents stock (10%), due to reduced risk of vertical transmission by elimination of Ms-positive flocks. Authors also found higher seroprevalence in meat production breeder stock (35%) than in meat rearing breeder stock. The accuracy of the estimated seroprevalence could have been influenced by frequency and number of birds sampled per flock, which was different for different poultry categories (Feberwee et al., 2008). In order to establish reliable Ms control and prevention program sampling methods, sample size and poultry categories must be determined in advance. High prevalence of Ms infection was found in Portuguese breeder farms, so the authors suggested that culling Ms-positive flocks with such a high prevalence is not an option (Moreira et al., 2015). On the other hand, elimination of positive breeder flocks is recommended, if possible, as one of the best solutions when there is a low Ms prevalence (Buim et al., 2009; Feberwee et al., 2008). The elimination of positive flocks is not carried out in Serbia, but infected flocks are treated with appropriate medications. The use of medications to treat Ms infection increases the production costs. Although vaccines and medication can reduce clinical signs and economic impact of Ms infection, medication cannot completely eliminate mycoplasma infections (Whithear, 1996; Kleven 2003a) and vaccines are not yet available in all countries, such as China and Portugal (Moreira et al., 2015; Xue et al., 2017) as well as Serbia. Ms are capable of establishing lifelong infections in poultry, and that can be one of the reasons for inability to eradicate Ms in commercial poultry flocks, especially in breeders that stay a long period in rearing and production sites.

CONCLUSION

It is evident that Ms is present on broiler breeder farms in South Bačka. In the absence of systematic monitoring of Ms infection in Serbia, further studies need to be done to obtain an overall picture of Ms prevalence in our country. Serological monitoring of Ms infection plays an important role in the prevention and control of this infection in poultry. Besides, it is also important to improve biosecurity measures on farms and to import broiler breeder chickens only from the farms that conduct vaccination against Ms. It is recommendable

to introduce vaccination against Ms in all broiler breeder farms in Serbia, as one of the most significant prevention measures.

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THE EFFECT OF FREEZING-THAWING AND MARINATION TIME ON COOKED CHICKEN BREAST MEAT QUALITY

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Abstract

Marination is often used to improve the yield and quality of chicken breast fillets. The effects of freezing-thawing prior to marination and marination holding time on the instrumental and sensory properties of cooked marinated chicken fillets were investigated. Fillets were marinated fresh or stored at -18 °C and then thawed prior to marination (frozen-thawed). Fillets were soaked in marinade for 1.5 h and 20 h and then cooked at 175 °C for 45 minutes for determination of cooking loss, Warner-Bratzler shear force, color properties, juiciness and sensory properties. Marinade uptake was greater in fillets marinated for 20 h, both fresh and frozen-thawed, than in fillets marinated for 1.5 h. Cooking loss was lowest for fresh unmarinated fillets (19.30%) and significantly lower ($P < 0.05$) comparing to frozen-thawed fillets marinated for 20 h (44.73%). The highest numerical value of color parameter lightness was found in fresh fillet marinated for 1.5 h. There were no significant differences between all examined fillets regarding color properties redness and yellowness. The fillets marinated for 20 h, both fresh and frozen-thawed were found to be significantly more tender ($P < 0.05$) as indicated by lower shear force values (13.96 N and 12.88 N, respectively) and higher sensory scores for tenderness (7.00 and 6.50, respectively) as compared to other investigated fillets. Furthermore, fresh fillets marinated for 20 h had the highest mark for the overall sensory acceptability (95.00% of maximum quality).

Keywords: chicken fillets, marinating, texture, sensory quality

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UTICAJ SMRZAVANJA-ODMRZAVANJA I VREMENA MARINIRANJA NA KVALITET TERMIČKI OBRAĐENOG PILEĆEG MESA

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Kratak sadržaj

Mariniranje se često koristi kako bi se povećao prinos i poboljšao ukupni kvalitet pilećeg mesa. U ovom radu je ispitivan uticaj smrzavanja-odmrzavanja pre mariniranja i vremena mariniranja na instrumentalna i senzorska svojstva termički obrađenih pilećih filea. Filei su marinirani sveži ili nakon odmrzavanja (skladišteni na -18 °C). Filei su bili potopljeni u marinadu u trajanju od 1,5 čas, odnosno 20 časova i termički obrađeni na 175 °C u trajanju od 45 minuta, radi određivanja kala termičke obrade, Warner-Bratzler sile presecanja, instrumentalnih parametara boje, sočnosti i senzorskih svojstava. U obe grupe uzoraka prinos mariniranjem je bio veći nakon 20 časova u poređenju sa fileima mariniranim 1,5 čas. Kalo termičke obrade je bio najmanji u svežim nemariniranim fileima (19.30%) i značajno niže vrednosti ($P < 0,05$) u poređenju sa odmrznutim fileima mariniranim u trajanju od 20 časova (44.73%). Najveća izmerena vrednost za svetloću zabeležena je u svežim fileima mariniranim 1,5 čas. Nije uočena značajna razlika među ispitivanim uzorcima filea *što se tiče* parametara udela crvene boje i udela žute boje. Obe grupe uzoraka, marinirane 20 časova, bile su značajno nežnije ($P < 0.05$) što su pokazale manje vrednosti sile presecanja (13,96 N i 12,88 N, respektivno) i veće vrednosti senzorskog svojstva nežnosti (7,00 i 6,50, respektivno) u poređenju sa ostalim ispitivanim fileima. Dalje, sveži filei marinirani 20 časova imali su najveću ocenu ukupne senzorske prihvatljivosti (95,00% maksimalnog senzorskog kvaliteta).

Ključne reči: pileći fileti, mariniranje, teksture, senzorski kvalitet

INTRODUCTION

Marination is the process of applying a water-based solution composed of ingredients such as salt, sugar, oil, organic acids, herbs and food additives such

as aroma enhancers, antioxidants and antimicrobials (Björkroth, 2005; Haute et al., 2016). Marinades are incorporated into meat by soaking, massaging, tumbling, or injecting (Parks et al., 2000). Originally, marinating was used to preserve the meat product over a longer time. Today, marinating is adopted by restaurants and industry in order to change the flavor profile of products, improve meat tenderness and juiciness, as well as to enhance yield by increasing retention and water content (Mielnik et al., 2008; Bianci et al., 2009) and is especially used for poultry products (Barbanti et al., 2005). Market forms of marinated poultry include whole birds, cut-up parts, boneless meat, and chopped and formed items. Many products sold in the raw, unmarinated state will be marinated by the retailer or by the consumer in the home prior to sale or consumption, respectively (Smith and Acton, 2010).

The functionality of marinades is dependent on the ingredients in aqueous solution. The most common ingredients in commercial marinades are NaCl, some type of phosphate and herbs and spices. Sodium chloride is a natural flavor enhancer that improves the taste and aroma of meat products. The addition of sodium chloride promotes uptake of water due to the electrostatic repulsion and partially solubilization of proteins (Bianci et al., 2009; Alvarado and McKee, 2007). Herbs and spices are usually added to provide a variety of flavors and aromas to marinades and to offset (harmonize) the bland chicken meat taste (Parks et al., 2000).

Some previous studies have investigated the influence of processing variables such as marinade ingredients and concentration, marination duration (the time course for marinade absorption), temperature, and chicken breast meat freezing-thawing on final product quality attributes (Heath and Owens, 1991; Xiong and Kupski, 1999; Bowker and Zhuang, 2017; Fenton et al.; Zheng et al., 2000). The formation of ice crystals during meat freezing can disrupt muscle cells and cause the loss of product structure that does not recover when thawed (Kaale and Eikevik, 2014; Leygonie et al., 2012).

The objective of this study was to determine the effects of fresh never-frozen chicken breast meat and breast meat that was frozen and thawed prior to marination and marination holding time on final product quality attributes.

MATERIAL AND METHODS

Raw materials

The chicken carcasses used in the present study were obtained from a local processing plant. Chilled chicken carcasses (n = 40) were cut to the basic anatomical parts. Fresh breast meat was divided into two halves, one half was im-

mediately marinated, and the other half was frozen and stored for 10 days, and marinated after defrosting. Chicken breast meat samples were distributed into 6 groups and the marinating processes were performed as shown in Table 1.

Table 1. Control and experimental groups of fillets

K_F – Control group	Fresh chicken breast meat
F_1 – Experimental group	Fresh chicken breast meat marinated for 1.5 h
F_2 - Experimental group	Fresh chicken breast meat marinated for 20 h
K_D - Control group	Defrosted chicken breast meat
D_1 - Experimental group	Defrosted chicken breast meat marinated for 1.5 h
D_2 - Experimental group	Defrosted chicken breast meat marinated for 20 h

Marinating process

Weighed and individually identified fresh and defrosted fillets were soaked in marinade in proportion of 1.25 kg marinade per 1.00 kg meat. The marinade consisted of salt (2.08%), sugar (4.00%), cinnamon powder (0.03%), dried thyme (0.03%), ginger powder (0.03%), mustard seed (0.17%) and pepper (0.17%). The content of spices is expressed in % in relation to water. The fillets were marinated at 2 °C for 1.5 h (F1 and D1) and for 20 h (F2 and D2) by soaking in plastic boxes. Chicken fillets from control groups were cooked without prior marination.

Marinade uptake

The samples weight was recorded before and after marinating. After the preset marinating time, the parts were removed from the marinade and drained for 10 min before determining the marinade retention. Calculation for marinade uptake was as follows: marinade uptake (%) = (marinated weight - raw weight) / raw weight x 100

Cooking procedure

Before cooking, the samples were individually weighed, enwrapped in aluminum foil and introduced in the convection air oven when temperature

reached the preset value. The cooking temperature was set at 175 °C for 45 min. After cooking, samples were cooled at room temperature for 1 h and then analyzed for cooking loss, color, texture and sensory characteristics. The calculation of cooking loss was as follows: cooking loss (%) = (marinated weight - cooked weight) / marinated weight x100

Color determination

The color was determined instrumentally, on the fresh cut of cooked and cooled fillet, with Minolta Chroma Meter CR-400. Color characteristics were expressed by *CIE L*a*b** system (lightness-*L**, redness and greenness - *a**; yellowness and blueness - *b**). Color measurements were made in duplicate.

Mechanical texture analysis

Shear force evaluation was conducted on cylinder samples taken from the center of each fillet, longitudinal to the muscle fibers. The cylinders were 1.27 cm in diameter. A Warner-Bratzler blade, using testing machine Texture Analyser TA XP (Stable Micro System, Godalming, England), was used to shear the samples across to the muscle fibers. Six measurements were performed on each sample to obtain mean values. A cross speed of 5 mm/s was applied using the 5 kg load cell. Mean values were expressed as shear force (N).

Juiciness

Juiciness was measured using the method of Gujral et al. (2002) with slight modifications. A meat sample (0.5 g) was taken from the center of the cooked and cold fillet and placed between a pair of pre-weighed filter paper and between two plexiglass plates and pressed for 1 min (maximally connected and tightened screws). The filter paper was weighed after pressing and the percentage of extracted juice was determined as follows: Juiciness (%) = (weight of filter paper after pressing - weight of filter paper before pressing/weight of sample) x 100

Sensory analysis

A panel consisting of nine trained members of different ages performed sensory evaluation. Evaluations were performed according to a 7-point scale descriptive system, from 1 to 7. Each mark was ascribed a distinctive quality

level, as presented in Table 2. The overall sensory quality was evaluated as total sum of mean scores for the sensory attributes.

