Brucellosis

Control Tools

Diagnostics availability

Commercial diagnostic kits available worldwide

Many commercial diagnostic kits are available worldwide (e.g. IDEX, Svanova, Pourquier, ID-Vet, Synbiotics, Prionics, VLA, INGENASA). These include iELISA, cELISA and FPA which all detect antibodies to S-LPS components of Brucella. In addition many diagnostic antigens are also commercially available worldwide such as Rose Bengal, complement fixation and serum agglutination antigens. Other assays are coming onto the market such as the Brucellacapt agglutination assay and lateral flow assays. Protein preparations for use in skin tests and in-vitro IFNg assays are available commercially but only through one company and supply is limited and vulnerable.

Reagents for the cultural identification and typing of Brucella are also available commercially and from OIE laboratories. These include phages and anti-sera. These are on a test/test basis more expensive than the serological reagents. There are commercially available PCR kits for typing isolates at species level.

GAPS:
- More effective selective enrichment and culture media are required. Conventional typing is difficult and poses reproducibility problems. Classical methods could be advantageously replaced by molecular methods.
- Methods for DNA detection on animal samples should be investigated.
- Although costs of tests are generally competitive, they are out of reach for many areas in Africa or Asia.
- Almost all kits require cold storage to maintain effectiveness. This may be a problem in some resource poorer regions.
- There are no commercially available PCR kits that claim to diagnose brucellosis.

Commercial diagnostic kits available in Europe

Most if not all of the kits available globally are also available in Europe (certainly those that are most well known in the brucellosis community are available).

Diagnostic kits validated by International, European or National Standards

Many of the kits available have been tested against International standards. Indeed in some cases, such as the development of the (Brucella abortus) OIE ELISA Standard Sera, the standards were set on the basis of the results obtained from commercially available diagnostic kits with good validation data and provenance.

Diagnostic method(s) described by International, European or National standards

These are well described in the last Edition of the OIE Manual for Diagnostic Tests and Vaccines: CFT, iELISA, cELISA and FPA are the currently prescribed tests for international trade in cattle. RBT, CFT, FPA and brucellin skin tests are the prescribed tests for international trade in small ruminants (B. melitensis infection). Only the CFT is the prescribed tests for B. ovis in sheep. RBT, iELISA, cELISA and FPA are the prescribed tests for B. suis in pigs.

GAPS:
- Updated regularly but criteria for inclusion-exclusion are not clear and properly documented. Probably lobbies and commercial interests, rather than intrinsically technical features of tests, play a major role.
- Some of the methodological descriptions are open to a variety of interpretations. Whilst this is advantageous in some respects, it can also lead to some drift in techniques between laboratories.

Commercial potential for diagnostic kits in Europe
Very high since eradication-surveillance is compulsory in the EU in most animal species. The market for standard commercial kits that meet the usual requirements (i.e. as already available) is fairly full. There may be an opportunity for niche kits to be developed to meet specific needs such as addressing false positives that arise from standard methods. However, such a market would be relatively small and research problems would need to be overcome before developing such a kit.

**GAP:** Possibly some space in the market for niche assays based on non-OPS antigens.

### DIVA tests required and/or available

A suitable and effective DIVA test would be of help in completing B. abortus eradication in countries that need vaccination (in the EU or elsewhere). The cELISA kit sold by Svanova markets itself as being able to differentiate between infected and vaccinated animals but this is not true in many cases and there is also information to indicate that this assay lacks sensitivity. A DIVA test would be more necessary for eradicating B. melitensis, since most infected countries need vaccination. Enabling a combined vaccination and test and slaughter programme would undoubtedly enhance the efficiency of disease eradication. Rev 1 vaccine (or other new vaccine to be developed) and an associated DIVA would be of interest in the case of B. ovis in the EU, which is an increasing problem in countries - regions in which B. melitensis has been eradicated and Rev 1 vaccine abandoned.

**GAPS:**
- Scarce information on the performance of serological tests in swine.
- Lack or only limited information on performance of tests in camelids, yaks, water buffaloes and wildlife.

### Opportunities for new developments

Good. Classical vaccines adequately tagged, new vaccine to be developed, both associated with an adequate DIVA tests; a B. ovis vaccine; a human vaccine; improved therapies for human brucellosis.

**GAPS:**
- More effective selective enrichment and culture media are required. Conventional typing is difficult and poses reproducibility problems. Classical methods could be advantageously replaced by molecular methods.
- Methods for DNA detection on animal samples should be investigated.
- Scarce information on the performance of serological tests in swine.
- Lack or only limited information on performance of tests in camelids, yaks, water buffaloes and wildlife.

### Vaccines availability

#### Commercial vaccines availability (globally)

Live attenuated vaccines S19, Rev 1 and RB51 and are the only vaccines recognised for use by the OIE. S19 and Rev vaccines can be produced without commercial infringement but should be extensively tested for efficacy and safety by recognised protocols before use. Whereas Rev 1 and RB51 are available globally, marketing of S19 has been discontinued in some countries.

**GAP:** No S19 for conjunctival route has been ever produced and marketed internationally.

#### Commercial vaccines authorised in Europe

Rev 1 (small ruminants) and S19 and RB51 (cattle) are authorised in the EU.

**GAP:** No B. ovis specific vaccine available in the EU (or elsewhere).

#### Marker vaccines available worldwide

None. Rev 1 vaccine and S19 have been deleted in a diagnostic marker (i.e. protein BP26) but associated DIVA tests are not sensitive enough. Since it interferes in iELISA, cELISA tests, R vaccines cannot be considered as marked vaccines.

**GAPS:** There is a critical need for new vaccines that are:
- more protective.
- able to generate immune responses easily differentiable from those of infected animals (DIVA assays required).
- less pathogenic for livestock (not abortifacient, etc.).
Marker vaccines authorised in Europe

None. Rev 1 vaccine and S19 have been deleted in a diagnostic marker (i.e., protein BP26) but associated DIVA tests are not sensitive enough. Since it interferes in iELISA, cELISA tests, R vaccines cannot be considered as marked vaccines.

Effectiveness of vaccines / Main shortcomings of current vaccines

Small ruminant vaccines:

B. melitensis Rev 1 induces strong immunity in sheep (against both B. melitensis and B. ovis infections) and goats (B. melitensis) and is safe enough when applied to young replacement animals but induces a serological response that interferes in serological tests, more markedly when applied by the subcutaneous route and to adult animals. Vaccination during pregnancy results in high numbers of abortions and vaccine excretion in milk. This is a serious inconvenient for applying mass vaccination campaigns, frequently the only suitable alternative in developing countries. Conjunctival vaccination of young (3-4 month old) animals minimise the diagnostic interferences and abortion and is the method of choice. Rev 1 is safe enough also in young rams or billy goats. Quality control is strictly necessary (Rev 1 shows instability). It is virulent in humans and is streptomycin resistant. R vaccines against B. melitensis in sheep have been investigated to solve the problem of the serological interference. None is as efficacious as Rev 1 and, although they do not interfere in RBT or CF, they elicit anti-core-oligosaccharide antibodies in (at least) iELISA and interfere in B. ovis serodiagnostic tests.

Cattle vaccines:

B. abortus S19. Is the best effective vaccine in cattle against B. abortus infection but provides no absolute protection (possibly less than Rev1 in sheep). It is also effective against B. melitensis infection in cattle, a relative frequent event in developing countries. The vaccine is safe enough when applied to young replacement females but not in males. Vaccination interferes with the diagnosis and complicates eradication. Conjunctival vaccination of young (3-4 month old) animals minimise diagnostic interferences and is the method of choice. Proven effective in many countries having eradicated B. abortus infection in cattle. Quality control is strictly necessary since S19 shows some instability. Not safe enough in bulls. Infectious for humans but less than Rev 1. Significantly safer than Rev 1 when used in adult cattle, including pregnant and lactating animals, but safety is not absolute. B. abortus RB51. R LPS mutant obtained to avoid the interference of vaccination in serological tests but, although it does not interfere in RBT or CF, it elicits anti-core-oligosaccharide antibodies in (at least) iELISA, cELISA and LFA. Considerably more expensive than S19. Lower protection than S19 against B. abortus in cattle. Ineffective in sheep, pigs and wildlife tested. Efficacy against B. melitensis in cattle unknown. Induces abortion and milk excretion when used in pregnant cattle. Not safe in bulls. It is virulent in humans and is rifampin resistant. Never proven more effective than S19 for eradicating B. abortus infection in cattle in any country.

Other animal vaccines:

B. abortus 45/20 and B. melitensis H38 (others) killed vaccines abandoned. B. suis Strain 2 (a biovar 1 strain attenuated in guinea pigs) has been used in China. In controlled experiments, S2 was inefficacious against B. melitensis or B. ovis. Information on alternative vaccines used in the former Soviet Union difficult to contrast. Many attempts to develop a B. ovis subcellular vaccine based on outer membrane components in different formulations (adjuvants, encapsulation, DNA, etc).

