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Canine brucellosis in Turkey from an environmental perspective

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Abstract: Brucellosis is a zoonotic disease that affects not only livestock with economical losses but also human beings by causing serious health problems worldwide. Canine brucellosis has largely been overlooked in the world. In Turkey, brucellosis has also been one of the major concerns for decades. Canine brucellosis in this country has been reviewed and discussed from an environmental perspective in this review. As a part of prevention measures, one of the urgent practices that should be applied is to expand the routine examinations to include serological testing for all animals on arrival to kennels and then to decide the fate of the seropositive animals. It is thought that by giving wider information about stray dogs and wild Canidae in a common but not quite large habitat like that in Turkey, new studies could provide more understanding of canine brucellosis in one common environment.

Key words: *Brucella canis*, environment, Turkey

1. Introduction

Brucellosis is an infection that affects both human and animals. *Brucella abortus*, *B. melitensis*, and *B. ovis* are the major species found in cattle and small ruminants. Porcine brucellosis is mainly caused by *B. suis*. The major impact of brucellosis in farm animals is economic and it represents a worldwide problem (1,2), except that Australia's status for *B. abortus* and *B. melitensis* is 'free', although infections by *B. ovis* and *B. canis* occur (3). All biovars of *B. abortus* and *B. melitensis* and biovars 1, 3, and 4 of *B. suis* are common pathogens with zoonotic potential (4,5). The disease is the world's most common bacterial zoonosis, resulting in about half a million cases per year. However, the actual number of cases, including those unreported, may be 10 times higher (6).

Before going further, other types of brucellosis in Turkey are briefly given below, since some possible connections between canine and livestock brucellosis have been documented (7,8). However, the main purpose of this review is to summarize canine and human data from Turkish sources produced to date and also to discuss measures for the control of this infection in the light of some environmental aspects.

2. Brucellosis in farm animals and humans is a major problem in Turkey

In Turkey, brucellosis caused by both *B. abortus* and *B. melitensis* in production animals and humans has been evidenced for years (9–11). *B. ovis* seems to be an imminent threat for rams in at least a part of Anatolia (9). No report on the incidence of *B. suis* in the country has been published to date. Regarding horse brucellosis, several serological studies have been conducted and the data were reviewed by Yilmaz and Wilson (12), who stated that the occurrence of brucellosis in equids could be up to 60.6%, with the exception of a single study that tested sera from various locations showing no evidence of seropositivity (13), suggesting that the disease may have been recently introduced (or even recently diagnosed) in Turkey.

3. Canine brucellosis and some concerns about the environment in Turkey

B. canis is the causative agent of brucellosis not only in domestic dogs but also in wild Canidae, and both were considered reservoirs of *B. canis*. In fact, brucellosis infection in dogs can be caused by four species of *Brucella* (*B. canis*, *B. abortus*, *B. melitensis*, and *B. suis*) (14,15), although the species other than *B. canis* as causative agents in dogs worldwide is still controversial (3,14). In Turkey, data on seropositivity for S-type brucellosis in dogs were published some time ago (16). *B. canis* was first described by Carmichael in 1966 (17), and Aras and Uçan (18) first detected the causative agent in dog samples in Turkey. Abortions, stillbirths, epididymitis, testicular atrophy, and generalized lymphadenitis can be observed in cases

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of canine brucellosis. Some dogs remain chronically infected for months (1,2,14). Transmission between dogs occurs by mucosal contact with infected material. Vaginal discharges, semen, and fluids and tissues related to birth and abortion are the sources of bacteria. Lower counts of bacteria are found in blood, saliva, milk, feces, and urine (19). Transmission of *B. canis* in dogs can be accomplished by ingestion; through genital, oronasal, and conjunctival mucosa; and even via broken skin. Fomites can spread *B. canis*. In some environmental conditions (like damp weather and low temperatures with no sunlight), *Brucella* spp. can remain viable for months in some tissues from infected dogs or in contaminated fomites (14). Infection in dogs can be diagnosed by isolation (1,18), molecular biology (18,24), and serology (1,20–22), including ELISA (15), and by immune enzyme analysis by dipstick assay (23).

