Are Italian–Polish veterinarians and breeders prepared to control an outbreak of Brucella canis infection in dogs?

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Abstract

Brucella canis infection is one of the most important causes of infertility in dogs and is a zoonosis for which no effective treatment or vaccines exist. It is not a mandatory notifiable disease. Following an increase of cases in Europe and worldwide, an investigation was performed to evaluate how much Italian and Polish veterinarians and breeders know about canine brucellosis and understand their perceptions of this infection. For this reason, two questionnaires were prepared, in Italian and Polish. Eighteen Italian and Polish veterinarians, specialists in canine reproduction, responded to the first survey and 44.4% of them affirmed having diagnosed canine brucellosis at least once in their clinical practice, and different perceptions emerged regarding the infection in the two countries. The second survey was completed by 145 Italian and Polish breeders; the disease was completely unknown to 22.8% of them, whereas 2.1% had diagnosed infection by B. canis in their kennels. In conclusion, knowledge of B. canis infection differs between these countries, with extremes ranging from diagnosed cases to complete underestimation of the presence of the problem. However, based on international data and reporting of a recent large outbreak in Italy, awareness of this contagious infectious disease and its management must be increased.

Key words: Brucella canis, dog, screening test, regulation, health requirement
Introduction

Reports of outbreaks of Brucella canis have progressively increased around the world in recent years (Lucero et al. 2010, Corrente et al. 2010, Gyuranecz et al. 2011, Holst et al. 2012, Keid et al. 2017, Hensel et al. 2018, Johnson et al. 2018, Buhmann et al. 2019, Galarce et al. 2020, Weese et al. 2020, Middlemiss 2021, Santos et al. 2021, Van Dijk et al. 2021). Commercial and large dog facilities are often involved, causing wide spreading of this infection (Keid et al. 2017, Johnson et al. 2018). B. canis is considered one of the most important agents for reproductive failures in dogs and is responsible for significant economic losses in infected kennels (Pickerill and Carmichael 1972, Hollett 2006, Holst et al. 2010). It is relevant also from a One Health perspective, being an agent of zoonosis. Human cases generally occur after exposure to infected dogs (vaginal discharges, abortion materials, fluids of bitches, semen and/or urine of male dogs) or accidental laboratory infections (Brower et al. 2007, Lucero et al. 2010); however, they are considered mild and uncommon, although critical cases have also been reported (Lucero et al. 2005, Lucero et al. 2010). As dogs are currently frequently treated as family members and live in close contact with humans, B. canis infection can spread easily and cause human infection. Moreover, clinical disease caused by these bacteria is generally associated with chronic infection and, as clinical signs are not typical, human and animal brucellosis are frequently misdiagnosed.

The control of the infection is difficult as no vaccines or effective treatment exist, and strict management of infected dogs is required. Accordingly, prevention is the real objective (Kauffman and Petersen 2019).

In the livestock sector there are rules that regulate breeding and movement of animals based on sanitary requirements, both at national and international level (Animal Health Law, AHL, European Union (EU) regulation 2016/429; Kauffman and Petersen 2019). In companion animals, this still occurs occasionally, limited to some infectious diseases. EU regulations exist for Brucella abortus, Brucella melitensis and Brucella suis at international level in large animals (AHL 2016/429, EU regulation 2020/686, EU regulation 2020/689), while the number of dedicated measures or regulations regarding B. canis on the movement of live animals and/or semen exchanges in dogs are currently limited and not systematic (Italian Ministry of Health-Veterinary certificate for introduction and import into Italy semen dog from third countries and UE countries 2021, EU regulation 2020/686). EU and extra-EU countries have adopted different regulations regarding B. canis, ranging from notification of infection, compulsory screening, euthanasia of infected dogs and checking of semen, to no control (Hollett 2006, Holst et al. 2012, Kaden et al. 2014, Hensel et al. 2018, Middlemiss 2021). Europe is also experiencing an increase in the number of outbreaks (Buhmann et al. 2019, De Massis et al. 2021, Middlemiss 2021, Van Dijk et al. 2021). To appropriately contain the spread of this infection, it is important to know the context in which to operate and the level of knowledge that stakeholders have of the problem.

