Review of bovine brucellosis surveillance in Europe in 2015

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Summary

Bovine brucellosis is a major zoonosis and is responsible for significant reproductive disorders and production losses in cattle. Surveillance and control are regulated at the European level with specific conditions to obtain and maintain the officially free status, which facilitates access to export markets. These European standards allow for harmonisation in brucellosis surveillance and diagnosis while leaving some flexibility to countries in the choice of measures to meet the desired objectives. This study reviews the bovine brucellosis surveillance systems currently in place across the European continent, according to countries' brucellosis status, based on a survey addressed to brucellosis diagnosis experts in the national reference laboratory of each country. Experts were asked to provide synthesised surveillance data and to describe technical conditions and screening tests carried out for the surveillance of abortions, serological

testing in herds, movement controls and any other surveillance components in 2015. Results were obtained for 34 out of 37 countries (92%). Surveillance systems included abortion surveillance (34 countries), routine herd screening (28 countries), movement testing (14 countries), routine testing at bull stations (9 countries), and screening tests at slaughterhouses (4 countries). The review highlighted variability in technical conditions and screening tests among countries. These results are discussed with regard to the European Union regulations, disease risks and epidemiological situations, with the aim of improving surveillance efficacy and efficiency.

Keywords

Abortion surveillance – Bovine brucellosis – Europe – Laboratory test – Movement testing – National Reference Laboratory – Programmed surveillance – Regulation – Surveillance system.

Introduction

Bovine brucellosis, caused by *Brucella abortus* or *B. melitensis*, is a major zoonosis and is responsible for significant reproductive disorders and production losses in cattle. The main clinical sign is abortion, most commonly in the last trimester of gestation (1). In Europe, there is a continuum in the health situation of countries regarding bovine brucellosis, ranging from countries with officially brucellosis free (OBF) status at country or regional scale to non-OBF countries still implementing control measures to eradicate the disease (2). While the OBF status facilitates access to export markets, it does not completely prevent brucellosis re-emergence, as demonstrated by the occurrence of *Brucella* outbreaks in cattle in Belgium in 2010–2013 (3) and in France in 2012 (4).

Bovine brucellosis surveillance is regulated at the European level by the Council Directive 64/432/EEC on animal health problems affecting intra-community trade in bovine animals and swine (5) (referred to hereafter as the Council Directive) and the *Terrestrial Animal Health Code* of the World Organisation for Animal Health

(OIE) (6). The Council Directive establishes the rules for obtaining and maintaining OBF status at country or regional scale. In particular, a Member State or region of a Member State is able to obtain (and retain) OBF status if 99.8% of herds have achieved OBF status for at least five years and no case of abortion due to *Brucella* infection has been recorded for at least three years. The conditions for obtaining or retaining this status rely on several surveillance components, including routine screening in herds, notification and investigation of cases of abortion, and serological screening at movements between herds (5). These European standards allow for harmonisation in brucellosis surveillance, control programmes and diagnosis while leaving some flexibility to countries in the choice of measures to meet the desired objectives (7).

Adaptations of European Union (EU) legislation to different epidemiological situations have resulted in the implementation of various surveillance systems. Although some details about specific surveillance components may be found for some countries in studies describing the epidemiology and management of bovine brucellosis (8, 9, 10), evaluating surveillance performance (11) or assessing brucellosis categorisation (12), there is no comprehensive overview of the surveillance systems in force in Europe. Yet, such information is a prerequisite to comparing surveillance systems among countries and further improving surveillance effectiveness and efficiency. The objective of this survey was to review the bovine brucellosis surveillance systems currently in place across the European continent, according to countries' brucellosis status and local situations.

Materials and methods

An online survey regarding the bovine brucellosis surveillance system in place in 2015 was sent to the Brucellosis EU Reference Laboratory (EURL) network, which comprises 36 countries from the European continent, including Member and non-Member States of the EU. For the United Kingdom, the distinction was made between Northern Ireland and Great Britain (considered as two countries). The survey

was carried out with Sphinx software and tested by two countries before being sent to all participants.

The survey started with questions about the epidemiological situation of the country regarding bovine brucellosis, followed by questions about the surveillance components in place in the country, including routine screening in herds (also denoted active surveillance), movement testing, abortion surveillance (clinical surveillance), as well as other country-specific surveillance components. The questions aimed to describe each surveillance component, including field implementation, laboratory tests and interpretation of test results. The survey is available from the corresponding author.

