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Brucellosis in Ruminant Animals and Adopted Strategy for its Control in Albania

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Abstract

Brucellosis is an important bacterial disease in animals and human. It is one of major neglected zoonotic diseases and more than half million humans are infected annually. In Albania, *Brucella melitensis* and *B. abortus* circulate in both large and small ruminants. Application of a national control strategy to control sheep and goat brucellosis and a recently applied control programme to control bovine brucellosis in large dairy herds have played a significant role in reducing the risk of disease transmission and the number of human cases. The correct application of the current approved strategy to control sheep and goat brucellosis, extending the present bovine brucellosis control programme to include small dairy herds, and designing an appropriate strategy to control bovine brucellosis in beef herds will ensure improved control of brucellosis.

Keywords: Bovine, sheep and goat brucellosis, *B. melitensis*, *B. abortus*, control strategy.

1. Introduction

Brucellosis is a bacterial contagious disease caused by various bacteria of the genus *Brucella*. Bovine brucellosis (*Brucella abortus)*, sheep and goat brucellosis (*B. melitensis)* and swine brucellosis (*B. suis*) are OIE listed and mandatory reported disease [15]. In general, each *Brucella* spp. is adapted to a specific host, however, most species of this genus can infect several species [17]. Brucellosis is widespread globally, however, the highest incidence is observed in the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru, and Mexico, while several countries in Western and Northern Europe, Canada, Japan, Australia and New Zealand are believed to be free from the agent [2].

Brucellosis in animals ranges from a mild disease with infected animals showing few signs to clinical cases in farm animals when the main clinical sign is abortion, which usually happen only once during the last trimester of pregnancy [17, 18]. Infected animals recover and may have subsequent successful pregnancies but continue to shed large numbers of bacteria in the birth fluids. *Brucella* spp. invade the udder tissue and can be shed in the milk, which presents a risk for animals and humans. Additional clinical signs described in animals include: infertility, retention of placenta, stillbirth and mastitis in female animals: orchitis and epididymitis in male animals, while arthritis may be present in males and females [5, 7, 20].

Brucella spp. are non-sporulating bacteria that persist for prolonged periods in the environment. *Brucella* spp. can be transmitted by the digestive route, and can infect animals and humans through skin abrasions, through mucous membranes and inhalation [17].

Brucellosis is a highly infectious zoonosis for humans causing a debilitating disease. Most severe human cases are caused by *B. melitensis*, *B. abortus* and *B. suis*, however, other *Brucella* spp also have zoonotic potential [5, 6, 17]. The most common symptoms in humans include intermittent fever, weight loss, profuse odour sweating, headache, weakness, hepatomegaly and splenomegaly. In addition, severe complications may occur: unilateral orchitis, spondylitis and in rare cases endocarditis and meningitis. Occupational exposure represents a risk factor: and veterinarians, farmers, butchers and laboratory workers are at high risk to infection [1,

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]21]. People can acquire infections through consumption of unpasteurized milk and dairy productsfrom infected animals. Brucellosis may be suspected usually based on prominent indicators a) abortion storm and b) human cases, but its confirmation is made through laboratory diagnosis based on serological tests, isolation and identification the bacteria and detection of *Brucella* nucleic acid. Control of brucellosis is based on scientific approaches, OIE standards and international best practice. National control programmes may adopt the most appropriate control strategy according to specific circumstances. In brucellosis-free countries/regions it is important to prevent the introduction of the infection, and this could be achieved by application of serological surveillance and biosecurity measures [15]. In endemic areas, prevalence rates play an important role in designing a rational control strategy; if prevalence rate is high, vaccination is a recommended approach accompanied with complementary measures. Standard vaccines for cattle are *B. abortus* S19 and *B. abortus* RB51 vaccinal strains, while *B. melitensis* Rev 1 strain remains the standard vaccine for sheep and goats [15, 3]. The preferred vaccination method with the Rev-1 vaccine is via the intra-conjunctival route as it offers at least three main advantages: the protective immune response is similar to subcutaneous injection; the antibody titre declines rapidly, and the bacterial vaccine strain is not shed in milk. In the countries/regions where the prevalence is low, or after control programmes have reduced infection rates, a test and slaughter approach may be used until the disease is eradicated. Control of *Brucella* infection in animals prevents human brucellosis: milk pasteurisation is an important measure to minimize the risk of infection to humans [5, 7, 16, 17].