Table 2. Sensory analysis of cooked chicken fillets

Value	Sensory attributes			
	Odor	Taste	Juiciness	Tenderness
1	Extremely bad	Extremely bad	Extremely bad	Extremely bad
2	Very bad	Very bad	Very bad	Very bad
3	Bad	Bad	Bad	Bad
4	Neither good nor bad	Neither good nor bad	Neither good nor bad	Neither good nor bad
5	Good	Good	Good	Good
6	Very good	Very good	Very good	Very good
7	Extremely good	Extremely good	Extremely good	Extremely good

Statistical analysis

The effects of sample type (fresh or frozen-thawed) and marination time on the variables studied were analyzed by Factorial ANOVA (Statistica 13.2 – Dell Inc., 2016). The Duncan's post hoc test was performed for comparison of mean values. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Increasing the marination time significantly ($P < 0.05$) increased the amount of marinade uptake by the chicken breast fillets, in both fresh and frozen-thawed fillets (Figures 1 and 2). Marinade uptake of frozen-thawed fillets was numerically lower in comparison with fresh fillets (14.27% and 16.50%, respectively) for longer marination time (20 h). Bowker and Zhuang (2017) found similar results for marinade absorption of fresh and frozen-thawed fillets vacuum-tumbled for 45 minutes. Chan et al. (2011) had shown that freezing significantly affected biochemical and functional properties of proteins in turkey breast meat, thus altering the functionality attributes of raw poultry meat.

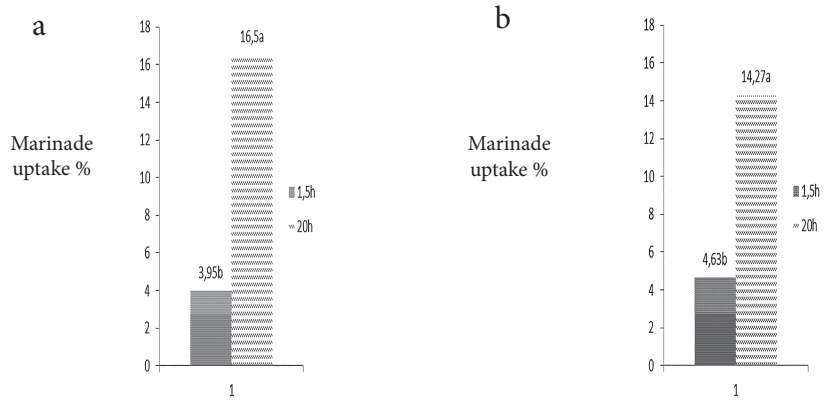

Figure 1. Marinade uptake (%) of fresh breast fillets (a) and frozen-thawed breast fillets (b)

Significant effect ($P < 0.05$) on cooking loss was observed for sample type (fresh or frozen-thawed fillets), marination time and interaction effect (sample type x marination time) (Table 3). Frozen-thawed fillets marinated for 20 h (44.73%) had the highest numerically cooking value.

Instrumental color parameters are presented in Table 3. Fresh chicken breast fillets unmarinated and marinated for 1.5 h differ significantly ($P < 0.05$) in comparison with frozen-thawed fillets unmarinated and marinated for 1.5 h, with respect to lightness values (CIE L^*). However, no significant difference was observed regarding fresh and frozen-thawed fillets marinated for 20 h (82.90 and 82.26, respectively). The lightness of all examined samples of fillets was dependent upon the sample type (Table 3). Redness and yellowness were not affected by sample type, marination time and interaction effect of these two factors.

Tenderness has been identified as the most important factor affecting consumer satisfaction and perception of taste (Naveena et al., 2004). Warner-Bratzler shear force (WBSF) is an important indicator related to meat tenderness (Zhao et al., 2012; He et al., 2015). One of the ingredients of three-spice powder mixture used for samples marination was ginger powder. Ginger, beside antioxidant and antimicrobial, has strong proteolytic activity (Bhaskar et al., 2006; Naveena et al., 2004). Significantly ($P < 0.05$) lower WBSH values were observed in both fillets marinated during 20 h compared to unmarinated fillets and fillets marinated for 1.5 h (Table 3). Longer marination time may have increased meat tenderness by enhancing muscle fiber disruption (Naveena et al., 2004; Bhaskar et al., 2006; He et al., 2015).

The results of juiciness show that the unmarinated fresh fillets had the highest value while the frozen-thawed fillets marinated for 20 h had the lowest value (41.86% and 26.67%, respectively) (Table 3). These results could be correlated with the results of cooking loss, thus, unmarinated fresh fillets had the lowest cooking loss, while the frozen-thawed fillets marinated for 20 h had the highest value of cooking loss (19.30% and 44.73%, respectively). Marination time did not have significant effect on cooked fillets juiciness, while sample type significantly affected the previously mentioned property.

Sensory property odor of examined fillet samples was in average evaluated as ”very good“, except frozen-thawed fillets marinated for 20 h which were ascribed a highest mark 7 („extreme good“) (Table 1). However, above mentioned sensory property was significantly influenced only by sample type. In general, other evaluated sensory properties of examined fillets were found to be significantly affected by the sample type. Taste of fresh fillets marinated for 20 h was marked significantly higher ($P < 0.05$) as compared to other examined fillets. Likewise, fresh fillets marinated for 20 h were evaluated significantly better ($P < 0.05$) than other samples with regard to sensory properties of juiciness and tenderness. Higher marks for juiciness and tenderness of previously mentioned fillets can be explained by higher marinade uptake. The higher sensory mark for tenderness for fresh fillets marinated for 20 h may have been in correlation with lower shear force value for these samples, than for other investigated samples. As well, aforementioned fillets had the highest (95.00% of maximum quality) and frozen-thawed unmarinated fillets had the lowest mark for the overall sensory acceptability (83.39% of maximum quality) (Figure 2).

Table 3. Cooking loss, color parameters (CIE *L* a* b*),* Warner-Bratzler shear force, juiciness and sensory properties of control and marinated breast fillets from fresh and frozen-thawed chicken breast meat.

Property		Fillet treatment						Factor significance		
		Fresh			Frozen-thawed			Sample (S)	Time (T)	SxT
		Marination time (h)								
		0	1,5	20	0	1,5	20			
Cooking loss (%)		19.30±4.64^{b}	21.49±2.67^{b}	39.87±5.13^{a}	25.66±8.2^{b}	39.25±2.53^{a}	44.73±5.35^{a}	*	*	*
Color	*L**	83.62±1.77^{a}	83.70±1.16^{a}	82.90±1.31^{ab}	82.09±1.9^{b}	81.87±1.24^{b}	82.26±1.95^{a} $_{b}$	*	ns	ns
	*a**	3.26±0.88	2.63±0.71	3.60±1.35	3.08±0.76	2.72±0.53	2.59±0.61	ns	ns	ns
	*b**	12.61±1.60	12.18±0.75	13.00±1.13	13.53±1.49^{a}	12.65±1.30	13.56±1.70	ns	ns	ns
WBSF (N)		19.78±3.38^{a}	18.05±3.42^{a}	13.96±1.87^{b}	20.71±8.95^{a}	19.80±5.65^{a}	12.88±3.82^{b}	ns	*	ns
Juiciness (%)		41.86±9.38^{a}	39.76±6.96^{ab}	38.09±5.08^{ab}	30.03±7.31^{c}	33.18±4.39^{bc}	26.67±3.87^{c}	*	ns	ns
Sensory properties	Odor	6.00±0^{c}	6.30±0.27^{b}	6.00±0^{c}	6.00±0^{c}	6.00±0^{c}	7.00±0^{a}	*	ns	ns
	Taste	5.95±0.28^{c}	6.30±0.27^{b}	6.70±0.27^{a}	5.95±0.16^{c}	6.00±0^{c}	6.50±0^{ab}	*	*	ns
	Juiciness	6.15±0.63^{b}	6.10±0.74^{b}	6.90±0.22^{a}	5.45±0.37^{c}	6.00±0^{bc}	6.40±0.42^{ab}	*	*	ns
	Tenderness	6.50±0.33^{b}	6.30±0.67^{bc}	7.00±0^{a}	5.95±0.16^{c}	6.00±0^{c}	6.50±0.35^{b}	*	*	ns

$^{a-c}$ Means within a row with different superscripts are significantly different ($P < 0.05$).
* $P < 0.05$, ns = not significant.

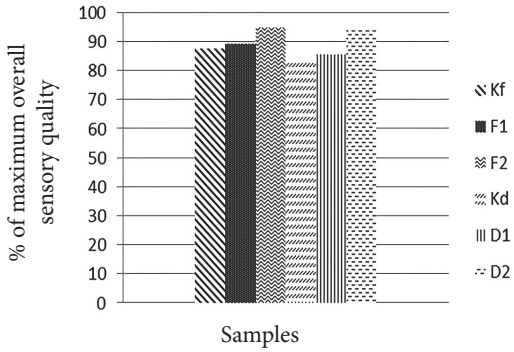

Figure 2. % of maximal overall sensory quality

CONCLUSION

This study evaluated the impact of sample type (fresh and frozen-thawed fillets) and marination time on cooking loss, color properties, Warner-Bratzler shear force, juiciness and sensory properties (odor, taste, juiciness and tenderness) of chicken breast meat. Marination time significantly affected cooking loss, WBSF values and most of the sensory properties (taste, juiciness and tenderness). However, sample type significantly affected cooking loss, lightness, juiciness and all investigated sensory properties.

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LABEL ANALYSIS OF SERBIAN HONEY: WHAT DOES (NOT) THE LABEL TELL US?

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Abstract

In order to differentiate between many honey types on Serbian market, consumers read labels, which represent an important aspect of packaging "catching" the consumer's eyes as well as carrying all the necessary information about the product, producer and seller. As consumers are more and more aware of health problems and nutrition they pay attention to the information written on the labels on different products. The aim of this study was to inspect labels on honey packages collected in an official monitoring during October 2017. In total 60 honey samples were collected and examined. Of all examined honey samples, 46 (76.67%) labels did not fulfill prescribed conditions according to Legislative on quality of honey and other bee products and Declaration, labeling and marketing of food ("Official Gazette RS", No. 101/2015; "Official Gazette RS" No. 85/13 and No. 101/13), while only 14 (23.33%) did. This results lead to indispensible need to inform and educate beekeepers about the actual laws and regulations in order to make labels that contain all the information required, which might possibly also raise the consumption of honey to higher level.

Key words: bee honey, bee products, market, monitoring

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PREGLED DEKLARACIJA SRPSKOG MEDA: ŠTA NAM (NE) GOVORE DEKLARACIJE?

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Kratak sadržaj

U svrhu razlikovanja mnogobrojnih vrsta medova na tržištu Srbije potrošači čitaju deklaracije koje predstavljaju važan deo pakovanja privlačeći potrošače izgledom i dajući im neophodne informacije o proizvodu, proizvođaču i prodavcu. Mnogi potrošači vode računa o informacijama koje se nalaze na deklaracijama zbog brige o svom zdravlju i ishrani. Cilj ovog istraživanja bio je pregled deklaracija koje se nalaze na pakovanjima meda u službenom monitoringu sprovedenom u oktobru mesecu 2017. godine. U ovom istraživanju ispitano je 60 uzoraka meda. Od svih pregledanih uzoraka meda 46 (76.67%) deklaracija nije bilo u skladu sa zahtevima Pravilnika o kvalitetu meda i drugih proizvoda pčela ("Službeni glasnik RS", broj 101/2015) i Pravilnika o deklarisanju, označavanju i reklamiranju hrane ("Službeni glasnik RS" broj 85/13 i 101/13), dok je samo 14 (23.33%) ispunjavalo zahteve. Ovi rezultati pokazuju potrebu za informisanje i edukaciju pčelara u skladu sa Pravilnicima. Naime, upotpunjavanje informacija na deklaracijama svakako može dovesti do povećanja konzumacije meda.

Ključne reči: pčelinji med, pčelinji proizvodi, tržište, monitoring

INTRODUCTION

Bees (*Apis mellifera)* collect nectar and transform it, combining it with their own specific substances, to the natural sweet substance called honey ("Official Gazette RS", 101/2015). The production of honey involves a wide range of factors acting together in perfect harmony (Prica et al., 2014). Moderate continental climate as well as floral and plant richness make perfect conditions for beekeeping in Serbia (Babić, 2014).

Honey and other bee products available on Serbian market should be labeled according to the Legislative on quality of honey and other bee products

and Declaration, labeling and marketing of food ("Official Gazette RS", No. 101/2015; "Official Gazette RS" No. 85/13 and No. 101/13). The label has to be written on Serbian language. It must be easy to understand, visible, clear and easy to read ("Official Gazette RS" No. 85/13 and No. 101/13).

