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Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): infection with *Brucella abortus, B. melitensis* and *B. suis*

EFSA Panel on Animal Health and Welfare (AHAW),
Simon More, Anette Bøtner, Andrew Butterworth, Paolo Calistri, Klaus Depner, Sandra Edwards, Bruno Garin-Bastuji, Margaret Good, Christian Gortázar Schmidt, Virginie Michel, Miguel Angel Miranda, Søren Saxmose Nielsen, Mohan Raj, Liisa Sihvonen, Hans Spoolder, Jan Arend Stegeman, Hans-Hermann Thulke, Antonio Velarde, Preben Willeberg, Christoph Winckler, Francesca Baldinelli, Alessandro Broglia, Frank Verdonck, Beatriz Beltrán Beck, Lisa Kohnle, Joana Morgado and Dominique Bicout

Abstract

The infection with *Brucella abortus*, *Brucella melitensis* and *Brucella suis* has been assessed according to the criteria of the Animal Health Law (AHL), in particular criteria of Article 7 on disease profile and impacts, Article 5 on the eligibility of the infection with *B. abortus*, *B. melitensis* and *B. suis* to be listed, Article 9 for the categorisation of the infection with *B. abortus*, *B. melitensis* and *B. suis* according to disease prevention and control rules as in Annex IV and Article 8 on the list of animal species related to the infection with *B. abortus*, *B. melitensis* and *B. suis*. The assessment has been performed following a methodology composed of information collection and compilation, expert judgement on each criterion at individual and, if no consensus was reached before, also at collective level. The output is composed of the categorical answer, and for the questions where no consensus was reached, the different supporting views are reported. Details on the methodology used for this assessment are explained in a separate opinion. According to the assessment performed, the infection with *B. abortus*, *B. melitensis* and *B. suis* can be considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL. The disease complies with the criteria as in Sections 2, 3, 4 and 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in points (b), (c), (d) and (e) of Article 9(1). The animal species to be listed for the infection with *B. abortus*, *B. melitensis* and *B. suis* according to Article 8(3) criteria are several mammal species, as indicated in the present opinion.

Keywords: *Brucella*, *B. abortus*, *B. melitensis*, *B. suis*, brucellosis, listing, categorisation

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Correspondence: alpha@efsa.europa.eu

Panel on Animal Health and Welfare (AHAW) members: Dominique Bicout, Anette Bøtner, Andrew Butterworth, Paolo Calistri, Klaus Depner, Sandra Edwards, Bruno Garin-Bastuji, Margaret Good, Christian Gortázar Schmidt, Virginie Michel, Miguel Angel Miranda, Simon More, Søren Saxmose Nielsen, Mohan Raj, Liisa Sihvonen, Hans Spoolder, Jan Arend Stegeman, Hans-Hermann Thulke, Antonio Velarde, Preben Willeberg, Christoph Winckler.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The background and Terms of Reference (ToR) as provided by the European Commission for the present document are reported in section 1.2 of the scientific opinion on the ad hoc methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Article 9, and 8 within the Animal Health Law (AHL) framework (EFSA AHAW Panel, 2017).

1.2. Interpretation of the Terms of Reference

The interpretation of the ToR is as in section 1.2 of the scientific opinion on the ad hoc methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Article 9, and 8 within the Animal Health Law (AHL) framework (EFSA AHAW Panel, 2017).

The present document reports the results of assessment on the infection with *Brucella abortus*, *Brucella melitensis* and *Brucella suis* according to the criteria of the AHL articles as follows:

- Article 7: the infection with *B. abortus*, *B. melitensis* and *B. suis* profile and impacts;
- Article 5: eligibility of the infection with *B. abortus*, *B. melitensis* and *B. suis* to be listed;
- Article 9: categorisation of the infection with *B. abortus*, *B. melitensis* and *B. suis* according to disease prevention and control rules as in Annex IV;
- Article 8: list of animal species related to the infection with *B. abortus*, *B. melitensis* and *B. suis*.

2. Data and methodologies

The methodology applied in this opinion is described in detail in a dedicated document about the ad hoc method developed for assessing any animal disease for the listing and categorisation of diseases within the AHL framework (EFSA AHAW Panel, 2017).

3. Assessment

3.1. Assessment according to Article 7 criteria

This section presents the assessment of the infection with *B. abortus*, *B. melitensis* and *B. suis* according to the Article 7 criteria of the AHL and related parameters (see Table 2 of the opinion on methodology (EFSA AHAW Panel, 2017)), based on the information contained in the fact-sheet as drafted by the selected disease scientist (see section 2.1 of the scientific opinion on the ad hoc methodology) and amended by the EFSA Panel on Animal Health and Welfare (AHAW).

3.1.1. Article 7(a) Disease Profile

3.1.1.1. Article 7(a)(i) Animal species concerned by the disease

Susceptible animal species

Parameter 1 – Naturally susceptible wildlife species (or family/orders)

B. abortus/B. melitensis

Most if not all wild mammals are theoretically susceptible, but ungulates are the most frequently affected wild animals, usually as a consequence of contact with infected livestock in extensive breeding systems. Infections have been reported in the one-humped (*Camelus dromedarius*) and two-humped (*Camelus bactrianus*) camels, llama (*Lama glama*), alpaca (*Vicugna pacos*), guanaco (*Lama guanicoe*), vicuña (*Vicugna vicugna*), water buffalo (*Bubalus bubalis*), European (*Bison bonasus*) and American bison (*Bison bison*), yak (*Bos grunniens*), African buffalo (*Syncerus caffer*), red deer (*Cervus elaphus*), moose (*Alces alces*), wild boar (*Sus scrofa*) and several species of African antelope, such as Kafue lechwe antelope (*Kobus leche kafuensis*), Arabian oryx (*Oryx leucoryx*) and sable antelope (*Hippotragus niger*) (Díaz-Aparicio, 2013; Godfroid et al., 2013). Sporadic cases have been reported also in carnivores like opossums (family Didelphidae), raccoons (*Procyon lotor*), coyotes

(*Canis latrans*), foxes (*Vulpes vulpes*) and wolves. In the European Union (EU), brucellosis due to both bacterial species has been reported only sporadically in wild ungulates such as ibex (*Capra ibex*), chamois (*Rupicapra* sp.), Spanish ibex (*Capra pyrenaica*) and red deer (*Cervus elaphus*) (Gortázar et al., 2007; Muñoz et al., 2010; Mick et al., 2014). However, these wild animals are considered occasional end hosts of brucellosis transmitted from infected livestock, rather than a true reservoir of the disease (Gortázar et al., 2007).

B. suis

This bacterial species is composed by five biovars (named from 1 to 5), with biovars 1, 2 and 3 causing brucellosis in domestic swine. This complexity accounts for the different types of epidemiological situations occurring in non-porcine and wild species. *B. suis* infection can occur in animals that are not the natural host of the particular infection through the ingestion of contaminated materials or by cohabitation with infected natural hosts. As examples, arctic foxes (*Vulpes lagopus*) and wolves (*Canis lupus*) may be infected by *B. suis* biovar 4 after eating infected reindeer. *B. suis* biovar 4 is a zoonotic agent and also causes a serious disease in wild or domesticated reindeer or caribou (*Rangifer tarandus* and its various subspecies) throughout the Arctic region, including Siberia, Canada and Alaska. Dogs and rodents, such as rats and mice, may acquire other *B. suis* biovars by cohabitation with infected hosts. In some cases, wildlife species are natural hosts for some *B. suis* biovars, as it has been reported in the former Soviet Union and the Baltic countries, where small rodents are infected by *B. suis* biovar 5 (EFSA, 2009; OIE, 2016). Infection by *B. suis* biovar 2 (which is a very rare zoonotic agent, see below) is reported frequently in the EU affecting the Eurasian wild boar (*Sus scrofa*) and the European brown hare (*Lepus europaeus*). Both wild species can transmit the infection to domestic pigs in outdoor farms, thus playing a relevant role as a reservoir of porcine brucellosis (EFSA, 2009; Muñoz et al., 2010). Outside the EU, feral pigs and peccaries (Tayassuidae) may maintain *B. suis* biovars 1 and 3 (both are important zoonotic agents) with the ensuing risks for both pigs and humans. These two last biovars are considered not to exist in the EU, with a unique and very rare exception reported for biovar 3 in horses in Croatia (Cvetnic et al., 2005), although this strain was finally identified as belonging to the biovar 1 (Fretin et al., 2008).

Parameter 2 – Naturally susceptible domestic species (or family/orders)

B. abortus

Cattle (primarily) and sheep and goats (very rarely). Sporadic cases have been reported also in farmed water buffalo (*Bubalus bubalis*) and, even more rarely, in horses and pigs (Cvetnic et al., 2005; OIE, 2016). Dogs belonging to infected herds are also found infected frequently. Outside the EU, in addition to the above domestic species, yak (*Bos grunniens*) and camels (both species) can be also found infected in mixed breeding systems (OIE, 2016).

B. melitensis

Sheep and goats (primarily) and cattle (less frequently and only when bovines and cattle cohabit with small ruminants (Verger et al., 1989; OIE, 2016)). Sporadic cases have been reported in farmed water buffaloes (*Bubalus bubalis*) and, more rarely, in horses and pigs (Cvetnic et al., 2005; OIE, 2016). Dogs belonging to infected flocks are also found infected frequently. Outside the EU, in addition to these domestic species, yak (*Bos grunniens*) and camels (both species) can be also found infected (OIE, 2016).

B. suis

As indicated above, the biovars 1, 2 and 3 infect domestic swine and are responsible for porcine brucellosis. *B. suis* biovar 2 infection is restricted to Continental Europe, and widespread in wildlife (see above) causing sporadic outbreaks in domestic swine reared in outdoor breeding systems. Very rare cases of *B. suis* biovar 2 infection have been reported in cattle, not followed by clinical signs (Fretin et al., 2013; Szulowski et al., 2013) and horses (Quaranta et al., 1995). *B. suis* biovars 1 and/or 3 are widespread in pigs in America, Asia, Oceania and probably Africa, causing also human brucellosis (unlike biovar 2, both biovars are important zoonotic agents). Infections by these biovars have been reported also in domestic dogs (Ramamoorthy et al., 2011). Biovar 3 is considered absent from the EU, while biovar 1 is very rare, it has been reported once in horses in Croatia (Fretin et al., 2008) and a clinical case has been reported in cattle in the USA (Ewalt et al., 1997).

Parameter 3 – Experimentally susceptible wildlife species (or family/orders)

Experimental infections have succeeded in several wild species, and probably both *B. abortus* and *B. melitensis* can be transmitted experimentally to most if not all wild mammals.

The experimental susceptibility of wild species to *B. suis* biovars remain to be established.

Parameter 4 – Experimentally susceptible domestic species (or family orders)

B. abortus and *B. melitensis* naturally induced infections have been reported in practically all domestic mammal species. Thus, both infections can be probably transmitted experimentally to most if not all domestic mammals.

Apart from pigs, cattle and horses, the domestic species experimentally susceptible to *B. suis* biovars remain to be properly established (with the exception of infection by biovar 4 in moose (Dieterich et al., 1991)).

However, infections in cattle by *B. suis* biovar 2 seem to be extremely rare, and in this case, at least considering the reported information, it seems that the infection is not followed by clinical (i.e. pathological) events (Fretin et al., 2013).

Reservoir animal species

Parameter 5 – Wild reservoir species (or family/orders)

B. abortus

No wild species have been proven as a natural reservoir. However, when human activity has impacted wildlife population dynamics, and where the infection is widespread in wildlife (i.e. the case of the bison and elk in the Yellowstone Park in the US – see comments in Section 3.1.2.4), some wild species could behave as a reservoir for both domestic cattle and wildlife (Cross et al., 2013).

B. melitensis

No wild species have been proven as a natural reservoir. In general, and particularly in Europe, wild ruminants are not regarded as reservoirs (maintenance hosts) for *Brucella* (Muñoz et al., 2010). However, exceptions may be found when human actions alter wildlife dynamics, such as in the Bargy Ibex case (Mick et al., 2014).

B. suis

This complex species is composed by five biovars, which reflects the different types of epidemiological occurrence in wild species. Wild boar is the natural reservoir of *B. suis* biovar 2. The infection is also frequent in European hares, although non-existent in other native hare species at least in the Iberian Peninsula (Muñoz et al., 2010), but the epidemiological role of hares remains unclear (Muñoz et al., 2010). The epidemiological relevance of the rare cases of *B. suis* biovar 2 in cattle and horses is unknown.

Feral pigs (*Sus scrofa*) and peccaries (Tayassuidae) are considered the natural reservoirs of *B. suis* biovars 1 and 3. These two last biovars are not present in the EU, with a rare exception for biovar 1 reported in horses (Fretin et al., 2008), and whose epidemiological significance is unknown.

Arctic foxes (*Vulpes lagopus*) and wolves (*Canis lupus*) may be infected by *B. suis* biovar 4 after eating infected reindeer (considered the reservoir of this biovar), but it is unknown if these carnivores are a reservoir or mere dead-end hosts. In the former Soviet Union and the Baltic countries, small rodents have been considered the natural reservoir of *B. suis* biovar 5, an infection of little epidemiological significance (EFSA, 2009; OIE, 2016). However, no recent reports are available.

Parameter 6 – Domestic reservoir species (or family/orders)

B. abortus: cattle (*Bos taurus*)

B. melitensis: sheep (*Ovis aries*) and goats (*Capra hircus*)

B. suis

- biovar 1, 2 and 3: pigs;
- biovar 4: domesticated reindeer/caribou;
- biovar 5: small rodents.

3.1.1.2. Article 7(a)(ii) The morbidity and mortality rates of the disease in animal populations

Morbidity

Parameter 1 – Prevalence/incidence

B. abortus*/*B. melitensis

B. abortus is found worldwide in cattle-raising regions except Japan, Canada, most European countries, most USA states, Australia and New Zealand, where it has been eradicated. *B. melitensis* is found in the Mediterranean countries, the Middle East, the Arabian Gulf, Latin America, Africa and Asia. Data on the prevalence and incidence of *B. abortus* or *B. melitensis* infections in domestic animals not submitted to official control are very difficult to get. The prevalence and incidence in domestic naïve populations which are neither vaccinated nor participating in any official sanitary intervention are usually very high, and depend largely on the time that has elapsed after the onset of disease in a given herd or flock. As example, in the early phases (before 1990) of official eradication programmes in Spain, herd prevalence ranged between 20% and 62% depending on the animal species and the regions considered. The individual prevalence (0.3–18.5%) depended on the region, animal species involved, and the technical characteristics of the programme (Blasco, 1986, 1990).

In contrast, when official interventions are applied, the prevalence/incidence figures are much lower but highly variable depending on the countries, the species, and the characteristics and degree of application of the programmes implemented. Suitable information on these parameters is available in EU Member States (MS) in which control and eradication campaigns are performed.

The last data available for 2016 (https://ec.europa.eu/food/funding/animal-health/national-veterinary-programmes_en) on the expected collective prevalence/incidence in these affected countries are summarised in Table 1.

