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CLOSTRIDIUM TERTIUM ISOLATED FROM FEED

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Abstract

Although *Clostridium tertium* is supposed to be a foodborne pathogen, the data on its detection in foodstuffs is scarce, and there are no reports on its isolation from feed. In this communication paper, the isolation of *C. tertium* from a sample of soya semolina is described. *C. tertium* may be important in differential diagnosis, when it is to be distinguished from *Clostridium perfringens*. It is a unique species due to the lack of key characteristics of the genus it belongs to because it grows in the presence of oxygen and does not produce toxins. It has been well-documented as a human pathogen, although its mechanisms of pathogenicity are still unknown. According to sporadic reports in veterinary medicine, it has been identified as a rare causative agent of infections in cattle, pigs, birds and marine mammals.

Keywords: *Clostridium tertium*, soya semolina, MALDI-TOF

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IZOLACIJA *CLOSTRIDIUM TERTIUM* IZ HRANE ZA ŽIVOTINJE

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Kratak sadržaj

Pretpostavka je da *Clostridium tertium* treba svrstati u patogene koji se mogu preneti hranom, ali je malo podataka o njegovom nalazu u namirnicama, dok o izolaciji iz hrane za životinje nema dostupnih izveštaja. U ovom saopštenju prikazujemo izolaciju *C. tertium* iz uzorka sojinog griza. *C. tertium* može biti od značaja u diferencijalnoj dijagnostici kod izolacije *Clostridium perfringens*. Specifična je vrsta jer ne poseduje ključne karakteristike roda kojem pripada: raste u prisustvu kiseonika i ne produkuje toksine. Dobro je dokumentovan kao humani patogen, iako su mehanizmi njegove virulencije i danas nepoznati. Na osnovu sporadičnih izveštaja u veterinarskoj medicini, identifikovan je retko kao uzročnik infekcija goveda, svinja, ptica i morskih sisara.

Ključne reči: *Clostridium tertium,* sojin griz, MALDI TOF

INTRODUCTION

Bacterium species of the *Clostridium* genus are endospore-forming, obligate anaerobes (or relatively oxygen-tolerant) widespread not only in solid and liquid environments (soil, sewage, surface waters, marine sediments, etc), but also in animal and human intestines and, eventually, in animal and plant products. Based on its rRNA structure, the genus comprises extremely heterogeneous species, many of which share phylogenetic similarities with some other bacterial genera (Collins et al., 1994). Owing to its capability to produce enteritis and enterotoxaemia in various domestic animals, *Clostridium perfringens* is the most important clostridium species in veterinary medicine. Animal feed is one of potential sources of infection. According to regulations on the microbiological criteria for animal feed quality, it is considered safe if

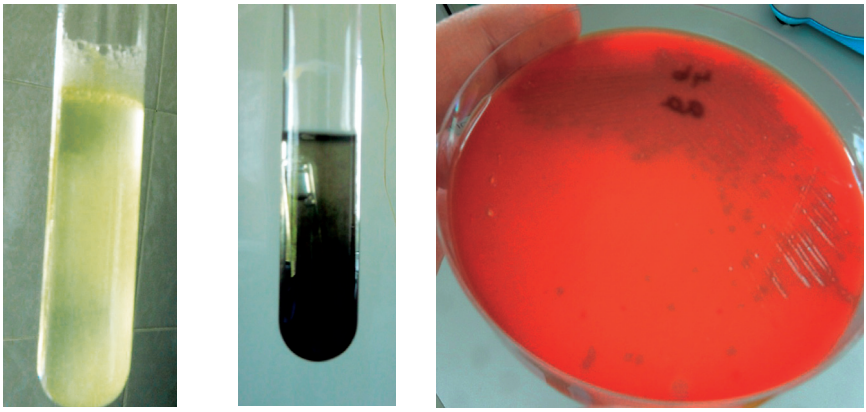
no *Clostridium perfringens* and *Clostridium botulinum* are detected in 50 g of a sample (Regulation on the Quality of Animal Feed, 2010). The isolation and identification of *C. perfringens* should be done in compliance with the EN ISO 7937 standard, which enables the precise identification and enumeration of the target species in food and animal feeding stuff. The identification of other members of *Clostridium* genus is not part of the routine procedure in laboratories for feed analysis in Serbia and is beyond their diagnostic capacity. For the above mentioned reasons, this case report is a result of an aspiration to satisfy the researchers' curiosity, discover the identity of certain *Clostridium* isolates from feed and to broaden the knowledge about bacterium species (other than *C. perfringens*) present in animal feedstuffs and feed. The isolation and identification of *Clostridium tertium* is presented in this communication paper. To the best of our knowledge, *Clostridium tertium* has not yet been detected in animal feed samples, although it is sometimes present in food of animal origin.

A REPORT ON A LABORATORY CASE

Sample: Soya semolina.

Isolation: Clostridia were isolated following the instructions given in the EN ISO 7937:2010 Standard. For further confirmation, five colonies black in colour due to sulphite reduction - grown on TSC (tryptone-sulfite-cycloserine) agar (Biokar Diagnostics, France) were chosen. They were inoculated into thioglycollate broth and incubated for 24 hours at 37°C (Fig 1.A). After incubation, 5 drops of thioglycollate culture was inoculated into lactose-sulfite (LS) broth (Biokar Diagnostics, France) for *C. perfringens* confirmation. After 24 hours of incubation at 46°C, LS was examined for gas production and the presence of black colour (sediment of iron sulfite). The formation of black colour has been observed, but Durham's tube was filled with gas to less than a 1/4 of its volume (Figure 1.B). According to ISO standard, the test in LS medium should be repeated in this case by transferring 5 drops of culture grown in LS broth to another test tube with the same medium, repeating the incubation in the same conditions. As the repeated test once again failed to confirm the presence of *Clostridium perfringens* species, the culture which grew in thioglycollate medium was transferred by streaking onto two plates with Columbia blood agar base with the addition of 5% of *defibrinated* sheep blood. The plates were incubated at 37°C, one in aerobic and the other in anaerobic conditions using GasPak EZ (Becton Dickinson and Company, USA). After 24 hours of incubation, the growth was noted only on the plate which was incubated in anaerobic conditions, which led to the conclusion that the species is a strict

anaerobe. However, after 48 hours, the growth was also observed in the dish incubated in aerobic conditions, which would have led to ambiguity if it had been a *Clostridium* species. The isolate formed little (about 1 mm in diameter), opaque colonies, surrounded by a zone of incomplete (a) haemolysis (Figure 1.C). It was confirmed that it was *Clostridium* genus by a negative test for catalase and the microscopic appearance of the Gram stained smears: Gram-positive rods with rounded ends were found and oval spores located terminally were rarely present in smears made from cultures grown in anaerobic conditions. Based on characteristic black colonies grown on sulfite cycloserine agar, Gram-stain morphology and negative catalase test results, the isolates were presumptively identified as *Clostridium* species.



A. B. C.

Figure 1. A. Growth of the isolate in thioglycollate medium; B. Test in lactose-sulfite medium; C. Colonies of *Clostridium* sp. isolate on blood agar.

Due to the lack of tests for biochemical and molecular typing of isolates of *Clostridium* species other than *C. perfringens* in our laboratory, the isolate was further processed at the Institute of Public Health of Vojvodina in Novi Sad. The identification was performed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS has been adapted to generate protein mass spectra from whole bacteria and other microorganisms. These spectra can be compared to a reference database for rapid and accurate taxonomic classification of unknown organisms at the genus, species, and, in some cases, at strain levels. The isolate was identified by MALDI TOF as *Clostridium tertium* (Fig 2).

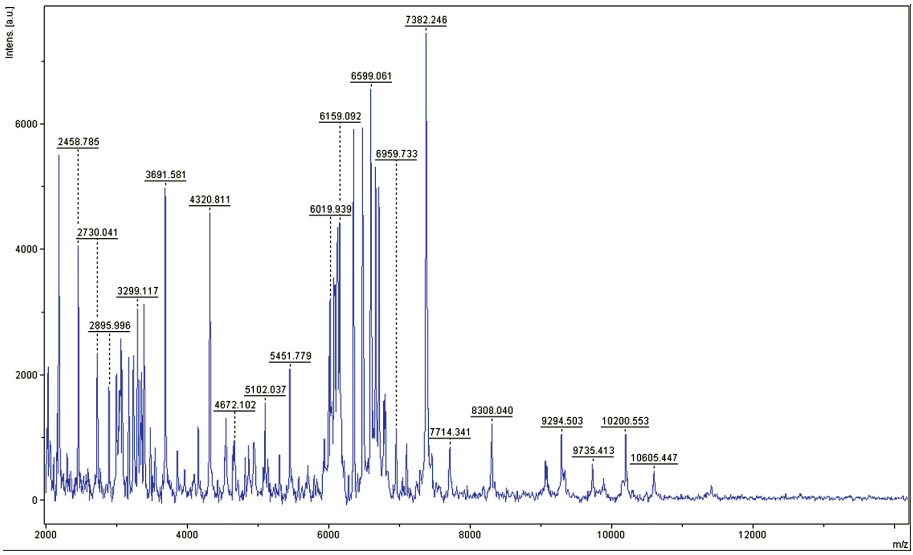


Figure 2. Spectra of *Clostridium tertium* isolate generated by MALDI-TOF Bruker flexControl software.

COMMENT

Clostridium tertium is abundant in soil, but is also found in animal and human intestines as well as in the commensal microbiome of the mouth cavity (Vanderhofstadt et al., 2010). It is a rare human pathogen. Reportedly, it was first described and its biochemical properties were studied in isolates from war wounds in the First World War (Ray et al., 2003). Therefore, *C. tertium* is considered capable of causing bacteraemia (Ray et al., 2003). In addition, it was found in persons with various ailments: meningitis, septic arthritis, enterocolitis, peritonitis, posttraumatic brain abscess, pneumonia, and necrotizing fasciitis and gangrene. *C. tertium* does not produce exotoxins and the mechanism of its virulence is not known (Ferrell and Tell, 2001; Ray et al., 2003; Vanderhofstadt et al., 2010). Moreover, its clinical importance is questionable since it is not entirely clear if it is a real pathogen or only a contaminant (Vanderhofstadt et al., 2010). It is supposed that *C. tertium* does damage to the gut mucosa when colonizing it (Ferrell and Tell, 2001), meaning it can penetrate into the bloodstream (Ray et al., 2003).

In veterinary medicine, *C. tertium* has been recognized as a causative agent of enteritis in cattle and pigs. AlMashat and Taylor in 1984 isolated similar

bacteria from cattle with enteritis and phenotypically identified it as *Sporolactobacillus* species (Silvera et al., 2003). In artificially infected cattle, this bacterium caused mild diarrhoea. Ferrell and Tell (2001) reported an isolation of *C. tertium* from faeces of *Trichoglossus moluccanus* that vomited and had blood in faeces. It was assumed that contaminated water was the source of infection and that a diet rich in carbohydrates is a favourable medium for bacterial fermentation. Šeol et al. (2006) were the first to accuse *C. tertium* of causing abscesses, osteomyelitis and, finally, death in a dolphin, which was the first detection of this bacterium in marine animals.

Postollec et al. (2012) detected nine *Clostridium* species in various foodstuff, but not *C. tertium* (although they reviewed certain data during its previous detection). A long time ago, in 1965, Goudkov and Sharpe first published a paper on *C. tertium* detected in cheese and milk. They claimed that in spite of unfavourable conditions for *C. tertium* growth in dairy products, it can spoil certain cheese types. Later (Fernández et al., 2015), this bacterium was listed as one of the three clostridium foodborne pathogens in cheese, along with *C. botulinum* and *C. perfringens*. Le Bourhis et al. (2005) even developed and validated PCR primers for *Clostridium* spp. detection in cheese.

C. tertium was also detected in meat samples (Ersöz and Coşansu, 2018). Search for *C. difficile* with the API20A (System for the identification of anaerobes and with serological Clostridium Difficile Test Kit) resulted in *C. tertium* detection in one out of 101 samples of meat products (beef and chicken) collected from the market (Ersöz and Coşansu, 2018). The same agent was successfully recovered from foie gras and was proved to be capable of growing during storage at 8°C (Prevost et al., 2013). It was confirmed that *C. tertium* spores can be inactivated in meat by hydrostatic pressure and bacteriocins (Kalchayanand et al., 2003).

C. tertium is resistant to high temperatures, it can grow under various atmospheric conditions, can cause diarrhea and is present in both healthy and diseased humans (Silvera et al., 2003). Since it is considered an intestinal commensal in animals, it remains unclear whether animal feed should be regarded as a potential source of infection.

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Authors' contributions

DM completed the microbiological analyses of Soya semolina samples and isolated *Clostridium tertium*. MĐ identified *C. pefringens* using MALDI-TOF. DM, NA, MV prepared the manuscript, and NA did the reviewing, editing and supervision.

Competing interest

Authors declared no conflict of interests regarding the present paper.

REFERENCES

1. Collins, M.D., Lawson, P.A., Willems, A., Cordoba, J.J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H., Farrow J.A. 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int J Syst Bacteriol, 44(4), 812-26. doi: 10.1099/00207713-44-4-812
2. ISO 7937:2010. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of *Clostridium perfringens* – Colony-count technique.
3. Ersöz, Ş.Ş., Coşansu, S. 2018. Prevalence of *Clostridium difficile* isolated from beef and chicken meat products in Turkey. Korean Journal for Food Science of Animal Resources, 38, 759-767. doi: 10.5851/kosfa.2018.e14
4. Ferrell, S.T. and Tell, L. 2001. *Clostridium tertium* infection in a rainbow lorikeet (*Trichoglossus haematodus haematodus*) with enteritis. Journal of Avian Medicine and Surgery, 15(3), 204-208.
5. Fernández, M., Hudson, J.A., Korpela, R., de los Reyes-Gavilán, C.G. 2015. Impact on human health of microorganisms present in fermented dairy products: an overview. BioMed Research International, Article ID 412714. http://dx.doi.org/10.1155/2015/412714
6. Goudkov, A.V., Sharpe, M.E. 1965. Clostridia in Dairying. Journal of Applied Microbiology, 28(1), 63 – 73.
7. Kalchayanand, N., Dunne, C.P., Sikes, A., Ray, B. 2003. Inactivation of bacterial endospores by combined action of hydrostatic pressure and bacteriocins in roast beef. Journal of Food Safety, 23, 219–233. doi: 10.1111/j.1745-4565.2003.tb00366.x
8. Le Bourhis, A.G., Saunier, K., Doré, J., Carlier, J.P., Chamba, J.F., Popoff, M.R., Tholozan, J.L. 2005. Development and validation of PCR primers to

assess the diversity of *Clostridium* spp. in cheese by temporal temperature gradient gel electrophoresis. Applied and Environmental Microbiology, 71, 29–38. doi:10.1128/AEM.71.1.29-38.2005

9. Postollec, F., Mathot, A.G., Bernard, M., Divanac'h, M.L., Pavan, S., Sohier, D. 2012. Tracking spore-forming bacteria in food: From natural biodiversity to selection by processes. International Journal of Food Microbiology, 158, 1-8. doi:10.1016/j.ijfoodmicro.2012.03.004
10. Prevost, S., Cayol, J.L., Zuber, F., Tholozan, J.L., Remize, F. 2013. Characterization of clostridial species and sulfite-reducing anaerobes isolated from foie gras with respect to microbial quality and safety. Food Control, 32, 222-227. doi : 10.1016/j.foodcont.2012.11.030
11. Regulation on the Quality of Animal Feed. 2010. Official Gazette of the Republic of Serbia, 4/10, p.71.
12. Ray, P., Das, A., Singh, K., Bhansali, A., Yadav, T.D. 2003. *Clostridium tertium* in necrotizing fasciitis and gangrene. Emerging Infectious Diseases, 9 (10), 1347 – 1348. doi: 10.3201/eid0910.030287
13. Silvera M., Finn B., Reynolds K. M., Taylor D. J. 2003. *Clostridium tertium* as a cause of enteritis in cattle. Veterinary Record 156: 60, 2003. doi:10.1136/vr.153.2.60
14. Šeol, B., Gomerčić, M.Đ., Naglić, T., Gomerčić T., Galov A., Gomerčić H. 2006. Isolation of *Clostridium tertium* from a striped dolphin (*Stenella coeruleoalba*) in the Adriatic Sea. Journal of Wildlife Diseases, 42(3), 709–711. doi:10.7589/0090-3558-42.3.709
15. Vanderhofstadt, M., Andre, M., Lonchay, C., Levecque, P., Holemans, X., Canon, J.L., D'Hondt, L. 2010. *Clostridium tertium* bacteremia: contamination or true pathogen? A report of two cases and a review of the literature. International Journal of Infectious Diseases, 14S, e335–e337. doi:10.1016/j.ijid.2010.03.004

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METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS*, A NEW THREAT IN HUMAN AND VETERINARY MEDICINE?

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Abstract

In this paper we briefly described the worldwide distribution of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in dogs and cats. The most common sequence type of MRSP strains in dogs is ST71 as it was detected in isolates on four continents (Europe, Asia, North and South America). However, several different MRSP sequence types are detected in small animals, and the presence of new genetic variants is continually reported. Sometimes isolates belonging to the same sequence type (ST) are detected in dogs, cats, their owners, and veterinarians. MRSP is often multidrug-resistant and its resistance patterns are usually linked to certain sequence types. The resistance to non-beta-lactam antibiotics such as erythromycin, clindamycin, tetracycline, gentamicin, enrofloxacin and sulfamethoxazole/trimethoprim is also recorded. Taking into account that MRSP tends to confer multidrug-resistant phenotype, it is quite challenging for veterinarians to give adequate therapy in clinically ill animals. It would seem as if the significance of MRSP in the clinical epidemiology of humans is not firmly established. However, the importance of MRSP in human medicine should not be underestimated given the fact that all methicillin-resistant *Staphylococcus* spp. carry resistance and virulence genes and have the potential to share their genetic elements with other bacteria.

Key words: antimicrobial resistance, cats, dogs, humans, MRSP, *Staphylococcus pseudintermedius*.

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METICILIN REZISTENTNI *STAPHYLOCOCCUS PSEUDINTERMEDIUS*, NOVA PRETNJA ZDRAVLJU LJUDI I ŽIVOTINJA?

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Kratak sadržaj

U radu prikazujemo relevantne činjenice o meticilin rezistentnim sojevima *Staphylococcus pseudintermedius* (MRSP) koji su široko rasprostranjeni u populaciji pasa i mačaka širom sveta. Metodom sekvenciranja genoma, kod izolata poreklom od pasa na teritoriji Evrope, Azije, Severne i Južne Amerike, najčešće je ustanovljen genetički tip ST71. Međutim, kod MRSP poreklom od malih životinja utvrđeno je više različitih genetičkih varijanti, a kontinuirano se izveštava o nalazu novih. Ponekada se isti genetički tip MRSP može naći kod pasa ili mačaka, njihovih vlasnika i veterinara. Izolati *Staphylococcus pseudintermedius* često poseduju rezistenciju na više klasa antibiotika, pri čemu je tip rezistencije u korelaciji sa određenim genotipom. Meticilin rezistentni sojevi *Staphylococcus pseudintermedius* takođe mogu biti rezistentni i na antibiotike koji ne pripadaju grupi beta-laktama, kao što su eritromicin, klindamicin, tetraciklin, gentamicin i trimetoprim/sulfametoksazol. Zbog toga terapija infekcija pasa i mačaka koje su izazvane sa MRSP, može predstavljati veliki izazov u veterinarskoj kliničkoj praksi. Iako meticilin rezistentni sojevi *Staphylococcus pseudintermedius* nisu od posebnog značaja u humanoj medicini, ne treba podceniti njihovu ulogu u potencijalnom transferu gena virulencije i gena rezistencije na druge srodne ili nesrodne vrste bakterija.

Ključne reči: rezistencija na antibiotike, mačke, psi, ljudi, MRSP, *Staphylococcus pseudintermedius*

INTRODUCTION

Shortly after methicillin was introduced into medical practice, the resistance to this antibiotic occurred in *Staphylococcus aureus* isolates. Not only did

MRSA become one of the most important nosocomial pathogens worldwide, but it was also a significant community-acquired and livestock-associated pathogen (Gordon and Lowy, 2008).

The resistance to methicillin occurs due to the presence of *mecA* gene that encodes penicillin-binding proteins (PBPs). This gene is integrated into a staphylococcal cassette chromosome *mec* (SCC*mec*) locus. The integration of the cassette is accomplished by the recombinase encoded by *ccr* gene. The sequence of *mec-scc* is used for the determination of SCC*mec* cassette type and the establishment of its epidemiological status (Gordon and Lowy, 2008, rev. Velhner et al., 2016). Other additional methods of MRSA molecular typing include spa typing, multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). Isolates causing nosocomial infections health care-associated MRSA (HA-MRSA) belong to SCC*mec* types I, II or III and less frequently to types IV and V, while community-acquired MRSA (CA-MRSA) belongs to SCC*mec* types V and VI (Kasai et al., 2016, rev. Velhner et al., 2016,).

Staphylococcus pseudintermedius has been recognized as a relatively new species since 2005 when it was isolated from a dog, a cat, a horse and a parrot by Devriese et al. (2005). Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) was first reported in Europe in 2006 and the whole genome sequence was available by 2013 (Moodley et al., 2013). MRSP belongs to *Staphylococcus intermedius* group and causes opportunistic infections in dogs and cats. There are six coagulase-positive staphylococcus species including *S. aureus*, *S. intermedius*, *S. schleiferi* subsp. *coagulans*, *S. hyicus*, *S. lutrae*, *S. delphini* and *S. pseudintermedius*. Among them, *S. intermedius*, *S. pseudintermedius* and *S. delphini* are very closely related (Sasaki et al., 2007).

MRSP produces coagulase the same as MRSA strains, and therefore laboratory identification can be a bit challenging (Sewid et al., 2018). However, molecular phylogenetic analysis (Sasaki et al., 2007), multiplex PCR (Sasaki et al., 2010) and MALDI-TOF MS diagnostic (Nisa et al., 2018) are overcoming difficulties of their phenotypic identification.

Staphylococcus pseudintermedius is often a part of normal flora in healthy dogs (Kjellman et al., 2015). However, it can cause skin infections, pyoderma, otitis externa and urinary tract infections in dogs and can be isolated from postsurgical sites of infection (Duijkeren et al., 2011, Somayja et al., 2016). Apart from dogs, MRSP can also be isolated from cats, less frequently from horses and humans (Devriese et al. 2005, Kadlec et al., 2010, Dos Santos et al., 2016). Even though it is sometimes a part of normal genital flora of bitches, *S. pseudintermedius* can cause reproductive problems leading to neonatal mortality (Ruzauskas et al., 2016, Corro et al., 2018).

In this brief review we described the worldwide distribution of MRSP specific sequence types, their antimicrobial resistance and the significance of MRSP in clinical epidemiology of humans and small animals, mainly dogs and cats.

SEQUENCE TYPE DISTRIBUTION OF THE MRSP

All MRSP isolates from dogs described until 2016 belong to 16 different sequence types and the most frequent clone worldwide is ST71. This clone was detected on four continents (Europe, Asia, North and South America). The most prevalent clone in the USA is ST68, while in Asia it is ST45/ST112. Other clones that are less abundant belong to sequence types ST45, ST258, ST261, ST112, ST265, ST68, ST169 and ST181. Interestingly, the genetic diversity among methicillin-sensitive *Staphylococcus pseudintermedius* (MSSP) is higher compared to MRSP and seven STs are present in both groups of isolates (Perreten et al., 2010, dos Santos et al., 2016). Clonal complex (CC) 71 is detected only in MRSP, while CC75 is exclusively found in MSSP strains. In addition, various SCC*mec* types were found in MRSP clonal lineages due to their independent acquisition (dos Santos et al., 2016). All MRSP isolates collected from felines in different European countries belonged to ST71, spa type 02 and SCC*mec* type II-III. A single MRSP isolate from Canada harbored SCC*mec* type V element (Kadlec et al., 2010). A research work carried out in Iran showed that MRSP can be isolated from the nostrils and perianal area of healthy dogs and cats and those isolates carried the cassette chromosome *SSCmec* II and V (that have been detected in two and 10 isolates respectively) (Tabatabaei et al., 2019).

