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EPIZOOTIOLOGICAL AND CLINICAL SIGNIFICANCE OF *STEPHANURUS DENTATUS* NEMATODE IN SWINE

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Abstract

Stephanuriosis is a disease of the urinary tract of both domestic pigs and wild boars occurring in tropical and subtropical regions, with a tendency to spread to other geographical areas. In endemic areas, the disease occurs more often in extensively bred herds, normally with nonspecific clinical picture. As a result of larval stage migrations and parasitism of adult forms of nematode *Stephanurus dentatus*, the damage occurs in the liver, kidneys, ureters, lungs, pancreas and perirenal adipose tissue. Damage to the liver is reminiscent of lesions caused by *Ascaris suum*, which is of particular clinical significance for differential diagnosis of parasitic swine diseases. *Stephanurus dentatus* is of great economic importance due to direct and indirect damage it causes. Infected animals delay in reaching market weight, their meat is declared unfit for consumption, and they are sent to forced slaughter. Examinations of potential presence of this parasite in Serbia should include populations of wild boars and domestic pigs from extensive traditional breeding outdoors. After intense climate changes that have occurred in recent decades, and also due to the possibility of importing infected animals from endemic areas, the proposed research would have both epizootiological and clinical significance.

Key words: pig, stephanuriosis, diagnosis, control, economic significance

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EPIZOOTIOLOŠKI I KLINIČKI ZNAČAJ NEMATODE *STEPHANURUS DENTATUS* KOD SVINJA

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Kratak sadržaj

Stefanurioza je oboljenje urinarnog trakta domaćih i divljih svinja u tropskim i suptropskim regijama, sa tendencijom širenja i na ostala geografska područja. U endemskim oblastima, oboljenje se češće javlja u zapatima koji se gaje ekstenzivno i protiče sa nespecifičnom kliničkom slikom. Kao posledica migracije larvenih stadijuma i parazitiranja odraslih oblika nematode *Stephanurus dentatus*, nastaju oštećenja na jetri, bubrezima, ureterima, plućima, pankreasu i perirenalnom masnom tkivu. Oštećenja koja nastaju u jetri podsećaju na lezije uzrokovane larvama *Ascaris suum*, što je od posebnog kliničkog značaja za diferencijalnu dijagnozu parazitskih oboljenja svinja. Zbog direktnih i indirektnih šteta koje prouzrokuje, *S. dentatus* ima veliki ekonomski značaj. Inficirane životinje kasne u postizanju tržišne težine, njihovo meso se proglašava neupotrebljivim i upućuju se na prinudno klanje. Ispitivanja eventualnog prisustva ovog parazita u Srbiji trebalo bi da obuhvate populaciju divljih svinja i domaće svinje iz ekstenzivnog tradicionalnog gajenja na otvorenim površinama. Nakon intenzivnih klimatskih promena koje su nastale u poslednjim decenijama, kao i zbog mogućnosti uvoza inficiranih životinja sa endemskih područja, predložena istraživanja bi imala epizootiološki i klinički značaj.

Ključne reči: svinja, stefanurioza, dijagnostika, kontrola, ekonomski značaj

INTRODUCTION

Stephanurus dentatus nematode parasitizes in the urinary tract of domestic pigs and wild boars, mostly causing the disease in tropical and subtropical areas (Gherman, 2013), and in pigs bred in traditional outdoor production systems (Fernández-Vizcaíno et al., 2021).

Larvae migration and parasitism of adult forms of *S. dentatus* leads to damage of internal organs, which results in significant direct economic loss (frequent forced slaughter of cachectic pigs, deposition of confiscates and meat during slaughter). Indirect damage is reflected in reduction of growth and weakening of the general resistance of infected pigs (Gherman, 2013). Since the migration of infectious larvae *S. dentatus* through the liver can cause severe hepatitis, with lesions reminiscent of those caused by *Ascaris suum* larvae (Constable et al., 2017), this helminthiasis has a particular clinical significance.

So far, stephanuriosis has been diagnosed in Africa, east and west India, Brazil, Hawaii, The Philippines, The United States of America and Australia (Constable et al., 2017). There is no relevant data on the presence of parasites in wild boars on the European continent, therefore additional research is necessary in order to define the role of wild boars as a potential natural reservoir of *S. dentatus* for domestic pigs (Fernández-Vizcaíno et al., 2021).

In the Republic of Serbia, the presence of the parasite *S. dentatus* has not been established during controls on the slaughter line in slaughterhouses. In the territory of the Republic of Serbia, there are reports related to problems of parasitic infections of the gastrointestinal tract in pigs (Ilić and Dimitrijević, 2005; Ilić and Dimitrijević, 2006; Ilić et al., 2011; Ilić et al., 2013; Ilić et al., 2021a), but there is no data in the literature on urinary tract parasitoses in this species of ungulate. Besides recent reports on urinary capillariasis of domestic and wild carnivores (Aleksić et al., 2020; Ilić et al., 2021b), not enough attention has been paid on studying urinary diseases of parasitic etiology of other animal species in previous research on animals in Serbia.

The aim of this paper is to point out the need for examination of potential presence of this parasite in the Republic of Serbia in wild boars and domestic pigs from extensive traditional breeding outdoors. The proposed examinations are of particular importance at a time of global climate change occurring over the last decade, as well as due to the intensification of commerce and pig trade, which pose a potential risk for import of infected animals.

ETIOLOGY

Stephanurus dentatus belongs to *Stephanurus* genus, Stephaurinae subfamily, *Syngamidae* family, *Strongylida* order and *Secernentea* class (Ballweber, 2001).

Morphological characteristics of the causative agent

Adult forms of parasite have a strong body and mottled appearance. They have a cuticle that is transparent and enables visualization of internal organs

(reproductive and intestinal system). Sex dimorphism is clearly stated. Females are longer (39.0 - 44.0 mm) and thicker (2.0 - 2.6 mm) compared to males who are 30.5 - 36.0 mm long and 1.2 - 1.4 mm thick (Gherman, 2013). At the anterior end of the body, they have buccal capsule with thick walls which contains teeth of different sizes. The parasite's esophagus is thinner in the front than in the back. The tail of a female sharply narrows and the vulva is located near the anus. At the posterior end of the body, a square-shaped bursa copulatrix is found in males, with two laterals and one dorsal lobe. The spicules are approximately the same size (0.70 to 0.98 mm). Eggs are broadly ellipsoid, with thin membranes and 32 to 64 blastomeres, with dimensions of 70 μm to 120 μm (Islam et al., 2015).

Life cycle

Adult parasites are mainly localized in the renal pelvis, in cysts around renal pelvis, in perirenal adipose tissue, ureter and in adipose tissue surrounding ureter. Other than these organs, they can rarely be found in the pancreas, lumbar muscles, spinal cord and lungs (Gherman, 2013).

Adult female parasites produce up to a million eggs a day, which are excreted in the urine of the host into the environment. Most eggs are excreted during the first morning urination. In optimal environmental conditions (warm, humid and shaded places), the eggs embryonate for 1 to 2 days and the larvae of the first stage (L1) are released from them. After two "sheddings" in the environment, 3 to 4 days later, larvae of the third stage (L3) are formed from the L1 larvae and they are infectious for the host. Infectious larvae can enter the pig organism directly (percutaneously or perorally) and indirectly by swallowing paratenic host- worms (Gherman, 2013).

In *per os* infections, L3 larvae, after ingestion, migrate from the digestive tract through the stomach wall and small intestine to the mesenteric lymph nodes, where "shedding" can occur from L3 to L4 larvae. A certain number of larvae migrate to the liver, and part of them goes to other organs (lungs, lymph nodes, pancreas and spleen) and in fetus in gravid sows. In the liver, L4 larvae migrate through parenchyma reaching the capsule which they penetrate three months after infection. Further migration through the peritoneal cavity, juvenile forms of the parasite mostly reach the perirenal and periurethral adipose tissue, kidneys and ureters, where a cyst is formed and in it adult forms parasitize. The cyst communicates with the organs of the urinary system through the fistula and through the urine the eggs reach the environment. Female parasite can start laying eggs six months after infecting the animal, but the prepatent period is usually longer (9 to 16 months). An infected animal can release eggs into the environment for three years (Constable et al., 2017).

Infectious (L3) larvae that enter the organism through the skin, migrate to the lungs through systemic circulation and then reach the trachea, pharynx and after swallowing, the intestines. Further migration is the same as with the peroral infection and the infectious larva reaches the liver after 1 to 6 weeks (Gherman, 2013).

An indirect infection occurs through worms that contain infectious L3 larvae. If the host ingests worms containing infectious larvae during feeding, they are released during the digestion and undergo further development, as in the case of direct infection, through the stomach wall and/or small intestine (Roepstorff and Nansen, 1998; Constable et al., 2017).

EPIZOOTIOLOGY

S. dentatus is greatly widespread in tropical and subtropical regions, with a tendency to spread to other geographical areas. It is most commonly found in Africa, The United States of America, India, Brazil, Hawaii, The Philippines and Australia, where the climate is mild enough to enable survival of eggs and larvae. Eggs and larvae are very sensitive to cold and drying (Constable et al., 2017).

In a dry environment, the eggs decay within an hour. Temperatures below 10 °C are harmful to eggs and temperatures below 4 °C and above 35 °C have an ovicidal effect. In optimal conditions of humidity, heat and sunlight, most larvae survive for up to three months, rarely up to five months. As a facultative parasite, larvae can survive in worms for a long period of time even when microclimate conditions are unfavorable. They can also develop in the ground at a depth of 25 cm and beneath the water surface as well (Gherman, 2013; Constable et al., 2017).

All age categories of pigs, including fetuses, are sensitive to parasites. Infection occurs per orally (by swallowing of infectious L3 larvae or worms that contain L3 larvae). This is, probably, the most common route for the onset of the infection. Infection can also occur percutaneously and prenatally (Gherman, 2013). Infection found in piglets less than five months of age was caused by L3 larvae during gravidity (Islam et al., 2017).

An area with adequate microclimate conditions, which favors parasite development, becomes endemic over time. Both wild boars and domestic pigs are susceptible to the disease. In domestic pigs the infection is more common in herds that are bred freely outdoors, when pastures are continuously contaminated. It also occurs in pigs that are bred extensively in closed facilities, with the possibility of staying at the outlet. The likelihood of the occurrence

of this parasite in intensive pig breeding is low on the farms where biosecurity measures are applied and with good zoo hygienic practice (Nansen and Roepstorff, 1999).

The prevalence of the infection varies in different parts of the world and primarily depends on climate conditions. According to data found in the literature, the disease has been established in 33% of pigs in Ghana, 42% in Belize (Central America), 9.7 to 40.5% in India and it is 62.5% in the south of China (Gherman, 2013). In an epizootiological study in Bangladesh the organs of slaughtered pigs aged 1 to 5 years were examined and the prevalence of stephanuriosis was determined to be 21%. The parasites were localized in renal pelvis, renal cortex, ureter and in perirenal adipose tissue of pigs that were four and five years old (Islam et al., 2015).

Examining parasite spreading in pigs on slaughter line in Benu region, Nigeria, the prevalence of *S. dentatus* was determined to be 13.2%. The authors deemed that this prevalence is a result of adequate microclimate conditions for development and survival of parasites in the external environment. Two climate seasons alternate in the examined region - rainy from April to October and dry from October to March (Obisike et al., 2018).

On the European continent *S. dentatus* can be found in different regions of the Iberian Peninsula - Cadiz, Granada, Madrid and Portugal (Moratal et al., 2018). By examining wild boars on several locations in southern and central Spain, an extremely high prevalence was determined (76.5%). These results suggest grouped distribution of infection theory in certain areas and are particularly important for this region, considering well developed pig production. Since stephanuriosis has not been previously identified as a health issue in pig breeding in this area, the authors suggest studying the importance of wild boars as a reservoir of disease in the nature and the possibility of transmission to domestic pigs (Fernández-Vizcaíno et al., 2021). During the study that was conducted in the Caribbean (St. Kitts), between 2012 and 2013, *S. dentatus* was diagnosed in 5. 9% of the shot wild boars (Morosco et al., 2017).

The presence of *S. dentatus* has not been established in regular inspection surveillance on the slaughter line in slaughterhouses in the Republic of Serbia. However, we should keep in mind the fact that pigs from the intensive breeding are mainly slaughtered in these facilities. Studying the potential presence of parasites in the Republic of Serbia should include examinations of both wild boars and domestic pigs from extensive breeding, particularly from herds that are bred outdoors. An additional risk factor may be the effects of global warming in the last decade and the possibility of presenting this nematode by importing infected animals.

PATHOGENESIS AND PATHOMORPHOLOGICAL CHANGES

In pigs infected by *S. dentatus* nematode, tissue damage occurs as a result of larval forms migrations and parasitism of adult forms of causative agents. Necrosis, fibrosis, cirrhosis and abscesses can form during the migration of larval forms. The liver is often enlarged with pronounced scars, and it can sometimes be accompanied by ascites. Extensive bleeding and irregular whitish changes called "milk spots" are present in the liver parenchyma. Thrombosis may be present in the blood vessels of the liver. As a result of the harmful effects of adult parasites, gray necrotic fields, infarcts and scars are present on the kidneys. The kidneys are edematous with a tense capsule. Frequent findings are pleurisy and peritonitis with numerous adhesions of internal organs (Islam et al., 2015; Constable et al., 2017).

In perirenal and periurethral adipose tissue cysts that are linked to organs of the urinary system by fistulas can be found (Gherman, 2013; Islam et al., 2015). The changes can also affect the mesenteric lymph nodes, spine, umbilical cord, pancreas, heart, lungs, spleen and skeletal muscles by forming eosinophilic nodules in these organs (Gherman, 2013).

Morosco et al. (2017) report on pathomorphological changes in the kidneys, perirenal adipose tissue and ureters of wild boars which were infected with *S. dentatus* nematode. Macroscopic examination found the cysts of thick walls in perirenal and periurethral adipose tissue as well as adult nematodes up to 3.5 cm long. Histopathological examination of the tissue surrounding the parasite revealed bleeding, edema, necrosis, erythrophagocytosis, hemosiderosis, fibrosis and a large number of eosinophil granulocytes. In addition to adult parasites, ellipsoid eggs of about 100 μm in size are occasionally observed in the renal pelvis and in cysts surrounding the ureter, too.

Eosinophilia is observed 2 to 3 weeks after infection, it reaches a peak in 6 to 7 weeks and is present even after 20 weeks. Although it often occurs, it has little specific diagnostic value because it is also present in other parasitic infections. In addition to dominant eosinophil granulocytes, lymphocytes, plasma cells, macrophages and occasionally multinucleated giant cells are also present in the tissue surrounding parasite and inside the cyst (Morosco et al., 2017).

As a result of interstitial nephritis, infiltration of eosinophilic granulocytes, neutrophilic granulocytes and mononuclear cells are observed in the kidney tissue (Islam et al., 2015).

It has been proved that the cattle can be a non-specific host for *S. dentatus* and that the parasite is rarely in them, mostly as a consequence of accidental infections. In experimentally infected calves, the liver and pancreas suf-

fer damage similar to those caused in pigs. Abscesses, fibrosis and eosinophil granulocytes infiltration are present. However, since parasite cannot complete its life cycle in cattle, pathomorphological changes in the organs where adult forms of the parasite should parasitize are absent (Morosco et al., 2017; Constable et al., 2017).

IMMUNITY

Along with innate and general resistance, the organism has specific humoral and cellular protection mechanisms that fight against parasites. The results of the study of the immune response of the animals infected with the *S. dentatus* are mostly based on the study of the serological response of experimentally infected animals.

In the cases of low-intensity infections, the level of antibodies is not measurable. Moreover, a direct link between the rate of serological response and the intensity of infection has not been proven. During the examination of the serological response of pigs experimentally infected with *S. dentatus* parasite, various tests were used: double immunodiffusion, immunoelectrophoresis and Enzyme Linked Immunosorbent Assay (ELISA) test. Double immunodiffusion and immunoelectrophoresis tests showed a positive reaction from the fourth week after the infection. A test of double immunodiffusion performed between 6th and 18th week and the application of immunoelectrophoresis between 6th and 15th week after the experimental infection showed that the percentage of positive reactions was over 60%. Both tests showed simultaneous onset of antibodies. ELISA test, which used rabbit antibody marked by alkaline phosphatase and gland extract to excrete parasites as antigen, proved high sensitivity of this method. The presence of antibodies against *S. dentatus* was established as early as two weeks after infection and the percentage of positive findings higher than 60% was obtained during the 20th week after infection. At the same time, the ELISA test is a quantitative method - based on the results we can define degree of infection; thus, it is recommended in conducting serodiagnostic research during prepatent period of *S. dentatus* (Partoutomo et al., 1983).

In experimental conditions, nine somatic antigens obtained from excretory glands of the adult *S. dentatus* parasite were used in the production of the *S. dentatus* vaccine. The efficacy of the produced vaccine was evaluated by monitoring the resulting lesions due to larvae and adult parasites migration in autopsied animals, after *per os* infection of pigs with infectious larvae. None of the produced vaccines completely prevented the migration of larvae through the liver as well as development of adult forms of parasites. However, the on-

set of the disease has been reduced as much as 92%. Therefore, it can be said that the vaccines are efficient, especially if combined with other disease control measures (Tromba and Romanowski, 1976).

CLINICAL PICTURE

Stephanuriosis is characterized by nonspecific clinical symptoms which usually occur as a consequence of migration of larvae forms as well as parasitism of adult forms of parasites (Gherman, 2013).

In the cases of low-intensity infections, the most common sign of the disease is delayed progress despite a good appetite. Numerous nodules can be observed on the skin and abdominal wall. Peripheral lymph nodes are enlarged and painful, while deaths are not common (Constable et al., 2017).

