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EPIZOOTOLOGICAL MONITORING OF SWINE BRUCELLOSIS IN UKRAINE: NATURAL RESERVOIRS, SPREAD RISKS, AND ADAPTATION OF EUROPEAN PREVENTION EXPERIENCE

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Summary. The article analyzes the epizootiological monitoring of swine brucellosis in Ukraine, focusing on the role of natural reservoirs of infection, such as wild boars and hares, in sustaining the epizootic process. The study presents data indicating that natural foci, particularly in southern regions, play a crucial role in the persistence and spread of brucellosis in certain areas. It highlights the involvement of wild boar and hare populations in maintaining the epizootic process among domestic pigs. Key information on the epizootiological monitoring of brucellosis is provided, highlighting its importance for farm and private livestock operations in Ukraine in recent years. Given the emergence of new international economic ties, including trade in livestock and animal relocation across borders, particular attention at the state level should be directed toward epizootiological surveillance. This is crucial for protecting farms and the livestock industry from the pathogen introduction through breeding animals and other genetic materials (sperm, embryos). Annual preventive serological screening of breeding livestock remains a fundamental component of epizootiological monitoring to ensure animal health regarding brucellosis. Alongside serological testing, clinical-epizootiological observations and assessments of potential pathways for animal and genetic material importation play a vital role. The study concludes that reducing the risk of introducing and potentially spreading the brucellosis pathogen among animals is primarily achievable through improved veterinary and sanitary control at customs and border checkpoints. The research identifies *Brucella suis* biovar 2 as the main infection reservoir in wildlife, causing miliary lesions, particularly in reproductive tissues, where abscess formation is frequently observed. The article also presents European strategies for planning and implementing preventive anti-epizootic measures against brucellosis and discusses their adaptation in Ukraine's pig farming sector. The adaptation of European prevention strategies is proposed, which includes implementing comprehensive measures to eradicate and prevent the spread of infection. The conclusions emphasize the necessity of improving Ukraine's national epizootiological monitoring system and standardizing diagnostic methods following international requirements

Keywords: biosecurity, transboundary risks, laboratory methods, epidemiological surveillance, pathogen properties and diagnostics, *Brucella suis*

Introduction. ***Relevance of the topic and analysis of recent studies and publications.*** Ukrainian pig farming is concluding 2024 with significant losses, primarily affecting the eastern regions of Ukraine, where parts of the industry have found themselves in active conflict zones.

Brucellosis in livestock is registered in many countries across all continents and holds significant economic and epidemiological importance. Despite advances in understanding the pathogen and developing preventive and diagnostic measures, animal brucellosis remains a pressing issue (Arroyo Carrera et al., 2006; Musalam et al., 2016; WOAH, 2022).

Currently, Ukraine faces substantial risks of introducing and spreading transboundary diseases from neighboring countries or those with close trade and economic ties. These challenges may pose potential threats to the country's veterinary and sanitary-epidemiological stability, as well as economic consequences, including restrictions on participation in international agricultural trade. These circumstances demand the creation and execution of an effective control system for transboundary infections in Ukrainian veterinary science. Monitoring studies conducted in the European Union focus on controlling emerging transboundary zoonoses (Cilia et al., 2021; Arroyo Carrera et al., 2006; SDVMMAU, 2000; Dawood et al., 2021). Establishing a system for monitoring, preventing, and controlling the spread of transboundary diseases should be considered a priority and mandatory for implementation in medical and biological practice. Such a system relies on the availability of specialized diagnostic tests, international reference laboratories for specific infections, and domestic capabilities for disease identification and typing.

In Europe, a specialized monitoring program and regulatory framework oversees research focused on the prevention, introduction, prediction, and eradication of dangerous transboundary diseases. These include EU directives on the control of anthrax, brucellosis, bluetongue, paratuberculosis, and others, along with instructional materials from the World Organisation for Animal Health (WOAH), the Sanitary Code, and additional guidelines (WOAH, 2022; Cilia et al., 2021; Dawood et al., 2021; Crichton and Medveczky, 1987).