It was established that the presence of MRSP-ST71 is slowly decreasing in dogs with skin and soft tissue infections in France (Bergot et al., 2018). Even though this trend was not statistically significant in the 2012-2013 period, the expanding decreasing trend was reported in the following years (2015-2016). The majority of MRSP isolates have their specific genetic background, except for a few clones such as 258. Also, of great importance is an emerging ST496 clone in France, previously identified in Australia, primarily in Sidney. In Finland, the examination of 1958 clinical isolates of *Staphylococcus pseudintermedius* showed that 266 isolates were oxacillin-resistant and 321 multidrug-resistant. The total number of 42 different sequence types was identified and among those 19 STs were new in a database. MRSP was predominantly diagnosed in private clinics in Finland since more patients with dermatological problems were admitted there compared to the Veterinary teaching hospital

(Grönthal et al., 2017). The collection of MRSP isolates from dogs (No 28) and cats (No 11) from Thailand was studied thoroughly in order to identify resistance genes, spa and dru types, MLST and PFGE. The most frequent MLST among the same PFGE cluster was ST45 followed by ST112, ST155, ST282, including three novel MLST types ST432, ST433 and ST434. The ST45, previously identified in Thailand, was found in 30 isolates that belong to the two most common PFGE patterns (Kadlec et al., 2016). In a research work from Japan (Kasai et al., 2016), clear differences were found between HA-MRSP and CA-MRSP isolates from dogs. The most homogenous group of isolates was SCC*mec* type III and most of them belong to the pulsotype A. Additionally, they have similar resistotypes and belong to the endemic clone ST71. SCC*mec* type V isolates were heterogeneous as they were classified into 25 pulsotypes. Furthermore, there were differences between MRSP and MSSP strains since MRSP was usually identified in older dogs after hospital admission. A survey conducted in a small animal hospital in Germany also contributed to the identification of SCC*mec* type II-III strains among MRSP isolates (60 out of 814 dogs included in the study were MRSP positive). MRSP identification has proved significant in dogs hospitalized and treated with antibiotics within six months before sampling. Samples had been collected before they entered the clinic. All isolates conferred high resistance rates to antibiotics (Nienhoff et al., 2011). A research carried out in Italy at the University of Bari revealed that out of 175 clinical samples from dogs, 151 were culture positive and 63 were identified as *S. pseudintermedius* by PCR detection of *nuc* gene. The most dominant clone was ST71, identified in 48% isolates. Single-locus variants of ST71 were ST410 (found in four isolates) and ST261 (two isolates), while one isolate was identified as a double-locus variant ST290. The new allelic profile, unrelated to ST71, was detected in one isolate and assigned as ST477. The SCCmec type II-III was identified in 67% isolates, while 33% isolates, including ST258, were SCC*mec* type IV (Ventrella et al., 2017). Three different sequence types ST71, ST252 and ST305 were identified among healthy dogs examined in small animal clinics in Oslo. This research also included 49 clinical isolates of MRSP that were classified into 15 different MLST groups. The majority of isolates belonged to the ST258 and ST71 and they have been found in the same geographical area in Norway (Kjellman et al., 2015).

RESISTANCE TO NON-BETA-LACTAM ANTIBIOTICS IN MRSP

What should be pointed out is that the resistance phenotype to non-beta-lactam antibiotics is often related to their clonal group. For instance, the iso-

lates from the most abundant clonal group CC71 were less resistant to amikacin, chloramphenicol, tetracycline and trimethoprim-sulfametoxasole while CC258 was less likely to be resistant to amikacin, enrofloxacin, gentamycin and chloramphenicol (dos Santos et al., 2016). In a research from Norway, it was shown that the clonal lineage ST71 was more resistant to antibiotics than other clonal groups. The highest resistance rates in ST71 included ciprofloxacin and gentamicin antibiotics as well (Kjellman et al., 2015). The clone ST496 is susceptible only to florfenicol and fusidic acid (Bergot et al., 2018). According to a study carried out in Finland, MRSP was more often multidrug-resistant than MSSP. The only exception is fusidic acid, to which both strains showed similar levels of resistance (around 24%). Additionally, a total number of 219 MRSP isolates (100%) were susceptible to amikacin (Grönthal et al., 2017).

In a research from Kasai et al. it was determined (2016) that therapy with minocycline was effective in the case of MRSP infection with SCC*mec* type III strains. It was found that the number of isolates belonging to the type V strains was susceptible to amoxicillin-clavulanic acid, cephalexin and cefazolin even if they were *mecA* positive, and in most cases, multidrug-resistant. SCC*mec* type III strains were more frequently resistant to oxacillin compared to type V isolates. In a research work conducted in Germany, it was established that MRSP isolates with high MIC to oxacillin were also frequently resistant to non-beta-lactam antibiotics such as erythromycin and other macrolides, clindamycin, tetracycline, enrofloxacin, sulfamethoxazole/trimethoprim (Nienhoff et al., 2011). In Poland, a total of 51 *S. pseudintermedius* isolates from the veterinary clinic and breeding kennels, mostly from dogs with pyoderma and bitches with reproductive disorders, were susceptible to vancomycin, daptomycin and linezolid. More than 50% isolates were resistant to penicillin, tetracycline and macrolides. Resistance to ciprofloxacin was detected in 31.4% isolates (Ruzauskas et al., 2016). In a research work from Italy (Ventrella et al., 2017) resistance rates to sulfamethoxasole/trimethoprim, clindamycin, ciprofloxacin and doxycyline were much more pronounced in MRSP isolates than in MSSP. Almost all isolates (except one) were susceptible to gentamicin. MRSP isolates from dogs and cats that were treated at a veterinary clinic in Iran, when resistant to oxacillin/cefoxitin, tend to develop additional resistance to gentamicin, tetracycline, lincomycin and erythromycin (Tabatabaei et al., 2019). MRSP isolates from two small clinics in Sydney showed increasing rates of resistance to fluoroquinolones, trimethoprim/sulfamethoxazole and erythromycin compared to MRSA isolates from the same clinic. It was concluded that the clonal lineage ST496 is the area of concern for public health since those isolates carry transferable genetic resistance determinants (Worthing et al., 2018). MRSP rarely causes health

problems in cats, unlike in dogs. In a research work conducted by Kadlec et al. (2010) isolates were collected from European countries and North America and antimicrobial resistance data was compared. The data showed that the isolates from Canada were resistant only to β-lactam antibiotics and tetracycline, while isolates from Europe were also resistant to macrolide/lincosamide, gentamicin, kanamycin, trimethoprim and ciprofloxacin. The majority of isolates were also resistant to chloramphenicol and tetracycline.

HUMANS AS MRSP HOSTS?

Some methicillin-resistant *Staphylococcus* (MRS) clones are spreading worldwide and this is the main reason why much attention is devoted to decreasing MRS infection in human and veterinary medicine. Furthermore, human infections with *S. pseudintermedius* have been recorded sporadically (Stegmann et al., 2010). Dogs have been indicated as a natural reservoir of *S. pseudintermedius* since this species can be isolated from healthy animals as much as the ill ones. Hanselman et al. (2009) pointed out that household hygiene (i.e. hand washing) can be a crucial step in preventing human infection with bacteria originating from pets, providing less chance for bacteria to switch from their commensal to pathogenic state and become a threat, both to humans and animals. In their research, indistinguishable *S. pseudintermedius* isolates were found in 4/9 households in humans and their pets (dogs). In another study conducted during a dog show in Berlin, nasal swabs were taken from dogs and their owners and it was elucidated that six owners and 13 dogs carried *S. pseudintermedius*. In addition, one human isolate was MRSP. For a total of 21 *S. pseudintermedius* isolates sequence type was defined and it was evident that isolates were heterogeneous and nine new sequence types were identified (Walther et al., 2012). On the other hand, in a research work in Sydney, conducted in two small veterinary clinics, MRSP was identified in 8% of personnel-owned dogs and 8% of veterinary personnel. Three MRSP isolates from personnel-owned dogs admitted to the clinic B were ST64 but the same ST was not identified in their owners. This finding implied that dogs got infected with *S. pseudintermedius* when being taken to clinic B but did not transfer it to their owners. Dogs with no visible skin lesions and generally regarded as healthy were included in the study. In these two clinics the most frequent MRSP isolate from dogs was ST469, but this clone was not identified in personnel-owned dogs, unlike ST64. Lack of human host tropism by MRSP was explained by a small sample size and the fact that dogs had to meet special selection criteria (Worthing et al., 2018). A research was conducted in

a small animal hospital in Germany. It included swabs from employees and the hospital environment for isolation and characterization of *Staphylococcus* spp. isolates resistant to oxacillin. MRSP was detected in nasal swabs of two persons, from the hands of two persons and in three environmental swabs (floor in the waiting area, clippers in the emergency room and a table in the intensive care unit). Two spa types (t02 and t06) were identified among MRSP isolates. In addition, all isolates had related PFGE patterns, the same antibiotic resistance profiles and the same resistance genes indicating that the transfer of MRSP between dogs and humans had occurred (Feβler et al., 2018). Even though zoonotic transmission of *S. pseudintermedius* from dogs to humans is rarely documented, there are many pieces of evidence that such transmissions can happen. It was documented in a case report from Scotland that *Clostridium perfringens* and *S. pseudintermedius* were isolated from a patient with an ecthyma-like lesion in a forehead. This patient was also an owner of three Siberian husky dogs and therefore swabs from nostrils, mouth, ear, forelimb, hind limb axillae between toes and anal margin were taken from his pets. *S. pseudintermedius* was isolated from dog swabs but these isolates were not in concordance with isolates from the patient. Moreover, they were genetically unrelated which was confirmed by MLST. The human isolate was resistant to penicillin while isolates from dogs were susceptible. All isolates were susceptible to oxacillin (Robb et al., 2017). However, in a case study from Spain, *S. pseudintermedius* isolated from patients and dogs of the same household had identical PFGE patterns, sequence types and antimicrobial phenotype and genotype even if isolates were susceptible to methicillin (Lozano et al., 2017). Early studies, conducted in Denmark by Guardabassi et al. (2004), have shown that *S. intermedius* isolates from dogs and their owners show identical or close related PFGE patterns, but between households these isolates were genetically unrelated. It was not certain if these strains were transmitted from dogs to owners or vice versa. However, authors postulated that resistance genes can be transferred from *S. intermedius* to human *S. aureus* and, therefore, present a serious risk for human health. In a study conducted in the Netherlands, MRSP was found in 15/20 households in 18 dogs and six cats. In addition, MRSP was identified in 5/14 contact dogs and 4/13 contact cats in six households. Nasal swabs were taken from 45 humans who owned pets and only two swabs taken from the same household were MRSP positive. These owners had a cat with cystitis caused by MRSP. A total number of 141 personnel employed in the veterinary clinic (13 clinics were included in the study) agreed to participate in the second part of the study and only four persons had MRSP. Thirty-one (16%) of environmental swabs was positive after the first sampling and after

the usual hygiene protocol only 14% were still MRSP positive. The authors concluded that there is a small likelihood of humans being colonized by MRSP in contaminated household and hospital environments (Duijkeren et al., 2011). Scientists drew a comparison between MRSP isolates from dogs, veterinarians and dog owners in Thailand based on sequence types and the result has shown that ST45, 112, 169, 178, 181 and 183 are shared between them. A new cassette SCC*mec*$_{57395}$ was identified in isolates from dogs and veterinarians which belong to ST45. However, this cassette was not found in isolates from dog owners (Chanchaithong et al., 2014).

CONCLUSION

In conclusion, MRSP therapy is difficult to undergo since most of the isolates are multidrug-resistant and resistant to beta-lactam antibiotics. The data about successful antibiotic therapy is scarce. Local treatment with fusidic acid should be considered, where possible, including the use of non-antibiotic substances for local and surgical wound treatment. Albeit there is little information about the role of MRSP in human infections, the nosocomial transmission and widespread contamination of the environment is an existing threat. Therefore, it is important to prevent contact with infected animals and have personal hygiene. In that way, the spread of MRSP between animals and humans will be restricted and antibiotic therapy, which is assigned as critical in human medicine, avoided.

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Authors' contributions

Authors contributed equally to this manuscript.

Competing interest

Authors declared no conflict of interests regarding the present paper.

REFERENCES

1. Bergot M., Martins-Simoes P., Kilian H., Châtre P., Worthing K.A., Norris J.M., Madec J.Y., Laurent F., Haenni M. Evolution of the population structure of *Staphylococcus pseudintermedius* in France. Frontiers in Microbiology, 9, 3055. doi: 10.3389/fmicb.2018.03055.
2. Chanchaithong P., Perreten V., Schwendener S., Tribuddharat C., Chongthaleong A., Niyomtham W., Prapasarakul N. 2014. Strain typing and antimicrobial susceptibility of methicillin-resistant coagulase-positive staphylococcal species in dogs and people associated with dogs in Thailand. Journal of Applied Microbiology, 117, 572-586.
3. Corro M., Skarin J., Börjesson S., Rota A. 2018. Occurrence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in successive parturitions of bitches and their puppies in two kennels in Italy. BMC Veterinary Research, 14, 308, https://doi.org/10.1186/s12917-018-1612-z.
4. van Duijkeren E., Kamphuis M., van der Mije I.C., Laarhoven L.M., Duim B., Wagenaar J.A., Houwers D.J. 2011. Transmission of methicillin-resistant *Staphylococcus pseudintermedius* between infected dogs and cats and contact pets, humans and the environment in households and veterinary clinics. Veterinary Microbiology, 150, 338-343.
5. Feβler A.T., Schuenemann R., Kadlec K., Hensel V., Brombach J., Murugaiyan J., Oechtering G., Burgener I.A. Schwarz S. 2018. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) among employees and in the environment of a small animal hospital. Veterinary Microbiology, 221, 153-158.
6. Gordon R.J., Lowy F.D. 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clinical Infectious Diseases, 46(Suppl 5), S350-S359.
7. Grönthal T., Eklund M., Thomson K., Piiparinen H., Sironen T., Rantala M. 2017. Antimicrobial resistance in *Staphylococcus pseudintermedius* and the molecular epidemiology of methicillin-resistant *S. pseudintermedius* in small animals in Finland. Journal of Antimicrobial Chemotherapy, 72, 1021-1030.
8. Guardabassi L., Loeber M.E., Jacobson A. 2004. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. Veterinary Microbiology, 98, 23-27.
9. Hanselman B.A., Kruth S.A., Rousseau J., Weese J.S. 2009. Coagulase positive staphylococcal colonization of humans and their household pets. Canadian Journal of Veterinary Medicine, 50, 954-958.

10. Kadlec K., Schwarz S., Perreten V., Andersson G.U., Finn M., Greko C., Moodley A., Kania S.A., Frank L.A., Bemis DA., Franco A., Iurescia M., Battisti A., Duim B., Wagenaar J.A., van Duijkeren E., Weese J.S., Fitzgerald J.R., Rossano A., Guardabassi L. 2010. Molecular analysis of methicillin resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. Journal of Antimicrobial Chemotherapy, 65, 1826-1837.
11. Kadlec K., Weiβ S., Wendlandt S., Schwarz S., Tonpitak W. 2016. Characterization of canine and feline methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) from Thailand. Veterinary Microbiology, 194, 93-97.
12. Kasai T., Saegusa S., Shirai M., Murakami M., Kato Y. 2016. New categories designated as healthcare-associated and community-associated methicillin-resistant *Staphylococcus pseudintermedius* in dogs. Microbiology and Immunology, 60, 540-551.
13. Kjellman E.E., Slettemeås J.S., Small H., Sunde M. 2015. Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) from healthy dogs in Norway-occurrence, genotypes and comparison to clinical MRSP. Microbiology Open, 4, 857-866.
14. Lozano C., Rezusta A., Ferrer I, Pérez-Laguna V., Zarazaga M., Ruiz-Ripa L., Revillo M.J., Torres C. 2017. *Staphylococcus pseudintermedius* human infection cases in Spain: Dog-to-human transmission. Vector Borne Zoonotic Diseases, 17, 268-270.
15. Moodley A., Riley M.C., Kania S.A., Guardabassi L. 2013. Genome sequence of *Staphylococcus pseudintermedius* strain E140, an ST71 European-associated methicillin-resistant isolate. Genome Announcements, 1, 2, e00207-12.
16. Nienhoff U., Kadlec K., Chaberny I.F., Verspohl J., Gerlach G.F., Kreienbrock L., Schwarz S., Simon D., Nolte I. 2011. Methicillin-resistant *Staphylococcus pseudintermedius* among dogs admitted to a small animal hospital. *Veterinary Microbiology*, 150, 191-197.
17. Nisa S., Bercker C., Midwinter A.C., Bruce I., Graham C.F., Venter P., Bell A., French N.P., Benschop J., Bailey K.M., Wilkinson D.A. 2019 Combining MALDI-TOF and genomics in the study of methicillin resistant and multidrug resistant *Staphylococcus pseudintermedius* in New Zealand. Scientific Reports, 9:1271, https://doi.org/10.1038/s41598-018-37503-9.
18. Perreten V., Kadlec K., Schwarz S., Andersson G.U., Finn M., Greko C., Moodley A., Kania S.A., Frank L.A, Bemis DA., Franco A., Iurescia M., Battisti A., Duim B., Wagenaar J.A., van Duijkeren E., Weese J.S., Fitzgerald J.R., Rossano A., Guardabassi L. 2010. Clonal spread of methicillin-re-

sistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. Journal of Antimicrobial Chemotherapy, 65, 1145-1154.

19. Robb A.R., Wright E.D., Foster A.M.E., Walker R., Malone C. 2017. Skin infection caused by a novel strain of *Staphylococcus pseudintermedius* in a Siberian husky dog owner. Journal of Medical Microbiology, 4, doi 10.1099/jmmcr.0.005087.
20. Ruzauskas M., Couto N., Pavilonis A., Klimiene I., Siugzdiniene R., Virgailis M., Vaskeviciute L., Anskiene L., Pomba C. 2016. Characterization of *Staphylococcus pseudintermedius* isolated from diseased dogs in Lithuania. Polish Journal of Veterinary Science, 19, 7-14.
21. Dos Santos T.P., Damborg P., Moodley A., Guardabassi L. 2016 Systematic review on global epidemiology of methicillin-resistant *Staphylococcus pseudintermedius*: Inference of population structure from multilocus sequence typing data. Frontiers in Microbiology, 7:1599. doi: 10.3389/fmicb.2016.01599.
22. Sasaki T., Kikuchi K., Tanaka Y., Takahashi N., Kamata S., Hiramatsu K. 2007. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. Journal of Clinical Microbiology, 45, 2770-2778.
23. Sasaki T., Tsubakishita S., Tanaka Y., Sakusabe A., Ohtsuka M., Hirotaki S., Kawakami T., Fukata T., Hiramatsu K. 2010. Multiplex-PCR method for species identification of coagulase-positive Staphylococci. Journal of Clinical Microbiology, 48, 765-769.
24. Sewid A.H., Hassan M.N., Ammar A.M., Bemis D.A., Kania S.A. 2018 Identification, cloning and characterization of *Staphylococcus pseudintermedius* coagulase. Infection and Immunity, 86:e00027-18. https://doi.org/10.1128/IAI.00027-18.
25. Somayaji R., Priyantha M.A.R., Rubin J.E., Church D. 2016. Human infection due to *Staphylococcus pseudintermedius*, an emerging zoonosis of canine origin: report of 24 cases. Diagnostic Microbiology and Infectious Disease, 85, 471-476.
26. Stegmann R., Burnens A., Maranta C.A., Perreten V. 2010. Human infection associated with methicillin-resistant *Staphylococcus pseudintermedius* ST71. Journal of Antimicrobial Chemotherapy, 65, 2047-2048.
27. Tabatabaei S., Najafifar A., Badouei A.M., Salehi Z.T., Tamai A.I., Khaksar E., Abbassi M.S., Ghazisaeedi F. 2019. Genetic characterization of methicillinj-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in pets and veterinary personnel in Iran: new insights into emerging methicillin-resistant *S. pseudintermedius* (MRSP). Journal of Global Antimicrobial Resistance, 16, 6-10.

28. Velhner M., Kozoderovic G., Suvajdzic Lj., Todorovic D., Petrovic J. 2016. Genetic mechanisms of methicillin resistance in *Staphylococcus aureus* (MRSA) and its capacity of transmission: a brief review. Arhiv Veterinarske Medicine, 9, 29-37.
29. Ventrella G., Moodley A., Grandolfo E., Parisis A., Corrente M., Buonavoglia D., Guardabassi L. 2017. Frequency, antimicrobial susceptibility and clonal distribution of methicillin-resistant *Staphylococcus pseudintermedius* in canine clinical samples submitted to a veterinary diagnostic laboratory in Italy: a 3-year retrospective investigation. Veterinary Microbiology, 211, 103-106.
30. Walther B., Hermes J., Cuny C., Wieler L.H., Vincze S., Elnaga Y.A., Stamm I., Kopp P.A., Kohn B., Witte W., Jansen A., Conraths F.J., Semmler T., Eckmanns T., Lübke-Becker A. 2012 Sharing more than friendship-nasal colonization with coagulase-positive Staphylococci (CPS) and co-habitation aspects of dogs and their owners. PLoS ONE, 7(4): e35197. doi:10.1371/journal.pone.0035197.
31. Worthing K.A., Brown J., Gerber L., Trott D.J., Abraham S., Norris J.M. 2018. Methicillin-resistant staphylococci amongst veterinary personel, personnel-owned pets, patients and the hospital environment of two small animal veterinary hospitals. Veterinary Microbiology, 223, 79-85.

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THERMOPHILIC *CAMPYLOBACTER* SPP. IN POULTRY MEAT PRODUCTION

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Abstract

Thermophilic *Campylobacter* spp. are the leading cause of zoonotic enteric disease in Europe and USA. In Serbia, it has an upward trend in human population. The disease is usually indirectly transmitted to humans through the consumption of food contaminated by the faeces of infected animals. The aim of this paper was to analyze data on the prevalence of *Campylobacter* spp. in poultry meat production chain and the risk for the development of the disease in humans. The *Campylobacter jejuni/coli* was identified at farm level in 73.3% of poultry, 66.6% calves and 58.3% pig samples of already ill or suspected cases. Clinical manifestation of the disease in birds can be expected if an additional immunosuppressive factor is present. Artificial infection of healthy chickens with 6.77 log cfu *C. jejuni* per chicken on day 21st of life leads to 5.26 log cfu/g faeces after only five days with a tendency to decrease during the next 18 days. Although chilling and freezing may significantly reduce *Campylobacter* contamination of carcasses, it cannot completely eliminate the initial contamination. According to our experimental results the prevalence of *Campylobacter* contaminated chickens from positive flock appears to drop from 100% live birds (with 3.02 log cfu/g faeces) to 50% of chicken carcasses. Contamination of the carcasses depends on initial contamination of live birds, good hygiene practices and good manufacturing practices. Therefore, high variability in contamination of carcasses can be considered; prevalence range from 11.43 to 90.00% of carcases was established in various slaughterhouses. At retail, *Campylobac-*

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ter was detected in 18.8% poultry meat samples and 10.0% samples of other meat types. *Campylobacter* is frequently found in the entire production chain of poultry meat and represents high risk for consumers' health.

Key words: *Campylobacter*, farm, slaughterhouse, retail

TERMOFILNE *CAMPYLOBACTER* VRSTE U LANCU PROIZVODNJE PILEĆEG MESA

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Kratak sadržaj

Termofilne *Campylobacter* vrste su jedan od vodećih uzročnika alimentarnih oboljenja u Evropi i USA. U Srbiji postoji rastući trend incidence u humanoj populaciji. Oboljenje se prenosi na čoveka putem hrane koje je kontaminirana fecesom inficiranih životinja. Cilj rada je analiziranje raširenosti *Campylobacter* spp. u lancu proizvodnje mesa živine i utvrđivanje značaja rizika za zdravlje ljudi. *Campylobacter jejuni/coli* je identifikovan na nivou farme u 73.3% uzoraka poreklom od živine, 66.6% teladi i 58.3% svinja. Uzorci u ovom istraživanju su poticali od bolesnih ili sumnjivih životinja. Klinička manifestacija oboljenja kod živine se očekuje samo u slučaju prisustva dodatnih, imunosupresivnih faktora. Veštačkom infekcijom zdravih brojlera sa 6.77 log cfu *C. jejuni* 21. dana života, već nakon pet dana dolazi do izlučivanja 5.26 log cfu/g fecesa sa tendencijom opadanja tokom narednih 18 dana. Iako hlađenje i zmrzavanje mogu značajno redukovati kontaminaciju živinskih trupova, nije moguće u potpunosti eliminisati početnu kontaminaciju. Prema našim eksperimentalnim podacima prevalenca *Campylobacter* kod veštački inficiranih pilića opada sa 100% živih jedinki (3.02 log cfu/g fecesa) na 50% klanično obrađenih trupova. Kontaminacija trupova zavisi od početne infekcije živih jedinki i dobre higijenske i dobre proizvodne prakse primenjene u klanici. Stoga nije neobično da postoji visoka varijabilnost u kontaminaciji trupova

poreklom iz različitih klanica, od 11.43 do 90.00%. U prometu prisustvo *Campylobacter* je detektovano kod 18.8% uzorka živinskom mesa i 10.0% uzorka ostalih vrsta mesa. *Campylobacter* ima visoku prevalencu u celom lancu proizvodnje živinskog mesa od farme, preko klanice pa sve do mesa u prometu i predstavlja značajan rizik za zdravlje potrošača.