Awareness of this infection for veterinarians and breeders is the first step to diagnosing and appropriately controlling it, as they are the professional figures who can propose diagnostic tests and manage infected and susceptible animals. For this reason, an investigation in two European countries, Italy and Poland (which apply general international rules against brucellosis, common to other European countries), was performed to determine how much veterinarians and breeders know and how they perceive B. canis infection and, consequently, to discuss what actions could be taken to reduce the risk of spreading this infection.

Materials and Methods

Two questionnaires, one for veterinarians and one for breeders, were prepared by four experts in epidemiology and infectious diseases of small animals and a graduating student. Subsequently, the questionnaires were administered to two veterinarians and two breeders to assess the clarity of the questions and validate them.

At the beginning of each questionnaire, a brief presentation of the questionnaire, explaining the purpose of the survey and ensuring the anonymity of the respondents, was included.

Questionnaires were prepared and administered in the period 15 December 2020 – 28 February 2021 by e-mail and social media, using Google Forms.

The national context of Italy and Poland, in which the questionnaires were administered, was that infection by B. abortus, B. melitensis and B. suis were notifiable, while specific requirement for B. canis exists occasionally to the knowledge of the authors (AHL 2016/429, EU regulation 2020/686, Italian Ministry of Health-Veterinary certificate 2021). Poland and certain provinces or regions of Italy are also officially recognised as free of bovine brucellosis, sheep and goat brucellosis, in accordance with the decision of the Commission of the European Communities 2006/169/WE and 2009/600/WE. Italy also supports the control of brucellosis in cattle, bubaline, sheep and goats with further laws (Italian national regulation 23 June 2021 “Extraordinary measures of the veterinary
police for tuberculosis, bovine and buffalo brucellosis, ovine-caprine brucellosis, enzootic bovine leukosis”).

The first questionnaire was designed for veterinarians specialised in small animal reproduction who were members of the European Veterinary Society for Small Animal Reproduction (EVSAAR). It consisted of nine questions (Qv), of which seven were multiple choice and two open-ended. The first two questions were to define the experience of the respondents (Qv. 1) and to determine how much of their job was devoted to small animal reproduction (Qv. 2). In the following questions, they were asked if they had ever diagnosed canine brucellosis (Qv. 3) and, if the answer was yes, they were to give an approximate number of diagnosed cases (Qv. 4) and describe their clinical signs (Qv. 5). The next questions were created to understand possible problems linked to performing laboratory diagnosis of canine brucellosis (Qv. 6 and Qv. 7). The following questions were asked to assess whether brucellosis, in their opinion, was an endemic disease characterised by high or low prevalence, determining clinical signs or asymptomatic infection or if it possibly was not present. The same question was asked for the national level, considering the respective country of the respondents, Italy or Poland (Qv. 8), and for the international level (Qv. 9). For the last question (Qv. 10), respondents were asked whether canine brucellosis, in their opinion, is an underestimated problem in their country.

The second questionnaire was designed for breeders belonging to national organisations recognised by the Ente Nazionale Cinofilia Italiana (FCI – ENCI) for Italian breeders and Związek Kynologiczny w Polsce (ZKwP) for Polish breeders. It was composed of seven questions, of which eight were multiple choice and one open-ended. The first three questions were created to estimate the experience of the breeder (Qb. 1), the quantity of dogs owned for each breeder (Qb. 2), and their breed (Qb. 3). It was not asked if the breeders owned other dogs, if they bred other dog breeds or if they owned any animal species other than the canine breed officially declared in the form. In the following questions, breeders were asked if they used artificial insemination (Qb. 4) and whether they knew of canine brucellosis (Qb. 5). Next, they were asked whether canine brucellosis had ever been diagnosed in their kennel (Qb. 6). The last two questions were introduced to understand if they suspected canine brucellosis when clinical signs suggestive of canine brucellosis, but very common in canine reproductive activity, were present; Qb. 7 and 8 asked if they had ever had specific clinical signs (abortion at the end of gestation, between 45-60 days, or stillbirth or poor vitality of puppies born, in the presence or without an enlargement of the lymph nodes, for the female, and sperm abnormalities or infertility for males, respectively) in female and male dogs in their kennels.