Ukraine currently maintains control systems for rabies, anthrax, foot-and-mouth disease, leptospirosis, brucellosis, salmonellosis, listeriosis, blackleg, and other diseases. Veterinary-sanitary regulations and legal documents related to veterinary oversight, epidemic prevention, biosafety, and biosecurity are outlined in specialized national laws (SDVMMAU, 2000) and other informational materials. These issues must remain a priority in national environmental policies and government operations. Unfortunately, commercial and business interests often take precedence, necessitating an emphasis on restoring rigorous veterinary control at border checkpoints, customs offices, and the quarantine service.

All these factors highlight the need to improve the animal brucellosis epizootiological monitoring system in response to the evolving epizootic situation. A regional assessment of the spatial-temporal and cause-and-effect patterns of livestock brucellosis outbreaks — especially among pigs — along with addressing false positive serodiagnostic results, evaluating the efficiency of epizootiological monitoring in eradication efforts, and enhancing screening and confirmatory diagnostic tests, remains relevant and holds scientific and practical significance in ensuring the long-term stability of the pig farming industry regarding brucellosis.

Objective and research tasks. This study aims to theoretically substantiate and analyze the existing system of epizootiological monitoring of swine brucellosis, which requires improvement through the optimization of traditional diagnostic tests following international standards. The research focuses on the development and implementation of innovative diagnostic technologies, as well as the adaptation of European experience in the prevention and control of the infection. The study is dedicated to formulating recommendations to enhance brucellosis control efficiency amidst increasing transboundary risks and the need to ensure epizootic stability in the pig farming sector.

Materials and methods. The materials for this study included literature sources from both foreign and domestic authors, as well as the authors' own research and observations. The research methods used were: dialectical, chronological, and the methods of analysis and synthesis.

Results and discussion. In pig farms, the initial introduction of the pathogen into a herd leads to rapid disease spread. An outbreak typically occurs after the introduction of infected animals or disease recurrence in previously sanitized farms due to hidden carriers of the pathogen. In chronically affected farms, the disease often manifests with subtle symptoms among the pig population.

Mating sows with chronically infected boars plays a particularly dangerous role in the spread of *Brucella suis*. Wild boars can also serve as a reservoir of infection for domestic pigs (Crichton and Medveczky, 1987; Ewalt et al., 1997; Grantina-Ievina et al., 2018; Lama and Bachoon, 2018; Szulowski et al., 2013; Olsen et al., 2019). Following transient bacteremia, *B. suis* colonizes the reproductive tract cells of both sexes: in sows, it infects the placenta and fetuses, while in boars, lesions appear in one or more of the following organs — testes, prostate gland, epididymis, seminal vesicles, or bulbourethral glands. In boars, infections often occur bilaterally, beginning with hyperplasia, which can progress to abscess formation. The final stage manifests as sclerosis and atrophy, and arthritis may develop in various joints, sometimes leading to spondylitis. Abortion is the most common manifestation of brucellosis in sows and can occur at any stage of pregnancy, most frequently between days 50 and 110 of gestation. Vaginal discharge is not a characteristic symptom, and in chronically infected herds, infertility becomes the primary concern rather than abortion. In boars, the disease often persists, leading to reproductive organ damage and temporary or permanent sexual dysfunction. *Brucella* bacteria can be present in boar semen without obvious reproductive organ damage or sexual dysfunction. Swelling of joints and tendon sheaths, lameness, and, in some cases, paralysis of the hind limbs may also occur in both sexes.

Transmission factors include aborted fetuses, placental membranes, genital secretions, urine, feces, milk, and other biological fluids, as well as contaminated feed, water, equipment, and veterinary tools. The pathogen is primarily transmitted via oral, airborne, contact, and sexual routes. Given the environmental conditions of pig farming, airborne and oral transmission are the most significant pathways. Males transmit *Brucella suis* through mating, including via contaminated semen. Blood-sucking insects and ticks can act as mechanical vectors, transferring the pathogen from infected to healthy animals. The primary entry points of infection include the mucous membranes of the mouth, respiratory tract, reproductive organs, conjunctiva, and skin (Corbel et al., 2006; Olsen et al., 2019).