Ključne reči: *Campylobacter*, farma, klanica, promet

INTRODUCTION

Campylobacter spp. are microaerophilic, Gram-negative, curved and motile rods, which commonly cannot grow below 30˚C; however, some low metabolic activity is detectable at 40˚C (EFSA, 2010). The most important foodborne *Campylobacter* species are thermophilic *C. jejuni* and *C. coli*. *C. jejuni* can be found in the intestines of a range of wild and domestic animals, especially birds. Its prevalence in birds could be attributed to the optimal growth temperature being 42˚C. *C. coli* can often be identified in the intestines of pigs. In the environment, the organism is isolated from the water, dust, soil, air and also in fish and vegetables (EFSA, 2010). Virulence factors of *Campylobacter* include motility, chemotaxis, adherence and invasion (Bolton 2015).

Thermophilic non-foodborne *Campylobacter* species are common causative agents of abortions in sheep and cattle and, occasionally, in other animal species. Thermophilic foodborne species can cause diarrhoea in animals and hepatitis in birds. *Campylobacter jejuni/coli* often colonize the alimentary tract of poultry and other domestic livestock, yet without development of diseases symptoms. The organisms can commonly be found in the caecum, colon and cloaca, colonizing the crypt and villus region, and are present in the mucus but not in the epithelium. Mucus is the environment that provides optimal *oxygen* level and improved motility of the bacteria (Mead, 2002). *Campylobacter jejuni/coli* are much more frequently detected in the caecum and rectum (70.0%) than in the reproductive system (uterus and magnum) (6.7%) of poultry (Stojanov et al., 2008).

CAMPYLOBACTER SPP. IN POULTRY FARMING

Campylobacter is present in farm surrounding environment, including the soil, water sources, dust, building surfaces, and the air (Ellis-Iversen et al., 2012). Major contamination sources include the environment, domestic and wild animals, water, partial depopulation (thinning) and carry over from

previous flocks (Barrios et al., 2006; Newell et al., 2011). The prevalence of *Campylobacter* positive flocks in Europe greatly differs between the countries and ranges between 5 and 90% depending on the geographic location, climatic differences, biosecurity measures as well as different research methodologies (Berndtson et al., 1996a,b).

If *Campylobacter* is introduced into the flock at an early phase of breeding, the colonization will most likely persist in all birds until slaughter, and the flock prevalence can reach even 100%. *Campylobacter* infection spreads within the flock via bird-to-bird transmission route. Such a rapid spread is a result of high expression level (8-9 log cfu/g faeces), coprophagia as well as contamination of drinking water and feeders (Thibodeau et al., 2015). The spread of *Campylobacter* is mediated also by the presence of flies, mice, and farm workers as the mechanical transmission vectors. Low flock prevalence of *Campylobacter* indicates recent colonization (Evans and Sayers, 2000).

Colonization occurs most commonly in birds at the age of 2-4 (van Gerwe et al., 2009). Despite extensive colonization, clinical symptoms in poultry are rare and unspecific and include watery diarrhoea often in the absence of pathoanatomical changes in the jejunal mucosa and the caecum. The intestinal bloating and distension consequent to the accumulation of watery and mucous contents are also diagnosed (Evans and Sayers, 2000). Macroscopic necrotic changes are visible in the liver of poultry (Lemos et al., 2015). Diarrhoea commonly occurs some 6 hours post infection, and can persist for up to 10 days. Clinical signs are greatly determined by the amount and virulence of the individual strain of *C. jejuni*, stress factors and immunosuppression (Evans and Sayers, 2000). Our research revealed that clinically manifest campylobacteriosis occurred in experimentally infected chickens with both *C. jejuni* and *Salmonella* Enteritidis or *C. jejuni* with simultaneous administration of live D78 Gumboro vaccine. Major clinical symptom was watery diarrhoea that varied in intensity between birds. Diarrhoea did not occur in chickens infected with only *C. jejuni* or only *Salmonella* Enteritidis. The administration of immunosuppressive agents induced clinically manifested signs associated with the occurrence of red or yellow spots on the liver (Stojanov et al., 2008). Immunosuppressive agents such as dexamethasone, ochratoxin A and secondary bacterial infections also promote clinical signs of campylobacteriosis (Stojanov et al., 2011).

In chicken categories older than three weeks, the infection with *C. jejuni* strains results in intestinal colonization, yet not in the clinical manifestations of the disease (Petrović et al., 2008; Petrović et al., 2012). Chickens inoculated with *C. jejuni* at the beginning of the fourth week of age (21st day of life) had

an average *C. jejuni* count of 5.26 log cfu/g on day 5 post-infection. A trend of *C. jejuni* count is displayed in Figure 1. after an initial growing tendency during first five days after infection, a continuous decrease has been observed during the period between day 11 and day 18 post-infection (from 4.97 to 3.02 log cfu/g) followed by an increase in *C. jejuni* count to 4.95 log cfu/g (day 28 post-infection).

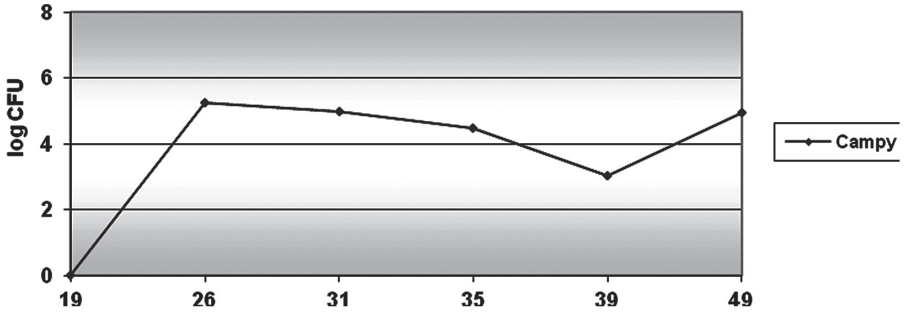

Figure 1. Average total count of *C. jejuni* in infected chickens (Petrović et al., 2012)

Surrounding environment including air distribution in the facility, open doors and windows, improper footwear, two or more workers in charge for the facility, presence of rodents and insects, two or more housing units at the farm, vicinity of farms with other domestic livestock, feed storage outside the facility and on-farm storage of broiler carcasses before distribution can represent important risk factors for the development of infection in broilers. Feedstuffs are not a suitable medium for multiplication of *Campylobacter* organisms, so drinking water remains far more important risk factor (Sibanda et al., 2018).

In cases when there are several units for flock accommodation at the same farm, *Campylobacter* infection can be identified in some flocks, while other ones remain *Campylobacter*-free. The susceptibility to colonization with *Campylobacter* strains increases with the age of the birds and flock size (Berndtson, 1996b). Gibbens et al. (2001) reported reduction of facility contamination by over 50% after application of relevant disinfection protocols. Poultry transportation equipment and cages are also potential sources of *Campylobacter*. Transportation negatively affects the poultry population and increases contamination due to the exposure to other birds' faeces (which is more likely to be watery due to stress conditions) (Dogan et al., 2019). The transmission of *Campylobacter* spp. across the farm occurs rapidly and the infection spreads flock-wide within only few days, which supports the theory that if the infection is present, every single bird in the flock is affected (Berndtson 1996b).

CAMPYLOBACTER SPP. IN SLAUGHTERHOUSES

Dominant route of *Campylobacter* contamination of poultry carcasses in a slaughterhouse is the rupture of intestines and consequent leakage and spread of the faeces. Another source is improper hygiene practice (cleaning and disinfection) in the facility (Hansson et al., 2005; Peryat et al., 2008). Carcass contamination is substantially influenced by biological properties of *Campylobacter.* Though microaerophilic and thus difficult to grow under common conditions in the food production chain, *Campylobacter* survives the acidative and oxidative stress and modified atmospheric packaging. This strongly indicates its high capability of adapting to unfavourable environmental conditions and surviving food processing procedures. Biological properties enabling the adaptability of this organism to stress include antioxidant defence, ability to enter to viable but not countable state (VBNC state), selection of virulent strains, antimicrobial resistance genes transfer and wide genetic variability (Meredith et al., 2014; Bolton 2015; Gomes et al., 2018).

Campylobacter contamination at slaughterhouse is closely related with the colonization status of broilers during rearing. Colonized chickens carry large amounts of *Campylobacter* in the caecum (5-8 log cfu) and intestines. Such chickens shed *Campylobacter* in the faeces during transportation and spread it into the environment (Dogan et al., 2019). Flock prevalence rate and *Campylobacter* concentration in the caecum along with the hygienic standards applied during processing strongly influence the amount of the pathogens in final poultry products and hence the exposure of the consumers to thermophilic *Campylobacter* species from poultry meat.

Major points of potential contamination in the slaughterhouse include picking, evisceration and some chilling processes. Leaking of faecal content during picking and evisceration process leads to carcass contamination; even very small amounts of the faeces can significantly elevate the *Campylobacter* counts on the carcasses. Immersion chilling with chlorinated water reduces contamination but without chlorine the contamination increases (Guerin et al., 2010; Dogan et al., 2019).

Contamination of carcasses could be reduced by scalding, washing and cold storage. During scalding, a part of *Campylobacter* organisms is washed out of the carcass surface. However, scalding water easily becomes contaminated with faecal contents and dust, thus causing recontamination of the carcass with certain amount of pathogens if scalding technology is not properly applied. The temperature of scalding water does not significantly influence the *Campylobacter* counts. Washing substantially reduces the concentration of *Campylobacter*

(90%), while airflow-chilling reduces *Campylobacter* counts depending on the temperature and humidity (Guerin et al., 2010; Dogan et al., 2019). *Campylobacter* contamination can be detected on all parts of broiler carcass neck skin, visceral cavity, even the region under the skin. The number of *Campylobacter* on the carcass commonly ranges around 2-3 log cfu/cm^2. Dry environment as well as the storage at 4°C or freezing result in the decrease of *Campylobacter* counts. Freezing at 18°C during 10 and 21 days will reduce the number of *Campylobacter* for 90% and 99%, respectively. However, there are reports on *Campylobacter* isolation from broiler carcasses even after 83 weeks of freezing. Even though current processing procedures reduce the *Campylobacter* count, the organism can often be found in final products at the range 1-4 log/cm^2. Cross-contamination at the slaughterhouse is considered possible yet limited, having in mind low concentration of *Campylobacter* on cross-contaminated carcasses as related to those originating from poultry that has previously been colonized with *Campylobacter*. Transmission of *Campylobacter* from cross-contaminated meat to the final products during processing is very unlikely, as well as the probability for consequent human infection and disease (EFSA, 2010).

The rate of carcass contamination at the slaughterhouse varies among poultry originating from *Campylobacter* colonized flocks. At certain production stages, the count of *Campylobacter* on the carcass can increase or decrease, and there are reports about complete elimination of all *Campylobacter* so the carcasses were free from *Campylobacter*. Various technological procedures applied at the slaughterhouses can affect cross-contamination and contamination of carcasses of poultry originating from *Campylobacter*-free flocks (Rosenquist et al., 2003). Slaughtering of flocks with 100% of infected birds results in 50% contaminated carcasses (Petrović et al., 2008). The prevalence of contamination significantly varies between individual slaughterhouses (Table 1).

Table 1. Occurrence of thermophilic *Campylobacter* spp. in poultry samples (Petrović et al., 2008)

Occurrence of *Campylobacter* spp. (%)	Abattoir mark						
	A	B	C	D	E	F	G
liver	40.00	5.00	8.56	6.00	34.28	2.86	5.71
carcasses	90.00	14.28	51.43	20.01	68.57	11.43	31.43

CAMPYLOBACTER SPP. AT RETAIL

The presence of thermophilic *Campylobacter* in meat on the market varies depending on the product type the highest risk is associated with whole poultry carcass, somewhat lower prevalence is evident in skin-off whole pieces. Minced meat is considered low-risk product because of beneficial effects of spices and oxidative stress as well as the range of poultry meat products (such as sausages, etc.) (Simone et al., 2017). In Serbian market, poultry meat is available as chilled (+4°C) or frozen (-20°C) product. Chilling does not significantly affect the survival of *Campylobacter*, whereas freezing reduces contamination (Reiersen et al., 2001). *Campylobacter* has been identified in 18.8% poultry meat samples and 10.0% of samples of other meat samples in a retail chain (Trajković et al., 2007).

Campylobacteriosis is the most commonly reported gastrointestinal human disease in the EU/EEA (EFSA and ECDC, 2018). Most common sources of human disease are undercooked or improperly heat treated meat, unpasteurized milk, dairy products and contaminated water. Some half of all cases of human campylobacteriosis in the USA is considered to be associated with consumption of poultry meat. After the incident with dioxin contamination in Belgium in 1999, poultry meat and relevant products were withdrawn from the market, which resulted in a 40% drop of the incidence of human campylobacteriosis. Grilling meat is at particular risk of infection development due to easy transmission of bacteria from raw meat to the hands and thus to other food while handling (Jorgensen, 2002). The risk of campylobacteriosis associated with consumption of pork and beef meat is considered relatively law; however, undertreated offal products still pose substantial risk. Important sources of human campylobacteriosis include drinking water and fresh products contaminated with campylobacteria from irrigation water. The presence of *Campylobacter spp.* in drinking and irrigation water is due to faecal contamination (EFSA, 2010).

CONTROL MEASURES

Qualitative risk analysis revealed that decrease in flock contamination level by 3 log cfu does not lead to significant decrease in the number of contaminated carcasses processed at the slaughterhouse; however, the *Campylobacter* count on the carcasses was significantly reduced (Reiersen et al., 2001). Quantitative risk analysis proved that even law reduction of *Campylobacter* spp. count in poultry faeces (2 log) decreases the incidence of human infection 30 times (Rosenquist et al., 2018) suggesting that control, that is, reduction of

Campylobacter in poultry faeces at the farms is a crucial measure for the prevention of human infections. Quantitative risk analysis also revealed a linear relationship between flock prevalence and number of contaminated carcasses after processing in the slaughterhouse, thus, compliance to strict hygienic barriers is the only effective approach to reducing the flock prevalence of *Campylobacter* (Reiersen et al., 2001).

Reduction of *Campylobacter* count on carcass surface can be accomplished by increasing scalding temperature, improving evisceration techniques, usage of large amounts of water across the entire production line, increasing the airflow at chilling and introducing relevant disinfection practices. Prevention of cross-contamination during transport of live chickens is of vital importance, also. However, all the aforementioned measures are of lesser influence on the decrease in rate of human infections as compared to the reduction of the incidence of *Campylobacter* in the flock.

CONCLUSION

Campylobacteriosis is one of the most important foodborne diseases mostly associated with consumption of contaminated poultry meat. In order to reduce the number of disease episodes, relevant measures should be applied across the entire poultry meat production chain and especially at poultry farms. Strict compliance with relevant biosecurity measures prevents the introduction of the pathogen into the production process at the farm. Adequate hygiene and sanitation practices in the slaughterhouses can prevent cross-contamination and decrease the contamination level. Ensuring of cold chain supply of poultry meat also contributes to an effective disease control. Successful protection of human population from alimentary diseases is closely associated with a range of preventive activities across the entire meat production chain and responsibility of each individual participant of this production chain.

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Author's contributions:

JP and RR made contributions to conception and design of the article, involved in data collection and drafting the manuscript. IS contributed with data about *Campylobacter* at farm level and manifestation of clinical campylobac-

teriosis. JP contributed with data about *Campylobacter* dynamics in artificially infected poultry and data about slaughterhouse prevalence. VG and JL contributed with data about *Campylobacter* prevalence at retail level. SM revised the manuscript critically and together with JP prepared the final draft of the manuscript. All authors read and approved the final manuscript.

Competing interest

Authors declared no conflict of interests regarding the present paper.

REFERENCES

1. Barrios P.R., Reuersen J., Lowman R., Bisaillon J.R., Michel P., Fridriksdottir V., Gunnarsson E., Stern N., McEwen O.S., Martin W. 2006. Risk factors for *Campylobacter* spp. colonization in broiler flocks in Iceland. Preventive Veterinary Medicine, 74, 4, 264-278.
2. Berndtson E., Danielsson-Tham M., Engvall A. 1996a. *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. International Journal of Food Microbiology 32, 35-47.
3. Berndtson E., Emanuelson U., Engvall A., Danielsson-Tham M. 1996b. A 1-year epidemiological study of campylobacter in 18 Swedish farms. Preventive Veterinary Medicine, 26, 167-185.
4. Bolton D.J. 2015. *Campylobacter* virulence and survival factors. Food Microbiology 48, 99-108.
5. Dogan O., Clarke J., Mattos F., Wang B. 2019. A quantitative microbial risk assessment model of *Campylobacter* in broiler chickens: Evaluating processing interventions. Food Control, 100, 97-110.
6. Guerin M., Sir C., Sargeant J., Waddell L., Oconnor A., Wils R. 2010. The change in prevalence of *Campylobacter* on chicken carcasses during processing: A systematic review. Poultry Science, 89, 1070-1084.
7. EFSA, European Food Safety Authority. 2010. Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in 2010. EFSA Journal 1437, 1, 1-89.
8. EFSA and ECDC, European Food Safety Authority and European Centre for Disease Prevention and Control. 2018. The European Union summary report on trends and sources of Zoonoses, zoometric agents and food borne outbreaks in 2017, EFSA Journal, 16, 12, 1-262.
9. Ellis-Iversen J., Ridley A., Morris V., Sowa A., Harris J., Atterbury R., Sparks N., Allen V. 2012. Persistent environmental reservoirs on farms as risk factors for *Campylobacter* in commercial poultry. Epidemiology and Infection, 140, 916 –924.

10. Evans S.J., Sayers A.R. 2000. A longitudinal study of *Campylobacter* infection of broiler flocks in Great Britain. Preventive Veterinary Medicine, 46, 209-223.
11. Gibbens J., Pascoe S., Evans S., Davies R., Sayers A. 2001. A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. Preventive Veterinary Medicine, 48, 2, 85-99.
12. Gomes C.N., Passaglia J., Vilela F.P., Pereira da Silva F.M.H.S., Duque S.S., Falcão J.P. 2018. High survival rates of *Campylobacter coli* under different stress conditions suggest that more rigorous food control measures might be needed in Brazil. Food Microbiology, 73, 327-333.
13. Hansson I., Ederoth M., Andersson L., Vågsholm I., Olsson E. 2005. Transmission of *Campylobacter* spp. to chickens during transport to slaughter. Journal of Applied Microbiology, 99, 5, 1149-1157.
14. Jorgensen F., Bailey R., Williams S., Henderson P., Wareing D., Bolton F., Frost A., Ward L., Humphrey T. 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. International Journal of Food Microbiology, 76, 154-164.
15. Lemos A., Morais L., da Conceição Fontes M., Pires I., Vieira-Pinto M. 2015. *Campylobacter* spp. isolation from infected poultry livers with and without necrotic lesions. Food Control, 50, 236-242.
16. Mead G. 2002. Factors affecting intestinal colonisation of poultry by *Campylobacter* and role of microflora in control. World's Poultry Science Journal, 58, 169-178.
17. Meredith H., Valdramidis V., Rotabakk B.T., Sivertsvik M., McDowell D., Bolton D.J. 2014. Effect of different modified atmospheric packaging S (MAP) gaseous combinations on *Campylobacter* and the shelf-life of chilled poultry fillets. Food Microbiology, 44, 196-203.
18. Newell K.T., Elvers D., Dopfer I., Hansson P., Jones S., James J., Gittins N.J., Stern R., Davies I., Conerton D., Pearson D., Salvat V.M. 2011. Biosecurity based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. Applied Environmental Microbiology, 77, 8605-8614.
19. Petrović J., Milanov D., Ratajac R. 2008. Savremeni trendovi u bezbednosti hrane: Rezistencija zoonotskih patogena prema antimikrobnim lekovima. Veterinarski glasnik, 62, 5-6, 257-406.
20. Petrović J., Stojanov I., Milanov D., Velhner M., Stojanović D. 2012. Four steps to risk assessment of *Campylobacter* contamination in broiler production in Serbia. In Proceedings, 6th Central European Congress on Food Institute for Food Technology, Novi Sad, Serbia 23-26.05.2012, 602-606.
21. Peyrat M.B., Soumet C., Maris P., Sanders P. 2008. Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and

disinfection procedures: analysis of a potential source of carcass contamination. International Journal of Food Microbiology, 124, 2, 188-194.
22. Reiersen H., Briem H., Hardardottir E., Gunnarsson F., Georgsson K.G. 2001. Human campylobacteriosis epidemic in Iceland 1998–2000 and effect of interventions aimed at poultry and humans. International Journal of Medical Microbiology, 291, 31,153.
23. Rosenquist H., Nielsen N.L., Sommer H.M., Nørrung B., Christensen B.B.: 2003. Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. International Journal of Food Microbiology, 83, 87-103.
24. Sibanda N., McKenna A., Richmond A., Ricke S.C., Callaway T., Stratakos A.C., Gundogdu O., Corcionvochi N. 2018. A Review of the Effect Management Practices on *Campylobacter* Prevalence in Poultry Farms. Frontiers in Microbiology doi: 10.3389/fmicb.2018.02002.
25. Simone S., Soncini G., Ziino G., Panebianco A., Giaccone V. 2017. Prevalence and quantification of thermophilic Campylobacter spp. in Italian retail poultry meat: Analysis of influencing factors. Food Microbiology, 62, 232-238.
26. Stojanov I., Milić N., Ašanin R., Vidić B., Grgić Ž., Prica N. 2008. Effects of some immunosuppressive factors on campylobacteriosis outbreaks in poultry. Acta Veterinaria 58, 2-3,167-177.
27. Stojanov I., Stojanović D., Milić N., Živkov-Baloš M., Kapetanov M. 2011. Influence of *Salmonella* Infection in Chickens on the Outcome of Campylobacteriosis in Experimental Conditions. Acta Veterinaria 61, 1, 57-65.
28. Thibodeau A., Fravalo P., Yergeau E., Arsenault J., Lahaye L., Letellier A. 2015. Chicken caecal microbiome modifications induced by *Campylobacter jejuni* colonization and by a non-antibiotic feed additive PLOS One, 10, Article 0131978. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0131978
29. Trajković-Pavlović L., Popović B., Novaković B., Gusman-Pasterko V., Jevtić M., Mirilov J. 2007. Occurrence of *Campylobacter, Salmonella, Yersinia enterocolitica* and *Listeria monocytogenes* in some retail food products in Novi Sad. Central European Journal of Public Health 15, 4, 167-171.
30. van Gerwe T., Miflin J.K., Templeton J.M., Bouma A., Wagenaar J.A., Jacobs-Reitsma W.F., Stegeman A., Klinkenberg D. 2009. Quantifying transmission of *Campylobacter jejuni* in commercial broiler flocks. Applied Environmental Microbiology, 75, 625-628.

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NOTOEDROSIS IN A HOUSEHOLD CAT - CASE REPORT

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Abstract

This paper describes a rare case of severe notoedrosis (notoedric mange) in a household cat from the suburban region of Banja Luka, Bosnia and Herzegovina. According to history, the male cat was in direct contact with stray kitten which had dermatological disorder. The examination of the skin revealed an intensely pruritic and hyperkeratotic dermatitis with typical scabby appearance. Deep scarification samples were collected from the altered skin area and macerated in 10% KOH. The microscopic examination revealed mites from genus *Notoedres*, later identified as *Notoedres cati* according to the morphological shape and size. The affected cat was treated with ivermectin 0.4 mg/kg, subcutaneously, two times with 7 day intervals. The first follow-up physical examination was done after seven days. This control showed that pruritic changes began to disappear. This case report confirms the presence of *Notoedres cati* in the cat population in Bosnia and Herzegovina.