Human vaccine:

Several unsuccessful/doubtful/obscure attempts, including attenuated, subcellular or DNA based vaccines.

GAPS: There is a critical need for new vaccines that are:

- more protective.
- able to generate immune responses easily differentiable from those of infected animals (DIVA assays required).
- less pathogenic for livestock (not abortifacient, etc.).
- attenuated in humans.
- more stable.
- affordable.

Commercial potential for vaccines in Europe

In most EU countries B. abortus has been eradicated and no vaccines are required. However, the S19 for conjunctival use (associated or not with potential DIVA tests) would be of great help (see 16.1) and interest in completing B. abortus eradication in PT, IT, ES and GR, since some regions in these countries need adequate vaccination programs.

New vaccines associated or not with DIVA tests would be necessary for eradicating B. melitensis, since most infected countries (P, IT, GR, ES) need vaccination. Rev 1 vaccine (or other new vaccines to be developed) and an associated DIVA would be of interest also in
the case of B. ovis infection, an increasing problem in regions in which B. melitensis has been eradicated and Rev 1 vaccine forbidden. Vaccines against B. suis are not required for industrial indoor breeding systems, but should be of great interest for outdoor breeding systems (at least in P and ES).

**GAP:** No possibilities for vaccines that do not solve the DIVA problem. New vaccines would be necessary to open the market.

### Regulatory and/or policy challenges to approval

It is estimated that a good vaccine would not face any unusual regulatory barriers.

### Commercial feasibility (e.g manufacturing)

Significantly improved vaccines would be very commercially feasible. For attenuated live vaccines, technology & experience gained in Rev 1 and S 19 production in the EU. After the production of the Master Seed Lot by the manufacturer a thorough control in vitro and in vivo of this pivotal biological starting material must be performed by a Reference Laboratory. After satisfactory control, the Master Seed Lot can be used by the manufacturer for the production of Brucella vaccines. However some basic processes must be respected.

1. The seedlot system must be respected in order to have a robust and validated technology to avoid qualitative difference from batch to batch.
2. These vaccines have to be produced in Category 3 confined area with adequate equipments as fermentor, centrifuge and negative pressure freeze - drier.
3. Beside the technology of production, rigour in process and batch release controls must be applied. This last test should be performed internally by the manufacturer and externally by Reference Laboratory. The question is how many batches have to be controlled in parallel by the manufacturer and the reference laboratory (10 first consecutive Industrial batches?).

### Opportunity for barrier protection

### Opportunity for new developments

Vaccines that are more protective, able to generate immune responses easily differentiable from those of infected animals (DIVA assays required), less pathogenic for livestock (not abortifacient, etc.), attenuated in humans, more stable and affordable, would have a good opportunity. These include tagged classical vaccines or new attenuated vaccines subunit vaccines (plus improved adjuvants and delivery systems), and DNA vaccines, all associated with DIVA system.

A specific need is a B. ovis vaccine; a B. suis vaccine may be useful under some circumstances in EU.

Some reformulations of classical vaccines (i.e. S19 conjunctival) may have a chance as well.

**GAPS:**

**Available resources**

- General constraints: availability of research teams with complementary skills (optimally, teams from public institutions in collaboration with Industry).
- Funding.

**Better understanding of host immunity-pathogen interactions and the pathology of the agent**

- Imperfect or no knowledge on (a), Brucella virulence mechanisms and genetic regulators for adapting to intracellular life; (b), interaction with immunity; (c), underlying mechanisms for host preference/specificity; (d), the pathology of the agent within each natural host other than humans, ruminants or swine (camelids, yaks, water buffaloes, etc.). Identifying these will help to understand the virulence mechanisms which in turn could help to generate improved treatments and vaccines.
- Others for specific approaches: tag immunogenic enough to be used in /DIVA test; identification of immunogenic/protection oligonucleotidic sequences (DNA vaccines) may be long and not necessarily successful.

**Legislation**

- Genetically Modified Organism legislation in Europe.

### Pharmaceutical availability

**Current therapy (curative and preventive)**
Seldom used in animals. However, B. suis infection in pigs could be treated with antibiotics when the infection affects large industrial premises since depopulation is unfeasible.

In humans, adults with acute brucellosis and no complications or focal disease should be treated with doxycycline-streptomycin or doxycycline-gentamicin combinations. In focal forms, the preferred regimen is the same but duration of therapy must be individualized. Surgery should be considered for patients with endocarditis, cerebral, epidural, spleen, hepatic or other abscesses not resolving with antibiotic therapy. During pregnancy tetracyclines and streptomycin must be avoided and a rifampin monotherapy is considered the regimen of choice. Trimethoprim-sulfamethoxazole (cotrimoxazole) plus rifampin is an alternative regimen but it is contraindicated before week 13 or after week 36 of pregnancy. Children less than 8 years old can be treated with rifampin-cotrimoxazole, or rifampin or cotrimoxazole plus gentamicin. Antibiotics have to be administered for long (usually 6 weeks but sometimes longer) times. Treatment is expensive and may pose compliance problems. Relapses occur in 5 to 30% of patients. Rifampin must be avoided in countries where tuberculosis is endemic.

**GAP: Human brucellosis**

More efficacious/cheaper antibiotics would be valued that could:

- avoid parental administration
- shorten the administration period
- avoid relapses.
- make treatment affordable.

**Future therapy**

Seldom used in animals. However, B. suis infection in pigs could be treated with antibiotics when the infection affects large industrial premises since depopulation is unfeasible.

In humans, adults with acute brucellosis and no complications or focal disease should be treated with doxycycline-streptomycin or doxycycline-gentamicin combinations. In focal forms, the preferred regimen is the same but duration of therapy must be individualized. Surgery should be considered for patients with endocarditis, cerebral, epidural, spleen, hepatic or other abscesses not resolving with antibiotic therapy. During pregnancy tetracyclines and streptomycin must be avoided and a rifampin monotherapy is considered the regimen of choice. Trimethoprim-sulfamethoxazole (cotrimoxazole) plus rifampin is an alternative regimen but it is contraindicated before week 13 or after week 36 of pregnancy. Children less than 8 years old can be treated with rifampin-cotrimoxazole, or rifampin or cotrimoxazole plus gentamicin. Antibiotics have to be administered for long (usually 6 weeks but sometimes longer) times. Treatment is expensive and may pose compliance problems. Relapses occur in 5 to 30% of patients. Rifampin must be avoided in countries where tuberculosis is endemic.

**GAP: Human brucellosis**

More efficacious/cheaper antibiotics would be valued that could:

- avoid parental administration
- shorten the administration period
- avoid relapses.
- make treatment affordable.

**Commercial potential for pharmaceuticals in Europe**

Reduced since brucellosis is in the process of being eradicated.

**Regulatory and/or policy challenges to approval**

**Commercial feasibility (e.g manufacturing)**

**Opportunities for new developments**

Human brucellosis: more efficacious/cheaper antibiotics would be valued that could:

- avoid parental administration
- shorten the administration period
- avoid relapses.
- make treatment affordable.
New developments for diagnostic tests

Requirements for diagnostics development

All serological tests need validation (cut-off optimization and sensitivity and specificity studies) according to local conditions (prevalence, breed, etc.) and specific animal host (both for domestic animals and wild life).

**GAP:** No validation studies (adequate to the particular country/conditions) for most commercial kits and animal species.

Time to develop new or improved diagnostics

Variable depending upon the animal species and resources. Optimally, gold standard serum collections should be based on bacteriological studies and these may be difficult in wild life.

Cost of developing new or improved diagnostics and their validation

Difficult to estimate.

Research requirements for new or improved diagnostics

More effective selective enrichment and culture media are required. Conventional typing is difficult and poses reproducibility problems. Classical methods could be advantageously replaced by molecular methods.

Methods for DNA detection on animal samples should be investigated.

Technology to determine virus freedom in animals

Vaccination is a more cost-effective policy than test and slaughter but as vaccination on its own is unlikely to eradicate disease, test and slaughter (with the associated compensation costs) is the only way to certify and maintain freedom from brucellosis.

New developments for vaccines

Requirements for vaccines development / main characteristics for improved vaccines

There is a critical need for new vaccines that are more protective, able to generate immune responses easily differentiable from those of infected animals (DIVA assays required), less pathogenic for livestock (not abortifacient, etc.), attenuated in humans, more stable and affordable.

Need for proper information on the use of Rev 1 and S19 in species other than small ruminants and cattle, respectively. Tests for specific protection and safety against infections by S brucellae in swine, camels, yaks, water buffaloes and others are needed. Need for specific B. ovis attenuated vaccine available when Rev 1 use is discontinued after eradication of B. melitensis. Need for B. canis vaccine.