In the cases of high intensity infections, a more severe form of disease occurs, and it is characterized by poor food conversion, weight loss and cachexia with a tendency to develop ascites. As a result of changes caused by aberrant larvae during migration, nonspecific clinical signs are manifested and are difficult to be linked to the presence of *S. dentatus.* Thrombi occur in larger blood vessels (*v. portae*, *a. hepatica*, *v. cava caudalis),* damaging lung tissue with consequential respiratory disorders, peritonitis, pleurisy, paresis and paralysis of the posterior parts of the body. As a result of peritonitis and intestinal intussusception, death of the animal can occur in 20 to 30 days after infection. A significant clinical symptom is weakness and paralysis of the hind legs, which occurs as a result of larval migration through the spinal cord (Cianciolo and Mohr, 2016; Constable et al., 2017). Unlike hypovitaminosis, fertility disorders were not observed in females /sows suffering from stephanuriosis. Blood test results of diseased animals revealed that eosinophilia and haematuria are also a common (Gherman, 2013).

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of stephanuriosis can be established by autopsy or parasitological examination of pig urine, which proves the presence of parasite eggs. By serological ELISA test, which is not a routine diagnostic procedure, it is possible to detect infection two weeks after infection with *S. dentatus* larvae. Young pigs with high intensity infection entirely by larvae (without the presence of adult parasites and organ damage), can be a major problem in disease diagnostic (Constable et al., 2017). Clinical examination is insignificant in the diagnosis of stephanuriosis due to non-specific symptoms occurring in infected pigs (Gherman, 2013).

The diagnosis is usually made in autopsy, by finding larval or adult forms of parasites, as well as lesions that are a result of migration or parasitism in organs. Pathomorphological changes present in the liver are of the greatest importance in endemic areas. The liver is enlarged with gray-white lesions, which are the signs of cirrhosis and the presence of abscesses. Lesions are usually more severe than those caused by *Ascaris suum* larvae. Abscesses may occur in the lungs and / or pancreas and phlebitis may occur in the portal vein with occasional thrombi. Mesenteric lymph nodes are enlarged. Pleurisy and peritonitis (sometimes with intestinal intussusception) may develop as a result of larval migration. The finding of adult forms of parasites in the kidneys, perirenal adipose tissue and ureter walls during autopsy, confirms the diagnosis (Gleyderson Silva et al., 2015).

The diagnosis is most often made by finding characteristic *S. dentatus* eggs by examining urine sediment of infected pigs. Most eggs are excreted during the first morning urination, and female parasites release up to a million eggs a day in the maximum potent phase in the external environment (Hendrix and Robinson, 2006; Gherman, 2013).

Reliable parasitological diagnostics is performed by applying methods for egg detection in urine, whose sensitivity is 100%. One of these techniques is the flotation technique (saturated aqueous solution of ZnSO4 with specific gravity of 1.200). Gravitational deposition technique – sedimentation (at a room temperature lasting for an hour) and centrifugal deposition technique (at 800 rmp for 5 minutes) allow detection of a larger number of *S. dentatus* eggs and are more reliable for a quantification of infection (determining the number of eggs in urine). However, the determined number of parasite eggs in urine does not correspond to a number of adult nematodes parasitizing in the same host (Fernández-Vizcaíno et al., 2021).

The number of determined eggs in 1 mL of urine can be influenced by various factors like number of adult forms, male and female parasites ratio, time of the day when sampling is performed, diagnostic method and the characteristics of a host (sex, age, nutritional status and mating season). Therefore, the numbers of eggs determined by the above-mentioned methods can only be used to roughly determine the numbers of adult parasites (Fernández-Vizcaíno et al., 2021).

In the differential diagnosis of stephanuriosis, it is necessary to consider the epizootiological anamnesis of a herd in detail. In clinical trial it is vital to identify all causes of delayed progress and weight loss. Poor and unbalanced diet, as well as chronic bacterial diseases (necrotic enteritis, swine dysentery), which may be accompanied by diarrhea, are also significant. In cases of paralysis and weakness of the posterior part of the body, it is necessary to eliminate

the following: vitamin A deficiency, rickets, osteomalacia, lumbar spine fractures, abscesses, spinal cord lymphomas, brucellosis, chronic form of erysipelas as well as infections with nematodes *Ascaris suum* and *Hyostrongylus rubidus* (Constable et al., 2017). The nematode *Dioctophyme renale*, leptospirosis and renal dysfunction of various etiologies are also important for differential diagnosis (Gherman, 2013).

THERAPY

Doramectin, ivermectine, fenbendazole, levamisole and flubendazole have good efficacy in the treatment of stephanuriosis. Doramectin administered at a dose of 0.3 mg per kg of body weight showed the efficacy of 100%. Ivermectine is administered at a dose of 30 μg per kg of body weight, achieving 100% efficacy, 14 to 21 days after the treatment. Fenbendazole mixed in food at a dose of 3 mg per kg of body weight, administered in the course of three days leads to healing in three weeks after the treatment. Levamisole at a dose of 10 mg per kg of body weight and flubendazole at a dose of 50 mg per kg of body weight are also effective in the treatment of stephanuriosis.

PROPHYLAXIS

The absence of stephanuriosis in intensive breeding systems is a result of several factors: a long prepatent period of the parasites**,** high sensitivity of preinfectious stages to the microclimate of the environment as well as the absence of paratenic hosts (Nansen and Roepstorff, 1999).

Disease control is based on general sanitary measures and improvement of pig breeding conditions. In infected herds, boars and gilts should be bred separately and used for only one cycle of breeding; after that they are moved out of the premises and excluded from breeding. During the prepatent period of the parasite (9 to 16 months), piglets are weaned from sows and transferred to another facility before the development of adult parasites whose eggs are eliminated in urine. After 3 to 4 seasons of farrowing, this method of breeding can eradicate the disease on farms (Gherman, 2013).

Young animals should be physically separated from adults and should not be moved into facilities where adults have stayed for three to six months after the adult animals have moved out. Due to the great importance of adult animals as a source of infection, early replacement of breeding material is crucial in disease prevention. Regular dehelminthization of all pigs with Fenbendazole or Ivermectine at intervals of four months is also significant (Constable et al., 2017).

In order to prevent the development of infectious forms of parasites in the free breeding of pigs outdoors, it is necessary to provide dry substrate without vegetation in the places where animals stay and sleep and absence of vegetation in the places where animals mostly urinate, because that reduces the potential of eggs to survive. It is necessary to regularly maintain the hygiene where the watering places are located and provide sufficient amounts of clean water. Since worms can be reservoirs in which parasite remains vital for at least a year, it is necessary to apply the measures aimed at reducing their number (Constable et al., 2017).

In all production systems, except on farms with organic production, regular application of anthelmintics in the prevention of stephanuriosis is the most common way to control this disease.

The first dehelmenthisation protocol is applied to adult animals. Gravid sows can be treated one to two weeks before farrowing with the aim of releasing adult parasites. This consequently reduces the possibility of intrauterine infection, infection of newborn piglets, as well as contamination of the environment where the newborn piglets will live. The second protocol is based on treating all farm animals simultaneously, at certain times of the year, which has proven to be a more economical solution due to reduced costs associated with hiring farm workers (Nansen and Roepstorff, 1999).

The control of parasitic infections in modern production must be based on monitoring of the conditions which favor their occurrence, maintenance and spreading in the herd and on reliable diagnostics (coprological surveillance, surveillance at the slaughter line in the slaughterhouses). The knowledge of the etiopathogenesis, prevalence and epizootiological characteristics of individual infections provide an adequate approach in their treatment and control (Nansen and Roepstorff, 1999).

Guided by these principles, a certain system of surveillance should be established for the presence of the parasite *S. dentatus* in the Republic of Serbia, with the purpose of its early detection, timely treatment and control measures.

CONCLUSION

S. dentatus is of great economic and clinical importance. The result of migration and parasitism of causative agents in the urinary tract of pigs is weight loss, delay in reaching market weight and frequent forced slaughter of cachectic pigs whose meat is declared unfit for human consumption. Due to the possibility of replacing this nematode with other parasites, veterinarians on the field should be warned about this helminthiasis when diagnosing parasitic diseases and diseases of the urinary tract of pigs.

To this day, no cases of this disease have been diagnosed in pigs in the Republic of Serbia. However, after the intense climate change that has happened in recent decades, as well as the possibility of importing infected animals from endemic areas, it is suggested that domestic pigs in traditional production systems should be examined for the possible presence of this parasite in the Republic of Serbia as well as wild boars as potential reservoirs of *S. dentatus*.

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Author's Contribution:

All authors (IT, DM, PR, and BD) contributed to manuscript design, performed literature searches, wrote and revised the article, and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests for a work presented in the Manuscript.

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Review article

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AFRICAN SWINE FEVER: A BIOSECURITY CHALLENGE FOR DOMESTIC PIG PRODUCTION IN SERBIA

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Abstract

African swine fever (ASF) is currently the most important challenge for domestic pig production worldwide. The virus reached Eurasia in 2007, and is today affecting more than half of European Union member countries. Among Western Balkan countries, Serbia suffered the first case of ASF in a backyard holding in 2019. Since then, numerous outbreaks in domestic pigs and wild boar have been reported throughout the country despite the efforts of the veterinary authorities to control the disease. The lack of an effective vaccine is one of the main constraints, and the only currently available option to prevent further ASF infections is the application of strict biosecurity measures. Regarding biosecurity, backyard pig producers and smallholding farmers in Serbia have substantial gaps in the knowledge and fail to comply with safe production behaviour that favours the spread of ASF virus. In the currently prevailing smallholder and backyards farming systems, farm biosecurity is largely non-existent. The aim of this review was to identify specific ASF-risks factors in the current pig production system and gaps in biosecurity measures related to the human activities recognised as social and cultural identity in Serbia. Moreover, the main risk factors for ASF spreading and transmission at the domestic/wild boar interface, biosecurity practices in different production systems, and possible future control measures and awareness campaigns are discussed.

Key words: African swine fever, domestic pig production, Serbia

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AFRIČKA KUGA SVINJA: BIOSIGURNOSNI IZAZOV ZA PROIZVODNJU DOMAĆIH SVINJA U SRBIJI

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Kratak sadržaj

Afrička kuga svinja (AKS) je trenutno najvažniji izazov za domaću proizvodnju svinja širom sveta. Virus je prvi put utvrđen u Evroaziji 2007. godine, a danas je prisutan u više od polovine država članica Evropske unije. Među državama zapadnog Balkana, Srbija je 2019. godine potvrdila prvi slučaj AKS u jednom seoskom gazdinstvu. Od tada se beleži pojava brojnih žarišta u populaciji domaćih svinja i divljih svinja širom zemlje uprkos preduzetim kontrolnim merama. Nedostatak efikasne vakcine je glavno ograničenje i jedina trenutno dostupna opcija za sprečavanje daljih infekcija je primena strogih mera biosigurnosti. Što se tiče biosigurnosti, proizvođači svinja u seoskim gazdinstvima i mali farmeri u Srbiji imaju značajne nedostatke u znanju i praksi i imaju različita rizična ponašanja koja mogu da doprinesu širenju AKS. U postojećem sistemu malih gazdinstava i dvorišta, biosigurnost se uglavnom ne primenjuje. Cilj preglednog rada je identifikacija specifičnih faktora rizika od AKS u postojećem sistemu proizvodnje svinja i nedostataka u biosigurnosnim merama koji se vezuju sa ljudskim aktivnostima, a koji su prepoznate kao svojevrsni društveni i kulturni identitet u Srbiji. Pored toga, analizirani su glavni faktori rizika od širenja i prenošenja virusa AKS između populacije domaćih i divljih svinja, praktičnih biosigurnosnih mera u različitim proizvodnim sistemima i mogućnosti kontrolnih mera i kampanje podizanja svesti.

Ključne reči: Afrička kuga svinja, proizvodnja domaćih svinja, Srbija

INTRODUCTION

African swine fever (ASF) is a viral disease of domestic pigs and wild boars that currently represents a major threat to the swine industry worldwide (Sanchez-Vizcaino et al., 2013; Bellini et al., 2021). The disease was first reported in Kenya in 1921, and several intercontinental transmissions have

occurred since then (Sánchez-Cordón et al., 2018; Ståhl et al., 2019; Liu et al., 2021). Within European continent, the first ASF incursion was reported in Portugal in 1957 (Boinas et al., 2011). It took until 1995 to officially eradicate the disease from the continent, with the exception of Sardinia Island, where ASF is still endemic from 1978 to this day (Cappaia et al., 2018). In 2007, a new introduction of the highly virulent genotype II of African swine fever virus (ASFV) was reported in Georgia, the Caucasus (Rowlands et al., 2008). Despite all preventive measures, in early 2014, ASF reached the territory of European Union (EU) with the first case reported in Lithuania followed by other Baltic countries (Blome et al., 2020; Malakauskas et al., 2022). Since then, disease has continuously been spreading, and it reached Asia (2018) and the Americas (2021), which identified ASF as the worst livestock pandemic of this century (Liu et al., 2021; de la Torre et al., 2022). Today, 15 years later, ASF is progressively spreading each year to the territories of new countries on 4 different continents. Indeed, at least two new countries have become affected each year in Europe: Czech Republic and Romania in 2017, Hungary, Bulgaria, Belgium in 2018, Slovakia and Serbia in 2019, Greece and Germany in 2020, Italy and Northern Macedonia in 2021. Only two countries (Belgium and Czech Republic), both with only wild boar population affected, have regained an ASF-free status (Schulz et al., 2019; Sauter-Louis et al., 2021; Sauter-Louis et al., 2022). The geographical expansion of ASF continues (Zani et al., 2019; EFSA, 2022), but there are a number of important differences reported regarding disease spreading in the different parts of European continent (EFSA, 2019; de la Torre et al., 2022). Excluding the Sardinia Island, from 2014 up to the end 2020, there have been 6037 ASF outbreaks in domestic pigs and 39,970 ASF notifications in wild boar across 12 EU countries (ADIS, 2021). Indeed, in 2014 when ASF arrived in the Baltic States and Poland, it became endemic in the wild boar population (Zani et al., 2019; Malakauskas et al., 2022). Wild boar has been the main affected species in all countries except for Romania, where most notifications occur in domestic pigs (Andraud et al., 2021; Ardelean et al., 2021). Also, on the other side of the world, by November 2021, China had reported 203 cases of ASF and culled in total 1.193 million domestic pigs (Liu et al., 2021). Today in Europe, except for Hungary, Belgium and the Czech Republic where ASF occurred only in wild boar, both domestic pigs and wild boars are affected (Lamberga et al., 2020; EFSA, 2022). Generally, the total number of reported wild boar cases in Europe has increased from year to year, whereas the number of outbreaks in domestic pigs has been limited (Chenais et al., 2018; Chenais et al., 2019a). However, in the European south-eastern countries, ASF is severely affecting small-scale domestic pig holders and backyards (Zani et al., 2019; Andraud et al., 2021; Ardelean et al., 2021). Indeed, in this part of Europe,

the observed epidemiological ASFV pattern is different and mostly associated with the characteristics of the domestic pig sector with large percentage of backyards with low levels of biosecurity where pig keeping represents an important source of livelihood and a traditional heritage (Chenais et al., 2019b; Gervasi and Guberti, 2021).

Since at present there is no treatment or vaccination, prevention and control of ASF rely on biosecurity measures (Jurado al., 2018; Bellini et al., 2021). It is well known that ASF virus can be transmitted to domestic pigs and wild boar through direct and/or indirect contact with infected animals, contaminated fomites and through the ingestion of contaminated meat products (Olesen et al., 2020; Bellini et al., 2021). The virus is highly resistant to environmental conditions and can persist in contaminated fomites and meat products for several months, contributing to the disease spread (Mazur-Panasiuk et al., 2019; Beato et al., 2022). In many cases, the exact route of introduction into domestic pig herds cannot be determined, but most introductions are attributed to indirect virus transmission (Ståhl et al., 2019; Olesen et al., 2020). However, in the current epidemic involving domestic pigs and wild boar population in Europe, disease transmission is frequently driven by human activities (EFSA, 2019). Indeed, the specific influence of humans and social, cultural factors on ASF epidemiology today is increasingly recognized (Jurado al., 2018; EFSA, 2022). It is important to mention that ASFV is the only known DNA virus that can be transmitted by vectors (Mazur-Panasiuk et al., 2019; Olesen et al., 2020). Thus far, only soft ticks of *Ornithodoros* spp. have been found to facilitate ASFV replication (Ståhl et al., 2019). The first documented case of ASFV isolation in ticks (*O. erraticus*) was recorded in Spain in the 1960s (Boinas et al., 2011). Since then, eight *Ornithodoros* species have been found to be involved in the ASFV transmission (Blome et al., 2020). However, a new type of ASFV that can infect hard ticks and transmitted from female adults to the first generation larvae was discovered in China. However, the conducted studies did not confirm that hard ticks are able to transmit ASFV to susceptible pigs (Liu et al., 2021). Other insects that may mechanically spread virus have also been reported, for example, the stable flies. In the study by Olesen et al. (2018), it was demonstrated that blood-feeding flies are capable of transporting infectious virus for at least 12 hours. Therefore, flies may play a role in the introduction of ASFV to pig farms, even those with high biosecurity (Liu et al., 2021; Štukelj et al., 2021). Indirect spread by vectors could help explain the introductions into large high-biosecurity farms and the seasonal pattern of transmission seen in domestic pig farms in EU countries (Estonia, Latvia) (Jurado et al., 2018; Olesen et al., 2020).

In this review, we explored the pig production systems in Serbia and identified specific ASF-challenge risks in biosecurity measures related to human activities recognised as social and cultural identity. Also, the main risk factors of disease spreading and transmission at the domestic/wild boar interface, the importance of biosecurity in different production systems, and control efforts that require further attention in awareness campaigns are discussed.