1.1 Human brucellosis in Albania

The first case of human brucellosis was detected in Albania in 1925 [13], while the positive results from a study based on *Brucella* DNA investigation carried out in human bones in southern Albania suggested that brucellosis has been endemic since at least the Middle Ages [14].

The incidence of human cases varies between regions and reflects the prevalence of disease in the animal population. During 1950, the incidence of human brucellosis was 23 per 100,000 inhabitants, while in 1960 it significantly increased to 37 per 100,000 inhabitants. During the following 15 years there was a significant reduction in reported human cases and during the 1976-1989 period, it remained almost constant at very low level. In 1990, when democracy emerged in Albania the incidence was at a comparatively low level *i.e.* 1.6 cases per 100,000 inhabitants [11, 13]. In 2004, the incidence was reported to be at 38 per 100,000 inhabitants, in 2011 the incidence dropped to 11.7 per 100,000 inhabitants again when mass vaccination of small ruminants started in 2012. In 2015, the number of cases reported was fewer than 6 per 100,000 inhabitants [10]. The effect of the nationwide vaccination programme in Albania since 2012 and the anticipated lower incidence in animals have reduced the incidence of disease in the human population. The annual total number of human cases fell from 440 in 2012 to 70 in 2018. In most districts, where the number of human cases was initially high at the start of the national vaccination campaign in 2012, the situation improved, and the number of human cases significantly decreased. However, at the same time, in some districts human brucellosis increased. The real number of human brucellosis cases may be higher, due to the potential for undiagnosed cases: mild cases are often misdiagnosed or not even reported. According to the World Health Organization, an approximation of the true number of cases in the Mediterranean countries can be obtained by multiplying by a factor of at least three the number of cases reported. Epidemiological investigations of 807 cases, reveal that 70% of infections were acquired by direct contact with infected animals [10]. This indicates that raising the public awareness, especially people at occupational risk will help to reduce the transmission of human brucellosis.

Brucellosis in sheep and goats

1.2 Small ruminants in Albania

Sheep and goats in Albania are kept mostly under extensive conditions where most farms are small. Lambing occurs mainly in the winter period when animals are kept indoors. Since massive excretion of bacteria takes place during *Brucella*-induced abortion and during normal parturition in infected animals, the disease is readily spread within a flock and to people who are in contact with infected animals: under these conditions, the environment becomes heavily contaminated. In smaller flocks, rams are kept with ewes throughout the year. As an effect of such husbandry, in some flocks the pregnancy status of animals is not completely clear, which complicates

implementation of mass vaccination because of known restrictions in the use of the Rev-1 vaccine in pregnant animals. Movement of small ruminants is frequent, and occurs for seasonal pasturing and trade. Movement of animals to abattoirs is less frequent. Commonly, during winter small ruminants are pastured near villages where flocks from the same commune are very likely to mix. During summer, large flocks are commonly moved to the better high pastures in the mountains. Farmers with smaller flocks occasionally merge their flocks with other small flocks to form a larger flock, which is then taken to the higher pastures: most prefer that their small flocks remain in the "lowlands". The time that animals are moved to higher pastures depends on the region and the prevailing temperatures. In any case, summer movement rarely happen before mid-May, sincethere is still sufficient grazing in lowlands in pastures near to villages; flocks do not usually return to from high pastures to lowland pastures before mid-October. These conditions favour the spread of brucellosis locally between flocks in affected villages, and also over greater distances through movement and livestock markets, therefore, specific measures to implement more stringent movement control have to be taken to minimize the spread of the disease [4, 16].