Proper labeling of honey and other bee products helps to provide the consumers with necessary information about products they purchase. Name of the food should be the one that is prescribed by law ("Official Gazette RS" No. 85/13 and No. 101/13). The product may only be called "honey" if it complies with the prescribed compositional standards and has no other ingredient added to it. According to the origin, honey is classified as: blossom or nectar honey (monofloral and polyfloral honey), honeydew and baker's honey. It is classified as comb honey, chunk honey, drained honey, extracted honey, pressed honey and filtered honey according to type of production and/or presentation on market. All honey types, except baker's honey and filtered honey, may be labeled with additional information relating to its floral origin, its regional, territorial or topographical origin or its specific quality criteria. The name of honey has to be characterized by specific blossom or plant it originates from in such a way that, if there is any reference to a particular blossom or plant, the honey has to come wholly or mainly from that blossom or plant. Likewise, if reference is made to a regional, territorial or topographical origin the honey must come wholly from that country or place. Baker's honey must be labeled with the words "*intended for cooking only*" in close proximity to the name ("Official Gazette RS", No. 101/2015).

Net mass on the label represents charging amount expressed with numerical value, either in weight liquid or units. Net mass of honey and honey products must be stated in grams (g) or kilograms (kg) as appropriate without the packaging (jar, lid, label, etc.). The mass marking should be shown in the same field of vision as the name of a food ("Official Gazette RS" No. 85/13 and No. 101/13).

The date of a minimum durability of certain food is defined as the date until which the food retains its specific properties if properly stored ("Official Gazette RS" No. 85/13 and No. 101/13). Honey should retain its specific properties for a number of years if correctly stored, therefore it's up to the beekeeper/honey packer to determine what a suitable shelf life for their product should be, taking into account the nature of the product. The optimal temperature for storing honey and bee products is 10-16°C with ambient relative humidity lower than 65% (Bogdanov, 2009). Still, recommended shelf life for honey stored at room temperature is 1 (one) year, while opened honey, stored in refrigerator has shelf life from 6 to 8 months (Roberts and Graham, 2004).

Every package of honey and/or bee product has to have a batch number on the label. Importance of batch number is shown in the definition of traceability. It represents the capacity to follow the whole life cycle (forward or backward) of any product, including food (Tatiana et al., 2013).

MATERIALS AND METHODS

In total, 58 honey samples and 2 bee products (acacia honey with curcuma and honey with walnuts) were collected from different retail chains in Vojvodina region (north Serbia). All samples were collected as a part of official monitoring of honey and bee products quality during October 2017.

All collected samples were, in their original packaging, transferred to the laboratory of Scientific Veterinary Institute "Novi Sad" for examinations. In order to analyze labels, every sample was inspected for the presence of name, classification according to origin and according to the ways of producing and placing on the market, net quantity, best use before/date of expiry and batch number.

A total of 60 investigated samples included 18 samples of acacia honey, 17 samples of meadow honey, 8 samples of floral honey, 6 samples of linden honey, 5 samples of honeydew honey, 2 sample of floral honey with honeydew, 2 bee products (acacia honey with curcuma and honey with walnuts), 1 sample of sunflower honey and 1 sample of acacia/baker's honey.

RESULTS AND DISCUSSION

According to both Legislative on quality of honey and other bee products and Declaration, labeling and marketing of food ("Official Gazette RS", No. 101/2015; "Official Gazette RS" No. 85/13 and No. 101/13), 46 (76.67%) labels did not fulfill prescribed conditions, while only 14 (23.33%) did.

In total, 44 (73.33%) labels lack the information about classification according to origin, while only 15 (25.00%) had that information. In this study, pollen analysis was not performed, but in studies carried out in Croatia during 2004 pollen analyses showed that 28.80% of honey samples did not match information about origin of honey provided on the labels, 14.60% in 2005 (Peternel et al. 2006) and 20% in 2012 (Hrga and Stjepanović, 2013). In our study, 1 (1.67%) label classification according to the origin had double meaning.

All 52 (86.67%) honey samples, which contained information about classification according to the type of production and/or presentation on market, have been labeled as extracted honey. The rest of the samples (8 (13.33%)) lack this information.

Net mass was indicated on 58 (96.67%) inspected labels, while 2 (3.33%) of them lacked this information.

Also, date of expiry was not indicated on 3 (5.00%) inspected labels, while 5 (8.33%) of them were with "*unlimited*" date of expiry. In previous studies it was found that the half life of diastase activity was 4 years at 20°C, while the time needed for formation of 40 mg/kg HMF (hydroxymethylfurfural) in honey was 2 to 4 years (Bogdanov, 2009). In addition, honey is highly hygroscopic substance, and its moisture content may vary depending on air humidity during storage (Prica et al., 2014).

Total of 13 (21.67%) labels were without information about the batch number. In a study from Romania, the authors have shown that approximately 80% of the respondents are ready to pay an extra bid for honey with a traceability system (Tatiana et al., 2013).

The lack of information on labels in Serbian market are probably because Serbian beekeepers usually use direct marketing, which allows them to keep most of the price for themselves and to make direct contact with customers, which lead to customer loyalty (Zarić et al., 2013). In a study conducted in Vojvodina region in 2014, 40.6% of the respondents said that they purchase honey directly from beekeepers. The reason for this was big confidence in the producers, lower price than in stores and belief in higher quality (Ćirić et al., 2015). In Poland, 78% of the respondents said that the honey from a beekeeper was somehow "better" than the ones offered in the stores (Roman et al., 2013).

Although none of the legislation considers honey as a major allergen and none of the inspected labels contained information about allergen (including honey with walnuts), many studies showed that both pollen and insect allergen activity was found in all types of honey (Helbling et al., 1992; Kiistala et al., 1995).

Earlier, honey was used in the nutrition of children, but lately there is a health concern for infants regarding the presence of *Clostridium botulinum* in honey (Bogdanov et al., 2008). According to several authors, honey should not be given to children under 12 months of age (Tanzi et al., 2002; Aureli et al., 2002). Although this information seems to be very important, both consumers and beekeepers from Serbia may not be informed enough, due to a lack of this information in public media and legislation. Consequently, none of the inspected labels contained any information about infant's consumption of the honey.

CONCLUSION

The importance of tracing and tracking honey and/or bee products by batch number in every step of its production life cycle as well as in the market to ensure the final safety and quality is important and useful in increasing consumers trust. The quality of honey and bee products depends on its origin. The active components in plants depend on various factors and climatic conditions in different geographical locations. Choosing the honey from a certain location could have many nutritional and health benefits. Honey, as the potentially allergenic substance containing pollen and insect allergens, can be the cause of serious health problems in some people/children. Honey is an excellent energy source, but not recommended in diets of children under 12 months of age because of potential presence of *Clostridium botulinum*.

The shelf life of honey has to be based on the analysis of the quality of honey such as diastase activity and HMF content. Beekeepers have to be aware of the potential risks with improper storage conditions of honey and bee products.

It is common knowledge that "the customers buy with their eyes". Therefore, it is important that offered honey and bee products are properly packaged in aesthetic, eye-catching packaging. A colored label completes the whole image of the product. It carries the necessary information on the composition of the product, and can bring the consumption of the honey to a higher level.

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DERMANYSSUS GALLINAE - OVERVIEW: LIFE CYCLE, MORPHOLOGY, PREVALENCE AND CONTROL MEASURES IN POULTRY FARMS

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Abstract

Dermanyssus gallinae or the poultry red mite is currently the most important ectoparasite affecting egg-laying hens in several countries causing reduced poultry welfare, mortality and even allergic reactions in poultry farms workers. Its short life cycle, which in optimal conditions can be completed within 7 days, and ability to survive in extreme circumstances without a blood meal up to 13 months, and the ability to infest new flock, makes it even more difficult to eradicate. *Dermanyssus gallinae* prevalence rates in different European countries, including Serbia, can reach up to 80-90%. Also, the poultry red mite is responsible in vector transmission of several bacterial and viral avian diseases, including *Salmonella* spp, *Chlamydia* spp., *Escherichia coli*, *Staphylococcus* spp., *Pasteurella multocida*, Newcastle disease and Fowl poxvirus. Besides that, the poultry red mite can also transfer antimicrobial resistance genes by carrying pathogenic bacterial flora. Control of *Dermanyssus gallinae* can be divided into conventional and alternative methods. Conventional methods are mostly focused on preventing infestations and/or killing *Dermanyssus gallinae*, while alternative methods include the use of essential oils, vaccines, light, odors, predatory mites, fungi, nematodes and bacterial endosymbionts, and temperature in order to eliminate the poultry red mite. Nevertheless, this small ectoparasite still makes millions worth damage to global poultry industry.

Key words: Poultry red mite, egg-laying hens, prevalence, vector transmission, prevention

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DERMANYSSUS GALLINAE - PRIKAZ: RAZVOJNI CIKLUS, MORFOLOGIJA, PREVALENCIJA I MERE KONTROLE NA ŽIVINARSKIM FARMAMA

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Kratak sadržaj

Dermanyssus gallinae ili crvena kokošija grinja je trenutno jedan od najbitnijih ektopatazita koji pogađa koke nosilje, dovodeći u nekoliko zemalja do narušavanja dobrobiti, mortaliteta, pa čak i do pojave alergijske reakcije kod farmera. Razvojni ciklus ovog parazita koji uz optimalne uslove može biti kompletiran unutar 7 dana, sposobnost preživljavanja u ekstremnim uslovima bez krvnog obroka do 13 meseci i mogućnost da inficira novo jato predstavlja veoma težak način za eradikaciju. Prevalencija *Dermanyssus gallinae* u mnogim evropskim zemljama, uključujući i Srbiju, može da dostigne čak 80 do 90 %. Takođe, crvena kokošija grinja je kao vektor odgovorna za prenos uzročnika raznih bakterijskih i virusnih bolesti, uključujući *Salmonella* spp., *Chlamydia* spp., *Escherichia coli*, *Staphylococcus* spp., *Pasteurella multocida*, virusa Newcastle bolesti i Fowl poxvirusa. Pored toga, crvena kokošija grinja ima mogućnost, prenošenjem patogenih bakterija, da prenese i gene antimikrobne rezistencije. Mere kontrole *Dermanyssus gallinae* se mogu podeliti na dve metode: konvencionalnu i alternativnu. Konvencionalna metoda je većinom fokusirana na prevenciji infestacije i/ili ubijanju parazita *Dermanyssus gallinae*, dok alternativna metoda podrazumeva upotrebu esencijalnih ulja, vakcina, svetla, mirisa, predatora, gljivica, nematodnih i bakterijskih endosimbioza, kao i temperature u cilju iskorenjivanja crvene kokošije grinje. Naime, ovaj ektoparazit i dalje dovodi do milionskih šteta u živinarskoj proizvodnji širom sveta.

Ključne reči: Crvena kokošija grinja, koke nosilje, prevalencija, vektorski prenosive bolesti, prevencija

INTRODUCTION

Dermanyssus gallinae (*Acari, Mesostigmata, Dermanyssoidea, Dermanyssidae*) or the poultry red mite is an obligatory blood-sucking parasite of both domestic and wild birds. The poultry red mite is a cosmopolitan parasite and has been confirmed on 30 avian and 20 mammal species (Nordenfors, 2000). This ectoparasite poses a significant threat to egg-laying hens in many parts of the world, including Europe (George et al., 2015). The cost of *Dermanyssus gallinae* is difficult to evaluate on a global level, but poultry farmers estimated the costs for preventive and control measures, as well as higher feed intake, higher mortality and lower egg quality due to the damage caused by poultry red mite to be millions of Euros/dollars in production and animal losses, treatments, veterinary bills, and lost working days (Van Emous et al., 2006).