Table 1: Herd prevalence and incidence in cattle, sheep and goat herds/flocks infected with *Brucella* in the EU Member State areas in which eradication programmes are cofinanced by the EU, hence do not include all herds and flocks in the country (in particular those of OBF areas) (year 2016)

Country	Bovine			Small ruminants		
	Herds tested	% Prevalence	% Incidence	Flocks tested	% Prevalence	% Incidence
Portugal	33,560	0.18	0.12	60,452	0.62	0.38
Spain	110,564	0.03	0.02	65,719	0.09	0.06
Italy	33,822(1)	0.48	0.48	38,158	0.78	0.61
Greece	Nd	Nd	Nd	22,950	0.02	0.01
Croatia	Nd	Nd	Nd	20,176	0.1	0.1

(1): Including buffaloes (1,282 herds; prevalence 1.01%; incidence 0.62%). Nd: not indicated.
Source: https://ec.europa.eu/food/funding/animal-health/national-veterinary-programmes_en

Individual prevalence/incidence is zero in the brucellosis officially free countries. The last data available (2016) on the expected individual prevalence are summarised in Table 2.

Table 2: Expected animal prevalence of *Brucella* infection in cattle, sheep and goat in the EU Member State areas in which eradication programmes are co-financed by the EU (year 2016)

Country	Bovine		Small ruminants	
	No. animals to be tested individually	% Prevalence	No. animals to be tested individually	% Prevalence
Portugal	899,600	0.02	1,558,000	0.1
Spain	3,874,200	0.002	8,490,600	0.01
Italy	1,004,279(1)	0.37	2,302,009	0.69

Country	Bovine		Small ruminants	
	No. animals to be tested individually	% Prevalence	No. animals to be tested individually	% Prevalence
Greece	Nd	Nd	4,279,000	0.02
Croatia	Nd	Nd	450,000	0.01

(1): Including buffaloes (260,375 animals; expected prevalence: 0.81%). Nd: not indicated.
Source: http://ec.europa.eu/dgs/health_foodsafety/funding/cff/animal_health/vet_progs_en.htm

In the absence of anthropogenic interventions in wildlife management that can modify the wildlife population dynamics (e.g. artificial winter feeding that can lead to increase of wildlife density and facilitates the animal contacts and the ensuing transmission), the prevalence of *B. abortus*/*B. melitensis* infections in wildlife species in the EU is considered very low or null (Muñoz et al., 2010; Garin-Bastuji et al., 2014; Mick et al., 2014).

B. suis

Infections caused by *B. suis* biovars 1 and 3 have been eradicated from domestic swine in several countries, including Canada, Australia and the USA. However, these infections still occur in wild and feral pigs, and can spread to domestic herds. In some studies, the individual prevalence in wild and feral swine ranged from 14% to 44%, and varied over time (CDC, 2005).

As the EU is considered as officially free from porcine brucellosis, data on the natural prevalence and incidence of *B. suis* biovar 2 infection in pigs are also scanty. The prevalence/incidence in intensive herds is considered insignificant (EFSA, 2009). However, outbreaks can be important in outdoor breeding herds due to spillover from infected wild boar. The percentage of infected pigs in naïve herds affected by a new outbreak is usually very high (15% to over 50%). The apparent prevalence of *B. suis* biovar 2 infection in wild boar is extremely high (25–45%) in the several EU Member States (Garin-Bastuji and Hars, 1999, 2001; EFSA, 2009; Muñoz et al., 2010).

Parameter 2 – Case-morbidity rate (% clinically diseased animals out of infected ones)

B. abortus*/*B. melitensis

As indicated above, suitable data on the natural case morbidity rate of brucellosis in naïve domestic animal populations are not available. Accordingly, only data obtained from artificially exposed animals are available. The most frequent clinical manifestation of *B. abortus*/*B. melitensis* infections is abortion. When brucellosis-free and unvaccinated cows are artificially exposed at the critical period of pregnancy (usually mid pregnancy) to *B. abortus* virulent challenge doses ($5 \times 10^7 - 5 \times 10^8$ CFU), 80–100% of the animals develop severe infections resulting in abortions in 75–100% of cows exposed (Moriyón et al., 2004). When challenge exposure is performed with similar *B. melitensis* doses in pregnant sheep and goats, the clinical consequences are very similar, with infection/abortion rates close to 100% (Verger et al., 1995; Barrio et al., 2009).

B. suis

In *B. suis*, biovar 2 naturally infected swine naïve herds, intraherd prevalence can be as high as 75–80%, with a very high proportion of abortions (25–60%) and infertility (30–80%) (Garin-Bastuji and Hars, 1999, 2001; EFSA, 2009; Dieste et al., 2011).

Mortality

Parameter 3 – Case-fatality rate

The case-fatality rate in naturally induced infections in domestic or wild animals is very low. Deaths are very rare in adult animals of most species; however, high *B. abortus* doses can be lethal in experimentally infected moose, and possibly also in bighorn sheep (Forbes et al., 1996). Fatality rate in humans is also very low (Pappas et al., 2006).

3.1.1.3. Article 7(a)(iii) The zoonotic character of the disease

Presence

Parameter 1 – Report of zoonotic human cases (anywhere)

Situation worldwide

Brucellosis is probably the commonest zoonotic infection worldwide, and whose global incidence has changed over the past decade because of various sanitary, socioeconomic, and political reasons, together with the evolution of international immigration and travel. Areas traditionally considered to be endemic (EU Mediterranean countries for example) have significantly improved the control of the disease, but the disease is still present. However, brucellosis is emerging strongly in the Near East and Asia (Pappas et al., 2006), and probably also in Africa (Ducrotoy et al., 2015). The incidence of human brucellosis worldwide was summarised in Dean et al. (2012a), with European estimates of 4-32 per 100,000 per year in Greece, 0.03 in Germany, 1.40 in Italy, and global reported maximum of 52-269 in Iraq.

Situation in the EU (see also Section 3.1.1.7 below)

A total of 437 confirmed human cases were reported in 2015 (EFSA, 2016) with a notification rate of 0.08 cases per 100,000 inhabitants. These primarily occurred in Greece (110 cases), Italy (106), Portugal (47), Spain (n = 39), Germany (n = 44), Bulgaria (37), France (n = 19), Sweden (n = 13) and the United Kingdom (n = 12) while other MS had less than 10 cases.

Brucella species information was missing for 71.5% of the 347 confirmed cases reported. Of cases in which bacterial isolation was conducted, 85.6% were due to *B. melitensis*, 2.1% to *B. abortus* and 12.4% to other *Brucella* species. In contrast to *B. suis* biovars 1, 3 and 4, *B. suis* biovar 2 has rarely been isolated from humans and non-porcine animal species. It can be considered less pathogenic to humans than other biovars and its zoonotic role is questioned in non-immunocompromised hosts (Godfroid et al., 2013; Mailles et al., 2016). Similarly, in cattle as well as in small ruminants, this biovar has been only isolated in singleton serological reactors in brucellosis officially free EU member states (Belgium (Fretin et al., 2013), France, Poland (Szulowski et al., 2013)), in the absence of any clinical sign or spread to other ruminants. However, precise information on the *Brucella* in cause is available only after its typing at biovar level; therefore at the initial confirmation of human or animal brucellosis, this will be difficult to assess and the control measures should be taken as it would be dealt with other more pathogenic species/biovars.

3.1.1.4. Article 7(a)(iv) The resistance to treatments, including antimicrobial resistance

Parameter 1 – Resistant strain to any treatment even at laboratory level

In contrast to other bacterial pathogens, selection for antibiotic resistance seems unimportant in brucellosis. This may relate to the absence of plasmids and lysogenic phages in the genus *Brucella* (Moreno, 1998). Moreover, due to economical, epidemiological and public health reasons, antibiotic treatment of brucellosis has been precluded in domestic animals, thus limiting the development of antibiotic resistance. Accordingly, resistance is not considered a significant issue in treating human brucellosis (Maves et al., 2011).

3.1.1.5. Article 7(a)(v) The persistence of the disease in an animal population or the environment

Animal population

Parameter 1 – Duration of infectious period in animals

Brucellae remain confined to the lymph nodes close to entry sites for 2–3 weeks, and then reach the blood via the efferent lymphatics; bacteraemia then leads to a generalised infection in reticuloendothelial organs, lymph nodes distant from entry sites, genital and extragenital organs and accessory sexual glands.

The precise duration of *B. abortus*, *B. melitensis* and *B. suis* infections has not been properly established in the different domestic species, but it is widely accepted that only a low proportion (10–15%) of infected animals develop a self-cure mechanism, while most remain infected for life, excreting the bacteria intermittently to the environment (Nicoletti, 1980; EFSA, 2009).

Parameter 2 – Presence and duration of latent infection period

The duration of the latent status until the animals develop the disease is highly variable, usually showing an abortion during their first or second pregnancy (Plommet et al., 1973; Lapraik and Moffat, 1982).

Brucella-induced latent infections are transmitted from infected dams to offspring either during pregnancy or perinatally (usually through milk). Latently infected animals are apparently healthy and show negative responses in the indirect immunological diagnostic tests, being thus very dangerous epidemiologically. Latent infections have been reported in up to up to 10% of the offspring born to *B. abortus* infected cattle (Plommet et al., 1973; Lapraik and Moffat, 1982), and also in *B. melitensis* infected goats (Renoux, 1962) and sheep (Grilló et al., 1997). Latent infections have not been reported in *B. suis* infected pigs, but they are believed to also occur these with variable frequency (EFSA, 2009).

Despite its low frequency, latent infection is one of the most frequent and dangerous causes of brucellosis transmission. If the objective is eradicating the infection, keeping replacements from *Brucella* positive animals should be avoided.

Parameter 4 – Length of survival (dpi) of the agent and/or detection of DNA in selected matrices (soil, water, air) from the environment (scenarios: high and low T)

Some *Brucella* species can survive for long periods outside the host. Dryness, high temperatures and direct sunlight exposure are very unfavourable for *Brucella* survival. Under favourable conditions, such as pH > 4, low temperatures, high humidity and the absence of direct sunlight, *Brucella* spp. may survive for relatively long periods in aborted fetuses and fetal membranes, faeces and liquid manure, water, wool and hay, as well as on equipment and clothes. *Brucella* spp. are able to withstand drying particularly in the presence of organic material and can then remain viable in dust and soil for relatively long periods. Survival is prolonged at low temperatures, especially in snow and ice. Since the presence of DNA in selected matrixes or the environment is not representative of the true survival ability of *Brucella*, no comments will be made on this topic. As an example, the persistence of *B. suis* on fomites varies from 4 h to 56 days (Ryan, 2010; US-EPA, 2010a,b; Calfee and Wendling, 2012).

Brucella species can persist for several weeks in soil (Franz et al., 1997; Charters, 1980) and in dust (Franz et al., 1997). *B. abortus* was reported to persist 66 days in wet soil, 48–73 days in soil at 90% humidity and < 4 days in dried soil (Nicoletti, 1980). *B. abortus* can persist in fetal tissues, soil or vegetation for several weeks or even months depending upon the season, temperature and sunlight (Aune et al., 2012). In bovine and bison fetuses deployed in February, March, April and May, *B. abortus* persistence was 81, 77, 69 and 25 days, respectively (Aune et al., 2012). *B. abortus* persisted 10–43 days at *B. abortus* contaminated sites associated with bison births or abortions for 7–26 days (Aune et al., 2012).

B. suis can persist at least 28 days in soil held at 5°C or 22°C (US-EPA, 2010a,b). However, in some study, this pathogen has been reported, surprisingly, to survive for 4 years in soil (Mollaret and Bourdin, 1973).

Depending on temperature and pH, *B. abortus* can persist in water from less than 1 to 77 days in water (Nicoletti, 1980; Falenski et al., 2011). *B. melitensis* and *B. suis* can persist for 1–7 days in dechlorinated water (Gilbert and Rose, 2012).

The survival of *Brucella* in milk and dairy products is highly variable from only few seconds to more than 4 months, depending on the *Brucella* species, the pH and temperature of preservation (for a review, see Garin-Bastuji and Blasco, 2016). Disinfectants reported to kill *Brucella* on contaminated surfaces include 2.5% sodium hypochlorite, quaternary ammonium compounds, 2–3% caustic soda, 20% freshly slaked lime suspension or 2% formaldehyde solution (all tested for 1 h). Ethanol, isopropanol, iodophores, substituted phenols or diluted hypochlorite solutions can be used on contaminated skin; alkyl quaternary ammonium compounds are not recommended for this purpose. Autoclaving (moist heat of 121°C for at least 15 min) can be used to kill *Brucella* species on contaminated equipment. These organisms can also be inactivated by dry heat (160–170°C) for at least 1 h. Boiling for 10 min is usually effective for liquids. Xylene (1 mL/L) and calcium cyanamide (20 kg/m^3) are reported to decontaminate liquid manure after 2–4 weeks. *Brucella* species can also be inactivated by gamma irradiation and pasteurisation (Ryan, 2010; Garin-Bastuji and Blasco, 2016).

3.1.1.6. Article 7(a)(vi) The routes and speed of transmission of the disease between animals, and, when relevant, between animals and humans

Routes of transmission

Parameter 1 – Types of routes of transmission from animal to animal (horizontal, vertical)

The main routes of transmission between animals and between animals and humans are summarised in Figure 1.

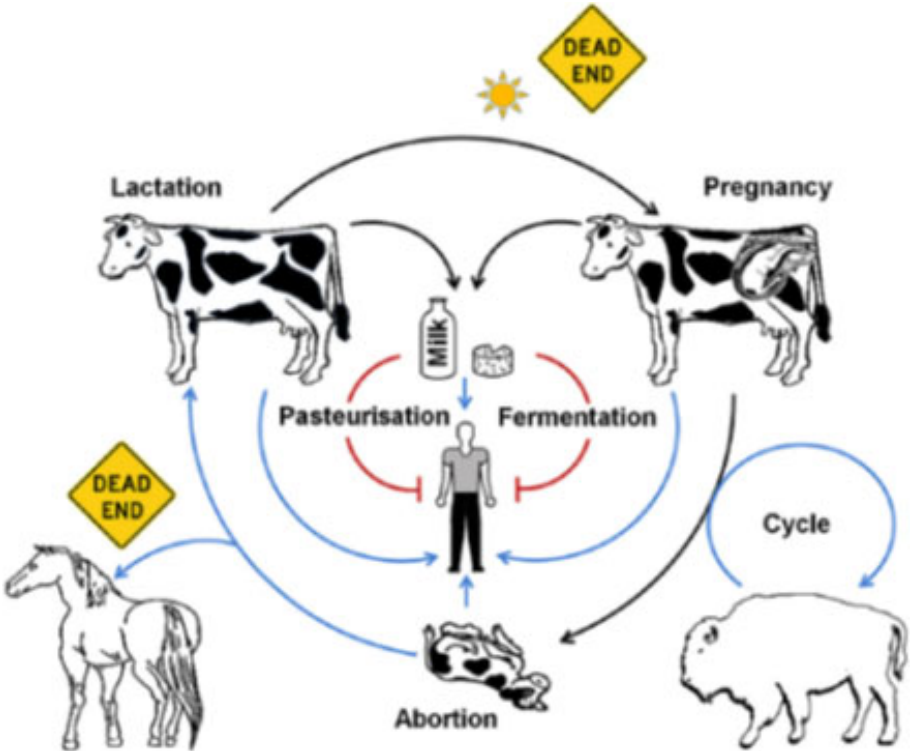

Figure 1: *Brucella* life host cycle (adapted from Moreno (2014))

Fetuses can be infected vertically, allowing the development of latent carriers. The heavily contaminated placenta and aborted fetuses become the main source of infection for humans (direct contact) and other animal hosts. Humans may also acquire infection eating unpasteurised dairy products, primarily or, exceptionally, raw or undercooked contaminated animal organs (like liver, blood and spleen) and meat.