Results

General information

A total of 34 out of 37 countries answered the survey, which corresponds to a response rate of 92%. The participants included 23 countries with OBF status in 2015 (Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Ireland, Latvia, Lithuania, Malta, Northern Ireland, Norway, Poland, Romania, Slovakia, Slovenia, Sweden, Switzerland and the Netherlands), nine which were non-OBF (Albania, Bosnia and Herzegovina, Croatia, Cyprus, the former Yugoslav Republic of Macedonia [FYROM], Hungary, Montenegro, Portugal and Serbia) and two with OBF areas, referred to as regionalised countries hereafter (Italy and Spain) (13).

Among the regionalised or non-OBF countries, six provided results about the presence of infected herds, with an estimated prevalence (calculated as the number of infected herds divided by the number of herds) ranging between 0.02% and 0.51%.

The most frequent surveillance component was abortion surveillance (implemented in 34/34 countries), followed by routine herd screening (28/34) and movement testing, including domestic trade and import/export certification (14/34) countries (Table I). Three countries

(all OBF) implemented only abortion surveillance. Two surveillance components were implemented in 20 countries, including abortion surveillance and either routine herd screening tests (17/34) or movement testing (3/34). The three surveillance components were in place in 11 countries. Additional surveillance methods were implemented in several OBF countries, including screening tests at the slaughterhouse (four countries), routine testing at bull stations (nine countries), wildlife surveillance (one country) or strengthened surveillance in dairy herds with an increased number of abortions (one country).

Ten countries reported that a portion of the cattle population is exempted from surveillance: fattening herds in nine countries and dairy cows in one country (Table II).

Insert Table I and Table II here

Programmed surveillance

Technical conditions

Routine screening tests in herds were implemented in 28 countries, including 17 OBF countries, nine non-OBF countries and two regionalised countries (Table III). All these countries, except Great Britain, conducted serological screening on blood samples and 16 used milk testing. While OBF and non-OBF countries set the screening frequency at a national scale, both countries with OBF regions applied a different frequency of testing depending on the brucellosis status of the geographical areas; these two countries were thus divided into OBF and non-OBF regions for the analysis of results regarding programmed surveillance.

Overall, for blood serology, OBF countries/regions applied annual sampling (15/18) or less frequent sampling (3/18), while non-OBF countries/regions implemented one (7/11) or two screenings a year (4/11). Four countries/regions (Cyprus, Northern Ireland, Portugal and Great Britain) applied a different sampling frequency in herds tested also by assays on milk. For example, in Northern Ireland, blood

serology was conducted every two years in beef herds but every five years in dairy herds because these herds were also tested monthly on milk.

The proportion of herds sampled at each screening varied among countries. Among OBF countries, screening concerned less than 25% of herds in 9 out of 14 countries and more than 75% of herds in 5 countries. Among non-OBF and regionalised countries, one out of 11 countries indicated a low screening rate (less than 25% of herds), 1 sampled between 26 and 50% of herds and nine sampled more than 75% of herds. Based on surveillance results provided by the countries, it was estimated that in countries that declared testing less than 25% of herds (by blood and/or milk tests), the actual number of herds tested in 2015 varied from 0% to 28% (nine countries) and in those that declared testing more than 75% of herds, the actual number of herds tested varied from 53% to 100% (12 countries).

All countries (except one) that used serological screening on serum samples tested between 75 and 100% of the animals under the programme per herd; the exception was France, with only 20% of animals tested per herd but with a large proportion of herds tested (75–100%). The minimum age for testing cattle using blood serological screening was six months in one out of 28 countries, 12 months in 11 countries and 24 months in 15 countries. In Portugal, the minimum age for screening was six months old in non-OBF herds and 24 months old in OBF herds.