Classical vaccines adequately tagged, new vaccine to be developed, both associated with an adequate DIVA tests; a B. ovis vaccine; a human vaccine; improved therapies for human brucellosis.

Time to develop new or improved vaccines

From the concept to the industrialisation and to EU marketing authorisation. It will take 5 to 10 years depending on whether improvement or development of completely new vaccines is considered. To develop a new vaccine (tagged, sub-unit, live modified) will take more time than the improvement of the existing Rev 1 and S19 vaccines. If the Master Seed Batch is changed, however, the registration of such an “improved” vaccine will have to go through all the development steps as for a new vaccine, and to build a registration dossier with new parts 2 (analytical), 3 (safety) and 4 (efficacy), parts. More attention if the vaccine is considered as Genetically Modified Organism.
Main steps for development of new vaccines:

1. Building project with relevant tasks. Bottle neck: Human resources and budget (see 15.7.).
2. Feasibility steps. Validation of Master Seed Batch / Working Seed Batch.
3. Labscale technology +analytical tools IPC and potency release test.
4. In this phase challenge trials in target animals must be performed.
5. Research to set the Minimal Protective Dose; production of the Pilot Batches
6. Industrialisation of the technology; field trials.

GAPS:

Available resources:
- Decisions concerning project management and adequate repartition of tasks between Public.
- Institutions/Laboratories and Industry.
- Category 3 facilities.

Legislation: Genetically Modified Organism legislation in Europe

Cost of developing new or improved vaccines and their validation

Cost will depend on the decision if it will be improvement or new development of vaccines with corresponding analytical and DIVA associated tests. Another factor which will dramatically increase the cost is if the development of new vaccine will require confined category 3 facilities (Laboratory, Industrial and challenge on target animals).

GAP: Category 3 facilities (Laboratory, Industrial and challenge on animals).

Research requirements for new or improved vaccines

Better understanding of host immunity-pathogen interactions and the pathology of the agent:

Imperfect or no knowledge on (a), Brucella virulence mechanisms and genetic regulators for adapting to intracellular life; (b), interaction with immunity; (c), underlying mechanisms for host preference/specificity; (d), the pathology of the agent within each natural host other than humans, ruminants or swine (camelids, yaks, water buffaloes, etc.). Identifying these will help to understand the virulence mechanisms which in turn could help to generate improved treatments and vaccines.

Classical vaccines adequately tagged, new vaccine to be developed, both associated with an adequate DIVA tests; a B. ovis vaccine; a human vaccine; improved therapies for human brucellosis.

New developments for pharmaceuticals

Requirements for pharmaceuticals development

Time to develop new or improved pharmaceuticals

Cost of developing new or improved pharmaceuticals and their validation

Research requirements for new or improved pharmaceuticals

Disease details

Description and characteristics.
The genus Brucella includes several recognized species: B. melitensis, B. suis, B. abortus, B. neotomae, B. canis, and B. ovis. Some are subdivided into biovars. In addition, isolates from marine mammals of the Atlantic have been grouped into two species: B. pinnipedialis and B. ceti; strains isolated from the common vole are proposed to belong to a new species: B. microti. Whereas B. melitensis, B. abortus, B. canis, and B. ovis have well-defined characteristics, B. suis shows a great internal diversity which could encompass the B. microti strains. Few isolates of B. neotomae have been studied, and the studies of the strains from marine mammals need to be complemented with additional strains from the Pacific Ocean. New Brucella strains that do not fit within the classical species have been described more recently.

**GAPS:** Taxonomy
- The internal taxonomy of the genus needs revision.
- It cannot be assumed that isolates from geographical areas other than Europe, the near East, and N. and S. America fall within the previously described species and biovars.

### Variability of the disease

The severity of brucellosis varies according to the host and the infective species and strain. In livestock and humans, the geographical range of the disease is well known but in wildlife, although there is some knowledge, there is much less information. The brucellae are highly clonal and stable in the hosts. However, two types can be distinguished naturally: rough (R) and smooth (S). R brucellae (B. canis and B. ovis) show a narrower host range (sheep and dogs) than the remaining Brucella species that all are S. The molecules responsible for these phenotypes are the cell envelope lipopolysaccharides. In S species, these molecules carry an O-polysaccharide not present in the R species. B. abortus preferentially infects cattle (and water buffalo); B. melitensis, sheep, and goats; B. ovis, sheep, and B. canis dogs. B. suis can be mainly isolated from swine (including wild swine), reindeer, hares, wild rodents, and some other wildlife species. Marine mammals harbour B. pinnipedialis and B. ceti. B. microti from common voles has been proposed as a new emerging pathogen, but clusters genetically with B. suis biovar 5. B. abortus, B. melitensis, and some B. suis biovars can infect animals other than their primary hosts, including wildlife, depending upon the epidemiological circumstances.

**GAPS:**
- Brucellosis in camellids, yaks, water buffaloes and other "exotic" animals.
- Swine brucellosis: epidemiology (interactions with wildlife), diagnosis, vaccines.
- Brucellosis in wildlife: epidemiology: role as reservoirs/carriers; diagnosis.

### Stability of the agent/pathogen in the environment

The brucellae do not multiply outside the hosts but may persist in the environment, mostly associated to animal products. Adverse environmental factors are high temperature, acid pH, dryness and exposure to sunlight. In temperate climates, particularly in winter, B. abortus may survive for several months in abortions, placenta, and tissues; in exudates and abortion discharges for less than a month; in liquid manure in fresh conditions for at least 8 months; in dairy products (milk, butter, cheese, cream, and ice cream), and depending upon pH and refrigeration, from one week to 4-5 months. In refrigerated organs, for at least 2 months; in water for up to 2 months. The data available suggest similar persistence for B. melitensis or B. suis.

**GAPS:**
- Information incomplete concerning non-pasteurized dairy products, particularly those obtained by traditional procedures (souring, etc.).
- Stability of Brucella in seawater mostly unknown (some studies have been performed).

### Species involved

#### Animal infected/carrier/disease

The brucellae infect a wide range of animals, especially the smooth strains, and the known range is getting wider all the time as the organism is looked for in more possible host species.

Cattle, yaks, water buffaloes, sheep, goats, reindeer, camellids, swine, horses, reindeer, hares, seals (pinnipeds), dolphins and porpoises (and other toothed whales), and dogs are susceptible. Poultry may be artificially infected but the disease is of little importance. But for hares and wild boars, these seem to be of little practical importance as reservoirs in Northern Europe, USA, Canada, and Mediterranean countries.

Any animal that has the disease poses a potential risk of spread to others.

Some animals (ruminants) are latently infected where the organism is present but at sub-clinical and sub-detectable levels. Whereas abortion is a common result of first pregnancies, this is not so in the second, third, etc. Calves born to these infected mothers can
acquire brucellosis without showing any symptom of the disease or being positive in diagnostic tests, but they usually abort during the first pregnancy and transmit the disease, thus acting as latent carriers. This has been shown experimentally, and there is much circumstantial evidence that latent infections are a source of re-introduction of brucellosis in flocks.

**GAPS:** Better understanding of host immunity-pathogen interactions and the pathology of the agent:

- A better understanding of latency and of detecting latently infected animals is important. The inability to screen out such animals during movement tests presents a risk to the disease free status of target destinations.
- The pathology of the agent within each host other than in humans and domesticated animals is not well known. Thus there is no evidence as to whether such animals should be considered diseased or carriers. Role of wild-life not well defined (spill-over? just carriers?) and possibly different in different breeding systems.

**Human infected/disease**

Human infection comes from direct or indirect contact with animals and animal products (no regular human to human transmission. B. melitensis, B. suis, and B. abortus cause human brucellosis. Indirect and fragmentary data show that B. melitensis and B. suis are more infectious and cause a more severe disease than B. abortus. Nevertheless, B. suis biovar 2 is not highly virulent in humans. B. canis is considerably less virulent. A few cases also show the virulence of the marine mammal strains for humans. No infections by B. ovis have been reported.

**GAP:** Data on the virulence of several B. suis biovars, B. neotomae, and B. microti for humans are fragmentary or do not exist.

**Vector cyclical/non-cyclical**

There are no true vectors. Conjunctival transmission by Musca domestica, Tabanus, spp., and Stomoxys calcitrans has been experimentally shown in cows, goats and sows. Blood-sucking insects and ticks that have fed on infected animals can harbour brucellae for a few days. There are reports on transovarial transmission in ticks. However, there is no evidence for a role of any of these arthropods in transmitting the disease.