**Statistical analysis**

Chi square or F tests were used as appropriate to compare the answers between the Italian and Polish questionnaires. A p<0.05 was considered significant.

**Results**

**Questionnaire for veterinarians**

The questionnaire was sent to 27 veterinarians of the EVSAAR, of which 12 were Polish and 15 Italian. It was completed and returned by 18 veterinarians (66.7%), of which eight were Polish veterinarians and ten Italian. Despite the small number, they were representative of the entire national territory of each country. Fifty per cent of the Italian veterinarians dedicated more than 75% of their time to small animal reproduction and another 47.5% dedicated 50-75%. Most of the Polish veterinarians (57.5%) dealt with small animal reproduction for more than 75% of their daily routine and another 15% for 50-75% of their time. Seventy per cent of Italian veterinarians and 62.5% of Polish ones had been qualified for 10 years or more.

Eight out of 18 (44.4%) veterinarians (3/8, 37.5% Polish and 5/10, 50% Italian) confirmed that they had diagnosed canine brucellosis in their clinical practice (Qv. 4) at least once.

The most frequently reported clinical signs reported by the total number of veterinarians (both Italian and Polish) who diagnosed canine brucellosis (Qv. 5) were vaginal discharge (indicated by 3 out of 7 veterinarians); orchitis, epididymitis, or fever (each of them indicated by 2 out of 7 veterinarians); prostatitis, azospermatia, hypofertility/infertility or abortion (each of them indicated by 1 out of 7 veterinarians). One diagnosis was referred to as an asymptomatic dog.

Similar percentages of Italian (40%) and Polish (37.5%) veterinarians noted that they had situations in which they suspected *B. canis* infection but for some reason a diagnostic confirmation was not possible (Qv. 6).

Both confirmed that the difficulty of diagnosing the infection of *B. canis* is mainly due to the cost of tests, but with significant differences between countries (Qv. 7, 40% for Italian and 75% for Polish, p<0.001). The Italian respondents also indicated technical problems (20%) and the current legislation (10%) as reasons for difficulty in diagnosis, but also other reasons, not explained (30%).
indicated generic other reasons for the remaining 25% of causes. In a few cases, after the survey, veterinarians explained that occasionally owners of dogs were not motivated to get the final diagnosis and, in addition to the problem of cost, it was also difficult admitting that there were some problems in their kennel.

Qv. 8 asked how they viewed the epidemiology of canine brucellosis in their respective countries and significant differences emerged from the answers (p<0.001). The perception of *B. canis* infection by veterinarians in their own country was very variable. Thirty per cent of the Italian veterinarians believed that canine brucellosis is an endemic disease, with a high prevalence, characterised by asymptomatic cases (30%). Another 30% of them believed it was present with clinical signs; 20% supposed it an endemic disease, characterised by low prevalence giving asymptomatic cases; and the last 20% thought it was not a problem. In the opinion of Polish veterinarians, it is an endemic disease with high prevalence, which gives asymptomatic cases (62.5%); it is an endemic disease with low prevalence, which gives asymptomatic cases (12.5%); it is not present (12.5%); and it is present with clinical cases (12.5%). These answers are as shown in Fig. 1.

The perception of the presence of this infection abroad by Italian veterinarians increased and was significantly different from Polish ones (Qv. 9, p<0.001). Italian veterinarians believed that canine brucellosis abroad is present and gives clinical signs (50%); 37.5% believe it an endemic disease with low prevalence and which gives asymptomatic cases; and none claim it is not being a problem abroad. Fig. 2 shows these results.

With Qv. 10, 70% of the Italian veterinarians thought that canine brucellosis is an underestimated problem in Italy, while only the 37.5% of the Polish ones believe it underestimated, indicating a significant discrepancy in the responses of Italian and Polish veterinarians (p<0.001).