Studies on canine brucellosis in Turkey have mostly addressed serological examinations to date. This is also the case in determining the disease in humans in the country (25–29). Data on *Brucella canis* infection in dogs and humans are shown in Tables 1 and 2. Some studies revealed that shelter dogs as well as workers at these facilities showed higher risk of the infection compared to dogs or people from the general population (Tables 1 and 2). These studies showed that dogs in a shelter in Konya showed the highest seroprevalence of brucellosis (25.5%) among the dog populations sampled from clinics or canine research units of faculties of veterinary medicine (29). Employees from this shelter also showed the highest prevalence (17%) of brucellosis (R-type) when compared to dog owners, veterinary medical staff (owners and staff together; n = 76), and human patients clinically suspected of S-type brucellosis (n = 1000) (29,30). Furthermore, some (2%) of the human patients clinically suspected of S-type brucellosis were actually infected by R-type *Brucella* in this city (29). More studies are needed to learn the current occurrence of the disease in man and dogs.

The occurrence of the disease is affected by environmental factors and types of approaches should be adopted and discussed in order to put an end to the occurrence of canine brucellosis. A candidate approach could be the "One Health" concept, although it has never been applied to any diseases like canine brucellosis before (i.e. a disease not causing a serious problem to public health). There is still a need for making a conceptual framework for the enzootic diseases in canines, although the concept of "One Health" has been discussed for

Table 1. *Brucella canis* infection in dogs in Turkey from past to present.

No.	Province	Number of serum samples	Method for antibody detection	Prevalence (%)	Reference
1	Ankara	134	2ME*	6.7	(20)
2	Not specified	222	2ME	6.3	(21)
3	İstanbul and İzmir	362	ELISA	7.45	(22)
4	Konya	135	i-ELISA**	21.5	(30)
5	Konya	35***	Isolation and PCR	0	(24)

*: 2-Mercaptoethanol test. **: Indirect ELISA. ***: These are vaginal swab samples only and tested by PCR and culture methods.

Table 2. *Brucella canis* infection in humans in Turkey from past to present.

No.	Province	Number of serum samples	Method for antibody detection	Prevalence (%)	Reference
1	Bursa	123	2ME*	1.6	(25)
2	Adana	514	2ME	8.3	(26)
3	Konya	76	MPAT**	9.2	(27)
4	Most of the country	1746	2ME	3.7	(28)
5	Konya	1000	MPAT	0.22	(29)

*: 2-Mercaptoethanol test. **: Modified plate agglutination test. The sera samples from the studies of 1, 2, and 5 were from patients with brucellosis-like symptoms. Sera of study 3 were from a human population at risk of R-type brucellosis.

emerging diseases with pandemic potential (31,32). In other words, the zoonotic potential of infections of dogs, with the exception of rabies, has been largely ignored throughout the world until recently. More than 60% of human pathogens are estimated to originate from animals. Feral dogs serve as a pool of microorganisms, some of which are also the causative agents of zoonotic diseases of humans (33). Therefore, zoonoses such as brucellosis (including the canine type), echinococcosis, rabies, and some others are insidious and continue to significantly affect human health (3–5). The main reasons for that are the close contact with animals by sharing the same environment and deficiencies in financial resources for controlling them.

Currently there is no definite figure for the number of stray (feral) dogs in Turkey, although estimated population sizes of owned and stray dogs in Ankara have recently been reported as 25,000–30,000 and 17,839, respectively (34,35). However, only very rough numbers for the stray dogs of several cities other than Ankara can indirectly be assumed due to a project called "The Project for National Control of Rabies in Turkey". The project aimed to establish three shelters for stray dogs and cats in three metropolitan municipalities (Ankara, İstanbul, and İzmir) in 2006, being the first ever established in the country (36). Since then, many other municipalities have constructed their own shelters for stray dogs and cats, which likely provided a source for canine brucellosis indirectly since sterilization operations are a possible cause of brucellosis spreading in dogs. In Konya's Metropolitan Municipality Shelter, 1620 stray dogs were examined, sterilized, and vaccinated by the shelter veterinarians in 2013 (37). In the country, the main issue for capturing and keeping stray dogs in animal shelters is to provide protection, care, and surveillance of the uncontrolled dogs and this has been used as one of the feasible solutions to resolve uncontrolled animal problems. Dogs collected from the city and its surroundings in this particular type of shelter are first clinically examined, and unless they are found to be sick, they are vaccinated for rabies, given medicines for parasites, operationally sterilized, and let to live in their new environment unless an adoption is requested. This is the main way of controlling stray dog populations (and maybe limiting the occurrence of some diseases indirectly, as well) in urban areas of Turkey. Thus, shelters may also contribute to the control of brucellosis transmission to some degree by neutering animals in the shelters because the amounts of secretions are decreased when the dogs are sterilized. Of course, that does not seem to be enough for inhibition of bacteria being transmitted to the nearby environment. On the contrary, a concern has emphasized that dogs in nature instinctively make packs comprising about a dozen individuals at the most. This can serve as one of the limiting factors for disease transmission among the various dog packs living outside the shelter. In crowded shelters, this natural behavior of dogs is forced to change by human-made alterations (by keeping large amounts of dogs in a single closed area or cages), leading to the shelter environment itself possibly becoming a perfect medium for spreading canine brucellosis among sheltered dogs.