Parameter 2 – Types of routes of transmission between animals and humans (direct, indirect, including food-borne)

See Figure 1 above.

Speed of transmission

Parameter 3 – Incidence between animals and, when relevant, between animals and humans

Data on the incidence of animal brucellosis have been commented above (see Section 3.1.1.2). In the absence of vaccination, the risk of transmission of brucellosis between animals in infected environments is very high. The incidence in humans has been reported to be highly correlated ($r = 0.82$) to the incidence of brucellosis in animals (Lee et al., 2013). Accordingly, when the disease is controlled in the animal hosts, a significant decrease is seen in human brucellosis incidence achieved.

Parameter 4 – Transmission rate (beta) (from R_0 and infectious period) between animals and, when relevant, between animals and humans

Several statistical methods have been applied to the quantitative study of brucellosis transmission in animals and humans. These methods have provided the basis for identifying key epidemiological parameters as the basic reproductive ratio (R_0) or the effective reproductive ratio (R_e), that indicates

the number of secondary infections for each infectious individual during ongoing transmission (for a review see Heffernan et al. (2005)). Several R_0 values have been reported or hypothesised for brucellosis (Table 3) but these figures are not straightforward because transmission dynamics is complicated by multiple interactions (Beauvais et al., 2016; Hou and Sun, 2016).

Table 3: Examples of reproductive ratio (R_0) figures calculated or hypothesised for animal brucellosis in different scenarios

R_0 values	Species	Country	Reference
1.75	Bison	USA	Hobbs et al. (2015)
1.2	Sheep	Mongolia	Zinsstag et al. (2005)
1.7	Cattle	Mongolia	Zinsstag et al. (2005)
2	Sheep	Hypothesised	Moreno (2014)
< 1 – > 3	Sheep/cattle	Mongolia	Racloz et al. (2013)

3.1.1.7. Article 7(a)(vii) The absence or presence and distribution of the disease in the Union, and, where the disease is not present in the Union, the risk of its introduction into the Union

Presence and distribution

Parameter 1 – Map where the disease is present in EU

Presence in animals

Bovine brucellosis

The list of countries and regions Officially Brucellosis-Free (OBF) is laid down in Annex II of the current version of Commission Implementing Decision (EU) 2016/168[1].

'not OBF yet'

For 2015, 938 positive or infected cattle herds were reported in total in the non-OBF regions of the non-OBF MS (967 in 2014), i.e. a herd prevalence of 0.28%.

Sheep and goat brucellosis

The list of countries and regions Officially *Brucella melitensis*-free (ObmF) is laid down in Annexes I and II to Commission Decision 2010/695/EU.[2]

Maps are provided in the European Union summary report on zoonoses (EFSA, 2016).

During the period 2005–2013, the overall proportion of sheep and goat flocks positive to *B. melitensis* in the EU showed a decreasing trend. In 2014, the decline continued from 0.11% in 2013 to 0.09% in 2014, the lowest prevalence ever reported. In the non-ObmF regions of the non-OBmF MSs, the overall prevalence of *B. melitensis*-positive sheep and goat flocks decreased from 0.45% in 2012 to 0.29% in 2015.

Porcine brucellosis

The EU is considered as officially free from this infection in domestic swine, although *B. suis* biovar 2 cases have been reported sporadically in several MSs in outdoor pig farms, in particular: Belgium, Croatia, Finland, France, Germany, Italy and Poland. Moreover, a relevant number of samples from wild boar (1,393 in Germany, 252 in Italy and 156 in Spain in 2014) and hares (16 in Germany in 2014) were reported to be infected by *B. suis* biovar 2. Maps are provided in the European Union summary report on zoonoses (EFSA, 2016).

[1] Commission Implementing Decision (EU) 2016/168 of 5 February 2016 amending the Annexes to Decision 2003/467/EC establishing the official tuberculosis, brucellosis and enzootic-bovine-leukosis-free status of certain Member States and regions of Member States as regards bovine herds. OJ L 32, 9.2.2016, p. 153–157.

[2] Commission Decision of 17 November 2010 amending the Annexes to Decision 93/52/EEC as regards the recognition of Estonia, Latvia and the Autonomous Community of the Balearic Islands in Spain as officially free of brucellosis (*B. melitensis*) and amending Annexes I and II to Decision 2003/467/EC as regards the declaration of Estonia as officially tuberculosis-free and officially brucellosis-free as regards bovine herds. OJ L 303, 19.11.2010, p. 14–17.

Presence in humans

A total of 449 human brucellosis cases (of which 437 were confirmed, and most due to *B. melitensis* and *B. abortus*), were reported in the EU in 2015 (EFSA, 2016).

Parameter 2 – Type of epidemiological occurrence (sporadic, epidemic, endemic) at MS level

See above in Sections 3.1.1.1 and 3.1.1.7 Parameter 1.

Risk of introduction

Not applicable. The animal disease is already present in several European countries. Control measures keep other countries Member States officially free.

3.1.1.8. Article 7(a)(viii) The existence of diagnostic and disease control tools

Diagnostic tools

Parameter 1 – Existence of diagnostic tools

Brucellosis caused by *B. abortus* (cattle and buffaloes), *B. melitensis* (small ruminants) and *B. suis* (swine) lacks pathognomonic symptoms and its diagnosis is based on the existing direct and indirect tests (OIE, 2016) (see Sections 3.1.4.1 and Tables A.1, A.2 and A.3 in Appendix A), the latter being those applied routinely in surveillance and control and eradication programmes. Detailed information of the availability, feasibility and effectiveness of the diagnostic tests is given below in Section 3.1.4.1.

Control tools

Parameter 2 – Existence of control tools

There are two possible strategies which exist to fight against brucellosis in ruminants: (1) a control programme based on mass (whole-flock/herd) vaccination or (2) an eradication programme based on test and cull, combined with vaccination. In both cases, the use of adequate vaccination procedures and diagnostic tests is of paramount importance. A detailed description is made in Section 3.1.4.2.

Blasco and Molina-Flores (2011) provide considerable detail on control and eradication of Brucellosis stating that Brucellosis can be first controlled (essentially by vaccination), and once the prevalence has decreased to reasonable levels the disease can be eradicated from a herd/flock by test and culling procedures (combined or not with vaccination), or by full depopulation. The authors provide a simple decision tree which should be taken into consideration by decision makers to select the most appropriate strategy according the different epidemiological situations. Independent of the prevalence of infection, the quality and degree of organisation of the national veterinary services is the limiting factor.

3.1.2. Article 7(b) The impact of diseases

3.1.2.1. Article 7(b)(i) The impact of the disease on agricultural and aquaculture production and other parts of the economy

The level of presence of the disease in the Union

Parameter 1 – Number of MSs where the disease is presence

See above in Section 3.1.1.7.

The loss of production due to the disease

Parameter 2 – Proportion of production losses (%) by epidemic/endemic situation (milk, growth, semen, meat, etc.)

There are very few well-documented studies on the production losses and the economic impact of brucellosis that take into account all aspects of the disease impacting the animal industry. Production losses can be divided broadly in direct (due to the pathological condition itself) and indirect (due to associated causes).

A summarised description of brucellosis losses is made in Figure 2.

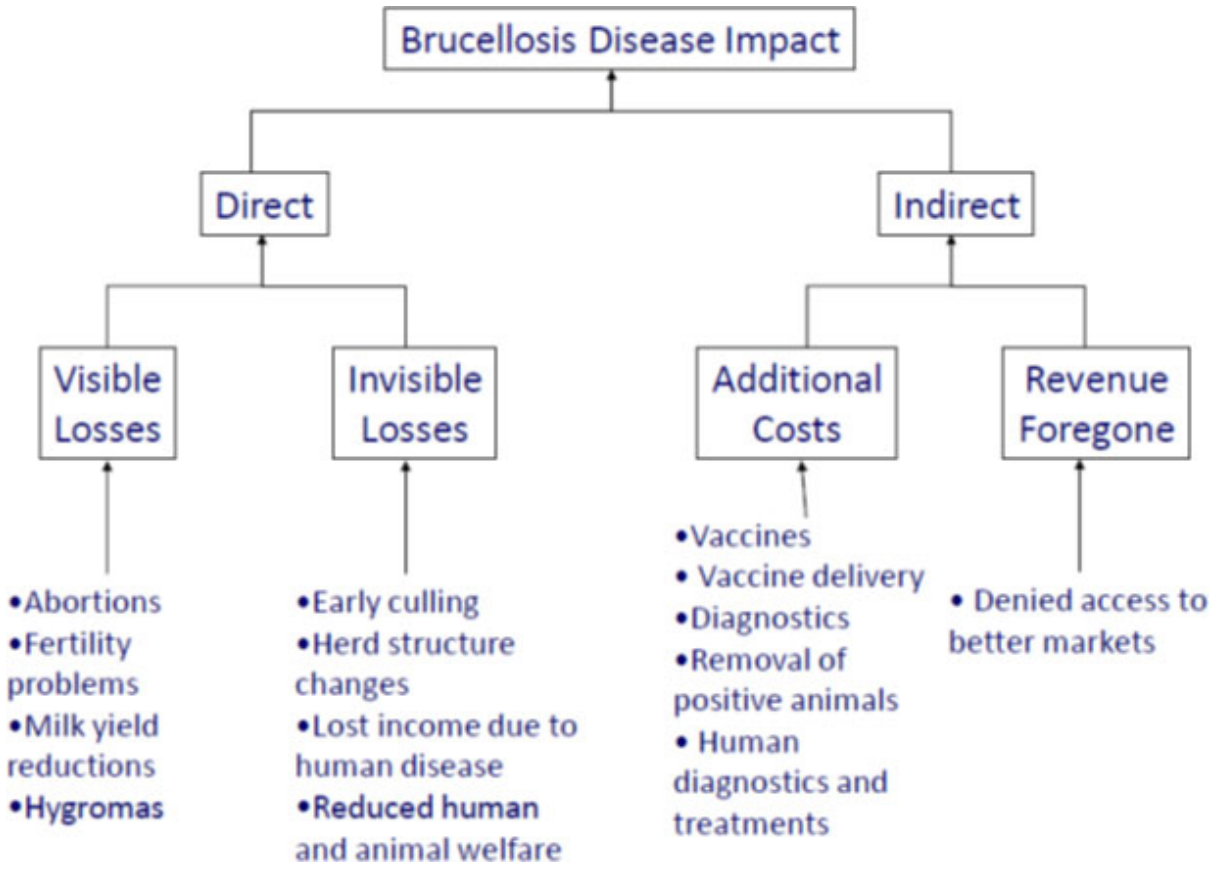

Figure 2: Direct and indirect costs of brucellosis (Bruce and Rushton, 2014)

Little is known about how brucellosis affects animal production quantitatively. A standardised methodology has been applied to the estimation of the direct disease costs and the human health and animal welfare impacts associated with 34 endemic diseases of livestock in Great Britain, but unfortunately, this study did not include brucellosis (Bennett and Ijpelaar, 2005).

The main clinical feature of brucellosis is late abortion in cattle, pigs, sheep and goats. Among the seropositive cattle, it has been estimated empirically that 10–50% abort and 20% of these remain infertile (Bernués et al., 1997). Having aborted, animals are often not milked and the entire lactation is lost. Besides abortions, perinatal mortality was also estimated empirically between 5% and 20%, and moreover, it was hypothesised that 1% of cows with abortions may die (Bernués et al., 1997). These authors estimated also theoretically a loss of 10–25% of total milk yield among the seropositive cows. These theoretical values were established many years ago in cattle in a particular epidemiological situation in the north of Spain. Despite this, these figures have been classically considered as the reference standard for losses caused for brucellosis no matter the animal species and the epidemiological conditions considered. In a study on bovine brucellosis (Santos et al., 2013), and based in part on these theoretical considerations, the following losses were estimated:

- 15% incidence of abortions in infected heifers and cows;
- an average of 2 months of temporary infertility for each infected cow and heifer, considering that 20% of cows that abort become sterile;
- an incidence rate of perinatal mortality of 10% for calves born from infected cows or heifers;
- 15% loss of the total milk yield of infected cows;
- 5% loss in meat production by infected cows;
- 1% mortality risk for infected cows that aborted (i.e. 0.15% of infected cows and heifers);
- an increase in the rate of replacement corresponding to 15% of the infected cows and heifers;
- replacement costs of infected bulls, considering roughly half of the seroprevalence of heifers, an average bull/cow ratio of 1/25, and same approach for calculating replacement as for females.

In summary, the direct production losses due to brucellosis have been estimated empirically, and only in cattle. Accordingly, the available losses reported are probably inaccurate, and precise information is lacking in the case of small ruminants and pigs.

Regarding porcine brucellosis, although it has been eradicated from the domestic pig population in Europe for decades, *B. suis* biovar 2 infection in wild boar (which is a sustainable infection in almost all European wild boar populations) is of major concern for pigs reared outdoors, should brucellosis control programmes in domestic pigs be implemented in the EU Member States (Godfroid et al., 2013).

3.1.2.2. Article 7(b)(ii) The impact of the disease on human health

Transmissibility between animals and humans

Parameter 1 – Types of routes of transmission between animals and humans

Brucella is easily transmitted from animals to humans. Humans become infected either through direct contact with infected animals or consumption of unpasteurised contaminated dairy products and selected raw or undercooked contaminated animal organs (like liver, blood and spleen). As the infectious dose is very low for humans, infection is usually an occupational risk for farmers, veterinarians, abattoir workers, laboratory personnel and others who work with animals and a risk for those who consume unpasteurised products. The increase in business and leisure travel to brucellosis-endemic countries has led to the importation of the human disease into non-endemic areas.

Parameter 2 – Incidence of zoonotic cases

The prevalence of brucellosis in humans depends upon several factors such as dietary habits, methods of processing milk and milk products, husbandry practices and environmental hygiene. Brucellosis causes more than 500,000 human infections per year worldwide. The disease has a limited geographic distribution, but it remains a major public health problem in the Mediterranean region, Western Asia, parts of Africa and Latin America (Pappas et al., 2006). The situation in the EU has been described in detail in Section 3.1.1.3.

Transmissibility between humans

Parameter 3 – Human to human transmission is sufficient to sustain sporadic cases or community-level outbreak

See comments in the next Parameter.

Parameter 4 – Sporadic, endemic, epidemic or pandemic potential

Human to human transmission of brucellosis is exceptional, and these exceptional cases have been reported. As an example, two physicians who assisted the surgical delivery of a placenta in an infected woman developed *Brucella melitensis* infection (Mesner et al., 2007). Sexually transmitted cases have been suspected but never really proven (Meltzer et al., 2010). Humans are considered thus a dead-end host of the disease.