MORPHOLOGY AND LIFE CYCLE

Dermanyssus gallinae is a small ectoparasitic mite approximately 1.5 mm in length, which varies in color from gray to brown/red depending on feeding status. The poultry red mite does not have eyes, but locates its hosts using a combination of several stimuli, including vibration, heat and carbon dioxide sensing through hair-like appendages called setae, normally clustered at the palpal or tarsal extremities (Kilpinen, 2005; Pritchard et al., 2015). Once on a host, the nymphs and females feed for short periods usually during darkness, while males do so very occasionally. *Dermanyssus gallinae* stays on the birds for only 0.5±1.5 h to feed, while the rest of time they are hidden under the conveyor belts of eggs and cage supports, under the rods, in nest boxes, beneath troughs and in small cracks and crevices in the poultry house walls.

Complete development of *Dermanyssus gallinae*, from egg, through one larval stage and two nymphal stages, to adult usually takes about one to two weeks depending on environmental factors. A six-legged white larva hatches from the egg under warm conditions at the temperature of 28±30°C within 2±3 days. After one day, the larva molts, without feeding, to a protonymph, which has 8 legs. After feeding, the protonymph transforms to a deutonymph, which feeds again and transforms to female or male adult. The temperatures in laying hen facilities generally maintained between 18 and 21 °C provide optimal conditions for the development of *Dermanyssus gallinae*. Under such conditions, the lifecycle of poultry red mite can be completed within 7 to 17 days (Maurer and Baumgärtner, 1992). Soon after molting, the adults mate. Within 12 h, females feed and deposit eggs several times. In a lifetime, female *Derman-*

yssus gallinae is able to lay about 30 eggs. The egg is oval shaped (400x270 μ), smooth and white (Chauve, 1998). In exploitation, which usually exceeds one full year, *Dermanyssus gallinae* may be present all the time, but highest density occurs during hot and humid seasons (Nordenfors and Hoglund, 2000). The reproductive potential enables *Dermanyssus gallinae* to triple its number in only 10 days. This includes all development stages (Pavlović et al., 2017). According to several authors the poultry red mite can persist in extreme conditions for up to 8, 9 or even 13 months without a meal (Chauve, 1998; Nordenfors, 2000; Pavlićević et al., 2007). Even when laying hens are removed from premises between production cycles the poultry red mite may survive long enough to infest new flock because of their ability to starve longer than the regular pause between production cycles. Only an extraordinary pause of 2 years should be long enough for the present poultry red mite to die out. Only in this circumstance, and with no alternative food sources, the required period for PRM to die out can be prolonged (Pavlićević et al., 2007).

PREVALENCE OF *DERMANYSSUS GALLINAE* IN EUROPEAN COUNTRIES

Some studies revealed higher prevalence rates in less intensive farming systems, including organic, free range farming and barns, which are due to the ability of *Dermanyssus gallinae* to hide in cracks and fissures and avoid control methods.

Variation in prevalence throughout Europe is shown in Table 1. In different European countries *Dermanyssus gallinae* prevalence rates can reach up to 80-90% as shown for the United Kingdom, France, The Netherlands, Serbia, Montenegro (Sparagano et al., 2009). Investigations that were carried out in laying hen farms, duckling and gosling broiler farms in Poland showed even higher prevalence (100%) (Cencek, 2003).

Table 1. Prevalence (%) of *Dermanyssus gallinae* in egg-laying hen systems (Sparagano et al., 2009)

Country	Prevalence (%) by production system				
	Cage	Barn	Free-range	Backyard	Organic
United Kingdom	8-88	33	60	-	-
Denmark	32	50	68	-	36
France	72	50	56	-	80
The Netherlands	82	83	-	-	78
Italy	74	-	-	-	-
Montenegro	30-80	-	-	-	-
Norway	23	-	-	-	-
Poland	100	100	-	-	-
Serbia	90	-	-	-	-
Sweden	4	33	-	67	-

"-" data not available

Hence, Höglund et al. (1995) have shown that the prevalence of *Dermanyssus gallinae* actually depends on the flock housing system, where the infestation rates were 4% in cage systems, 33% in alternative systems and 67% in backyard flocks.

Studies in industrial egg production systems in Portugal have shown the presence of *Dermanyssus gallinae* in 94% of the laying hen units sampled (Waap et al., 2017).

Large populations of *Dermanyssus gallinae* in laying hen facilities may result in decreased egg production, egg quality, increased stress, mortality and morbidity (Mul et al., 2009).

PATHOGENS ASSOCIATED WITH *DERMANYSSUS GALLINAE*

Dermanyssus gallinae can act as a vector for bacteria and viruses. It is well established that *Dermanyssus gallinae* can be implicated in the transmission of vector-borne diseases. However, their role in the natural transmission cycles of pathogenic agents is poorly known; thus, they are mostly ignored as vectors of human or animal diseases. Bacterial and viral avian diseases potentially associated with *Dermanyssus gallinae* as a vector (according to relevant scientific literature) are reviewed in Table 2.

Table 2. Bacterial and viral avian diseases which are transmitted by *Dermanyssus gallinae* (Moro et al., 2009)

	Details	Pathogen
Bacteria	Isolated from mites	*Salmonella* Gallinarum
		Chlamydia spp.
		Escherichia coli
		Staphylococcus spp.
	Transmission demonstrated	*Salmonella* Enteritidis
		Pasteurella multocida
Viruses	Isolated from mites	Newcastle disease
	Transmission demonstrated	Fowl poxvirus

The use of antimicrobial drugs for prophylactic purposes or therapy can lead to the formation of resistant strains of different bacterial species. These resistant bacterial strains are excreted by animal excrements. In this incidence PRM come in contact with those strains and become vectors who transmit the antimicrobial resistance genes (Stojanov et al., 2017). Some studies have shown that *Escherichia coli, Enterobacter* sp. *Citrobacter* sp., and *Pseudomonas aeruginosa* strains found in *Dermanyssus gallinae* were resistant to amoxicillin, ampicillin, and colistin (Stojanov et al., 2016).

Moro et al. (2009) demonstrated that, immediately after the experimental infection, *Salmonella* spp. was found in 29% of mites infected by a blood meal and in 55% of mites infected by cuticular contact. Earlier studies showed that *Salmonella* spp. could survive inside the mites for up to 4 months (Zeman et al., 1982). In humans, *Dermanyssus gallinae* may cause allergic reactions (Chauve, 1998).

CONTROL STRATEGIES

Despite many methods, the control of *Dermanyssus gallinae* is difficult for numerous reasons. First of all, mites spend most of the time in inaccessible cervices and leave their resting place only to eat, which happens for only 30 to 60 minutes every few days. This makes the control difficult to manage, especially if using acaricides that require the contact the target to be effective.

The control of *Dermanyssus gallinae* can be divided into two parts: conventional methods and alternative methods. The conventional methods mostly focus on killing or preventing infestations by maintaining good hygiene

practices and regular cleaning of poultry facilities, which can remove large proportions of the poultry red mite populations, as well as their eggs (Nordenfors and Höglund, 2000). Worldwide, *Dermanyssus gallinae* has typically been controlled using several synthetic acaricides including organochlorines, organophosphates, pyrethrin, pyrethroids, carbamates, amitraz, and endectocides (Chauve, 1998). The use of synthetic acaricides is limited in many European countries by imposing stricter legislation regarding active ingredients. Another problem associated with acaricide use is the possibility of poultry red mite to develop resistance (Sparagano et al., 2014), as well as the risk of exposing eggs, poultry and humans to their residues (Hamscher et al., 2003).

Another useful product in controlling poultry red mite is silica dust. The main benefit of this product is its ability to immobilize poultry red mite by adhering to its body and to cause damage to its cuticle, which leads to severe dehydration and death (Mul et al., 2009).

Many alternative methods have been announced and used in the control of poultry red mite for years, including essential oils, vaccines, light, odors, predatory mites, fungi, nematodes and bacterial endosymbionts (Mul et al., 2009; Sparagano et al., 2014), but none of them have yet given relevant contribution in clinical practice. Development of products based on SiO2 (Kilpinen and Steenberg, 2009; Schulz, 2014) is the most important, but insufficient in clinical practice of *Dermanyssus gallinae* control. The use of insecticides still remains dominant (Sparagano et al., 2014; Pritchard et al., 2015).

However, important improvement in *Dermanyssus gallinae* control has been noticed in 2017. A novel systemic acaricide Exzolt™ (10 mg/mL fluralaner solution, MSD Animal Health) appeared in the market. This product is based on active substance fluralaner and can be administrated *per os*, making it easy and safe in practical use on farms (Dolz, 2017).

In order to provide a safe, rational and highly efficient *Dermanyssus gallinae* control mechanical methods have been improved and combined with a range of existing applications and formulations, including new oil-based formulation (Pavlićević et al., 2017).

CONCLUSION

The fact that *Dermanyssus gallinae* causes huge problems worldwide manifested as poor egg quality and decreased production, mortality of poultry and transmission of several bacterial and viral diseases emphasizes the need and importance of future investigation and development of novel control methods. The monitoring of *Dermanyssus gallinae* is an important instrument in

recognizing the risk and taking appropriate measures on time. Cooperation between veterinarians, scientists and farmers can help to identify effective new control and eradication methods.

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AN INNOVATIVE FORMULATION OF PARAFFIN AND SILICONE OILS FOR THE CONTROL OF THE RED POULTRY MITE *(Dermanyssus gallinae)* – EXAMINATION OF THE EFFICIENCY UNDER LABORATORY CONDITIONS

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Abstract

The past decades of clinical practice in poultry farming are characterized by inadequate control of one of the most important poultry ectoparasite, the red poultry mite (*Dermanyssus gallinae*). Therefore, the program for control of *D. gallinae* based exclusively on a physical mechanisms of action of acaricides has been developed in Serbia since 2012. By the beginning of 2017, a synergistic relationship between paraffin and silicone oils was observed by our team, and its efficiency in preventing the red poultry mite from respiring was examined. For laboratory examination the Petri dish and tin-box methods were used. Direct, full 1-minute exposure of adult mites to the recommended (15 and 20%) or even much lower concentration of the formulation resulted in 100% mortality after 24 hours. Subsequent 1-hour exposure (24 hours after application onto treated non-absorbent surface of adult mite) resulted in 100% mortality with the same concentrations. The long term effect depends on the surface quality. The concentrations of 15 and 20% provide 100% long-term effect after a 1-hour exposure period, and remains fully effective over the following 4-month period. In the following months (8 months), the formulation continues to work with a slightly lesser, however, still significant effect. The formulation is most effective on a plastic surface, where the full effect is achieved with significantly lower concentrations. The formulation has no effect on eggs; however, after the short development cycle is completed in the egg (2-6 days) and exit from

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the shell, the larva is immediately exposed to the effects of the formulation and eliminated. High efficiency of the paraffin and silicone oils formulation, which has been established in laboratory conditions, justifies its use to the purpose of D. *gallinae* control in cages and equipment before flock settlement as well as in transport cages after cleaning and disinfection.

Key words: *D. gallinae,* paraffin and silicone oil, laboratory examination, efficiency

INOVATIVNA FORMULACIJA PARAFINSKOG I SILIKONSKIH ULjA ZA KONTROLU CRVENE KOKOŠIJE GRINjE (*Dermanyssus gallinae)* – LABORATORIJSKO ISPITIVANJE EFIKASNOSTI

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Kratak sadržaj

Protekle decenije kliničke prakse u živinarstvu karakterišu se pogrešnim pristupom i neadekvatnom kontrolom najznačajnijeg ektoparazita živine, crvene kokošije grinje (*Dermanyssus gallinae*). Iz tog razloga se od 2012. godine u Srbiji razvija programska kontrola *D. gallinae,* bazirana isključivo na fizičkom dejstvu. Ograničenosti postojećih preparata i metoda iziskivali su unapređenja. Početkom 2017. godine našim istraživanjima je utvrđen sinergizam parafinskog i silikonskog ulja i ispitivana je njenu efikasnost u sprečavanju respiracije crvene kokošije grinje. U laboratorijskom ispitivanju korišćena je metoda petrijeve šolje i limenih kutija. Direktnim, punim izlaganjem adulta u ekspoziciji od 1 minut, nakon 24 časa, utvrđen je 100% mortalitet, kod preporučenih (15 i 20%), ali i znatno nižih koncetracija formulacije. Naknadnim izlaganjem (24 sata od nanošenja) na tretiranu ne upijajuću površinu adulta u trajanju ekspozicije od 1 sat utvrđen je 100% mortalitet, kod preporučenih koncetracija 15 i 20%. Produženo delovanje je u zavisnosti od kvaliteta podloge. Formulacija je najefikasnija na plastičnoj podlozi, gde se pun efekat ostvaruje i u značajno nižim koncetracijama.

