The Russian experience in brucellosis veterinary public health

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Summary
Russia has extensive experience in controlling brucellosis in large and small animals, based on systematic animal health and disease control measures, combined with the use of specific prophylaxis. Widespread application of the live brucellosis vaccine *Brucella abortus* strain 82 has led to a dramatic reduction in the prevalence of brucellosis. Among the distinctive properties of this vaccine are weak agglutinogenicity and high anti-epizootic efficacy, which allow differentiation between infected animals and vaccinated animals, combined with protection against brucellosis infection. In this paper, the authors review brucellosis epidemiology, diagnostics, the application of vaccines and management procedures that allow the Russian Federation to reduce the overall prevalence of brucellosis.

Keywords
Brucella – Brucellosis – Control – Diagnostics – Epidemiology – Epizootiology – Eradication – Russia – Vaccination – Vaccines.

Epidemiology

The former Soviet Union (FSU) and Russia have more than 40 years' experience in controlling large and small animal brucellosis through the implementation of complex, well-organised, routine animal health and anti-epizootic measures, based on the principle of controlling the epizootic process (12). The systematic study of brucellosis in the FSU began in the 1930s when animal and human brucellosis were included in the group of infectious diseases that were subject to government regulation. Brucellosis control at that time was limited and focused only on preventing the transmission of infection to new farms or territories, by selective examination of cattle (mainly by the allergenic method), slaughter of serological reactors and animals that aborted, and animal health and hygiene procedures at farm facilities and within territories.

However, these measures did not lead to a decline in the number of brucellosis cases and the prevalence of cattle brucellosis in the FSU increased markedly. By 1952, the number of brucellosis-infected sites (individual farms, collective farms and/or larger geographical areas) had increased to 13,580, with over 280,000 detected cases of cattle brucellosis. Only after the development of the *Brucella abortus* strain 19 live vaccine, and its wide application across the FSU from 1953 till 1970, did the number of sites affected by cattle brucellosis decrease to 3,519 (53, 54). The subsequent development and introduction of the Russian *B. abortus* strain 82 live vaccine into the national brucellosis control programme was the key factor for continued progress in reducing cattle brucellosis (2, 34, 35, 36).

At present, brucellosis is still reported from all types of farms and domestic livestock in Russia, including strains of *B. abortus*, *B. melitensis*, *B. suis* and *B. ovis*.

Between 1999 and 2009, the number of infected sites slightly increased, ranging from 54 in 2001 to 112 in 2009 (Fig. 1). The number of sites affected with ovine brucellosis also remained low: 13 in 2004 and 21 in 2009 (Fig. 2).

However, the relatively low number of brucellosis-infected areas does not diminish the epizootic and epidemic significance of sheep and goats in the transmission of brucellosis. Since the Soviet Union's dissolution at the beginning of the 1990s, social and economic changes in Russia have led to huge changes in the management and ownership of agricultural animals. About 80% of all sheep and goats, and more than 50% of all cattle, were transferred from state to private farms. New livestock owners were largely unable to implement costly anti-brucellosis measures. As these private farms now included multiple livestock species, favourable conditions arose for the transmission of *B. melitensis* to cattle, leading to a dramatic rise in new cases of brucellosis in humans (adults and children), due to this pathogen (46) (Fig. 3). Over the last two decades, large and small animal brucellosis has become a considerable problem within the Southern Federal District, the Southern Volga region, and the Siberian Federal District (6).

In recent years, the epizootic situation of brucellosis in Russia has been complicated by:

– economic difficulties that led to drastic reductions in regulatory efforts to stem brucellosis

– less effective veterinary and Customs controls in areas bordering brucellosis-infected territories (Azerbaijan, Kazakhstan, Kirghizia, Mongolia)

– a decline in serological testing and vaccination of animals

– a reduction in microbiological evaluation of animal infection, leading to a lack of knowledge about the origin of infection

– a reduction in the ability to plan and implement brucellosis control policies in infected territories.

Diagnosis

Current brucellosis programmes in the Russian Federation use a combined diagnostic approach, based on the results of clinical–epizootiological, allergic and serological, bacteriological, molecular and genetic laboratory examinations (33). During the mass testing of animals, serological tests include:

– reaction of agglutination (RA)

– the complement fixation test (CFT)

– the Rose Bengal test

– ring reaction, with milk (24, 26).