Key words: household cat; *Notoedres cati*, Bosnia and Herzegovina;

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NOTOEDROZA KOD KUĆNE MAČKE – PRIKAZ SLUČAJA

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Kratak sadržaj

Ovaj rad opisuje rijedak slučaj teške notoedroze (notoedres šuge) kod kućne mačke iz suburbanog područja Banja Luke, Bosna i Hercegovina. Prema anamnezi, mačak je bio u direktnom kontaktu sa mačetom lutalicom koje je imalo dermatološke poremećaje. Dermatološkim pregledom je ustanovljen visoko pruritični i hiperkeratotični dermatitis sa tipičnom krustoznom prezentacijom. Uzeti su skarifikati dubljih slojeva kože sa promjenjenih dijelova kože i macerirani u 10% KOH. Mikroskopskim pregledom su ustanovljene grinje iz roda *Notoedres*, koje su identifikovane kao *Notoedres cati* na osnovu morfologije i veličine. Oboljeli mačak je terapiran potkožno sa ivermektinom u dozi od 0.4 mg/kg dva puta, u razmaku od sedam dana. Prvi kontrolni pregled je izvršen nakon sedam dana. Već na osnovu prvog kontrolnog pregleda je ustanovljeno da su se pruritične lezije počele povlačiti. Ovaj rad potvrđuje prisustvo *Notoedres cati* u populaciji mačaka u Bosni i Hercegovini

Ključne riječi: kućna mačka, *Notoedres cati*, Bosna i Hercegovina

INTRODUCTION

Notoedrosis or notoedric feline mange is a rare but potentially fatal disease of domestic and wild felids caused by obligate sarcoptic mite species *Notoedres cati* (Foley et al., 2016). Members of the genus *Notoedres* are related to the *Sarcoptes* genus, but they differ with regard to some morphological characteristics such as shape or development of setae so that these traits were initially sufficient to separate mites into individual genera (Foley et al., 2016). *Notoedres cati* is extremely contagious, and it spreads rapidly via direct contact among cats, other mammals (hedgehogs, guinea pigs, European rabbits) and humans

(Foley et al., 2016; Klompen, 1992). Notoedric mange was documented in dogs (Leone, 2007) but there are scarce data explaining how dogs can serve as definitive hosts for *Notoedres cati* or if dogs can serve as a source of infection for cats in the transmission chain. It is accepted that *Notoedres cati* has a cosmopolitan worldwide distribution in felid population with several reported endemic foci in North and South America; however, epidemiological studies on mite distribution and mange prevalence are scarce (Foley et al., 2016). So far, a limited number of clinical case reports indicate that *Notoedres cati* is present and widespread in Europe (Foley et al., 2016). In the Balkans, *Notoedres cati* was reported in stray cats in Slovenia (Rataj et al., 2004), Bosnia and Herzegovina (Vuković, 1959), Greece (Lefkaditis et al., 2015) and rabbits in Serbia (Ilić et al., 2018). In today's small veterinary practices, clinical cases of notoedric mange can be rarely seen because the current use of 'new' antiparasitic drugs and indoor keeping of domestic cats reduced the prevalence of this skin disease.

This case report describes clinically manifested notoedrosis (notoedric feline mange) in a household domestic cat from the suburban region of Banja Luka, Bosnia and Herzegovina.

In mid-February 2019, a male, one-year castrated domestic shorthair cat was brought to the veterinary practice „BL vet" Banja Luka for a physical and dermatological examination due to intense and frequent itching. The cat lived in a house, but it used to go outdoors regularly and was often in direct contact with other stray cats in the area of Rakovačke bare (44.796082; 17.183279), Banja Luka. According to the owner, 14 days before he went on a business trip, he noticed that the cat was in direct contact (they often lay together outside) with the stray kitten with an advanced skin disorder. Afterwards (approximately 5 - 10 days) the cat began to scratch itself constantly around the head and ears until the first lesions appeared. There was no history of vaccination and deworming of the owned cat.

The physical examination revealed good body condition and mild lethargy with normal respiration, heart rate and body temperature. Visible gingival mucosa and conjunctiva were mildly anaemic. There were indications of mild dehydration. The skin was dry and dull with the hair coat of poor quality. Alopecia and secondary scratches were present on the skin of the ears, head and face (Figure 1.).

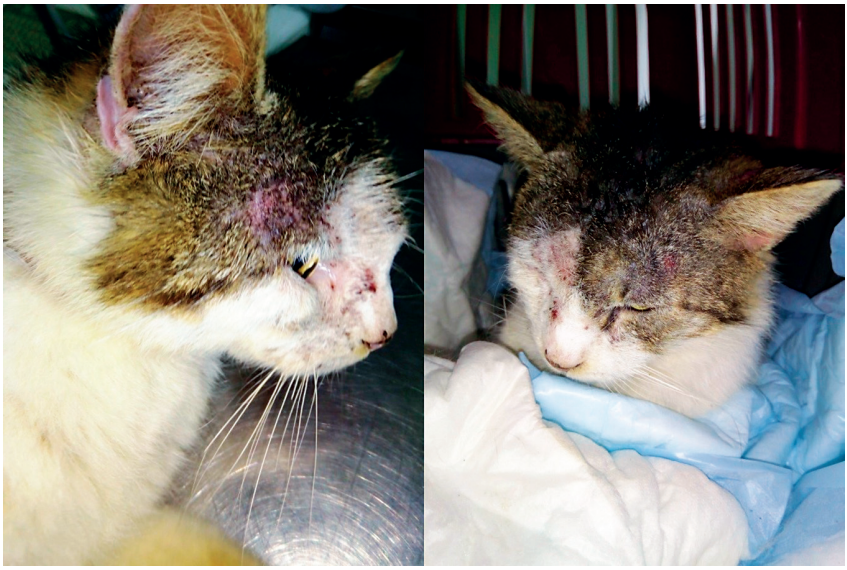

Figure 1. Pruritic head lesions in a household cat (original photos)

Due to intense pruritus, scales and crusts on the face, ear pine, head and front legs were observed. Pruritus was intense in the periocular area so that the eyes were sometimes closed during the episodes of scratching and consequent self/mutilation dermatitis. The reactive skin on the head was hyperkeratotic, hyperpigmented/grey of scabby appearance (Figure 2).

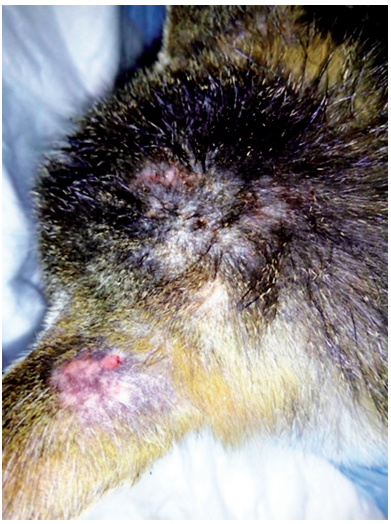

Figure 2. Hyperpigmented and hyperkeratotic changes on ear pine and head in a cat (original photo)

After clinical and dermatological examinations, clinical suspicion to notoedric mange was established. According to Hellmann et al. (2013), skin lesions were scored as 3rd-grade ones – severe lesions affecting an area wider than the region of the head, severe alopecia, a thick/crusty and scabby appearance of the skin, intensive scratching, inducing to self-trauma injury.

Several deep skin scrapings from the altered skin area of ear pine and head were taken with a scalpel blade. The collected skin material was macerated in 10% KOH (potassium hydroxide) and observed under the microscope (4x, 10x, 20x, 40x) in the veterinary practice "BL vet". The microscopic examination revealed a high number of adults and eggs of small burrowing mites from the family Sarcoptidae. At higher magnifications, oval idiosoma with longer front legs, short pretarsi with long pedicel and terminal sucker (p), long bristle or terminal setae on the third and fourth leg were observed in female mites – Tse (Figure 3).

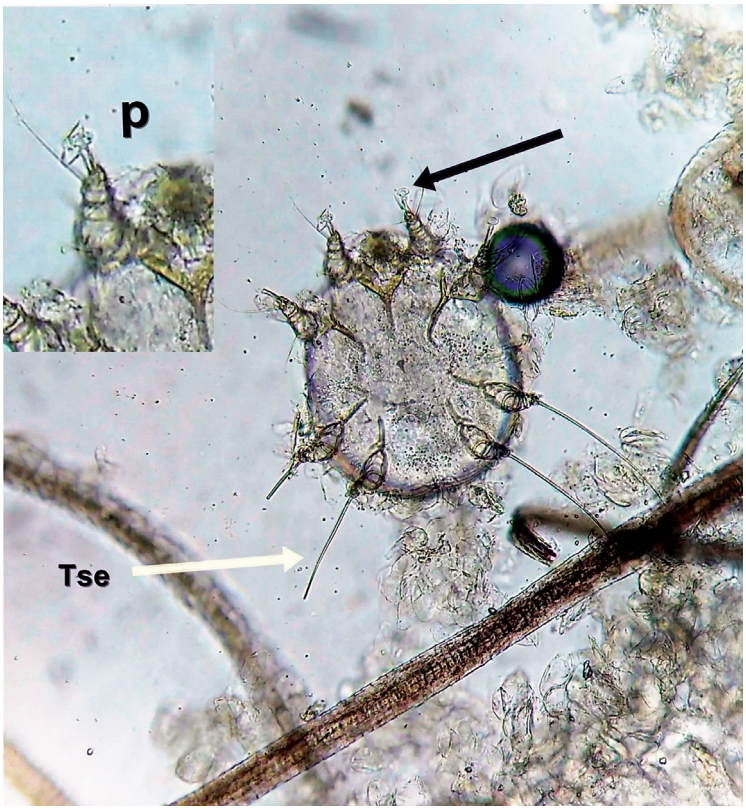

Figure 3. Ventral view of female *Notoedres cati*: (p) black arrow – pedicel; (Tse) white arrow – terminal setae (original photo)

At the dorsal surface, the idiosoma had concentric, fingerprint-like striations (St), the anus surrounded by blunt spines (Sp) and dorsal setae (Se) (Figure 4). According to the morphological and morphometric examinations (Bowman, 2008; Klompen, 1992) the definitive diagnosis was *Notoedres cati*.

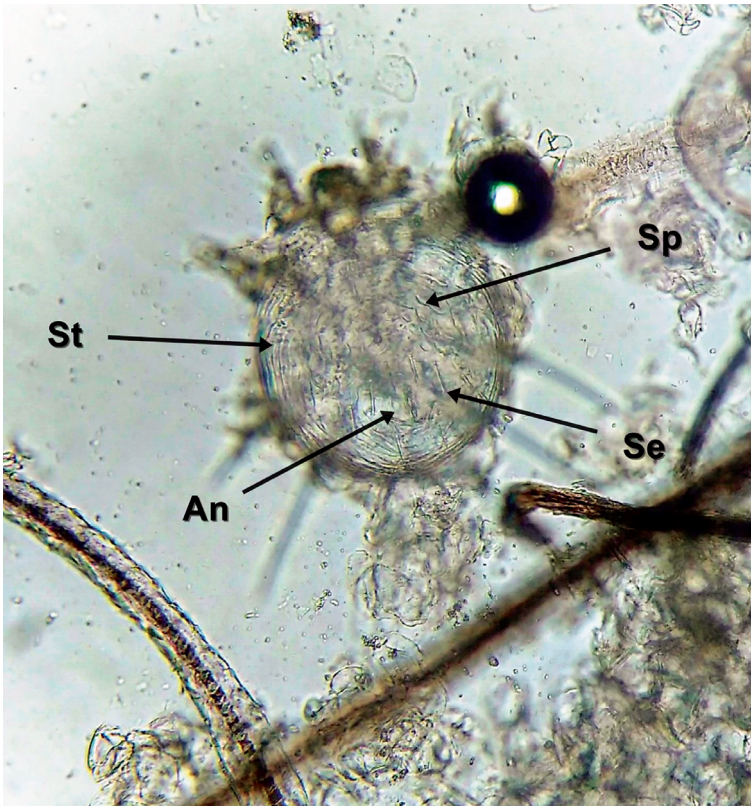

Figure 4. Dorsal view of female *Notoedres cati*; An – anus; Se – setae; Sp – „blunt" spines; St – 'finger-like' striations (original photo)

The affected cat was treated with ivermectin 0.4 mg/kg, subcutaneously, in 7 days intervals. In addition, dexamethasone 0.2 mg/kg, intramuscularly, was administered. The first follow-up physical examination was done after the first seven days. The severe pruritus decreased and the skin lesions started to heal, and hence the treatment with ivermectin was repeated using the same dose to finishing the treatment protocol. No adverse effects of ivermectin were observed in this case. No further follow-up examinations of the cat were performed.

DISCUSSION

The occurrence of notoedrosis in household cats is extremely rare in Bosnia and Herzegovina, as evidenced by the fact that the last written evidence of its occurrence dates back to 1959 (Vuković, 1959). In this first report, there was no precise information on the clinical impact of *Notoedres cati* to cats and other hosts; however, the author noticed only a low prevalence of mites in carnivores (Vuković, 1959). This report suggests that this mite species is still present in the population of domestic cats and that it can cause severe disease in cats – especially among stray animals without regular monitoring of health condition.

Given the data obtained from the anamnesis in this case, it can be assumed that the current mite infection in the cat was probably developed as a consequence of direct contact with a stray kitten. Therefore, it is possible that the disease is present in a wider area of this part of the city. In order to confirm the suspicion of potential spread of this parasite it is necessary to conduct an extensive epidemiological investigation not only in Banja Luka but in a wide area of the country.

Earlier surveys suggest that subclinical infection with *Notoedres cati* is possible, especially in stray cats (Rataj et al., 2004). The advanced infection found in our case is less common in the literature and the fact is that this condition, if left untreated, can cause serious health disorders and, in some cases, the death of diseased animals (Hellmann et al., 2013). The clinical manifestations of the disease described in the literature can be divided into several degrees in relation to the distribution of lesions (Hellmann et al., 2013). In the described case, the established degree of the lesion is described as extremely "difficult" and "advanced". There are several strategies for feline scabies therapy that mainly depend on the availability of active antiparasitics and the severity of the disease. The systemic use of ivermectin administered in our case was often implemented in the treatment of feline mites (Sampaio et al., 2017). It should be noted that, in the meantime, new "spot-on" formulations of safer antiparasitics based on imidacloprid 10% / moxidectin 1% for cats have been developed that are more accessible on markets (Hellmann et al., 2013). Ivermectin is toxic to kittens (Kirkpatrick and Megella, 1987), which must be taken into account when selecting the therapy. Supportive therapy with corticosteroids and antibiotics is justified in severe cases to reduce intense itching and prevent secondary pyoderma. Dexamethasone was applied in our case due to intense itching which could lead to self-mutilation.

This case represents a rare description of notoedrosis in a household cat, and confirms the presence of this 'forgotten' parasite in the territory of Bosnia and Herzegovina. In daily clinical practice, deep skin scarification samples should be taken from cats with manifest pruritus and tested for the presence of *Notoedres cati*.

Authors' contributions

OS examined cat and diagnosed Notoedric mange, drafted first version of the manuscript; DV, MD, DN and IT drafted the final version of the manuscript

Competing interest

Authors declared no conflict of interests regarding the present paper.

REFERENCES:

1. Bowman D.D., Hendrix C.M., Lindsay D.S., Barr S.C. 2008. Feline clinical parasitology. John Wiley & Sons, 469.
2. Foley J., Serieys L.E.K., Stephenson N., Riley S., Foley C., Jennings M., Wengert M., Vickers W., Boydston E., Lyren L., Moriarty J., Clifford D.L. 2016. A synthetic review of notoedres species mites and mange. Parasitology, 143, 1847-1861.
3. Hellmann K., Petry G., Capari B., Cvejic D., Kramer F. 2013. Treatment of naturally *Notoedres cati* -infested cats with a combination of imidacloprid 10%/moxidectin 1% spot-on (Advocate/Advantage Multi, Bayer). Parasitol Res, 112, 57–66.
4. Ilić T., Stepanović P., Nenadović K., Dimitrijević S. 2018. Improving agricultural production of domestic rabbits in Serbia by a follow-up study of their parasitic infections. Iran J Vet Res, 19, 290-297.
5. Kirkpatrick C.E., Megella C. 1987. Use of ivermectin in the treatment of *Aelurostrongylus abstrusus* and *Toxocara cati* infection in a cat. J Am Vet Med Assoc, 190, 1309-1310.
6. Klompen J.S.H. 1992. Phylogenetic Relationships in the Mite Family Sarcoptidae (Acari: Astigmata). Museum of Zoology, The University of Michigan, 131.
7. Lefkaditis M.A., Sossidou A.V., Panorias A.H., Koukeri S.E., Pastiu A.L., Athanasiou L.V. 2015. Urban stray cats infested by ectoparasites with zoonotic potential in Greece. Parasitol Res, 114, 3931-3934.

8. Leone F. 2007. Canine notoedric mange: a case report. Veterinary Dermatology, 18, 127–129.
9. Rataj A.V., Posedi J., Bidovec A. 2004. Ectoparasites: *Otodectes cynotis, Felicola subrostratus* and *Notoedres cati* in the ear of cats. Slov Vet Res, 41, 89-92.
10. Sampaio K.O., de Oliveira L.M., Burmann P.M., Sousa F.R.P., Evangelista J.S., Cunha M.G. 2017. Acetate tape impression test for diagnosis of notoedric mange in cats. J Feline Med Surg, 19, 702-705.
11. Vuković V. 1959. Prilog poznavanju parazitarnih oboljenja mesojeda. Veterinaria (Sarajevo), 8, 605-610.

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PHYSICOCHEMICAL CHARACTERISTICS OF SERBIAN HONEYDEW HONEY

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Abstract

The aim of this study was to investigate the composition and quality of Serbian honeydew honey. For this purpose, the physicochemical characteristics of 14 honeydew samples were analyzed. The physicochemical characteristics of all honeydew honeys from Serbia analyzed in this research can be considered to be within the parameters prescribed for honeydew in general. The sum value of glucose and fructose, the content of sucrose, water, hydroxymethylfurfural, acidity, and diastase activity were in line with European and national regulations for honey, for all investigated honeydew samples. Out of a total of 14 tested honey samples, 1 sample did not comply with the national regulations for honey regarding electrical conductivity. According to our results, in most of investigated samples the fructose/glucose (F/G) ratio was greater than 1.11 and glucose/water (G/W) ratio was close to 2. This means that they can be categorized as medium-crystallizing honeys. The results obtained in this study indicate excellent quality, absence of undesirable fermentation, acceptable freshness and proper manipulation of Serbian honeydews.

Key words: honeydew honey, physicochemical characteristics

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FIZIČKO-HEMIJSKE KARAKTERISTIKE MEDLJIKOVCA POREKLOM IZ REPUBLIKE SRBIJE

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Kratak sadržaj

U ovom radu ispitivan je sastav i kvalitet srpskog medljikovca (šumskog meda). Rezultati ispitivanja fizičko-hemijskih karakteristika medljikovca iz Srbije nalaze se u granicama očekivanih vrednosti za medljikovac. Vrednosti dobijene za sadržaj redukujućuh šećera (glukoza i fruktoza), saharoze, vode, hidroksimetilfurfurola, kao i kiselost i aktivnosti dijastaze su u skladu sa evropskim i nacionalnim propisima koji se odnose na kvalitet meda. Od ukupno 14 ispitanih uzoraka medljikovca, 1 uzorak nije odgovarao odredbama propisa u pogledu električne provodljivosti. Prema rezultatima ispitivanja, u većini ispitivanih uzoraka odnos F/G je bio veći od 1,11, a odnos G/V bio je blizu 2. Na osnovu ovih rezultata, medljikovac se može okarakterisati kao med sa srednjom brzinom kristalizacije. Rezultati dobijeni u našem istraživanju ukazuju na odličan kvalitet, odsustvo neželjene fermentacije, prihvatljivu svežinu i pravilnu manipulaciju srpskim medljikovcem.

Ključne reči: medljikovac, fizičko-hemijske karakteristike

Introduction

Honeydew is honey produced by bees (*Apis mellifera*) from secretions of living parts of plants or excretions of plant-sucking insects (European Commission, 2002). Secretions of living parts of plants are sweet substances that appear periodically on the leaves of some plants, most commonly on fir, pine, spruce, willow, oak, soft chestnut and other evergreen and deciduous trees (Primorac et el., 2009; Vasić et al. 2019).

The interest in honeydew has increased because of its nutritional, sensorial and potential therapeutic properties (Sergalio et al., 2019). Honeydew is a distinctive honey compared to blossom honey since it usually has higher electrical conductivity, pH values, ashes, higher content of disaccharides, trisaccharides, and lower content of monosaccharides (Sergalio et al., 2019; Živkov Baloš et al., 2018, 2019a). Darker color and different sensory features compared

to blossom honey are also the characteristics of this type of honey (Escuerdo et al., 2013; Flores et al., 2015; Živkov Baloš et al., 2019b). Numerous studies have established that honeydew contains a number of bioactive compounds, such as proteins, amino acids and phenolics (Bogdanov et al., 2004; Escuerdo et al., 2013; Silva et al., 2018; Vasić et al., 2019), compared to other types of honey, which classifies it as health-promoting food.

Basically, honey is a concentrated water solution of fructose and glucose, with small amounts of various complex sugars (Escuredo et al., 2014; Valdés-Silverio et al., 2018). Fructose and glucose are present in nectar or in the carbohydrate excretion of insects that suck the fluid from the phloem (Lazarević et al., 2017). As a result of high concentration of sugar, honey crystallizes over time. Some components of honey, such as other carbohydrates, pollen grains, air bubbles, and particles can have an impact on the crystallization of honey. Fructose/glucose (G/F) ratio and glucose/water (G/W) ratio can be used to estimate the rate of honey crystallization. Generally, honeys with low G/W and F/G ratio do not crystallize easily (Escuredo et al., 2014).

Crystallization of honey is an undesirable process. During the crystallization changes occur in the textural properties, making honey less appealing to consumers, who prefer liquid and transparent product (Kabbani et al., 2011). Crystallization of honey affects the processing of honey during extraction, filtration, mixing and bottling (Dobre et al., 2012; Laos et al., 2011).

Honeydew honey is a natural product with complex composition, which depends on bee species, geographical region, available floral source and storage conditions (Karabagias et al., 2014). Considering all the factors mentioned above and the number of possible floral sources in particular, it is understandable that no two honeys are the same (Kirs et al., 2011). Honeydew physicochemical quality criteria are well specified by the European Legislation (European Commission, 2002) and the regulation concerning the quality of honey in the Republic of Serbia (Official Gazette RS, 101/2015). The major criteria for honey in both of those standards are sugar content (sucrose and sum of fructose and glucose), moisture content, water-insoluble content, electrical conductivity, free acidity, diastase activity and hydroxymethylfurfural (HMF) content.

The aim of this study was to investigate the composition of Serbian honeydew honey, in order to obtain the information about the honey quality, and gain an insight into its nutritional suitability.

MATERIAL AND METHODS

A total of 14 honeydew samples were collected from beekeepers at different regions of Serbia. All collected samples were in their original packaging

and transferred to the laboratory of Scientific Veterinary Institute "Novi Sad" for examination. Manufacturers used field observations for botanical origin determination. Our research included only the samples with confirmed botanical origin stated on the manufacturing specification label. All the selected samples were produced by *Apis mellifera*. Honey analyses were carried out immediately after sampling. All samples were analyzed in duplicate by methods prescribed in Harmonised methods of the International Honey Commission Methods (2009).

Water content analysis

Water content was determined by refractometry, measuring the refractive index (RI) using a standard model Abbetype refractometer at 20° C. Water content (%) was then obtained from the Chataway table.

Electrical conductivity

Electrical conductivity was measured at 20° C in solutions of honey samples (20.0 g dry matter of honey in volume solution in 100 ml distilled water) using a conductometer Crison (Type Basic 30).

Free acidity

The acidity of honey was determined by volumetric method. Ten grams of honey was dissolved in 75 ml of water and solution was titrated with 0.1 M NaOH to pH 8.30. Acidity is expressed in milliequivalents/kg honey (mEq/kg).

Water-insoluble matter

Insoluble matter was determined by gravimetric method. The insoluble matter was collected on filter of specified pore size by rinsing with warm water. Dried residues (135° C) were weighed until constant weight was obtained.

