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Incidence of reproductive pathogens in breeding pigs in the Republic of Srpska

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Abstract

The aim of the study was to examine the incidence of reproductive pathogens in the breeding pig populations on selected farms in the Republic of Srpska. The study included a total of 11 farms from the territory of the Republic of Srpska (Bijeljina - 4, Gradiška - 3, Prnjavor - 2, Foča - 1, Ljubinje - 1), from which a total of 50 blood samples of breeding sows and boars were taken. All blood samples were serologically negative for the causative agents of leptospirosis and brucellosis. The presence of the PRRS virus was found on 54.55% of examined farms and 40% of blood samples, circovirus on 81.18% farms and 66% blood samples, and Aujecki's disease virus on 9.09% of the farms and 10% of blood samples. The results of this study indicate the presence of mixed infections with the mentioned causative agents, which results in an unclear clinical picture in the pigs on the examined farms, as well as the need to implement systematic monitoring and active surveillance of these diseases, with the aim of reducing their negative impact on the production and reproductive parameters of pigs. Also, the results of this study indicate an insufficient level of knowledge and awareness of farmers about the importance of applying preventive measures in controlling the presence of the mentioned and other pathogens in farm pigs, and in the following period it is necessary to work on educating farmers in this regard.

Key words: breeding pigs, reproductive pathogens, monitoring

Introduction

The breeding herd, which consists of boars, sows, and gilts, represents the basis of the functioning of pig farms, especially those with a rounded production cycle, from the production of piglets and breeding animals, all the way to the rearing of fatteners, as the final product of pig farms (Uzelac & Vasiljević, 2011, Jotanović & Savić, 2017). Reproduction, as a key segment of pig production, is influenced by numerous factors, the most important of which are the way of keeping, the location of the farm, nutrition, microclimatic conditions, the season, the implementation of biosecurity measures, the herd health (control of the presence of breeding, parasitic, and infectious diseases), herd size, body condition, as well as the application of artificial insemination or natural service (Lončarević et al., 1997). The technology of modern pig production is based on rearing a large number of animals in a limited space and the widespread use of artificial insemination of sows and gilts, which, in addition to a positive effect on production results (Gerrits et al., 2005), can represent a significant biosecurity risk if the control of the health of breeding animals is not at the appropriate level (Jotanović & Savić, 2017). Particularly important diseases of breeding pigs are those that attack the reproductive organs, because they lead to a decrease in reproductive efficiency, a decrease in the number of piglets, and fattening pigs produced, and directly affect the economic effect of production. Trade in reproductive material, whether it is breeding gilts and boars or insemination doses of semen, can be a significant source of the spread of various diseases from one farm to another, and even from one country to another (Thacker et al., 1984, Stanković et al., 2005, Knox, 2016). In this sense, special attention should be paid to monitoring the health of the breeding herd within the framework of creating a biosecurity program and managing the pig herd health on the farm (Jotanović & Savić, 2017). Also, the presence of these diseases in breeding animals has a negative impact on the health status of other categories of pigs on the farm, primarily through the reduction of their general resistance, which makes them more sensitive to pathogens from the environment and affects their health and achieved production results (Alexopoulos et al., 2003, Bojkovski, 2015).

According to the results of previous studies (Lealklevezas et al., 1995, MacMilan et al., 2006, Foti et al., 2008, Wasiński & Pejsak, 2010, Duinhof et al., 2011, Kim et al., 2018, Segales et al., 2019, Arent & Ellis, 2019), as particularly important diseases that attack breeding pigs, and at the same time are often transmitted through reproductive material, whether it is breeding boars, sows, and gilts or semen for artificial insemination, the following ones stand out brucellosis, leptospirosis, porcine reproductive-respiratory syndrome (PRRS), parvovirus, circovirus, and Aujecki's disease. In addition to the above, a number of other causative agents of infectious diseases can be transmitted in this way,

whether they originate from the organism of an infected animal or they contaminate the reproductive material secondarily.