**Reservoir (animal, environmental)**

**Domestic livestock:** The animal disease is endemic in many areas and this makes the establishment of free areas very difficult as all surrounding areas may still have infections. History is filled with prevalence reduction and disease eradication programmes which succeed in the short term but fail in the long term due to reintroduction of the disease from neighbouring infected areas or importation by animal trade. In such cases prevalence may rapidly reach or exceed previous levels.

**Wildlife:** The presence of the disease in wildlife is a large possible reservoir of infection. In Europe, the wild boar and hare populations contain B. suis infected animals. But for marine mammals, wild boar, hares and some wild rodents, brucellosis in most forms of wildlife seems to be a spill-over disease originated through contacts with domestic livestock. Wild boars, however, are a source of brucellosis for pigs bred extensively. In some areas of the US, bison (Bison bison) and elk (Cervus canadensis) are infected by B. abortus as a result of contacts with cattle and, after eradication of brucellosis in the latter, act as a potential reservoir.

**GAP:** Greater understanding of the wildlife reservoir.

**Description of infection & disease in natural hosts**

**Transmissibility**

Brucellosis by S. brucellae is highly transmissible, and spreads very rapidly in immunologically naive flocks. The main routes of transmission are well known. Infected ruminants and swine may shed brucellae via urine, but the aborted foetus, foetal membranes and fluids, genital discharges and milk are the most important sources of contagion. Semen produced during the acute early stages of infection is also a source of contagion, and artificial insemination spreads the disease more readily than natural insemination.

The disease is usually transmitted in humans and animals by ingestion of infective organisms. However ocular infection via the mucus membrane may occur and infection may take place via inhalation due to aerosols.

At least B. abortus, B. melitensis and some B. suis biovars can infect livestock other than their preferential hosts. Breeding systems using several animal species (transhumance, some forms of pastoralism) favour the interspecies transmission.

**GAPS:** Brucellosis in camelids, yaks, water buffaloes and other "exotic" animals:

- Description of infection & disease in natural hosts
- Epidemiology of brucellosis in mixed breeding systems (cattle, camels, sheep and goats together) and the risk of perpetuation of the infection by
Brucella species in hosts other than the preferential ones (for example, B. abortus in sheep).

Epidemiology in domestic animals: Definition of epidemiological units (should take into account the high possibility of cross-species infections) often inadequate.

Pathogenic life cycle stages

None.

Signs/Morbidity

Brucellosis lacks pathognomonic symptoms/signs. Abortion, birth of weak offspring infertility and genital lesions in males are the most common but not specific manifestations of brucellosis, and are not constantly present. Encephalitis is relatively frequent in infected marine mammals.

Incubation period

It seems to vary considerably (from weeks to months) depending upon several factors, including the infective dose and strain, the host species and individual susceptibility. In cattle and small ruminants, these include the immunologically status (previous exposures and vaccination), challenge size (conversely related) and physiological status, particularly the state of pregnancy (shorter incubations times as infection happens closer to mid pregnancy). This may vary according to many factors. In experimental infections in these animals, antibodies appear between 10-20 days but this may be different in natural infections.

Mortality

After infection with B. abortus/B. melitensis/B. suis there is low mortality of infected animals however there is a very high mortality of unborn foetuses especially during the first pregnancy when the animal has the disease. Thus, the rate of abortions varies between 0 to 40% in cattle, sheep, goats and swine, depending upon whether the disease has been recently introduced in a flock or the flock is chronically infected. Perinatal mortality is estimated between 0-20%. Adult mortality seems low or very low, although exact figures are difficult to find. In cattle, 1% of cows with abortions may die due to metritis and other secondary complications. (In many countries with a test and slaughter control program mortality may be considered high as all animals identified as infected are slaughtered and ‘at risk’ or ‘contact’ animals are also frequently culled.)

Shedding kinetic patterns

In cattle, small ruminants and swine shedding through vaginal fluids is very intense after abortion / parturition (see 2.1.) and wanes in several weeks in most cases. However, excretion in milk in these species is very frequent and may last for several years. A significant proportion of infected males may excrete brucellae in semen for several months/years. The main period of shedding of infective organisms is at birth or abortion when the material exuded is highly infections containing many infective doses. Infected females may also shed Brucella in milk although this appears to be intermittent. It has also been reported that a small but significant number of animals shed significantly higher levels of Brucella in their milk and may be responsible for most of an infected herds overall shedding via this route.

GAP: Better understanding of host immunity-pathogen interactions and the pathology of the agent:

- The shedding of Brucella in milk appears to be transient and variable between individuals but the reasons for this are not well understood.

Mechanism of pathogenicity

The brucellae are facultative intracellular parasites. They partially escape detection by innate immunity. This opens a time window to reach the intracellular niche (an endoplasmic reticulum-derived vacuole) before adaptive immunity is activated. Several virulence factors, including a type IV secretion system and genetic regulators are known to be used to reach the intracellular niche. The brucellae block apoptosis and thus reproduce in massive numbers in several types of cells (dendritic, macrophages, epithelial, trophoblasts, etc.).

After penetrating the mucosa, the organism localizes in the lymph nodes nearest to the portal of entry and then spreads to other lymphoid tissues and organs. Bacteraemia develops at the beginning, and becomes intermittently later, often recurring at abortion/parturition. In the pregnant animal the uterus is invaded by way of the endometrium and uterine glands; then the infection spreads into placental cotyledons. Invasion of the allantochorion leads to infection of foetal blood vessels, placental fluids and foetus.
itself. Erythritol and/or partial immunosuppression/tolerance in placenta may be factors accounting for this particular tropism. Abortion is the outcome, with abundant bacterial shedding and spreading of the infection to supramammary lymph nodes (and others) and milk.

**GAP:** Better understanding of host immunity-pathogen interactions and the pathology of the agent:

- Imperfect or no knowledge on (a), Brucella virulence mechanisms and genetic regulators for adapting to intracellular life; (b), interaction with immunity; (c), underlying mechanisms for host preference/specificity; (d), the pathology of the agent within each natural host other than humans, ruminants or swine (camels, yaks, water buffaloes, etc.). Identifying these will help to understand the virulence mechanisms which in turn could help to generate improved treatments and vaccines

### Zoonotic potential

#### Reported incidence in humans

Very high. A figure of 500,000 new cases/year worldwide is often quoted but the source of this figure is unknown. There are no reliable data for most countries. The prevalence varies (annual incidence per million of population) Between 0.2 and 148 for countries of the Mediterranean basin and Near East (year 2006).

**GAP:** Brucellosis is a highly underreported disease. It is often not diagnosed and/or reported, especially in developing areas. Under reporting is probably also common in the new states of the European Union. Underreporting is associated to scarcity of medical services and lack of a keen awareness of the possible disease.

#### Estimated level of under-reporting in humans

Because it is seldom deadly and lacks specific symptoms, it is largely underreported. For developing countries, it has been suggested that the actual number of cases can be 10 times higher than that reported.

#### Risk of occurrence in humans, populations at risk, specific risk factors

The populations at greatest risk are those that regularly come into contact with infected animals and those that consume unpasteurised dairy products. Such populations therefore include farm workers, especially subsistence farmers, veterinarians and slaughterhouse workers in endemic areas. Laboratory workers in endemic countries are also at risk.

#### Symptoms described in humans

Symptoms vary with the population studied and the Brucella species involved. The incubation period can be from one week to up to a year but two weeks is probably common. In general terms, B. melitensis causes more severe disease, followed by B. suis and then B. abortus. Patients with acute brucellosis may manifest a wide spectrum of symptoms including fever (undulant or not), sweats, malaise, anorexia, headache, arthralgias, myalgias, backache and weight loss. Lymphadenopathy, splenomegaly and hepatomegaly are found in some cases. Complications can occur anywhere in the body. They include spondylitis, sacroilitis, osteomyelitis, meningitis, orchitis and abscesses. The mortality rate is low (2% - 5%) although morbidity is significant. Endocarditis is the primary cause of mortality. Increased rates of spontaneous abortion, premature delivery and intrauterine infection with foetal death have been described, but it is unclear whether these occur at rates higher than in other bacterial diseases.

#### Likelihood of spread in humans

Not significant under normal circumstances. Human to human transmission has been rarely associated with blood transfusion, bone marrow transplantation, transplacental or perinatal exposure, sexual intercourse and breast feeding. However, there is limited data for all these claims. This lack of data shows that these routes of transmission are not significant compared to those from animals to humans.

#### Impact on animal welfare and biodiversity

**Both disease and prevention/control measures related**
Animal movement tracking, husbandry and sanitary practices and vaccination policies help to prevent and control spread. Rare breads of domesticated animals are threatened, often not by the disease directly but by slaughter prescribed by surveillance and eradication schemes. At least in EU countries, several bovine, ovine, caprine and porcine endangered local breeds can be seriously affected by the disease since stamping out measures have to be implemented compulsorily for eradication.