OVERVIEW OF ASF SITUATION IN SERBIA (2019-2021)

In 2019, ASF was for the first time detected in domestic swine backyard population in central part of Serbia (Milićević et al., 2019). Following the first one, several outbreaks were confirmed in the villages of one municipality. Disease outbreaks were registered in the next 2 months in the backyard domestic pig population. According to the epidemiological investigation, almost all cases were firstly detected as health disorders in sows (anorexia, abortion, death) (Polaček et al., 2021). However, the last outbreak in 2019 was confirmed in another region, at the border with Romania in the south-east area of Vojvodina province (Petrović et al., 2021). The distance between the first and the last outbreaks was estimated to about 185-190 km. According to the results of epidemiological investigations, it was concluded that these outbreaks represent most probably two separate introductions from a neighbouring country - Romania (Polaček et al., 2021). In Europe, the long distance ASF transmission has been associated with the disposal of infected waste, meat or meat products in wild boar habitat, for example, in the Czech Republic, where the closest ASF cases were about 400–500 km away (Jurado et al., 2018; Bellini et al., 2021). Similarly, in 2017, in Romania, the first detection of ASF was confirmed in a domestic pig backyard, and contaminated Ukrainian meat products were suspected as the most likely source of ASFV (Zani et al., 2019). Subsequently, the Romanian domestic pig sector was affected with more than 3800 outbreaks from 2017 to 2020 (Ardelean et al., 2021). According to the results of epidemiological investigation in Serbia, it has been suggested that ASFV was most probably brought in with the different meat products from the affected neighbouring country - Romania (Milićević et al., 2019; Nešković et al., 2021; Polaček et al., 2021).

At the beginning of 2020, new cases of ASF outbreaks were registered in the south-eastern region of Serbia in a wild boar population. From then on, there have been continuous outbreaks in domestic pigs and wild boars along the country border with Romania and Bulgaria (Petrović et al, 2021; Polaček et al., 2021). At the beginning of 2021, a disease outbreak was confirmed in domestic pigs but for the first time on a large commercial pig farm, near the

border with Romania. The first clinical signs were notified in the gestating stable: high fever and mass abortions in pregnant sows, regardless of gestation stage were detected (Nešković et al., 2021). Similar findings were reported in the ASF outbreaks on a commercial pig farms in other countries (Lamberga et al., 2020; Liu et al., 2021). Afterwards, ASF was spreading to numbers of smallholdings and backyards and wild boars in different localities in the south-eastern and central part of Serbia. Additionally, in 2021 the ASF was detected 2021 in the wild boar population in the immediate neighbouring area with Romania, South Banat region of Vojvodina Province. This was the first case of ASFV confirmation in wild boar population located in one organised enclosed hunting ground (Petrović et al., 2021). From then on, ASF is continuously present in the south Banat, in backyards population located in several villages and wild boars located in open hunting grounds. The epidemiological situation in Serbia is somehow different comparing the EU countries (Chenais et al., 2018): the occurrence of ASF in wild boars seems to have a strong connection with the presence of the virus in backyards and immediate environment in/around villages. Indeed, in the most cases ASF occurred in wild boars located in areas which have previously been declared as an infected area, i.e., the first cases in domestic pigs were firstly notified (Polaček et al., 2021).

THE ORGANISATIONAL STRUCTURE OF DOMESTIC PIG PRODUCTION SECTOR IN SERBIA

In the Western Balkan region, the existence of highly variable pig farming system was reported (Prodanov-Radulović et al., 2015; EFSA, 2019). Considering the situation in Serbia, the main and highly important difference as compared to the EU member countries is related to the structure and organisation of domestic pig sector (Prodanov-Radulović et al., 2020; Prodanov-Radulović et al., 2020b). Serbia has the highest pig density of all countries in the Balkan region, with a total population of 2.7 million pigs (EFSA, 2019). However, the estimated number of pigs has fluctuated over the years, and nowadays it shows constant decreasing. Based on the official data, there were 2,983,102 pigs in 2020, and from that number 1,260,970 were in Vojvodina Province (Polaček et al., 2021). The official data of the Veterinary Directorate differ from the above due to the fact that only marked individuals are recorded in the database. However, in terms of percentage, over 50% of the domestic pig population is located in production units with very low or no biosecurity measures (Prodanov-Radulović et al., 2020a; Polaček et al., 2021). According to the official data, more than 40% of the people live in rural areas, and 36% of the total population is poor or at risk for poverty (EFSA, 2019). The above data indicate

that this could be the actual percentage of human population oriented towards extensive pig production sector. Serbia has started to harmonise the legislation related to animal diseases with the EU, but it is still ranked among the most vulnerable for disease outbreaks in Europe (EFSA, 2019; EFSA, 2022).

In the EU, pig farms are classified into three categories: non-commercial farms (pigs kept only for fattening for own consumption and neither pigs nor any of their products leave the holding); commercial farms (sell the pigs or move pig products off the holding) and outdoor farms (pigs kept temporarily or permanently outdoors) (Bellini et al., 2021). Rather than taking into account the farm size, this classification considers the commercial attitude of the holdings. In this way, it controls two important facts: the risk of ASF spreading by trading pigs and the risk for the farm of being exposed to source of infection (EFSA, 2019; EFSA, 2022). However, the pig farming in Serbia include five different pig production holdings: commercial pig farm (industrial pig production); family farm of type A (farm with more than 10 animals and high level of biosecurity); family farm of type B (farm with more than 10 animals and low level of biosecurity); backyards (few animals/less than 10 pigs kept mainly for self-consumption, with a low or total absence of biosecurity) (Prodanov-Radulović, et al., 2020; Prodanov-Radulović et al., 2021). Finally, the last type includes free-range and semi-free-range pig keeping, where domestic pigs often share the habitat with wild boars (Prodanov-Radulović et al., 2015; Polaček et al., 2021). From the aspects of domestic pig population density, farms are present through the whole country territory, with the highest densities in northern parts (Vojvodina Province) along the borders with Romania, Hungary and Croatia (Prodanov-Radulović et al., 2018; Prodanov-Radulović et al., 2020a). Beside industrial pig farms, backyards and smallholdings are a kind of traditional family type units. Indeed, in some parts of the Vojvodina region (especially in Srem and Mačva district), domestic pigs are dominantly managed under a backyard and smallholdings system (Prodanov-Radulović et al., 2015; Prodanov-Radulović et al., 2020b). Certainly, the key biosecurity measures are not easy to implement in this type of farming system (Chenais et al., 2019b; Polaček et al., 2021). According to the epidemiological investigations, the majority of the extensive units are incompletely or only partially fenced, and access of other persons is not controlled. The introduction and purchase of new animals (piglets, breeding animals) does not imply quarantine measures and health controls. Moreover, due to stables' construction characteristics, the vehicles that transport food or animals to the slaughterhouse have to enter directly the farm perimeter (Prodanov-Radulović et al., 2017a). The most important risk factors that promote the introduction and spread of the ASFV

are multiple: poor farming practices and low biosecurity, introduction of purchased pigs from internal trade with no quarantine measures, human activities and factors often related to society and the cultural background of the farmers (EFSA, 2019; Zani et al., 2019).

BIOSECURITY CHALLENGE IN COMMERCIAL PIG PRODUCTION SECTOR IN SERBIA

Despite the fact that all the mentioned different pig production units are highly diverse, according to the Serbian Law, they are in the category of commercial holdings. Actually, anyone who has a registered pig holding (regardless of number/type of production) and carries out the official pig labelling can obtain the necessary veterinary health certificate and sell pigs on the country market (Prodanov-Radulović et al., 2015; Prodanov-Radulović et al., 2020a). Of course, the actual commercial production implies intensive pig production from farrow-to-finish and/or farrow-to-piglets or fattening only. Lately, the commercial fattening units can be found quite frequently in the villages, where weaned pigs imported from ASF-free EU member countries are placed (Prodanov-Radulović et al., 2017a; Prodanov-Radulović et al., 2020b). Within the actual pig unit classification, there are substantial differences in only one classified group, i.e., in the commercial pig holdings. In general, the true commercial pig farms include the classical pig farm structure according to the technological pig production process. Again, there is a difference between old types of pig holdings (built in the period 1970-1980s) and farms built after 2000, where the breeding pigs imported from EU are mainly located. The number of these farms is actually small, and they are usually a part of the regional companies (Prodanov-Radulović et al., 2020b). On the other hand, there are commercial pig holdings, which in were earlier owned by the state (socialist model of governance). In the 1990s, farms were privatized but the new owners have not been obligated to invest in the modern concept of biosecurity. Indeed, today we have examples of a large production system, with capacity from 450 to 1300 sows that is still managed by the old type of farrow-to-finish production system. Some of the biosecurity measures that we know today, which are essential for sustainable pig production, are not possible to be implement without major investments (Prodanov-Radulović et al., 2020a; Prodanov-Radulović et al., 2021). Indeed, the external biosecurity measures cannot always be applied in these systems (entrance the transport vehicles in farm perimeter, farm workers with direct contact with the backyard population, entering the different sources of food for employees) (Prodanov-Radulović et al.,

2021; Polaček et al., 2021). The problem of transport vehicles stands out, and the situation is similar on most of the farms, i.e., the vehicles enter the farm perimeter (animal feed transport, public rendering services, slurry transport and transport to the slaughterhouse). Nowadays, a major problem from the biosecurity aspect is the lack of workers. It is very difficult to find workers who do not have domestic pigs in their backyards or have any kind of contact with domestics and/or wild boar after working time (Prodanov-Radulović et al., 2017b; Nešković et al., 2021; Angeloni et al., 2022). So, the request for workers not have any contact with domestic pigs outside of their employment is very difficult to implement.

In Serbia, the biosecurity measures are not officially required by the Law, and are only specified in a form of general recommendations. Indeed, in the latest Government Instruction for ASF it is only requested that commercial and family farms need to have officially written and implemented biosecurity plan. However, this does not include the details what the biosecurity plan needs to include (Polaček et al., 2021). In the EU member countries, formal biosecurity plans are generally focused on commercial holdings that exceed a specific size defined by the national legislation (Bellini et al., 2021). Regardless of the type of production, high levels of farm biosecurity are considered the most important tools for preventing introduction of ASFV. Biosecurity measures on farms and especially at the farm entrance (thorough cleaning and disinfection and personal protective equipment) have an important role in any type of pig production (Bellini et al., 2021; Beato et al., 2022). Lamberga et al. (2020) recently described an ASF outbreak at a large commercial pig farm in Latvia, where the weakest points identified were the entrances of the farm. The other risk factors identified in this study are similar as the present ones in Serbia: different vehicles entering the farm perimeter and the possibility that farm employees were involved in activities linked to wild boars (Lamberga et al., 2020). Pig farms need to pay special attention to the infrastructure construction of the staff entrance and sanitary lock (Jurado et al., 2018; Lamberga et al., 2022). The tenacity and resistance to inactivation of ASFV are important aspects that make external biosecurity control hard to manage. It is well-known that ASFV survives for 11 days in faeces at room temperature, one month in contaminated pen, 18 months in blood stored at 4 °C, and several years in frozen meat (Mazur-Panasiuk et al., 2019; Štukelj et al., 2021). Effective disinfection against ASFV can only be achieved when recommended concentration of disinfectant is used and contact time is ensured (De Lorenzi et al, 2020; Liu et al., 2021; Beato et al., 2022).

BIOSECURITY CHALLENGE IN BACKYARD AND SMALLHOLDER PIG PRODUCTION IN SERBIA

Rearing domestic pigs in backyards is a common and traditional practice in the south-eastern countries of Europe (EFSA, 2019; Zani et al., 2019; Andraud et al., 2021). Smallholdings and backyards represent about 60% of the domestic pig holdings in Serbia. In the villages, mixed backyard farming systems are common, with different livestock (pigs, sheep, cattle, chicken) and cropping systems, and with a focus on subsistence farming (Prodanov-Radulović et al., 2015; Prodanov-Radulović et al., 2017a). The primary purpose for keeping pigs is regular family meat supply but also an extra income. The majority of households have one or two sows together with the piglets and fatteners (Polaček et al., 2021). The designation 'backyard holding' stands for a quite heterogeneous family-run small-scale pig farming system with low biosecurity (Zani et al., 2019; Mutua and Dione, 2021; Ardelean et al., 2021). In EU countries, this type of holdings is classified as non-commercial pig sector with farms including 10 pigs per holding (Bellini et al., 2021). Backyard farms with their low biosecurity standards are considered prone to ASF introduction and thus are of particular interest in disease prevention and control (Sanchez-Vizcaıno et al., 2013; Zani et al., 2019; Mutua and Dione, 2021).

In backyards and smallholders, pig feeding strategies depend on feed resources availability and the ability of farmers to buy ingredients. In these systems, domestic pigs frequently have access to swill (i.e., the kitchen leftover food from owners and restaurants) (Chenais et al., 2019a; Mutua and Dione, 2021). According to the law, swill feeding is banned in Serbia; however, it is difficult to control in practice. Similarly, swill feeding was banned in the EU in 2002, but the epidemiological studies of ASF outbreaks have shown that this practice is still used (Boklund et al., 2020). Such practices can facilitate ASFV spread, as happened in 2012, when ASF was introduced in Ukraine due to the use of contaminated pork products in swill feeding (EFSA, 2019; Bellini et al., 2021). In general, swill is considered to be the most likely source of ASF introduction in Georgia (de la Torre et al., 2022), Bulgaria (Zani et al., 2019) and Romania (Ardelean et al., 2021). Swill feeding represents an important risk for indirect ASF transmission because of the long term survival of the virus in pig meat (Bellini et al., 2021). The ASFV can survive in chilled meat or carcasses for up to 6 months, and at 4 ˚C even for two years. Similarly, virus can survive for long periods of time in smoked and salted pork products (Sanchez-Vizcaıno et al., 2013; Sánchez-Cordón et al., 2018).

The existence of breeding animals in the backyards (gilts, sows, boars) is highly-risky situation in the extensive production system. Moreover, in Ser-

bia, breeding boars are frequently found in the small-scale holdings, and they are moved around for breeding purposes (Prodanov-Radulović et al., 2015; Polaček et al., 2021). The critical point from this aspect is animal loan practices, i.e., sharing of boars for natural mating in several villages (Mutua and Dione, 2021). This can be significant risk factor contributing to the transmission of ASFV through direct pig-to-pig contact (Olesen et al., 2020). The aforementioned specific circumstances are likely to contribute to the introduction and establishment of ASFV in Serbian vulnerable pig production system thus promoting the disease spreading. On the contrary, according to the EU regulations, sows or boars cannot be held on non-commercial farms for mating purposes (Cappaia et al., 2018; Bellini et al., 2021).

Another important risky activity in backyards and smallholdings is related to home-slaughtering practice. Backyard pigs are mostly slaughtered at home, usually before Christmas or whenever new meat supplies for family are needed (Polaček et al., 2021). Home-slaughtering is considered as a feature of non-professional pig production, which is a known constraint to ASF control (EFSA, 2019; Bellini et al., 2021). Such practices contribute to the spread of ASFV due to improper disposal of offal, often in the immediate environment of the village, and the use of slaughter waste for feeding other domestic animals in the yard (dogs, cats). Finally, home-slaughtering could be a driver for the spread of the other infectious and zoonotic diseases (Petrović et al., 2019; Petrović et al., 2022). However, home slaughtering of domestic pigs is allowed in Serbia.

It is well-known that implementation of biosecurity is a key to successful pig production in an ASF-endemic environment (Bellini et al., 2021; Gervasi and Guberti, 2021). Several studies have recommended training of pig farmers on biosecurity measures as a means of mitigating ASF. In a specific smallholder pig sector such as Serbian, additional costs for application of biosecurity, availability of funds are key barriers to adopt better practices (Polaček et al., 2021; Angeloni et al., 2022). In the current systems, farmers rely on cheap biosecurity and animal management measures to sustain their pig production; however, these practices are not sufficient to stop ASF transmission (Andraud et al., 2021; Ardelean et al., 2021). Relevant stakeholders need to be educated about implementation of biosecurity measures in an effort to mitigate the risks (Mutua and Dione, 2021). Further, backyards could facilitate the introduction of ASFV from wild boar population to domestic pigs and vice versa (Gervasi and Guberti, 2021). Low biosecurity farms and the human factor that creates link to wild boars around the villages are deemed to be the most dangerous combinations for the spread and persistence of ASFV in domestic pig sector (Nešković et al., 2021). Indirect contact through visiting the yard by the

neighbours or via shared different mechanical equipment or vehicles cannot be excluded. Thus, direct or indirect contact to contaminated fomites, which entered the stable via human activities, is regarded as the most likely source of infection (Zani et al., 2019; Bellini et al., 2021). In the case of ASF outbreak in one backyard, the whole village needs to be regarded as one epidemiological unit. The clinical phase starts usually after an incubation period of about 3–5 days (Blome et al., 2020), which would be the earliest time point during the ASF infection when the owner might suspect that one of his pigs is sick. In the backyard context, detecting suspect animals depends nearly exclusively on the pig owner. However, ASF could remain unreported for longer period due to a constant supply of susceptible pigs (Liu et al., 2021). The slow spread of the disease from pig to pig hampers early disease detection as it leads to initially low mortalities (Schulz et al., 2019).

Outdoor keeping, semi-free range or free range pigs is common in some regions in Serbia (Prodanov-Radulović et al., 2015). This type of pig keeping represents one of the weakest links in the biosecurity chain and the biggest risk factor for ASF introduction. The interaction between wild boars and pigs can prolong ASFV circulation, as observed in Sardinia (Cappaia et al., 2018). The free-ranging pigs can act as a bridge in transmitting ASFV between wild boars and domestic pigs (Sauter-Louis et al., 2021; Ardelean et al., 2021). Above all, allowing domestic pigs to roam freely is a concern, for not only ASF but also for diseases of public health importance (Petrović et al, 2019; Petrović et al., 2022). In the EU, concerning current ASF situation, the EU Commission has banned outdoor keeping of pigs as the main strategy to avoid ASF spread (EFSA, 2022).