**Questionnaire for breeders**

The questionnaire was sent to 186 breeders of which 115 were Polish and 71 Italian. It was completed and returned by 145 breeders (78%), 95 Polish breeders (82%) and 50 Italian breeders (70%). They were representative of the entire national territory of each country. Overall, 47.7% of breeders (56.8% of Italian and 44.2% of Polish breeders) had been registered in the respective national organisation (ENCI or ZKwP) for more than 15 years, and 32.6% (27% of Italian and 34.7% of Polish breeders) for 7-15 years. This indicates that the group of breeders interviewed were experienced and had been breeding dogs for many years. In total, the respondents were breeders of at least 56 different breeds, very different in size and characteristics, including herding, terrier, hound, pinscher, toy, and non-sporting groups. Although not explicitly asked, 3 out of 50 (6%) of the Italian breeders and 17 out of 95 (17.9%) of the Polish breeders reported breeding more than one breed.

Significant differences in kennel size were observed for Italian and Polish breeders (Qb. 3, p<0.001), with 50.5% of the Italian kennels having less than six
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Discussion

The questionnaire was intended to indicate whether veterinarians and breeders, both Italian and Polish, had knowledge of B. canis infection and to identify what actions could be taken to reduce the risk of the spreading of this infection. More different responses between Italians and Poles were found in veterinarians than in breeders, leading to more homogeneous results.

Based on the years of practice for veterinarians and the long enrolment time of the owner to the corresponding national registers of breeders, the respondents were veterinarians and breeders who were experts in canine reproduction and dog breeding. Accordingly, they represented a population that had greater likelihood of noticing reproductive problems, of which one possible aetiology may be canine brucellosis, and should also be more sensitive to the health aspects associated with this activity.

Questions used to verify the presence of the infection (Qv. 3–5 for veterinarians and Qb. 5 for breeders) indicated, for both veterinarians and breeders, that B. canis infection is circulating and has been diagnosed: 8 out of 18 (44.4%) veterinarians confirmed having diagnosed it at least once in their clinical practice and three kennels out of 145 (2%) had received a diagnosis of canine brucellosis. This, associated with the evidence of the presence of documented outbreaks in Europe and around the world, is sufficient to indicate that the infection must be controlled in this context.

In most of the veterinary diagnoses, veterinarians reported the presence of clinical signs previously associated with the infection. In one case (12.5%), the infection was asymptomatic, confirming that it would be better to investigate it as an aetiologic agent in cases of infertility but also to actively conduct screening to detect asymptomatic infections early. Consequently, veterinarians indicated the costs, specified as the main problem for 75% of Polish veterinarians and 40% of the Italians, as a limit to diagnosis. It should be considered that, if the diagnostic investigation for canine brucellosis is not compulsory by law and is the responsibility of the owner, and if it is not a requirement to certify the breeding, the search for the bacterium becomes the will of the owner, who must be motivated to do it and spend money. Italian veterinarians also associated technical aspects (20%) as a limit for diagnosis: probably with the diagnosis...
of canine brucellosis being very specific, not all laboratories are able to make diagnoses, which is challenging to investigate because routine microbiological diagnosis of brucellosis is based on brucellae having smooth colonies (B. abortus, B. melitensis and B. suis) and it can easily not detect brucellae having rough colonies, like B. canis or Brucella ovis, both serologically and by direct search (Lucero et al. 2005, Lucero et al. 2010). Another problem selected by 10% of Italian veterinarians was the ongoing law; this aspect could mainly depend on the fear of the consequences of the application of restrictions on breeding and dogs in Italy, as applied in other outbreaks, for which the breeder can be prevented from breeding and selling. Moreover, this condition can create a competing interest for a private veterinarian hired by the owner. Another 30% of the Italian and 25% of Polish veterinarians declared ‘other’ as a reason for problems in diagnosing canine brucellosis: this answer should be further investigated to understand the reasons.