Hydroxymethylfurfural

HMF was determined by an HPLC Dionex UltiMate 3000 Series system with UV detection (Thermo Scientific, Germany). Honey sample (1 g) was dissolved in 25 mL of water, filtered through a 0.45 μm nylon filter and injected (10 μl) into the HPLC system. The HPLC column was 150x3 mm Hyperilsil GOLD, with particle size of 3 μm. The mobile phase was methanol: water (10:90, v/v), at flow rate of 1 mL/min. All measurements were conducted at room temperature. The system was controlled by Chromeleon® 7 software (Thermo Scientific, Germany).

The external calibration curves produced by standard solutions were used to quantify the amount of HMF in the samples.

Sugar Composition determination

The sugar composition (fructose, glucose, sucrose) was determined by an HPLC Dionex UltiMate 3000 Series system (Thermo Scientific, Germany) equipped with a refractive index detector RefractoMax521 (ERC Inc, Japan) at 35°C. Honey sample (1 g) was dissolved in 25 mL 25% methanol, filtered through a 0.22 μm nylon filter and injected (5 μl) into the HPLC system. The HPLC column was Hypersil GOLD Amino 150x3 mm (particle size 3 μm), fitted with a guard column Hypersil GOLD Amino 10x3 mm (particle size 3 μm). The mobile phase was acetonitrile: water (8:2, v/v) filtered through 0.22 μm membrane filter, at a flow rate of 1 mL/min. All measurements were performed at room temperature. The system was controlled by Chromeleon® 7 software (Thermo Scientific, Germany). The external calibration curves produced by standard solutions were used to quantify the amount of sugars in the samples. Honey sugars were identified and quantified by comparing their retention times and peak areas with those of standard sugar solutions.

Diastase activity

Diastase activity was determined by spectrophotometric method (Megazyme International Ireland, 2014). Two grams (2.00 g) of sample were dissolved in sodium maleate buffer and adjusted volume to the mark of volumetric flask with water. Amylazyme tablets (Megazyme International, Ireland) were added to buffer solution. In the presence of α-amylase, the substrate is hydrolysed and soluble dyed products are released. The reaction was terminated and, following the filtration, the absorbance of the filtrate is measured at 590 nm. The absorbance is directly proportional to the diastase activity of the sample. The diastase activity is calculated as diastase number (DN).

Statistical analysis

Statistical analysis was performed by the PAST software package, version 2.12, Oslo, Norway. The data were grouped according to the samples of honeydew and presented as mean, standard deviation, minimum, maximum values, and coefficient of variation.

Results

The results of physicochemical analysis of Serbian honeydew are shown in Tables 1 and 2. The water content in all investigated honeydew samples was below 20%, which is the maximum permissible level set by national regulations for honey (Official Gazette, 101/2015).

The results of sugar profile analysis by HPLC-RI are shown in Table 1. Our study revealed that in all examined honeydew samples, the percentage of fructose and glucose ranged from 28.51 to 38.83 and from 24.92 to 34.09%, respectively. The sum value of glucose and fructose was in line with European and national regulations (EU, 2002; Official Gazette RS, 101/2015) with the value of over 45 g/100 g for all honeydew samples. Sucrose content in all investigated honey samples was below 5 g/kg honey, which is the maximum permissible level set by the European Legislation (EU, 2002) and national regulations for honey (Official Gazette RS, 101/2015). In 13 out of the total of 14 investigated samples (93%), sucrose content was below the detection limit of the applied method. Detection limit of the applied method is 0.25 %. The F/G ratio and G/W ratios were calculated for all samples of honeydew. The mean F/G ratio was 1.22 and ranged from 1.06 to 1.50. The mean G/W ratio was 1.89 and ranged from 1.50 to 2.32.

Table 1. Moisture content and carbohydrate concentrations of honeydew samples

Sample	Water[1] (%)	Fructose[2] (%)	Glucose[3] (%)	Sucrose (%)	F + G (%)	Ratio F/G	Ratio G/W
1	17.0	33.99	30.37	n.d.[4]	64.36	1.12	1.79
2	15.4	38.32	34.09	n.d.	72.41	1.12	2.21
3	16.6	36.02	24.98	n.d.	61.00	1.44	1.50
4	15.8	37.18	24.92	n.d.	62.10	1.49	1.58
5	15.4	37.57	25.01	n.d.	62.58	1.50	1.62
6	14.2	35.76	29.44	n.d.	65.20	1.21	2.07
7	16.4	31.94	26.32	n.d.	58.26	1.21	1.60
8	14.4	28.91	27.18	n.d.	56.09	1.06	1.89
9	13.6	34.70	31.51	n.d.	66.21	1.10	2.32
10	16.2	36.10	31.74	n.d.	67.84	1.14	1.96
11	16.6	36.03	31.78	n.d.	67.81	1.13	1.91
12	14.8	36.31	28.48	n.d.	64.79	1.27	1.92
13	14.1	34.14	31.73	0.30	65.87	1.08	2.25
14	18.2	38.83	33.41	n.d.	72.24	1.16	1.84
Minimum	13.6	28.91	24.92	< 0.25	56.09	1.06	1.50
Maximum	18.2	38.83	34.09	0.30	72.41	1.50	2.32
STDEV[5]	1.3	2.61	3.21	/	4.64	0.15	0.26
Mean	15.6	35.41	29.35	< 0.25	64.77	1.22	1.89
CV (%)[6]	8.35	7.37	10.94		7.17	12.51	13.67

[1]W – Water. [2]F – Fructose. [3]G – Glucose. n.d.[4] – unquantifiable value (less than detection limit); STDEV[5] - standard deviation; CV[6] - coefficient of variation

In accordance with the regulation concerning the quality of honey in the Republic of Serbia (Official Gazette, 101/2015), minimum electrical conductivity in honeydew put in the market is fixed to 0.8 mS/cm. The *values of electrical conductivity* in the investigated honeydew samples *were between 0.61 and 1.99* ms/cm. Out of a total of 14 tested honey samples, 1 sample (No. 11) did not comply with the national regulations for honey.

Maximum value of free acidity in all types of honey (except in baker's honey) was 50 mEq/kg, as is set by regulation (Official Gazette, 101/2015). Free acidity in all tested honeydew samples was below 50 mEq/kg. These data indicate the absence of undesirable fermentation. The mean acidity value in the investigated samples was 21.29 mEq/kg.

Table 2. Electrical conductivity, free acidity, 5-HMF and diastase activity in honeydew samples

Sample	Electrical conductivity (mS cm^{-1})	Free acidity (meq kg^{-1})	5-HMF[1] (mg kg^{-1})	Diastase (DN)
1	1.22	9.6	5.21	25.16
2	0.87	7.4	0.50	17.19
3	0.88	5.4	11.72	17.74
4	0.82	4.6	1.35	13.43
5	0.89	4.6	0.81	18.64
6	1.15	30.0	1.85	22.12
7	1.60	48.4	6.55	12.08
8	1.85	44.0	1.69	20.87
9	1.15	33.0	2.77	16.23
10	1.55	29.0	11.20	14.25
11	0.61	18.0	18.08	19.01
12	1.33	15.5	1.48	28.55
13	1.99	21.5	1.41	23.33
14	1.15	27.0	26.01	26.47
Minimum	0.61	4.6	0.50	12.08
Maximum	1.99	48.40	26.01	28.55
STDEV	0.41	14.52	7.70	5.00
Mean	1.22	21.29	6.47	18.43
CV (%)	33.37	68.20	118.92	25.46

5-HMF[1]: 5-hydroxymethylfurfural

The regulation concerning the quality of honey in the Republic of Serbia established the maximum 5-HMF content (40 mg/kg) and minimum diastase activity (8 DN). Generally, all tested samples were in compliance with the provisions of the Regulations regarding the content of 5-HMF and diastase activity. Mean 5-HMF content in investigated honeydew samples was 6.47 mg/kg, with the range 0.50 to 26.01 mg/kg. Minimum value of diastase activity was 12.08 DN and mean activity was 18.43 DN (Table 2).

Discussion

Honey is a highly viscous solution of sugars, dominantly glucose and fructose in about equal concentrations (Venir et al., 2010). The most prevalent sugar in honeydew is fructose, followed by glucose and sucrose. These results are in accordance with other literature data (Kirs et al., 2011; Kivrak et al., 2017; Sousa et al., 2016; Sergalio et al., 2019; Seijo et al., 2019). In general, the glucose content was lower than the fructose content, which indicated the natural feeding of bee colonies and confirmed the high quality of the examined honeydew honeys. Sucrose content can also be used as an indicator of artificial feeding of honeybees, if beekeepers overfeed the bees with sugar during the spring. High concentration of sucrose means an early harvest of honey (Ouchemoukh et al., 2007; Pasias et al., 2017; Saxena et al., 2010).

F/G and G/W ratios are important parameters for predicting the crystallization tendency of honey. Fructose/glucose ratio shows the degree of honey crystallization, because glucose is less water soluble than fructose (Laos et al., 2011). Honey samples in which F/G ratio is greater than 1.33 do not crystallize for a long period of time. If the F/G ratio is less than 1.11, honey crystallizes quickly (Escuerdo et al., 2014). Crystallization process is slower or null when G/W ratio is less than 1.7, and it is faster when the ratio is higher than 2 (Dobre et al., 2012) or 2.10 (Venir et al., 2010). According to our results (Table 1), in most of investigated samples, F/G ratio was greater than 1.11 and G/W ratio was close to 2, so they can be categorized as medium-crystallizing honey.

Water is the second largest component of honeydew. Honey moisture depends on the production season, floral source, abundance of nectar flow, soil, ventilation of beehives, colony strength, meteorological conditions in the area of honey production (primarily air humidity), and maturation and honey harvest time (Escuerdo et al., 2014; Kirs et al., 2011; Lazarević et al., 2017; Sousa et al., 2016; Živkov Baloš et al., 2019b). Moisture significantly affects the physical properties of honey such as crystallization, viscosity, and rheological behaviour. Although there are differences in moisture content, it can be assumed

that the tested honeydews had adequate maturity since moisture was generally lower than the maximum permissible value (20%). As shown in Table 1., moisture of honeydew samples is fairly homogeneous, and this parameter is characterized by a low coefficient of variation.

The electrical conductivity is often used in routine quality control of honey. The conductivity is related to the concentration of soluble minerals, organic acids and proteins. It is a useful tool for distinguishing honeys of different botanical origin. Storage time can also affect the electrical conductivity of honey. The most adequate parameters for distinguishing honeys of different geographical origin are those which described the patterns of pH and electrical conductivity with changes of honey concentration (Acquarone et al., 2007). Honeydew honey is characterized by higher electrical conductivity than blossom honeys, which is a good parameter to distinguish between the two types of honey. The values lower than 0.8 mS/cm may indicate adulteration or mixtures with other types of honey (Sergalio et al., 2019).

Free acidity in the examined samples ranged between 4.6 and 48.4 mEq/kg (CV = 68.2%) (Table 2). The acidity of honey is caused by organic acids (tartaric, citric, oxalic, acetic, etc.), nectar or bee secretions (Yadata, 2014). The acidity value varies depending on the floral source and bee species (Sousa et al., 2016). *Our earlier results (Živkov Baloš et al., 2018) demonstrated high acidity of forest honey, as compared with other honey types. These results were also reported by Bergamo et al. (2019) and Primorac et al. (2009).* The natural acidity of honey can be increased by the storage and ripening of honey, as well as during honey fermentation. Honey adulterated with sugar syrup has acidity lower than 1, while honey that is adulterated with invert sugar has a pronounced high acidity (Yadata, 2014).

5-Hydroxymethylfurfural is formed as an intermediate product in the Maillard reaction from the direct dehydration of sugars under acidic conditions during thermal processing of foods (Pasias et al., 2017). This compound is formed slowly and naturally during honey storage. Thus, it is considered an indicator of honey freshness (Serglaio et al., 2019). Diastase activity is closely related to 5-HMF. This parameter is sensitive to heat and long storage period. Diastase activity signifies a possible overheating of honey, above 60° C, as well as prolonged storage (Bergamo et al., 2019). Considering the 5-HMF content and diastase activity found for investigated honeydew honeys, all samples were in accordance with the established limits. These results indicate acceptable freshness and proper manipulation of honeydews.

Conclusion

The physicochemical characteristics of all honeydew honeys from Serbia analysed in this research are within the parameters expected for honeydew in general. Additionally, it can be concluded that Serbian honeydew is characterized by good quality as honey samples were within limits established by the European and national Legislation.

Therefore, further research on honeydew physicochemical and therapeutic properties is required to confirm the quality and authenticity of this product and for better understanding of the value of this honey.

Author's Contribution:

M.Ž.B. drafted the manuscript and made substantial contributions to the basic idea; S.J. carried out the HPLC analysis and was involved in drafting of the manuscript; N.P. carried out other physicochemical analysis and performed statistical analysis; D.LJ.P and S.V.K. were involved in drafting of the manuscript; M.P. collected the data; V.P. and D.M. revised the manuscript.

Competing interest

Authors declared no conflict of interests regarding the present paper.

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REFERENCES

1. Acquarone C., Buera P., Elizalde B. 2007. Pattern of pH and electric conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. Food chemistry, 101, 2, 695-703. https://doi.org/10.1016/j.foodchem.2006.01.058.
2. Bergamo G., Sergalio S.K.T., Gonzaga L.V., Fett R., Costa A.C.O. 2019. Physicochemical characteristics of bracatinga honeydew honey and blossom honey produced in the state of Santa Catarina: An approach to honey differentiation. Food Research International, 116, 745-754. https://doi.org/10.1016/j.foodres.2018.09.007.

3. Bogdanov S., Ruoff K., Persano-Oddo L. 2004. Physico-chemical methods for the characterisation of unifloral honeys: A review. Apidologie, 35, 4-17. doi: 10.1051/apido:2004047.
4. Dobre I., Georgescu I.A., Alexe P., Escuerdo O., Seijo M.C. 2012. Rheological behavior of different honey types from Romania. Food Research International, 49, 1, 126–132. https://doi.org/10.1016/j.foodres.2012.08.009.
5. Escuerdo O., Miguez M., Fernández-González M., Seijo M. 2013. Nutritional value and antioxidant activity of honeys produced in European Atlantic area. Food Chemistry, 138, 2-3, 851-856. https://doi.org/10.1016/j.foodchem.2012.11.015.
6. Escuredo O., Dobre I., Fernández-Gonzálezs M., Seijo M.C. 2014. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. Food Chemistry, 149, 84–90. https://doi.org/10.1016/j.foodchem.2013.10.097.
7. European Union. 2002. European Commission Council Directive 2001/110/EC of 20. December 2001, relating to honey. Official Journal of the European Communities, L10:47.https://eur-lex.europa.eu/legal- content/EN/TXT/PDF/?uri= CELEX: 32001L0110&from=EN
8. Flores M.S.R., Escuerdo O., Seijo M.C. 2015. Assessment of physicochemical and antioxidant characteristics of *Quercus pyrenaica* honeydew honeys. Food Chemistry, 166, 101-106. https://doi.org/10.1016/j.foodchem.2014.06.005.
9. International Honey Commission Methods. 2009. Harmonised methods of the International Honey Commission Methods, Swiss Bee Research Centre, FAM, Liebefeld, Switzeland.
10. Kabbani D., Sepulcre F., Wedekind J. 2011. Ultrasound-assisted liquefaction of rosemary honey: Influence on rheology and crystal content. Journal of Food Engineering, 107, 2, 173–178. https://doi.org/10.1016/j.jfoodeng.2011.06.027.
11. Karabagias I.K., Badeka A.V., Kontakos S., Karabournioti S., Kontominas M.G. 2014. Botanical discrimination of Greek unifloral honeys with physico-and chemometric analyses. Food Chemistry, 165, 181-190. https://doi.org/10.1016/j.foodchem.2014.05.033.
12. Kirs E., Pall R., Martverk K., Laos K. 2011. Physicochemical and melissopalynological characterization of Estonian summer honeys. Procedia Food Science 1, 1, 616–624. https://doi.org/10.1016/j.profoo.2011.09.093.
13. Kivrak Ş., Kivrak I., Karababa E. 2017. Characterization of Turkish regarding of physicochemical properties, and their adulteration analysis. Food Science and Technology (Campinas), 37, 1, 80–89. http://dx.doi.org/10.1590/1678-457x.07916.

14. Laos K., Kirs E., Pall R., Martverk K. 2011. The crystallization behaviour of Estonian honeys. Agronomy Research, 9, 427–432.
15. Lazarević K.B., Jovetić M.S., Tešić Ž.Lj. 2017. Physicochemical parameters as a tool for the assessment of origin of honey. Journal of AOAC International, 100, 4, 840–851. https://doi.org/10.5740/jaoacint.17-0143.
16. Megazyme Interantional Ireland. 2014. Diastase activity (α-Amylase) in honey, Assay procedure, T-AMZHY, 05/14, www.megazyme.com
17. Official Gazette RS. 2015. Rulebook on quality of honey and other bee products, No. 101.
18. Ouchemoukh S., Louaileche H., Schweitzer P. 2007. Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food Control, 18, 1, 52–58. https://doi.org/10.1016/j.foodcont.2005.08.007.
19. Pasias I.N., Kiriakou I.K., Proestos, C. 2017. HMF and diastase activity in honeys: a fully validated approach and a chemometric analysis for identification of honey freshness and adulteration. Food Chemistry, 229, 425-431. https://dx.doi. org/10.1016/j.foodchem.2017.02.084.
20. Primorac Lj., Angelkov B., Mandić M., Kenjerić D., Nedeljko M., Flanjak I., Perl Pirički A., Arapceska M. 2009. Comparison of the Croatian and Macedonian honeydew honey. Journal of Central European Agriculture, 10, 3, 263-270.
21. Saxena S., Gautam S., Sharma, A. 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chemistry, 118, 2, 391–397. https://doi.org/10.1016/j.foodchem.2009.05.001.
22. Seijo M.C., Escuerdo O., Rodríguez-Flores M. 2019. Physicochemical properties and pollen profile of oak honeydew and evergreen oak honeydew honeys from Spain: A comparative study. Foods, 8, 4, 126. https://doi.org/10.3390/foods8040126.
23. Sergalio K.T.S., Silva B., Bergamo G., Brugnerotto P., Gonzaga L. V., Fett R., Costa A.C.O. 2019. An overview of physicochemical characteristics and health-promoting properties of honeydew honey. Food Research International, 119, 44-60. https://doi.org/10.1016/j.foodres.2019.01.028.
24. Silva B., Gonzaga L.V., Fett R., Costa A.C.O. 2018. Simplex-centroid design and Derringers desirability function approach for simultaneous separation of phenolic compounds from *Mimosa scabrella* Bentham honeydew honeys by HPLC-DAD. Journal of Chromatography A, 1585, 182-191. https://doi. org/10.1016/j.chroma.2018.11.072.
25. Sousa J.M.B., Soza L.E., Marques G., Benassi M.T., Gullon B., Pintado M.M. 2016. Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-

arid region. LWT - Food Science and Technology, 65, 645-651. https://doi.org/10.1016/j.lwt.2015.08.058.
26. Valdés-Silverio L.A., Iturralde G., García-Tenesaca M., Paredes-Moreta J., Narváez-Narváez D.A., Rojas-Carrillo M., Tejera E., Beltrán-Ayala P., Giampieri F., Alvarez-Suarez J.M. 2018. Physicochemical parameters, chemical composition, antioxidant capacity, microbial contamination and antimicrobial activity of Eucalyptus honey from the Andean region of Ecuador. Journal of Apicultural Research, 57(3), 382–394. https://doi.org/10.1080/00218839.2018.1426349.
27. Vasić V., Gašić U., Stanković D., Lušić D., Vukić-Lušić D., Milojković-Opsenica D., Tešić Ž., Trifković J. 2019. Towards better quality criteria of European honeydew honey: phenolic profile and antioxidant capacity. Food Chemistry, 274, 629-641. https://doi.org/10.1016/j.foodchem.2018.09.045.
28. Venir E., Spaziani M., Maltini E. 2010. Crystallization in "Tarassaco" Italian honey studied by DSC. Food Chemistry, 122, 2, 410–415. https://doi.org/10.1016/j.foodchem.2009.04.012.
29. Yadata D. 2014. Detection of the electrical conductivity and acidity of honey from different areas of Tepi. Food Science and Technology, 2, 5, 59-63. DOI: 10.13189/fst.2014.020501.
30. Živkov Baloš M., Jakšić S., Mihaljev Ž., Popov N., Ljubojević Pelić D., Vidaković S. 2019a. Sugar profile of honeys from Serbia. Abstract book, Second International Congress on Hygiene and Preventive Medicine "Challenges and Public Health Interventions", Novi Sad, 10-13. April 2018, Faculty of Medicine, University of Novi Sad, 108-109, ISBN 978-86-7197-577-3.
31. Živkov Baloš M., Jakšić S., Popov N., Mihaljev Ž., Ljubojević Pelić D. 2019b. Comparative study of water content in honey produced in different years. Archives of Veterinary Medicine, 12, 1, 43-53.
32. Živkov Baloš M., Popov N., Vidaković S., Ljubojević Pelić D., Pelić M., Mihaljev Ž., Jakšić S. 2018. Electrical conductivity and acidity of honey. Archives of Veterinary Medicine, 11, 1, 91-101.

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MYCOPLASMA SYNOVIAE INFECTION IN LAYERS: DIAGNOSIS AND CONTROL MEASURES – A REVIEW

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Abstract

Mycoplasmas are widespread bacteria in domestic and wild birds. Among the important species in laying hen, *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, are considered as an emergent pathogen in the last few years worldwide, causing considerable economic losses as a result of falling eggs and the decrease in egg quality. Transmission of *M. synoviae* occurs horizontally, more rapidly in multi-age sites, and vertically, leading to a decline in hatchability in breeding farms. The interaction between *M. synoviae* and the host's immune system explains the immunosuppression induced by this pathogen. Inside the cell, *M. synoviae* can escape the immune system by implementing several mechanisms.

Subclinical respiratory infection is often associated to *M. synoviae.* However, severe disease may be observed in the presence of other factors (respiratory viruses, stressors). The emergence of a new form of clinical manifestation of disease associated to *M. synoviae* infection has been described since the 2000s. Eggshell apex abnormalities of the produced eggs, associated to high risk of cracks and breakage, is described.

The diagnosis of *M. synoviae* infection is based on various tests, including serology, culture and biomolecular methods. Control is based on the acquisition of free mycoplasma birds, biosecurity, regular monitoring and vaccination. Management of other risk factors is essential.

Keywords: antibiotic, biosecurity, eggshell apex abnormality, layer hen, *Mycoplasma synoviae*, PCR; serology, vaccination

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MYCOPLASMA SYNOVIAE INFEKCIJA KOD NOSILJA: DIJAGNOZA I MERE KONTROLE – PREGLED LITERATURE

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Kratak sadržaj

Mikoplazme su široko rasprostranjene bakterije kod domaćih i divljih ptica. Tokom poslednjih nekoliko godina, *Mycoplasma gallisepticum* i *Mycoplasma synoviae* smatraju se pretećim patogenima širom sveta, koji dovode do značajnih ekonomskih gubitaka zbog smanjene nosivosti i opadanja kvaliteta jaja. Do prenošenja *M. synoviae* dolazi horizontalno, brže na mestima gde se uzgaja živina različite starosti i vertikalno, što dovodi do smanjenja izleganja pilića na priplodnim farmama. Interakcija između *M. synoviae* i imunog sistema domaćina izaziva imunosupresiju. Unutar ćelije, *M. synoviae* koristi različite mehanizme da zaobiđe imuni sistem domaćina.

Subklinička respiratorna infekcija se često povezuje sa *M. synoviae.* Međutim, do ozbiljnih kliničkih manifestacija bolesti dolazi u prisustvu drugih faktora (respiratorni virusi, različiti stresogeni). Pojava nove kliničke manifestacije bolesti koja se dovodi u vezu sa infekcijom *M. synoviae* opisuje se od 2000-ih godina. Opisane su nepravilnosti apeksa ljuske jajeta povezanih sa visokim rizikom od pucanja i lomljenja ljuske.

Dijagnoza infekcije *M. synoviae* zasniva se na raznim testovima, uključujući serologiju, kultivisanje i biomolekularne metode. Kontrola se sprovodi redovnim monitoringom, vakcinacijom, biosigurnosnim merama i nabavkom živine koja je slobodna od mikoplazme. Upravljanje drugim rizicima je od suštinske važnosti.