It is well known that in domestic animals, brucellosis occurs in a chronic form, with long-term intracellular persistence of the pathogen in lymphoid organs and reproductive glands.

The traditional serological methods used worldwide for the diagnosis of brucellosis include the tube agglutination test (AT), Rose Bengal test (RBT), complement fixation test (CFT), milk ring test (MRT), as well as more modern and sensitive methods such as the indirect enzyme-linked immunosorbent assay (iELISA), competitive ELISA (cELISA), and the fluorescence polarization assay (FPA). However, according to literature sources, none of these methods provide completely reliable results or a definitive diagnostic assessment for detecting the disease in individual animals. In each case of a positive-reacting animal, further diagnostic clarification must be performed under the national system (Van Aert et al., 1984). Serological diagnostic methods are classified into screening and confirmatory tests. Screening methods include RBT, MRT, and iELISA, while confirmatory methods include CFT, cELISA, as well as bacteriological and molecular genetic studies. The diagnostic evaluation of serological reactions, determination of the epidemiological situation regarding brucellosis in a specific farm, or differential diagnosis is carried out using multiple tests, as regulated by current national and international standards (SDVMMAU, 2000; WOAH, 2022).

Screening serological studies serve as the methodological foundation for conducting epidemiological monitoring and ensure rapid and effective control of the epizootic well-being regarding animal brucellosis. According to current national and international regulatory documents, the Rose Bengal Test (RBT) and Enzyme-Linked Immunosorbent Assay (ELISA) are considered the primary screening methods for diagnosing brucellosis in livestock. However, the interpretation of serological diagnostic results is complicated by the antigenic similarity of *Brucella* to other Gram-negative microorganisms, particularly *Yersinia enterocolitica*, which leads to false positive reactions. These false positive results hinder an objective assessment of the epidemiological situation and can result in the unnecessary culling of not only positively reacting animals but also entire herds, causing significant economic losses (Skulin et al., 1981). A comparative evaluation of the sensitivity and specificity parameters of the traditional screening test (RBT) and the alternative method (ELISA) in brucellosis diagnostics has both theoretical and practical significance. Additionally, understanding the spatial-temporal and cause-effect relationships is crucial in refining the diagnosis when isolated cases of seropositive animals are detected (Stack et al., 1999).

Recently, advanced technologies have been developed and implemented in laboratory diagnostics, including enzyme-linked immunosorbent assay (ELISA), the polarized fluorescence method, and polymerase chain reaction (PCR) (Van Aert et al., 1984). In comparative studies, Van Aert et al. (1984) found that in infected herds, ELISA detected 13.6% more seropositive animals than the tube agglutination test (SAT), 19.2% more than the complement fixation test (CFT), and 21.7% more than the Rose Bengal test (RBT) (Alhaji, Wungak and Bertu, 2016).

French researchers demonstrated significantly higher sensitivity of ELISA, showing that the milk ring test (MRT) detects 2.5 IU/mL of antibodies, while ELISA detects as little as 0.0075 IU/mL. The specificity of ELISA and CFT in healthy herds exceeded 0.998. However, when testing 1,511 serum samples from infected farms, ELISA had lower specificity than CFT but significantly higher sensitivity (Alhaji, Wungak and Bertu, 2016; Beauvais, Musallam and Guitian, 2016). Nielsen et al. (2006) found that ELISA and the polarized fluorescence method were the most sensitive and specific in comparative studies.

According to literature sources (Akhvlediani et al., 2017; Nielsen et al., 2006; Kurmanov et al., 2022), unexpected false positive brucellosis reactions are recorded under conditions of high-density young animal housing, drinking from stagnant water sources, feed contamination with animal excrement, poor-quality feed, and anamnesis-related reactions after vaccinations. Generally, cross-reactive antibody titers with *Brucella* antigens are low, do not tend to spread, and decrease or disappear within 2–4 weeks.