**GAP:** Legislation/rules for eradication should be modified (at least in the EU) in the case of endangered species or breeds and control methods (vaccination) different from depopulation considered.

### Endangered wild species affected or not (estimation for Europe / worldwide)

None known (at least in the EU) but possible. Many species can be infected including sea mammals. The disease may affect the fecundity of many wildlife species.

**GAP:** The prevalence and effect of the disease in wildlife.

### Slaughter necessity according to EU rules or other regions

**Domestic livestock:** it is necessary/highly recommended (a), in the last steps of eradication when test and slaughter programmes are implemented; (b) in outbreaks in free areas (however, vaccination [generally forbidden in the EU] could be effective and the best alternative in the case of endangered breeds).

**Wildlife:** there is a high necessity for slaughter if the wildlife presents a significant threat of infection to valuable livestock.

**GAPS:** Legislation:
- Environmental problems with destruction of slaughtered animals (EU).
- It would be convenient to allow Brucella free but vaccinated animals (M3 or B3 status) some trade movements (i.e., destination feedlots and slaughterhouses). This would avoid premature abandon of vaccination.

### Geographical distribution and spread

#### Current occurrence/distribution

Global occurrence. Less than 20 countries (including Northern European countries and France) are free of brucellosis in livestock, and many of these countries maintain a wildlife reservoir. In Europe the disease is highest in the Southern and Mediterranean regions. The distribution of the disease is well described by the geographical occurrence of human brucellosis.

**GAPS:**
- Reporting, surveillance & awareness: no reliable data for parts of the Near East, Asia, Africa and Latin America.
- Brucellosis in wildlife: the prevalence and effect of the disease in wildlife.

#### Epizootic/endemic - if epidemic frequency of outbreaks

Brucellosis is endemic in many areas of the world. In many such areas new outbreaks or re-emergence occurs frequently. Brucellosis epizootic outbreaks occur when infection first enters an immunologically naïve flock/area, or several years after eradication.

**GAP:** Brucellosis in camelids, yaks, water buffaloes and other "exotic" animals – Geographical distribution and spread.

#### Seasonal cycle (seasonality)

The disease progression is linked to the breeding cycles of the primary hosts. When the breeding cycles are seasonal, so is the disease cycle. Once established, brucellosis is permanent in natural host population.

Human brucellosis may show a seasonal cycle associated with breeding practices, as contagion is more likely to occur during calving and lambing and milking.

#### Speed of spatial spread during an outbreak
The main spread of disease is normally through direct contact with infected materials (rather than through aerosol). Spread can therefore occur rapidly when there are no physical barriers between breeding animals and herds. Spread also occurs through the movement of animals and the speed of spread is therefore strongly linked to the speed and spread of infected animals. The disease can also be spread through transported fomites that come into contact with infected animals. It usually high in immunologically naïve flock/area.

**Transboundary potential of the disease**

The disease is widely spread and is already very 'transboundary'. New outbreaks may readily spread across boundaries when there is unrestricted movement of animals across them. Higher where extensive breeding is used.

**Seasonal cycle linked to climate**

In some climates, seasonal cycle is linked to the breeding cycle.

**Distribution of disease or vector linked to climate**

Present in all climates inhabited by humans. None or insignificant spread of disease by vector.

**Outbreaks linked to extreme weather**

Not really.

**Sensitivity of disease or vectors to the effects of climate change (environmental changes/land use)**

Little other than direct impact of climate change on hosts.

**Route of Transmission**

**Usual mode of transmission (introduction, means of spread)**

Ingestion needs large doses and infection seems occurs through the mucous membranes of the oropharynx; animals are readily infected through the conjunctiva under experimental conditions, and this may be a common natural route; inhalation is also possible but seems rare; transmission can occurs through broken skin (it might be possible through the intact skin but much less effectively). Infection may also be gained through the udder trough automatic milking. Natural/artificial insemination may be also a mode of transmission. Congenital infection is known to occur in cattle, sheep and goats.

**GAP:** Route of transmission in camelids, yaks, water buffaloes and other "exotic" animals.

**Occasional mode of transmission**

Infection by aerosol is occasional but highly significant in some circumstances such as slaughter houses and laboratories.

**Conditions that favour spread**

Large flocks, extensive breeding, sharing common pastures, poor management practices (particularly in intensive breeding), poor hygiene, and unawareness of the disease unregulated movement of infected animals; low standards of animal sanitation and husbandry; no segregation of birthing animals and clean-up of post birth tissues and fluids; incorrect disposal of aborted foetuses and associated materials.

In the case of ruminants (and, therefore, humans), absence of vaccination could be considered a main condition.
GAPS: Legislation: it would be convenient to allow Brucella free but vaccinated animals some trade movements (i.e., destination feedlots and slaughterhouses). This would avoid premature abandon of vaccination.

Detection and Immune response to infection

Mechanism of host response

Brucella triggers both antibody and cell-mediated responses. In primary infections, antibodies are not effective, and overcoming the infection depends largely on the cellular immunoresponse. Antibodies, however, may play a role in the protection provided by vaccines and when transferred via colostrum and milk. Brucella can invade and persist in macrophages that are in a non activated state at the time of entry but do not seem to survive in pre-activated macrophages. The route of entry into these cells is therefore important. The infective strategy of brucellosis is believed to be one of stealth whereby it establishes itself into its favoured niche prior to the host raising an effective immune response. The host may respond by increasing the inflammatory action of macrophages but this may come too late and lead to a failure of clearance that results in the recurrent febrile episodes seen in humans.

GAPS: Imperfect or no knowledge on (a), Brucella virulence mechanisms and genetic regulators for adapting to intracellular life; (b), interaction with immunity; (c), underlying mechanisms for host preference/specificity; (d), the pathology of the agent within each natural host other than humans, ruminants or swine (camelids, yaks, water buffaloes, etc.). Identifying these will help to understand the virulence mechanisms which in turn could help to generate improved treatments and vaccines.

Immunological basis of diagnosis

Detection of antibodies and/or cellular responses.

Main means of prevention, detection and control

Sanitary measures

Sanitary measures are effective at containing and limiting the spread of disease. But are only effective or applicable in low prevalence situations They include: Culling of infected animals. Separating off birthing areas from the rest of the herd and decontaminating this area once used. Ensuring that any birth or abortion material is rapidly and effectively removed is also extremely important in limiting disease spread. Rigorous cleaning of fomites also helps to limit the spread of disease. Treatment of slurry with lime prior to spreading may also reduce disease spread if infection is present. Brucellae are susceptible to a number of commonly available disinfectants (2.5% sodium hypochlorite, 2-3% caustic soda, 70% ethanol) that can be used to clear areas and premises.

Mechanical and biological control

Isolation of infected animals and herds via restriction of animal movements is important in limiting the spread. Movement restrictions on animals identified as linked by epidemiological studies is also important. The control of animals in physically and geographically linked zones is also helpful.

Diagnostic tools

Direct tests:

1. Bacteriology. The only unequivocal diagnostic method, especially important in non-endemic areas. Culture is slow, expensive and presents significant risks to diagnosticians. Selective media are necessary. Several selective media have to be combined for optimal sensitivity. Sensitivity is usually low (it depends on the type and number of samples, their adequate conservation and the amount of bacteria in the sample). Identification and typing by classical method is difficult and only suitable in reference laboratories.
2. Several PCR protocols have been optimized for analytical sensitivity & specificity under laboratory conditions but are insufficiently sensitive on accessible clinical material. These methods are currently also expensive and inaccessible to some laboratories although cheaper alternatives are in development.

GAPS: Direct tests

- More effective selective enrichment and culture media are required. Conventional typing is difficult and poses reproducibility problems. Classical methods could be advantageously replaced by molecular methods.
Methods for DNA detection on animal samples should be investigated.

Indirect (immunological) tests:

- Intrinsic limitations are: (a), immune-response proves exposure to Brucella but not necessarily infection; (b), these tests may fail at early stages of infection or in old or immuno-suppressed animals. The basis of immunodiagnosis is largely down to the detection of anti-Brucella antibodies.

1. Blood serum tests in infections due to S Brucella:

   i) Smooth-Lipopolysaccharide (S-LPS) tests. These tests use S-LPS or the polysaccharide (O-chain polysaccharide-core oligosaccharide LPS sections [PS]). The Rose Bengal test (RBT) and the Complement Fixation test (CFT) have been standardized for the diagnosis of cattle and small ruminant brucellosis, and used successfully in eradication programs. There are also ELISA assays available that are suitable for high throughput serology. Several indirect ELISA (iELISA) show sensitivity equal or higher than that of RBT, higher than that of the CFT, can be automated, and are suitable for domestic ruminants. To improve the specificity in vaccinated animals, a competitive ELISA (cELISA) has been developed but its sensitivity does not seem optimal. A fluorescence polarization assay (FPA) is also available. Purse serological assays, such as lateral flow assays (LFA), are also in development but are not yet in validation trials. Specificity hampered by: (a), Vaccination with Rev 1 or S19; (b), cross-reacting bacteria that cause false positive serological reactions (FPSR) (Y. enterocolitica O:9; E. coli O:157 and a few other bacteria) are a problem in some areas, particularly in brucellosis free countries). Despite claims by some manufacturers, no test is able to fully differentiate between infected and vaccinated animals.