Pocinkovani lim zahteva povećanje koncetracije formulacije koje su optimalizovane za pripremu kaveza i opreme na 20%. Ova koncetracija formulacije obezbeđuje 100% efekat produženog delovanja u ekspoziciji od 1 sat, koje se nesmanjeno manifestuje u periodu od 4 meseca. U narednim mesecima (8 meseci), formulacija nastavlja da deluje nešto manjim, ali i danje značajnim dejstvom. Formulacija ne deluje na jaja. Međutim, posle završetka kratkog razvojnog ciklusa u jajetu (2-6 dana) i izlaska, larva biva odmah izložena dejstvu formulacije i eliminisana. Visoka efikasnost formulacije parafinskog i silikonskog ulja koja je utvrđena u laboratorijskim uslovima, opravdava njenu primenu na kavezima i opremi pred naseljavanje jata, i transportnim kavezima posle pranja i dezinfekcije, u cilju kontrole *D. gallinae*.

Ključne reči: *Dermanyssus gallinae*, parafinsko i silikonsko ulje, laboratorijsko ispitivanje, efikasnost

INTRODUCTION

The past decades of clinical practice in poultry farming are characterized by a wrong approach and inadequate control of the most important poultry ectoparasite, the red poultry mite (*Dermanyssus gallinae*). The evidence and consequence of such inadequate control is reflected in the current situation: high prevalence rates (Sparagano et al., 2009; Flochlay et al., 2017; Pavlićević et al., 2017; Pavlićević et al., 2017a), severe direct and indirect damages, negative effects on flock's health status and production results, and especially important toxicological risk in egg production such as the most recent fipronil incident (Pavlićević et al., 2017b).

Until now, synthetic chemical compounds manifesting neurotoxic effects (acaricides, in a broader sense - insecticides) were the dominant method of controlling *D. gallinae* (Wang et al., 2010; Sparagano, 2014; Flochlay et al., 2017). Moreover, their application has often been uncritical and almost regularly incompetent, which resulted in consequent spreading and progression of *Dermanyssosis* disease. Moreover, there is a problem of emerging resistance of *D. gallinae* (Liebisch, 2003; Marangi et al., 2009; Pavlićević et al., 2016). Only the recent international fipronil incident has drawn the attention to a huge toxicological risk to which poultry farming was exposed, especially egg production.

Although there is a wide selection of various products and methods on the market, only selected SiO_2 formulations offered an acceptable and safe al-

ternative for the control of *D. gallinae* (Pavlićević et al, 2017c). However, SiO_2 formulations have a far smaller range of practical uses than synthetic chemical unities with neurotoxic effects, and their drawbacks are evident. All aforementioned facts strongly suggest the necessity of improvement of *D. gallinae* control strategy.

Contrary to the generally accepted practices, programmed control of *D. gallinae* has been developing in Serbia since 2000 (P-441/01; Pavlićević et al., 2003; 2003a; 2007; 2007a; 2008; 2016; 2017a; 2017b; 2017c), and since 2012, the programme has exclusively been based on products with a physical effect (Pavlićević et al., 2017c). An innovative, professional, and full-scale program enabled the elimination of all risky substances, especially synthetic chemicals with a neurotoxic effect, from egg production. Beside the optimisation of SiO_2 application we offered a completely new approach to physical control (Patent No P 547/17). By combining medicinal paraffin and silicone oil we determined a synergy which can provide essential product characteristics required for successful control of red poultry mite.

The aim of this research is to determine the efficiency of paraffin and silicone oils formulation (Pavlićević and Kovačević, 2017 (Patent No P 547/17)) on the red poultry mite (*Dermanyssus gallinae)* in laboratory conditions.

MATERIALS AND METHODS

The subject of testing was oil formulation consisting of paraffin and silicone oil (Patent No P 547/17) intended for preparation of cages before flock settlement in poultry industry. The formulation was applied in the form of aqueous emulsion at working concentration of 15-20%. Laboratory tests encompassed the recommended concentrations (15-20%), but also a range of lower concentrations such as 1, 2, 5, 10, and 12%.

Biological test was performed on adults of the laboratory strain of *D. gallinae* (i.e., well-nourished female individuals) and their eggs. The laboratory strain of *D gallinae* was grown in isolation chambers of the laboratory. Chambers were designed to enable favourable conditions for life and reproduction while still preventing their escape. Isolation chambers contained the cardboard traps, on which the mites were collected for the use in biological testing. The number of individuals per testing was 100, arranged in 5 dishes of 20 individuals each. Furthermore, the control group included 20 individuals per test. The mites were transferred using the needle, by grasping the ambulacra.

Laboratory examination implicated exposures of 1 minute, 1 hour, 24 hours and a continuous exposure (efficiency on *D. gallinae* eggs). In case of

1-minute exposure, we performed the full exposure of individual mites, which were placed into a dish and the investigated product with a sufficient acaricidal effect was added onto them. Such exposition simulates the situation when a working emulsion is directly applied onto mites, resulting in a full exposure of the whole body. When the exposure was complete, the mites were transferred into a dish for observation (result analysis) and placed onto the filter paper (which covers the bottom of a clean Petri dish) in order to remove excess liquid and prevent asphyxiation. The mites were held in the same dish until the end of the test. The 1-hour exposure is considered the most important test as it defines the prolonged effect of the formulation. In case of 1 and 24-hour exposure, the sufficient amount of examined product at a desired concentration was introduced into the cylinder formed of Petri dishes, evenly spread by shaking, and the leftover was drained by turning and filtering. The dishes were dried at room temperature. The mites were introduced to the test dishes after applying the product and subsequent drying at room temperature for 24 hours.

When investigating the prolonged effect, repeated tests were performed on prepared test dishes at desired intervals. When the exposure was completed, the mites were transferred to a dish for observation (result analysis). After transferring, immediately after the exposure of mites to the product with an acaricidal effect, a knock-down effect was read. The mortality of *D. gallinae* in observation dishes was determined once a day during a 10-day period by visual examination utilizing a magnifier and a microscope. The mortality was assessed according the position of extremities and the body, motility, and morphological changes. The retention factor indicates to what extent the formulation inhibits the adults to escape the treated surface. The mortality was estimated 24 hours after exposure of mites to treated surface.

In this study, the sides of a plastic Petri dish or tin box were used as the testing surface. The materials used included plastic and galvanized sheet metal, which is commonly the material for the production of cages and equipment in poultry farming. Ambient conditions for testing were within the following ranges: temperature 17-26°C (average 21.62°C), and humidity 39-69% (average 51.81%).

Laboratory examination in plastic Petri dishes

The test dishes are formed from plastic Petri dishes with dimension of 55 mm (outside measure) in a way that the identical rim sides of the Petri dish come together. In this manner, tall-form and short-form cylindrical dishes were created, in which the mites were tested for the acaricidal properties of

the product. Cylinders made of high-rim sides were used for exposure, and those made of low-rim sides for observing the effects after the exposure. The preparation of the dishes made of high-rim side Petri dishes was done in a way to obtain a rough surface to enable even application of the examined solution, suspension, or powder. The preparation was performed using a wire attachment on the drill. After the mechanical preparation, the surface was wiped with concentrated alcohol for further degreasing and drying and used according to the exposure plan.

The effects of the formulation on egg viability were tested using plastic Petri dishes into which previously fed female mites were placed. After lying of eggs, the females were removed from the dish. The eggs were treated with the investigated formulation at high concentration (20%) and subjected to continuous formulation exposure.

Laboratory examination in tin boxes

Two-part dishes made of galvanized sheet metal, which are put together to form a box were used. Dimensions of one part were 79 x 100 x 62 mm, and the sides had an outside groove so that they would fit together. Tin boxes were used unprocessed and according the same method as the one used for the Petri dish. The examined formulation and mite individuals were placed inside the box.

Separate records were kept for each test - a report of laboratory examinations, where all important information was recorded.

RESULTS

The results of the experiment of direct exposure of *D. gallinae* to the oil formulation of paraffin and silicone oil (Patent No P 547/17) for the exposure time of 1 minute that simulates direct exposure during application are shown in Table 1.

Table 1. Exposure of *D. gallinae* to the oil formulation of paraffin and silicone oil (Patent No P 547/17) for 1 minute

Working concentration of the emulsion (%)	1	2	5	10	15	20
Mortality of the experiment group (%)	100	100	98	100	100	100
Mortality of the control group (%)	0	5	5	0	0	0
Efficiency (%)	100	95	93	100	100	100

The result of the experiment of direct exposure of *D. gallinae* to 1% emulsion of the oil formulation of paraffin and silicone oil (Patent No P 547/17) for the exposure time of 1 hour that simulate the occasion when *D. gallinae,* which are hidden during application, come into the contact with the formulation during activity, is shown in Table 2.

Table 2. The result of further exposure of *D. gallinae* to 1% emulsion of the oil formulation of paraffin and silicone oil (Patent No P 547/17) for 1 hour in plastic Petri dishes

Day (month) of the residual effect	1	84	85	91 (3)	98	189	214 (7)	244 (8)
Mortality of the experiment group (%)	100	100	100	99	99	96	89	79
Mortality of the control group (%)	0	1	0	0	1	0	0	0
Efficiency (%)	100	95	100	99	94	96	89	79

Mite deceased due to the effect of the investigated formulation, (Scanning electron micrograph (SEM)) is shown in Figure 1.

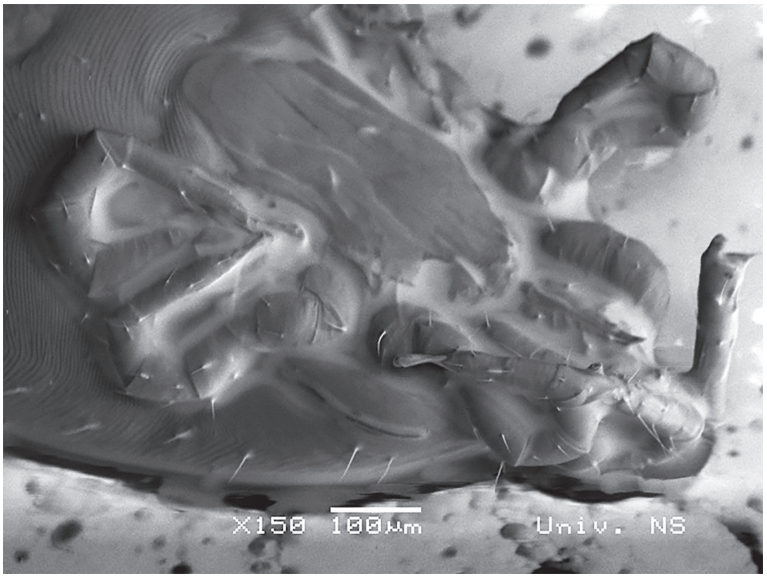

Figure 1. Mite died due to the effect of the investigated formulation, Scanning electron micrograph (SEM).

The importance of the surface type for the residual effect of oil formulation of paraffin and silicone oil (Patent No P 547/17) is presented in Table 3.

Table 3. The result of further exposure of *D. gallinae* to 1% emulsion of the oil formulation of paraffin and silicone oil (Patent No P 547/17) for 1 hour, different surfaces

Surface	Plastic Petri dish	Galvanized sheet metal
Mortality of the experiment group (%)	98	29
Mortality of the control group (%)	0	0
Efficiency (%)	98	29

The result of the experiment of direct exposure of *D. gallinae* to different % of emulsion of the oil formulation of paraffin and silicone oil (Patent No P 547/17) for the exposure time (defined timeframes minute/hours) on galvanized sheet metal, are shown in Tables 4, 5 and 6.