The severity of human forms of the disease

A specific meta-analysis has been conducted on this topic (Dean et al., 2012a). Brucellosis is a multisystemic disease with a broad spectrum of symptoms. Asymptomatic infections are common. In symptomatic cases, the disease is extremely variable and the clinical signs may appear insidiously or abruptly. Typically, brucellosis begins as an acute febrile illness with nonspecific flu-like signs. Splenomegaly, hepatomegaly, coughing and pleuritic chest pain are sometimes seen. Gastrointestinal signs including anorexia, nausea, vomiting, diarrhoea and constipation occur frequently in adults but less often in children.

The disease is considered non-fatal (mortality rate of around 1% or less). However, when the diagnosis or treatments are not made properly, severe complications are common. Brucellosis causes one case of endocarditis and four neurological cases per 100 patients. One in 10 infected males suffers from epididymo-orchitis. Arthralgia, myalgia and back pain affect around half of the patients (65%, 47% and 45%, respectively).

Parameter 5 – Disability-adjusted life year (DALY)

DALY for a given disease are calculated by summing the years of life lost due to premature mortality (YLL) in the population and the equivalent healthy years lost due to disability for incident cases of the health condition (YLD). DALY estimates for brucellosis are based on the fact that is a non-fatal disease. So, the YLL part of calculations goes to zero. A brucellosis disability weighting of 0.2 has been proposed for DALY calculation, based on the pain and impaired productivity known to result from infection, and a median duration of untreated brucellosis of 3.1 years (Roth et al., 2003). Under this assumption, the cost-effectiveness and economic benefit for human society and the agricultural sector of a mass vaccination programme against ruminant brucellosis was modelled (Roth et al., 2003). The intervention consisted of a 10-year mass vaccination of Mongolian livestock using Rev.1 vaccine in small ruminants and S19 vaccine in cattle. Cost-effectiveness, expressed as cost per DALY averted, was the primary outcome. In a scenario of 52% reduction of brucellosis transmission between animals achieved by mass vaccination, it was estimated that a total of 49,027 DALY could be averted.

A DALY estimation was made in Kenya (http://es.slideshare.net/ILRI/brucellosis-inkenya) based on a reported number of 77,937 brucellosis cases in year 2012, and assuming average disease duration of six months, a disability weight of 0.19, and no mortality. The total DALY lost was 7,352 (i.e. 0.190 DALY per 1,000 people). DALY lost were 4,862 in males (i.e. 0.253 DALY per 1,000 people) and 2,490 in females (0.128 per 1,000 people). Extrapolating these Kenyan incidence data, the DALY for human brucellosis in Sub-Saharan countries ranged from 140,220 (considering only the reported cases) to 632,400 (considering 50% underreporting).

Another DALY estimate in Sudan using a 0.2 disability weight, concluded that the healthy years lost by an infected person accounted 7.5 DALY (Elkhansaa and Angara, 2014).

The availability of effective prevention or medical treatment in humans

Parameter 6 – Availability of medical treatment and their effectiveness (therapeutic effect and any resistance)

There are suitable treatments for human brucellosis. The prophylaxis of human brucellosis has to be based on three essential measures:

- awareness campaigns to educate the population on the major transmission risks (essentially, direct contact with animals and eating raw milk products);
- suitable food safety legislation and implementing measures avoiding the arrival to the markets of contaminated dairy products;
- the reduction/elimination of the infection in the animal hosts through suitable control and eradication campaigns. This last measure is, without any doubt, the most relevant for the definitive eradication of human brucellosis.

In addition, the antibiotic treatment is prolonged. A review of the efficacy of the FAO/WHO recommendations is reported by Ariza et al. (2007).

Parameter 7 – Availability of vaccines and their effectiveness (reduced morbidity)

No vaccines are available for human brucellosis.

3.1.2.3. Article 7(b)(iii) The impact of the disease on animal welfare

Parameter 1 – Severity of clinical signs at case level and related level and duration of impairment

Brucellosis is considered as a major contributor to animal suffering, causing fever, abortions, stillbirths and the birth of weak offspring, with the ensuing increase of perinatal mortality. Animals that abort may retain the placenta and develop endometritis and infertility. Animals usually abort only once, but subsequent chronic infertility is quite frequent. Milk yield is significantly reduced in infected animals but clinical signs of mastitis are uncommon. Acute orchitis and epididymitis can occur in males, and may result also in infertility. Arthritis and hygromas are seen occasionally. Deaths are very rare except in the fetus or new-born.

Studies on the impact of brucellosis on animal welfare beyond the clinical symptoms described above are lacking.

3.1.2.4. Article 7(b)(iv) The impact of the disease on biodiversity and the environment

Biodiversity

Parameter 1 – Endangered wild species affected: listed species as in CITES and/or IUCN list

In the EU, brucellosis due to *B. abortus* or *B. melitensis* have been reported only sporadically in wild ungulates such as ibex (*Capra ibex*), chamois (*Rupicapra* sp.) and red deer (*Cervus elaphus*) (Gortázar et al., 2007; Mick et al., 2014). However, these wild animals are considered occasional end hosts of brucellosis transmitted from infected livestock, rather than a true reservoir of the disease (Gortázar et al., 2007). Moreover, both infections are usually non-lethal in these wild species, which appear as of 'Least concern' in the IUCN list.

Brucellosis due to *B. suis* biovar 2 is reported frequently in the EU affecting the Eurasian wild boar (*Sus scrofa*) and the European brown hare (*Lepus europaeus*), which are considered the natural reservoirs of porcine brucellosis in Continental Europe. This infection is non-lethal in these wild species. Moreover, none of these two wild species appear as endangered or vulnerable in the IUCN list.

Parameter 2 – Mortality in wild species

Brucellosis is a non-fatal disease, being this issue of minor concern.

Environment

Parameter 3 – Capacity of the pathogen to persist in the environment and cause mortality in wildlife

B. suis biovar 2 infection is well established and persists naturally in the Eurasian wild boar (*Sus scrofa*) and the European brown hare (*Lepus europaeus*), these wild species being the natural reservoir of this infection in Continental Europe, and the main reservoir for domestic pigs. This infection is non-lethal in any of these wild species.

It has been questioned if *B. abortus* and *B. melitensis* can persist naturally in wildlife, being thus a reservoir for both human and domestic animals (Rhyan et al., 2013).

3.1.3. Article 7(c) Its potential to generate a crisis situation and its potential use in bioterrorism

Brucella species can be easily cultured from infected animals. Also, these bacteria can be transferred, multiplied and stored easily. An intentional contamination of food or water with pathogenic *Brucella* species could pose a threat with a low mortality rate but with a very high attack rate. Some *Brucella* (particularly *B. melitensis* and *B. suis* biovars 1 and 3) are highly infectious through oral/aerosol routes and, moreover, low doses are infective for humans and also for animals. Altogether, these characteristics make of this pathogen an attractive candidate to be used as a potential agent for biological warfare purposes (Doganay and Doganay, 2013).

Parameter 1 – Listed in OIE/CFSPH classification of pathogens

The *Brucella* genus is listed in the OIE list of notifiable terrestrial and aquatic animal diseases and in the CFSPH bioterrorism pathogens. See http://www.cfsph.iastate.edu/Products/resources/WallChart.pdf

Parameter 2 – Listed in the Encyclopaedia of Bioterrorism Defence of Australia Group

Pathogenic Brucellae are listed in the Encyclopaedia of Bioterrorism Defence of Australia Group. See: http://www.defence.gov.au/health/infocentre/journals/ADFHJ_sep00/ADFHealthSep00_1_3_099_106.pdf

Parameter 3 – Included in any other list of potential bioagroterrorism agents

Pathogenic *Brucella* are specified in the select agent rules implemented by USDA APHIS and the Centers for Disease Control and Prevention (CDC) of the Department of Health and Human Services (HHS) of the US and overlap with diseases and agents/toxins listed by APHIS, CDC and Code of Federal Regulations of the US (CFR) 121.4. See: https://www.fas.org/sgp/crs/terror/RL32521.pdf

3.1.4. Article 7(d) The feasibility, availability and effectiveness of the following disease prevention and control measures

3.1.4.1. Article 7(d)(i) Diagnostic tools and capacities

Availability

Parameter 1 – Officially/internationally recognised diagnostic tool, OIE certified

As indicated above (see Section 3.1.1.8), several diagnostic tests are available, recognised by the OIE, and used for surveillance/eradication in most countries worldwide (EFSA, 2009). These OIE tests and their main applications are summarised in Tables A.1, A.2 and A.3 (Appendix A).

Direct bacteriological isolation is the most specific test for the confirmation of *Brucella.* Both molecular and microbiological tests can be used for identification and typing. The vaccine strains (*B. abortus* strains S19 and RB51 and *B. melitensis* Rev.1) can be distinguished from their corresponding virulent field counterparts by both molecular (polymerase chain reaction (PCR) and related molecular tests) and classic microbiological criteria. Despite their usefulness for identification and typing, molecular tests are not fully suitable for the direct diagnosis from field samples. Serology is the most adequate for diagnosis at population level and is used for a presumptive diagnosis or for surveillance to screen herds/flocks. Serological tests used in sheep, goats, cattle and pigs include the buffered *Brucella* antigen tests (Rose Bengal and buffered plate agglutination tests), complement fixation, indirect or competitive enzyme-linked immunosorbent assays (ELISAs) and the fluorescence polarisation assay (FPA) (EFSA, 2006; EFSA, 2009; OIE, 2016). Other serological tests include gel precipitation tests with Native Hapten (NH) or cytosolic proteins (suitable for ruminants), the serum agglutination test (a test used in all animal species with controversial results), and the brucellin skin test (for use in all animal species). ELISAs or the *Brucella* milk ring test (MRT – this only for use in cattle) can be used also to screen dairy herds/flocks by detecting antibodies in milk (OIE, 2016).

In vaccinated cattle, the NH-based gel precipitation tests are sometimes used to diagnose infected animals in vaccination contexts (OIE, 2016).

The brucellin allergic skin test can be used to test unvaccinated animals for discriminating the true brucellosis infected animals from those giving false positive serological reactions (FPSR) in serological tests (generally due to cross-reactions with other gram-negative bacteria) (OIE, 2016).

Effectiveness

Parameter 2 – Se and Sp of diagnostic test

Direct tests (see Table 4) are very specific (particularly the classical bacteriology) for the diagnosis of brucellosis. However, since only the indirect tests are used for surveillance/eradication programmes, comments on diagnostic accuracy (diagnostic sensitivity (DSe) and diagnostic specificity (DSp)) will be focused exclusively in the immunological tests.

Because of different background flora, sera from *Brucella*-free animals can produce significantly different background reactivity depending upon their origin. Therefore, cut-offs obtained with animals from *Brucella*-free countries and infected animals from endemic areas as negative and positive controls, respectively (Nielsen et al., 1998, 2007), must be interpreted bearing in mind this problem.

Tests for diagnosing brucellosis in small ruminants and cattle (*B. melitensis* and *B. abortus* infections)

The EFSA meta-analytic report (EFSA, 2006) as well as literature data (using essentially the same inclusion/exclusion criteria that those used in EFSA (2006)) were used to produce the diagnostic accuracies summarised in Tables A.1 (Appendix A – sheep), A.2 (Appendix A – cattle) and A.3 (Appendix A – goats). Sensitive enough and reasonably specific tests are available, with the possibility to combine more than one of these tests for a suitable diagnosis and culling policy (EFSA, 2006).

Table 4: OIE test methods available for the diagnosis of *B. abortus*, *B. melitensis* and *B. suis* infections (Source: adapted from OIE (2016). Infection with *Brucella abortus, B. melitensis* and *B. suis*)

Method	OIE test prescription	Purpose					
		Population freedom from infection	Individual animal freedom from infection[a]	Efficiency of eradication policies[b]	Confirmation of clinical cases[c]	Confirmation of suspect cases[d]	Herd/flock prevalence of infection – surveillance
Direct	**Agent identification**						
Staining methods	–	–	–	–	+	–	–
Culture[k]	–	–	–	–	+++	+++/++[d]	–
PCR[e]	–	–	–	–	+	+/+[d]	–
Indirect	**Detection of immune response[e],[f]**						
BBAT (RBT, BPAT, Card test)	Prescribed for trade	+++	++	+++	+	+	+++
FPA	Prescribed for trade	++	++	+	++	+	++
CFT	Prescribed for trade	++	++	+++/+[j]	++	+	++
I-ELISA	Prescribed for trade	+++	++	+++	++	+	+++
C-ELISA	Prescribed for trade	+	–	–	+	+	+
BST	Other tests	+++	–	+	+++	+++	++
SAT	Other tests	+	+	+	–	–	+
NH and cytosol protein-based tests[g]	Other tests	–	–	+/++[i]	++	++	–
Bulk milk tests[h] Milk I-ELISA or Milk ring-test	Other tests	+++	–	+++	–	–	+++

+++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose. CFT: complement fixation test; I-/C-ELISA: indirect/competitive enzyme-linked immunosorbent assay; FPA: fluorescence polarisation assay; BST: Brucellin skin-test; SAT: standard tube agglutination test; NH: native hapten.

(a): This applies only to herds/flocks, countries or zones free from infection with *Brucella.*

(b): In order to improve the efficiency of eradication policies in infected herds/flocks, it is recommended to associate tests in parallel to increase the sensitivity of the diagnosis, i.e. two serological tests at least, e.g. BBAT or FPA and CFT or I-ELISA. The sensitivity is further increased by a parallel testing in both serology and BST.

(c): In low-prevalence or almost-free zones, the predictive value of positive results to serological tests may be very low. In such situation, the agent identification is usually needed for confirming clinical cases.

(d): In infected herds/flocks, a positive result to any serological test may be considered as a confirmation of a clinical case: (1) In infected herds/flocks, any reactor in any serological test should be considered as infected, (2) In low-prevalence or almost-free zones, singleton serological reactors may be confirmed by culture or BST (BST individual sensitivity is not 100%; BST is, however, highly effective when interpreted at herd/flock level), (3) In free countries or zones, suspect animals are those positive to both a screening and a confirmatory serological test (tests in series) and may be confirmed by culture (and or PCR) and/or BST.

(e): False-positive and false negative results may occur.

(f): The sensitivity and specificity of serological tests are far lower in pigs than in ruminants. Therefore, it is almost impossible to implement a large-scale serosurveillance in a pig population (due to the lack of specificity). In addition, in non-endemic areas, clinical or serological suspicions must almost always be confirmed by culture (and or PCR) and/or BST.
(g): In zones where subcutaneous S19 or Rev.1 vaccination is practiced, NH test may help in differentiating antibodies due to vaccination from those due to infection. In free countries or zones affected by the FPSR, both NH and cytosol-based tests are suitable for differentiating FPSR from true infections.
(h): Dairy cattle only.
(i): NH tests particularly useful for testing and slaughtering purposes when adult vaccination with S19 (cattle) or Rev 1 (small ruminants) is performed. Cytosol-protein based tests particularly useful when false positive serological reactions occur.
(j): Of limited efficacy when testing pigs.
(k): The best selective culture media are the Farrell's and CITA's media (see De Miguel et al., 2011); PCR: polymerase chain reaction; BBAT: buffered *Brucella* antigen tests (i.e. Rose Bengal test (RBT) and buffered plate agglutination test (BPAT)).