The perception of the infection epidemiology within the country and abroad varied significantly: Italian veterinarians expected any epidemiological scenario in their territory (high and low endemicity, with or without clinical signs), while Polish veterinarians considered the infection hyperendemic but asymptomatic, especially in their own country. For Italians, B. canis infection is predominantly asymptomatic abroad, hyper- or hypoendemic, and even not present for some of them. For Polish veterinarians, it is certainly present abroad, both with clinical and asymptomatic signs, however with a variable expected prevalence. The reason for this different perception of the infection by veterinarians, inside the country and abroad, is probably derived from the fragmentary information they received over time and from their personal experience. For this reason, structured epidemiological studies are needed to understand the real extent of the distribution of B. canis infection in the individual country and Europe, and accurate information must be communicated to veterinarians and breeders. Results of epidemiological investigations could be very useful as they motivate the active detection of the bacterium if endemic conditions occur. This is also important from the One Health perspective, as in these scenarios human practitioners should be more motivated to know and diagnose this infection in humans, in which unspecific clinical signs appear. Previous reports of human infections have underlined how the awareness of human medical personnel about this infection must be increased to avoid the non-diagnosis or misdiagnosis generally associated with B. canis infection, which is very difficult to identify in the laboratory if appropriate tests are not used, and which must be specifically investigated (Lucero et al. 2005, Lucero et al. 2010).

In brief, for 70% of Italian veterinarians, canine brucellosis is an underestimated problem, while Polish veterinarians consider it a real problem. The reasons for these answers should be further investigated to explain this difference, and if the lack of awareness is linked to breeders or the limited monitoring of the sanitary system. In any case, this underestimation of the problem was confirmed when, in Italy, a large outbreak was recently notified publicly, involving more than 800 dogs in a large commercial breeding enterprise located in Central Italy (De Massis et al. 2021). As the first outbreak occurred in Italy, the initial lack of awareness in the management of the infection caused a delay in its control, allowing an increase of cases within the initial outbreak and some dogs from this kennel had been previously sold in the rest of Italy, resulting in notifications and alerts in various parts of the Italian territory. However, the outbreak had not yet been publicly notified when this survey was conducted and after this event, new plans, rules and recommendations were applied by local and national authorities and are being continuously updated. A previous study of multi-state outbreaks in the United States reported that the absence of universally accepted guidelines for control and prevention of B. canis infection delayed the responses of the veterinarians during outbreaks because initially they spent time and effort developing and implementing the protocols necessary for infection control (Johnson et al. 2018).

Brucellosis in animals is one of the infectious diseases studied during the university course of veterinary medicine, with special attention paid to B. abortus and B. melitensis causing infections in ruminants and considered a relevant zoonosis transmitted by infected animals and their products, and for which national and international eradication plans are ongoing. Canine brucellosis is generally studied less thoroughly during the same course. Given the current epidemiological situation, it is necessary to provide more specific training already to students of veterinary medicine, emphasizing the diagnostic requirements necessary for this infection, even in the presence of asymptomatic conditions or nonspecific clinical signs in dogs, and the healthcare management protocol to be applied in kennels. In this regard, some universities have already taken this action (personal communication). Moreover, the support of public health institutions in controlling brucellosis should also be extended to small animals, providing strategies to increase the demand for diagnostic tests,
maybe also with partial financial support for owners and veterinarians, given that the cost of the tests was considered a relevant limiting factor for diagnosis in the survey. For this infection, which is a public concern, early diagnosis would make it possible to avoid the spread of an outbreak as occurred in Central Italy, where the costs for the maintenance of the seized dogs, the economic damage due to the blocking of breeding activity and the reduced conditions of animal welfare probably exceeded the costs of preventive screening tests, as previously noted (Kauffman and Petersen 2019).

More than three quarters of breeders, who manage different breeding conditions between Poland and Italy, were aware of the disease. However, reversing the sentence, based on the answers to Qb. 5, the almost one out of four (22.8%) of the respondent breeders, considered as expert in canine reproduction, had never heard of the infection. In addition, suspicion of infection by breeders in the presence of typical signs was very low, both in Italy and Poland (Qb.7 and 8). Italian breeders were more sensitive to identifying clinical signs suggestive of canine brucellosis in female dogs, which is rarely considered by Polish breeders. On the other hand, clinical signs in male dogs were probably underestimated and less noticed. B. canis testing is highly recommended as part of any infertility screening program, even in low-risk situations (Kauffman and Petersen 2019).