Table 4. Working concentration of 10% emulsion of oil formulation of paraffin and silicone oil (Patent No P 547/17) on galvanized sheet metal

Day (month) of the residual effect	1	1	12	30	35	42	(3)	(4)	(6)
Mortality of the experiment group (%)	97	96	99	100	99	100	96	90	90
Mortality of the control group (%)	0	1	0	0	0	0	2	0	0
Efficiency (%)	97	91	99	100	99	100	85	90	90

Table 5. Working concentration of 15% emulsion of oil formulation of paraffin and silicone oil (Patent No P 547/17) on galvanized sheet metal

Day (month) of the residual effect	1	1	10	21	30	67	(3)	115	(4)	(5)
Mortality of the experiment group (%)	97	100	99	98	99	97	94	100	87	88
Mortality of the control group (%)	0	5	0	0	10	0	0	0	0	0
Efficiency (%)	97	95	99	98	89	97	94	100	87	88

Table 6. Working concentration of 20% emulsion of oil formulation of paraffin and silicone oil (Patent No P 547/17) on galvanized sheet metal

Day (month) of the residual effect	1	67	(3)	(4)
Mortality of the experiment group (%)	100	100	100	100
Mortality of the control group (%)	0	0	0	0
Efficiency (%))	100	100	100	100*

* the full mortality is achieved 4 days after exposure, in comparison to previous tests in which it is 1 day

In Table 7, the experiment results for a 24-hour exposure of oil formulation of paraffin and silicone oil (Patent No P 547/17) on galvanized sheet metal are presented.

Table 7. The experiment results for a 24-hour exposure on galvanized sheet metal

Concentration (%)	10	12	15	20
Mortality of the experiment group (%)	100	100	100	100
Mortality of the control group (%)	0	0	0	0
Efficiency (%)	100	100	100	100

The results of testing different concentrations of the oil formulation of paraffin and silicone oil (Patent No P 547/17) on plastic surface are shown in Tables 8 and 9, and the efficacy of the formulation on the eggs is shown in Table 10.

Table 8. Testing different concentrations of the working emulsion: 1, 5, 10, and 15% on a plastic surface

Time of observation (hours)	Water	1%	5%	10%	15%
1	24	79	87	100	100
24	3	78	98	100	100
Temperature (C)	22	25-27	25-27	25-27	25-27
Moisture (%)	51-56	37-61	37-61	37-61	37-61

Table 9. Testing concentrations of 15 and 20% emulsion on a tin surface, over a period of 30 days, with analysis on a weekly basis.

Day	1			7			14			21			30		
Concen. of the working emulsion (%)	%	T*	Hum**	%	T	Hum.	%	T	Hum.	%	T	Hum.	%	T	Hum.
15	100	22	51-56	95	20	50-47	92	20	46-47	94	19-20	49	93	19	43-46
20	100	22	51-56	98	20	47-50	92	20	46-47	97	19-20	49	97	19	43-46

* Temperature showed in °C

** Humidity showed in %

Table 10. Results of the laboratory experiment, signifying the viability of eggs exposed to the formulation emulsion

Test day	No. of eggs	Larvae		Protonymphs		Ambient Conditions	
		Live	Dead	Live	Dead	Temperature	Moisture
1	29	-	-	-	-	18	42
2	29	-	-	-	-	18	48
3	21	8	-	-	-	17	42
4	13	7	9	-	-	17	40
5	7	3	19	-	-	17	42
6	3	1	25	-	-	17	44
7	3	0	26	-	-	17	42
8	3	0	26	-	-	17	43

tThe image of the egg and larvae (light microscope (200x)) used in the research are shown in Figures 2. and 3.

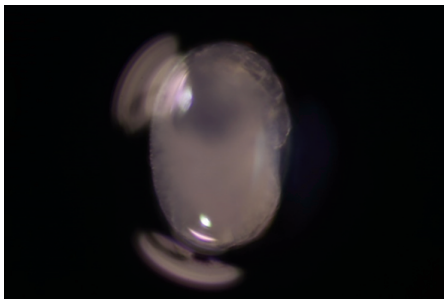

Figure 2. Egg, light microscope (200x)

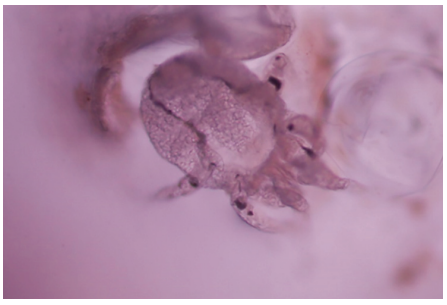

Figure 3. Larvae, light microscope (200x).

DISCUSSION

According to our knowledge, there is no standardized method for laboratory and clinical examination of the efficiency of different acaricides on *D. gallinae*. The testing of biological efficiency of the formulation is highly challenging with regard to reproducibility and particularly to application in practice. We have been using the Petri dish method since 2000, and the quality of laboratory results has been checked in practice.

The biological test, Petri dish method, is one of the fundamental elements in the program for control of red poultry mite (*Dermanyssus gallinae*). The Petri dish method provides conditions for rational pharmacotherapy through selection of products with acaricidal properties, describing the pharmaco-toxicological profile, and monitoring the resistance, i.e., sensitivity - Patent No 441/01 (Pavličević, 2001).

In the presented study, the recommended concentrations for cage preparation before settlement were tested along with some lower concentrations in order to obtain more comprehensive idea of the formulation and its action.

The working concentration of formulation is used rather than a precise dose. The reason for this is to create the simulation of practical conditions. Testing in laboratory conditions commonly implicates testing of acaricides at highly precise doses (defined concentration and amounts per unit of surface), but the same precision cannot be repeated in practice, and thus, exact results cannot be expected. In practice, the dosage oscillates depending on the ambient conditions. Thus, the level of acceptance is crucial. Despite all of the above, laboratory studies generally should be an important guideline for further clinical trials. One-minute exposure provides information on the efficiency on directly exposed individuals during the very application of the formulation. This is the easiest "task" for the product intended for the control of *D. gallinae*. The laboratory test revealed 100% efficiency of product concentration of 10-20%. However, a very high efficiency is evident even at 20 x lower concentration.

One-hour exposure provides information about mites which escaped the direct application of the formulation, yet come in contact with it after leaving their shelter. This exposure is the most important one for efficiency assessment in laboratory conditions. It is evident that the absorptivity of the surface onto which the product is applied affects the effectiveness of formulation. On quickly absorbing surfaces, there is no residual effect. Preliminary application of the formulation on a concrete surface (regular concrete) resulted in quick drying of the surface, which did not justify the testing of a prolonged effect, thus, such tests have not been performed.

On non-absorbable surfaces (galvanized metal and plastic), a layer that has a prolonged effect is created. The formulation is most efficient on a plastic surface (i.e., the less absorbable surface), where the full effect is achieved with lower concentrations. Galvanized metal requires an increased formulation concentration, which were optimized to 20% for the preparation of cages and equipment. This concentration provides 100% prolonged effect with an exposition of 1 hour which remains stable and consistent over a period of 4 months. During the following months (8 months), the formulation remains active to somewhat lesser, but still significant effect. Slightly lesser efficiency is observed with lower concentrations of the emulsion. However, concentrations of 10 and 15% demonstrate satisfactory efficacy that is applicable for practical control.

A 24-hour exposure does not simulate a situation in practical conditions, thus, it was used only for determining the tendency and comparability with other examinations (Sybil et al., 2011; Van Sauers, 2009). The full (100%) effects of the control are observed at the examined concentrations of 10-20% on a galvanized metal surface.

Aside from the efficiency on directly exposed mites and a prolonged working on non-absorbable surfaces, the formulation also manifest particularly pronounced retention factor, which inhibits the escape of mites from the treated surface. The retention factor depends on the concentration of the formulation. It is optimally expressed at concentrations of 15-20%. It is most expressed during the day one; however, its effects remain during at least 1 month.

We haven't confirmed any significant effect on eggs continually exposed to the formulation. Also, even mites that are lethally exposed to the effects of formulation can lay eggs until the moment of death. In case of sub-lethally exposed mites, we haven't noticed any significant influence on reproduction. However, the formulation remains active and affects the eggs, different developmental stages of mite as well as the reproduction cycle. The environment covered with a layer of formulation with prolonged effect is a trap for newly hatched larvae, which are prevented from moving and thus killed. The insecticidal effect of petroleum-derived spray oils has been known for more than one century (Agnello, 2002). Mineral oil works in a physical way by stopping respiration. Aside from a number of excellent properties, a lack of a prolonged effect was determined (Buteler and Stadler, 2011). However, this property itself is crucial for an efficient control of *D. gallinae*. The paraffin and silicone oil formulation was designed with the purpose of securing a prolonged effect. The morphological position of stigmas with *D. gallinae* offers the possibility of being inhibited by slightly oiled surfaces. However, at the same, time there is a need to avoid excessive dirt on the cages and equipment. Otherwise, excessive

build-up of dirt on treated surfaces would prevent the prolonged effect thus creating unhygienic environmental conditions.

Mauer et al. (2009) determined certain level of efficiency of diesel oil. Also, they referred to the research of Guimaraes and Tucci (1992) who reported 100% efficiency of mineral oil after 2-hour exposure of directly treated *D. gallinae*. The investigations did not include the prolonged effect. In addition, the importance of the prolonged effect was neglected by many authors who addressed the topic of the effectiveness of essential oils (Van Sauers, 2009; Locher et al., 2010). Some examples of the confirmed prolonged effect are as following: azadirachtin - 11 days on impregnated filter paper (Locher, 2009); fluralaner (administered in drinking water) – persists for 15 days in the blood of the poultry (Heckeroth et al., 2015; Prohaczik, et al., 2017); spinosad - 21 days on different metal surfaces (George et al., 2010). An exceptional prolonged effect of carbaryl (70 days) was lost due to a development of resistance (Pavlićević et al., 2016).

Differently designed formulations, methods of application, and a non-standardized methodology of laboratory examination does not allow proper comparability on the basis of laboratory experiments with contemporary active matters, such as fluralaner (Thomas et al., 2017), spinosad (George et al., 2010), phoxim and others (Locher, 2009; Sybil et al., 2011). In that respect, the comparability of the efficiency and profitability is to be further addressed and confirmed by clinical research.

Contrary to synthetic chemical substances with a neurotoxic properties (acaricides, in broader sense - insecticides), the development of the resistance is not likely due to the physical acaricidal effect of the product. Certain fluctuations in the efficiency do exist, and can be attributed to ideal coverage of the surface with the examined formulation.

The paraffin and silicone oil formulation works in a physical way, and is designed to maximally express its properties required for the control of *D. gallinae*. Having in mind the actual control methods (Sparagano et al., 2014; Flochlay et al., 2017), prolonged long-term effect of silicone-paraffin oil layer is considered a new and innovative approach (Patent No P 547/17) to the control of the red poultry mite. An approval of high efficiency and the aforementioned characteristics recommend the formulation for preparation of cages and equipment in poultry farming, before the flock settlement, as well as for transport cages. With a thorough preparation (cleaning and washing) and a rest of the facility, and subsequent professional application of the product, we can expect high efficiency of the formulation. An adequate preparation of the facility is considered crucial in preventive veterinary medicine and the control of the red

poultry mite. The success of the control will be additionally increased by placing a layer of product with a prolonged effect on absorbable surfaces. One of potential solutions is spreading diatomic earth onto the floor. We expect that proper use of the formulation will enable a yearly exploitation of a layer flock. The properties of the formulation identified in this research through laboratory examination strongly suggest the possibility of an increased efficiency of eradication within the control program of *D. gallinae*. Further clinical examinations are needed to confirm the results acquired in laboratory conditions.

CONCLUSION

The high efficiency of the formulation based on paraffin and silicone oil (Pavlićević A. and Kovačević P., 2017 (Patent No P 547/17)), which has been determined under laboratory conditions, justifies its application for the control of *D. gallinae* on cages and equipment at the farm before settling the flock, and transport cages after thorough washing and disinfection (before using it for flock transport). The formulation has a potential for a high level of suppression, and as a part of a well-designed program, also for the eradication of *D. gallinae* from production facilities, i.e., poultry farms.