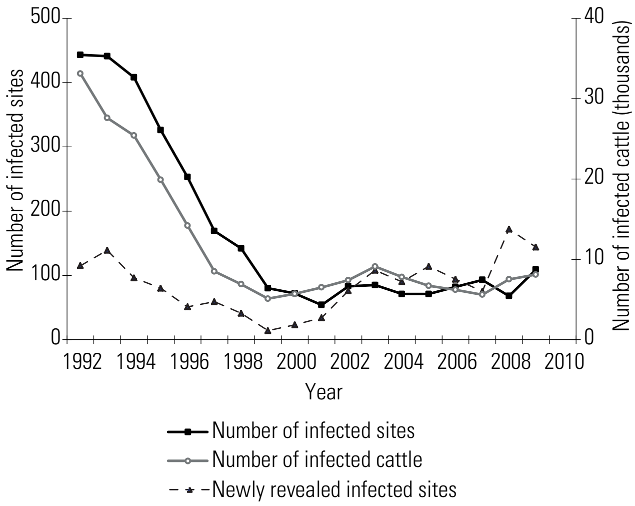

Fig. 1
Cattle brucellosis in the Russian Federation, 1992 to 2009

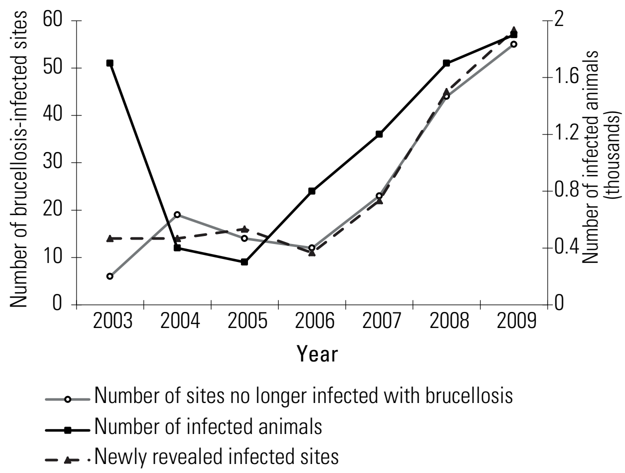

Fig. 2
Ovine brucellosis in the Russian Federation, 2003 to 2009

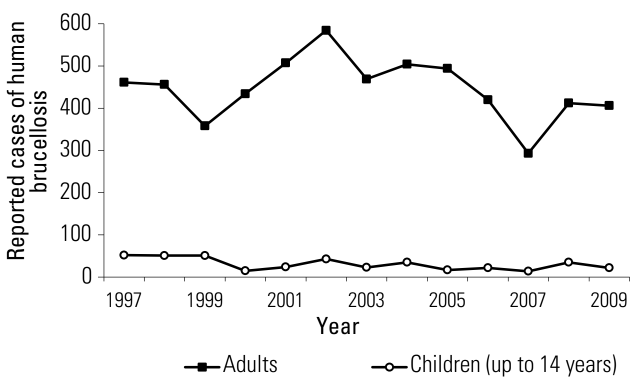

Fig. 3
Human brucellosis morbidity in the Russian Federation (first detected cases)

Recently, the indirect reaction of haemagglutination with a new erythrocyte antigen has been added. For differential diagnostics of brucellosis in cattle, sheep, goats and reindeer in vaccinated herds, immunodiffusion, with the brucellosis O-polysaccharide antigen, is also used, in addition to the RA and CFT serological tests (31).

The choice of the diagnostic tests used under field conditions is influenced by the properties of the pathogen, the route and dose of infection, the phase of the infection process and the species and age of the animal, among other factors. However, the serological tests available could not effectively detect infected animals during all phases of the infection, or were not suitable for use both on infected farms and in brucellosis-free areas. In recent times, new tests for brucellosis diagnostics, such as enzyme-linked immunosorbent assay (ELISA) tests based on monoclonal antibodies (28, 39, 52), fluorescent polarisation (22, 27), and polymerase chain reaction (PCR) (49), were developed and introduced into veterinary use. The ELISA tests developed for cattle brucellosis include tests which can differentiate between antibodies to the smooth (S) and rough (R) forms of *Brucella* and antibodies to the S-LPS antigen of *B. abortus*, *B. melitensis* and *B. suis* (52). As an alternative to bacteriological testing, the 'BRU-COM' test system was developed and introduced to detect *Brucella* by PCR. This test is highly sensitive and allows the detection of *Brucella* DNA when the sample contains an amount as small as ten colony-forming units (CFU) in physiological solutions, 25 CFU in cattle blood or milk, and 100 CFU in semen (48).

Methods used to diagnose animal brucellosis in the Russian Federation are coordinated with the recommendations of the World Organisation for Animal Health (OIE).