Some characteristics of the kennels of the breeders interviewed, such as the large size of the kennels (Keid et al. 2017, Johnson et al. 2018, Kauffman and Petersen 2019, Weese et al. 2020), were previously recognised as a risk factor for infection. Moreover, artificial insemination and management of large kennels indicate experience, high technical specialisation and performance in canine breeding. At the same time, the high level of breeding generally requires the use of specific genetic lines, and for this reason travelling to breed dogs in another country is more frequent than in the past. This is a context in which a contagious disease, like canine brucellosis, can spread. This kind of breeding, requiring veterinary assistance, is increasing. However, in parallel with technical improvements, health certification of the kennel should be required. Indeed, there is a part of the breeding industry that remains more subject to economic and commercial criteria and less careful of health requirements.

At present, in a situation where the search for infection in a kennel is voluntary, the breeder must be made aware of the problem: the infection can be present without clinical signs; probably B. canis infection is underestimated especially in male dogs; the breeders and their families are the first to be exposed to this zoonotic risk. Low awareness of canine brucellosis among Swedish kennel owners, associated with an increase in litters from non-Swedish stud dogs, was considered the risk factor introducing canine brucellosis in previous outbreaks in Sweden (Kade 2014). In endemic areas, low awareness concerning the risk of canine brucellosis in humans, and especially its transmission, was considered a cause for its spread and human cases; campaigns for information and education of the community have been carried out to control infection (Lucero et al. 2010, Marzetti et al. 2013).

It would be appropriate for politicians to make research on the bacterium compulsory, as is already the case in some countries (Hollett 2006, Lucero et al. 2010, Reynes et al. 2012, Cosford 2018, Hensel et al. 2018, Johnson et al. 2018, Kauffman and Petersen 2019). The breeder must be helped in this action: diagnosis should be encouraged, with economic support or with certification for the brucellosis-free status of the kennel. Veterinarians must be trained to provide immediate practical support and know the protocols to help the breeders to control the infection in kennels. There have been several attempts by the EU to create responsible small animal breeders (EU Platform on Animal Welfare, Responsible dog breeding guidelines, 2020). Health requirements, as a certification for brucellosis-free kennels, if not mandatory, could be proposed as good breeding practice to be certified, as previously performed in some countries (Johnson et al. 2018). Recommendations for testing before mating dogs from foreign countries should be followed to prevent infection, as suggested in some EU countries or applied in other susceptible species (Kade 2014, EU Regulation 2020/686). Moreover, the health standard of the breeding could be a new commercial requirement that a stakeholder may request when purchasing a dog.

Investigating the infection voluntarily or making this research mandatory could limit the spread of the infection, which otherwise spreads subtly and without showing signs, especially in an initial phase. Identifying this infection and being prepared to control it, are fundamental to managing it appropriately.

One of the limits of the research was the small number of veterinarians who filled out the questionnaire. However, it was preferred to interview experts in the sector, leading to a reduced number of respondents, those considered the most able to identify the problems, perceptions and critical points compared to more generalist veterinarians, who may know less about the issue. Another limit of the study was that some responses to the questionnaire would have
required a more detailed answer to better understand some reasons, but a simple questionnaire with few questions was preferred to obtain a good response rate.

Conclusions

Recent research from around the world confirms that outbreaks of *B. canis* infection are increasing. The reason for this rise in cases has not been identified, and probably many different factors are involved, such as the more frequent movements of dogs around the world, the lack of routine health screening and poor knowledge of reproductive infectious diseases. Moreover, *B. canis* infection is a zoonosis, so the management of the kennel in the case of a positive diagnosis must be very restrictive to ensure the safety of people and animals. It is necessary to train veterinarians; make breeders aware; motivate them to search for infection, especially if they are professionals; increase detection of infection through diagnosis; introduce routine screening; support the breeder for the cost of diagnosis to appropriately control the infection; and require health standards for breeding when purchasing or selling a dog.

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