1.3 Control measures and the epidemiological situation with brucellosis in sheep and goats

Brucellosis in small ruminants in Albania was considered to be widespread with prevalence varying from region to region. In the absence of sustained surveillance during 1990-2004, there was anecdotal evidence that the disease spread to the districts, which were considered to be free. A wide range of control measures were applied: a combination of test and slaughter; mass vaccination in specified areas; vaccination of replacement animals; and, not intervening to control infection; sometimes all measures were applied to the same population. Based on the assumed risk of the disease spreading when movement control and the animal identification and registration (IR) system were not enforced, the veterinary department decided in 2011 to adopt a national brucellosis control programme in 2012 when the first mass vaccination campaign was implemented. In 2013 and 2017, mass vaccination campaigns were repeated. According to the adopted strategy, from 2014 onwards (excluding 2017) the brucellosis control programme in small ruminants was continued by vaccination of replacement animals only. Post vaccination monitoring (PVM) was implemented to assess the efficacy of vaccination. The general conclusion - based on results of the PVM - indicated that, on average, in more than 90% of the vaccinated flocks over 80% of the animals tested were serologically positive in the Rose Bengal Plate test, which indicated good immunity. These results also suggested that the procedures for handling the vaccine, including the cold chain, and the administration of the vaccine were generally effective. The numbers of reported abortions and suspected cases of brucellosis were low and only few suspect cases were reported [16, 22].

1.4 Bovine brucellosis

Cattle management systems in Albania are not intensive, nevertheless dairy cattle form a most important sector for milk production. The vast majority of herds have only one or two animals per holding producing milk for households; there are fewer than 100 dairy herds of 50 or more cattle. Animals in small herds are usually fed within the farm with some grazing in nearby village pastures where cattle mix with cattle from other households - both cattle and small ruminants graze together. Breeding is by both natural and artificial means, depending on the breed and value of the animal. Animals in intensive herds are kept on the farm and do not have daily contact with other animals. New introductions are commonly home bred, however, buying animals from other farms or livestock markets is frequently practised. Breeding is almost always by artificial insemination although sometimes bulls on the farm are used for breeding cattle belonging to other owners in the village. Of particular interest for the bovine brucellosis are the so-called "beef" production herds, especially in the southern parts of the country. Often, these farms have two principle holdings: summer and winter holdings and the management practices are similar to the common practice for large sheep flocks. When the weather conditions in spring improve, cattle are moved to higher pastures where they are exclusively kept outside and remain there until late autumn when they return to the "winter" holding. Breeding in these animals is natural and seasonal. The calving season is in early spring in the "winter" holdings where housing and hygiene conditions are usually poor. Milk is used for feeding calves and animals are either not milked or only milked for household consumption. Calves are usually slaughtered at the age of about 6 months.

Trading of cattle is very frequent: animals are commonly bought and sold at livestock markets, which are largely uncontrolled, or directly from farm to farm. Evidence from field investigations indicates that unauthorized movement of cattle and buying animals of unknown health status were the predominant sources of infection in the introduction of brucellosis in previously *Brucella*-free holdings 16].