   GAPS: Smooth-Lipopolysaccharide (S-LPS) tests:

   - Scarcity information on the performance of serological tests in swine.
   - Lack or only limited information on performance of tests in camels, yaks, water buffaloes and wildlife.
   - RBT antigen standardisation against a single international standard serum may not be optimal.
   - CFT is not sensitive enough and cumbersome to perform.
   - In general, very few validation studies have been performed for iELISAs, cELISAs, FPA or LFA in the different animal species.
   - No S-LPS test solves the FPSR problem satisfactorily.

   ii) non S-LPS tests Native hapten (NH). In precipitation tests, NH is able to differentiate the immunological responses of infected and vaccinated small ruminants and cow. NH tests are simple and highly specific since no false positive serological reactions are caused by Y. enterocolitica O:9, E. coli O:157 and other bacteria that cross-react with the Brucella S-LPS. Protein tests. Several assays with crude protein extracts/fractions or iELISA with cloned, immunogenic protein species has been described. They are not susceptible to false positive serological reactions caused by Y. enterocolitica O:9, E. coli O:157 and other cross-reacting bacteria. However such assays are not commonly employed and have not been rigorously validated.

   GAPS: non S-LPS tests Native hapten (NH):

   - Less sensitivity than S-LPS tests. Lack of studies in species other than small ruminants and cattle.
   - Antigen availability. Lower sensitivity than S-LPS tests. Lack of studies in species other than small ruminants and cattle.

2. Blood serum tests in infections due to R Brucella R Brucella Gel precipitation and several indirect ELISA (iELISA): available for diagnosing B. ovis and B. canis infections.

   GAPS: Blood serum tests in infections due to R Brucella R Brucella Gel precipitation and several indirect ELISA (iELISA):

   - There are no optimal tests.
   - No test combines 100% sensitivity and 100% specificity.
   - Very few validation studies.
   - Lack of international standards.
   - Limited information on false positive reaction due to crossreactive bacteria (a problem identified in B. ovis and B. canis).

3. Milk tests:

Tests developed include the Milk Ring Test (only suitable in cattle), and several iELISA in cattle, sheep and goats.

   GAPS: Milk tests: Studies are required on iELISA to:

   - Standardize these tests in milk.
   - Validate (specificity and sensitivity) these tests, particularly in pooled milk.
   - Investigate the specificity in the context of infections due to bacteria cross-reacting with Brucella S-LPS.

4. Cellular immunity tests in infections by S or R Brucella.

   i) in vivo (allergic tests). Skin tests with cytosolic protein (brucelline or Brucellergene) extracts have been proven successful, particularly when interpreted at herd level. Skin tests show only moderate sensitivity for individual diagnosis. Not suitable when vaccination (any vaccine type) is applied. Highly specific in cases of FPSR caused by Y. enterocolitica O:9, E. coli O:157 and cross-reacting bacteria.

   ii) The laboratory measurement of cytokines (gamma-interferon) following in vitro stimulation of immune blood cells has also been tried but suffers from an unsatisfactory specificity at individual animal level. The gamma-interferon test is expensive and there are not enough validation and sensitivity/specificity studies.
GAPS: Cellular immunity tests in infections by S or R Brucella:

- Allergen availability.
- Scarce information on the performance in swine. Unknown in camelids, yaks, water buffaloes and others.
- Laboratory diagnosis based on the cellular immune response might be developed by investigating stimulation with more specific antigens and the detection of a wider range of cytokines.

Vaccines

Only available against B. abortus (cattle) and B. melitensis or B. ovis (small ruminants) infections. Several attempts to produce effective subcellular or DNA based vaccines but none resulted as practical and/or effective as the current vaccines. The effective vaccines are, for the moment, live attenuated strains. New live generation vaccines have been tested, but none proven as effective as the existing ones. Currently there are differences in the quality of the vaccines. Different hypothesis could be mentioned: 1. Use of Master Seed Lot (none validated by Reference Laboratory). 2. Not conform equipment and facilities are used, 3. Personnel not mastering the technology and the controls (IPC and release tests).

There is a critical need for new vaccines that are:

- more protective.
- able to generate immune responses easily differentiable from those of infected animals (DIVA assays required).
- less pathogenic for livestock (not abortifacient, etc.).
- attenuated in humans.
- more stable.
- affordable.

Therapeutics

Seldom used in animals. However, B. suis infection in pigs could be treated with antibiotics when the infection affects large industrial premises since depopulation is unfeasible.

In humans, adults with acute brucellosis and no complications or focal disease should be treated with doxycycline-streptomycin or doxycycline-gentamicin combinations. In focal forms, the preferred regimen is the same but duration of therapy must be individualized. Surgery should be considered for patients with endocarditis, cerebral, epidural, spleen, hepatic or other abscesses not resolving with antibiotic therapy. During pregnancy tetracyclines and streptomycin must be avoided and a rifampin monotherapy is considered the regimen of choice. Trimethoprim-sulfamethoxazole (cotrimoxazole) plus rifampin is an alternative regimen but it is contraindicated before week 13 or after week 36 of pregnancy. Children less than 8 years old can be treated with rifampin-cotrimoxazole, or rifampin or cotrimoxazole plus gentamicin. Antibiotics have to be administered for long (usually 6 weeks but sometimes longer) times. Treatment is expensive and may pose compliance problems. Relapses occur in 5 to 30% of patients. Rifampin must be avoided in countries where tuberculosis is endemic.

GAPS: Human brucellosis

More efficacious/cheaper antibiotics would be valued that could:

- avoid parental administration
- shorten the administration period
- avoid relapses.
- make treatment affordable.

Biosecurity measures effective as a preventive measure

All Brucella species are classified as B3 pathogens. Appropriate biosecurity measures are known for outbreaks.

GAP: B. ovis is not infectious for humans and B. canis shows a markedly reduced virulence. At least B. ovis should not be included among B3 pathogens.

Border/trade/movement control sufficient for control

Necessary and implemented at international level. The movement of infected animals is the main mechanism for the spread of disease between herds. Diagnostic testing procedures are in place for the movement of animals between nations which are based upon the methods described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

Prevention tools
In areas free of brucellosis, the best preventative measure to take is to ensure that imported animals are disease free. This is mainly done through the application of serodiagnosis and by selecting areas for importation that are certified disease free.

When brucellosis is present in a herd then sanitation measures and vaccination can be put in place as described above. Where livestock are exposed to wildlife that may be carrying the disease then an effective physical barrier is the best means of prevention.

**GAP:** The spread of brucellosis from wildlife to livestock is not fully understood. Improved high resolution epidemiological tools would assist in such investigations.

### Surveillance

Necessary and implemented in (developed) countries free of brucellosis. Surveillance is usually conducted through serology using the tests recognised by the OIE. Serological testing of milk is also frequently performed. The number and proportion of animals tested can vary tremendously depending on the objective of the surveillance, the historical prevalence of the disease and the threat of spread and/or introduction. This sero-surveillance is often supported by alternative assays such as the skin test and final confirmation of disease is sometimes only made after positive culture. An additional surveillance method is to report and examine abortions by culture to detect Brucella. Surveillance is only effective if supported by a strong veterinary infrastructure to take the appropriate samples and deliver the appropriate measures.

**GAP:** The level and type of surveillance performed across the globe (not possible where structural weaknesses exist) is very different and is frequently intertwined with vaccination programmes and thus significant complications ensue.

### Past experiences on success (and failures) of prevention, control, eradication in regions outside Europe

Brucellosis is a difficult disease to control in livestock owing to several factors. Amongst these are the lack of outward clinical signs of disease other than abortion and fertility reduction meaning that detection is difficult without a sustained and expensive surveillance programme. Proficient Veterinary Services, stakeholder engagement, and appropriate budgets are essential.

With a few exceptions, all successful control and elimination programs in cattle and sheep have been carried out with attenuated S strains, S19 and Rev 1 respectively. Vaccination is not 100% effective and is generally not capable of eradicating the disease completely. Re-introduction of disease into previously free areas can easily occur via animal movements. Without constant surveillance the disease can rapidly spread.

Reservoirs of infection from wildlife that threaten livestock also exist in many areas. The low number of countries that are completely disease free is testimony to the difficulties of eradication, prevention and control.