Pig brucellosis is an infectious disease caused by the *Brucella suis*, which is transmitted by direct contact between a sick and a healthy animal, usually through coitus (Lord et al., 1997). The symptoms of brucellosis are abortions, repeat breeding and infertility of sows, swelling of the testicles, and infertility of boars (Olsen et al., 2019). The presence of the causative agent is detected serologically, as well as during examination of the semen during its preparation for artificial insemination (Lealklevezas et al., 1995). Abortions in sows and gilts can occur as early as the 17th day after mating with a boar that had *B. suis* in its semen, and infected boars may lose sexual drive (Vandeplassche et al., 1967).

Leptospirosis is an infectious disease, caused by different strains of bacteria from the genus *Leptospira*, which causes reproductive problems such as abortions, stillbirths, and infertility in sows. Clinical symptoms and infertility can also occur in acutely infected boars, although the disease usually passes without significant clinical symptoms (Wasiński & Pejsak, 2010). Leptospires settle in the kidneys and genital tract of sows and boars, and are excreted through urine and genital secretions (Ellis et al., 1986, Arent & Ellis, 2019). The source of infection with leptospira on pig farms can also include rodents, primarily rats, which contaminate food for pigs with their urine and faeces, and are often eaten by pigs, so they become infected (Stanković et al., 2008).

Guerin and Pozzi (2005) proposed a categorization of viral diseases that can possibly be transmitted to sows and gilts via boar semen during artificial insemination. The first category includes viruses that are transmitted by semen, but without the risk of transmission through artificial insemination, due to the existence of national and international eradication programs. This group of viral diseases includes foot-and-mouth disease, Aujecki's disease, classical swine fever, and African swine fever. The second category includes viral diseases that are often transmitted through semen, such as reproductive-respiratory syndrome (PRRS) and parvovirus. The third category includes viruses that can be found in semen, but more scientific evidence is needed to better assess the risk of transmission via semen, and the representative of this category are circoviruses (PCV-2, Porcine Circovirus-2).

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease that is widespread on pig farms, so that a small number of farms are free from this disease (Duinhof et al., 2011, Ruiz-Fons, 2012). In addition to the direct effect of the virus, which manifests in abortions, reduced fertility and vitality of newborn piglets, the presence of this virus on farms results in a decrease in the general resistance of the organism of infected pigs, due to which the frequency of secondary infections is increased (Lealklevezas et al., 1995; Nodelijk, 2002, Šarić et al., 2011). Althouse (2007) states that the presence of PRRS at the farm

level is manifested by a reduced percentage of conception from artificial insemination, a decrease in fertility, an increased percentage of neonatal piglet mortality, frequent occurrence of respiratory diseases, and reduced growth. Prieto and Castro (2005) highlight high contagiousness and the tendency of the virus to be transmitted both directly and indirectly as the main characteristic of PRRS, which makes it difficult to maintain the status of a PRRS-free herd. The virus is easily transmissible between animals, as it is excreted through nasal secretions, urine, faeces, and semen (Swenson et al., 1994, Prieto et al., 1996). Yaeger et al. (1993) and Swenson et al. (1994) state that the semen of infected boars is not always the route of transmission of PRRS, and Pileri and Mateu (2016) point out that the possibility of virus transmission in this way largely depends on the amount of virus present in the semen. The period of viremia, during which the virus is present in the blood of infected boars, is considered as the most critical time for its transmission via semen, while in the later stages of the disease this possibility decreases, but is not absent (Christopher-Hennings et al., 2001).