The issue of cross-serological reactions is critical for diagnostic research and herd health improvement. It is essential to determine the causes of false positive results, requiring additional diagnostic measures. In brucellosis eradication programs, even isolated animals reacting positively with low titers must not be overlooked by specialists.

Thus, in Ukraine, positive results from a single test must be confirmed through additional testing, particularly with CFT, under the national system for brucellosis diagnosis refinement and differential diagnosis.

The prevention and eradication of porcine brucellosis are based on general farm management and veterinary-sanitary measures. These measures aim to prevent the introduction of the *Brucella* pathogen into healthy herds, ensure effective disease control, and promptly detect infections if an outbreak occurs. The scientifically grounded national strategy for maintaining stable epizootic well-being regarding brucellosis includes:

— strict veterinary control over the importation of animals from other farms, regions, and countries, with mandatory preventive quarantine and serological testing;

— routine screening of livestock populations for brucellosis;

— timely diagnosis in suspected cases and prompt decision-making for disease eradication without the use of anti-brucellosis vaccines;

— identification of the source and transmission routes of the pathogen, studying its biology, species classification, and geographic distribution;

— quality control of eradication measures, elimination of epizootic outbreaks, and prevention of reinfections;

— comprehensive epizootiological and epidemiological investigation of brucellosis outbreaks;

— objective interpretation of large-scale preventive testing results, diagnosis, differential diagnosis, and bacteriological studies in cases of inconclusive serological findings;

— monitoring the brucellosis epizootic situation in wildlife, and conducting serological and bacteriological diagnostics of wild animals if necessary;

— short-term forecasting of the epizootic situation in the region.

Epizootiological surveillance of brucellosis is carried out through annual preventive testing of breeding livestock and monitoring to prevent the introduction of animals and breeding material from affected farms and regions.

According to the national state strategy adopted in Ukraine and similar strategies in European countries, brucellosis surveillance in domestic and wild animal populations is conducted at the state level to prevent the introduction of the pathogen into livestock populations. In cases of suspected infection, the diagnosis is promptly confirmed, and the boundaries of the epizootic outbreak and the threatened zone are determined. The elimination of brucellosis outbreaks and the eradication of the pathogen in the epizootic focus are carried out in the shortest possible time by completely replacing the affected livestock, without the use of anti-brucellosis vaccinations (Kurmanov et al., 2022; Blasco et al., 2023; Busol et al., 2023; Charypkhan and Rüegg, 2022; Gong et al, 2021; Erdenebaatar et al., 2003; Godfroid et al., 2002).

Thus, effective epizootiological monitoring and enhanced screening methods are crucial for ensuring the long-term stability of Ukraine's livestock sector regarding brucellosis. It has been proposed to adapt European experience in brucellosis prevention to Ukrainian pig farms, specifically by implementing comprehensive measures aimed at eradicating the infection and preventing its spread (WOAH, 2022; Cilia et al., 2021; Kurmanov et al., 2022). The planning and organization of preventive and anti-epizootic measures for swine brucellosis are carried out according to the following scheme:

1. Phase of High or Unknown Prevalence Without Control Programs

During this phase, the scale and spread of the problem should be determined as previously described.

On-Farm Surveillance

— voluntary investigation of cases of abortions and weak piglets, as well as submission to a diagnostic laboratory for culture testing (passive surveillance);

— examination of pigs for clinical signs, including orchitis (passive surveillance);

— serological surveillance using buffered antigen *Brucella* tests only as herd-level tests (active surveillance);

— Brucellin tests are also used to identify infected herds (active surveillance);

— sampling of contact wild pigs (active surveillance).

Off-Farm Surveillance

— monitoring the percentage of abortions and other tissues from which *B. suis* has been isolated (passive surveillance);

— bacteriological examination of tissues (submandibular, gastro-hepatic, internal iliac, and inguinal lymph nodes) and blood for serological testing of breeding-age pigs at slaughter (active surveillance).

