



# The Role of Various Species and Biovars of Animal Brucellae in Brucellosis Epidemiology

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**Abstract** | Three species from *Brucella* genus; *B. abortus*, *B. melitensis*, and *B. suis* are mainly the reason for abortion in natural hosts (cattle, sheep, goats, pigs and deer), causing huge economic losses. Likewise, they considered the major cause of brucellosis cases in humans. The role of *B. ovis* and *B. neotomae* in the epidemiology of brucellosis has not been proven. It has been previously reported that the brucellosis in marine mammals mainly attributed to *B. ceti* and *B. pinnipedialis* which are pathogenic for humans and of public health importance. Given the large number of reports about the high epidemic risk of brucellosis in dogs; a series of experiments were carried out to study the virulent properties of *B. canis* compared to the other *Brucella* species (*B. abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. ovis*). As a result of the research, it was found that the virulent properties (infecting ability) of brucellae from the *B. ovis*-64 species significantly exceeded the indicators of brucellae of the *B. canis* species. However, it is believed that *B. ovis* is not zoonotic to human. In Russia, there is no stationary foci for pig brucellosis; only sporadic cases were reported in some regions. In this regard, the probability of humans' infection from infected pigs is extremely low. At the same time, there are stationary foci of reindeer brucellosis that able to infect between 10 to 20% or more from the population engaged in reindeer herding. Given the above, a situation arises when people in large numbers become infected with brucellosis from reindeer, the name of the pathogen of which has been reserved for pigs. In conclusions, the epidemiological hazard not only depend on the virulence of brucellae but also on the frequency of human contact with the carrier animals.

**Keywords** | Brucellosis, Brucellae, Cultures, Biovar, Antibodies, Immunogenicity, Virulence, Antigenicity

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## INTRODUCTION

According to the International Office of Epizootics (IOE), the recent classification of *Brucella* species is mainly based on the differences in the pathogenesis and

the specificity to a particular host (Albertyan et al., 2013). For instance, *B. abortus* species are mainly detected in cattle, while *B. melitensis* in small cattle, and *B. suis* in pigs (Ignatov, 2010). At the same time, brucellae can often spillover to a host which is nonspecific to them (Fedorov et al., 2006).

Previous genetic and immunological studies reported that all members of the *Brucella* genus were closely related and should probably be considered as variants of a separate species (Godfroid, 2017). However, based on the real differences in the specificity to a particular host and the epidemiological hazard, as well as according to the molecular studies of the genomic variations in the main species, the International Committee on Systematics of Prokaryotes, the Subcommittee on Brucella Taxonomy took a clear position in 2005 in favor of returning the Taxonomic Brucella systematics to the nomenclature of 1986 (Albertyan et al., 2013; Fedorov et al., 2006; Ignatov, 2010). In this regard, six classical *Brucella* species; *B. abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. ovis*, and *B. canis* with the relevant recognized biovars have been reapproved.

Of all the species of the *Brucella* genus, *B. abortus* (cattle), *B. melitensis* (sheep and goats) and *B. suis* (pigs and deer) cause the greatest damage to agricultural production, as they cause abortion of animals, leading to huge economic losses.

The role of *B. canis* in the epidemiology has not been sufficiently studied, although as early as in 1936, Lange reported about human infection by brucellosis from a sick dog (Ignatov, 2010). In 1995, the Chinese Journal of Epidemiology published some data about two people infected with brucellosis from a dog (Wang et al., 1995). It should be noted that brucellosis caused by *B. canis* species is reported in countries where brucellosis did not report from other animals as Great Britain, Germany, Czech Republic, Slovakia, and Japan. Since the beginning of the third millennium, the usage and application of new technologies leads to rapid progress for understanding the diversity of brucellae. Currently, eleven species are recognized within the *Brucella* genus: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, *B. ceti*, *B. pinnipedialis*, *B. microti*, *B. inopinata*, and *B. papionis* (Godfroid, 2017).

*B. ceti* and *B. pinnipedialis* cultures were isolated in 2007 from marine mammals. and are pathogenic to humans and can pose an epidemic hazard (Godfroid, 2017). In early 2008, the ninth *Brucella* species was added to the *Brucella* genus, and named *B. microti*, while two strains of this species had been isolated from infected wild voles in South Moravia, the Czech Republic (Vinokurov et al., 2019). In 2010, the tenth *Brucella* species *B. inopinata nov.* was added to the *Brucella* genus, had been isolated from a breast implant of patient with clinical manifestations of brucellosis (Albertyan et al., 2013). Likewise, *B. papionis* was isolated in 2014 from stillborn fetus of a baboon (Godfroid, 2017). Finally, strains that have not yet been approved as new *Brucella* species, were isolated from rodents, foxes and frogs and were characterized as atypical *Brucella* strains that differed from the currently existing

species (Fedorov et al., 2006).