Ključne reči: antibiotik, biosigurnost, apeks jajčane ljuske, koke nosilje, *Mycoplasma synoviae*, PCR; serologija, vakcinacija

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INTRODUCTION

Mycoplasmosis are worldwide infections of domestic and wild birds. Historically, infections were described firstly in turkeys in 1926, then in chicken in 1936 (Nascimento et al., 2005; Kleven 2008). More than 120 species, isolated in mammals, birds, reptiles and fishes, were determined in *Mycoplasma* genus. Currently, more than 20 species of mycoplasma are considered pathogenic in poultry (Nascimento et al., 2005; Purswell et al., 2011).

Mycoplasma gallisepticum (*M. gallisepticum*) (chicken and turkey), *Mycoplasma synoviae* (*M. synoviae*) (chicken and turkey), *Mycoplasma meleagridis* (*M. meleagridis*) (turkey) and *Mycoplasma iowae* (M. iowae) (turkey) (Stipkovits et Kempf 1996) (in Sprygin et al., 2011) are the most important species.

Mycoplasmosis caused by *M. synoviae* was firstly described in turkeys in 1926 and in chicken in 1936 (Nascimento et al., 2005, Kleven 2008). *M. synoviae* is responsible of locomotor disorders, including arthritis, tendinitis and synovitis. However, contrarily to *M. gallisepticum*, *M. synoviae* may be incriminated in a subclinical respiratory infection, which can predispose birds to the chronic respiratory disease following interactions with other pathogens (Newcastle disease virus, infectious bronchitis virus, *Ornithobacterium rhinotracheale* and *Escherichia coli*).

In last decade, the situation of mycoplasma infections is characterized by the emergence of *M. synoviae* compared to *M. gallisepticum* and a more pronounced respiratory tropism (aerosacculitis) than the articular tropism, especially in chicken and secondarily in turkey (Khalifa et al., 2013). In laying hens, *M. synoviae* has been causing serious egg drop problems since the 2000s with alteration of the eggshell quality.

ECONOMIC IMPACT

Economic impact of *Mycoplasma* infection in layer hen flocks are well documented regarding to *M. gallisepticum*. Whereas, the economic significance of *M. synoviae* has been a subject of debate for many years. While the increasing occurrence worldwide of arthropathic and amyloidogenic *M. synoviae* strains in poultry as well as strains that induce eggshell apex abnormalities (EAA) and egg production losses (Landman and Feberwee, 2001, 2004; van Beek et al., 2002; Feberwee et al., 2007), has increased attentiveness of the clinical and economic impact of infection with this *Mycoplasma*. Prevalence of *M. synoviae* infection in layers in some countries is summarized in Table 1.

Table 1. Worldwide prevalence of *M. synoviae* in layers

	Country	Region / period of study	Analysis	Prevalence	Reference(s)
Europe	*France*	Côtes d'Armor, Brittany / 2002-2003	Culture	68%	Dufour-Gesbert et al. (2006)
	Germany	-	PCR	75%	Kohn et al. (2009)
	Netherlands	-	Serology	73%	Landman and Feberwee (2001); Feberwee et al. (2008, 2009)
	Poland	16 provinces / 2010-2016	PCR	29%	Kursa et al. (2019)
	Turkey	Konya region / 2010	PCR Serology	25% 22.5%	Aras and Sayin (2014)
	UK	-	Serology	78.6%	Hagan et al. (2004)
Africa	*Algeria*	Eastern regions	Serology	26.7%	Aimeur et al. (2010)
	Ghana	Ga-East district, Accra region	Serology	75%	Matilda et al. (2018)
	Libya	Region of Al-Jabal Al-Gharbi	Serology	28%*§	Kammon et al. (2017)
	Morocco	Different regions	Serology PCR	100%**	Nassik et al. (2014)
	Tunisia	Region of Cap-Bon (6 districts)	Serology Culture	28.5% 9.5%	Boussetta et al. (1997)
Asia	*China*	21 provinces / 2010-2015	Serology	41.19%*	Xue et al. (2017)
	Pakistan	Different regions in Rawalpindi / 2016-2017	Serology	42.6%	Shoaib et al. (2019)
Oceania	*Australia*	Two provinces	Serology	69%	Gole et al. (2012)
America	*USA*	Southern and central California	Serology	32-91%	Mohammed et al. (1986)
	Brazil	Three States / 2001-2004	PCR	22.85%	Buim et al. (2009)

PCR: Polymerase Chain Reaction;
UK: United Kingdom; USA: United States of America
**different categories of poultry production; ** broiler breeder flocks; §MG and MS*

Currently, it is demonstrated that *M. synoviae* infection causes severe economic losses due to vertical transmission of the germ, resulting in death of embryo, consequently a decrease in hatch rate, significant post-hatch mortality, bacteria diffusion in the hatchery, and quality degradation of day-old chicks. Moreover, *M. synoviae* may induce transient immunosuppression, an increase in mortality of 1-4%, particularly in broiler chickens, a decrease of 5-10% in egg production rate, and a decrease of 5-7% in hatch rate (Mohammed et al., 1987; Stipkovits and Kempf 1996).

Mycoplasmosis due to *M. synoviae* occurred in layer hens flocks are associated to a decrease in the egg quality (Table 2). Egg-production level can decrease from 86% to 79% after three weeks in 54 weeks old layer hens (Jeon et al., 2014). Losses in infected hens are estimated at eight eggs per hen compared to healthy flocks (Mohammed et al., 1987). Additional losses are mentioned in affected flocks due to therapeutic and prophylactic means (Ferguson-Noel and Williams 2015). Similar reports are observed in layer hens flocks in Tunisia. Indeed, a decrease of 5-20% in eggs production is mentioned, according to the farms registers. The prevalence of *M. synoviae* infections in Tunisian poultry industry remains relatively high. Overall sero-prevalence of 19% (23 flocks / 63 visited) of all types of production in the Cap-Bon region (north-eastern of Tunisia) is mentioned (Boussetta et al., 1997). The prevalence in layer hens flocks is estimated to 28.5% (6 flocks / 21 visited). Currently, it appears that this prevalence is higher according to field findings, in the absence of official publications.

Table 2. Performance data for negative and positive *M. synoviae* layer flocks (Hussein, 2017)

	***M. synoviae* free**	***M. synoviae* positive**
Egg per hen housed	321	300
B-Grade percentage	2.87%	3.76%
FCR	2.36	2.47
Mortality	5%	12.60%
Point of lay pullet price	£3.90	£3.90
Price feed per ton	£ 210	£ 210

FCR: Feed conversion ratio

Positive layers do not achieve the optimal weight during the breeding phase allowing them to lay eggs period. Consequently, the production level, the eggs size and the eggshell quality are significantly affected later.

A new syndrome, called "Eggshell Apex Abnormality" (EAA), has been identified since 2000 in broiler-type breeders and layer hen's flocks. This new form is due to infection by certain strains of *M. synoviae* that multiply in the hen's genital tract. The presence of such eggs type in layer's flocks in Tunisia has also been reported in many integrations.

The upper part of the eggshell appears translucent, thin and very fragile, so it is easy to break. The demarcation is clear between the normal and the affected parts of the shell (Feberwee et al., 2009; Jeon et al., 2014). Eggshell discoloration was also observed in positive flocks, which further increasing the decommissioning rate of eggs. This rate may rise from 2.6% to 8.3% (Jeon et al., 2014). However, other causes, such as infectious bronchitis virus (IBV) and egg drop syndrome'76 virus (EDS'76) may induce more than 25% of egg quality degradation. Eggs from infected flocks are smaller, with a low commercial value. Because of possible eggshell cracks presence, interior components contamination is reported. The penetration of potential pathogens into affected eggs has been accompanied with the increase of embryo mortality (Hunton 2005).

TRANSMISSION

One of the ways in which poultry mycoplasma are disseminated is through egg transmission. This mode of transmission eased by the oviduct contamination is mainly observed for *M. meleagridis* and *M. iowae*. Whereas, the contamination of embryonated eggs with *M. gallisepticum* and *M. synoviae* is mainly due to the contiguity of the oviduct and contaminated air sacs (Kempf 1997; Dufour-Gesbert et al., 2006). Possible transmission via contaminated semen may also occur during artificial insemination in turkeys.

Transmission of *M. gallisepticum* and *M. synoviae* is mainly the consequence of the direct and close contact between animals, where bacteria may penetrate via respiratory and/or conjunctival pathways. Transmission can also occur through indirect contact, due to possible persistence of *Mycoplasma* for several days in the environment. The involvement of several types of animated (people, wild birds) and inanimate vectors (vehicles, food, water...) has been also established (Figure 1).

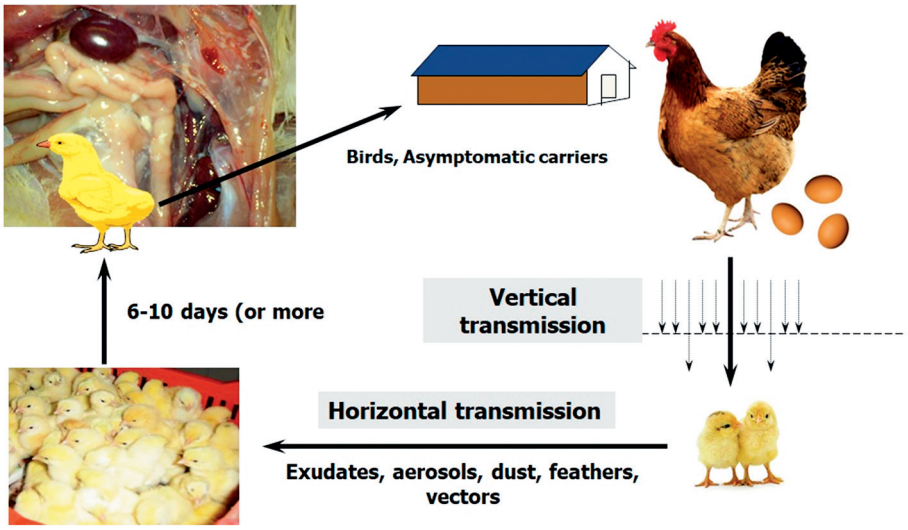

Figure 1. Transmission ways of *Mycoplasma synoviae* in poultry

Once infected, birds may be able to carry asymptomatically the bacteria even throughout the production period. Furthermore, because of the extension of poultry farms and the concentration of large integrations with multi-age flocks in restricted geographical areas, maintaining the free-status of flocks becomes very difficult.

PATHOGENICITY AND INTERACTION WITH OTHER PATHOGENS

M. synoviae is involved in several types of disorders: respiratory, articular and genital. In general, this bacteria causes subclinical respiratory infection (Kleven 2008). However, it may evolve progressively and will induce air sacculitis lesions, particularly when mycoplasma infection is exacerbated by other pathogens such as Newcastle disease virus (NDV), IBV and avian influenza virus (AIV), or when birds are infected with highly virulent *M. synoviae* strains (Lockaby et al., 1999; Santos et al., 2014; Umar et al., 2017). Experimental infection of breeder hens with virulent *M. synoviae* strain can induce EAA eggs production from the sixth day after a single intra-tracheal inoculation if preceded by an infection with IBV. In addition, the mean daily egg production per hen was significantly reduced by the *M. synoviae* EAA strain in SPF egg layers (Feberwee and Landman, 2010).

It is currently well established that mycoplasmas cause immunosuppression in infected birds. Indeed, these bacteria cause excessive release of pro-inflammatory cytokines, inhibit phagocytosis, and affect the B-cells and T-cells functions (Stipkovits et al., 2012).

Several escape mechanisms to the host immune response were described in mycoplasmas to explain the existence of chronic infections and some therapeutic failures. These include intracellular location, molecular mimicry (recognition of mycoplasmas surface epitopes as well as the self by the immune system) and antigenic variability (Bencina et al., 1994; Garcia et al., 1994; Kleven 1998; Nascimento et al., 2005).

In systemic infection, *M. synoviae* may cause articular disorders. The first cases of infectious synovitis have been described on broiler chickens since the 1950s and 1960s. While in layer hens, *M. synoviae* was found to be involved in lesions of ovaritis, salpingitis and peritonitis. Co-infection of layers with virulent strains of *M. synoviae* and *E. coli* increase significantly the mortality. Virulent MS strains can act as a complicating factor in the layer *E. coli* peritonitis syndrome (Raviv et al., 2007).

Moreover, *M. synoviae* vaccines may predispose poultry to severe viral and bacterial infections. Immunosuppressed layer hens have been shown high levels of viral particles in tissues caused by AIV, subtype H9N2 (Kwon et al., 2008; Umar et al., 2017). Similarly, immunosuppression can increase the susceptibility of hens to bacteria and viruses infections (Subler et al., 2006). More severe clinical signs and lesions following inoculation of chickens by *M. gallisepticum* and H3N8, compared to animals infected with *M. gallisepticum* or H3N8 alone are illustrated (Stipkovits et al., 2012).

DIAGNOSIS

Field diagnosis of *M. synoviae* infection is difficult because of the nonspecific clinical signs, lesions and the numerous similar diseases. That is why, laboratory investigations are very important to confirm clinical suspicion. Rapid detection of infection is demanded to prevent spread and reduce economic losses. Several direct and indirect diagnostic methods are available (Table 3).

Table 3. Comparative elements of the main diagnostic techniques of *M. synoviae* infections

	Method	Application domain	Detected element	SP / SE	Advantages	Disadvantages
Direct diagnosis	Bacteriology	Research	Colonies	SP +++ SE++	-Strain isolation -Depth study of strains -Antimicrobial susceptibility studies	-Low time limit: 2-3 weeks -Cost (identification method) -Culture media very complex -Sometimes, growth inhibition in favor to other bacteria
	PCR	-Diagnostic -Research	DNA	SP+++ SE+++	-Affordable cost -Transport of samples less restrictive -Rapid response	Detection of dead bacteria
Indirect diagnosis	RPA	Flock's Surveillance	IgM (IgG)	SP+/- SE++	-Early detection (7-10 days) -Low cost -Very rapid -Applicability in farms	-Test repetition needed -Risk of false-positives
	ELISA	-Surveillance -Diagnosis	IgG	SP+ SE++	-Facile use -Automatable -Rapid response	-Cost -Availability

DN : Deoxyribonucleic Acid; RPA: Rapid Plate Agglutination;
ELISA : Enzyme Linked ImmunoSorbent Assay;
Ig: Immunoglobulin; PCR : Polymerase Chain Reaction; SE : Sensitivity; SP : Specificity

The mycoplasma infection diagnosis has been based on three techniques for several decades: bacteria isolation and identification, detection of specific antibodies and detection of bacterial DNA (deoxyribonucleic acid) by PCR (Polymerase Chain Reaction) (Dufour-Zavala et al., 2008; OIE 2008; Qasem et al., 2015).

Serological investigations are widely used for preliminary diagnosis and screening. The most commonly used tests are rapid plate agglutination (RPA) and the immuno-enzymatic technique, ELISA (enzyme linked immunosorbent assay). The hemagglutination inhibition (HI) test is generally performed to confirm positive results (Gole et al., 2012; Khalifa et al., 2013; Michiels et al., 2015). Furthermore, positive serology must be verified by bacteria isolation and identification or by PCR, because of false-positive results and cross-reaction problems, which can be expected in about any serologic test (Feberwee et al., 2005; Heleili et al., 2012). Therefore, it is not advisable to rely completely on one diagnosis test only.

The ELISA test is used for the detection of specific antibodies against *M. gallisepticum* and *M. synoviae* in serum and egg yolk. This allows the evaluation of population immunity and transferred passive immunity (Hagan et al., 2004). Association between ELISA serological status for *M. synoviae* and egg quality parameters, such as translucency, shell breaking strength, percentage of shell reflectivity and shell deformation, has been established (Gole et al., 2012). Similarly, RPA can be applied to egg yolks, where the results are considered positive, negative and uncertain for antibody titles greater than or equal to 1:10, 1:5 and less than 1:5, respectively (Heleili et al., 2012; Nadeem et al., 2014).

The *Mycoplasma* culture is laborious, slow and expensive laboratory method, requiring sterile conditions. Problems with culture include the proliferation of faster-growing Mycoplasma species and the growth of other saprophytic organisms, such as *M. gallinarum* and *M. gallinaceum*. The detection of first colonies may take up to four weeks and, even in this case, negative culture or compromised result by mixed infections can be reported (Ferguson-Noel and Williams 2015).

Due to the disadvantages of conventional diagnosis techniques, the use of molecular methods is very helpful. The PCR, a rapid test, is characterized by high sensitivity and specificity. This technique allows the mycoplasma detection in clinical samples even from asymptomatic infected and/or treated birds (Evens et al., 2005; Peebles et al., 2014; Kursa et al., 2019). *M. synoviae* may be detected in different types of clinical samples issued from layer hens flock producing eggs with abnormal apex (Catania et al., 2010). Different variants of PCR are used for the detection of the four common mycoplasma species (*M. gallisepticum, M. synoviae, M. meleagridis* and *M. iowae*) in the same sample, including real-time PCR (Bagheri et al., 2011; Fraga et al., 2013). Furthermore, PCR has the advantage of detecting co-infections with numerous field and vaccine strains of respiratory pathogens (*M. synoviae*, *M. gallisepticum*, IBV, avian metapneumovirus) (Buim et al., 2009; Hutton et al., 2017; Ball et al., 2018; Fujisawa et al., 2019). The use of random amplification of polymorphic DNA to establish the DNA profile may be used for epidemiological investigations and/or for rapid identification of strains (Charlton et al., 1999; Aras and Sayin 2014).

CONTROL

Control of *Mycoplasma* infections is based in general, on three complementary approaches: biosecurity, antibiotics preventive treatments and vaccination. Due to the vertical transmission and the absence of walls of the germs, making them more fragile in the outside environment, control of *M. synoviae*

infections is theoretically easy. Voluntary programs of *M. synoviae* eradication are implemented in some countries, such as the United States and the United Kingdom. Similarly, because of the increase in *M. synoviae* infections prevalence in poultry farms, Germany started a mandated eradication control program in January 2013 in all types of production, excepted of broiler flocks (Feberwee et al., 2017).

The destruction of grandparents and *M. gallisepticum* confirmed positive breeders is a measure implemented in some countries, including the Netherlands. However, the control of *M. synoviae* is currently limited to the application of biosecurity programs, the screening of breeder's flocks and voluntary slaughter of infected grandparents in some countries. Vaccination against *M. synoviae* can be performed as an additional measure to reduce infection pressure in multi-age flocks of commercial layer hens.

The first defence barrier is the application of the all-in-all-out band, associated to good biosecurity and monitoring program. However, in a multi-age system, layers flocks are usually infected by *M. gallisepticum* and *M. synoviae* in most regions of the world, which presents a potential risk of infection transmission to broiler flocks.

The first step in controlling *Mycoplasma* infections is the acquisition of fertile eggs and *Mycoplasma*-free birds. Treatment of hatching eggs by heating (46°C for 12-14 hours), or more effectively, by injecting antibiotics, either by an in-ovo injector or by dipping into an antibiotic solution, are different methods used for the eradication of the infection in grandparents (Nascimento and Nascimento 1994; Stipkovits and Kempf 1996).

National certification programs have contributed to the control of Mycoplasma infections in many countries, such as the United States (USA 1997), Brazil (Villa, 1998) (in Nascimento et al., 2005) and France (Official Hygienic and Sanitary Control) (Stipkovits and Kempf 1996). Monitoring is carried out by serological methods such as SPA, ELISA and/or HI. In Tunisia, the control of *M. gallisepticum*, *M. synoviae* and *M. meleagridis* is also based on an OHSC using the SPA technique. *Mycoplasma* detection is frequently confirmed by PCR (Nascimento et al., 1994; Nascimento et al., 1998).

Mycoplasma monitoring is targeting breeding and commercial laying farms. Analyses are performed on a number of animals, chosen randomly, that vary according to the incidence of infection. Two samples are taken from breeding animals in Tunisia, between 10-12 weeks and between 20-24 weeks, on 2.5% of the total effective for *M. gallisepticum* and 5% for *M. synoviae.*

Antibiotic treatments can be administered in contaminated environments as a preventive measure, especially during stress period, or as part of a curative

treatment. The administration of antibiotic as preventive tool is very common in several countries, including Tunisia. Several antibiotic molecules are used; these include macrolides (tylosin, tylvalosin, tiamulin, tilmycosin), tetracyclines (oxytetcracycline, doxicycline) and aminosides (spectinomycin) (Bébéar and Kempf 2005); Kreizinger et al., 2017). Tetracyclines, due to their relatively low cost, are primary antibiotics in the treatment of avian mycoplasmosis.

The implemented programs vary widely across countries, regions and farms. However, the emergence of antimicrobial resistance in *M. synoviae* limits the use of this control approach. Therefore, the study of the sensitivity profiles of field-isolated strains to antibiotic molecules is a fundamental step towards improving the effectiveness of medical protocols. However, although the treatments reduce significantly clinical signs, mycoplasma may be further isolated after cessation of antibiotic administration, when animals are infected by resistant strains (Reinhardt et al., 2005; Carrou et al., 2006).

Vaccination against *M. synoviae* is performed especially in breeders and layers to prevent clinical signs and bacteria spread. Currently there are two commercialized live attenuated vaccines available against *M. synoviae*: the temperature sensitive MS-H vaccine strain and the NAD independent MS1 vaccine strain (Kreizinger et al., 2018). Interaction of these vaccines with other respiratory pathogens is documented. Indeed, *M. synoviae* vaccine strains can modify AIV replication and immune responses. Furthermore, live vaccines can act as a complicating factor during respiratory co-infection in layers, which may subsequently lead to vaccination strategies advance against poultry respiratory pathogens, in general (Umar et al., 2017).

The immunization of animals against *M. synoviae* must consider the following properties:

- It is shown that vaccination minimizes vertical and horizontal transmission of *M. synoviae*. Active immunization can prevent and/or reduce clinical signs but does not prevent colonization of internal organs by wild strains (Jones et al., 2006; Noormohammadi et al., 2007; Feberwee et al., 2009).
- Immunization may be accompanied by seroconversion, which must be assessed through a monitoring system that does not interfere with other official control programs (Markham et al., 1998; Feberwee et al., 2009). Thus, differentiation of vaccine strains from field isolates is essential during vaccination and eradication programs. It is essential to establish a Differentiating Infected from Vaccinated Animals (DIVA) system based on multi-technique approach (Dijkman et al., 2016; Moronato et al., 2018).

Differentiation between vaccine and wild strain was investigated using nested PCR. This technique allows the presence of an adenine in a nucleotide at position 468 of the oppF-1 gene of the vaccine strain of *M. synoviae*-H. Indeed, the above-mentioned authors show the exclusivity of this mutation in the vaccine strain, compared to wild *M. synoviae* strains isolated in Australia (Zhu et al., 2017). Recently, differentiation between *M. synoviae* vaccine and field strains was performed with indirect ELISA based on OppF-C gene (Kordafshari et al., 2019). Furthermore, a melt-curve and agarose gel-based mismatch amplification mutation assays (MAMA) was recently provided to discriminate the MS1 vaccine strain from the MS-H vaccine strain and wild-type *M. synoviae* isolates (Kreizinger et al., 2018). However, these assays are limited by the available facilities and the cost.

CONCLUSION

Despite the lack of official data on the incidence of *M. synoviae* and its economic impact in layers, it appears that this mycoplasma is gaining increasing interest around the world. Although *M. synoviae* cause usually a subclinical respiratory infection, it is responsible for several articular and genital disorders. Moreover, the interaction between field and vaccine strains of *M. synoviae* and other viral and bacterial infections increase usually the severity of clinical signs and lesions, and consequently the economic losses. In layer hens, *M. synoviae* infection has been accompanied since the beginning of 2000s by a new form, characterized by EAA eggs with a very fragile apex shell. Significant withdrawing rates for affected eggs, justifies the importance of implementing appropriate control measures. In this sense, control of *M. synoviae* infection in layers should be based on an integrated approach involving biosecurity, vaccination, and regular surveillance. These should be well performed to limit the problem of mycoplasmas persistence in infected flocks, especially in multi-age integrations, and minimize economic losses.

Authors' contributions

Authors contributed equally to this manuscript.

Competing interest

Authors declared no conflict of interests regarding the present paper.