2. Mass Vaccination Phase

There is no data on countries using swine brucellosis vaccines to support any serological surveillance programs. Off-farm surveillance remains the same as in Phase 1. While vaccines may have demonstrated good efficacy, they have never been widely used in pigs.

3. Testing and Removal, Segregation, or Slaughter Phase

Since none of the existing serological tests are reliable for individual pigs, herd infection diagnosis relies on buffered antigen *Brucella* tests (including the Card test) (Brown et al., 2015). However, some countries attempt herd eradication by testing all eligible animals (typically older than six months) every 30 days and removing positive reactors until the entire herd tests negative. If this option fails, depopulation (slaughter sale) is carried out 30 days after facility cleaning and disinfection, followed by repopulation with animals from brucellosis-free herds.

An alternative approach is offspring segregation, where piglets are separated from sows at approximately one month of age and raised separately. These animals must be tested 30 days before breeding.

Cases of abortion, movement testing, tracking of neighboring herds, and epidemiological investigation of infected herds can be controlled in the same way as in bovine brucellosis.

Off-Farm Surveillance

— if it is possible to trace the herd's origin from markets or slaughterhouses using temporary or permanent identifiers, all breeding-age pigs should be regularly tested;

— if wild pigs are in contact with farmed pigs, selective testing should continue;

— periodic bacteriological surveillance of positive-reactor animals from infected herds or randomly selected pigs or herds at slaughter should be conducted, with monitoring of *B. suis* isolation, as in Phase 1.

4. Release Phase

The OIE Terrestrial Animal Health Code does not specify conditions for countries free of swine brucellosis. However, several countries have achieved or are in the process of achieving this status.

On-Farm Testing

The OIE defines a herd as free from swine brucellosis if it meets the following requirements:

1. The herd is under official veterinary supervision.
2. No animals in the herd have had brucellosis in the past three years, and all suspected cases undergo laboratory testing.
3. All pigs housed in the same facility are officially free from brucellosis.

Although not explicitly stated, these herds should not have direct contact with wild pigs.

Breeding pig herds (all animals older than six months) can be certified as brucellosis-free if:

I. The entire herd is tested and found to be seronegative; or II. Stepwise testing is conducted as follows:

— selective testing of 25% of pigs every three months or 10% monthly, with all results seronegative;

— no pig should be tested twice in the same year;

— to maintain free status, herds should be re-tested every 12 months;

— continuous monitoring for clinical signs is required;

— all movements into *B. suis*-free herds must come from *B. suis* -free herds, or if not, the animals must test seronegative 30 days before movement, be isolated upon arrival, and be re-tested after 30–60 days;

— if artificial insemination is used, all semen must come from boars in *Brucella* sp.-free herds.

Off-Farm Testing

Periodic bacteriological and serological examination of any positive-reacting or suspected pig sent for slaughter.

Conclusions. 1. Brucellosis remains an important social and medical issue for countries with developed animal husbandry and economic conditions based on private ownership in agriculture.

2. The emergence of brucellosis outbreaks in areas previously considered safe from this disease remains a pressing issue due to cross-border movements of livestock in the absence of effective veterinary and customs control.

3. In addition to bacteriological studies, a comprehensive set of laboratory tests (ELISA and PCR) should be used to ensure timely diagnosis of brucellosis.

4. The existing epizootiological monitoring system requires improvement by optimizing the use of traditional diagnostic tests, determining their diagnostic value, developing and implementing advanced technologies for producing brucellosis diagnostic agents for animals, and standardizing diagnostic studies following international standards.

5. In brucellosis eradication programs for pigs, even isolated cases of animals testing positive with low titers should not be overlooked by specialists.

6. Due to the scale, complexity, and multifaceted nature of biological security and biosafety issues, it is necessary to develop a unified methodology for creating a national biosafety and biosecurity system, which various institutions and organizations currently represent.

7. In Ukraine, positive results from a single test must be confirmed by additional studies using other tests, particularly the complement fixation test (CFT) according to the national system for brucellosis diagnosis verification and differential diagnostics.

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