Circovirus infections with PCV-2 can manifest clinically as circovirus pneumonia within the Porcine Respiratory Disease Complex (PRDC), circovirus enteritis, and reproductive disorders caused by circoviruses (Firth et al. 2009, Kim et al., 2018, Segales et al., 2019, Li et al., 2023). Also, according to Harding et al. (1998), one of the forms of circovirus infections in pigs, called PMWS (Porcine multisystemic wasting syndrome), manifests itself in progressive weight loss, rapid breathing, profuse watery diarrhoea, stunting, cyanosis of the ears, icterus, lymphadenopathy, and eyelid swelling. For the second form of circovirus infections, PDNS (Porcine dermatitis nephropathy syndrome), Paolo (1998) and Thibault et al. (1998) point to the appearance of dermatitis in the area of the chest, abdomen, thighs, and front legs that occurs in the form of purple-red islands of different shapes and sizes, as the most significant symptoms.

Aujecki's disease virus in pigs is transmitted mainly by cohabitation, which is why it can be said that sperm or the mating is not the primary way of spreading this disease (Castro et al., 1992). However, the replication of the virus also takes place in the genital tract, so that the transmission of the disease can also occur during the act of mating or artificial insemination, due to exposure to contaminated vaginal mucosa or sperm (Leontides et al., 1994). It is characteristic of this disease in pigs that itching is absent, as a key clinical symptom that appears in other types of animals, possibly present on pig farms, primarily dogs and cats. Dieuzy et al. (1987) point out that boars infected with Aujecki's disease often refuse to jump on a phantom, while sows inseminated with infected semen may develop vaginitis or endometritis resulting in increased embryonic mortality. Althouse (2007) and Foti et al. (2008) state that, at the farm level, the presence of Aujecki's disease is often asymptomatic, although

reproductive disorders, a high degree of neonatal mortality, and growth retardation of piglets may occur. In relation to the category of pigs, Mettenleiter et al. (2019) state that pregnant sows often abort after infection with Aujecki's disease, or give dead, mummified or avital piglets, which die in the first days after farrowing. According to these authors, in piglets younger than two weeks, Aujecki's disease manifests itself in rapid and sudden deaths within a few hours, even before the clinical symptoms of the disease develop. In older piglets, until weaning, an increase in body temperature, loss of appetite, depression, vomiting, respiratory, and nervous symptoms (uncoordinated movement, paralysis, spasms, muscle tremors) are observed, with high mortality. Respiratory symptoms, in the form of cough, difficulty breathing, and conjunctivitis are dominant symptoms in weaned piglets, with mortality being significantly lower compared to younger categories. In adult pigs, Aujecki's disease usually causes mild respiratory symptoms, but mostly without deaths (Mettenleiter et al., 2019).

Bearing in mind that systematic monitoring of the previously mentioned diseases has not been carried out in the Republic of Srpska, the aim of this study was to monitor the presence of the causative agents of diseases important for the reproduction of pigs in the population of pigs on farms and breeding centres in the Republic of Srpska.

Material and Methods

The study, carried out in 2019, included a total of 11 pig farms from the entire territory of the Republic of Srpska (Bijeljina - 4, Gradiška - 3, Prnjavor - 2, Foča - 1, Ljubinje - 1), where a fully rounded production system is present, from raising piglets and rearing breeding animals to fattening pigs. In order to gain a complete insight into the state of the frequency of the presence of reproductive pathogens in the population of breeding pigs in the Republic of Srpska, farms with a larger capacity, which sell breeding material (gilts, boars, and insemination doses of boar semen), as well as those that have a special significance for pig production in the given region (Herzegovina) were selected.

Blood samples for examination of the reproductive pathogens presence (n=50) were taken from breeding sows and boars, according to the principle of a random sample representative for given farms, by puncture of *v. cava cranialis*, in sterile vacutainers without anticoagulants. After spontaneous coagulation at room temperature, the blood samples were transported in a hand-held refrigerator to the PI Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka. After centrifugation and separation of blood serum, the samples were serologically tested (ELISA, microagglutination, BAB test) for brucellosis, leptospirosis, PRRS, Circovirus, and Aujecki's disease, and the data obtained are presented in tables and graphs below.