1.5 Control measures and the epidemiological situation with bovine brucellosis

The first national programme to control bovine brucellosis was adopted in March 2016 when its implementation commenced. The Bovine Brucellosis Control Programme (BBCP) consisted of a series of actions toward progressive control of the disease including active surveillance, passive surveillance and control measures, in both infected and uninfected herds. Given the small size of farms, a more comprehensive and structured approach, aligned to the European Union regulation, was adopted in those holdings. Active surveillance based on repeated quarterly bulk milk testing was introduced in herds of 21 or more cattle. Individual animal testing was conducted in all positive herds, after which the standard control measures including slaughtering of positive and in-contact animals and cleaning and disinfection were implemented. Supplementary measures, including enforcement of passive reporting and traceability, were also implemented. In 2017, herds with 11-20 cattle were included in the active surveillance scheme. The results of the implementation of the BBCP indicated a 2% prevalence [8], which suggests an overall low prevalence on a herd basis. A serological survey done in 38 randomly selected beef herds in southern Albania in late 2017 suggested a much more complicated epidemiological situation in this distinctive sub-population than in the larger dairy herds. The results of the survey indicated very high overall herd prevalence of 55% (40% - 71% of 95% CI). The average 'within herd' prevalence in infected herds was 45%. Bacteriological isolation and typing of the causal organism from specimens collected from 16 slaughtered sero-positive animals confirmed the presence of *B. abortus* (under review). In 2019, another serological survey of 120 small herds of cattle (3 to 9 animals per herd), in Lushnja district, suggested no infection or very low herd prevalence of bovine brucellosis in this sub–population (unpublished data).

1.6 Gap analysis

Despite successful implementation of a range of control measures, that reduced abortions and human cases of brucellosis we identified several gaps that must be addressed in the short-, medium- and long-term. Identified gaps regarding brucellosis in sheep and goats were: reduced vaccination coverage on both village and flock levels compared with initial stage of the national control programme; reduced numbers of vaccinated flocks tested for assessment of seroconversion; delays in reporting vaccination; problems related to animal identification; underreporting and laboratory investigation of abortions in sheep and goats. As in small ruminants, a number of gaps were identified regarding bovine brucellosis: farmer awareness related to importance of disease and their roles and responsibilities according to the Albanian veterinary law: non-compliance with bovine animal identification and registration regulations; general absence of effective cattle movement control, confirmed during outbreak investigations conducted in 2016, 2017 and 2018; abortion cases were not well reported; and, a lack of laboratory investigation [19].

1.7 Rational strategy for control and eradications of brucellosis in Albania

Control of brucellosis is a challenge, even for well-developed countries. There are several strategies, however, each country has specific circumstances that must considered before approving any control programme. Control of brucellosis is a multidisciplinary activity involving a range of public institutions, in particular the public health authority and authority responsible for food safety; their contributions are essential for effective control. The Ministry of Agriculture plays a lead role in coordination of the activities towards achieving the goal of control and eradication.

Control of brucellosis in sheep and goats will be based on the current control programme which will continue, and emphasis will be given to achieving full vaccination coverage (Table 1). Generally, the highest priority for bovine brucellosis is the adoption of the most appropriate control approach adjusted to the local epidemiological conditions. The epidemiological situation in dairy herds seems to be favourable. Effective control requires full enforcement of the

control measures, which will result in rapid elimination of the infection from bovine population, including enforcement of preventive measures to keep the bovine population free of the infection. Given the very high prevalence in beef herds, the only applicable control approach is by mass vaccination, following the pattern adopted for sheep and goats. Under these conditions, use of intra-conjunctival S19 is most appropriate. Control of brucellosis is not possible without having a reliable identification and registration system, movement control, inspection and monitoring capacity, laboratory capacity, financial and human resources, and an electronic database that is fit for purpose. These preconditions are applicable to the control of brucellosis in both small and large ruminants (Table 1). The encouraging trend of the decreasing number of human cases achieved by mass vaccination has to be maintained and further improved by strict enforcement of veterinary public health measures. Human cases have to be investigated jointly by veterinary authorities, Public Health Authority and National Food Authority to establish epidemiological links between humans and animals, to assess the risk of transmission of infection by food and to assure that all measures in relation to food safety are in place. In this regard, the Ministry of Agricultural and Rural Development [19, 22] should initiate the establishment of a common, formal procedure for cooperation, collaboration and coordination including establishing of joint teams for outbreak investigation. Public awareness campaigns must be undertaken and maintained to achieve close collaboration with the target groups of stakeholders. It is important to enforce surveillance programmes, which will include: data collection from vaccinated flocks and herds; assessing the efficacy and coverage of vaccination campaign; epidemiological investigation of abortions; investigation of confirmed human cases; maintaining and improving animal identification and registration system and ensuring the effective control of animal movement; maintaining the current laboratory capacities; establishing a differential diagnostic panel for abortion. It is also essential to introduce new diagnostic methods such as gel diffusion precipitation test using native hapten gene, and Flourescence Polarisation Assay, which are important for the differentiation of vaccinated and infected animals.