Recently, many reports have reported the high epidemic danger of dog brucellosis caused by *B. canis*, which is reflecting the normative documents regulating the anti-brucellosis measures (Godfroid, 2017; Kanouté et al., 2017; Vinokurov et al., 2019). The aim of the current study is to study the virulence properties of *B. canis*, compared to other *Brucella* species that had been stored in the freeze-dried form for several years in the VIEV museum collection, stored at the experimental base in the Vyshny Volochek branch of the Federal State Budgetary Institution Federal Scientific Center VIEV of RAS on the Lisiy Island.

## MATERIALS AND METHODS

The authors made a series of experiments within the state research program of the State Academy of Sciences upon assignment No. 0578-2014-0001.

The current research aims to study the role of various species and biovars of the animal brucellae in epidemiology. Laboratory experiments with Guinea pigs were planned for studying the antigenic, virulent, and other biological properties of the museum brucellae cultures. To study the general biological properties of the brucellae cultures from the museum collection, seven groups of guinea pigs; five animals in each group, were housed according to the principle of analogs and infected with various *Brucella* species.

One month after the infection, the guinea pigs were euthanized for pathological and bacteriological examination. The blood sera was collected 15 and 30 days after the infection.

## RESULTS AND DISCUSSION

Studying the *Brucella* species from the museum collection did not reveal any significant changes in their cultural, morphological, tinctorial, and biological properties, depending on the duration of storage in the freeze-dried state. As the research results show, 25 cultures from the museum collection did not lose their properties. Some cultures of *B. abortus* species remained viable after being stored in a refrigerator for over 60 years in the freeze-dried state. For studying the general biological properties of the museum brucellae cultures, the authors performed a series of experiments on guinea pigs for studying the antigenic properties, are shown in Table 2.

Our results revealed that the most pronounced serological reactions in AT, CFT, and RBT in guinea pigs were groups I, V, and VII that had been infected with *B. neotomae*, *B.*

*abortus-191* and *B. abortus-191*, respectively. In groups II and VI that were infected with the R- form of *B. ovis* and *B. canis*, CFT were carried out with the R- antigen of *B. ovis* culture, and the reaction in the animals in group II was more pronounced, however, due to the significant scattering of variables, the difference was not statistically significant (Fedorov et al., 2018a, 2018b). The novelty of the research consisted in the study of the serological reaction during AT and CFT in the selected strains.

Pathological and bacteriological studies revealed that the most pronounced pathomorphological changes were noticed in groups II and VII that infected with brucellae of *B. ovis-64* and *B. abortus 54*, respectively. The regional and peripheral lymphatic nodes and parenchymal organs were enlarged with pronounced foci of hyperemia and hemorrhage. The animals of group V that infected with *B. abortus 191*, showed clear pathological changes. While, the animals in groups I and VI; infected with *B. neotoma* and *B. canis*, respectively, there was any recorded pathological changes.

The infection index during the bacteriological study was the most pronounced in group VII (II = 95 %) while group V infected with *B. abortus - 191* culture isolated from a sick horse and stored in the freeze-dried state for over 40 years,

a high infection index was recorded (II = 89 %). Group II, the infection index was 25 % with a more pronounced pathoanatomical picture compared to group V. Finally, groups III and IV, the infection index was 12 %, and for groups I and VI 6 %.

Thus, it was found that the two brucellae cultures that were persistent dissociants, i.e., in the R- form, virulent properties of *B. ovis-64* species significantly exceeded similar indicators of the *B. canis* species. At the same time, no one cannot deny the fact that humans get infected with brucellosis from dogs with brucellae of *B. canis*, while from rams with brucellae of *B. ovis*, human cannot be infected. In our opinions, the reason thereto is not related to the virulent properties of a particular pathogen, but in the social factor, i.e., the different approaches within the society and direct human communication with animals which means that no one contacts sheep so closely as contacting pets including dogs. A differentiated approach should be used for assessing the earlier reports of human infection with brucellosis from dogs. Likewise, consideration should be given to the possibility of spillover of brucellae to other species including nonspecific hosts. For example, *B. melitensis* is often isolated from shepherd dogs, the pathological process in humans proceeds with numerous complications till disability compared to other *Brucella* species.