Serbia has a central geographical position in the Balkans (EFSA, 2019), surrounded by the ASF positive countries on three sides: north, east and south (Hungary, Romania, Bulgaria, and recently North Macedonia) (EFSA, 2022). The global dimension of the current epidemic shows that all countries are at risk: human-mediated dispersal to domestic or wild boar populations can occur at any time and to any country, regardless of the distance from on-going infections (Chenais et al., 2018; Chenais et al., 2019a; de la Torre et al., 2022). Cooperation among countries and information exchange plays a key role in better understanding ASF epidemiology, and in implementing timely and appropriate preventive measures.

CONCLUSION

Pig production in Europe is highly heterogeneous with different biosecurity standards. However, in the pig systems like the one in Serbia, farmers rely

on cheap biosecurity and animal management measures, which are often not sufficient to prevent or control ASF. Farmers still have significant knowledge gaps in view of ASF and practice various risky behaviours that might favour disease spread. The ongoing practices of natural mating, home-slaughtering and swill feeding can be identified as main challenging biosecurity risk factors.

ASFV has a history of more than 100 years worldwide. It is anticipated that it will continue to threaten the pig industry in countries around the world for a long time in the future. Our work reviewed the main risk factors involved in the introduction and spread of ASF in Serbia and this information can be relevant in assessing the risk level of different holdings in order to plan specific preventive measures. We can conclude that different types of risks affect different types of farming systems, and they need to be considered when preparing a biosecurity program. In the future, the study with insights into the ASF knowledge of backyard farmers can be helpful to identify predominant risky practices carried out by them. These insights may help to better understand the role of backyard farmers in the ASF epidemic in Serbia and to improve future evidence-based policies, including the development of new public awareness activities.

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JPR and MJ made substantial contributions to conception and design of the review, wrote the main part of the article; BB and JM helped in formatting and translating the text, and were involved in drafting of the manuscript, VP revised the manuscript critically and gave the final approval of the manuscript to be published.

Competing interest

The authors declare that they have no competing interest.

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THE MOST COMMON ANTHROPOZOONOSES IN THE REPUBLIC OF SRPSKA IN THE PERIOD 2015 – 2020

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Abstract

Zoonotic diseases are increasingly becoming an emerging public health threat, partially due to the risk of spillover events at the human-wildlife interface. Their potential for infecting people with exotic pathogens originating from unusual pets should not be overlooked. The aim of the study is to present and analyze the trend of zoonoses in the 2015-2020 period using the descriptive method. The source of data is reports of single cases of infectious diseases, which is in accordance with the applicable legislation governing this area. The incidence of anthropozoonoses was the highest in 2017 amounting 16.5/100,000, while the lowest value in this five-year period was in 2020, with 1.1/100,000. The share of anthropozoonoses in the total incidence of infectious diseases was also the lowest in 2020, with the value of 0.02%, while the highest share of this group of diseases was recorded in 2017 with a value of 1.42%. In the specified period, the three most commonly reported anthropozoonoses are Q-febris, leptospirosis, and brucellosis. In 2020, the most frequently registered anthropozoonosis was toxoplasmosis, while in the previous 5 years, this disease was not reported among the three most common. It is necessary to raise awareness about the presence of zoonoses in the overall incidence of infectious diseases in the Republic of Srpska, because due to the common non-specific clinical picture, zoonoses are not the first to be considered in differential diagnosis. In the fight against zoonoses, a coordinated approach to "One Health" is necessary, which will enable design and implementation of programs, policies, legislation and research in the area of public health.

Key words: zoonosis, incidence, One Health, Republic of Srpska

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NAJČEŠĆE ANTROPOZOONOZE U REPUBLICI SRPSKOJ U PERIODU 2015 - 2020

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Kratak sadržaj

Zoonotske bolesti sve više postaju nova prijetnja javnom zdravlju, djelomično zbog rizika od bliskog kontakta i narušavanja granice čovjeka i divljeg svijeta. Ne treba zanemariti ni mogućnost zaražavanja ljudi egzotičnim patogenima porijeklom od neobičnih kućnih ljubimaca. Cilj studije je prikazati i analizirati kretanje zoonoza u periodu 2015-2020. godine korišćenjem deskriptivnog metoda. Izvor podataka su pojedinačne prijave slučajeva zaraznih bolesti, što je u skladu sa važećom zakonskom regulativom koja uređuje ovu oblast. Incidencija antropozoonoza je najviša bila 2017. godine i iznosila je 16,5/100.000, dok je najniža vrijednost u ovom petogodišnjem periodu bila u 2020.godini i iznosila je 1,1/100.000. Učešće antropozoonoza u ukupnom obolijevanju od zaraznih bolesti najniže je bilo takođe u 2020. godini i iznosilo je 0,02%, dok je najveće učešće ove grupe oboljenja zabilježeno 2017.godine sa procentom učešća od 1,42%. U posmatranom periodu, tri najčešće prijavljivane antropozoonoze su Q-febris, leptospiroza i bruceloza. U 2020. godini je najčešće registrovana antropozoonoza bila toksoplazmoza, dok u prethodnih 5 godina ova bolest nije bila prijavljena ni u tri najčešće. Neophodno je podizanje svjesnosti o prisustvu zoonoza u ukupnom obolijevanju od zaraznih bolesti u Republici Srpskoj, jer zbog veoma često ne specifične kliničke slike, zoonoze nisu prve u razmatranju u diferencijalnoj dijagnostici. U borbi protiv zoonoza neophodan je koordinisan pristup strategiji „Jedno zdravlje" koji će omogućiti dizajniranje i primjenu programa, politika, zakonodavstva i istraživanja u javnom zdravlju.

Ključne riječi: zoonoze, incidencija, Jedno zdravlje, Republika Srpska

INTRODUCTION

Six in ten human cases of infectious disease arise from animal transmission (Center for Disease Control, 2018). Fifty years ago, following the wide-

scale manufacture and use of antibiotics and vaccines, it seemed that the battle against infections was being won for the human population. Since then, however, and in addition to increasing antimicrobial resistance among bacterial pathogens, there has been an increase in the emergence of zoonotic diseases originating from wildlife, sometimes causing fatal outbreaks of epidemic proportions. Zoonosis is defined as any infection naturally transmissible from vertebrate animals to humans. In addition, many of the newly discovered diseases have a zoonotic origin. Due to globalization and urbanization, some of these diseases have already spread all over the world, caused by the international flow of goods, people and animals. However, special attention should be paid to farm animals since, apart from the direct contact, humans consume their products, such as meat, eggs, and milk. Therefore, zoonoses such as salmonellosis, campylobacteriosis, tuberculosis, swine and avian influenza, Q fever, brucellosis, STEC infections, and listeriosis are crucial for both veterinary and human medicine. Consequently, in the suspicion of any zoonoses outbreak, the medical and veterinary services should closely cooperate to protect the public health (Libera et al., 2022). Zoonotic diseases, particularly those associated with livestock and poultry, are becoming an increasing threat for public health for various reasons. For example, the predictions suggest that the global human population will constantly increase and reach almost 10 billion by 2050. Consequently, it will result in a higher food demand (United Nations, 2019). One Health is an effective approach for the management of zoonotic disease in humans, animals and environments. Examples of the management of bacterial zoonoses in Europe and across the globe demonstrate that One Health approaches of international surveillance, information-sharing and appropriate intervention methods are required to successfully prevent and control disease outbreaks in both endemic and non-endemic regions. Additionally, One Health approach enables effective preparation and response to bioterrorism threats (Cross, 2018).

Diagnostics plays a key role in disease surveillance. Misdiagnosis results in inappropriate treatment, or missed opportunities to prevent further disease transmission. The zoonoses discussed in this paper often present as undifferentiated febrile illnesses, and so a detailed history is key to diagnosis. More common ailments with similar symptoms are initially suspected, and diagnosis may be missed altogether in self-limiting cases (Gunaratnam et al, 2014).

It is almost certain that large-scale zoonotic disease outbreaks will almost certainly continue to occur regularly in the future. Therefore, a better general understanding of the factors affecting variation in the severity of outbreaks is critical for well-being of the global community (Stephens et al, 2021). En-

demic zoonoses continue to be relatively neglected, often with a lack of local and international realization of the extent to which they impact human health and well-being. This is partly due to the issues surrounding local capacity and knowledge and partly because, unlike emerging infectious diseases, they are not seen as a threat to people in the developed world. Both EIDs and endemic zoonoses, however, can be tackled using the One Health approach, which includes the identification and mitigation of human activities that lead to disease emergence and spread (Cunningham et al, 2017). In the fight against zoonoses, a coordinated approach to "One Health" is necessary, as it will enable the design and implementation of programs, policies, legislation and research in the field of public health.

The aim of the study is to present and analyze the trend in zoonoses during the 2015-2020 period using the descriptive method. The source of data is reports of single cases of infectious diseases, which is in accordance with the applicable legislation governing this area.

MATERIAL AND METHODS

No ethical approval was obtained because this study did not involve laboratory animals. Only non-invasive procedures were used.

As part of epidemiological surveillance, an analysis of the data obtained from monitoring the trend of anthropozoonosis according to European Union (EU) case definitions was performed (European Commission, 2018). EU definitions have been part of the national legislation for years now, and they are regulated by law. Using the descriptive method, the data obtained from all 54 primary health centers, as well as 10 hospitals in Republic of Srpska, were analyzed. The data were obtained through the Notification of Infectious Diseases, which is an official and binding document for every doctor who registers and thus reports an infectious disease, which is regulated by regulations and law. The disease reports are sent from these institutions to the Public Health Institute of the Republic of Srpska which analyzes the data and generates official reports. The trend of anthropozoonoses in the mentioned period is described and the three most common anthropozoonoses for each year are determined. The case of each disease is classified as possible - probable - confirmed on the basis of the national case definition criteria. Using statistical analysis with statistical software SPSS 23, we compared the incidences of these diseases, while patient demographics were analyzed and statistically processed using chi-squared (χ^2) test. This test was used to determine whether there is a statistically significant difference between the observed frequencies of the three

diseases in the observed groups and the frequencies of the same groups in the general population.

RESULTS

The incidence of anthropozoonoses was the highest in 2017 with 16.5/100,000, while the lowest value in this six-year period was in 2020, amounting to 1.1/100,000 (Figure 1).

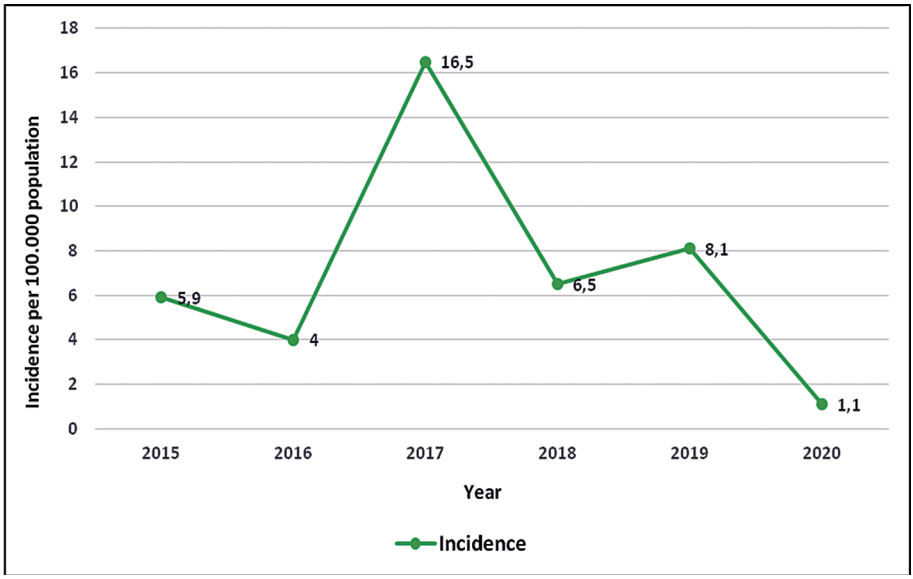

Figure 1. Incidence of anthropozoonosis per 100,000 in the Republic of Srpska 2015-2020

The share of anthropozoonoses in the total incidence of infectious diseases was also the lowest in 2020 and amounted to 0.02%, while the highest share of this group of diseases was recorded in 2017 with a value of 1.42%. In the specified period, the three most commonly reported anthropozoonoses were Q-febris, leptospirosis, and brucellosis.

The analysis of the collected data from epidemiological surveillance in the mentioned period, showed that there was a total of 283 cases of these three diseases, and the incidence trend shows that the incidence was the highest in 2017, high in 2016, 2018 and 2019, while in 2020 the incidence was very low (Table 1).

Table 1. Number of reported zoonoses, the percentage and incidence of the 3 most common zoonosis by year

Year	Total No. of zoono-sis	Brucellosis		Leptospirosis		Q-febris	
		% share in all reported zoonosis	Incidence	% share in all reported zoonosis	Incidence	% share in all reported zoonosis	Inci-dence
2015	83	14.45	1.03	15.66	1.12	10.84	0.77
2016	56	16.07	0.78	14.28	0.69	57.14	2.76
2017	191	6.8	1.13	16.75	2.78	14.65	2.43
2018	75	45.33	2.96	6.66	0.44	21.33	1.39
2019	93	9.67	0.79	25.8	2.10	30.1	2.45
2020	12	50	0.53	33.3	0.35	8.33	0.09

If we separate the incidence for each of these three diseases individually, we come to the following information: a total of 83 cases of brucellosis were reported in that period, and the incidence trend shows that the incidence of brucellosis was the highest in 2018 (2.96% 000), and significantly lower in other seasons. In the observed period, a total of 86 cases of leptospirosis were reported, and the incidence trend was the highest in 2017, with the incidence of 2.78% 000, high in the 2019 season with the incidence of 2.1% 000 and significantly lower in other seasons. 114 cases of Q-fever were reported in the same period, with the incidence trend that was highest in the 2016 season and an incidence of 2.76% 000, high in the 2017 (2.43% 000) and 2019 seasons (2.45% 000) and significantly lower in other seasons (Figure 2).

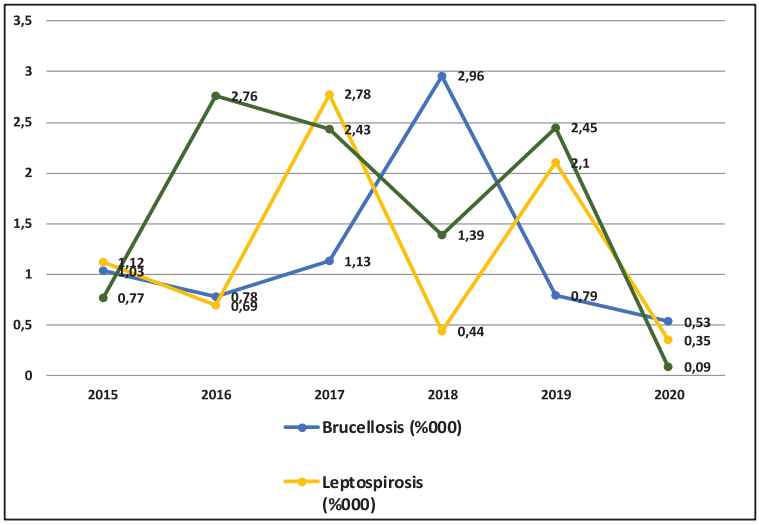

Figure 2. Incidence of Brucellosis, Leptospirosis and Q-Febris in the Republic of Srpska 2015-2020

Based on the results of the χ ^ 2 test (χ ^ 2 = 56.993; p = 0.000), it can be concluded that there was a statistically significant difference in the total number of patients of all three diseases by sex. Statistically significant (p =0.000, < 0.05) there was a higher number of male patients than the number of female patients, compared to the number of men and women in general population observed for all three diseases together as well as for each of the diseases individually (Figure 3).

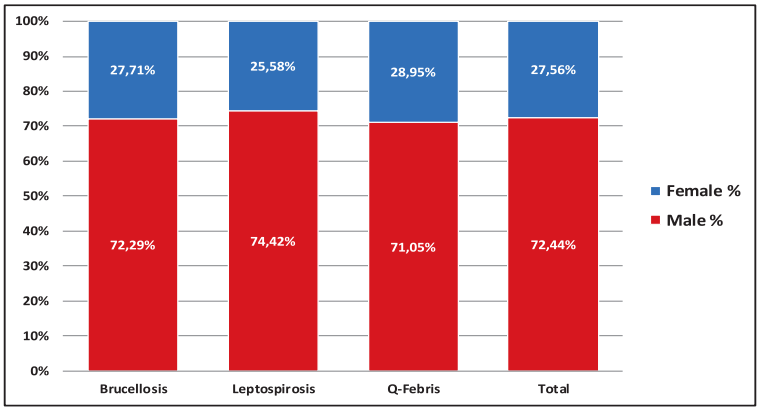

Figure 3. Sex distribution (%) of Brucellosis, Leptospirosis and Q-febris cases in the Republic of Srpska 2015-2020

The data obtained from epidemiological population surveillance from 2015 to 2020 in the Republic of Srpska show that out of a total of 283 patients with brucellosis, leptospirosis and Q-fever, 155 or 54.77% live in urban areas and 128 or 45.23% in rural areas. However, statistical analysis of data for each of the diseases separately reveals that there are significant differences in this regard between these three diseases. Namely, the results of the χ ^ 2 test for brucellosis (χ ^ 2 = 1.157; p = 0.282) and leptospirosis (χ ^ 2 = 2.110; p = 0.146) show that there was no statistically significant difference in the number of patients according to the type of settlement in which they lived, while in patients with Q fever (χ ^ 2 = 33,778; p = 0,000) this statistical difference is significant, as indicated by the relatively high value of the χ ^ 2 test and the high probability (p = 0.000, <0.05) for the accuracy of that statistical difference (Figure 4).