Table 1. Most important elements of proposed rational strategy for brucellosis control in cattle, sheep and goats in Albania

Control measures according control strategy	**Bovine brucellosis**		**Sheep & goat brucellosis**	**Comments**
	Dairy cattle	**Beef cattle**		
Vaccination	NA	NA	Yes	*B. melitensis* Rev 1 strain is used for sheep and goats, applied "a drop in the eye".
Post-vaccination monitoring	NA	NA	Yes	
Collection of data on vaccinated flocks & animals	NA	NA	Yes	For control of *B. abortus* S19 and RB51 vaccine strains available.
Surveillance of the efficacy of the vaccination campaign	NA	NA	Yes	
Collection of data on vaccination coverage	NA	NA	Yes	Use of vaccine in cattle should be strictly regulated
Active and passive surveillance	Active	Passive	Passive	Surveillance is of essential importance to monitor the success of the control programme.
Compulsory testing of all slaughtered animals at the abattoirs	Yes	Yes	NA	Tracing back of positive cases will identify positive herds, particularly beef cattle
Epidemiological investigations of abortions	Reporting of abortions is a crucial method of surveillance for brucellosis, especially when active surveillance is absent or interferes with mass vaccination programme.			
Investigation after reports of human cases	Often neglected. Confirmed human cases of brucellosis may be a sign of an active presence of the disease in animals			
Movement control	It is not fully enforced.			

Animal Identification and registration	Strengthening and reinforcement of I&R System for ruminant animals, their movement control and tested and positive animals shall be promptly reported to the RUDA database.
Public awareness	Implementation of a publicity campaign programme promoting the prevention, detection and reporting of the disease.
Laboratory tests available and in use. There is a need for establishing differential diagnosis panel for abortion cases: Q – fever, Toxoplasmosis; Salmonellosis; Enzootic abortion in ewes, Campylobacteriosis.	
1. MRT Milk ring test	MRT is used as screening test of dairy herds by using bulk milk. All animals of positive herds will be tested by RBPT
2. Milk ELISA	
3. RBPT	RBPT (Rose Bengal Plate Test) is accredited by ISO/IEC 17 025, use as a screening test at individual level (sheep, goats and cattle).
4. CFT	CFT (Complement Fixation Test) and ELISA are accredited by ISO/IEC 17025. They are used with RBT as confirmatory test (cattle, sheep and goats).
5. iELISA	
6. ELISA	
7. Bacteriological isolation	It is used for confirmation of abortion cases.
8. PCR	Polymerase Chain Reaction. It is used to identify isolates.
9. GDPT (Gel diffusion precipitation test)	GDPT and FPA can distinguish vaccinated from infected animals. FPA (Florescence Polarization Assay) is available at Faculty of Veterinary Medicine, Tirana.
10. FPA	
Stamping out and farmer compensation	Positive animals tested in confirmatory test eliminate within 30 days and farmer will be compensate
Cleaning and disinfections	Positive premises will be clean and target disinfection will apply.

2. Conclusions

The successful implementation of the current brucellosis control strategy in sheep and goats, and the extension of the programme to control bovine brucellosis to the entire national dairy herd and the control of disease in the beef cattle will create supportive conditions for Albania to achieve brucellosis-free status. Achieving brucellosis-free status is long-term objective; it will have a great impact for controlling the disease in the human population and will facilitate exporting of live animals and products of animal origin to the European market.

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