Management

Eliminating brucellosis on cattle-breeding farms is the most effective way of reducing the economic costs associated with brucellosis infections. The most effective method on farms is to isolate any infected animals and remove them from the herd within two weeks of identification of the pathogen. Sheep or goats which abort should be removed immediately. Only highly productive or pure-bred seropositive cattle can be kept in isolation. Because complete elimination of brucellosis from infected areas takes a long time (from three to five or six years), all infected farms are registered and undergo systematic monitoring. Brucellosis-infected pastures are neutralised by not using them for two to three months; hay collected from infected sites is not used for two months; and water sources are not used for three months. Faecal material is heat-treated for two months. Contaminated areas from the sites of abortion or parturition are disinfected twice, with a mechanical cleaning after the first disinfection. Contaminated bedding is burned. For other materials, such as equipment, shoes or clothes, disinfection procedures are used which employ 20% slack lime solution, 2% formaldehyde solution, 5% solution of soap-carbolic mixture, or 5% solution of phenolic creoline.

To prevent the transmission of brucellosis through animal products, the following measures are taken:

– the sale of milk and milk products from brucellosis-infected farms is forbidden

– meat from brucellosis-infected animals is processed in meat-packing plants in such a way (mainly by meat heat-treating) that the *Brucella* cells are inactivated

– hides from brucellosis-infected animals are salted and stored for two months, and wool is held on the farms for two months or inactivated by a hot washing procedure.

Vaccination

Over 50 *Brucella* vaccine strains have been evaluated in the Russian Federation, with more than 30 of them reviewed by certification boards (Table I). One of the first vaccines widely used in cattle was the live vaccine, *B. abortus* strain 19 (50). This vaccine had high stability and immunogenicity and protected cattle against infection and abortion (23, 25). It is still used as a standard for comparison when studying the immunogenic properties of new brucellosis vaccines. Despite the fact that vaccination with strain 19 had a positive effect in reducing brucellosis within the Russian Federation, its use in cattle was discontinued as it did not provide complete protection against abortion in infected herds, and also did not provide a long-term reduction in herd seroprevalence. Another argument against the wide use of this vaccine was the persistent titres in immunised animals, which cross-reacted with brucellosis diagnostic tests. Cross-reacting titres prevented the differentiation of animals infected with field strains of *B. abortus* from vaccinated animals and thus impaired the characterisation of disease prevalence (5, 7, 15, 25, 51). Others have reported that strain 19 can cause abortions in pregnant cattle (11, 40). A *B. abortus* isolate from an aborted fetus in the Moscow region in 1929, strain 104-M, was also evaluated as a vaccine. This smooth strain was stable and, when compared to strain 19, low doses of strain 104-M induced reduced titres and greater immunity in laboratory animals, sheep and cattle (18, 19, 42, 47). The 104-M vaccine was successfully evaluated in cattle in the Volgograd and Novosibirsk regions of the Russian Federation, as well as in Georgia, Kirghizia and Kazakhstan, and was recommended for field use.

Between 1955 and 1980, the live *B. abortus* strain 19 vaccine was widely used in small animals and then, between 1980 and 1988, the live *B. melitensis* Rev.1 vaccine strain

Table I
List of brucellosis vaccine strains which were used or accepted for veterinary practice in the Russian Federation

Strain	Year of discovery	Author(s)	Animal species	Incorporated into veterinary practice
B. abortus 19	1952	J.M. Buck	Cattle, sheep	+
B. abortus 104-M	1970	K.V. Shumilov, H.S. Kotlyarova	Cattle, sheep	+
B. abortus 82	1960	K.M. Salmakov	Cattle	+
B. abortus 75/79-AB	1996	K.V. Shumilov, I.P. Nikiforov *et.al.*	Cattle	+
B. abortus 45/20	1922	A.D. McEven	Cattle	–
B. abortus KB 17/100	1997	K.V. Shumilov, V.V. Kalmikov	Cattle	+
B. abortus 21	1960	V.S. Ryaguzov	Cattle	–
B. abortus B-8	1955	P.N. Jovanik	Cattle	–
B. abortus 7/26	1980	P.N. Jovanik	Cattle	–
B. abortus 519	1966	I.A. Kosilov	Cattle	–
B. abortus 82-PS (penicillin sensitive)	1979	K.M. Salmakov, G.A. Belozerova	Cattle	–
B. abortus B-1	1948	E.S. Orlov	Cattle	–
B. abortus 4004/1	1962	E.S. Orlov	Cattle	–
B. abortus 16/4	1967 to 1971	P.A. Trilenko	Cattle	–
B. abortus 70	1971	K.P. Studentsov	Cattle	–
B. melitensis 56	1973	E.S. Orlov, A.A. Klochkov	Cattle	–
B. melitensis 53H38	1960	G. Renoux	Cattle, sheep	–
B. melitensis K-24	1971	P.A. Trilenko	Sheep, goats	–
B. melitensis 'Nevskyi 12'	1959	I.N. Nevskyi, M.S. Abidjanov	Cattle	–
B. melitensis 'Nevskyi 13'	1980	R.G. Yaraev, K.V. Shumilov *et al.*	Cattle	–
B. melitensis 89/23	1964	L.V. Kirilov	Cattle	–
B. suis 61	1955	M.K. Yuskovets	Cattle	–