Tests for brucellosis in pigs (*B. suis* infection)

Comments on diagnostic performance of the OIE tests in pigs are based on the meta-analytical EFSA estimates (EFSA, 2009), as well as on more recent work (Muñoz et al., 2012; Dieste-Pérez et al., 2015a). Highly sensitive and reasonably specific tests with the potential to combine more than one are available (EFSA, 2009). However, serological testing in pigs is mainly useful to monitor herd status but is not reliable enough for identifying individual animals infected. Evidence from the systematic review suggests that indirect and competitive ELISAs could be suitable candidates because of their high DSe and DSp (EFSA, 2009). However, these ELISA tests have not been fully evaluated and standardised in pigs, and highly variable results are obtained when applied in the same population.

A major concern in porcine brucellosis officially free countries is the presence of false positive serological reactions (FPSR) in S-LPS-based tests. Little is known about the causes of FPSR in pigs, but infections by *Yersinia enterocolitica* O:9 are considered the main responsible (EFSA, 2009). Neither S-LPS- nor R-LPS-based tests are fully specific for differentiating porcine brucellosis from FPSR (Appendix A). In addition to bacteriology and molecular tools, the brucellin skin test (BST) is considered the only confirmatory test discriminating between brucellosis and the infections caused by *Y. enterocolitica* O:9 or other cross-reacting bacteria (EFSA, 2009; Dieste-Pérez et al., 2014). However, some serological tests based on cytosolic proteins can be a suitable and more practical alternative to the BST (Dieste-Pérez et al., 2015a).

Feasibility

Parameter 3 – Type of sample matrix to be tested (blood, tissue, etc.)

A large variety of sample matrices can be cultured. Milk samples and vaginal swabs from aborted animals are particularly useful for diagnosis in live animals. *Brucella* can also be cultured from aborted fetuses (stomach contents, spleen and lung) or the placenta. The spleen, the whole lymph nodes, udder and late pregnant or early post-parturient uterus, testis/epididymides, and accessory male sex glands are the most reliable samples to collect at necropsy (De Miguel et al., 2011). These samples are also suitable for direct PCR diagnostic procedures.

3.1.4.2. Article 7(d)(ii) Vaccination

Availability

Parameter 1 – Types of vaccines available on the market (live, inactivated, DIVA, etc.)

Three live attenuated vaccines are currently available: *B. abortus* S19 and *B. abortus* RB51 (for brucellosis cattle) and *B. melitensis* Rev.1 (for brucellosis in sheep and goats).

There are no vaccines against brucellosis induced by *B. abortus*/*B. melitensis* in camels, water buffaloes and yaks, which are important domestic species cohabiting with small ruminants and cattle in several breeding systems outside the EU.

The *B. abortus* S19 but not RB51 has been also proven effective against *B. melitensis* infection in cattle (Jiménez de Bagüés et al., 1991; Peniche Cardeña et al., 2009) and partially protective in red deer (Arenas-Gamboa et al., 2009). *B. abortus* S19 vaccine is used empirically in water buffalo and yak for the prophylaxis of brucellosis in some countries (e.g. Mongolia). However, no protection experiments have been conducted in camel, water buffalo, yak, swine and other domestic livestock. Developments in this area are required urgently. A major drawback of the S19 vaccine is that it is difficult to distinguish between cattle naturally infected from those vaccinated as adults, which is possible with the RB51 vaccine (Yang et al., 2013). Another disadvantage of the S19 and RB51 vaccines is that they can cause abortion if given to pregnant cows (Smith and Ficht, 1990; Yang et al., 2013) and their virulence for

humans (Spink et al., 1962; Ashford et al., 2004). Another disadvantage of RB51 is that it is resistant to rifampicin, which is often used for treating brucellosis (Gulsun et al., 2011).

Large-scale Rev.1 vaccination programmes proved its capacity to reduce the *Brucella* seroprevalence (Ward et al., 2012; SCOFCAH, 2011). On the other hand, *Brucella* has been occasionally detected in milk of Rev.1-vaccinated goats (Banai, 2002), the vaccine strain is virulent to humans (Blasco and Diaz, 1993) and differentiating naturally infected with *B. melitensis* and *B. ovis* from Rev.1-vaccinated animals might be difficult in vaccine is applied subcutaneously or in adults (Fensterbank et al., 1982).

The general characteristics of the currently available vaccines for the prophylaxis of animal brucellosis are summarised in Table 5.

Table 5: General characteristics of currently available brucellosis vaccines (Adapted from Blasco et al. (2016))

Vaccine	Advantages	Disadvantages	Comments
***B. melitensis* Rev.1**	Proved efficacy in control/ eradication programmes (France, Italy, Portugal, Spain) Effective against both *B. melitensis* and *B. ovis* Safe in young replacements (males and females) Single dose affords useful protection for life Biological quality control feasible (OIE protocol)	Highly abortifacient when used in pregnant animals Serological interference in classical serological tests (RBT, CFT), indirect and competitive ELISAs, fluorescence polarisation assay, and other S-LPS tests Low virulence for humans; streptomycin resistant	Safety issues minimised by avoiding use in mid-pregnancy animals by the conjunctival route Serological interference is minimised when applied to young replacements by the conjunctival route Human infections can be diagnosed using standard serological tests; treatment requires regimes that do not include streptomycin
***B. abortus* S19**	Proved efficacy in control/ eradication programmes (EU countries, USA and Australia) Protects cattle against both *B. abortus* and *B. melitensis* Single dose affords useful protection for life Biological quality control feasible (OIE protocol)	Depending on the dose and vaccination route can be abortifacient when used in pregnant cattle Used subcutaneously is not safe in bulls (unknown when applied by the conjunctival route) Serological interference in classical serological tests (RBT, CFT), indirect and competitive ELISAs, fluorescence polarisation assay, and other S-LPS tests Low virulence for humans	Serological interference is minimised when reduced doses are applied to young animals (particularly by the conjunctival route) Abortive effect minimised when reduced doses are applied by the conjunctival route Human infections can be diagnosed using standard serological tests; standard antibiotic treatment effective
***B. abortus* RB51**	• No interference in classical serological tests (RBT, CFT)	• Not recommended in pregnant cattle (abortifacient) or bulls. • Less effective than S19 in inducing protective immunity (efficacy or revaccination unknown). • Unknown protection span. • Protection of cattle against *B. melitensis* unknown. • Serological interference in indirect and competitive ELISAs and fluorescence polarisation assay. • Low virulence for humans; rifampicin resistant	• No proved efficacy for eradication. • No appropriate serological diagnostic tests to diagnose human infections; treatment requires regimes that do not include rifampicin

B. suis

With the exception of a live attenuated Chinese vaccine (Xin, 1986), whose efficacy for brucellosis has been disproven in controlled experiments in ruminants (Bosseray and Plommet, 1990; Blasco et al., 1993; Verger et al., 1995), no vaccines are available against brucellosis in pigs.

Parameter 2 – Availability/production capacity (per year)

The three vaccines currently available are widely produced worldwide with important regional differences.

In the EU, the production capacity of the S19 vaccine is limited (only three manufacturing companies exist in Spain), as it is also the case of RB51 (only one company exists in Spain). In the case of *B. melitensis* Rev.1 vaccine, the situation is similar with a single manufacturing company in France (Hungary) and three more companies in Spain. Italy produces also a reduced amount of Rev.1 manufactured by a single official laboratory but not by private companies. As a consequence of the effective control/eradication of brucellosis in the EU MS, these manufacturing companies have reduced significantly the overall production capacity, which is essentially maintained for the export market. However, manufacturing technology is currently well implemented and the production capacity of these remaining companies could be significantly increased to cover an emergency situation, at least at medium term.

Effectiveness

Parameter 3 – Field protection as reduced morbidity (as reduced susceptibility to infection and/or to disease)

B. abortus S19 and *B. melitensis* Rev.1 remain the most effective vaccines, and when combined with test and slaughter have been instrumental in almost all successful cases of eradication. A single dose of these vaccines confers from 50% to 100% protection against challenges infecting 80–100% of unvaccinated controls (Nicoletti, 1990; Jacques et al., 2007; Barrio et al., 2009; Dorneles et al., 2015). However, quality of product is essential and thus there are internationally accepted quality control protocols for both vaccines (Grilló et al., 2000; OIE, 2016).

Although RB51 vaccine also protects cattle against mild *B. abortus* challenges (Moriyón et al., 2004; Dorneles et al., 2015), the protective life span of this vaccine is unknown, and field infections in vaccinated animals have been observed even in revaccinated cattle (Moriyón et al., 2004; Herrera-López et al., 2010). Under controlled conditions, the few valid comparisons strongly suggest that RB51 is inferior to S19 (Moriyón et al., 2004).

Whether RB51 protection is useful under field conditions has been a matter of debate.

Parameter 4 – Duration of protection

It has been proven experimentally that Rev.1 induces suitable protection against *B. melitensis* in sheep for at least two consecutive pregnancies (Verger et al., 1995). The protection lapse span of Rev.1 against *B. melitensis* in goats is very long at 4–5 years (Alton, 1990; Díaz-Aparicio et al., 2004).

The duration of immunity induced by S19 in cattle vaccinated as calves has been proven to be also quite long, covering almost the entire productive lifespan of the animals, and it has been reported that the immunity in cattle vaccinated between 6 and 8 months of age does not decrease from the first through the fifth pregnancy (Dorneles et al., 2015). Revaccination with S19 demonstrated no apparent benefit in cattle and as in the case of the Rev.1 vaccine, a single vaccination is considered effective enough for the whole productive lifespan of the animals (Dorneles et al., 2015).

The protective lapse span of RB51 vaccine is unknown.

3.1.4.3. Article 7(d)(iii) Medical treatments

Availability

For economic, epidemiological and public health reasons, treatment with antibiotics has been generally precluded in animals infected with brucellosis. However, several therapeutic regimens have been evaluated successfully for brucellosis in cattle, sheep, pigs and dogs (Nicoletti et al., 1985; Marín et al., 1989; Jiménez de Bagüés et al., 1991; Grilló et al., 2006; Dieste et al., 2011; Dieste-Pérez et al., 2014).

The therapeutic effect has been reported as 67% using oxytetracycline combined with streptomycin, and as approximately 20% using oxytetracycline alone (Nicoletti et al., 1985).

3.1.4.4. Article 7(d)(iv) Biosecurity measures

Availability

Parameter 1 - Available biosecurity measures

Brucellosis is mainly spread through:

- animals moving between and within farms and, in particular, the introduction of new animals with unknown or poorly certified officially free status (the entry of latent carriers in particular);
- direct contact with neighbours' animals/farms infected with brucellosis;
- sharing equipment, feed, water and bedding between farms;
- direct contacts with wildlife (relevant in the case of porcine brucellosis due to *B. suis* biovar 2).

Measures avoiding these risk factors should contribute to minimise brucellosis spread between infected and healthy herds/flocks. Biosecurity would be focused essentially on controlling and reducing movements of animals (particularly the purchase of replacements avoiding latent carriers), feed, water, people and equipment to and from areas where livestock is kept.

Effectiveness

Parameter 2 – Effectiveness of biosecurity measures in preventing the pathogen introduction

While the above measures are feasible and effective for minimising brucellosis spread in highly intensified farming systems (usually ruminant dairy herds/flocks and swine reared intensively), they are very difficult to implement in outdoor or fully extensive/transhumant breeding systems, in particular for preventing porcine brucellosis. In the case of brucellosis in domestic ruminants, and at least in the EU, rules on zoning and on restriction of animal movements according the available EU Directives, have been proven very effective to minimise the spread of *B. abortus* and *B. melitensis* infections.

In contrast, biosecurity measures minimising the spread of porcine brucellosis due *B. suis* biovar 2 in the EU are very difficult (if not impossible) to implement. As this infection is widespread in wildlife, the only feasible biosecurity measures are: (i) reducing the wildlife density and (ii) avoiding the contacts between wildlife and domestic swine though suitable fencing (EFSA, 2009).

Feasibility

Parameter 3 – Feasibility of biosecurity measures

Biosecurity measures for avoiding the spread of *B. abortus* and *B. melitensis* infections in domestic ruminants based on the current rules on zoning and on restriction of animal movements according the available EU Directives, have been proven feasible and very effective.

However, biosecurity measures minimising the spread of porcine brucellosis due *B. suis* biovar 2 in the EU are very difficult (if not impossible) to implement. Reducing the density of wild boar (the main reservoir of infection) can be attempted but results are uncertain given the high capacity for movements of these wild animals. The total depopulation of wild reservoir species seems neither feasible nor ethically justified. The most effective way to prevent contacts between wild boar and domestic pigs is a suitable fencing system (EFSA, 2009). However, while this suitable (usually very expensive) fencing is feasible for some particular backyard farms and industrial outdoor breeding holdings, it cannot be implemented in fully extensive systems like those typical for Iberian pigs in the Iberian Peninsula and other similar outdoor pig breeding systems.

3.1.4.5. Article 7(d)(v) Restrictions on the movement of animals and products

Availability

Parameter 1 – Available movement restriction measures

Council Directive 64/432/EEC[3], Council Directive 91/68/EEC[4] and Commission Decisions lay down conditions applying to imports of live animals and products from third countries. The SPS Agreement recognises the OIE as the relevant international organisation responsible for the development and promotion of international animal health standards, guidelines, and recommendations affecting trade in

[3] Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine. OJ 121, 29.7.1964, p. 1977–2012.

[4] Council Directive 91/68/EEC of 28 January 1991 on animal health conditions governing intra-Community trade in ovine and caprine animals. OJ L 46, 19.2.1991, p. 19–36.

live animals and animal products. These internationally accepted rules are contained in the OIE Terrestrial Animal Health Code (http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/).

Effectiveness

Parameter 2 – Effectiveness of restriction of animal movement in preventing the between farm spread

The existing EU Directives on the restriction of movements have proven to be highly effective in preventing the spread of brucellosis between farms in the MS in which the disease yet exists. However, these rules allow that animals born from infected dams but showing false negative results in the official tests and that may be latently infected may qualify for OBF status. To avoid this possibility, MS currently involved in the EU official eradication programmes state generally require animals born from infected dams to be also culled in the infected holdings that are not submitted to full depopulation.

Feasibility

Parameter 3 – Feasibility of restriction of animal movement

The Council Directives dealing with restrictions of animal movement (see Parameter above) have been successfully implemented by MS many years ago, and have proven to be of paramount importance in the successful eradication of brucellosis and to reduce the spread of the disease in the countries in which the disease is yet present. Accordingly, these measures have a wide acceptance in the EU MS and have been proven feasible. Computerised management of livestock movements constitutes a further step in the management of health hazards associated with the movement of animals. To facilitate such control, the EU require national computerised registers containing basic details about any national movement of animals and, in some cases, international movement. Furthermore, the Trade Control and Expert System (TRACES) has been widely implemented in EU MS. This is an on-line system which comprises a network of Veterinary Authorities and economic operators of Member States (Commission Decision 2003/623/EC[5]).