Results and Discussion

The presence of reproductive pathogens can be a significant limiting factor for achieving high production and economic results, development, and improvement of pig production on farms, and in some cases can even call into question the survival of farms. Having this in mind, it can be said that monitoring of breeding pigs' health, with biosecurity measures on farms, is a key element in controlling the presence of these pathogens, as well as their harmful effect on the pigs' health and production results of farms (Stanković et al., 2005, Knox, 2016, Jotanović & Savić, 2017). This study included a total of 11 pig farms from all over the Republic of Srpska that produced breeding material for market, such as boars, gilts, or insemination doses of semen. Representative blood samples were taken using a random sample method from breeding animals (boars and sows), in order to establish the possible presence of individuals seropositive for the presence of reproductive pathogens. The reason for such an approach when designing the methodology of this research was the fact that the presence of reproductive pathogens on these farms, through the sale of reproductive material, can have a greater impact on the spread of these diseases to other farms than is the case with farms that produce reproductive material themselves and do not purchase animals from other sources. When selecting a representative sample of animals on each of the examined farms, special attention was focused on the health status of breeding boars, where the possibility of transmitting the disease to the wider population of pigs on the same or other farms via semen is far greater than in sows and gilts (Jotanović & Savić, 2017). Blood samples were tested using serological diagnostic methods for the presence of antibodies to the causative agents of brucellosis, leptospirosis, PRRS virus, circoviruses, and Aujecki's disease virus (Table 1). When interpreting the obtained results, it should be borne in mind that the current legislation (Program of Animal Health Protection Measures at the state level) at the time of the study did not order active surveillance, i.e., regular serological diagnosis of any of the three mentioned viral diseases, while at the same time it ordered the taking of blood samples and surveillance of the occurrence of brucellosis and leptospirosis in case of suspicion of these diseases, especially in breeding animals and after abortions.

Tab 1. Results of serological examination of breeding pigs on the examined farms (n=11) for the presence of reproductive pathogens

Disease	Result				
		Number of farms	%	Number of samples	%
Brucellosis	Positive	0/11	0.00	0/50	0.00
	Negative	11/11	100.00	50/50	100.00
Leptospirosis	Positive	0/11	0.00	0/50	0.00
	Negative	11/11	100.00	50/50	100.00
Porcine Reproductive Respiratory Syndrome (PRRS)	Positive	6/11	54.55	20/50	40.00
	Negative	5/11	45.45	30/50	60.00
Circovirus (Porcine Circovirus -2. PCV-2)	Positive	9/11	82.82	33/50	66.00
	Negative	2/11	18.18	17/50	34.00
Aujeczky disease	Positive	1/11	9.09	5/50	10.00
	Negative	10/11	91.91	45/50	90.00

Lord et al. (1997), MacMilan et al., (2006), and Althouse (2007) mark abortions, repeat breeding, and infertility in sows and boars as key symptoms of brucellosis, as well as swelling of the testicles in boars, while Vandeplassche et al. (1967) point out that boars positive for brucellosis may lack libido. Brucellosis is a disease that is untreatable and is controlled by law, so the current legislation in the Republic of Srpska stipulates that all pigs positive for this disease, regardless of whether they show clinical symptoms or not, should be culled and euthanised. In order to control this disease, the legislation in the Republic of Srpska provides for mandatory serological testing of breeding boars, as well as clinical, laboratory, and serological diagnostics in all cases where there is suspicion of this disease, especially after sow abortion. Ellis et al. (1986) and Arent and Ellis (2019) mark abortions, stillborn piglets, and infertility in sows and boars as reproductive problems caused by leptospirosis, and in particular point to the fact that the causative agents of this disease can be retained in the kidneys and genital tract of sows and boars, and excreted through urine or genital fluids. In order to monitor the occurrence of leptospirosis, current legislation also orders mandatory serological testing of breeding boars, as well as clinical,