was introduced. Data suggested that vaccination with Rev.1 induced greater titres and twofold higher immunity in sheep, as compared to the strain 19 vaccine (3, 55). Further investigation demonstrated that a single immunisation of young ewes with Rev.1 did not induce long-term protection against experimental challenge with a high dose of virulent *Brucella*, and so re-immunisation of ewes with Rev.1 at two-yearly intervals was approved. At present, it is difficult to determine the true effects of Rev.1 or strain 19 on the prevalence of brucellosis in sheep, as both these vaccines are still used in some parts of the Russian Federation. In 2009 and 2010, approximately 4.995 and 3.95 million sheep, respectively, were immunised with *B. melitensis* Rev.1, whereas 1.82 million sheep were vaccinated with strain 19 in 2009, and 1.69 million in 2010.

Numerous studies by Russian scientists demonstrated that brucellosis vaccines from R-forms had weak immunogenic properties and did not protect cattle from infection (44, 47). Therefore, further studies for new brucellosis vaccines focused on strains with high immunogenicity, weak responses on agglutination tests and low pathogenicity in humans. One of the most promising strains identified during this work was the SR-*B. abortus* strain 82. This strain was identified by Salmakov in 1961 by selecting colonies originally isolated from an aborted bovine fetus (34). Strain 82 combines the desired weak agglutination test response with high immunogenicity and comparatively high efficacy against brucellosis (9, 34, 35, 36, 37, 38). Strain 82 has low residual virulence, is not shed, and is stable during *in vitro* passage in media, or *in vivo* passage in guinea pigs and cattle, including pregnant cows. Some data suggest that strain 82 can be abortogenic in pregnant cattle (4, 10, 17). Strain 82 was tested in laboratory animals, and cattle under experimental and field conditions, and was officially approved for use as a vaccine for cattle in 1974 (1). A national regulatory control programme for cattle brucellosis was then developed, based on strain 82 vaccination, and targeted at regions where the prevalence was high. Vaccination is not used in areas that have been free of brucellosis for at least four years. In areas where there is a risk of resurgence or reintroduction of brucellosis, heifers are vaccinated at four to five months of age (10^{11} CFU subcutaneously) and booster vaccinated at ten months of age. In brucellosis-free regions that border brucellosis-infected areas, cows are re-immunised after calving, and every two years thereafter. In brucellosis-infected areas, heifers are vaccinated twice with strain 82 and adult cows are re-vaccinated after calving and every one to two years thereafter, until brucellosis has been eliminated and the region shares no boundary with areas of infection.

More than 40 years of widespread vaccination with strain 82 in the Russian Federation has provided improved herd immunity in cattle and allowed earlier use of serological diagnostics after vaccination (three to six months after strain 82 vaccination versus two to three years after strain 19 vaccination); leading to the eradication of cattle brucellosis in many areas of Russia.

Another vaccine strain with weak agglutinogenic properties, which was used under field conditions in the Russian Federation, was *B. abortus* strain 75/79-AB. This strain was isolated in 1979 from a seropositive cow that had been immunised twice (1974 and 1976) with strain 82 after *in vitro* passages in media, guinea pigs and heifers. Strain 75/79-AB is an RS-form strain. It was found to have satisfactory immunogenicity and was not abortogenic, which allowed the vaccination of pregnant cattle, irrespective of the stage of gestation, and also permitted serological testing within three months of immunisation (29, 30, 44). Evaluating the efficacy of this vaccine strain in 130,000 cattle in 41 administrative regions of Altai Krai in the Russian Federation demonstrated that the vaccine induces protective immunity against infection with brucellosis (45). Recently, comparative studies of strain 75/79-AB have shown a genetic relationship with strain 82 (20). These studies indicate that strain 75/79-AB is a dissociated form of strain 82. Strain 75/79-AB has been used as a vaccine in Altai Krai and in 13 republics and regions of the Russian Federation since 1997.