**GAP:** Although some argue that the tools required to control the disease are already known are available and are effective if properly applied, superior tools are needed that enable control at lower costs that are currently required as these costs are unsustainable for most economies where brucellosis is prevalent (unsustained control amounts to no control).

### Costs of above measures

Such measures are high cost. United States Department of Agriculture sources estimate that in the 1990s, on average about US$150 million was spent each year in the US. Vaccination is a more cost-effective policy than test and slaughter but as vaccination on its own is unlikely to eradicate disease, test and slaughter (with the associated compensation costs) is the only way to certify and maintain freedom from brucellosis.

Due to the few clear clinical symptoms in livestock effective surveillance requires the testing of a relatively high proportion of animals by serology. The costs of sample collection and testing alone (without including any slaughter and compensation costs) can easily extend from tens of thousands into millions of Euros per annum dependent upon population size.

The costs of the serological testing are a low proportion of these costs once animal compensation and logistical costs are also weighed in. Very few studies have assessed the benefit-cost ratio of controlling animal brucellosis. For sheep (B. melitensis brucellosis) under extensive management and with a 52% protection (Rev 1 vaccination), the benefit–cost ratio for society has been estimated in 3.2 (range 2.27–4.37) when all aspects of this zoonosis are included (see 11.2.). This suggests that brucellosis control is one of the most cost-effective interventions (comparable to tuberculosis treatments).

**GAP:** Although some argue that the tools required to control the disease are already known are available and are effective if properly applied, superior tools are needed that enable control at lower costs that are currently required as these costs are unsustainable for most economies where brucellosis is prevalent (unsustained control amounts to no control).
Disease information from the OIE

**Disease notifiable to the OIE**

Yes.

**OIE disease card available**

[http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/BCLS-EN.pdf](http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/BCLS-EN.pdf)

**Bovine:**
[http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.11.3.htm](http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.11.3.htm)

**Caprine and Ovine:**

**Brucella ovis:**

**Porcine:**
[http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.15.3.htm](http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.15.3.htm)

**OIE Terrestrial Animal Health Code (reference)**

Bovine:
[http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf)

Caprine and Ovine:
[http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.02_CAPRINE_OVINE_BRUC.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.02_CAPRINE_OVINE_BRUC.pdf)

Brucella Ovis:

Porcine:
[http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.05_PORCINE_BRUC.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.05_PORCINE_BRUC.pdf)

**OIE Terrestrial Manual (reference)**

Bovine:
[http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf)

Caprine and Ovine:
[http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.02_CAPRINE_OVINE_BRUC.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.02_CAPRINE_OVINE_BRUC.pdf)

Brucella Ovis:

Porcine:
[http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.05_PORCINE_BRUC.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.05_PORCINE_BRUC.pdf)

**Socio-economic impact**

**Zoonosis: Impact on affected individuals and/or aggregated DALY figures**

Presently, the main impacts are produced in low income and developing countries. It is clearly a disease related with the poverty. Morbidity rates are very high in infected individuals. Only a study (in Mongolia) has estimated median DALY figure (years of healthy life lost) per case of brucellosis was 0.945.

**GAP:** Socio-economic studies under different situations to prioritize interventions in developing countries.

**Zoonosis: cost of treatment and control of the disease in humans**

Usually very high in developed countries (in the EU, treatment with doxycycline & rifampicine, a minimum of 60-70€; diagnosis [blood culture & serology] 240€). Generally inapplicable in developing countries.

**Direct impact (a) on production**

Not well documented, particularly in developing countries. Mortality negligible. The impact depends on country, breeding system, etc. Estimates of the economic impact of brucellosis in livestock are not easy but studies in Mexico and Argentina, which both depend heavily on the sale of livestock domestically and internationally, estimate annual costs to be US$60 million and $200 million respectively.

**GAP:** Socio-economic studies under different situations to prioritize interventions in developing countries.
Direct impact (b) cost of private and public control measures

Usually very high in developed countries. Over 20 million euro dedicated yearly by the EU to eradication in member countries, and only to finance 50% of operative costs.

**GAP:** Socio-economic studies under different situations to prioritize interventions in developing countries.

**GAP:** Socio-economic studies under different situations to prioritize interventions in developing countries.

Indirect impact

Very high. Infected animals cannot be marketed. Several endangered breeds in some extensive breeding systems seriously affected by eradication campaigns.

**GAP:** Socio-economic studies under different situations to prioritize interventions in developing countries.

Trade implications

**Impact on international trade/exports from the EU due to existing regulations**

Very high. Trade restrictions are placed on animals that come from nonbrucellosis free regions and nations especially when trading with free nations.

**GAP:** It would be convenient to allow Brucella free but vaccinated animals some trade movements (i.e., destination feedlots and slaughterhouses). This would avoid premature abandon of vaccination.

**Impact on EU intra-community trade due to existing EU regulations**

Very high. Infected animals cannot be marketed. Regulations do restrict trade but are necessary to maintain freedom in free areas.

**GAP:** It would be convenient to allow Brucella free but vaccinated animals some trade movements (i.e., destination feedlots and slaughterhouses). This would avoid premature abandon of vaccination.

**Impact on national trade due to existing regulations**

Very high. Infected animals cannot be marketed.

**GAP:** It would be convenient to allow Brucella free but vaccinated animals some trade movements (i.e., destination feedlots and slaughterhouses). This would avoid premature abandon of vaccination.

**Main perceived obstacles for effective prevention and control**

A lack of effective DIVA vaccines and assays for all species. A lack of control on animal movements in some countries/regions. Low resolution of epidemiological markers. High costs associated with cultural confirmation of disease. Impact of latently infected animals not well understood but infected animals that are undetectable are sure to pose some risk of spread. Legislative measures inappropriate. Healthy but vaccinated animals cannot be marketed. This forces the premature abandon of vaccination. Legislation should be modified allowing free but vaccinated animals similar status than brucellosis free animals, at least for some trade movements (i.e., destination feedlots and slaughterhouses).

**GAPs:**

- It would be convenient to allow Brucella free but vaccinated animals some trade movements (i.e., destination feedlots and slaughterhouses). This would avoid premature abandon of vaccination.
- More effective selective enrichment and culture media are required. Conventional typing is difficult and poses reproducibility problems. Classical methods could be advantageously replaced by molecular methods.
- Methods for DNA detection on animal samples should be investigated.
- Scarce information on the performance of serological tests in swine.
- Lack or only limited information on performance of tests in camelids, yaks, water buffaloes and wildlife.
There is a critical need for new vaccines that are:

- more protective.
- able to generate immune responses easily differentiable from those of infected animals (DIVA assays required).
- less pathogenic for livestock (not abortifacient, etc.).
- attenuated in humans.
- more stable.
- affordable.

- No proper information is available on the use of Rev 1 and S19 in species other than small ruminants and cattle, respectively.
- None available and tested for specific protection and safety against infections by S. brucellae in swine, camelids, yaks, water buffaloes and others.
- No specific B. ovis attenuated vaccine available when Rev 1 use is discontinued after eradication of B. melitensis.
- No B. canis vaccine.

More efficacious/cheaper antibiotics would be valued that could:

- avoid parental administration
- shorten the administration period
- avoid relapses.
- make treatment affordable.

Main perceived facilitators for effective prevention and control

A lack of effective DIVA vaccines and assays for all species. A lack of control on animal movements in some countries/regions. Low resolution of epidemiological markers. High costs associated with cultural confirmation of disease. Impact of latently infected animals not well understood but infected animals that are undetectable are sure to pose some risk of spread. Legislative measures inappropriate. Healthy but vaccinated animals cannot be marketed. This forces the premature abandon of vaccination. Legislation should be modified allowing free but vaccinated animals similar status than brucellosis free animals, at least for some trade movements (i.e., destination feedlots and slaughterhouses).

**GAPS:**

- It would be convenient to allow Brucella free but vaccinated animals some trade movements (i.e., destination feedlots and slaughterhouses). This would avoid premature abandon of vaccination.
- More effective selective enrichment and culture media are required. Conventional typing is difficult and poses reproducibility problems. Classical methods could be advantageously replaced by molecular methods.
- Methods for DNA detection on animal samples should be investigated.
- Scarce information on the performance of serological tests in swine.
- Lack or only limited information on performance of tests in camelids, yaks, water buffaloes and wildlife.

There is a critical need for new vaccines that are:

- more protective.
- able to generate immune responses easily differentiable from those of infected animals (DIVA assays required).
- less pathogenic for livestock (not abortifacient, etc.).
- attenuated in humans.
- more stable.
- affordable.

- No proper information is available on the use of Rev 1 and S19 in species other than small ruminants and cattle, respectively.
- None available and tested for specific protection and safety against infections by S. brucellae in swine, camelids, yaks, water buffaloes and others.
- No specific B. ovis attenuated vaccine available when Rev 1 use is discontinued after eradication of B. melitensis.
- No B. canis vaccine.