The final evaluation of the formulation will be possible after analysing the effects of *D. gallinae* control after preparation of cages for flock settlement throughout a one-year period of flock exploitation, as well as after comparing the results with comparative controls.

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LYME BORRELIOSIS IN NORTH BACKA DISTRICT

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Abstract

Lyme disease is the most common vector borne disease in regions with moderate climate. The cause of the disease is *Borrelia burgdorferi sensu lato (B. burgdorferi s.l.)*, whereas infection is spread via bite from a tick carrying the causative agent. The objective of this work is the examination of descriptive-epidemiological characteristics of Lyme disease in North Backa region in the period from 2012 to 2016. In terms of classification, Lyme disease does not fall into the category of diseases that must be reported when infection occurs among animals. The average rate of incidence in humans during the observed five-year period is 7/100.000. The highest incidence of this disease among humans was observed in 2013 (In 12.2/100,000) with 22 reported cases, whereas the lowest incidence was in 2015 (In 5.0/100,000) with 9 reported cases. The highest rate of incidence was recorded in Bačka Topola municipality – 15.9/100.000; followed by Mali Iđoš with 7.2/100.000; while the lowest rate was recorded in Subotica being 5.0 cases per 100,000 residents. The presence of the disease was confirmed in all patients via laboratory (serological) tests. Women were more likely to become infected, with the male-female patient ratio being 41%-59%. The disease was documented predominantly among people aged 50-69. The majority of cases were recorded during spring and summer, in the period from May to June (71%). Among animals, the disease was documented in Subotica and Mali Iđoš municipality, with average prevalence rates among dogs and horses being 0.07%; and 1.52% respectively. Subotica municipality has the most accurate records of cases and a prevalence of 0.06% among dogs, and 1.43% among horses, while Mali Iđoš municipality has higher prevalence, being 2.99% among horses and 0.14% among dogs. The highest occurrence of Lyme dis-

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ease among animals was documented in 2015, with a prevalence of 2.85% among horses. The presence of causative agents among examined ticks in Mali Iđoš municipality was 16.28% on average, predominantly in 2015 (in 21.21% of examined ticks).

Keywords: Lyme disease, incidence, sub-registration, prevalence, horses, dogs, ticks

LAJM BORELIOZA U SEVERNOBAČKOM OKRUGU

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Kratak sadržaj

Lajmska bolest je najčešća vektorska bolest u područjima sa umerenom klimom. Uzročnik bolesti je *Borrelia burgdorferi* sensu lato (*B burgdorferi* s.l.), a infekcija se ostvaruje ubodom zaraženog krpelja. Cilj rada je sagledati deskriptivno-epidemiološke karakteristike lajmske bolesti u Severnobačkom okrugu u periodu od 2012-2016.godine. Kod životinja, oboljenje ne podleže obavezi prijavljivanja. U posmatranom petogodišnjem periodu registruje se prosečna stopa incidencije od 7/100 000. Najviša incidencija ovog oboljenja registrovana je 2013. godine (In 12.2 / 100 000), kada su prijavljene 22 obolele osobe, a najniža 2015 (In 5.0 / 100 000), sa registrovanih 9 slučajeva ovog oboljenja. Najviša prosečna stopa incidencije registrovana je u opštini Bačka Topola 15.9 / 100000, sledi opština Mali Iđoš sa 7.2 / 100000 i najniža u opštini Subotica, 5.0 / 100000 stanovnika. Svi oboleli su imali laboratorijsku (serološku) potvrdu bolesti. Češće su oboljevale žene, u odnosu Ž:M 59% - 41%. Oboljenje je najčešće registrovano u uzrastu od 50-69 godina. Većina slučajeva zabeležena je u prolećnim i letnjim mesecima u periodu od maja do jula (71%). Kod životinja oboljenje je registrovano u opštinama Subotica i Mali Iđoš, kod pasa sa prosečnom godišnjom prevalencom 0,07%, i konja 1,52%. Prevalenca u opštini Subotica kod pasa je 0,06%, kod konja 1,43%. Najviše obolelih životinja registrovano je 2015.godine, sa prevalencom od 2,85% kod konja. Iz ispitanih

krpelja u Opštini Mali Iđoš prisustvo uzročnika je utvrđeno prosečno u 16,28% ispitanih krpelja uzetih sa ljudi i / ili životinja, najviše tokom 2015 (21,21% pregledanih krpelja).

Ključne reči: Lajmska bolest, incidencija, subregistracija, prevalenca, konji, psi, krpelji

INRODUCTION

Lyme disease is the most frequent vector borne disease in the regions with moderate climate. Causative agent of the disease is *Borrelia burgdorferi sensu lato* complex, from the family of *Spirochaetacea*. Within this complex, there are several borrelia genotypes, but for some of them, their pathogenicity for humans and / or animals has still not been determined. In Europe, Lyme borreliosis is caused by three similar Borrelia types: *B. burgdorferi sensu strico, B.garinii* and *B. afzelii* (Margos et al., 2008). The disease is transmitted by the Ixodide ticks. In the USA, the major vectors are *Ixodes scapularis* and *Ixodes pacificus*, in Europe *Ixodes ricinus* and in Asia *Ixodes persulcatus* (Gray, 1998, Steere et al., 2004). Global geographical distribution of Lyme disease is in correlation with the prevalence of Ixodide ticks infected with *Borrelia burgdorferi*. Mice and mice-like rodents are considered as the major reservoirs of infection even though the disease has been found in many animal species so far (Pavlović et al., 2006).

In humans, this disease is manifested with a broad spectrum of clinical symptoms, with sub acute, acute or chronic form of the disease. Some of them are very characteristic (erythema migrans), so that with the history of tick bite it is sufficient to establish the diagnosis. The conformation of other forms of the disease requires identification of the causative agent or specific antibodies. In the initial stage of the disease, one third or half of the infected people give seropositive response. In later stages of the disease seropositivity increases to 90–100% (Aguero-Rosenfeld et al., 2005). Besides in humans, Lyme disease can be found in dogs, horses, cattle and sheep (Infectious diseases in Province of Vojvodina in 2015, annual report; Mladenović 2014.).

In Serbia first studies on Lyme disease were started in 1987. In the region of North Bačka Lyme borreliosis was firstly found in 1994.

There were two main aims of the study. The first aim of the study was to survey the descriptive epidemiological characteristics of Lyme disease in North Bačka region during the period from 2012 to 2016. The second one

was to collect and analyse epizootiological characteristics of Lyme disease in animals of North Bačka County in the time frame from 2012 to 2016. This was shown with the following data:

- Number of confirmed clinical cases in dogs and horses,
- Study on infection in ticks.

MATERIAL AND METHODS

In this study, a descriptive epidemiological method was used. The source of the information and data were health reports for infectious disease. The indicators of frequency of Lyme disease occurrence was the ratio of proportion and rate.

The source of data in animals was the database of confirmed clinical cases of Lyme disease in daily protocols of veterinary practices from North Bačka District during the period from 2012 – 2016, as well as laboratory reports of the Specialist Veterinary Institute of „Subotica" and Scientific veterinary institute „Novi Sad" (used for confirmation of clinical diagnosis).

The prevalence of the disease was expressed in relation to the number of registered dogs or horses at the level of a municipality. Data on findings in tick samples collected from people and animals were from the municipality of Mali Iđoš, taken from the archives of Veterinary Practice „Anidok".

Diagnosis of Lyme disease in dogs and horses was established based on the anamnesis, epidemiological data on the exposure of sick animal to a tick bite, clinical signs in horses and dogs (fever, arthritis, lameness, joint oedema, etc) and a positive reaction to a specific antibiotic treatment (tetracycline, doxycycline). Laboratory confirmation of clinical diagnosis (3-6 weeks after infection) was done using several methods.

In dogs: with commercial fast serological tests for the detection of C6 antibodies

- CaniV-4 (Bionote); Se:93%, Sp: 98%, or
- SNAP 4Dx (Idexx); Se:94%, Sp:96%

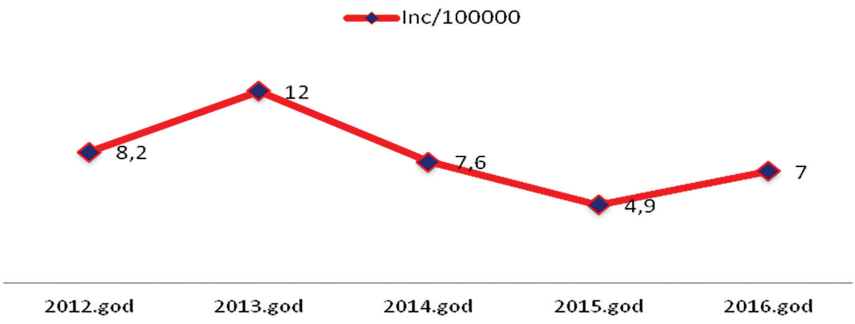
In horses, the conformation of clinical diagnosis was done using IgG ELISA test.

The ticks collected from humans and animals were examined only upon the demand of the owner in the region of Mali Idjoš, applying Fassisi® BoTick membran immunoassay fast test for a direct confirmation of the causative agent in a tick.

The abdominal content of free ticks collected from near neighbouring municipalities – Ada, Novi Bečej and Novi Sad, was examined by dark field microscopy. From ticks that were identified as positive for the presence of *B. burgdorferi s.l* spirochaetae, cultivation was performed in commercial media (BSK-H Medium Complete, Sigma) (Cutler et al., 2017).

RESULTS

During the study period, 73 cases of Lyme disease were registered in humans in North Bačka District, with an average incidence of 7.8/100000. The highest incidence of this disease was registered in 2013, (Incidence 12.2/100000) when 22 sick persons were reported, and the lowest incidence was in 2015 (Incidence 5.0/100000), with only 9 registered cases of Lyme disease in humans (Graph 1).



Graph 1. Lyme disease incidence in North Bačka District from years 2012 - 2016

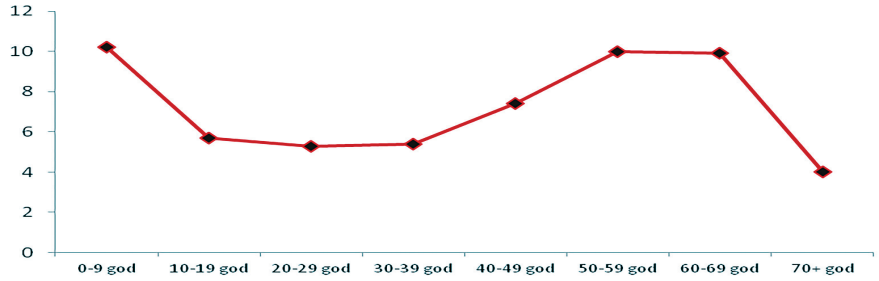
During the period of study, the disease was registered in all three municipalities of the North Bačka District. The highest average level of incidence was found in the municipality of Bačka Topola (15.9/100000), then in municipality of Mali Iđoš (7.2 /100000) and the lowest one was in the municipality of Subotica (5.0 /100000).

Table 1. Geographical distribution of Lyme disease in North Bačka District from 2012-2016

Municipality	Number of sick people	Average incidence/100000
Subotica	39	5
Bačka Topola	29	15.9
Mali Iđoš	5	7.2
Total	73	7.8

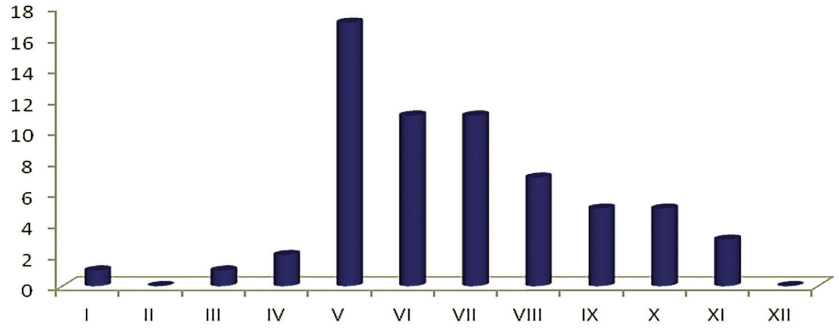
During the study period, 43 of the patients with Lyme disease were females (Inc 8.8/100000) and 30 were male (Inc 6.8/100000)

The graph showing age specific levels of incidence has a bimodal shape. The highest age specific incidence was found in children (0-9 years), but also in older population (in sixth and seventh decade of life).