REFERENCES

1. Aimeur R., Bouaziz O., Kabouia R., Bererhi E.H. 2010. Prévalence de Mycoplasma gallisepticum et de Mycoplasma synoviae dans les élevages avicoles de l'Est Algérien. Revue Médecine Vétérinaire, 161(3), 141-145.
2. Aras Z. and Sayin Z. 2014. Molecular Epidemiology of Mycoplasma synoviae Infection in Commercial Layers. Kafkas Univ Vet Fak Derg, 20 (1), 83-87. doi: 10.9775/kvfd.2013.9488.
3. Bagheri H., Doosti A., Arshi A. 2011. Detection of Mycoplasma gallisepticum in Chaharmahal Va Bakhtiari Province poultry using PCR. Global Veterinaria, 7, 54-59.
4. Ball C., Forrester A., Ganapathy K. 2018. Co-circulation of genetically diverse population of vaccine related and unrelated respiratory mycoplasmas and viruses in UK poultry flocks with health or production problems. Veterinary Microbiology, 225, 132-138. doi.org/10.1016/j.vetmic.2018.09.009
5. Bébéar C.M. and Kempf I. 2005. Antimicrobial therapy and antimicrobial resistance. In *Mycoplasmas. Molecular biology, pathogenicity and strategy for control*. Eds. A. Blanchard and G.F. Browning, Horizon Bioscience, Norfolk pp.535–568.
6. Bencina D., Kleven S.H., Elfaki M.G., Snoj A., Dovc P., Dorrer D., Russ I. 1994. Variable expression of epitopes on the surface of Mycoplasma gallisepticum demonstrated with monoclonal antibodies. Avian Pathology, 23, 19-36.
7. Boussetta M., Chaouachi N., Mlik B. 1997. Etude sérologique et bactériologique des mycoplasmoses aviaires dans la région du Cap Bon en Tunisie. Revue Élev Méd vét Pays trop, 50, 93-96.
8. Buim M.R., Mettifogo E., Timenetsky J., Kleven S., Piantino Ferreira A.J. 2009. Epidemiological survey on Mycoplasma gallisepticum and M. synoviae by multiplex PCR in commercial poultry. Pesq Vet Bras, 29(7), 552-556.
9. Catania S., Bilato D., Gobbo F., Granato A., Terregino C., Iob L., Nicholas R.A. 2010. Treatment of eggshell abnormalities and reduced egg production caused by Mycoplasma synoviae infection. Avian Dis, 54, 961-964.
10. Dijkman R., Feberwee A., Landman W.J.M. 2017. Development, validation and field evaluation of a quantitative real-time PCR able to differentiate between field Mycoplasma synoviae and the MS-H-live vaccine strain. Avian Pathology, 46, 403-415. doi: 10.1080/03079457.2017.1296105
11. Dufour-Gesbert F., Dheilly A., Marois C., Kempf I. 2006. Epidemiological study on Mycoplasma synoviae infection in layers. Veterinary Microbiology, 114 (1-2), 148-154. doi.org/10.1016/j.vetmic.2005.10.040
12. Dufour-Zavala L., Swayne D.E., Glisson J.R., Pearson J.E., Reed W.M., Jackwood M.W., Woolcock P.R. 2008. A laboratory manual for the isolati-

on, identification and characterization of avian pathogens. 5th eds. American Association of Avian Pathologists, Athens, GA. pp. 59-64.
13. Evans J.D., Leigh S.A., Branton S.L., Collier S.D., Pharr G.T., Bearson S.M.D. 2005. Mycoplasma gallisepticum: Current and developing means to control the Avian pathogen. Journal of Applied Poultry Research, 14, 757-763.
14. Feberwee A. and Landman W.J., 2010. Induction of eggshell apex abnormalities in broiler breeder hens. Avian Pathology, 39(2), 133-137. doi: 10.1080/03079451003657637.
15. Feberwee A., de Vries T., Landman W.J.M. 2008. Seroprevalence of Mycoplasma synoviae in Dutch commercial poultry farms. Avian Pathology, 37 (06), 629-633.
16. Feberwee A., de Wit J.J., Landman W.J.M. 2007. Mycoplasma synoviae-associated eggshell apex abnormalities. In *Proceedings*, 15th World Veterinary Poultry Congress, Beijing, China p. 234.
17. Feberwee A., de Wit J.J., Landman W.J.M. 2009. Induction of eggshell apex abnormalities by Mycoplasma synoviae: field and experimental studies. Avian Pathology, 38, 77-85.
18. Feberwee A., Dijkman R., Klinkenberg D., Landman W.J.M. 2017. Quantification of the horizontal transmission of Mycoplasma synoviae in non-vaccinated and M. Synoviae-H vaccinated layers. Avian Pathology, 46, 346-358, doi: 10.1080/03079457.2017.1282602.
19. Feberwee A., Mekkes D.R., de Wit J.J., Hartman E.G., Pijpers A. 2005. Comparison of culture, PCR, and different serologic tests for detection of Mycoplasma gallisepticum and Mycoplasma synoviae infections. Avian Diseases, 49(2), 260-268.
20. Ferguson-Noel N.M. and Williams S.M. 2015. The efficacy of Mycoplasma gallisepticum K-strain live vaccine in broiler and layer chickens. Avian Pathology, 44, 75-80.
21. Fraga A.P., De Vargas T., Ikuta N., Fonseca A.S., Celmer Á.J., Marques E.K., Lunge V.R. 2013. A Multiplex real-time PCR for detection of Mycoplasma gallisepticum and Mycoplasma synoviae in clinical samples from Brazilian commercial poultry flocks. Brazilian Journal of Microbiology, 44, 505-510.
22. Fujisawa S., Murata S., Takehara M., Katakura K., Hmoon M.M., Win S.Y., Ohashi K. 2019. Molecular detection and genetic characterization of Mycoplasma gallisepticum, Mycoplama synoviae, and infectious bronchitis virus in poultry in Myanmar. BMC Veterinary Research, 15, 261. doi. org/10.1186/s12917-019-2018-2.
23. Garcia M., Elfaki M.G., Kleven S.H. 1994. Analysis of the variability in expression of Mycoplasma gallisepticum surface antigens. Veterinary Microbiology, 42, 147-158.

24. Gole V.C., Chousalkar K.K., Roberts J.R. 2012. Prevalence of antibodies to Mycoplasma synoviae in laying hens and possible effects on eggshell quality. Preventive Veterinary Medicine, 106(1), 75-78. doi: 10.1016/j.prevetmed.2012.02.018.
25. Hagan J.C., Ashton N.J., Bradbury J.M., Morgan K.L. 2004. Evaluation of an egg yolk enzyme-linked immunosorbent assay antibody test and its use to assess the prevalence of Mycoplasma synoviae in UK laying hens. Avian Pathology, 33(1), 93-97.
26. Heleili N., Ayachi A., Mamache M., Chelihi A. 2012. Seroprevalence of Mycoplasma synoviae and Mycoplasma gallisepticum at Batna commercial poultry farms in Algeria. Veterinary World, 5, 709-712.
27. Hunton P. 2005. Research on eggshell structure and quality: an historical overview. Brazilian Journal Poultry Science, 7(2), 67-71.
28. Hussein K. 2017. True cost of Mycoplasma synoviae in commercial layers. Poultry World, May 22 (https://www.poultryworld.net/Health/Articles/2017/5/True-cost-of-Mycoplasma-synoviae-in-commercial-layers-135827E/)
29. Hutton S., Bettridge J., Christley R., Habte T., Ganapathy K. 2017. Detection of infectious bronchitis virus 793B, avian metapneumovirus, Mycoplasma gallisepticum and Mycoplasma synoviae in poultry in Ethiopia. Trop Anim Health Prod, 49, 317-322. DOI 10.1007/s11250-016-1195-2.
30. Jeon E.O., Kim J.N., Lee H.R., Koo B.S., Min K.C., Han M.S., Lee S.B., Bae Y.J., Mo J.S., Cho S.H., Lee C.H., Mo I.P. 2014. Eggshell apex abnormalities associated with Mycoplasma synoviae infection in layers. Journal Veterinary Science, 15(4), 579-582.
31. Jones J.F., Whithear K.G., Scott P.C., Noormohammadi A.H. 2006. Duration of immunity with Mycoplasma synoviae: comparison of the live attenuated vaccine MS-H (Vaxsafe MS) with its wild-type parent strain, 86079/7NS. Avian Diseases, 50, 228-231.
32. Kammon A., Mulatti P., Lorenzetto M., Ferre N., Sharif M., Eldaghayes I., Dayhum A. 2017. Biosecurity and geospatial analysis of mycoplasma infections in poultry farms at Al-Jabal Al-Gharbi region of Libya. Open Veterinary Journal, 7(2), 81-85. DOI: 10.4314/ovj.v7i2.1
33. Kempf I. 1997. Les mycoplasmoses aviaires. Le Point Vétérinaire, 28(182), 41-48.
34. Khalifa K.A., Abdelrahim E.S., Badwi M., Mohamed A.M. 2013; Isolation and molecular characterisation of Mycoplasma gallisepticum and Mycoplasma synoviae in chickens in Sudan. Journal of Veterinary Medicine, Article ID 208026, doi:10.1155/2013/208026.

35. Kleven S.H. 1998. Mycoplasmas in the etiology of multifactorial respiratory disease. Poultry science, 77, 1146-1149.
36. Kleven S.H. 2008. Control of avian mycoplasma infections in commercial poultry. Avian Diseases, 52, 367-374.
37. Köhn S., Spergser J., Ahlers C., Voss M., Bartels T., Rosengarten R., Krautwald-Junghanns M.E. 2009. Prevalence of Mycoplasmas in commercial layer flocks during laying period. Berl Munch Tierarztl Wochenschr, 122(5-6), 186-192.
38. Kordafshari S., Marenda M.S., O'Rourke D., Shil P., Noormohammadi A.H. 2019. Mutation of oppF gene in the Mycoplasma synoviae MS-H vaccine strain and its implication for differential serological responses to vaccination versus field challenge. Veterinary Microbiology, 231, 48-55. doi: 10.1016/j.vetmic.2019.02.029.
39. Kreizinger Z., Grózner D., Sulyok K.M., Nilsson K., Hrivnák V., Benčina D., Gyuranecz M. 2017. Antibiotic susceptibility profiles of Mycoplasma synoviae strains originating from Central and Eastern Europe. BMC Veterinary Research, 13, 342. doi 10.1186/s12917-017-1266-2.
40. Kreizinger Z., Sulyok K.M., Grózner D., Bekő K., Dán Á., Szabó Z., Gyuranecz M. 2018. Development of mismatch amplification mutation assays for the differentiation of MS1 vaccine strain from wild-type Mycoplasma synoviae and MS-H vaccine strains. PLoS One, 12(4), e0175969. doi: 10.1371/journal.pone.0175969.
41. Kursa O., Tomczyk G., Sawicka A. 2019. Prevalence and phylogenetic analysis of Mycoplasma Synoviae strains isolated from Polish chicken layer flocks. Journal Veterinary Research, 63(1), 41-49. doi: 10.2478/jvetres-2019-0010
42. Kwon J-S., Lee H-J., Lee D-H., Lee Y-J., Mo I-P., Nahm S-S., Kim M-J., Lee J-B., Park S-Y., Choi I-S., Song C-S. 2008. Immune responses and pathogenesis in immunocompromised chickens in response to infection with the H9N2 low pathogenic avian influenza virus. Virus Research, 133, 187-194.
43. Landman W.J.M. and Feberwee A. 2001. Field studies on the association between amyloid arthropathy and Mycoplasma synoviae infection, and experimental reproduction of the condition in brown layers. Avian Pathology, 30, 629-639.
44. Landman W.J.M. and Feberwee A. 2004. Aerosol-induced Mycoplasma synoviae arthritis: the synergistic effect of infectious bronchitis virus infection. Avian Pathology, 33, 591-598.
45. Le Carrou J., Reinhardt A.K., Kempf I., Gautier-Bouchardon A.V. 2006. Persistence of Mycoplasma synoviae in hens after two enrofloxacin treatments and detection of mutations in the par C gene. Vet Res, 37, 1-24.

46. Lockaby S.B., Hoerr F.J., Lauerman L.H., Smith B.F., Samoylov A.M., Toivio-Kinnucan M.A., Kleven S.H. 1999. Factors associated with virulence of Mycoplasma synoviae. Avian Diseases, 43, 251-261.
47. Markham P.F., Glew M.D., Browning G.F., Whithear K.G., Walker I.D. 1998. Expression of two members of the pMGA gene family of Mycoplasma gallisepticum oscillates and is influenced by -specific antibodies. Infect Immun, 66, 2845-2853.
48. Matilda A.A., Linda A.A., Cornelius A., Korley K.N., Paa T.A. 2018. Activity of Mycoplasma synoviae in Commercial Chickens in Ghana: Serological Evidence. Advances in Life Science and Technology, 62.
49. Michiels T., Welby S., Vanrobaeys M., Quinet C., Rouffaer L., Lens L., Martel A., Butaye P. 2015. Prevalence of Mycoplasma gallisepticum and Mycoplasma synoviae in commercial poultry, racing pigeons and wild birds in Belgium. Avian Pathology, 45, 244-252. doi.org/10.1080/03079457.2016.1145354.
50. Mohammed H.O., Carpenter T.E., Yamamoto R. 1987. Economic impact of Mycoplasma gallisepticum and M. synoviae in commercial layer flocks. Avian Diseases, 3, 477-482.
51. Mohammed H.O., Carpenter T.E., Yamamoto R., McMartin D.A. 1986. Prevalence of Mycoplasma gallisepticum and M. synoviae in commercial layers in southern and central California. Avian Diseases, 30, 519-526.
52. Moronato M.L., Cecchinato M., Facchetti G., Mainenti M., Gobbo F., Catania S. 2018. Application of different laboratory techniques to monitor the behaviour of a Mycoplasma synoviae vaccine (MS-H) in broiler breeders. BMC Veterinary Research, 14(1), 357. doi: 10.1186/s12917-018-1669-8.
53. Nadeem M., Yousaf A., Iqbal Z., Awais M.M., Pervez B.A. 2014. Prevalence, diagnosis and treatment of mycoplasmosis in game birds. World's Poultry Science Journal, 70, 69-80.
54. Nascimento E.R. and Nascimento M.G.F. 1994. Eradication of Mycoplasma gallisepticum and M. synoviae from a chicken flock in Brasil In: The 3th Western Poultry Disease Conference; Sacramento, Califórnia, USA. p.58.
55. Nascimento E.R., Nascimento M.G.F., Danelli M.G.M., Machado S.L., Lignon G.B., Polo P.A. 1998. Comparison of PCR kits for the detection of Mycoplasma gallisepticum and M. synoviae (MS) in MS infected and uninfected chickens. In: *Proceedings*, 47th Western Poultry sease Conference; Sacramento, Califórnia, USA. p84-86.
56. Nascimento E.R., Pereira V.L.A., Nascimento M.G.F., Barreto M.L. 2005. Avian Mycoplasmosis update. Brazilian Journal of Poultry Science, 7, 01-09.
57. Nassik S., Aboukhalid R., Azzam F., Rahmatallah N., Lahlou-Amine I., Fassi-Fihri O., El Houadfi M. 2014. Detection of Mycoplasma synoviae In-

fection in Broiler Breeder Farms of Morocco Using Serological Assays and Real Time PCR. Journal of Life Sciences, 8, 815-821. doi: 10.17265/1934-7391/2014.10.004

58. Noormohammadi A.H., Hemmatzadeh F., Whithear K.G. 2007. Safety and efficacy of the Mycoplasma synoviae MS-H Vaccine in Turkeys. Avian Diseases, 51, 550-554.
59. Organisation Mondiale de la Santé Animale (OIE) 2008. Avian mycoplasmosis (Mycoplasma gallisepticum, Mycoplasma synoviae). In: Manual of diagnostic tests and vaccines for terrestrial animals pp. 525-541.
60. Peebles E.D., Jacob R., Branton S.L., Gerard P.D. 2014. Effects of Mycoplasma gallisepticum vaccination on serum α1-acid glycoprotein concentrations in commercial layer chickens. Poultry Science, 93, 1396-402.
61. Purswell J.L., Evans J.D., Branton S.L. 2011. Serologic response of roosters to gradient dosage levels of a commercially available live F strain-derived Mycoplasma gallisepticum vaccine over time. Avian Diseases, 55, 490-494.
62. Qasem J.A., Al-Mouqati S.A., Al-Ali E.M., Ben-Haji A. 2015. Application of molecular and serological methods for rapid detection of Mycoplasma gallisepticum Infection (Avian mycoplasmosis). Pakistan Journal of Biological Sciences, 18, 81-87.
63. Raviv Z., Ferguson-Noel N., Laibinis V., Wooten R., Kleven S.H. 2007. Role of Mycoplasma synoviae in Commercial Layer Escherichia coli Peritonitis Syndrome. Avian Diseases, 51(3), 685-690.
64. Reinhardt A.K., Gautier-Bouchardon A.V., Gicquel-Bruneau M., Kobisch M., Kempf I. 2005. Persistence of Mycoplasma gallisepticum in chickens after treatment with enrofloxacin without development of resistance. Vet Microbiol, 106, 129-137.
65. Santos F.C., Brandão M.D.M., Silva C.C., Machado L.S., Soares M.V., Barreto M.L., Nascimento E.R., Pereira V.L.A. 2014. Eggshell apex abnormalities in a free-range hen farm with Mycoplasma synoviae and infectious bronchitis virus in Rio de Janeiro state, Brazil. Brazilian Journal of Poultry Science, 16: 101-104.
66. Shoaib M., Riaz A., ul Hassan M., Yousaf A., Ur Rehman S., Zafar M.A., Kamran M., Amir R.M., Malik A.M. 2019. Sero-Prevalence and Associated Risk Factors of Mycoplasma Gallisepticum, Mycoplasma Synoviae and Salmonella Pullorum / Gallinarium in poultry. Pakistan Veterinary Journal, in press. doi: 10.29261/pakvetj/2019.097
67. Sprygin A.V., Elatkin N.P., Kolotilov A.N., Volkov M.S., Sorokina M.I., Borisova A.V., Andreychuk D.B., Mudrak N.S., Irza V.N., Borisov A.V., Drygin V.V. 2011. Biological characterisation of Russian Mycoplasma gallisepticum field isolates. Avian Pathology, 40, 213-219.

68. Stipkovits L. and Kempf I. 1996. Mycoplasmoses in poultry. Revue Scientifique et Technique, Office International des Epizooties, 15, 1495-525.
69. Stipkovits L., Glavits R., Palfi V., Beres A., Egyed L., Denes B., Somogyi M., Szathmary S. 2012. Pathologic lesions caused by coinfection of Mycoplasma gallisepticum and H3N8 low pathogenic avian influenza virus in chickens. Veterinary Pathology, 49,273-283.
70. Subler K.A., Mickael C.S., Jackwood D.J. 2006. Infectious bursal disease virus-induced immunosuppression exacerbates Campylobacter jejuni colonization and shedding in chickens. Avian Diseases, 50, 179-184.
71. Umar S., Tanweer M., Iqbal M., Shahzad A., Hassan F., Usman M., Sarwar F., Qadir H., Asif S., Nisa Q., Younus M., Ali A., Akbar M., Towakal F., Shah M.A. 2017. Mycoplasma synoviae vaccine modifies virus shedding and immune responses of avian influenza (H9N2) infection in commercial layers. Poultry Science, 96, 3086-3095. doi.org/10.3382/ps/pex149
72. Van Beek P., Feberwee A., Fabri T., Heijmans M.J.H.M. 2002. Longitudinal field study on the presence of Mycoplasma synoviae in meat-turkey flocks with arthritis. In *Proceedings,* 4th International Symposium on Turkey Diseases, Institute of Poultry Diseases, Free University of Berlin, Berlin, Germany, pp.177-178.
73. Xue J., Xu M.Y., Ma Z.J., Zhao J., Jin N., Zhang G.Z. 2017. Serological investigation of Mycoplasma synoviae infection in China from 2010 to 2015. Poultry Science, 96, 3109-3112. doi: 10.3382/ps/pex134
74. Zhu L., Konsak B.M., Olagoun O.M., Agnew-Crumptona R., Kanci A., Marenda M.S., Browning G.F., Noormohammadi A.H. 2017. Identification of a new genetic marker in Mycoplasma synoviae vaccine strain MS-H and development of a strategy using polymerase chain reaction and high-resolution melting curve analysis for differentiating MS-H from field strains. Veterinary Microbiology, 210, 49-55. doi.org/10.1016/j.vetmic.2017.08.021

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CHEMICAL COMPOSITION AND MICROBIAL SAFETY OF PORK MEAT PRODUCTS ORIGINATING FROM HERZEGOVINA

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Abstract

This paper presents the results of chemical composition and microbiological safety testing of dry cured meat products, fermented and semi-dry or pasteurized sausages produced by several meat industries from Herzegovina. In the period from 2016 to 2018, a total of 85 meat products were sampled. These included 20 samples of fermented pork sausages, 10 samples of dry-cured pork loin, 5 samples of dry-cured pork neck and 50 samples of heat-treated sausages. Quality control testing of the samples included determination of meat protein, crude fat, moisture, sodium nitrite and polyphosphates content in sausages, using standard analytical methods. It was confirmed that all analysed samples meet the requirements in terms of chemical composition, level of additives and microbiological safety. In comparison with dry cured meat products and sausages analyses results, with minimum requirements that are set forth in regulations for that product category. The above mentioned suggests that the analysed samples are good quality products and they are in compliance with the regulations.

Keywords: dry cured products, sausages, chemical composition, microbiological safety

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HEMIJSKI SASTAV I MIKROBIOLOŠKA BEZBEDNOST PROIZVODA OD MESA SVINJA POREKLOM IZ HERCEGOVINE

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Kratak sadržaj

U ovom radu prikazani su rezultati ispitivanja kemijskog sastava i mikrobiološke ispravnosti trajnih suhomesnatih proizvoda, fermentiranih i toplinski obrađenih kobasica podrijetlom iz više mesnih industrija sa područja Hercegovine. U razdoblju od 2016. do 2018. ukupno je uzorkovano 85 mesnih proizvoda, od toga 20 uzoraka fermentiranih suhih trajnih kobasica od svinjskog mesa, 10 uzoraka svinjske pečenice, 5 uzoraka suhog svinjskog vrata - buđole i 50 uzoraka toplinski obrađenih kobasica. U cilju ispitivanja kakvoće, u uzorcima trajnih suhomesnatih proizvoda i kobasica određena je količina bjelančevina, sirovih masti, vode, te aditiva natrijevog nitrita i polifosfata primjenom standardnih analitičkih metoda. Usporedbom rezultata analiza uzoraka s zakonski propisanim uvjetima, utvrđeno je da svi analizirani proizvodi udovoljavaju propisanim uvjetima, u pogledu kemijskog sastava i mikrobiološke ispravnosti. Svi uzorci kobasica udovoljavali su zahtjevima Pravilnika, u pogledu sadržaja aditiva. Iz svega navedenog možemo zaključiti da su analizirani uzorci proizvodi dobre kvalitete i odgovaraju propisanim zakonskim normativima.

Ključne riječi: trajni proizvodi, kobasice, kemijski sastav, mikrobiološka ispravnost

INTRODUCTION

Food legislation lays down general principles and requirements for meat products and ensures that only safe products can be placed on the market.

According to current regulations, the quality of foodstuffs including meat products is determined according to their chemical composition and product specification, i.e., specification sheet. Production process and quality of food products is systematically addressed by relevant legislative and inspection authorities, but it is also in the spotlight of consumers. Increased health awareness and knowledge about food-related risks strongly affects current consumer demand trends. An accurate insurance of the safety of food products requires determination of product composition by application of appropriate chemical and microbiological analyses (Aćimović et al., 2014).

Dry cured meat products are common traditional foods, which have been produced and consumed worldwide over the centuries. They contain a variety of flavours and textures and are considered an important segment of local economy and an essential part of the cultural and gastronomic heritage. Technology of production of dry cured products, regardless of the type, involves salting, drying and ripening procedures (Krvavica et al., 2012). However, the quality of these products relies upon the selection of raw materials and processing technologies. Knowledge and understanding of highly complex chemical reactions in the tissues facilitate improved control of the processing system and the quality of finished products as well. Cured meat products are widely accepted and popular among consumers. However, the adverse effects of dry cured meat products and sausages such as high contents of salt, saturated fats and cholesterol, etc. must not be neglected (Popelka, 2016; Čop, 2016). Traditional manufacturing process might be associated with certain risks related to environmental conditions, hygiene, animal health, welfare on the farms, etc. (Oiki et al., 2016). Sausages are produced by stuffing selected ground mixture (meat, fat tissue, skin, offal, connective tissue and other ingredients) into natural or artificial casings (Kovačević, 2001). Dry fermented sausages are considered products of highest quality. In the region of Herzegovina, such sausages are commonly produced from pork meat, sometimes mixed with certain amount of beef, which undergo fermentation, smoking and drying process. Fermented dry sausages from Bosnia and Herzegovina are usually put on the market under commercial names "kulen", "zimska salama", "srijemska kobasica", "sudžuk" and "čajna kobasica" and a wide range of similar products is also available. According to the Regulation for minced meat, semi-finished meat products and meat products (Anonymous 2013 a,b,; Anonymous 2017) heat-treated sausages are defined as meat products manufactured from the mixture of minced meat, mechanically deboned meat, fat and adipose tissue, offal, blood and blood products of different mincing degree and additional non-meat ingredients. A portion of filling for heat-treated sausages, i.e. meat batter

or meat emulsion, is shaped into natural or artificial casing, pouch, mold, and subjected to heat treatment - pasteurization with/without smoking or boiling and sterilization. In Bosnia and Herzegovina, heat-treated sausages are marketed as finely minced or roughly minced boiled semi-durable sausages, whole-cut meat sausages or boiled and other heat-treated sausages.