3.1.4.6. Article 7(d)(vi) Killing of animals

Availability

Parameter 1 – Available methods for killing animals

Brucellosis eradication requires the identification of infected animals, their progressive elimination from the herd/flock and replacement with non-infected animals (Crespo Léon et al., 2012). Council Directives 64/432/EEC (amended and updated) – for bovines – and 91/68/EEC – for sheep and goats – establish the procedures for gaining, maintaining, suspending, withdrawing or regaining the OBF status in the EU. The culling of infected animals (and even the full depopulation of the affected herd/flock) is properly stated and justified in both Directives, and this has been a tool of critical importance for the successful eradication of brucellosis in some MS, as well as for the significant reduction of spread in those MS in which brucellosis yet exists.

In the case of identifying and confirming *B. suis* infection in holdings belonging to MS, the slaughter of infected pigs is recommended. However, in these cases it is more advisable to adopt whole-herd depopulation (the measure currently applied in the few cases in which *B. suis* outbreaks have been confirmed in EU MS) as this would reduce the risk of spread to other holdings (EFSA, 2009).

Effectiveness

Parameter 2 – Effectiveness of killing animals (at farm level or within the farm) for reducing /stopping spread of the disease

The killing of infected animals or the whole depopulation of infected holdings has been successfully implemented in EU MS for many years, and proven to be of paramount importance in the successful eradication of brucellosis in some MS as well as to reduce the spread of the disease in MS in which the disease is still present.

[5] 2003/623/EC: Commission Decision of 19 August 2003 concerning the development of an integrated computerised veterinary system known as Traces. OJ L 216, 28.8.2003, p. 58–59.

Feasibility

Parameter 3 – Feasibility of killing animals

These measures have a wide acceptance in the EU and have been proven effective and feasible after many years of application.

3.1.4.7. Article 7(d)(vii) Disposal of carcasses and other relevant animal by-products

Availability

Parameter 1 – Available disposal option

At the EU level, the killing of infected animals (or the whole herd/flock) can be made by several procedures, all proven feasible and effective after many years of application of the official eradication programmes. The slaughter of infected animals/whole holdings can be made at field level in the affected holding or, alternatively, in selected places authorised for this purpose. Once killed, the animals have to be sent to authorised disposal centres, according the rules stated in Regulation (EC) No 1069/2009[6]. Alternatively, the infected animals (or the whole herd/flock affected) can be sent to specially authorised slaughterhouses, and the carcasses can be authorised for human consumption according the rules described in Regulations (CE) No 853/2004[7] and (EC) No 854/2004[8]. In the EU Food Law, brucellosis in animals is listed as a specific hazard and detailed provisions for the disease to ensure safety of meat and to protect public health have been established therein. Chapter IX (F) of Section IV of Annex I to Regulation (EC) No 854/2004 lays down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

Effectiveness

Parameter 2 – Effectiveness of disposal option

The above options have been successfully applied by EU MS for many years and proven safe and effective enough for the disposal of brucellosis infected animals/holdings.

Feasibility

Parameter 3 – Feasibility of disposal option

The above options have been successfully applied in EU MS for many years, and proven feasible for the disposal of brucellosis infected animals/holdings.

3.1.5. Article 7(e) The impact of disease prevention and control measures

3.1.5.1. Article 7(e)(i) The direct and indirect costs for the affected sectors and the economy as a whole

There are very few well-documented studies on the production losses and the economic impact of brucellosis taking into account all aspects of the disease. In particular, little is known about how brucellosis affects animal production quantitatively, and the figures used as a reference were established empirically many years ago (Bernués et al., 1997). Moreover, well-documented studies on the indirect costs of brucellosis are also scarce. A review of the studies available concludes that brucellosis is in all likelihood a major problem in low-income countries of Africa and Asia (McDermott et al., 2013).

An overall brucellosis framework that considers costs of illness, costs of prevention, and opportunity costs (both public and private) at household, livestock sector, health sector and broader societal levels is shown in Table 6.

It can be concluded that the cost estimates published for brucellosis are of limited value.

[6] Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). OJ L 300, 14.11.2009, p. 1–33.

[7] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

[8] Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. OJ L 139, 30.4.2004, p. 206–320.

Table 6: Costs to be considered and estimated when planning brucellosis control and eradication programmes (McDermott et al., 2013)

	Actors	Cost of illness	Cost of prevention	Intangible and opportunity costs
Private	Individuals and households	Treatment (e.g. medication), loss of household production	Risk mitigation (e.g. boiling milk)	Disutility of ill health per individual (DALYs)
	Livestock sector	Treatment, herd slaughter, market loss due to risk of infected meat and milk, mortality, morbidity, lower production, loss of exports	Biosecurity, vaccination* and procedures to control disease along the value chain (e.g. pasteurisation)	Disutility of ill health for friends, family, etc. Future emerging disease Loss of animal genetic resources Loss of opportunities occasioned by spending on disease prevention and cure
Public	Health sector (human and animal)	Treatment (hospital provision, etc.) Outbreak costs, movement restrictions, culling, vaccination	Risk mitigation (e.g. movement control and vaccination*), disease surveillance, research	
	Economy	Indirect effects on economic development, ecosystem services and tourism	Biosecurity, avoiding wildlife and vectors, disease surveillance, research	

Dark grey boxes: market prices available and commonly included in economic assessments of disease. Light grey boxes: market prices not available so costs need to be estimated through other methods. White boxes: prevention costs reflect efficiency and effectiveness of public and private service provision. Usually there are few data and only rough estimates are made. Black box: included in health metrics (DALYs: disability-adjusted life years).

*: A number of costs (for example, vaccination) produce benefits for both the private sector (better livestock production) and the public sector (fewer human infections).

Parameter 1 – Cost of control (e.g. treatment/vaccine, biosecurity)

In rich countries, an important part of successful brucellosis eradication has been the official compensations given to farmers for the culled livestock. However, in high prevalence conditions and in most resource-limited countries, eradication is not feasible, and applying control programmes is the only suitable option (Blasco and Molina-Flores, 2011). To attempt control (usually through mass vaccination of the whole susceptible population), the benefits to public health and society need to be demonstrated, particularly in countries with limited resources. The costs of these control programmes are highly variable and depend essentially of the country, the animal species involved, the vaccination programme selected (for a review see Blasco and Molina-Flores (2011) and Blasco et al. (2016)), and the vaccine used. Accordingly, well-documented economic analyses of brucellosis control programmes are also scarce.

An interesting cost-benefit study of a control programme based on vaccination of small ruminants with the Rev.1 vaccine was conducted in Portugal (Coelho et al., 2011). The most relevant costs saved and expensed when applying a mass-vaccination programme with Rev.1 are shown in Table 7.

Table 7: Total incremental costs in US$ for *Brucella* control using a strategy based on mass vaccination of small ruminant with Rev.1 vaccine in Trás-os-Montes e Alto Douro (2000–2005), Portugal (Coelho et al., 2011)

Difference between the years	Compensation costs paid to farmers		Vaccine costs		Cost of patient hospitalisation		Total	
	Spent	Saved	Spent	Saved	Spent	Saved	Spent	Saved
2000–2001	115,357	–	19,592	–	–	24,451	110,498	–
2000–2002	–	331,148	29,843	–	–	89,411	–	390,716
2000–2003	–	1,069,300	39,681	–	–	206,507	–	1,236,125

Difference between the years	Compensation costs paid to farmers		Vaccine costs		Cost of patient hospitalisation		Total	
	Spent	Saved	Spent	Saved	Spent	Saved	Spent	Saved
2000–2004	–	1,870,307	55,826	–	–	341,399	–	2,155,879
2000–2005	–	2,577,002	60,788	–	–	502,356	–	3,018,569
Mean/year	–	515,400	12,158	–	–	100,741	–	603,714

In the five years of study, more than US$ 3,000,000 were saved after mass vaccination with Rev.1 with respect to the costs generated by an eradication programme (consisting in the vaccination of only young replacements and testing and culling the adult sheep and goats) with an annual average reduction in monetary costs of US$ 603,714. The annual average saving in costs of compensation paid to farmers and cost of patient hospitalisation in hospitals were US$ 515,400 and US$ 100,471, respectively (Coelho et al., 2011).

Parameter 2 – Cost of eradication (culling, compensation)

Due to the important variations in the prevalence and in the costs of diagnostic tools/vaccines and of the operative costs of the involved private/public veterinary services in the different countries, no internationally recognised costs have been defined for eradication, monitoring and surveillance. However, precise figures, updated yearly and available on line (http://ec.europa.eu/dgs/health_food-safety/funding/cff/animal_health/index_en.htm, exist in the case of EU MS in which brucellosis is yet present.

Sheep and goats

As an example, the costs of the eradication programme for brucellosis in small ruminants submitted to the EU by Portugal (http://ec.europa.eu/dgs/health_food-safety/funding/cff/docs/animal_vet-progs_2016-7_dec-2015-3609-ec_ov-cap-brucellosis_prt.pdf) are for year 2016 €2,394,916. This was calculated considering the following relevant figures: 60,452 flocks to be tested, 0.62% expected collective (flock) prevalence, 2,193,200 animals to be tested, 0.1% expected individual prevalence, and 48,830 animals to be vaccinated with the Rev.1 vaccine.

Cattle

The total cost of the brucellosis eradication programme to be carried out in bovines in Italy for year 2016 is €11,219,302. This was calculated considering the following relevant figures: 33,822 flocks to be tested, 0.71% expected collective (herd) prevalence, 1,004,279 animals to be tested, and 0.37% expected individual prevalence.

Between 2007 and 2011, the total cost incurred by the Health Service of Lazio Region (Italy) for the eradication of brucellosis in cattle was estimated in 5,996.809 EUR, of those 4,797,389 were the cost of the veterinarians labour, 8,864 for the transport, 23,908 for disposal and compensation for culled animals (Caminiti et al., 2016).

Parameter 3 – Cost of surveillance and monitoring

See above.

Parameter 4 – Trade loss (bans, embargoes, sanctions) by animal product

See next Parameter.

Parameter 5 – Importance of the disease for the affected sector (% loss or € lost compared to business amount of the sector)

Economic losses due to a serious and unexpected brucellosis outbreak occurring in an officially free MS could be large and widespread if no official intervention is conducted. First, losses would include the value of lost production, the cost of destroying diseased or potentially diseased animals, and the costs of containment (diagnostics, compensation to producers for destroyed animals, disposal costs and costs of veterinary services). Second, and very important, export markets could be lost if importing countries place restrictions on products to prevent the possibility of disease spread. Third, multiplier effects could ripple through the economy due to decreased sales by agriculturally dependent businesses (farm input suppliers, food manufacturing, transportation, retail grocery, and food service).

Finally, as brucellosis is a zoonosis, tourism could be affected also if negative perceptions for food or personal safety exist. Depending on the erosion of consumers' confidence and export sales, market prices of the affected commodities (essentially live animals and meat) may drop. This would affect not only to the owners of the affected area but also to producers whose herds were not directly infected, making the event national in scale even if the disease itself is contained to a small region.

Fortunately, the expected impact of a brucellosis outbreak in a given officially free MS in the current epidemiological situation should not be very high due to the existence of zoning or regionalisation in the EU, and the continuous surveillance of the brucellosis free areas, allowing an early response in the eventual detection of any brucellosis outbreak. Zone/region means a clearly defined part of a country containing an animal subpopulation with a distinct health status with respect to a specific disease (brucellosis in this case) for which required surveillance, control and biosecurity measures have been applied for trade purposes. Accordingly, the EU defines clearly and regularly the MS regions with the different brucellosis status inside the national boundaries for the purposes of international trade or disease control strategies. Having in consideration the favourable evolution of brucellosis even in the MS in which infection persists, and the epidemiological nature of brucellosis (low capacity of spreading at the short term), any unexpected local brucellosis outbreak should not have important consequences for the sector if interventions are applied over a short period. An exception could be porcine brucellosis since the EU is the main exporter of swine/swine meat to the international markets (around 37% of the total exports worldwide). If trade were limited by third countries, the consequences for the EU swine industry could be very important.

3.1.5.2. Article 7(e)(ii) The societal acceptance of disease prevention and control measures

Consumer confidence in government may be tested depending on the scale of the eradication effort and the means used for the disposal the animals culled. The need to slaughter thousands of animals could generate public criticism if culling methods are considered inhumane or if the destruction of carcasses is questioned environmentally. This should thus be avoided. Fortunately, the currently applied culling and disposal systems used in the EU eradication programmes for brucellosis have been widely accepted by the affected owners and the society in general.

3.1.5.3. Article 7(e)(iii) The welfare of affected subpopulations of kept and wild animals

Parameter 1 – Welfare impact of control measures on domestic animals

Whenever properly managed by competent veterinarians, the measures implemented for controlling brucellosis (usually through vaccination), do not pose a relevant issue from the animal welfare standpoint.

Parameter 2 – Wildlife depopulation as control measure

This is not an issue in the case of *B. abortus* and *B. melitensis* infections since wild animals are very rarely affected and, in the case of sporadic infections, this does not have any epidemiological significance. In the very rare event that high prevalence of these infections is found in wildlife (i.e. the case of the Alpine Ibex infected by *B. melitensis* in a very restricted area of the French Alps (Mick et al., 2014), the depopulation of the affected wild animals could be considered whenever this be feasible, socially acceptable, and the animal species involved is not endangered.

In contrast, this could be considered in the case of the infection caused by *B. suis* biovar 2 since both wild boar and hares are the wild reservoir of porcine brucellosis in the EU and the prevalence is very high in both species (EFSA, 2009; Muñoz et al., 2010). The precise role played by hares in the transmission of infection to swine remains unclear (EFSA, 2009). However, available evidence suggests that wild boar is the main source of infection for domestic pigs bred in outdoor rearing systems, even on fenced premises (EFSA, 2009; Muñoz et al., 2010). This outdoor housing is used in open air semi-intensive systems or in extensive systems (as the free ranging Iberian pig system), which have a moderate to high but permanent risk of infection via contacts with infected wild boar (EFSA, 2009; Muñoz et al., 2010). As full depopulation of wild boar in the EU is unfeasible, reducing the population density of wild boar could be a suitable alternative to reduce the risk of transmission of *B. suis* biovar 2 to domestic pigs reared in these outdoor breeding systems.

3.1.5.4. Article 7(e)(iv) The environment and biodiversity

Environment

Parameter 1 – Use and potential residuals of biocides or medical drugs in environmental compartments (soil, water, feed, manure)

No drugs/chemicals other than the common and legally accepted disinfectants are used in the current control/eradication campaigns in the EU.

Biodiversity

Parameter 2 – Mortality in wild species

Wild animals are not affected significantly by the disease in the EU, with the exception of *B. suis* biovar 2 affecting wild boar and hares. However, brucellosis is a non-fatal disease for wildlife and domestic animals.

3.2. Assessment according to Article 5 criteria

This section presents the results of the expert judgement on the criteria of Article 5 of the AHL about the infection with *B. abortus*, *B. melitensis* and *B. suis* (Table 8). The expert judgement was based on Individual and Collective Behavioural Aggregation (ICBA) approach described in detail in the opinion on the methodology (EFSA AHAW Panel, 2017). Experts have been provided with information of the disease fact-sheet mapped into Article 5 criteria (see supporting information, Annex A), based on that the experts indicate their Y/N or 'na' judgement on each criterion of Article 5, and the reasoning supporting their judgement.