Graph 2. Age specific incidence levels in Lyme disease.

Lyme disease has a characteristic seasonal distribution. The highest number of cases (71%) is registered in spring and summer months, from May to July, or more specifically during the period of six months, from May to October, (even 90% of all reported cases are Lyme disease) (Graph 3).

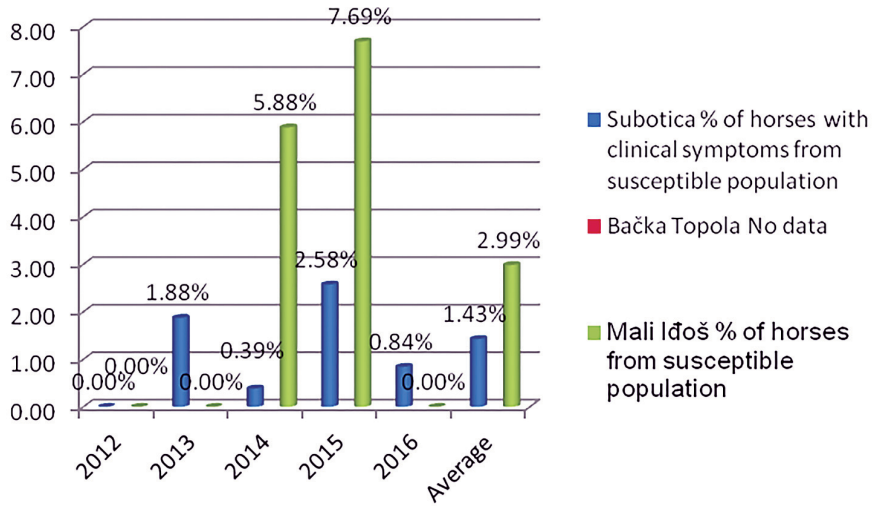


Graph 3. Distribution of Lyme disease patients in months.

Table 2. Registered cases of Lyme disease in animals in the region of North Bačka from 2012 to 2016

Municipality	Species	Year															2012-2016 average prevalence (%)*
		2012			2013			2014			**2015**			2016			
		"n"	"n" sick	Prevalence (%)	n	"n" sick	Prevalence (%)	no	"n" sick	Prevalence (%)	n	"n" sick	**Prevalence (%)**	n	"n" sick	Prevalence (%)	
Subotica	dogs	12148	6	0.049	11772	9	0.08	10766	5	0.046	10967	6	0.055	10080	6	0.060	0.06
	horses	360	0	0.000	320	6	1.88	259	1	0.386	233	6	2.575	237	2	0.844	1.43
Bačka Topola	dogs	985		0.000	1024		0.00	957		0.000	908		0.000	928		0.000	0.00
	horses	128		0.000	97		0.00	18		0.000	119		0.000	128		0.000	0.00
Mali Iđoš	dogs	1630	2	0.123	1576	0	0.00	1156	0	0.000	850	3	0.353	1137	4	0.352	0.14
	horses	46	0	0.000	22	0	0.00	17	1	5.882	13	1	7.692	15	0	0.000	2.99
Total horses:		406	0	0.000	342	6	1.75	276	2	0.725	246	7	**2.846**	252	2	0.794	1.52
Total dogs:		13778	8	0.058	13348	9	0.07	11922	5	0.042	11817	9	0.076	11217	10	0.089	0.07
Total all species:		13778	8	0.058	13690	15	0.11	12198	7	0.057	12063	16	0.133	11469	12	0.105	0.09

* Average prevalence of sick dogs and horses with positive laboratory finding (%)

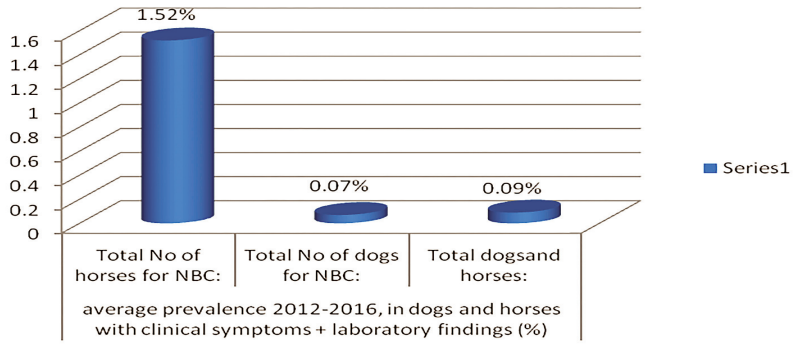


Graph 4. Results of the study on prevalence of borreliosis in dogs, by the municipalities of North Bačka District

During the study period from 2012-2016, in the municipalities of North Bačka region, human cases of Lyme borreliosis were registered with the average incidence rate of 7.8/100000. The highest incidence has been registered in 2013 (In 12.2 / 100000), when there were 22 human cases of the disease reported and the lowest incidence was in 2015 (In 5.0 / 100000), with 9 registered cases of human borreliosis (Graph 1).

Table 3. The average number of registered Lyme disease cases in animals in North Bačka District for the period 2012-2016

Municipality	Species	Average number of animals	Average number of registered sick animals	Average prevalence from 2012-2016, in dogs and horses, confirmed with laboratory finding (%)
Subotica	Dogs	9130.6	**5.2**	**0.06**
	Horses	234.4	**2.6**	**1.43**
Bačka Topola	Dogs	774.8	0	**No data**
	Horses	72.4	0	
Mali Iđoš	Dogs	1042.4	**1**	**0.14**
	Horses	19.6	**0.4**	**2.99**
Total for horses:				**1.52**
Total for dogs:				**0.07**
Total for dogs and horses:				**0.09% (9 / 10.000)**



Graph 6. The average number of registered animal cases of Lyme borreliosis in the region of North Bačka District during the period 2012-2016

Findings on the presence of causative agent of Lyme disease in ticks on the territory of Mali Iđoš municipality obtained using Fassisi® BoTick membran immunoassay are shown in Table 4.

Table 4. Findings on the presence of causative agent of Lyme disease in ticks on the territory of Mali Iđoš municipality (Fassisi® BoTick membran immunoassay)

Year:	2012	2013	2014	**2015**	2016	**Average**
No. of analysed ticks	26	30	40	33		**32.25**
No. of positive	2	4	8	7	Not analysed	**5.25**
% ticks positive for B. burgdorferi s.l.	7.69	13.33	20.00	**21.21%**		**16.28%**

There are no data on planned sampling of ticks from urban green areas in any of the municipalities in North Bačka region. The number of analysed *I.ricinus* ticks, positive for the presence of *B. burgdorferi s.l.* has been compared to the previously reported results from the near neighbouring regions. In those regions, the analysis was done by dark field microscopy examination of abdominal content of live ticks (Table 5).

Table 5: Comparison with near neighbouring regions (Mali Iđoš = 16.28%)

Number of analysed and positive ticks *I. ricinus* for the presence of *B. burgdorferi* in different regions during 2008 (Savić et al.)

Region	Total No of examined *I. ricinus* ticks	No of ticks positive for the presence of *B. burgdorferi s.l.*	% of ticks positive for the presence of *B. burgdorferi s.l.*
Region Ada – Mol	78	19	24.3
Region Novi Bečej	25	0	0
Region of Novi Sad city with surrounding	96	28	29.2

DISCUSSION

Global geographic distribution of Lyme disease is in correlation with the spreading of *Ixodes* ticks. Infection foci can be found in the regions with moderate climate which fits the biological cycle of this vector.

During the study period 2012- 2016, 73 human cases of Lyme disease were registered in North Bačka region, with an average annual incidence of 7.8/100000. The highest incidence of this disease was found in 2013 (In 12.2/100000), when 22 sick persons were registered, while the lowest incidence was found in 2015 (In 5.0 /100000), with 9 registered cases of human Lyme disease. During the period of study, the disease was registered in all three municipalities of North Bačka region. The highest average incidence was found in Bačka Topola municipality (In 15.9/100000), than in municipality of Mali Iđoš (In 7,2/100.000) and Subotica where the average incidence found was 5.0/100000 residents.

At the same time, in the Republic of Serbia and Vojvodina, average annual incidences were 11.3/100000 and 9.9/100000 residents respectively (Infectious diseases in AP Vojvodina 2015, the annual report). In whole Europe, 85.000 cases of human Lyme disease are registered annually. It is estimated that significant number of Lyme disease cases pass unrecognized and unregistered. Significantly high incidence is registered in Austria (300/100000) and Slovenia (155/100000), and the lowest incidence can be found in Great Britain (0.7/100000) and Ireland (0.6/100000) (Orloski, 2000; Rizzoli, 2011). Many experts support the opinion that a significant number of cases remain unreported and unregistered, and some of them even estimate that the real incidence is 2-3 times higher than the one officially reported (Ropac et al., 2013).

These big differences in the incidence confirm that Lyme disease keeps the well-known focal distribution on large continental levels, as well as in small countries, to the level of region and municipalities, which is caused by the distribution of vectors. In our study we have also confirmed that three times higher incidence was found in the municipality of Bačka Topola as compared to Subotica.

Out of the total number of cases of human Lyme disease, females were more infected than males 43:30 (1.33:1). Most of the authors have established similar gender distribution among infected persons (Pavlović et al., 2006; Infectious diseases of AP Vojvodina, the 2015 annual report 10). On the contrary, some authors such as Ropac et al, have found three times higher incidence of the disease in men as compared to woman e.g., in Bjelovarsko-Bilogorska County in Croatia (Ropac et al., 2013).

The disease has been registered in all age groups and the most age specific rates can be found in children (0-9 years) as well as in the elderly group (sixth and seventh decade of life). Distribution of the disease by age group is bimodal like in most of the countries, where the primary (lower) maximum is found in children from 5–9 years old and secondary (higher) maximum in adults from 50–64 years old (Infectious diseases in AP Vojvodina 2015, annual report). The explanation of these differences in disease occurrence among different age groups can be associated with specific activities and behaviour of these groups during their free time outside (Mladenović, 2014).

Lyme disease (*Erythema migrans*) has a characteristic seasonal distribution. The highest percentage of cases (71%) is registered during the spring and summer months, from May to July. The increase in number of sick people is superposed with seasonal activity of ticks and more frequent stay of the people outside. Therefore, this disease has a seasonal character in all regions with continental climate. The majority of the quoted authors have come to the same results in their research (Pavlović et al., 2006; Infectious diseases of AP Vojvodina 2015, the annual report; Ropac et al., 2013).

CONCLUSION

Based on the findings obtained during the study period 2012 – 2016, in the region of North Bačka District, the number of registered human cases of Lyme disease shows a decreasing tendency. Significant differences in incidence rates were found between different municipalities and also large oscillations in number of registered cases per year are most probably due to miss registration of cases and failures in reporting to the relevant public health authorities or epidemiological competent services and less likely, to improved epidemiological situation. The possible reasons for failures in reporting cases may vary, but some of them certainly include omissions in keeping records, ineffectiveness of laboratory diagnostic etc. Regarding the prevalence of registered clinical cases, it can be assumed that large number of unexamined dogs and horses is seropositive and pass undiagnosed as subclinical cases.

Despite the fact that the total number of Lyme disease - sick persons is small with no death cases reported, the understanding of a true epidemiological and epizootiological situation of this disease is a prerequisite for organized planning and implementation of measures for suppression of the disease and adequate Public health protection.

For the purpose of determining the actual situation concerning Lyme disease in the region, further studies in the field of infection prevalence in ticks

and determination of entomological risk index are needed to identify the season and region(s) with infected vectors that represent potential risk for the occurrence of infection or disease in humans or animals.

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

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

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

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

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

Conclusion. Here the authors gives his final conclusions.

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

Examples of references:

1. **Articles in journals:**

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

2. **Books:**

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

3. **Chapters in books:**

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

4. **Articles in proceedings:**

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

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

Note

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

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