At the present time, vaccination of cattle against brucellosis in the Russian Federation is fairly evenly distributed between the strain 82 and the strain 75/79-AB vaccines. In 2009 and 2010, respectively, about 1.99 and 1.72 million animals were vaccinated with strain 82; approximately 151,000 and 135,000 were vaccinated with strain 75/79-AB; and approximately 14,000 and 12,500 animals with strain 19. Over the last few years, agglutinogenic or weakly agglutinogenic adjuvant-vaccines have been evaluated (8, 13, 16, 21, 32). A new strain, KB 17/100, was developed by selecting isolates based on cultural, morphological, biochemical and antigenic properties from a culture of the highly immunogenic *B. abortus* 104-M. KB 17/100 is in R-form, is stable after ten *in vitro* passages on nutrient media, has low residual virulence, and is adjuvanted in a 'water-in-oil' emulsion (14, 41, 43).

Testing strain KB 17/100 in chronically infected cattle herds on 124 farms in the Saratov Oblast region suggested that this vaccine did not induce titres during diagnostic testing in naïve animals; did induce titres in cattle latently infected with brucellosis, allowing the rapid removal of infected cattle; is not abortogenic; and, as an inactivated vaccine, is ecologically safe. Despite their success to date in controlling brucellosis in cattle in the Russian Federation, researchers continue to work to develop new vaccines, and improve currently used vaccines. Using targeted genetic studies and evaluating the effects of various factors on *Brucella* cultures (bacteriophages, high temperature, ultraviolet rays, gamma radiation, ultrasound, antibiotics and others), new vaccine strains continue to be identified and evaluated in *in vivo* studies.

Conclusion

Long experience in Russia has demonstrated that a system of organised animal health measures, combined with disease control steps, is the foundation stone in controlling animal brucellosis. The most efficient means of control are brucellosis vaccines that induce weak serological titres. Official brucellosis control programmes in Russia include the widespread vaccination of cattle with *B. abortus* strain 82 to reduce disease prevalence. Since strain 82 has weak agglutinogenic properties, it does not induce long-term titres in standard brucellosis tests, and thus allows the identification of virulent field strains of *Brucella* soon after vaccination (9). As a result of the widespread use of strain 82 vaccine across the Russian Federation, the number of disease-infected sites has dramatically declined and many regions are now classified as free of cattle brucellosis. In association with the decline in cattle brucellosis, there has also been a sharp decline in human brucellosis in Russia. The authors' experience is that optimising the use of strain 82 as part of a system of sanitary and control measures has been successful in reducing brucellosis in cattle.

Brucellose et santé publique vétérinaire : l'expérience de la Fédération de Russie

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Résumé

La Russie a une longue expérience dans la lutte contre la brucellose chez les grands et les petits ruminants, qui repose sur des mesures systématiques de santé animale et de contrôle de la maladie, associées à l'application de prophylaxies spécifiques. La vaccination à grande échelle avec un vaccin utilisant la souche vivante 82 de *Brucella abortus* a considérablement réduit la prévalence de la brucellose. Les propriétés marquantes de ce vaccin sont sa faible agglutinogénicité et sa grande efficacité anti-épizootique, qui permet la différenciation entre animaux infectés et animaux vaccinés (stratégie DIVA) tout en conférant une protection contre l'infection brucellique. Les auteurs font le point sur l'épidémiologie et le diagnostic de la brucellose, sur l'application de la vaccination et sur les procédures de gestion qui ont permis à la Fédération de Russie de réduire la prévalence globale de la brucellose.

Mots-clés

Brucella – Brucellose – Contrôle – Diagnostic – Épidémiologie – Épizootiologie – Éradication – Russie – Vaccin.

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La experiencia rusa de lucha contra la brucelosis como parte de la salud pública veterinaria

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Resumen

Rusia cuenta con gran experiencia en la lucha contra la brucelosis en grandes y pequeños rumiantes, con un método que combina una serie de medidas sistemáticas de sanidad animal y control de la enfermedad con el uso de profilaxis específica. La aplicación generalizada de la vacuna antibrucélica a base de organismos vivos de la cepa 82 de *Brucella abortus* ha deparado una espectacular reducción de la prevalencia de la enfermedad. Entre las propiedades características de esta vacuna están su escasa aglutinogenicidad y su gran eficacia anti-epizoótica, lo que permite distinguir entre los animales infectados y los vacunados, además de conferir protección contra la infección. Los autores pasan revista a la epidemiología y el diagnóstico de la brucelosis, a la aplicación de vacunas y a los protocolos de gestión que han servido a la Federación de Rusia para reducir la prevalencia general de brucelosis.

Palabras clave

Brucella – Brucelosis – Control – Diagnóstico – Epidemiología – Epizootiología – Erradicación – Rusia – Vacuna.

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