The minimum number of judges in the judgement was 10. The expert judgement was conducted as described in the methodological opinion (EFSA AHAW Panel, 2017). For details on the interpretation of the questions, see Appendix B of the methodological opinion (EFSA AHAW Panel, 2017).

Table 8: Outcome of the expert judgement on the Article 5 criteria for the infection with *Brucella abortus*, *B. melitensis* and *B. suis*

Criteria to be met by the disease: According to AHL, a disease shall be included in the list referred to in point (b) of paragraph 1 of Article 5 if it has been assessed in accordance with Article 7 and meets all of the following criteria		**Final outcome**
A(i)	The disease is transmissible	Y
A(ii)	Animal species are either susceptible to the disease or vectors and reservoirs thereof exist in the Union	Y
A(iii)	The disease causes negative effects on animal health or poses a risk to public health due to its zoonotic character	Y
A(iv)	Diagnostic tools are available for the disease	Y
A(v)	Risk-mitigating measures and, where relevant, surveillance of the disease are effective and proportionate to the risks posed by the disease in the Union	Y
At least one criterion to be met by the disease: In addition to the criteria set out above at point A(i)-A(v), the disease needs to fulfil at least one of the following criteria		
B(i)	The disease causes or could cause significant negative effects in the Union on animal health, or poses or could pose a significant risk to public health due to its zoonotic character	Y
B(ii)	The disease agent has developed resistance to treatments and poses a significant danger to public and/or animal health in the Union	N
B(iii)	The disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union	Y
B(iv)	The disease has the potential to generate a crisis or the disease agent could be used for the purpose of bioterrorism	Y
B(v)	The disease has or could have a significant negative impact on the environment, including biodiversity, of the Union	N

Colour code: green = consensus (Yes/No).

3.2.1. Outcome of the assessment of the infection with *Brucella abortus*, *B. melitensis* and *B. suis* according to criteria of Article 5(3) of the AHL on its eligibility to be listed

As from the legal text of the AHL, a disease is considered eligible to be listed as laid down in Article 5 if it fulfils all criteria of the first set from A(i) to A(v) and at least one of the second set of criteria from B(i) to B(v). According to the assessment methodology (EFSA AHAW Panel, 2017), a criterion is considered fulfilled when the outcome is 'Yes'. According to the results shown in Table 8, the infection with *B. abortus*, *B. melitensis* and *B. suis* complies with all criteria of the first set and with three criteria of the second set, therefore it is considered eligible to be listed as laid down in Article 5 of the AHL.

3.3. Assessment according to Article 9 criteria

This section presents the results of the expert judgement on the criteria of Annex IV referring to categories as in Article 9 of the AHL about the infection with *B. abortus*, *B. melitensis* and *B. suis* (Tables 9, 10, 11, 12 and 13). The expert judgement was based on ICBA approach described in detail in the opinion on the methodology. Experts have been provided with information of the disease fact-sheet mapped into Article 9 criteria (see supporting information, Annex A), based on that the experts indicate their Y/N or 'na' judgement on each criterion of Article 9, and the reasoning supporting their judgement.

The minimum number of judges in the judgement was ten. The expert judgement was conducted as described in the methodological opinion (EFSA AHAW Panel, 2017). For details on the interpretation of the questions see Appendix B of the methodological opinion (EFSA AHAW Panel, 2017).

Table 9: Outcome of the expert judgement related to the criteria of Section 1 of Annex IV (category A of Article 9) for the infection with *Brucella abortus*, *B. melitensis* and *B. suis*

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Final outcome**
1	The disease is not present in the territory of the Union OR present only in exceptional cases (irregular introductions) OR present in only in a very limited part of the territory of the Union	N
2.1	The disease is highly transmissible	N
2.2	There be possibilities of airborne or waterborne or vector-borne spread	Y
2.3	The disease affects multiple species of kept and wild animals OR single species of kept animals of economic importance	Y
2.4	The disease may result in high morbidity and significant mortality rates	N
At least one criterion to be met by the disease: In addition to the criteria set out above at point 1-2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, including epidemic or pandemic potential OR possible significant threats to food safety	N
4	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	NC
5(a)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC
5(c)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(d)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N

Colour code: green = consensus (Yes/No), yellow = no consensus (NC).

Table 10: Outcome of the expert judgement related to the criteria of Section 2 of Annex IV (category B of Article 9) for the infection with *Brucella abortus*, *B. melitensis* and *B. suis*

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Final outcome**
1	The disease is present in the whole OR part of the Union territory with an endemic character AND (at the same time) several Member States or zones of the Union are free of the disease	Y
2.1	The disease is moderately to highly transmissible	Y
2.2	There be possibilities of airborne or waterborne or vector-borne spread	Y
2.3	The disease affects single or multiple species	Y
2.4	the disease may result in high morbidity with in general low mortality	Y
At least one criterion to be met by the disease: In addition to the criteria set out above at point 1-2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, including epidemic potential OR possible significant threats to food safety	Y
4	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	NC
5(a)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC
5(c)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(d)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N

Colour code: green = consensus (Yes/No), yellow = no consensus (NC).

Table 11: Outcome of the expert judgement related to the criteria of Section 3 of Annex IV (category C of Article 9) for the infection with *Brucella abortus*, *B. melitensis* and *B. suis*

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Final outcome**
1	The disease is present in the whole OR part of the Union territory with an endemic character	Y
2.1	The disease is moderately to highly transmissible	Y
2.2	The disease is transmitted mainly by direct or indirect transmission	Y
2.3	The disease affects single or multiple species	Y
2.4	The disease usually does not result in high morbidity and has negligible or no mortality AND often the most observed effect of the disease is production loss	Y
At least one criterion to be met by the disease: In addition to the criteria set out above at point 1-2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, or possible significant threats to food safety	Y
4	The disease has a significant impact on the economy of the Union, mainly related to its direct impact on certain types of animal production systems	N
5(a)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC
5(c)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(d)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N

Colour code: green = consensus (Yes/No), yellow = no consensus (NC).

Table 12: Outcome of the expert judgement related to the criteria of Section 4 of Annex IV (category D of Article 9) for the infection with *Brucella abortus*, *B. melitensis* and *B. suis*

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria	**Final outcome**
The risk posed by the disease in question can be effectively and proportionately mitigated by measures concerning movements of animals and products in order to prevent or limit its occurrence and spread	Y
The disease fulfils criteria of section 1, 2, 3 or 5 of Annex IV of AHL	Y

Colour code: green = consensus (Yes/No).

Table 13: Outcome of the expert judgement related to the criteria of Section 5 of Annex IV (category E of Article 9) for the infection with *Brucella abortus*, *B. melitensis* and *B. suis*

Diseases in category E need to fulfil criteria of section 1, 2 or 3 of Annex IV of AHL and/or the following:		**Final outcome**
E	Surveillance of the disease is necessary for reasons relating to animal health, animal welfare, human health, the economy, society or the environment (If a disease fulfils the criteria as in Article 5, thus being eligible to be listed, consequently category E would apply.)	Y

Colour code: green = consensus (Yes/No).

3.3.1. Non-consensus questions

This section displays the assessment related to each criterion of Annex IV referring to the categories of Article 9 of the AHL where no consensus was achieved in form of tables (Tables 14, 15 and 16). The proportion of Y, N or 'na' answers are reported, followed by the list of different supporting views for each answer.

Table 14: Outcome of the expert judgement related to criterion 2.1 of Article 9

Question		**Final outcome**	**Response**		
			Y (%)	**N (%)**	**na (%)**
2.1 (cat.A)	The disease is highly transmissible	NC	50	50	0
2.1 (cat.B,C)	The disease is moderately to highly transmissible	NC	50	50	0

Number of judges: 10; NC: non-consensus.

Reasoning supporting the judgement

supporting Yes for 2.1 (cat.A):

- Within-herd animal prevalence rapidly reaches 30–100% when infection is first introduced into a herd or flock.
- The infective dose is low and thus it is the most frequent and easily acquired zoonotic occupational infection.
- Latency with negative serology and disease recrudescence during pregnancy are common and responsible for many incidents of transmission to large numbers of animals.
- The estimation of R_0 is not straightforward because transmission dynamics are complicated by multiple interactions between animals.
- supporting Yes for 2.1 (cat.B,C):
- Published R (R_0, R_e) values are relatively low (less than 2 in most cases).

After discussion, the AHAW Panel reached consensus about the disease being at least moderately transmissible.

Table 15: Outcome of the expert judgement related to criterion 4 of Article 9

Question*		Final outcome	Response		
			Y (%)	N (%)	na (%)
4 (cat.A,B)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	NC	90	10	0

Number of judges: 10; NC: non-consensus.
*: At the time of the collective judgement, the assessment of the current impact considering the control measures in place was considered.

Reasoning supporting the judgement

supporting Yes:

- The disease has a substantial impact in infected areas, as a consequence of abortion and infertility.
- The disease can affect all productions systems for each susceptible species.
- The loss of the officially free status may have important economic consequences due to the ban on trade of live animals for breeding or slaughter (breeders in particular) as well as semen, embryos and raw-milk products (OIE code provisions).

supporting No:

- In the current situation and given the control measures in place in the EU, the impact is not significant on the economy of the whole Union due to actual low prevalence and incidence in MSs (maximum 0.78% prevalence and 0.61% incidence in Italy in 2016 in small ruminants (expected).

Table 16: Outcome of the expert judgement related to criterion 5(b) of Article 9

Question*		Final outcome	Response		
			Y (%)	N (%)	na (%)
5(b)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC	70	30	0

Number of judges: 10; NC: non-consensus.
*: At the time of the collective judgement, the assessment of the current impact considering the control measures in place was considered.

Reasoning supporting the judgement

supporting Yes:

- Brucellosis is considered a major contributor to animal suffering, causing fever, abortions, stillbirths and the birth of weak offspring, with the ensuing increase of perinatal mortality.
- It is present in more than in 3 countries.

supporting No:

- In the current situation and given the control measures in place in the EU, there is no welfare impact on large number of animals because of the low prevalence.

3.3.2. Outcome of the assessment of criteria in Annex IV for the infection with *Brucella abortus*, *B. melitensis* and *B. suis* for the purpose of categorisation as in Article 9 of the AHL

As from the legal text of the AHL, a disease is considered fitting in a certain category (A, B, C, D or E corresponding to point (a) to point (e) of Article 9(1) of the AHL) if it is eligible to be listed for Union intervention as laid down in Article 5(3) and fulfils all criteria of the first set from 1 to 2.4 and at least one of the second set of criteria from 3 to 5(d) as shown in Tables 9–13. According to the assessment methodology (EFSA AHAW Panel, 2017), a criterion is considered fulfilled when the outcome is 'Yes'.

A description of the outcome of the assessment of criteria in Annex IV for the infection with *B. abortus*, *B. melitensis* and *B. suis* for the purpose of categorisation as in Article 9 of the AHL is presented in Table 17.

Table 17: Outcome of the assessment of criteria in Annex IV for the infection with *Brucella abortus*, *B. melitensis* and *B. suis* for the purpose of categorisation as in Article 9 of the AHL

Category	Article 9 criteria										
	1°set of criteria					2°set of criteria					
	1	2.1	2.2	2.3	2.4	3	4	5a	5b	5c	5d
	Geographical distribution	Transmissibility	Routes of transmission	Multiple species	Morbidity and mortality	Zoonotic potential	Impact on economy	Impact on society	Impact on animal welfare	Impact on environment	Impact on biodiversity
A	N	N	Y	Y	N	N	NC	N	NC	N	N
B	Y	Y	Y	Y	Y	Y	NC	N	NC	N	N
C	Y	Y	Y	Y	Y	Y	N	N	NC	N	N
D						Y					
E						Y					

According to the assessment here performed, the infection with *B. abortus*, *B. melitensis* and *B. suis* complies with the following criteria of the sections 1–5 of Annex IV of the AHL for the application of the disease prevention and control rules referred to in points (a) to (e) of Article 9(1):

1) For being assigned to category A, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment brucellosis complies with criteria 2.2, 2.3, but not with 1, 2.1 and 2.4. For being eligible for category A, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and brucellosis does not comply with criteria 3, 5a, 5c and 5d and the assessment is inconclusive on compliance with criteria 4 and 5b.
2) For being assigned to category B, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment brucellosis complies with all criteria. For being eligible for category B, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and brucellosis complies with criterion 3, does not comply with criteria 5a, 5c and 5d and the assessment is inconclusive on compliance with criteria 4 and 5b.
3) For being assigned to category C, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment brucellosis complies with all criteria. For being eligible for category C, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and brucellosis complies with criterion 3, does not comply with criteria 4, 5a, 5c and 5d and the assessment is inconclusive on compliance with criterion 5b.
4) For being assigned to category D, a disease needs to comply with criteria of section 1, 2, 3 or 5 of Annex IV of the AHL and with the specific criterion D of section 4, which brucellosis complies with.
5) For being assigned to category E, a disease needs to comply with criteria of section 1, 2 or 3 of Annex IV of the AHL and/or the surveillance of the disease is necessary for reasons relating to animal health, animal welfare, human health, the economy, society or the environment. The latter is applicable if a disease fulfils the criteria as in Article 5, which brucellosis complies with.

3.4. Assessment of Article 8

This section presents the results of the assessment on the criteria of Article 8(3) of the AHL about the infection with *B. abortus*, *B. melitensis* and *B. suis*. The Article 8(3) criteria are about animal species to be listed, as it reads below:

'3. Animal species or groups of animal species shall be added to this list if they are affected or if they pose a risk for the spread of a specific listed disease because:

a) they are susceptible for a specific listed disease or scientific evidence indicates that such susceptibility is likely; or
b) they are vector species or reservoirs for that disease, or scientific evidence indicates that such role is likely'.

For this reason, the assessment on Article 8 criteria is based on the evidence as extrapolated from the relevant criteria of Article 7, i.e. the ones related to susceptible and reservoir species or routes of transmission, which cover also possible role of biological or mechanical vectors.[9] According to the mapping, as presented in Table 5, section 3.2 of the scientific opinion on the ad hoc methodology (EFSA AHAW Panel, 2017), the main animal species to be listed for the infection with *B. abortus*, *B. melitensis* and *B. suis* according to the criteria of Article 8(3) of the AHL are as displayed in Tables 18 and 19.