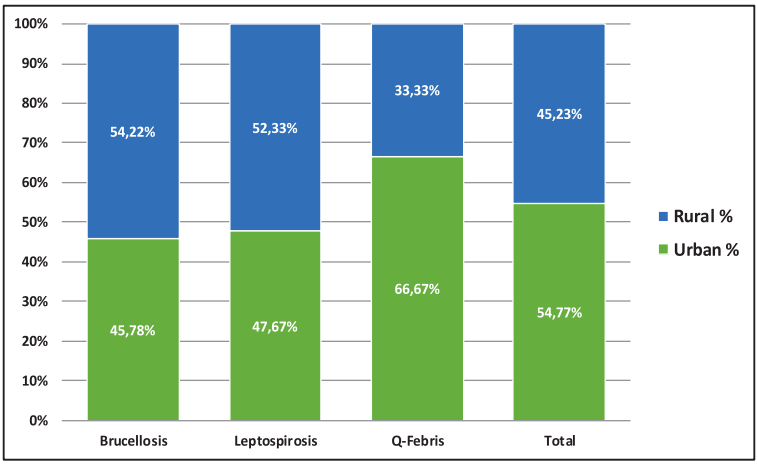

Figure 4. Urban/rural cases distribution of Brucellosis, Leptospirosis and Q-febris in the Republic of Srpska 2015-2020

The average age of patients with brucellosis, leptospirosis and Q-fever in the Republic of Srpska from 2015 to 2020 was 48.34 (± 16.96) years. The largest number of patients at the time of illness in the total number and individually in relation to each disease belonged to the age group of 50 - 64 year (34.98%), followed by groups of 30 - 49 years (30.74%), 65 - 79 years (16, 61%) and the 20 - 29 age group (11.31%). These data show that the working age group of population aged 20 to 64 suffered the most (77.03%) (Figure 5).

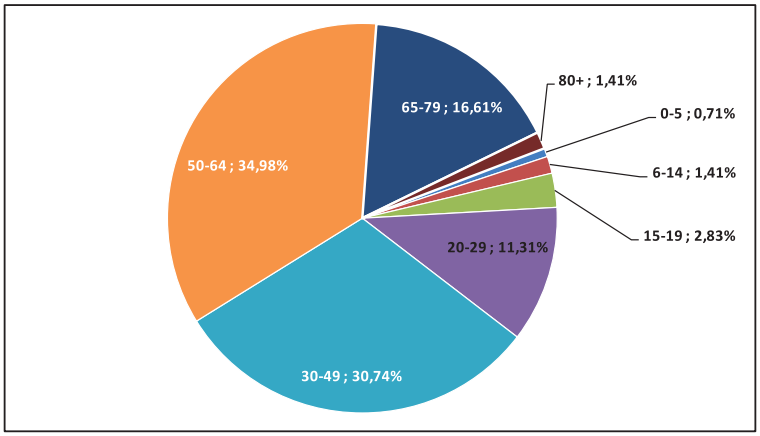

Figure 5. Age distribution of Brucellosis, Leptospirosis and Q-febris cases in the Republic of Srpska 2015-2020

The analysis of the regional distribution of patients by each of the three diseases, and in total, we come to the result that the largest number of reported cases was registered in the Banja Luka region, and lowest in the Trebinje region. When it comes to the reports on the outbreaks of these three infectious diseases in the given period, one epidemic of Q fever was reported in 2016 in Banja Luka, where 30 patients were registered and that correlates with the trend.

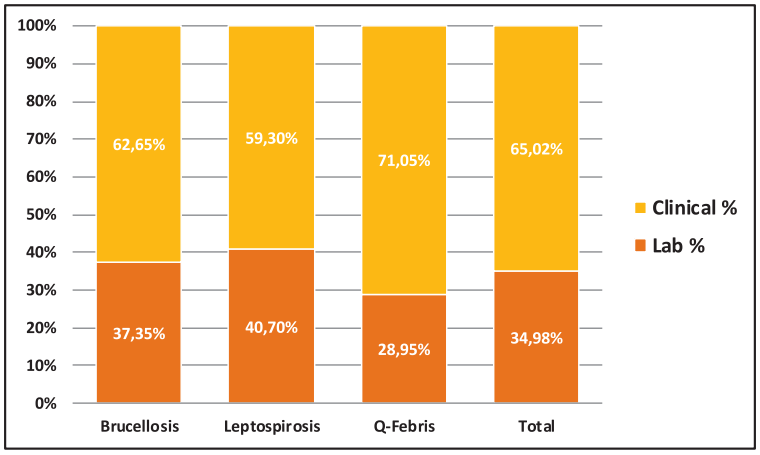

Figure 6. Brucellosis, Leptospirosis and Q-febris cases confirmation in the Republic of Srpska 2015-2020

According to data from population surveillance, the diagnosis of brucellosis, leptospirosis and Q-fever was clinically established in 184 cases (65.02%) (probable cases), which is significantly more than 99 cases (34.98%) in which the diagnosis was confirmed in the laboratory (confirmed cases). There is a similar relationship in terms of the method of establishing the diagnosis for each of the three diseases, with the number and share of clinically made diagnoses (probable cases) being highest in patients with Q fever (81 cases and 71.05%) while there were 33 confirmed cases (28.95%). The number and share of laboratory confirmed cases is the highest in patients with leptospirosis (35 cases and 40.7%), while there were 51 probable cases (59.3%). The number and share of probable cases of brucellosis was 52 (62.65%), while there were 31 confirmed cases (37.35%) (Figure 6).

DISCUSSION

Communicable disease epidemiology is closely linked to pathogen ecology, environmental and social determinants, economic factors, access to care, as well as the state of country development (McMichael, 2012). Climate change continues to have both direct and indirect effects on communicable diseases, often in combination with other drivers, such as increased global travel and trade. The frequency, duration, and intensity of heat waves has increased across Europe, and the last decade was the warmest ever recorded (World Meteorological Organization, 2013).

A global, integrated zoonotic disease surveillance system needs to detect disease emergence in human or animal populations anywhere in the world at the earliest time possible. An effective global, integrated zoonotic disease surveillance system requires effective surveillance at national, regional, and international levels, because information from outbreak investigations is used by human and animal health officials at all levels to implement response measures and evaluate the effectiveness of those responses.

Emerging zoonotic diseases can occur any time in any part of the world. Therefore, it is difficult to predict which pathogens may emerge, which human and or animal populations it may affect, or how these pathogens may spread. From a growing number of experiences, the world has learned that it is crucial to detect and report emerging zoonotic disease outbreaks that occur in a single country or region. Early detection and reporting at the local level give the international community an opportunity to assist national authorities and implement effective response measures (Keusch et al, 2009).

Q fever is a severe, zoonotic disease spread worldwide and caused by *Cox-*

iella burnetii. This disease was first described by Derrick in 1937 following an epidemic fever outbreak among employees at a slaughterhouse in Brisbane, Australia (Derrick, 1937). Q fever can manifest as an acute disease, usually as a self-limited febrile illness, pneumonia, or hepatitis. It may also occur as a persistent focalized infection with endocarditis. The main reservoirs of *C. burnetii* are cattle, sheep, and goats, but infections were detected in other animals such as domestic mammals, marine mammals, reptiles, birds, and ticks (Eldin et al, 2017). *C. burnetii* is most abundant in aborted fetuses, amniotic fluid and placenta after stillbirth or normal birth of offspring from infected mothers, and in the urine, feces and milk of infected animals. Transmission to humans most commonly occurs through inhalation of aerosolized bacteria from the placenta (delivery or abortion), feces, or urine of infected animals. Human-to-human transmission is extremely rare. In humans, the diagnosis of Q fever is mainly established by serology, microbiological cultures, or PCR tests (Pechstein et al, 2018; España et al, 2020).

Brucellosis is caused by the intracellular pathogens from *Brucella* genus. Four *Brucella* species can infect humans: *B. abortus, B. canis, B. melitensis*, and *B. suis*. Brucellosis may be transmitted to humans through contaminated food and dairy products, by occupational contact, or inhalation of infected aerosols. Another important route of infection is the contamination of mucous membranes or open wounds with fetal fluids, making veterinarians, farmers, and abattoir workers the most susceptible to infection. In other cases, transmission from animals to humans is mainly associated with drinking contaminated milk (Amjadi et al, 2019).

Leptospirosis is a widespread bacterial zoonosis occurring most commonly in low-income populations living in tropical and subtropical regions, both in urban and in rural environments. Rodents are known as the main reservoir animals, but other mammals may also significantly contribute to human infections in some settings. Clinical presentation of leptospirosis is nonspecific and variable, and most of the early signs and symptoms point to the so-called 'acute fever of unknown origin' (Goarant, 2016; Adler et al, 2009).

The implementation of the case definition is significant because all reported cases need to be categorized in the same way in accordance with international regulations (Nichols et al, 2014). The introduction of a case definition facilitated the early recognition of these diseases as well as the appropriate direction in their diagnosis and confirmation. This also enables the evaluation of surveillance system through the analysis of the report of each individual case (Mohamed et al, 2019; Katelaris et al, 2019; Debeljak et al, 2018; Boden et al, 2014).

Standards for good laboratory practices overlap with standards for good laboratory network operations. Good laboratory practice principles are simply applied to laboratory facilities that meet proper standards for testing, safety, and security; employ a trained and proficiency-tested staff; have standardized operating procedures, validated test protocols, and properly functioning equipment; and use a communication system that relies on common platforms and accurately and reliably reports test results in a timely manner.

The Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (WOAH – ex OIE), and the World Health Organization (WHO) recognize a joint responsibility to minimize the health, social and economic impact of diseases arising at the human-animal interface by preventing, detecting, controlling, eliminating or reducing disease risks to humans originating directly or indirectly from domestic or wild animals, and their environments. An important aspect of efforts to mitigate potential health threats at the human-animal ecosystems interface is early warning, supported by robust risk assessment to inform decisions, actions, and timely communication between agencies and sectors responsible for human health, animal health, wildlife, and food safety. In 2006, in response to health threats such as H5N1 highly pathogenic avian influenza (HPAI) and the severe acute respiratory syndrome (SARS), the three organizations consolidated their efforts to establish a Global Early Warning System for Major Animal Diseases Including Zoonosis (GLEWS). GLEWS became one of the mechanisms used by the WOAH, FAO, and WHO together for monitoring data from existing event-based surveillance systems and track and verify relevant animal and zoonotic events (FAO-WOAH-WHO, 2010).

Based on the results of the study, it can be seen that the incidence of these diseases was the lowest in 2020. The cause of this drastic decline is largely the outbreak of the COVID 19 pandemic and the fact that it cast a shadow on other diseases due to the enormous burden it imposed on the health system. Pandemic certainly did not change the course of these diseases, but it did make their reporting, adequate diagnosis and anti-epidemic action very difficult. But despite the epidemic, doctors who are the first to receive and treat patients do not consider zoonoses as the first option in differential diagnosis, especially because most of them do not have specific symptoms at the beginning of the disease, and in the most severe clinical phase fever, malaise, headache, muscle aches, pneumonia or even meningitis are the symptoms of many other non-zoonotic diseases. Our doctors were somewhat more cautious about zoonoses in the first few years after the catastrophic floods that hit this region in 2014, but that has changed over time due to the impact of several factors.

In the observed period, out of the three most reported diseases, namely brucellosis, leptospirosis and Q fever, the largest number of reported cases were Q fever cases, with the highest incidence in 2016. The reason for this is the registered outbreak of this disease in the area of Banja Luka. Furthermore, a significantly higher number of people with disease fell ill in urban areas than in rural areas. These data may lead to the conclusion that contact of the urban population with the villages through excursions, hiking, visits to rural families and many other activities that bring this population into contact with the rural area, allows immunologically incompetent population contact with *Coxiella* spores. The fact is close contact with the reservoir animals of this disease is not necessary- it is enough to breathe air with spores that carry this causative agent. Thus, in the area of the city of Banja Luka, there were several outbreaks of this disease in the period before 2010.

A statistically significantly higher number of men contracted these three diseases than women, which traditionally described these zoonoses as "male diseases", mostly because men are more likely to engage in livestock, agriculture and other activities in the nature and with animals.

In terms of age groups, the largest number of patients falls in the range of 20-64 year olds, which leads us to the conclusion that these diseases affected the working population the most, namely those who come into contact with animals and their products through agriculture, livestock, etc.

Based on the results of the analysis, the largest number of patients was registered in the region of Banja Luka, which is also the most populated area in the Republic of Srpska. Hospital and diagnostic capacities are the largest in this region, so the increased reporting of these diseases can be related to that fact.

A significantly higher percentage of reported cases of brucellosis, leptospirosis and Q fever was reported based on clinical criteria. This is certainly something that makes a weak point of the system of control over anthropozoonoses and represents a link that requires significant improvements. Adequate and precise diagnostics are necessary to confirm the case of any infectious disease, which makes the system of supervision and control of these diseases stronger and more reliable. Another weakness of the system is the absence of a unique electronic system for reporting of infectious diseases- health institutions are not connected into IT network which would obtain a flow of information on reports of infectious diseases, outbreaks and all other data that is necessary to analyze the situation or other unexpected health events. For this reason, it is impossible to get all the information about each patient, because most of the reporting and data sharing is paper based or via e-mail at our request. That is why establishing a network of health institutions with the Public Health Insti-

tute of the Republic of Srpska would be one of the main factors of the improvement and strengthening of the infectious disease surveillance system.

CONCLUSIONS

The incidence of anthropozoonoses in the Republic of Srpska in the 2015-2020 period was highest in 2017, and the lowest in 2020. The three most commonly reported diseases were brucellosis, leptospirosis, and Q fever. The reported cases of these three diseases were more common among urban population, the patients being mostly male and a majority of them belonged to the working population. The largest number of cases has been reported as probable without microbiological confirmation, which is stated in the case definition for each disease. It is necessary to improve the reporting of zoonoses in the Republic of Srpska in terms of case confirmation, as well as raising awareness of the frequency and importance of anthropozoonoses of all physicians, especially those who first treat patients.

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Author's Contribution:

NRV – drafted the manuscript and made substantial contributions to the basic idea, JA – made contributions to the basic idea, JĐD - revised the manuscript critically.

Competing interest

The authors declare that they have no competing interests.

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PREVALENCE OF TICKS AND RISK FACTORS ASSOCIATED WITH THE INFESTATION OF SHEEP IN RIVER NILE STATE, SUDAN

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Abstract

A cross-sectional study was conducted in River Nile State, Sudan, between June to August 2018 to determine the prevalence of tick infestation on sheep and the potential risk factors associated with the infestation. A total of 135 sheep from five different localities (Shendi, Al Matamah, Ad-Damer, Atbara, and Berber) were examined and, of these, 90 were tick-infested (66.7%). A total of 340 ticks (male 185, female 155) were collected and identified using zoological taxonomic keys. The most dominant tick species collected in this survey were *Rhipicephalus eversti* Neumann, 1897 (38%), followed by *Hyalomma anatolicum* Koch, 1844 (23.8%), *Rhipicephalus sanguineus* Latreille, 1806 (20.6%), *Rhipicephalus praetextatus* Gerstacker, 1873 (16.4%), and *Hyalomma dromedarii* Koch, 1844 (1.2%). The chi-square analysis showed that there was a significant association ($p \leq 0.05$) between tick infestation and localities, housing type, sex, control of ticks, and removal of manure. The highest prevalence rate was recorded in Atbara, in the open housing type, in females, in farms that did not use acaricides and did not remove the manure frequently. On the other hand, there was no significant association ($p > 0.05$) between tick infestation and herd size, rearing system, breed, age and colour of coat (p=0.846). This study expanded the knowledge on tick fauna and associated risk factors in the River Nile State, and it demonstrated that multiple tick species are infesting sheep with the potential to transmit several tick-borne diseases.

Key words: Ticks, risk factors, sheep, Sudan

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PREVALENCIJA KRPELJA I FAKTORI RIZIKA POVEZANI SA INFESTACIJOM OVACA U DRŽAVI REKA NIL, SUDAN

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Kratak sadržaj

Istraživanje je sprovedeno kao studija preseka u državi Reka Nil, Sudan, u periodu od juna do avgusta 2018. Cilj istraživanja bio je da se odredi prevalencija infestacije krpeljima kod ovaca i identifikuju potencijalni faktori rizika vezani za ovu infestaciju. Ispitano je ukupno 135 ovaca sa pet različitih lokacija (Šemdo, Al Matamah, Ad-Damer, Atbara, i Berber) od kojih je 90 (66.7%) bilo zaraženo krpeljima. Sakupljeno je ukupno 340 krpelja (185 muških i 155 ženskih jedinki), a identifikacija je izvršena primenom zooloških taksonomskih ključeva. Najdominantnije identifikovane vrste sakupljenih krpelja bile su *Rhipicephalus eversti* Neumann, 1897 (38%), zatim *Hyalomma anatolicum* Koch, 1844 (23.8%), *Rhipicephalus sanguineus* Latreille, 1806 (20.6%), *Rhipicephalus praetextatus* Gerstacker, 1873 (16.4%) i *Hyalomma dromedarii* Koch, 1844 (1.2%). Hi-kvadratni test pokazao je značajnu povezanost ($p \leq 0.05$) između infestacije i lokaliteta, načina držanja, pola, mera kontrole krpelja i uklanjanja stajnjaka. Najviša stopa prevalencije zabeležena je u oblasti Atbara, u otvorenim smeštajnim jedinicama, kod ženskih jedinki na farmama koje nisu primenjivale acaricide i nisu redovno uklanjale stajnjak. S druge strane, nije bilo značajne povezanosti ($p > 0.05$) između infestacije krpeljima i veličine zapata, sistema uzgoja, rase, starosti i boje krzna ($p = 0.846$). Ovo istraživanje proširilo je znanja o fauni krpelja i relevantnim faktorima rizika u državi Reka Nil i pokazalo da više vrsta krpelja napadaju ovce i potencijalno prenose ozbiljna oboljenja izazvana krpeljima.