Some secretions (saliva, lacrimal secretions, etc.) and excretions (urine, feces) produced by stray dogs that live free in the environment (those never caught for shelter rehabilitation, or those that visited a shelter only once) are physiologically released to the environment in some degree. A mean fecal output of 0.34 kg per day per dog was estimated. That means that each dog defecates 124.1 kg annually. The average urine excretion per dog (12 kg annually) is about 750 mL per day (38). Feces and urine in environment are good media for bacteria and other harmful organisms. To imagine the possible impact of feces excreted by stray dogs living within a city containing about 5000 stray dogs, the dogs can be assumed to produce more than 620 t of feces annually. This suggests that the least possible impact on such an environment needs to be verified, although these disposals are removed by mostly biodegradation (meaning that they remain for some time in the environment) (32). There should be a balance between fecal contamination by dogs and natural decontamination by the environment that they share. However, a question raised is how many feral dogs can be tolerated by a particular environment in an acceptable period of time. The answer to this remains unknown.

To understand any infection by the epidemiological triad, interactions in habitats shared by wild and domestic animals and humans to some degree should be considered together. Situated between Europe and Asia, Turkey spans a diverse territory consisting of two parts different in size and natural features: the main part, Anatolia, on the east, borders Georgia, Armenia, Azerbaijan, Iran, Iraq, and Syria. The other part, Thrace, to the west, is isolated from Anatolia by the Sea of Marmara and two straits and shares boundaries with Bulgaria and Greece. The wild habitat in the eastern part of Turkey is diversified with a limited number of wolves, which naturally encounter dogs from time to time (39,40). Like in any part of the world, confrontations (contact that inevitably occurs) between domestic dogs and wolves (or even other wild Canidae like foxes or jackals, which are also common in Turkey) can be expected, leading to injury or death and further leading to transmission of some infections from one species to another. In the case of canine brucellosis, if domestic animals pose a threat to wildlife (since edible parts of infected animals can be expected to infect predators), a proposed scheme of transmission will become more clear (Figure).

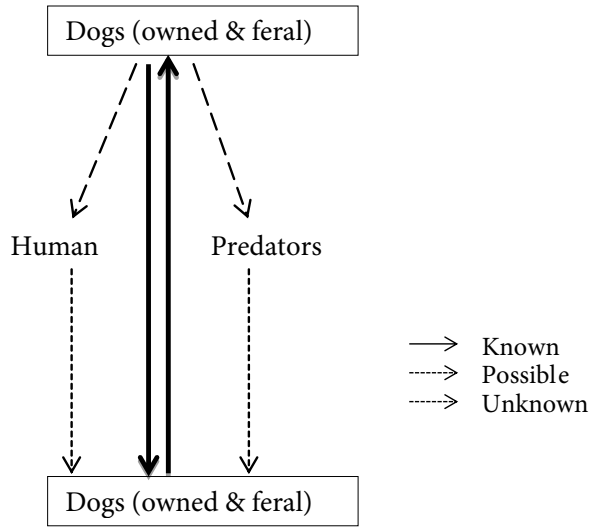

Figure. A flowchart for current understanding of the disease's transmission between mammals.

It is known that wolves use the Iranian areas neighboring Turkey as a habitat (41). In Armenia, a controversial strategy of wolf control was announced by an ecologist (42), evidencing the presence of wolves in Armenia, as well. A project on a wildlife corridor to study the ecological and habitat use of northeastern Turkey by wolves and some other large carnivore species was launched on land covering 23,500 ha (40). The author suggested that this corridor may provide a link to the isolated populations of wolves in this region and outside Turkey. It can be speculated that tracing wolves in this area should provide some evidence on transboundary movement of these animals soon. As for the west, Zlatanova and Popova (43) published a dataset on a wolf population located in Bulgaria near the Turkish border. Wolves from Greece along with those from Bulgaria formed a unique cluster based on 67K single nucleotide polymorphism analysis (44). Despite no confirmation of the association of landscape connectivity and canine brucellosis in Europe, diagnosing the diseases, if any, in wild animals in the future would help us to understand the role of wild animals in disease spread in a common environment. The number of wild Canidae as well as the prevalence (and incidence) of the disease that might occur in these habitats remains to be elucidated at present.