laboratory, and serological diagnostics in all cases where this disease is suspected, especially after sow abortion. In contrast to brucellosis, for which medical treatment in animals is not allowed, animals suffering from leptospirosis can be treated with antibiotics, and the effect of therapy is assessed based on the results of serological testing, i.e., changes in the antibody titer in the prescribed sampling intervals. Also, a significant measure in controlling the presence and harmful effects of leptospirosis is the vaccination of breeding pigs with commercially available vaccines (Wasiński & Pejsak, 2010). The results of this research have shown that all examined farms were free from brucellosis and leptospirosis, which is encouraging information, considering the harmful effects that these two diseases can cause on pig farms in terms of reproductive and other health disorders (abortions, farrowing, birth of dead piglets, etc.), economic losses due to euthanasia of brucellosis-positive pigs, as well as the costs of leptospirosis treatment in positive animals. This finding is also important bearing in mind that brucellosis is a zoonosis, so there is a possibility of its transmission to people who work on farms and with pigs.

The porcine reproductive-respiratory syndrome virus (PRRS) is widespread on pig farms in the world, primarily due to its very high degree of contagiousness, which allows it to be transmitted from one farm to another directly, via infected animals, but also indirectly, via people, vehicles or equipment (Duinhof et al., 2011, Šarić et al., 2011, Ruiz-Fons, 2012). The high degree of contagiousness of PRRS is indicated by Prieto and Castro (2005), who consider this characteristic of the virus and this disease to be crucial in making it difficult to achieve the status of a herd free from this disease. A particularly common way of transmitting this virus is through semen doses for artificial insemination as a frequent item of trade between farms (Pileri & Mateu, 2016). Nodelijk (2002) and Althouse (2007) state that infections with the PRRS virus are associated with reproductive problems in sows (abortions, repeated breeding, stillbirths), and lead to piglet mortality and respiratory diseases in pigs of all categories, while adult boars usually do not have any symptoms caused by the presence of the PRRS virus or they are quite mild. The presence of this virus on pig farms, in addition to its direct harmful effect, also results in a decrease in the general resistance of the pig organism to other pathogens from the environment and an increased frequency of health disorders of other aetiologies, especially in facilities where microclimatic and other conditions are not optimal, which is why reduction of the harmful effect of the presence of this virus can be achieved by improving housing, feeding, and care conditions, as well as by controlling secondary bacterial and other infections (Lealklevezas et al., 1995; Alexopoulos et al., 2003, Bojkovski, 2015). The results of this study showed that 54.55% of farms and 40.00% of blood samples were positive for the presence of the PRRS virus, which indicates a significant degree of spread of this virus on our farms,

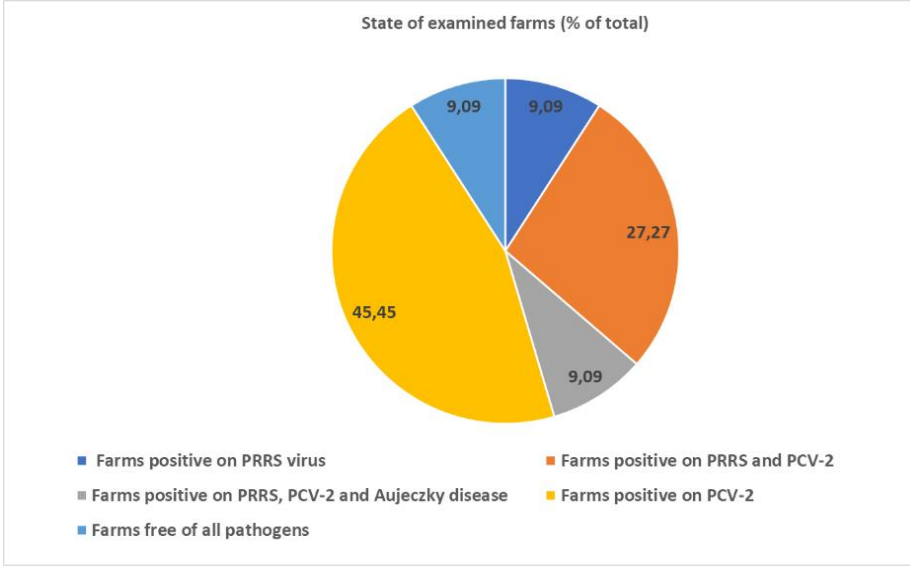
in which uncontrolled purchase of semen originating from boars of unknown serological status, but also the purchase of breeding gilts from farms positive for this disease, certainly played a significant role. It is important to emphasize that there are currently no commercial vaccines against this disease available on our market, which makes its control difficult, and reduces it to the implementation of general biosecurity measures and the maintenance of optimal animal hygiene conditions on farms, with the simultaneous control of secondary bacterial infections (Alexopoulos et al., 2003). Bojkovski, 2015). An additional factor in controlling this disease when establishing new farms can be the purchase of breeding gilts and boars or semen doses for artificial insemination from farms free of this disease, in addition to the previously mentioned measures (Jotanović & Savić, 2017).