Ključne reči: krpelji, faktori rizika, ovce, Sudan

INTRODUCTION

Ticks, belonging to the family *Ixodidae*, are the most important vectors of a wide variety of pathogens including protozoa, bacteria, helminths, and viruses, which affect domestic animals and humans (Jongejan and Uilenberg, 2004). During feeding, ticks may cause direct or indirect effects on their hosts. The direct effects of ticks include the sucking of blood, which in turn leads to anaemia and damage to the skin. Consequently, these effects result in significant losses in productivity, fertility, body weight, milk and meat production, and mortality (Mapholi et al., 2014). The indirect losses of ticks are related to the infectious agents transmitted by them and the costs associated with the treatment and control (Hurtado and Giraldo-Ríos, 2018).

Ticks and tick-borne diseases are prevalent in Sudan, cause notable economic losses, and constitute major barriers to the development of animal production. Among these diseases, Theileriosis, Babesiosis, and Anaplasmosis are considered the most important diseases (El Hussein et al., 2004).

In Sudan, more than 70 tick species were identified including the most economically important ticks in Africa (Hassan, 2003).

According to Hoogstraal (1956) and Osman et al. (1982) ticks infesting livestock in Sudan are mainly *Hyalomma anatolicum, Hyalomma dromedarii, Hyalomma rufipes, Hyalomma impressum, Hyalomma impeltatum, Hyalomma truncatum, Rhipicephalus evertsi, Rhipicephalus sanguineus, Rhipicephalus praetextatus, Rhipicephalus decoloratus, Rhipicephalus annulatus, Amblyomma lepidum* and *Amblyomma variegatum*.

In a recent study conducted in Khartoum and East Darfur State, the authors reported that the most prevalent tick species were *H. anatolicum* (57.3%) and *H. rufipes* (29%), respectively (Mossaad et al., 2021).

R. evertsi was the dominant tick species (51.6%) in Al Gezira State (Hayati et al., 2020), while *H. anatolicum* was the predominant species (73.6%) in River Nile State (Ahmed et al., 2005). In White Nile State, *A. lepidum* was the most abundant tick species (Guma et al., 2015), whereas *H. rufipes* is the most frequent one in North Kordofan State (Mohammed-Ahmed et al., 2018). Clearly, there is a large variation in the tick prevalence among the states.

Although the distribution and prevalence rate of tick infestation have been documented previously in several states in Sudan, these data are changing dramatically due to climate changes and animal movement (Hassan and Salih, 2013). Therefore, an annual investigation is recommended to update our data and to predict which kind of "new" or emergent infectious diseases could occur in the State. Additionally, only a few studies have investigated the risk factors associated with tick infestation on livestock farms in Sudan. Therefore,

this study was aimed to estimate the prevalence of tick infestation and to determine the potential risk factors associated with tick infestation in sheep in River Nile State.

MATERIAL AND METHODS

Ethical approval

All animal procedures were carried out following the ethical standards established by the Institutional Ethics Committee of Sudan University of Science and Technology, Sudan.

Study area

The study was conducted in River Nile State, which is located in the northern part of Sudan between latitude 16 - 22 °N and longitude 30 - 32 °E. The state is bordered by Khartoum State to the south, the Arab Republic of Egypt to the north, Kassala State and the Red Sea State to the east and Kordofan State to the west. Generally, the climate is semi-desert, and the temperatures range between 47 °C in summer and 8 °C in winter. The mean rainfall is between 150 and 25 mm.

Study design

A cross-sectional study was performed over the time from June to August 2018 in River Nile State, Sudan (Figure 1). Five different localities, namely: Shendi, Al Matamah, Ad-Damer, Atbara, and Berber were conveniently selected and individual sheep were randomly sampled.

Figure 1. Shows the study area (River Nile State in red area).
(https://en.wikipedia.org/wiki/River Nile %28state%29)

Meanwhile, individual animal data including age, sex, breed and coat colour was recorded in a questionnaire. Moreover, the data regarding locality, herd size, housing type, rearing system, tick control, and removal of manure were also documented.

The animals were classified into three age groups, young animals (age < 6 months), adults (age between 6 months and 3 years) and old (age > 3 years). The breed of sheep was classified into two categories: crossbreed (Local breed × Saanen) and local breed. The coat colour of the examined sheep was classified into four groups, white, black, brown, and mixed colour (more than one colour). The herd size was classified into three groups: small (less than 70 animals), medium (70 - 140 animals) and large (more than 140 animals). The housing type was categorized into two groups: the open and semi-closed. The rearing system was classified into two groups: one-species rearing system (one species of animal) and a mixed rearing system (more than one species of animals).

Sample size

The overall number of animals to be included in the study was calculated using a formula of Thrusfield (2007):

$$n = (1.96)^2 \times P_{exp} \times (1- P_{exp}) / d^2$$

Accordingly, the sample size of 135 sheep was determined.

Collection and identification of ticks

Ticks were collected from the predilection sites of sheep's bodies (preferred regions for ticks), which included ears, tails, udders and testicles. The collection was performed using a pair of blunt forceps, and the ticks were transferred into labelled tubes that contained 70% ethanol. The tick specimen was identified using morphological keys (Hoogstraal, 1956; Walker et al., 2003).

Data analysis

The statistical software program (SPSS version 16.0) was used to analyse the data. The association between the tick infestation and risk factors was analysed using the Chi-square test. In all analyses, a 95% the confidence interval (CI) was held and the *p*-value less than 0.05 ($p > 0.05$) was set for statistical significance.

RESULTS

The overall prevalence of tick infestation

Out of 135 examined sheep, 90 sheep were infested with ticks (66.7%) while 45 sheep were non-infested (33.3%).

Tick species

A total of 340 ixoid ticks (185 male and 155 female) were collected. Five tick species of the two genera were identified during this study. *R. eversti* was the most abundant among the tick species collected from study animals (38%), followed by *H. anatolicum* (23.8%), *R. sanguineus* (20.6%), *R. praetextatus* (16.4%), and *H. dromedarii* (1.2%) (Table 1).

Table 1. Prevalence of tick species

Tick species	No of ticks			Prevalence	Overall Male/ Female ratio
	Male	Female	Total		
Hyalomma anatolicum	46	35	81	23.8%	1.3 : 1
Hyalomma dromedarii	1	3	4	1.2%	0.3 : 1
Rhipicephalus eversti	76	53	129	38%	1.4 : 1
Rhipicephalus sanguineus	34	36	70	20.6%	0.9 : 1
Rhipicephalus praetextatus	28	28	56	16.4%	1 : 1
Total	185	155	340	-	-

Prevalence of tick infestation based on risk factors

There was significant variation ($p \leq 0.05$) in the prevalence of tick infestation among the surveyed localities. The highest prevalence rate was reported in Atbara (100%), while the lowest prevalence was documented in Al Matamah (28%) (Table 2).

Table 2. Prevalence of tick infestation on the basis of locality, herd size, housing type, raring system, breed, sex, age, colour coat, tick control, and remove of manure in sheep in River Nile State.

Risk factor	No. of sheep	No. of infested sheep (%)	df	Chi-Square (X^2)	p-value
Locality:					
Shendi	63	48 (76.2 %)	4	26.164	0.000
Al Matamah	25	7 (28 %)			
Ad- Damer	22	18 (81.8 %)			
Atbara	7	7 (100 %)			
Berber	18	10 (55.6 %)			

Risk factor	No. of sheep	No. of infested sheep (%)	df	Chi-Square (X^2)	p-value
Herd size:					
Small	72	51 (70.8%)			
Medium	45	27 (60%)	2	1.463	0.481
Large	18	12 (66.7%)			
Housing type					
Open	90	70 (77.8%)	1	15.000	0.000
Semi-closed	45	20 (44.4%)			
Rearing system					
One species rearing system	35	27 (77.1%)	1	2.334	0.127
Mixed rearing system	100	63 (63%)			
Animal breed					
Local breed	135	90 (66.7%)	-	-	-
Sex					
Male	65	37 (56.9%)	1	5.355	0.021
Female	70	53 (75.7%)			
Age					
Young	20	12 (60%)			
Adult	111	75 (67.6%)	2	0.566	0.754
Old	4	3 (75%)			
Coat colour					
White	47	29 (61.7%)			
Black	13	9 (69.2%)	3	0.815	0.846
Brown	68	47 (69.1%)			
Mixed	7	5 (71.4%)			
Tick control (acaricide)					
Yes	47	23 (48.9%)	1	10.200	0.001
No	88	67 (76.1%)			
Removal of manure					
Weekly	14	4 (28.6%)			
Monthly	31	14 (45.2%)	2	22.794	0.000
Irregularly	90	72 (80%)			

The housing type and sex were also significantly associated ($p \leq 0.05$) with the prevalence of tick infestation. The prevalence of infestation in the open types was higher (77.8%) than in the semi-closed types (44.4%). Females showed the highest prevalence of infestation (75.7%) compared with males (56.9%).

Both factors, i.e., tick control and removal of the manure, were also significantly associated with the tick infestation. The highest rate of ticks infestation was observed in farms that did not use acaricide (76.1%) compared with farms that used acaricide as a control measure (48.9%). Moreover, a higher infestation of ticks was detected in farms that did not remove the manure regularly (80%) as compared with other ones.

Risk factors such as herd size, rearing system, breed, age, and coat colour were not significantly associated with the prevalence of tick infestation ($p > 0.05$) (Table 2). However, the prevalence was higher in small heard size (70.8%) compared with medium (60%) and large size (66.7%). The highest prevalence rate of infestation was observed in one-species rearing system (77.1%) than in mixed rearing system (63%). Animals of the young age (age < 6 months) showed the lowest prevalence rate (60%) followed by adult animals (age between 6 months and 3 years) (67.6%), whereas animals of old age (age > 3 years) showed the highest prevalence rate (75%) (Table 2).

During the sampling, all sheep examined were local breed. With respect to the coat colour, the prevalence rates of tick infestation ranged between 61.7% and 71.4% (Table 2).

DISCUSSION

In Sudan, out of 70 tick species documented, 34 species are known to infest sheep and goats. These species belong to the genera *Amblyomma, Hyalomma, Rhipicephalus* (*Boophilus*) (Osman, 1997). During the current study, five species of ticks were found to infest sheep in River Nile State, Sudan. These include (in order of abundance), *R. eversti*, *H. anatolicum*, *R. sanguineus*, *R. praetextatus*, and *H. dromedarii*. These species were previously reported in sheep from River Nile State (Ahmed et al., 2005) and from Khartoum (Gad Elrab, 1986). On the other hand, Yagoub et al., (2015) identified ten tick species that infesting sheep and goats in Nyala town, South Darfur. A similar finding was also reported in North Kordofan and Kassala States, in which ten species of ticks that infest sheep were recognized (Springer et al., 2020). It is evident that the climate and ecological conditions of River Nile State do not allow for the existence and reproduction of several other ticks species that infest sheep such as *Rhipicephalus* (*Boophilus*) species and *Amblyomma* species, which exist in other states of Sudan (Osman et al., 1982).

The overall prevalence of tick infestation in sheep in the Nile River State was 66.7%. This result was comparable to those reported by Mathewos et al., (2021) in Ethiopia (68.33%) and higher than the 51.97% reported by Khan et

al., (2022) in Pakistan. The variation in the infestation rate may be attributed to varying environmental conditions, production and management factors that in turn affect the ticks' population (Norval et al., 1992).

In general, male ticks represented the majority of all species collected except for *H. dromedarii* and *R. sanguineus*. This confirms the finding of Ahmed et al. (2005) who reported that the number of male ticks usually exceeded the number of females among all species infesting sheep.

In the current study, risk factors such as locality, housing type, sex, control of ticks and removal of manure were significantly associated with tick infestation. A significant variation in the tick infestation was observed among the localities where the highest prevalence was observed in Atbara (100%), while the lowest one was reported in Al Matamah (28%). This finding confirms the results of Fesseha et al. (2022) who observed a high frequency of tick infestation in Dasenech in Ethiopia (54.9%) compared to results of Salamago (45.1%), with a statistically significant association. This could be due to the distinctions in the agroclimatic conditions of the study areas, and also to the time of sample collection. It is well recognized that tick activity can be influenced by altitude, season, rainfall, and atmospheric relative humidity (Ayalew et al., 2014).

Higher tick infestation was reported in the open type (77.8%) compared with the semi-closed type (44.4%) housing. Contrary to that, a lower prevalence of tick infestation has been observed on farms with open houses type in Pakistan (Rehman et al., 2017). One possible explanation to this difference is the construction of open housing types in Sudan. Namely, the construction of these houses is very poor, using traditional materials such as mud and wood full of cracks, which provides an optimal environment for the ticks to survive. Moreover, the animals that were raised in close or semi-closed housing types in Sudan received a proper veterinary service and more attention since the majority of them was owned by the companies. This hypothesis was confirmed by our results revealing that the prevalence of tick infestation was higher in farms that did not apply acaricide (76.1%) compared with the farm that used acaricide (48.9%). In addition, the prevalence was higher in the farms that did not regularly remove manure (80%) compared with other ones. As we know, ticks have a free-living stage, in which they are dropping down to the ground in order to complete their life cycle (Walker et al., 2003). During this time, ticks become more susceptible to many factors such as hygiene measures, which in turn reduce the tick population.

The analysis of infestation according to sex revealed that there were more tick infestations on female sheep than on male animals. Similar finding was also reported in previous study, which established that the tick infestation was

higher in females (78.1%) than in males (58.42%) (Mathewos et al., 2021). The logical explanation for this finding is that the stress factors such as pregnancy and lactation may have made the female animals more susceptible to infestation with ticks as compared with males. Adding to that, the females are generally kept for a long time for birth-giving pursuits, which raises the possibility of being infested with ticks (Mathewos et al., 2021).

This study found no significant association ($p > 0.05$) between tick prevalence and factors such as herd size, rearing system, breed, age, and coat colour. However, the proportion of tick infestation was higher in old animals (age ≥ 3 years) (75%) than in the adult (67.6%) and young (60%) age groups. This finding was strengthened by the findings of Asmaa et al. (2014) who reported higher infestation in animals aged more than 3 years. A higher prevalence of tick infestation may be due to low immunity in older animals and the long-mileage movement of older animals searching for food, which increases the probability of infesting with ticks (Fesseha et al., 2022).

Although there is no association between the herd size and tick infestation, the prevalence of infestation was higher in the small-size herds (less than 70 animals) (70.8%) as compared with the medium (60%) and large size (66.7%) herds. This was comparable with the study conducted by Sajid et al. (2020) in which they found that herds having 40 - 60 goats showed the highest ticks infestation. This might be due to the fact that a large number of ticks were fed on a few animals, which increased the rate of infestation.

No significant differences in the tick infestation rate were found between the sheep reared with other ruminants (mixed rearing system) and those reared separately (one species rearing system). This finding was in agreement with the report of Sajid et al. (2020) who found no association between the tick infestation and rearing type (with other ruminants or separately).

In the present study, sheep coat colour did not significantly influence the prevalence of tick infestation since the prevalence ranged between 61.7% and 71.4%. Contrary to that, Hayati et al., (2020) and Hassan (1997) found that the coat colour of cattle had a significant influence on the tick burdens, where animals with light coat colour carried more ticks compared to the animals with dark coat colour. The author suggested that ticks picked by cattle with dark coat colours such as black and brown die or leave before the attachment, due to the rather increased temperature in the animal skin microenvironment produced by the dark coat colour (Hayati et al., 2020). Probably such kind of effect does not exist in sheep due to the difference between sheep coats and cattle coats.

CONCLUSION

The current study revealed a high prevalence of tick infestation in sheep in River Nile State, Sudan, and this causes a major health restraint, which results in huge economic losses. This study showed that *Rhipicephalus eversti* Neumann, 1897 was the predominant tick species followed by *Hyalomma anatolicum* Koch, 1844, *Rhipicephalus sanguineus Latreille, 1806*, *Rhipicephalus praetextatus* Gerstacker, 1877, and *Hyalomma dromedarii* Koch, 1844. The risk factor analyses showed that localities, housing type, sex, control of ticks, and removal of manure significantly affected the prevalence of tick infestations. While factors such as herd size, rearing system, breed, age and colour of coat had no influence on the prevalence of tick infestation. Due to the high prevalence of sheep ticks in the study area, the State requires prompt attention at all levels to decrease the impact of tick and tick-borne disease on the health and production of animals and thereby enhance their productivity.

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Author's Contribution:

This work was carried out in collaboration among all authors. H.K.K. carried out the experiments. S.B.M. planned the experiments, analysed the data, wrote the manuscript and supervised the project. K.M.T contributed to the final version of the manuscript. All authors read and approved the final manuscript.

Competing interest

The authors declare no conflicts of interest in relation to this work.

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EPIDEMIOLOGICAL CHARACTERISTICS OF HUMAN LISTERIOSIS IN VOJVODINA, SERBIA, IN THE PERIOD 2005-2020

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Abstract

In the Autonomous Province of Vojvodina, listeriosis has been reported to health authorities since 2005. In this study, we retrospectively analysed the data of listeriosis collected from January 1, 2005 to December 31, 2020. Descriptive method was applied. Demographic, chronological and topographical distribution of the disease in Autonomous Province of Vojvodina was reviewed. In the study period, a total of 52 cases of listeriosis were registered. The average annual incidence rate of reported cases was 0.16/100,000. The largest annual number of cases (n = 10) was reported in 2020. The highest incidence rate registered in youngest age group < 1 year was (5.4/100,000). Gender distribution of the infected population showed no difference with respect to number of infected males and females. Listeriosis was registered throughout the year, but most frequently in October (21.15%). Topographical distribution revealed that listeriosis was more frequently reported in South Bačka county (2.4/100,000 inhabitants). The overall case fatality rate of reported *Listeria monocytogenes* infection was 23.08%. The highest case fatality rate was in the age group 19 - 59 years (29.41%), and fatal outcome in males was twofold the fatality rate in females. Our data analysis suggests that our practitioners, gynaecologists,

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paediatricians, oncologists and other clinicians should take listeriosis into consideration much more often in differential diagnosis in order to improve the diagnosis of the disease and further on epidemiological investigation on source of infection.