As there is lack of relevant information on meat products manufactured in B&H, the aim of this research was to identify basic nutritional parameters and microbial safety of nine different types of semi-dry and dry meat products manufactured in several meat factories from the region of western Herzegovina and sampled over a three-year period. An overview of the results of analyses of chemical composition and microbial safety of finished products from the group of fermented dry sausages, dry cured meat products and heat-treated sausages (finely minced boiled, semi-dry, whole-cut meat sausages) is presented. For each sample, the contents of proteins, crude fat and water were determined and the microbial population were determined. Quantification of sodium nitrite and polyphosphates was performed for all the samples from the category of dry fermented and heat-treated sausages. The obtained results were compared with the reference values laid down in the national regulations with the aim to evaluate the quality and microbial safety of meat products as well as to determinate the content of nitrites and polyphosphates (Anonymous 2013a,b,c, 2016, Anonymous 2017; Anonymous 2018a,b).

MATERIAL AND METHODS

This research was conducted on 85 samples of semi-dry and dry processed meat products manufactured by several meat companies. According to the classification laid down by the Regulations (Anonymous 2013a and 2017), the samples were categorized into five categories – 20 samples of fermented dry sausages ("čajna kobasica" and "zimska salama"), 15 samples of dry cured meat products (dry-cured pork tenderloin and dry-cured pork collar – "budjola"), 10 samples of heat-treated sausages (finely minced cooked sausages – frankfurter), 20 samples of roughly minced cooked sausages ("kranjska kobasica" and "narodna kobasica") and 20 samples of sausages made of whole cuts of meat ("kuhana šunka" and "pica šunka"). The sampling was performed randomly over a 3-year period (2016-2018) in the territory of western Herzegovina – municipalities of Široki Brijeg, Ljubuški, Grude, Čitluk and Posušje as well as in the city of Mostar.

Sampling and sample preparation were conducted according to the standard procedure (ISO 3100-1:1991). All analyses were performed in the labora-

tories of the Public Institution "Veterinary Institute Bihać" applying accredited methodologies. Chemical quality of the samples was determined by quantifying total lipid contents (BAS ISO 1443:2007), moisture contents (BAS ISO 1442:2007) and total protein contents (BAS ISO 937:2007). In sausage samples, nitrite (BAS ISO 2918:2007) and polyphosphate (BAS ISO 13730:2008) contents were determined. Microbial safety of samples was determined by standard BAS ISO methods for detection and/or enumeration: *Salmonella* spp. (BAS EN ISO 6579-1:2018), *E. coli* (BAS ISO 16649-1:2019), *Listeria monocytogenes* applying (BAS EN ISO 11290-2:2018); *Enterobacteriaceae* (BAS ISO 21528-2:2013); sulphitoreducing clostridia (BAS ISO 15213:2008); *Staphylococcus aureus* (BAS EN ISO 6888-1:2008); the counts of aerobic colonies applying (BAS EN ISO 4883-1:2014).

Statistical data analyses

The results are presented as a mean value± standard deviation. Descriptive statistics (minimum, maximum, mean value, standard deviation, variance, modus and median) was calculated for all analysed variables. The data were processed using Microsoft Office Excel 2016 (Microsoft, SAD) software package.

RESULTS

In dry cured products, water content varied between 29.0% in dry pork collar and 52.1% in dry-cured pork tenderloin. Total fat content ranged from minimum value in pork tenderloin (15.5%) to a maximum of 45.0% in dry-cured pork collar. The highest average protein content (21.8%) was determined in a dry-cured pork collar "budjola" (Figures 1 and 2). In dry fermented sausages, the highest protein and fat contents were determined in "zimska slama", amounting29.7% and 43.4%, respectively. High water content (27.7%) was found in "čajna kobasica". The highest protein and fat contents in the group of semi-dry sausages were determined in sausages "narodna kobasica" and "kranjska kobasica" amounting 28.6% and 53.3%, respectively. Water content ranged between 24.3% in "kranjska kobasica" and 29.8% in "narodna kobasica". The average contents of proteins, fat and water in frankfurter sausage were 17.6%, 24.1% and 58.3%, respectively. The analyses of sausages made of whole cuts of meat revealed an average protein content of 13.2% in cooked ham and 14.7% in „pizza šunka", whereas lower fat content (9.0%) was determined in „pizza šunka" and higher moisture content (73.3%) was found in cooked ham.

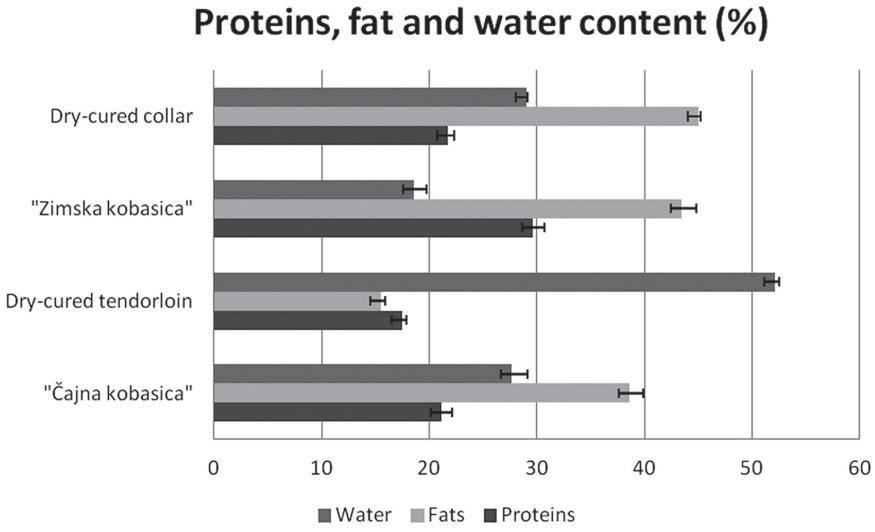

Figure 1. Mean values (±SD) of protein, fat and moisture determined in the dry cured products

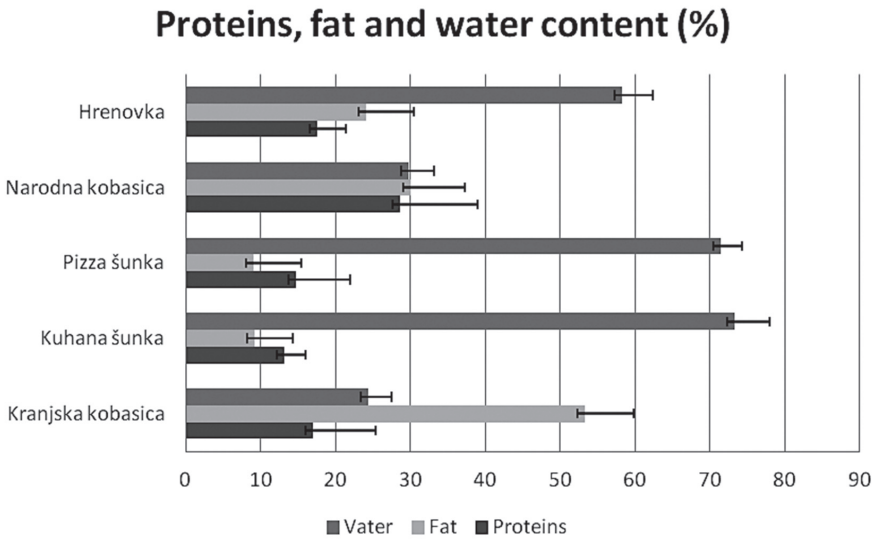

Figure 2. Mean values (±SD) of protein, fat and water determined in the semi-dry and pasteurised sausages

Food additives are used in meat industry with the aim to improve sensory properties of food, enhance processing procedures or extend durability of the products, results are presented in Table 1. These results revealed high variation of sodium nitrite concentration between dry sausages and heat-treated sausages comparing to low variation between polyphosphates content.

Table 1. Mean values (±SD) of sodium nitrite and polyphosphates content

Product type	Sodium nitrite mg kg^{-1}	Polyphosphates g kg^{-1}
Čajna kobasica	4.9 ± 4.5	3.0 ± 0.6
Zimska salama	5.7 ± 4.3	3.6 ± 0.4
Kranjska kobasica	40.9 ± 14.9	2,1 ± 0.2
Narodna kobasica	42.3 ± 15.6	3.4 ± 0.4
Frankfurter	29.2 ± 8.4	4.2 ± 0.6
Kuvana šunka	37.3 ± 19.6	2.8 ± 0.7
Piza šunka	31.8 ± 17.3	3.2 ± 0.7

Our research did not reveal the presence of food borne pathogens *Salmonella* spp., *L. monocytogenes* in any of the analysed samples. The indicators of process hygiene - total counts of aerobic mesophilic bacteria, *Enterobacteriaceae*, sulphitoreducing clostridia, *E. coli* and *Staphylococcus aureus* and other coagulase positive staphylococci were less than 10 colony forming units per 1g products, which is in compliance with the minimum requirements laid down in the Regulation.

DISCUSSION

Chemical composition of meat products from Herzegovina is similar to industrial products from neighbouring countries, where similar processing technologies are applied (Croatia, Slovenia and Serbia). The water, protein and fat content in dry cured products is typical and comparable with the results for similar types of products from Bosnia and Herzegovina (B&H) and Croatia (Pleadin et al., 2013; Pleadin et al., 2017). The fat content in dry fermented sausages in our research is similar with contents in sausages from Croatia and Serbia (38-41%) (Pleadin et al., 2009; Pleadin et al., 2013; Dučić et al., 2018), but it is lower than average fat content (40-50%) in similar, industrialy produced sausages from Spain (Olivares et al., 2010). The average protein content is similar with contents in sausages from Croatia and Serbia (26.2 - 47.9%) (Pleadin et al., 2009; Pleadin et al., 2013; Dučić et al., 2018). Fat and protein content in

industrial products in our research is higher than content in traditional homemade product from Croatia (15.4% and 19.5%, respectively) (Kozačinski et al., 2008). Pleadin et al., (2009; 2017) reported somewhat lower contents of proteins (15.1%) and fat (24.6%) for semi-dry sausages from B&H, which could be attributed to different recipes used by specific manufacturers (the amount of added bacon, the use of more or less lean meat). The results of this study for sausages made of whole cuts of meat correspond with previously reported results (Pleadin et al., 2009) for similar heat-treated sausages from Croatia.

All the analysed products were in accordance with requirements of the Regulation No. 82/13 (Anonymous 2013a) with regard to their chemical composition. The results of this research suggest that dry sausages are products of highest quality and commercial value due to their ingredients and nutritive value, i.e. the selection of best-quality meat and long ripening period. Compared to semi-dry and cooked sausages, dry products are characterized by lower moisture content.

This research revealed low concentration of sodium nitrite and polyphosphates. Kovačević et al. (2016) and Pleadin et al. (2009) reported somewhat higher values for sodium nitrite (42.0 mg kg-1 and 44.8 mg kg-1) for similar products from the category of heat-treated sausages available on Croatian market. Kovačević et al. (2016), Pleadin et al. (2009) and Dučić et al. (2018) reported similar results (7.0 mg kg-1, 5.4 mg kg-1 and 7.1-9.1 mg kg-1) for dry sausages from Croatia and Serbia. These vallues are within values recommended for stability during the storage period (5-15 mg kg-1) (Sindelar and Milkowski 2011). All analysed samples were in compliance with the requirements of the Regulation No 33/18 (Anonymous 2018a) agreed with the European Commission (EC) Regulation No. 1333/2008 (EC, 2008). According to this Regulation, a maximum permitted level for sodium nitrite (mg kg-1) in dry-fermented sausages is 50 mg kg-1, 100 mg kg-1 for heat-treated sausages, 50 mg kg-1 for dry-cured meat products and 100 mg kg-1 for semi- dry-cured meat products. Maximum permissible level for polyphosphates is 5 g kg-1 for all meat products.

From a public health perspective, fermented and heat-treated meat products can be analysed for their technological, biochemical, toxicological, nutritive and other properties. Complex production process and diverse health aspects of fermented meat products require a multidisciplinary approach enabling an insight into potential risks and control thereof (Zdolec, 2016). Meat and meat products are highly suitable medium for microbial growth. Thus, meat products can contain organisms that are responsible for desirable specific taste of fermented products (sensory properties), harmful agents that can

cause undesired changes of the texture, colour or taste of fermented products as well as pathogens, which can harm the health of consumers. Some of the most important foodborne pathogens that can be found in fermented meat products and heat-treated sausages are *Salmonella* spp, *L. monocytogenes*, *S. aureus* and *E. coli* (De Cesare et al., 2007; Stojanac et al., 2015). Fermented meat products are a food vehicle in the outbreaks usually caused by *Salmonella* spp. or verocytotoxigenic *E. coli* (Paramithiotis and Drosinos, 2016). Fermented meat products with a pH ≤ 4.4 and water activity (aw) ≤ 0.92, or with pH ≤ 5.0 and aw ≤ 0.94 do not enhance the growth of pathogenic bacteria. However, although considered formally safe, the risk still exists and is associated with post-processing contamination – e.g. during slicing or packaging. Regarding the aforementioned, fermented meat products could still be considered potentially risky food. The results of microbiological analyses confirmed the microbiological safety of pork meat products originating from Herzegovina.

CONCLUSION

According to the results obtained in this research and data from the literature, we can conclude that fermented dry sausages, dry cured meat products and heat-treated sausages have chemical composition similar to products from other parts of B&H, Croatia, Serbia and Slovenia. The analyses of the obtained results strongly indicate that all product samples are of good quality and in compliance with relevant legislation.

Authors' contributions

LP, DK and KM made contributions to conception and design of the article, involved in data collection and drafting the manuscript. LP, JG, BK and MJG contributed with data about chemical composition, additive content and microbiological results. JP revised the manuscript critically and together with LP prepared the final draft of the manuscript. All authors read and approved the final manuscript.

Competing interest

Authors declared no conflict of interests regarding the present paper.

REFERENCES

1. Anonymous, 2013a. Regulation of minced meat, semi-finished meat products and meat products [in Bosnian]. Official Gazette of the Bi&H 82.
2. Anonymous, 2013b. Regulation of minced meat, semi-finished meat products and meat products – Amendment [in Bosnian]. Official Gazette of B&H' 84.
3. Anonymous, 2013c. Regulation on microbiological criteria in food [in Bosnian]. Official Gazette of B&H 11.
4. Anonymous, 2016. Regulation on maximal permited of contaminants in food [in Bosnian]. Official Gazette of B&H 79.
5. Anonymous, 2018a. Regulation on microbiological criteria in food – Amendment [in Bosnian]. Official Gazette of B&H 54.
6. Anonymous, 2018b. Regulation on food aditives [in Bosnian]. Official Gazette of B&H 33.
7. Aćimović M., Kozačinski L., Njari B., Cvrtila Fleck Ž: Usporedni prikaz propisa o kakvoći mesnih proizvoda. Meso 16, 4, 342-345, 2014.
8. BAS ISO. 2007. Meat and meat products - Determination of total fat content. No 1443.
9. BAS ISO. 2007. Meat and meat products - Determination of moisture content. No 1442.
10. BAS ISO. 2007. Meat and meat products - Determination of nitrogen content. No 937.
11. BAS ISO. 2007. Meat and meat products -Determination of nitrite content. No 2918.
12. BAS ISO: Meat and meat products- Determination of total phosphorus content - Spectrometric method. No 13730, 2008.
13. BAS ISO. 2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of sulfite- reducing bacteria growing under anaerobic conditions. No 15213.
14. BAS ISO. 2013. Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method. No 21528-2.
15. BAS ISO. 2019. Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. No 16649-1.
16. BAS EN ISO. 2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci

(Staphylococcus aureus and other species) - Part 1: Technique using Baird-Parker agar medium. No 6888-1.
17. BAS EN ISO. 2014. Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique. No 4883-1.
18. BAS EN ISO. 2018. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. No 6579-1.
19. Čop M. 2016. Utjecaj Enterococcus faecalis 101 na kakvoću trajnih kobasica iz domaćinstva. Diplomski rad. Veterinarski fakultet Sveučilišta u Zagrebu.
20. De Cesare A., Mioni R., Manfreda G. 2007. Prevalence of Listeria monocytogenes in fresh and fermented Italian sausages and ribotyping of contaminating strains. International Journal of Food Microbiology 12, 124-130.
21. Dučić M., Vranješ D., Baltić M.Ž. 2018. Selected physico-chemical properties of Serbian dry fermented sausages in different meat industries. Meat Technology 59,2, 120-126.
22. EC (European Commission): Regulation on food additives No. 1333, 2008.
23. ISO (International Organisation for Standardization), 1991. Meat and meat products - Sampling and preparation of test samples -Part 1: Sampling. No 3100-1
24. Kovačević D. 2001. Kemija i tehnologija mesa i ribe. Sveučilište Josipa Jurja Strossmayera u Osijeku, Prehrambeno tehnološki fakultet, Osijek.
25. Kovačević D., Mastanjević K., Ćosić K., Pleadin J. 2016. Količina nitrita i nitrata u mesnim proizvodima s hrvatskog tržišta. Meso 2, 18, 40-46.
26. Kozačinski L., Hadžiosmanović M., Cvrtila Fleck Ž., Zdolec N., Filipović I., Kozačinski Z. 2008. Kakvoća trajnih kobasica i češnjovki iz individualnih domaćinstava. Meso 10, 1, 45-52.
27. Krvavica M., Mioč B., Friganović E., Kegalj A., Ljubičić I. 2012. Sušenje i zrenje – temeljni tehnološki procesi u proizvodnji trajnih suhomesnatih proizvoda. Meso14, 2, 138-144.
28. Oiki H., Kimura H., Zdolec N. 2016. Traditional production of fermented meats and related risk. In: Fermented meat products: health aspects. N. Zdolec (ed.). Taylor & Francis, Boca Raton, USA, 49-57.
29. Olivares A., Navarro, J. L., Salvador, A., Flores, M. 2010. Sensory acceptability of slow fermented sausages based on fat content and ripening time. Meat Science, 86, 251–257.
30. Paramithiotis S., Drosinos E.H. 2016. Foodbone pathogens of fermented meat products. In: Fermented meat products: heaalth aspects. N. Zdolec (ed.). Taylor&Francis, Boca Raton, USA, 196-227.

31. Pleadin, J., Perši N., Vulić A., Đugum J. 2009. Kakvoća trajnih, polutrajnih i obarenih kobasica na hrvatskom tržištu. Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam 4 (3-4), 104-108.
32. Pleadin J., Vahčić N., Perši N., Kovačević D. 2013. Varijabilnost fizikalno-kemijskih i senzorskih svojstava autohtonih mesnih proizvoda između proizvodnih domaćinstava. Meso 15, 2, 122-131.
33. Pleadin J., Vasilj V., Lešić T., Frece J., Markov K., Krešić G., Vulić A., Bogdanović T., Zadravec M., Vahčić N. 2017. Kemijski sastav i pojavnost mikotoksina u tradicionalnim mesnim proizvodima podrijetlom s domaćinstava Bosne i Hercegovine. Meso, 19, 4, 309-316.
34. Popelka P. 2016. Fermented meats composition – health and nutrition aspects. In: Fermented meat products: health aspects. N. Zdolec (ed.). Taylor & Francis, Boca Raton, USA, 389-416.
35. Sindelar J.J., Milkowski A.L. 2011. Sodium nitrite in processed meat and poultry meats: A review of curing and examining the risk/benefit of its use. American Meat Science Association. AMSA white paper series, No. 3.
36. Stojanac N., Stevančević O., Blagojević B., Davidov I., Stojanac V. 2015. Značaj i kontrola infekcije Salmonella spp. u proizvodnji svinja. Letopis naučnih radova 39, 1, 58-65.
37. Zdolec N. 2016. Fermented Meat Products: Health Aspects. N. Zdolec (ed.). CRC Press Taylor & Francis, Boca Raton, Florida, USA, 572.

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Examples of references:

Articles in journals:

1. Stojanović D., Maličević Ž., Ašanin R. 2002. The use a new model for the investigation of sepsis. Acta Veterinaria, 52, 2/3, 125-131.
2. Chen J. and McClane B.A. 2015. Characterization of *Clostridium perfringens* TpeL toxin gene carriage, production, cytotoxic contributions, and trypsin sensitivity. Infection and Immunity, 83, 2369–2381. doi:10.1128/IAI.03136-14.
3. Williams R.B. 2015. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathology, 34, 159-180. doi: 10.1080/03079450500112195.
4. Bailey M.A., Macklin K.S., Krehling J.T. 2013. Use of a multiplex PCR for the detection of toxin-encoding genes *netB* and *tpeL* in strains of *Clostridium perfringens.* ISRN Veterinary Science, Article ID 865702, 1-4. doi:10.1155/2013/865702.

Books:

5. Ficken, M. D. and Wages, D. P. 1997. Necrotic enteritis in Diseases of Poultry, Eds. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, Iowa State University Press, Ames, Iowa, USA, 10th edition, ISBN xxx-xxx-xxxxx-xx-x.

Chapters in books:

6. Plumb J.A. and Hanson L.A. 2011. Sturgeon viruses. In *Health maintenance and principal microbial diseases of cultured fishes*. Eds. J.A. Plumb, L.A. Hanson, 3rd edition, Blackwell Publishing, 219-225.

Articles in proceedings:

7. Giangaspero A., Marangi M., Pati S., Cafiero M.A., Camarda C., Sparagano O.A.E. 2011. Investigating the presence of acaricide residues in laying hens naturally infected by the red mite *Dermanyssus gallinae*. In *Book of Abstracts*, The 12th Asian food conference 2011, BITEC Bangna, Bangkok, Thailand, 27.
8. Vidanović D., Petrović T., Šekler M., Debeljak Z., Vasković N., Matović K., Plavšić

B., Dmitrić M. 2018. Avian influenza in Serbia: epidemiological situation during 2016–2017. In *Programme and Abstract book*, 11th International Congress for Veterinary Virology, 12th Annual Meeting of EPIZONE, 27-30.08.2018, University of Veterinary Medicine Vienna, Vienna, Austria, 118 (p187).

9. Lazić G., Lazić S., Bugarski D., Grubač S., Lupulović D., Samojlović M., Petrović T. 2018. Human enteroviruses in river water and sewage in Vojvodina. In *Book of Abstracts*, International Scientific Conference "Green economy and environment protection", Belgrade, 23-25. April 2018, edited by Larisa Jovanović, Belgrade, Naučno stručno društvo za zaštitu životne sredine "ECOLOGICA", 95-96. ISBN 978-86-89061-11-6.

Lows and Regulations:

10. European Union. 2003. Commission Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition, Official Journal of the European Union, L 268:29. **https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2003R1831:20100901:EN:PDF**

Citations with organisations as authors:

11. European Food Safety Authority. 2016. Peer review of the pesticide risk assessment of the active substance benzoic acid. EFSA Journal, 14(12):4657-n/a. **http://dx.doi.org/10.2903/j.efsa.2016.4657.**

Software:

12. Statistica (Data Analysis Software System). 2006. v.7.1., StatSoft, Inc., USA (**www.statsoft.com**).

Web Links:

13. OIE: Animal Diseases. Available at: **http://www.oie.int/en/animal-health-in-the-world/information-on-aquatic-and-terrestrial-animal-diseases/.** Accessed 07.08.2019.
14. European Centre for Disease Prevention and Control (ECDC). Historical data by year - West Nile fever seasonal surveillance. Available at: https://ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical Accessed 31.07.2019.

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