More efficacious/cheaper antibiotics would be valued that could:

- avoid parental administration
- shorten the administration period
- avoid relapses.
- make treatment affordable.
**Risk**

The major risk is reintroduction of the disease in countries/areas where it has been eradicated and vaccination has been discontinued. In non-protected animals, the disease spreads very quickly.

**Main critical gaps**

**Gap 1. Taxonomy:**
- The internal taxonomy of the genus needs revision.
- It cannot be assumed that isolates from geographical areas other than Europe, the near East and N. and S. America fall within the previously described species and biovars.

**Gap 2. Brucellosis in camelids, yaks, water buffaloes and other "exotic" animals:**
- Description of infection & disease in natural hosts.
- Epidemiology of brucellosis in mixed breeding systems and the risk of perpetuation of the infection by Brucella species in hosts other than the preferential ones.
- Geographical distribution and spread.
- Route of transmission.

**Gap 3. Swine brucellosis:** Epidemiology (interactions with wildlife), diagnosis, vaccines.

**Gap 4. Brucellosis in wildlife:**
- Epidemiology; role as reservoirs/carriers; diagnosis.
- Greater understanding of the wildlife reservoir.
- The prevalence and effect of the disease in wildlife.
- The spread of brucellosis from wildlife to livestock is not fully understood. Improved high resolution epidemiological tools would assist in such investigations.

**Gap 5. Stability of the agent/pathogen in the environment:**
- Information incomplete concerning non-pasteurized dairy products, particularly those obtained by traditional procedures (souring, etc.).
- Stability of Brucella in seawater mostly unknown (some studies have been performed).

**Gap 6. Better understanding of host immunity-pathogen interactions and the pathology of the agent:**
- A better understanding of latency and of detecting latently infected animals is important. The inability to screen out such animals during movement tests presents a risk to the disease free status of target destinations.
- Connection with gaps 2 and 4. The pathology of the agent within each host other than in humans and domesticated animals is not well known. Thus there is no evidence as to whether such animals should be considered diseased or carriers. Role of wild-life not well defined (spill-over? just carriers?) and possibly different in different breeding systems.
- The shedding of Brucella in milk appears to be transient and variable between individuals but the reasons for this are not well understood.
- Imperfect or no knowledge on (a), Brucella virulence mechanisms and genetic regulators for adapting to intracellular life; (b), interaction with immunity; (c), underlying mechanisms for host preference/specificity; (d), the pathology of the agent within each natural host other than humans, ruminants or swine (camelids, yaks, water buffaloes, etc.). Identifying these will help to understand the virulence mechanisms which in turn could help to generate improved treatments and vaccines.

**Gap 7. Human brucellosis:**
- Data on the virulence of several B. suis biovars, B. neotomae, and B. microti for humans are fragmentary or do not exist.
- More efficacious/cheaper antibiotics would be valued that could: avoid parental administration, shorten the administration period, avoid relapses and make treatment affordable.

**Gap 8. Epidemiology in domestic animals:** definition of epidemiological units (should take into account the high possibility of cross-species infections) often inadequate.

**Gap 9. Reporting, surveillance & awareness:**
- Brucellosis is a highly underreported disease. It is often not diagnosed and/or reported, especially in developing areas. Underreporting is associated to scarcity of medical services and lack of a keen awareness of the possible disease.
- No reliable data for parts of the Near East, Asia, Africa and Latin America.
- The level and type of surveillance performed across the globe (not possible where structural weaknesses exist) is very different and is frequently intertwined with vaccination programmes and thus significant complications ensue.
- Studies on the prevalence of the disease in developing countries, especially in most of Africa and some parts of Asia and Latin America.
- Lack of adequate technical and economic support by international bodies (FAO/WHO). Lack of truly experienced international specialists in designing and conducting control strategies.

**Gap 10. Legislation:**
• Legislation/rules for eradication should be modified (at least in the EU) in the case of endangered species or breeds and control methods (vaccination) different from depopulation considered.
• Environmental problems with destruction of slaughtered animals (EU).
• It would be convenient to allow Brucella free but vaccinated animals some trade movements (i.e., destination feedlots and slaughterhouses). This would avoid premature abandon of vaccination.
• B. ovis is not infectious for humans and B. canis shows a markedly reduced virulence. At least B. ovis should not be included among B3 pathogens.
• Genetically Modified Organism legislation in Europe.

Gap 11. Diagnosis:

• More effective selective enrichment and culture media are required. Conventional typing is difficult and poses reproducibility problems. Classical methods could be advantageously replaced by molecular methods.
• Methods for DNA detection on animal samples should be investigated.
• Scarce information on the performance of serological tests in swine.
• Lack or only limited information on performance of tests in camelids, yaks, water buffaloes and wildlife.
• No test combines 100% sensitivity and 100% specificity, particularly when vaccination is implemented.
• RBT antigen standardisation against a single international standard serum may not be optimal.
• CFT is not sensitive enough and cumbersome to perform.
• RBT and CFT have not been optimized for serodiagnosis in small ruminants or swine.
• In general, very few validation studies have been performed for i-ELISAs, cELISAs, FPA or LFA in the different animal species.
• No S-LPS test solves the FPSR problem satisfactorily.
• There are no optimal tests.
• No test combines 100% sensitivity and 100% specificity.
• Very few validation studies.
• Lack of international standards.
• Limited information on false positive reaction due to crossreactive bacteria (a problem identified in B. ovis and B. canis).
• Studies are required on iELISA to:
  • Standardize these tests in milk.
  • Validate (specificity and sensitivity) these tests, particularly in pooled milk.
  • Investigate the specificity in the context of infections due to bacteria cross-reacting with Brucella S-LPS.
• Allergen availability.
• Scarce information on the performance of Cellular immunity tests in infections by S or R Brucella in swine. Unknown in camelids, yaks, water buffaloes and others.
• Laboratory diagnosis based on the cellular immune response might be developed by investigating stimulation with more specific antigens and the detection of a wider range of cytokines.
• Although some argue that the tools required to control the disease are already known are available and are effective if properly applied, superior tools are needed that enable control at lower costs that are currently required as these costs are unsustainable for most economies where brucellosis is prevalent (unsustained control amounts to no control).
• Although costs of tests are generally competitive, they are out of reach for many areas in Africa or Asia.
• Almost all kits require cold storage to maintain effectiveness. This may be a problem in some resource poorer regions.
• There are no commercially available PCR kits that claim to diagnose brucellosis.
• Updated regularly but criteria for inclusion-exclusion are not clear and properly documented. Probably lobbies and commercial interests, rather than intrinsically technical features of tests, play a major role.
• Some of the methodological descriptions are open to a variety of interpretations. Whilst this is advantageous in some respects, it can also lead to some drift in techniques between laboratories.
• Possibly some space in the market for niche assays based on non-OPS antigens.
• No validation studies (adequate to the particular country/conditions) for most commercial kits and animal species.

Gap 12. Vaccines:

• There is a critical need for new vaccines that are: more protective, able to generate immune responses easily differentiable from those of infected animals (DIVA assays required), less pathogenic for livestock (not abortifacient, etc.), attenuated in humans, more stable and affordable.
• No proper information is available on the use of Rev 1 and S19 in species other than small ruminants and cattle, respectively.
• None available and tested for specific protection and safety against infections by S brucellae in swine, camelids, yaks, water buffaloes and others.
• No specific B. ovis attenuated vaccine available when Rev 1 use is discontinued after eradication of B. melitensis.
• No B. canis vaccine.
• No human vaccine.
• No S19 for conjunctival route has been ever produced and marketed internationally.
• No B. ovis specific vaccine available in the EU (or elsewhere).
• No possibilities for vaccines that do not solve the DIVA problem. New vaccines would be necessary to open the market.

Gap 13. Cost and socio-economic analysis: Socio-economic studies under different situations to prioritize interventions in developing countries.

Gap 14. Available resources

• General constraints: availability of research teams with complementary skills (optimally, teams from public institutions in collaboration with
Industry). Funding.

- Decisions concerning project management and adequate repartition of tasks between Public Institutions/Laboratories and Industry.
- Category 3 facilities.

Conclusion

1. Superior tools are needed that enable control at costs lower than are currently required as these costs are unsustainable for most economies where brucellosis is prevalent.
2. The diagnosis and immunoprophylaxis of brucellosis in exotic animals and developing countries and in wild-life needs investigation.
3. There is a need for basic research on virulence/immunity for developing new vaccines.
4. New immunologically tagged vaccines and complementary DIVA tests are necessary.
5. Current Genetically Modified Organism EU legislation may be a problem in the development of new vaccines.
6. Little knowledge on brucellosis (including epidemiology) in wildlife.

Sources of information

Name of expert group leader

Expert group members are included where permission has been given

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