Circovirus infections of pigs on farms, caused by PCV-2, most often appear as circovirus pneumonia within the Porcine Respiratory Disease Complex (PRDC), as circovirus enteritis or reproductive disorders caused by circoviruses. Reproductive disorders caused by circovirus infections in sows and gilts can be seen as abortions, repeat breeding, and the stillbirths, while boars generally do not show any clinical symptoms of diseases of the reproductive organs (Firth et al. 2009, Kim et al., 2018, Segales et al., 2019, Li et al., 2023). Also, circovirus infections can occur as PMWS (Porcine multisystemic wasting syndrome) in piglets, with progressive weakening, rapid breathing, profuse diarrhoea, stunting, cyanosis of ears, icterus, swelling of lymph nodes and eyelids (Harding et al., 1998), or as PDNS (Porcine dermatitis nephropathy syndrome), with dermatitis on the chest, stomach, thighs, and front legs in the form of purple-red islands of different sizes (Paolo, 1998, Thibault et al., 1998). The results of this study show that a very high percentage of examined farms (82.82%) and two thirds of examined blood samples (66.00%) were positive for the presence of circovirus, which indicates that circovirus infections represent a significant health problem on our pig farms, and that much more attention should be paid to their detection and suppression. In order to control circovirus infections in pigs, in addition to early detection, a significant role can be played by the introduction of vaccination against circovirus with commercially available vaccines, which is already sporadically carried out on farms, with the implementation of biosecurity measures, maintenance of good hygiene conditions in pig facilities, and control of secondary bacterial infections (Alexopoulos et al., 2003, Bojkovski, 2015).

The presence of Aujecki's disease on pig farms is most often manifested by respiratory symptoms, growth retardation, a high degree of neonatal mortality, and problems in reproduction, such as frequent repeated breeding, the birth of stillborn, and avital piglets, while boars usually do not show clinical symptoms (Althouse, 2007, Foti et al., 2008). Given that all the above symptoms in pigs are insufficiently specific, Aujecki's disease in pigs farms is most often detected

indirectly, after it appears in other animals around the farm, in which the characteristic itching occurs, especially in dogs and cats, as they are often fed with the meat of dead or slaughtered pigs (Ehlers et al., 2019). The virus of Aujecki's disease in domestic pigs is transmitted mainly through cohabitation, and the replication of the virus takes place in the mucous membrane of the nose and throat, as well as in the genital organs, which is why the transmission of the disease can occur during the act of mating or artificial insemination (Leontides et al., 1994). The results of this study showed that only one of the farms examined was positive for the presence of this virus (9.09%), and that all the samples from this farm were positive, which makes 10.00% of the total number of tested blood samples. Control of Aujecki's disease is carried out by raising the level of biosecurity on farms, controlling the entry of other species of animals that can be carriers of the virus, especially rodents, and using commercially available vaccines against this disease on farms where this disease is present (Foti, 2008, Ehlers et al., 2019).