Table 18: Animal species to be listed for the infection with *Brucella abortus* and *B. melitensis* according to criteria of Article 8 (*source*: data reported in Section 3.1.1.1)

	Order	Family	Genus/species
Susceptible	Artiodactyla	Camelidae	*Camelus dromedarius*, *Camelus bactrianus*, *Lama glama*, *Vicugna pacos*, *Lama guanicoe*, *Vicugna vicugna*
		Cervidae	*Cervus elaphus*, *Alces alces*
		Suidae	*Sus scrofa*
		Bovidae	*Bos taurus*, *Bubalus bubalis*, *Bison bonasus*, *Bison bison*, *Bos grunniens*, *Syncerus caffer*, *Capra hircus*, *Ovis aries*, *Capra ibex*, *Rupicapra* spp., *Capra pyrenaica*, *Kobus leche kafuensis*, *Hippotragus niger*, *Oryx leucoryx*
	Carnivora	Canidae	*Canis lupus*, *Canis latrans*, *Vulpes vulpes*
		Procyonidae	*Procyon lotor*
	Didelphimorphia	Didelphidae	Not specified
	Perissodactyla	Equidae	*Equus caballus*
Reservoir	Artiodactyla		*Bos taurus* (*B. abortus*) *Ovis aries*, *Capra hircus* (*B. melitensis*)
Vectors	None		

Table 19: Animal species to be listed for the infection with *Brucella suis* according to criteria of Article 8 (*source*: data reported in Section 3.1.1.1)

	Order	Family	Genus/species
Susceptible	Artiodactyla	Suidae	*Sus scrofa*
		Bovidae	Not specified
		Cervidae	*Rangifer tarandus*
		Tayassuidae	Peccaries (all genera)
	Perissodactyla	Equidae	*Equus caballus*
	Carnivora	Canidae	*Vulpes lagopus*, *Canis lupus*
	Rodentia	Not specified	
	Lagomorpha	Leporidae	*Lepus europaeus*
Reservoir	Artiodactyla	Suidae	*Sus scrofa* (biovars 1, 2 and 3)
		Tayassuidae	Peccaries (all genera) (biovars 1 and 3)
		Cervidae	*Rangifer tarandus* (biovar 4)
	Rodentia	Small rodents (several species) (biovars 2 and 5)	
Vectors	None		

[9] A vector is a living organism that transmits an infectious agent from an infected animal to a human or another animal. Vectors are frequently arthropods. Biological vectors may carry pathogens that can multiply within their bodies and be delivered to new hosts, usually by biting. In mechanical vectors the pathogens do not multiply within the vector, which usually remains infected for shorter time than in biological vectors.

4. Conclusions

TOR 1: *for each of those diseases an assessment, following the criteria laid down in Article 7 of the AHL, on its eligibility of being listed for Union intervention as laid down in Article 5(3) of the AHL;*

- According to the assessment here performed, the infection with *B. abortus*, *B. melitensis* and *B. suis* complies with all criteria of the first set and with three criteria of the second set and therefore can be considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL.

TOR 2a: *for each of the diseases which was found eligible to be listed for Union intervention, an assessment of its compliance with each of the criteria in Annex IV to the AHL for the purpose of categorisation of diseases in accordance with Article 9 of the AHL;*

- According to the assessment here performed, the infection with *B. abortus*, *B. melitensis* and *B. suis* meets the criteria as in sections 2, 3, 4, and 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in points (b), (c), (d), and (e) of Article 9(1) of the AHL.

TOR 2b: *for each of the diseases which was found eligible to be listed for Union intervention, a list of animal species that should be considered candidates for listing in accordance with Article 8 of the AHL.*

- According to the assessment here performed, the animal species that can be considered to be listed for the infection with *B. abortus*, *B. melitensis* and *B. suis* according to Article 8(3) of the AHL are several mammal species, as reported in Tables 18 and 19 in Section 3.4 of the present document.

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Abbreviations

AHAW	EFSA Panel on Animal Health and Welfare
AHL	Animal Health Law
BBAT	Buffered Brucella antigen test
BPAT	buffered plate agglutination test
BST	Brucellin skin-test
CDC	Centers for Disease Control and Prevention
CFSPH	Center for Food Security and Public Health
CFR	Code of Federal Regulations of the US
CFT	complement fixation test
CFU	colony forming unit
CITES	the Convention on International Trade in Endangered Species of Wild Fauna and Flora
DALY	Disability-adjusted life year
DSe	diagnostic sensitivity
DSp	diagnostic specificity
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FPA	fluorescence polarisation assay
FPSR	false positive serological reactions
ICBA	Individual and Collective Behavioural Aggregation
IUCN	International Union for Conservation of Nature
HHS	Department of Health and Human Services
MRT	milk ring test
MS	Member State
NH	native hapten
OBF	Officially Brucellosis-Free
ObmF	Officially *Brucella melitensis*-free
OIE	World Health Organization for Animal Health
PCR	polymerase chain reaction
RBT	Rose Bengal test
R_0	basic reproductive ratio
R_e	effective reproductive ratio
SAT	standard tube agglutination test
ToR	Terms of Reference
TRACES	Trade Control and Expert System
WHO	World Health Organization
YLD	years lost due to disability
YLL	years of life lost due to premature mortality
USDA APHIS	US Department of Agriculture's Animal and Plant Health Inspection Service

Appendix A – Tables

Table A.1: Diagnostic sensitivity (DSe) and specificity (DSp) of serological tests for the diagnosis of *B. melitensis* infection in sheep (n.d.: not determined)

Tests	% DSe (no. tested) [95% CI] with sera from		%DSp (no. tested) [95% CI] with sera from		
	Brucella infected	*Brucella* free	% DSp (no. animals/age in months at /months post-Rev 1 vaccination) [95% CI]		Reference
			Conjunctival route	Subcutaneous route	
stRBT	85.9 (135) [80.0–91.8]	100 (416) [98.9–100]	n.d.	n.d.	Blasco et al. (1994a)
	100 (77) [94.1–100]	100 (77) [94.1–100]	90.9 (11/3/4) [57.1–99.5]	0.0 (11/3/4) [0–32.1]	Jiménez de Bagüés et al. (1992)
			90.0 (10/13/4) [54.1–99.5]	10.0 (10/13/4) [5.2–45.9]	
mRBT	88.1 (135) [82.7–93.6]	100 (416) [98.9–100]	n.d.	n.d.	Blasco et al. (1994a)
	94.0 (135) [90.0–98.0]				
	100 (55) [91.9–100]	100 (60) [92.5–100]	81.8 (11/3/4) [47.8–96.8]	38.9 (18/3/6) [18.3–63.9]	Marín et al. (1999)
			66.0 (56/14/5) [52.1–77.8]	14.9 (47/14/5) [6.7–28.9]	
BPAT	77.5 (71) [65.7–86.2]	99.8 (1,286) [99.3–99.9]	n.d.	n.d.	Nielsen and Gall (2001)
CFT	92 (55) [81.6–97.6]	100 (60) [92.5–100]	100.0 (11/3/4) [67.9–100]	77.8 (18/3/6) [51.9–92.6]	Marín et al. (1999)
			69.6 (56/14/5) [55.7–80.8]	44.7 (47/14/5) [30.5–59.8]	
	83.1 (71) [71.9–90.6]	99.5 (1,286) [98.9–99.8]	n.d.	n.d.	Nielsen and Gall (2001)
	100 (77) [94.1–100]	100 (77) [94.1–100]	100 (11/3/4) [67.9–100]	0.0 (11/3/4) [0–32.1]	Jiménez de Bagüés et al. (1992)
			60.0 (10/13/4) [27.4–86.3]	60.0 (10/13/4) [27.4–86.3]	
iELISA -S/LPS	100 (140) [96.7–100]	100 (100) [95.4–100]	n.d.	n.d.	Blasco et al. (1994b)
	100 (55) [91.9–100]	100 (60) [92.5–100]	72.7 (11/3/4) [39.3–92.7]	11.1 (18/3/6) [1.9–36.1]	Marín et al. (1999)
			32.1 (56/14/5) [20.6–46.1]	10.6 (47/14/5) [4.0–23.9]	
	100 (77) [94.1–100]	100 (77) [94.1–100]	100.0 (11/3/4) [67.9–100]	54.6 (11/3/4) [24.6–81.9]	Jiménez de Bagüés et al. (1992)
			90.0 (10/13/4) [54.1–99.5]	80.0 (10/13/4) [44.2–96.4]	
	93.0 (71) [83.7–97.4]	97.6 (1,286) [96.6–98.3]			Nielsen and Gall (2001)

Tests	% DSe (no. tested) [95% CI] with sera from		%DSp (no. tested) [95% CI] with sera from		
	***Brucella* infected**	***Brucella* free**	**% DSp (no. animals/age in months at /months post-Rev 1 vaccination) [95% CI]**		**Reference**
			Conjunctival route	**Subcutaneous route**	
cELISA-S/LPS	96 (55) [86.4–99.4]	100 (60) [92.5–100]	89.5 (11/3/4) [57.1–99.5]	88.9 (18/3/6) [63.9–98.1]	Marín et al. (1999)
			89.3 (56/14/5) [77.4–95.6]	87.2 (47/14/5) [73.6–94.7]	
	76.1 (71) [64.2–85.1]	99.7 (1,286) [99.1–99.9]	n.d.	n.d.	Nielsen and Gall (2001)
FPA	91.5 (71) [81.9–96.5]	98.6 (1,286) [97.8–99.1]	n.d.	n.d.	Nielsen and Gall (2001)
GD-NH	90 (55) [77.4–95.6]	100 (60) [92.5–100]	100.0 (11/3/4) [67.9–100]	100.0 (18/3/6) [78.1–100]	Marín et al. (1999)
			96.4 (56/14/5) [86.6–99.4]	85.1 (47/14/5) [71.1–93.3]	
RID-NH	96.1 (77) [88.3–99.0]	100 (77) [94.1–100]	100 (11/3/4) [67.9–100]	81.8 (11/3/4) [47.8–96.8]	Jiménez de Bagüés et al. (1992)
			90.0 (10/13/4) [54.1–99.5]	80.0 (10/13/4) [44.2–96.4]	

Table A.2: Diagnostic sensitivity (DSe) and specificity (DSp) of serological tests for the diagnosis of brucellosis in cattle (n.d.: not determined)

Tests	% DSe (no. tested) [95% CI] with sera from	%DSp (no. tested) [95% CI] with sera from			
	***Brucella* infected**	***Brucella* free**	**S19 vaccinated (no. calves/ months after vaccination) [95% CI]**	**Reference**	**Affected by FPSR (data from Muñoz et al. 2004)**
stRBT	100 (112) [95.9–100]	100 (95) [95.2–100]	n.d.	Díaz-Aparicio et al. (1993)	n.d.
BPAT	97.8 (636) [96.2–98.7]	98.7 (1,446) [97.9–99.2]	48.7 (261/n.d.) [42.5–54.9]	Nielsen et al. (1995)	n.d.
	n.d.	n.d	50 (56/4) [36.5–63.5]	Lord and Cherwonogrodzky (1992)	n.d.
SAT	81.5 (146) [74.1–87.3]	98.9 (995) [98.0–99.4]	n.d.	McGiven et al. (2003)	n.d.
	n.d.	n.d.	81 (56/4) [67.2–89.3]	Lord and Cherwonogrodzky (1992)	n.d.

Tests	% DSe (no. tested) [95% CI] with sera from	%DSp (no. tested) [95% CI] with sera from			
	***Brucella* infected**	***Brucella* free**	**S19 vaccinated (no. calves/ months after vaccination) [95% CI]**	**Reference**	**Affected by FPSR (data from Muñoz et al. 2004)**
CFT	91.8 (146) [85.8–95.5]	99.9 (995) [99.3–100]	n.d.	McGiven et al. (2003)	n.d.
	97.6 (636) [96.0–98.6]	99.9 (1,446) [99.6–100]	n.d.	Nielsen et al. (1995)	n.d.
	94.5 (147) [89.2–97.4]	100 (640) [99.3–100]	n.d.	Stack et al. (1999)	n.d.
	n.d.	n.d	49 (56/4) [34.8–61.8]	Lord and Cherwonogrodzky (1992)	n.d.
	100 (112) [95.9–100]	100 (95) [95.2–100]	48 (40/2 [31.8–63.7]	Díaz-Aparicio et al. (1993)	n.d.
iELISA -NH	100 (75) [93.9–100]	97.7 (130) [92.9–99.4]	85.2 (61/3) [73.3–92.6]	Abalos et al. (2000)	n.d.
iELISA -S/LPS	97.2 (146) [92.7–99.1]	97.8 (6,957) [97.4–98.1]	n.d.	McGiven et al. (2003)	n.d.
	100 (424) [99.0–100]	99.7 (15,715) [99.6–99.8]	56.3 (261/n.d.) [50.1–62.4]	Nielsen et al. (1995)	n.d.
	100 (98–100 [189])	100 (96.7–100 [112])	n.d.	Muñoz et al. (2004)	58.4 (49.2–67.1 [125])
cELISA-S/LPS	95.2 (146) [90.0–97.9]	99.7 (1,440) [99.2–99.9]	n.d.	McGiven et al. (2003)	n.d.
	100 (636) [99.3–100]	99.7 (1,446) [99.2–99.9]	100 (261/n.d.) [98.2–100]	Nielsen et al. (1995)	n.d.
	84.4 (78.3–89.4 [180])	100 (95.9–100 [90])	n.d.	Muñoz et al. (2004)	88.8 (81.9.3–93.7 [125])
FPA	96.6 (146) [91.8–98.7]	99.1 (1,947) [98.5–99.4)	n.d.	McGiven et al. (2003)	n.d.
	99.3 (1,084) [98.5–99.7]	100 (23,755) [99.98–100]	n.d.	Nielsen and Gall (2001)	n.d.
	n.d.	n.d	99.2 (248/n.d.) [96.8–99.9]	Nielsen et al. (1998)	n.d.
GD-S/LPS	n.d.	n.d.	81 (56/4) [67.2–89.3]	Lord and Cherwonogrodzky (1992)	n.d.
GD-NH	n.d.	n.d.	100 (432/6) [98.9–100]	Lord et al. (1989)	n.d.
RID-NH	92.0 (112) [84.9–96.0]	100 (95) [95.2–100]	100 (40/2) [89.1–100]	Díaz-Aparicio et al. (1993)	100 (97.1–100 [125])

Table A.3: Diagnostic performance of serological tests for the diagnosis of *B. melitensis* infection in goats (n.d.: not determined)

Reference	Test	%DSe[(1)]	% DSp (no./age in months/months after Rev 1 vaccination) [95% CI]		
			Brucella free[(1)]	Conjunctival	Subcutaneous
Stournara et al. (2007)	RBT	90.3	99.6	100 (48/≤ 3/4) [90.8–100]	n.d.
				64 (106/≥ 24/4) [54.2–73.1]	n.d.
	mRBT	97.5	98.1	100 (48/≤ 3/4) [90.8–100]	n.d.
				47 (106/≥ 24/4) [37.5–57.0]	n.d.
	CFT	98.7	100	100 (48/≤ 3/4) [90.8–100]	n.d.
				86.0 (106/≥ 24/4) [77.4–91.6]	n.d.
	iELISA	97.5	100	100 (48/≤ 3/4) [90.8–100]	n.d.
				50.0 (106/≥ 24/4) [40.2–59.8]	n.d.
	cELISA	96.3	99.4	100 (48/≤ 3/4) [90.8–100]	n.d.
				90 (106/≥ 24/4) [81.8–94.5]	n.d.
	FPA	97.5	98.9	98 (48/≤ 3/4) [87.5–99.9]	n.d.
				81 (106/≥ 24/4) [72.1–87.8]	n.d.
Salih-Alj Debbarh et al. (1996)	iELISA CP28	89	100	100(10/3/1) [65.5–100]	n.d.
Díaz-Aparicio et al. (1994)	RID-NH	93.4	100	100 (11/3/4) [67.9–100]	n.d.
				90 (10/adults/4) [54.1–99.5]	10/adults/4) [44.2–96.5]

(1): Taken from the corresponding study.