**Table 1:** The experimental setup for studying virulent, antigenic, reactogenic and other biological properties of the brucellae cultures from the museum collection.

Group No.	The number of live animals	Brucella species	Brucellae dosage	Brucellae injection location
1	5	<i>B. neotomae</i>	1 billion m.c.	Subcutaneously in the inguinal area
2	5	<i>B. ovis-64</i>	1 billion m.c.	- // -
3	5	<i>B. suis Tomsen</i>	1 billion m.c.	- // -
4	5	<i>B. melitensis - 565</i>	1 billion m.c.	- // -
5	5	<i>B. abortus - 191</i>	1 billion m.c.	- // -
6	5	<i>B. canis K01</i>	1 billion m.c.	- // -
7	5	<i>B. abortus 54</i>	1 billion m.c.	- // -

**Table 2:** The results of studying the antigenic properties of the museum brucellae cultures.

Group No.	The number of live animals	Brucella species	The results of serological studies one month after the infection		
			Rose Bengal test (RBT)	Antibodies titer in	
				Agglutination Test (AT)	Complement-Fixation Test (CFT)
I	5	<i>B. neotomae</i>	+	176 ± 48	22 ± 6
II	5	<i>B. ovis-64</i>	-	-	23 ± 7*
III	5	<i>B. suis Tomsen</i>	+	80 ± 24	20 ± 6
IV	5	<i>B. melitensis - 565</i>	+	64 ± 8	15 ± 3
V	5	<i>B. abortus - 191</i>	+	208 ± 48	22 ± 6
VI	5	<i>B. canis K01</i>	-	-	10 ± 3*
VII	5	<i>B. abortus 54</i>	+	160 ± 48	20 ± 6

\*: Serological diagnostics in CFT with the antigen to *B. ovis*

In the latter case, the frequency of human contacts with the animal carrier of the pathogen plays an important role rather than the virulent properties of the microorganism (Fedorov et al., 2018b). For instance, it was reported that the *B. ceti* and *B. pinnipedialis* cultures isolated from marine mammals posed an epidemic danger due to their pathogenicity to humans (Godfroid, 2017), however, it is known that humans get infected with brucellosis through contact with sick animals or animal byproducts (Khoch and Sleptsov, 2001). It is officially assumed that since the beginning of the third Millennium, the usage of new technologies lead to rapid progress for understanding the diversity of brucellae, and, after many years of interruption, the process of expanding *Brucella* species continues (Ignatov, 2010). However, in the 60s of the past century, Soviet scientists proposed to legitimize yet another species *B. rangiferi* which is currently associated with the fourth biovar *B. suis*, although it is commonly isolated from reindeer and has clear markers that may be used for differentiating it from the other species and biovars. Under various pretexts, *Brucella rangiferi* species has not been officially adopted. This situation may be explained by the state of the Cold War at that time when many initiatives emanating from the Soviet Union were bureaucratically delayed and blocked in the end. Such a consideration remains in place given that after the collapse of the USSR, avalanche-like identification of new *Brucella* species occurred (Fedorov et al., 2006).

In Russia, there are no stationary foci of pig brucellosis, only sporadic cases are noted in some regions in some years. In this regard, the probability of human infection from infected pigs is extremely low. At the same time, there is stationary foci of reindeer brucellosis between 10 to 20% or more of the population engaged in reindeer herding have been reacting to brucellosis (Khoch et al., 2001). So, the situation arises when large number of people become infected with brucellosis from reindeer, the name of the pathogen of which has been reserved for pigs.

## CONCLUSION

To conclude, out of the two brucellae cultures that are persistent dissociants, i.e., contained in the R- form, the virulent properties of some strains of *B. ovis* species significantly exceeded similar indicators of *B. canis* species. The epidemiological hazard of various species and biovars of brucellae are not depending only on the virulence but also on the frequency of contacts with the animal carriers and their byproducts. For instance, humans get infected with brucellosis from dogs, by *B. canis*, while they are not infected from the rams by *B. ovis*. In the regions of the

Russian Federation with developed reindeer husbandry, people often get infected with brucellosis caused by *B. suis* pathogen (pig pathogen). In the future, the authors propose to attribute the causative agent of reindeer brucellosis to a separate species called *B. rangiferi*, since it has clear markers by which it can be differentiated from other species and biovars of *Brucella*.

## AUTHORS CONTRIBUTION

All authors contributed equally.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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