The results of the frequency of occurrence of viral pathogens important for the reproduction of pigs on the examined farms are shown in Graph 1.



Graph. 1. State of the examined farms (n=11) regarding the presence of viral pathogens important for pig reproduction

In addition to the above-mentioned data on the incidence of individual pathogens important for the reproduction of pigs on the examined farms, the

results of this study (Chart 1.) showed that simultaneous presence of several pathogens, i.e., mixed infections with different viruses that can affect the reproduction of pigs, was found on most of the examined farms. The presence of these pathogens on pig farms, in addition to breeding pigs, which were in the focus of this research, also affects the health and production performance of other categories of pigs, through the potentiation of the pathogenic action of the pathogens present, the formation of an insufficiently clear clinical picture in different categories of pigs on the examined farms, which makes it difficult to establish a diagnosis and take the necessary measures to control the presence of these diseases (Alexopoulos et al., 2003, Bojkovski, 2015).

Conclusion

The results of this research indicate frequent presence of pathogens that can be transmitted through reproductive material, and manifest their harmful effects in relation to the reproductive and production performance of pigs on farms. A special problem in their presence on farms is created by the fact that they mostly manifest themselves with insufficiently specific symptoms, which is why they are not often identified on farms at all. Also, the results of this research indicate the simultaneous presence of several pathogens on the same farm, which is why their harmful effects overlap and potentiate each other, giving an insufficiently clear clinical picture, so their presence can only be detected by laboratory testing. A big problem in the detection and control of these diseases is the lack of their systematic monitoring, especially in boars for the production of insemination doses of semen, which are often sold without control of origin and health status. In this sense, it is necessary to create monitoring programs for these and other pig diseases, and to integrate them into the program of mandatory animal health protection measures financed by the state, and to provide support to producers of reproductive material in terms of monitoring the health of the breeding herd, as a key category of animals in the improvement of farm functioning. Also, the support of the state and competent institutions is necessary in the creation and implementation of biosecurity plans on pig farms, as well as the control of animal movement, as an important factor in controlling the occurrence and spread of pig diseases.

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Учесталост присуства репродуктивних патогена код приплодних свиња у Републици Српској

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Сажетак

Циљ рада био је да се установи учесталост присуства репродуктивних патогена у популацији приплодних свиња на одабраним фармама у Републици Српској. Истраживањем је обухваћено укупно 11 фарми са подручја Републике Српске (Бијељина - 4, Градишка - 3, Прњавор - 2, Фоча - 1, Љубиње - 1), са којих је узето укупно 50 узорака крви крмача и нерастова. Сви испитани узорци били су серолошки негативни на узрочнике лептоспирозе и бруцелозе. Присуство вируса ПРРС установљено је на 54,55% испитаних фарми и 40% испитаних узорака крви, цирковируса на 81,18% фарми и 66% испитаних узорака крви, а вируса Аујецкијеве болести на 9,09% фарми и 10% испитаних узорака крви. Резултати овог истраживања указују на присуство мијешаних инфекција наведеним узрочницима, што има за посљедицу нејасну клиничку слику код свиња на испитаним фармама, као и потребу спровођења систематског мониторинга и активног надзора наведених болести, са циљем да се умање негативни утицаји њиховог присуства и ширења на остварене производне и репродуктивне параметре свиња. Такође, резултати овог истраживања указују и на недовољан ниво знања и свијести фармера о значају примјене превентивних мјера у контроли присуства наведених и других патогена свиња на фармама, те је у наредном периоду неопходно радити на едукацији фармера у овом погледу.

Кључне ријечи: приплодне свиње, репродуктивни патогени, праћење

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