Key words: Listeriosis, epidemiology, Vojvodina, APV, epidemiological characteristics

EPIDEMIOLOŠKE KARAKTERISTIKE LISTERIOZE KOD LJUDI U VOJVODINI, SRBIJA, U PERIODU 2005-2020.

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Kratak sadržaj

U Autonomnoj Pokrajini Vojvodini listerioza se prijavljuje nadležnim zdravstvenim organima od 2005. godine. U ovoj studiji smo retrospektivno analizirali podatke o listeriozi prikupljene od 1. januara 2005. do 31. decembra 2020. godine. Primenjena je deskriptivna metoda. Prikazana je demografska, hronološka i topografska distribucija bolesti u Autonomnoj Pokrajini Vojvodini. U periodu istraživanja registrovana su ukupno 52 slučaja listerioze. Prosečna godišnja stopa incidencije prijavljenih slučajeva ove bolesti bila je 0,16/100.000. Najveći godišnji broj slučajeva (10) prijavljen je 2020. godine. Najveća stopa incidencije registrovana je u najmlađoj starosnoj grupi < 1 godine (5,40/100.000). Polna distribucija obolelih ne pokazuje značajnu razliku između broja obolelih muškaraca i obolelih žena. Listerioza se registrovala tokom svih meseci u godini, ali najčešće u oktobru (21,15%). Topografska distribucija pokazuje da je listerioza češće prijavljivana u Južnobačkom okrugu (2,4/100.000 stanovnika). Letalitet kod registrovanih slučajeva inficiranih bakterijom *Listeria monocitogenes* iznosio je 23,08%. Najveći letalitet bio je u starosnoj grupi 19-59 godina (29,41%). Smrtni ishod je bio dvostruko češći kod muškaraca nego kod žena. Naši

rezultati ukazuju na to da bi ginekolozi, pedijatri, onkolozi i drugi lekari u diferencijalnoj dijagnozi trebalo mnogo češće da uzimaju u obzir listeriozu, kako bi se unapredilo otkrivanje ove bolesti i dalje epidemiološko ispitivanje izvora infekcije.

Ključne reči: listerioza, epidemiologija, Vojvodina, APV, epidemiološke karakteristike

INTRODUCTION

Listeriosis is an infectious disease from a group of zoonoses caused by the bacteria *Listeria monocytogenes*. Although 14 serotypes of these Gram-positive bacteria have been detected so far, only four have been characterized as pathogenic (1/2a, 1/2b, 1/2c and 4b) (Muchaamba et al., 2022). *Listeria monocytogenes* is ubiquitous in nature and can be found in surface waters, soil, vegetation, fodder, silage, sewage and in the digestive tract of many mammals (Bubonja et al., 2007; Mathews, 2008; Hernandez-Milian and Payeras-Cifre, 2014). Animals are mainly asymptomatic carriers of Listeria *monocytogenes*, who shed the bacteria through their faeces and thus can contaminate food sources for other animals and humans (WHO, 2018; Ryser and Marth, 2007). That is why the disease is classified as foodborne.

The main route of transmission of the organism to humans is alimentary, i.e., by consuming contaminated food such as insufficiently pasteurized milk, soft cheeses, uncooked meat delicacies, raw vegetables, frozen foods, raw fish, etc. (Hernandez-Milian and Payeras-Cifre, 2014; Štivić and Tomić Paradžik, 2018; Kiš et al., 2019). *Listeria* can also be transmitted to humans through direct contact with infected animal tissues, especially during lambing or calving (Ryser and Marth, 2007). In addition, transplacental transmission of Listeria *monocytogenes* and transmission during passage of the newborn through the birth canal are important because it can lead to miscarriage, premature birth, neonatal infection and neonatal death (Jackson et al., 2010).

Incubation period lasts from one to 90 days, mostly about three weeks (McLauchlin et al., 2020; Dimitrijević et al., 2008). Two clinical forms of listeriosis can be distinguished: non-invasive and invasive (WHO, 2018). Non-invasive listeriosis is also called febrile listerial gastroenteritis, which is manifested by diarrhoea and flu-like symptoms, and rarely can be registered in this form. Invasive form of the disease is severe condition with a clinical presentation of septicaemia, meningitis or encephalitis. This form of listeriosis is accompanied by high case fatality rate (CFR) of as much as 20 - 30% (WHO, 2018). Pregnant women, neonates, adults over the age of 65 and persons with a compromised

immune system are at risk of developing severe, invasive forms of the disease (Doganay, 2003). Listeriosis is mostly reported sporadically, while outbreaks occur occasionally. The incidence rate across the world ranges from 0.1 to 11.3 cases per 100,000 inhabitants per year (Jeffs et al., 2020). This disease was the fifth most frequently reported zoonosis in the European Union (EU) in 2020, with 1,876 confirmed cases, giving an incidence rate of 0.42 per 100,000 European inhabitants (ECDC, 2020a).

In our province, listeriosis has been reported to health authorities since 2005. The Register of reports of diseases, including listeriosis, is settled at the Institute of Public Health of Vojvodina (IPHV), and it is the only official source of data and evidence on human listeriosis in Autonomous Province of Vojvodina (APV).

APV is located in the north of the Republic of Serbia. It covers an area of 21,506 square kilometres with the population of almost two million inhabitants (RZS, 2014). Vojvodina is territorially divided into seven administrative counties and 45 municipalities.

The aim of this paper was to describe the epidemiological characteristics of listeriosis in APV, Serbia, in the period from 2005 to 2020, for the first time since the disease data have been collected.

MATERIAL AND METHODS

Data collection

The study extended over a sixteen-year period, from January 1, 2005 to December 31, 2020. The collected data of listeriosis were retrospectively analysed using a descriptive method. The data sources included individual reports of infectious diseases cases, epidemiological surveys, and monthly and annual reports. Demographic, chronological and topographical distribution as well as the clinical forms of the listeriosis in APV were reviewed.

Laboratory procedures

Listeria monocytogenes was isolated from blood and cerebrospinal fluid of the patients, or in some occasions from the placenta, amniotic fluid and uterus swab. Classical cultivation of the causative agent on standard media was performed, and the CAMP-test (named for the original authors: Christie, Atkins, and Munch-Petersen) with *Rhodococcus equi* and *Staphylococcus aureus* strains were used to distinguish haemolytic *Listeria* species.

Statistical methods

Data are presented as mean value with the standard deviation (SD), median with interquartile range (IQR) and minimum and maximum values across the groups. The normality assumption was tested using Shapiro-Wilk test with the skewed statistics. To examine the distribution of listeriosis incidence rates throughout the analysed 16-year period between different age groups and county of residence, we used Mann-Whitney U test and Kruskal-Wallis H test with the Dunn's pairwise post hoc comparison using Bonferroni adjustment. All statistical analyses were performed using Stata v.16 (StataCorp LLC. 2019). Results were considered statistically significant when the p-value was < 0.05.

RESULTS

In the period from 2005 to 2020, a total of 52 cases of listeriosis were registered in APV. Of the total 52 cases included in the study, 12 deaths were registered. The largest annual number of cases (n = 10) was reported at the end of the study period, in 2020. The cases of listeriosis in the observed period were reported sporadically and mainly in the form of the severe clinical presentations, with the diagnoses of *listeriosis septica* (40%), *meningitis et meningoencephalitis listerialis* (35%) and *listeriosis non specificata* (25%).

The average annual incidence rate ranged from 0.05/100,000 in 2006 to 0.52/100,000 in 2020 showing an apparent increasing trend during the study period (Figure 1).

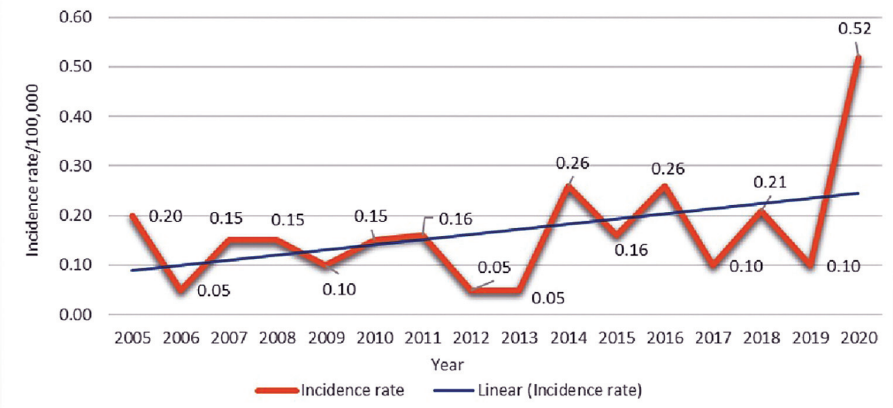

Figure 1. Incidence rate of human listeriosis in APV, Serbia, in the period 2005-2020

The highest age-specific incidence rate was recorded in youngest age group < 1 year (5.40/100,000) (Table 1). Gender distribution of the infected individuals showed no difference between males and females (M : F = 1.08 : 1). The gender-specific incidence rate peaks in age group < 1 among males (6.27/100,000) and the same age group among females (4.44/100,000).

Table 1. Mean incidence rates of listeriosis by age groups and gender in APV, Serbia, in the period 2005-2020

Age group	**No of cases**	**Σ**	**incidence rate/100000**	**Σ**
< 1	male 9	15	male 6.27	5.40
	female 6		female 4.44	
1-18	male 0	1	male 0	0.02
	female 1		female 0.14	
19-59	male 10	17	male 0.47	0.10
	female 7		female 0.32	
60+	male 8	19	male 0.25	0.25
	female 11		female 0.27	
Total	male 27	**52**	male 0.17	**0.16**
	female 25		female 0.15	

The difference between incidence rates of listeriosis among the four age groups (< 1, 1 - 18, 19 - 59 and 60 +) was statistically significant, ($\chi^2 = 18.06$; $p < 0.01$) (Table 2). Multiple comparisons showed that there was a statistically significant difference between age groups < 1 and 1 - 18 ($p < 0.01$) and groups 1-18 and 60 + ($p < 0.01$).

Table 2. Multiple comparisons of incidence rates of listeriosis by age-groups, APV, Serbia, in the period 2005-2020.

Age group (years)	**mean incidence**	**SD**	**median**	**IQR**		**min**	**max**	**χ2**	**p-value¶**
<1	5.40	7.15	5.70	0	5.80	0	28.90	18.06	**0,0004**
1-18	0.02	0.08	0	0	0	0	0.30		
19-59	0.11	0.12	0.10	0	0.15	0	0.40		
60+	0.25	0.26	0.20	0	0.40	0	0.90		

SD= standard deviation; IQR = interquartile range
Indicators of significance between groups using Kruskal-Wallis H test. Dunn's pairwise post hoc comparison was conducted with Bonferroni adjustment.

In APV, listeriosis has been registered throughout the year, but the largest number of cases was reported in October, followed by May, August and September (Figure 2).

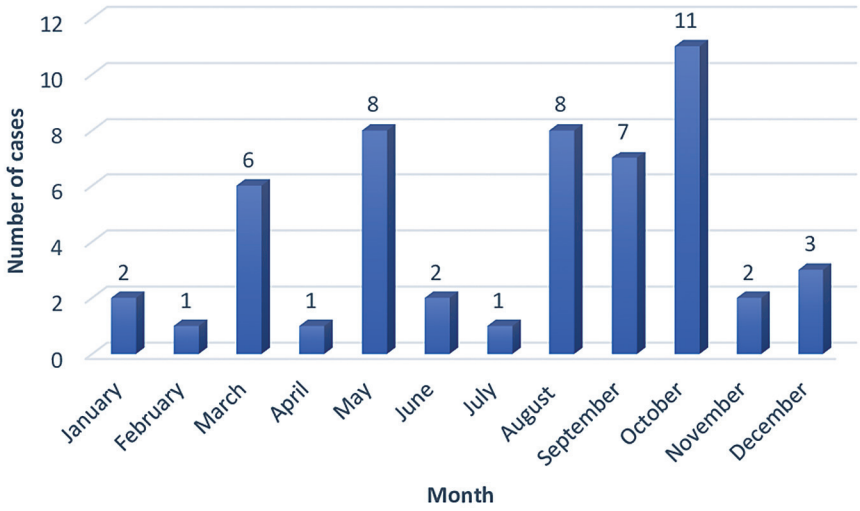

Figure 2. Seasonal distribution of human listeriosis in APV, Serbia, in the period 2005-2020

Topographical distribution revealed that listeriosis was more frequently reported in South Bačka county (incidence rate 2.4/100,000 inhabitants) (Figure 3). Although the incidence rate of listeriosis was higher in South Bačka county than in all other counties there was no statistically significant difference between the counties ($p = 0.55$).

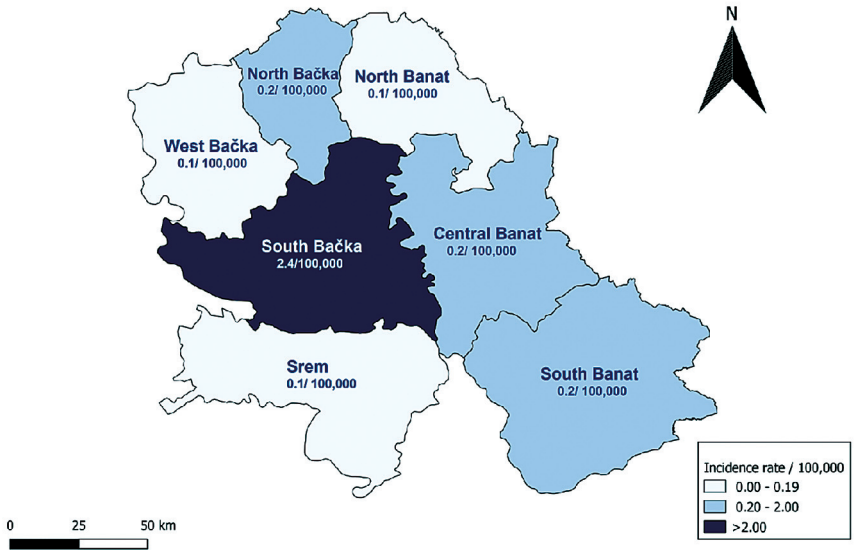

Figure 3. Distribution of human listeriosis in APV, Serbia, by counties in the period 2005-2020

The overall case fatality rate of reported *Listeria monocytogenes* infection was 23.08%. The highest CFR was in age group 19 - 59 (29.41%), and fatal outcome was twice as frequent in males as in females (M : F = 2 : 1).

Table 3. Case fatality rates of listeriosis by age groups and gender, APV, Serbia, in the period 2005-2020

Age group	No of deaths	Σ	Case fatality rate (%)	Σ
< 1	male 0	2	male 0	13.33
	female 2		female 33.33	
1-18	male 0	0	male 0	0
	female 0		female 0	
19-59	male 5	5	male 50	29.41
	female 0		female 0	
60+	male 3	5	male 37.50	26.31
	female 2		female 18.18	
Total	male 8	**12**	male 29.63	**23.08**
	female 4		female 16.00	

DISCUSSION

Listeriosis occurs worldwide. It could be a very serious infection with severe clinical symptoms in certain vulnerable groups and with a high case-fatality rate. The highest numbers of cases worldwide have been reported in high-income countries, but the highest burden of the disease is estimated to be in some Latin American countries (Noordhout et al., 2014). To the best of our knowledge, this is the first study conducted in Serbia that summarizes the chronological, topographical and demographic characteristics of human listeriosis in APV.

Our results revealed that the average annual incidence rate of reported invasive cases was 0.16/100,000 inhabitants in APV, which is in accordance with the incidence of the entire territory of Serbia, but lower than the average incidence (0.43/100,000 inhabitants) reported in the EU between 2011 and 2020 (IPHS, 2020; ECDC Atlas, 2020). In the EU, over a seven-year period from 2008 to 2015, the notification rate of invasive cases of listeriosis increased from 0.30 to 0.46 cases per 100,000 inhabitants (EFSA, 2018).

Furthermore, the average incidence recorded in our study is almost two times lower than the annual incidence in the United States (0.28/100,000) between 2008 and 2016 among the general population excluding pregnant women, and four times lower than the average incidence in Germany in the period 2010-2019 (0.69/100,000) (Pohl et al., 2019; Wilking et al., 2021). Due to the high proportion of mild and asymptomatic forms of the disease and their insufficient recognition, the incidence of listeriosis is considered to be significantly higher in APV.

In 2020, an incidence rate of listeriosis in APV reached the highest value of 0.52/100,000. A total of 10 cases were reported during that year, and all of them were investigated; however, due to a long period of incubation and poor anamnestic data the source of the infection and epidemiological association of cases could not be determined, thus, the cause of this increase of the incidence rate remains unclear. It is likely that during the COVID-19 pandemic the clinicians were paying more attention to infectious diseases, including listeriosis. Even though the notification rate of listeriosis in 2020 has improved in the APV, it is still lower than that reported by some European countries. In the same year, the highest incidence rates in the EU were reported in Finland, Slovenia, Malta and Sweden with 1.70, 1.20, 0.97 and 0.85 cases per 100,000 inhabitants, respectively (ECDC, 2020a). Finland reported the highest number of cases of listeriosis per year in Europe, most probably due to the high consumption of cold-smoked and dried salmon (Pasonena et al., 2019).

All cases of listeriosis reported in Vojvodina during the 16-year study period occurred sporadically, with no epidemiological association established. In the EU, from 2010 to 2016, 63 outbreaks were reported (ECDC, 2018). There is an example of the outbreak in South Africa, showing that it could be very massive and fatal with 1050 persons infected and 216 deaths (CIPH, 2020).

We further established that the highest age-specific incidence was registered at the age of < 1 year, while according to the ECDC data the largest portion of human listeriosis cases was observed in the age group over 64 years (ECDC, 2020a). A study conducted in Germany in the period 2010-2019 revealed that the highest incidence rate was recorded among adults ≥ 85 years (Wilking et al., 2021). Our findings showed that listeriosis occurred almost equally in males and females, while in Austria, in the period from 1997 to 2007, the male to female ratio of listeriosis was 1.23 : 1 (Kasper et al., 2009). A similar sex ratio was registered in the EU (ECDC, 2020b).