On the other hand, the term 'transboundary animal diseases' (TADs) is defined by the FAO as those epidemic diseases with high transmissibility, irrespective of national borders, and causing serious social, economic, and public health consequences. Brucellosis is not one of the TADs listed by the FAO; some examples of TADs are rinderpest, influenza A (H7N9, H1N1), and avian influenza (45). However, a human case of brucellosis was recently reported emphasizing beyond-borders transmission (46). This case did not fit the typical requirements for a TAD. However, this does not mean that brucellosis (and maybe canine brucellosis, too) might not have some common features with TADs to some degree in the future, which would suggest that it might be one of the candidate diseases to be listed if brucellosis epidemics with common transboundary features appear, since excess presence of stray dogs within the same environment with humans could place stress on some features of bacterial behaviors, although this is merely speculative at present.

From the perspective of One Health, national agencies are proposed to share the One Health concepts to monitor the disease, not only for humans, but also for Canidae from companion and wild sources. Some suggestions were recommended by Uçan and Aras (31) for introducing the One Health approach to the curriculum of medical and veterinary medicine faculties throughout the country as a first step. A model core curriculum for veterinary education including One Health concerns has already been published (47). Veterinary and medical academic professionals should also be asked to be involved in education and even in determining One Health curriculum issues in disciplines from environmental to agricultural sciences. This would allow veterinary professionals to gain a wider and deeper approach to host–agent–environment interactions.

A national monitoring (even as voluntary for beginning) system can be set up and implemented. This may be further arranged to include a passive surveillance system that screens both canine brucellosis in man and animals, including wild canines, wherever possible. In this approach, case definitions in man (as probable, suspected, or confirmed) and in canine species (as positive, negative, and undefined) can be described first and the use of serology for monitoring dogs should be verified. A routine data flow to a national coordinated authority that may be formed can be initiated by voluntary submission of the cases by veterinarians and medical doctors. All these would provide some basic pool of data on frequency of the cases by provinces, counties, and rural areas. Some projections on animal contact and occurrence of the diseases in man could also be analyzed.

More effectively, formal national health organizations should be encouraged to work together and allocated a budget. A national project for awareness by health professionals can be launched to draw more attention to One Health key factors like medicine, ecology, policy, culture, economics, management, and education. In any case, firm coordination between veterinary and medical services should be established. The Turkish National Committee for Zoonoses has already been established

but apparently requires more activity to play more critical roles.

In the light of One Health, although the approach cannot be readily established nationwide, some specific measures can still be implemented. More of the regulatory policies can be specified and implemented by local authorities (e.g., in kennels, all dogs should undergo repeated testing to diagnose canine brucellosis, removal of infected animals should be encouraged, and infected dogs should be neither sold nor used for breeding purposes).

Primary prevention measures are of great importance since there is no vaccine commercially available for *B. canis*. Obviously, treatment of canine brucellosis by selected antibiotics in dogs would not be widely applicable due to resistance concerns in humans, since physicians and veterinarians have very few options in making a choice about the antibiotics that they could use in the treatment. A veterinarian's final decision on treatment of an infected owned dog should only be made after interviewing the owner; the veterinarian should focus on what the pet in question means for the owner. We think that this particular type of evaluation in this specific case is an uncommon practice in veterinary medicine. Thus, veterinarians practicing medicine on pets should be further educated to gain enough academic background on methods of psychological evaluations.

Education of dog owners, including children, for awareness about the infection in the local environment through media on special international days (World Animal Day, World Veterinary Day, World Health Day, etc.) can be voluntarily conducted by health professionals or even by veterinary students.

Campaigns for companion animal owners to make them more enthusiastic about welfare and self-contributions to managing environmental health (e.g., to retrieve and dispose of feces deposited on public property without being forced by a law) can be designed and started.

Serological examination of brucellosis (S-type) suspected patients who have already presented to health practitioners or hospitals should be conducted for both types (S and R) of the disease.

In conclusion, canine brucellosis seems to be a threat for dogs and human in Turkey. The presence of infected free-ranging dogs appears to be major reason for the disease in the environment. However, control of any of the endemic diseases among dogs is difficult and sometimes impracticable. Despite difficulties, One Health may establish a guarantee for controlling infections in dogs as well as in humans since it places more focus on environmental issues. It can be speculated that the most priority for accomplishing the objective of controlling the disease, both in Canidae and humans, should be given to how to control stray dogs and finding out more about habitats, conservation, and management of wild Canidae whenever possible. The infection occurrence, however, can be decreased by taking all the measures from primary to tertiary preventions and ensuring the euthanasia, isolation, or removal (in some cases) of infected dogs in both developing and developed countries.

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