Our results revealed that the diagnosis of septic listeriosis was dominant in the age group < 1 (86.67%), and the diagnosis of listeriosis meningitis prevailed in the age group 60 + (73.68%). In a study conducted in Turkey between 1987 and 2001, sepsis was diagnosed in 21 - 43% of cases, and 56% of central nervous system infections (Doganay, 2003).

Out of 25 female cases with the notification of *Listeria monocytogenes* in this study, seven were pregnant, although, six were detected during postpartum period. In the study conducted in the United States covering the period 2004-2007, about 16% of reported listeriosis cases were in pregnant woman (Jackson et al., 2010). These data are not surprising considering that pregnant women have greater risk of *L. monocytogenes* infection due to the immunosuppression during pregnancy. Also, infection with this bacterium can cause premature births, miscarriages or stillbirths, thus, pregnant women and women in the postpartum period are more often laboratory tested for listeriosis (Jeffs et al., 2020).

In APV, listeriosis was registered throughout the year, but the largest number of cases was reported in May, August, September and especially in October. A study conducted in Israel from 1997 to 2007 reported almost the same seasonal distribution, and the largest proportion of cases (64.2%) was reported from May to October (Vasilev et al., 2009). Almost the same seasonal summer peak was reported by ECDC (ECDC, 2020b). It is assumed that more frequent occurrence of other gastrointestinal infections during the warmer months is related to the increase in the number of listeriosis patients, as the causative agents of these infections may cause immunosuppression and facilitate the entry and spread of *L. monocytogenes* in the gastrointestinal tract (Schwartz et al., 1989; Vasilev et al., 2009).

The largest number of registered cases in the South Bačka county can be explained by the fact that the main administrative centre and the largest city in APV (Novi Sad) is situated on the territory of this county, where diagnostic possibilities, disease reporting and service networks are more developed.

Overall case fatality rate of 23.1% recorded during our study period, as well as 33.3% in 2019 in the entire Serbia and 13.0% in 2020 in EU, make listeriosis one of the most serious human foodborne diseases (IPHS, 2020; ECDC, 2020a).

CONCLUSION

Listeriosis is an important public health problem in Vojvodina. Control measures of listeriosis rely on the safe production, distribution and preparation of food of animal origin, as well as on health education in the general population, primarily in vulnerable groups.

The analysis of our data shows that our practitioners, gynaecologists, paediatricians, oncologists and other clinicians should take listeriosis into consideration much more often in differential diagnosis in order to improve the diagnosis of the disease and further on epidemiological investigation on source of infection. Education and raising awareness about this disease at all levels of health care are essential for recognition and better notification of listeriosis. Timely exchange of information and institutional collaboration between public health and veterinary services is also very important to improve listeriosis surveillance.

Author's Contribution:

NJ, TP and GD made contributions to the basic idea, conception and design of the article; NJ and SR were involved in the data collection; NJ drafted the manuscript; GD coordinated the work and revised the manuscript and participated in the final draft of the manuscript; VV performed statistical analysis; NJ, TP, VV, SR and GD contributed in data analysis and interpretation of results. All authors have read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interest.

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EVALUATION OF THE EFFICACY OF ANTIMICROBIAL ACTIVITY OF CONTACT LENS CARE SOLUTION

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Abstract

Contact lens care solutions are necessary for proper hygiene of contact lenses. Numerous microorganisms can be transmitted through them, leading to serious eye infections, while blindness can occur in particularly severe cases. A direct link between ocular infections and contact lens care solutions has been proven. Commercial preparations must meet a number of safety and antimicrobial efficacy requirements. This paper presents examinations of antimicrobial efficacy of commercial preparation called LENS CARE. The examinations were carried out according to ISO 14729:2012 standard Stand-alone test. It has been found that commercial preparation LENS CARE within proscribed contact lens care regimen of 8h meets the primary criteria of the Stand-alone test for *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* with the results of 3.4, 3.6 and 1.0 log10 reductions, respectively. If used in accordance with the manufacturer's instructions, the tested preparation has the desired antimicrobial effect against the tested pathogens.

Key words: medical device, stand-alone test

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ISPITIVANJE EFIKASNOSTI ANTIMIKROBNE AKTIVNOSTI PREPARATA ZA ODRŽAVANJE KONTAKTNIH SOČIVA

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Kratak sadržaj

Rastvori za održavanje kontaktnih sočiva su neophodna za pravilno održavanje higijene kontaktnih sočiva. Preko kontaktnih sočiva se mogu preneti brojni mikroorganizmi koji dovode do ozbiljnih infekcija oka, a u naročito ozbiljnim slučajevima može nastati slepilo. Postoje dokazi o direktnoj povezanosti epidemija okularnih infekcija sa sredstvima za održavanje kontaktnih sočiva. Komercijalni preparati moraju da ispunjavaju brojne zahteve legislative u pogledu bezbednosti i antimikrobne efikasnosti. U radu je opisano ispitivanje antimikrobne efikasnosti komercijalnog preparata pod nazivom LENS CARE. Ispitivanja su izvršena prema zahtevima standarda ISO 14729:2012 - Stand-alone test i ustanovljeno je da komercijalni preparat LENS CARE u propisanom vremenu delovanja od 8h ispunjava primarne kriterijume Stand-alone testa za *Pseudomonas aeruginosa, Staphylococcus aureus* kao i *Candida albicans* sa rezultatima od 3,4, 3,6 i 1,0 log10 redukcija. Ukoliko se koristi u skladu sa uputstvom proizvođača preparat ima efikasno antimikrobno dejstvo prema ispitanim patogenima.

Ključne reči: medicinsko sredstvo, stand-alone test

INTRODUCTION

Contact lenses are medical devices that make life much easier for people who need vision correction, they are applied directly to the cornea and can affect eye health. Microbial keratitis – corneal infection is a sight-threating emergent ophthalmic disease. The disease is a treatable infection but can lead to a number of complications such as corneal perforation, opacification, endophthalmitis (Jin et al., 2017). The occurrence of microbial keratitis caused by *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Fusarium* spp. linked with contact lenses has been directly proved (Jalbert et al., 2000; Ma et al., 2009).

Proper use of contact lenses and their care ensures eye health. Wearing lenses during the night, wearing them for multiple days, improper cleaning

and disinfection, their improper storage, poor hygiene of lens storage box, and poor personal hygiene of patients can lead to eye infections (Agi et al., 2021). A great risk factor for the occurrence of infections is the sterility and efficiency of lens care solutions. The outbreak of fungal keratitis occurred during 2005 and 2006 in the USA, Hong Kong and Singapore. More than 200 people became ill and the infection occurred only in people who wore contact lenses and used lens care solution "B&L ReNu with MoistureLock solution". During the epidemiological investigation, it was determined that the product was sterile, leading to a conclusion that weak antimicrobial activity of the product in combination with poor hygienic practices of the patients caused the infection. It is also believed that storage conditions after opening of the solution led to a significant reduction in its antimicrobial activity, temperature and number of days of storing the open product (Ma et al., 2009).

Table 1. Stand-alone test criteria (SRPS EN ISO 14729:2012)

Microorganism	**Criteria**	**Average log 10 reductions at contact lens care regimen**
Bacteria	Primary criteria	3.0
	Secondary criteria	The minimum acceptable log reduction for any single bacterial type is 1.0
Fungi	Primary criteria	1.0
	Secondary criteria	Stasis at contact lens care regimen

It is necessary to prove that lens care solutions are suitable for intended use, i.e. it is necessary to prove that the product has an adequate biocidal effect. In the case of agents intended for cleaning and disinfection of contact lenses, it is necessary to prove that the level of antimicrobial activity is appropriate, i.e. that the contact lens care regimen is designed to meet at least minimal performance (secondary) criteria of the Stand-alone test - ISO 14729:2012. The criteria are shown in Table 1. Primary criteria of 3.0 log reductions means that mean microorganism reduction is at least 99.9% and secondary criteria of 1.0 log reduction means that mean microorganism reduction is at least 90%.

The paper presents the results of testing the efficiency of the commercial preparation LENS CARE during the contact lens care regimen (8h).

MATERIAL AND METHODS

Product

The paper presents the results of examination of a commercial preparation called LENS CARE, which is intended for washing, removing proteins and lipids, disinfecting, moisturizing and storing all types of soft contact lenses. The preparation is sterile on the basis of 0.0001% polyhexanide. Three product lots were examined (Lot 1, Lot 2, and Lot 3). Manufacturer recommends contact lens care regimen of 8h.

Methods

The tests were performed according to the procedure described in SRPS EN ISO 14729: 2012 Ophthalmic optics — Contact lens care products — Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses and European Pharmacopeia 8.0. Stand-alone test is inoculum challenge test. This test challenges a disinfecting product with a standard inoculum of microorganisms and establishes the extent of their viability loss (log of number of decimal reductions) at recommended contact lens care regimen.

The following challenge organisms were used in the tests - reference microorganisms: *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 9027 and *C. albicans* ATCC 10231. The microorganisms were grown on TSA (Trypticase Soy Agar) at 30 – 35 °C for 24 h (bacteria) and on SAB (Sabouraud agar) 20 – 25 °C for 24 – 48 h (*C. albicans*). Stock inoculums in peptone salt solutions were made from reference microorganisms and they were assigned total aerobic bacteria/mold count (TAMC/TYMC) and then they were added to the LENS CARE solution in order to achieve the final concentration of 5 log cfu/mL LENS CARE. The total count of challenge organisms was enumerated after 8 h. Three lots of products were tested, each challenge organism was tested in three samples and the determination of the number of microorganisms was performed in duplicate by seeding on TSA/SAB media. These media were incubated at 30 - 35 °C for 72 h (TSA) and at 20-25 °C for 5 days (SAB).

Total count of challenge organisms is shown according to log10 base. Descriptive statistics and p value was calculated using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). The results of the statistical tests were considered significant for $p < 0.05$.

RESULTS AND DISCUSION

In the past, several disinfectants were used for disinfection of contact lenses, for example first unpreserved saline and then disinfectants. Nowadays, all in one multipurpose product is mainly used for cleaning, disinfection and rewet in one use. Modern preparations are usually based on polyhexamethylene biguanide (PHMB) with polyaminopropyl biguanine (ReNu) or polyquaternium-1 with myristamidopropyl dimethylamine (Opti-Free), while some preparations contain only one biocide polyexanid or polyhexamethylene biguanide (Lite, Complete Easy Rub, SOLO-care) (McAnally et al. 2021). LENS CARE is in the group of preparations with one biocide. In order to compare the results with other studies, it must be taken into account whether Stand-alone or Regimen test is used for testing, since lens care solutions have lower antimicrobial activity in the presence of contact lenses (Regimen test), depending on the type of the microorganism being tested. Commercial solutions based on PHMB reacts most with silicone hydrogel and hydrophilic soft contact lenses, which leads to a decrease in the concentration of PHMB and a decrease in antimicrobial activity (Gabriel et al. 2018; McAnally et al. 2021).

The decline in antimicrobial activity in the presence of lenses is one of the reasons why the requirements for the Stand-alone test (primary criteria) are stricter compared to the Regimen test (secondary criteria), where only one decimal reduction is sufficient (Table 1.). The results of the LENS CARE solution efficiency test are shown in Table 2 expressed as a number of decimal reductions after the contact lens care regimen (8h). TAMC/TYMC vallues are expressed an average vallues of duplicate enumeration.

Table 2. Efficacy results of three lots of LANS CARE solution – Stand-alone test

Lot	No of microorganisms (log10 cfu/mL)	*S.aureus* ATCC 25923	*P. aerugi-nosa* ATCC 9027	*C. albicans* ATCC 10231
Lot 1	Initial concentration	5.9	5.3	4.7
	TAMC/TYMC	2.1	2.2	3.7
	No of decimal reductions	3.8	3.1	1.0
Lot 2	Initial concentration	5.6	4.9	4.2
	TAMC/TYMC	1.9	1.9	3.2
	No of decimal reductions	3.7	3.0	1.0
Lot 3	Initial concentration	5.1	5.5	4.6
	TAMC/TYMC	1.7	1.4	3.5
	No of decimal reductions	3.4	4.1	1.1
Average ± SD	No of decimal reductions	3.6 ± 0.2	3.4 ± 0.6	1.0 ± 0.1

TAMC – total aerobic microbial count, TYMC – total combined yeasts/moulds count, cfu – colony forming unit

Commercial lens care solutions are effective against most major eye pathogens. Antimicrobial activity depends on the type and amount of biocide contained in the preparation, and also on the method of use (Zhu et al., 2011; Gabriel et al. 2018).

S. aureus is a pathogen that can lead to eye infection, it is most commonly transmitted by hands. However, eye infections caused by the use of contact lenses have also been proven (Jalbert et al., 2000). *S. aureus* has the ability to form a biofilm on various surfaces and thus significantly reduces the effectiveness of detergents and disinfectants. Compared with the results of testing of other commercial preparations (Ewbank 2000; Rosenthal et al., 2003; Kal et al., 2017; Gabriel et al., 2018), it was found that LENS CARE provides a slightly higher number of decimal reductions of *S. aureus* after the recommended time (3.6 log reductions) than Opti-Free preparation (3.2 – 3.3 log reductions) and a slightly lower than SOLO-care preparation (4.2 - 4.9 log reductions) and ReNu preparation (3.9 - 5.0 log reductions). However, there are significant differences between the studies - Kal et al., (2017) found that Opti-Free provides 1.9 log reductions of *S. aureus*, unlike Gabriel et al. (2018) who identified 4.0 –5.0

log reductions. Although they used the same commercial preparation (Opti-Free Express), the same recommended time (6h) and the same test (Regimen), there were differences in the results. A possible explanation could be the differences in the contact lenses on which the test was performed or the differences in the design and implementation of the study itself.

Keratitis caused by *P. aeruginosa* is the most common type of infection transmitted through contact lenses (Agi et al., 2021). Corneal infection can develop in just a few hours (Wilson et al., 1981), so it is essential that contact lens care products are effective against this microorganism. In relation to antimicrobial activity against *P. aeruginosa*, LENS CARE is slightly less effective (3.4 log reductions) than SOLO-care (3.5 - 3.8 log reductions), ReNu and Opti–Free (4.0 – 5.0 log reductions), according to data presented by Kal et al., (2017), Ewbank (2000) and Gabriel et al. (2018). However, in a study by Kal et al., (2017) it was reported that ReNu Multi Plus leads to less than 2.9 log reductions after the recommended time (4h), while, using the same Regimen test, Gabriel et al. (2018) found that ReNu Fresh provides 4.2 log reductions, although ReNu Multi Plus contains 0.001% polyaminopropyl biguanide and ReNu Fresh 0.0001%.

SOLO-care preparation is the most similar in composition to LENS CARE preparation (0.0001% polyhexanide). Producers recommend a different contact lens care regimen for SOLO-care (4 hours), while it is 8 hours for LENS CARE. SOLO-care is somewhat more effective against *S. aureus* and *P. aeruginosa* compared with data of Ewbank 2000 and Kal et al., 2017.

C. albicans can cause fungal keratitis, although somewhat less frequently (14%) compared to *Fusarium* species (41%) (Iyer et al., 2006). *Candida* eye infections usually occur either after trauma or after the use of corticosteroids, and it is more common in colder climates. Still, the main risk factor for all fungal keratitis is wearing contact lenses (Iyer et al., 2006, Imamura et al, 2008). Transmission of *C. albicans* through contact lenses is facilitated by their ability to form biofilms (Imamura et al., 2008). The biofilm that *Candida* forms is tightly bound to hydrogel and silicone contact lenses, so the effect of lens care solutions is much weaker against *Candida* compared to bacteria (Zhu et al., 2011). The effect of LENS CARE contact lens care products achieves at least one decimal reduction of *C. albicans* after recommended time, which is statistically significantly less effective than challenge bacteria but still meets the primary criteria of the Standalone test. Similar results were obtained by Imamura et al (2008) for ReNu Moisture Loc and ReNu Multi Plus, while Gabriel et al. (2018) found that there is less than one decimal reduction for the ReNu Fresh preparation. Preparations based on polyquaternium and myristamidopropyl

dimethylamine have much higher antimicrobial activity (1.5- 4.5 log reductions) but only on the stand-alone test while their activity drops below 1 log reduction in the presence of lenses (Gabriel et al., 2018).

There are no statistically significant differences between the effect of the LENS CARE solution on *S. aureus* compared to *P. aeruginosa* ($p = 1.0$), but the number of decimal reductions of *C. albicans* is significantly lower than *S. aureus* ($p = 0.00003$) and *P. aeruginosa* ($p = 0.00001$).

CONCLUSION

LENS CARE solution is sufficiently effective and meets the requirements of the Stand-alone test in terms of antimicrobial efficacy against to *S. aureus, P. aeruginosa* and *C. albicans* at the recommended time of 8 h. In order for the preparation to show its maximum efficiency, it is necessary to follow the labelling instructions of the manufacturers.

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Author's Contribution

This paper was written as a review paper which dealt with a series of papers in the field of microbial quality of pharmaceutical products. All authors of this paper have participated in the writing of this paper and previous research. Writing paper JP and RR, examinations JP and IS, and data processing JP and NV.

Competing interest

The authors declare that they have no competing interest.

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Headings in the paper are: **INTRODUCTION, MATERIAL and METHODS, RESULTS, DISCUSSION (or RESULTS and DISCUSSION for Short Communications), CONCLUSION and REFERENCES**. For Review articles instead M&M, Results and Discussion section, the Main text section should subdivided by different subheadings describing topics presented/discussed/analysed. For Case studies this part could be the same or replaced by Case presentation part with or without different subheadings.

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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.
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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.

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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.
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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.**

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