Insert Table III here

Diagnostic tests

The most commonly used first-line screening test for blood samples was the Rose Bengal test (RBT) (19/28 countries). Other tests utilised were the indirect enzyme-linked immunosorbent assay (iELISA) on individual bovine serum, iELISA on pooled bovine sera, complement fixation test (CFT) and serum agglutination test (SAT) (Tables III and IV). In 19 out of 28 countries, a single screening method was used. In six countries diagnostic laboratories could select the screening test

among two different methods and in three countries among three different methods. Following a positive result on serology, confirmatory tests included CFT, iELISA on individual bovine serum, competitive enzyme-linked immunosorbent assay (cELISA), RBT, SAT and brucellin skin tests. The number of alternative methods for confirmation varied among countries: a single method was allowed in seven countries (out of 28), two methods in six countries, three methods in 11 countries and four methods in four countries.

Screening on milk samples was performed primarily by iELISA on bulk milk (14/16 countries); other tests included iELISA on individual milk, the ring test and iELISA on pooled bovine milk. Most countries (14 countries) used only one method for first-line screening on milk samples; in other countries, two methods were allowed. Six countries indicated that they performed confirmatory tests on milk samples, by iELISA on individual samples, iELISA on bulk milk, culture or ring test. A single confirmatory method was used in four out of six countries and two tests in other countries.

In four out of 28 countries, first-line screening tests for bovine brucellosis included culture on post-mortem samples or on vaginal swabs. For confirmation, seven countries used *Brucella* culture, on post-mortem tissues or vaginal swabs, or polymerase chain reaction (PCR) on tissue samples.

Following the first-line screening and the confirmatory test conducted at the local laboratory, two countries indicated that the final decision is based on the result of that confirmatory test, and 26 countries indicated that an additional (final) confirmatory test is performed, by the national reference laboratory (NRL) in 17 countries or by either the NRL or a local laboratory in three countries (six countries did not mention where this final test is conducted). *Brucella* culture and PCR were the most common methods applied to definitely confirm an infection and were implemented in 23 countries and 14 countries, respectively; serological tests (iELISA, cELISA, CFT, milk ring test) were also used for confirmation in four out of 26 countries.

Insert Table IV here

Abortion surveillance

Technical conditions

The following (non-exclusive) choices were proposed as possible events considered as an abortion: loss of pregnancy, expulsion of a foetus, stillbirth, death within 12 hours of birth, death within 24 hours of birth, death within 48 hours of birth, and premature birth (Table V). Thirty-two countries supplied a definition of abortion; Sweden indicated that there was no official definition of an abortion and Croatia did not report the official definition. The expulsion of a foetus was considered as an abortion in 28 out of 32 countries with clinical surveillance. Some countries restricted the definition to the expulsion of a foetus between 43 and 260 days of pregnancy (Denmark), 42 and 210 days (Estonia), 100 and 260 days (Netherlands) or during the last third of pregnancy (Germany). Loss of pregnancy and stillbirth were considered as an abortion in 16 countries. The death of a live calf within 12, 24 or 48 hours of birth was considered as an abortion in one, two and three countries, respectively. Premature birth with a live calf was considered as an abortion in seven countries.

The notification of abortion(s) was not mandatory in three (out of 34) countries, mandatory after a single abortion in 26 countries, or mandatory after a series of two or more abortions in three countries; in two countries (Spain and Switzerland), the protocol varied among herds. In Spain, the procedure depended on the geographical area: in areas of nil prevalence, the notification was mandatory after a series of abortions, while in areas where prevalence is not nil, the notification of all abortions was mandatory. In Switzerland, the notification was mandatory after a series of abortions except for traders or mountain pasture farms, which are required to notify all abortions. For two countries with mandatory notification of abortions, the obligation referred to abortions within the last trimester (Germany) or abortions suspected of being due to brucellosis (Slovenia).

In the case of notification after a series of abortions, the protocol varied among countries: a series was defined as four or five abortions ‘at the same time’ (Bulgaria); two or more abortions within four

months (Switzerland); more than one abortion within a year (Norway); two abortions or more in a month or three abortions throughout the year in herds with fewer than 100 animals, or if more than 4% of abortions occurred in the year in herds with more than 100 animals (Spain). In FYROM, the number of abortions was not precisely defined, but should be 'unusually high' (considering the herd health status from previous years).

Based on notification results for 2015, the median value of the ratio of the number of abortion notifications to the number of herds indicated that 2.6% of herds (interquartile interval [IQI] 0.1–10.1) notified abortion(s) in countries where the notification of all abortions is mandatory (N = 21), 0.5% of herds (IQI 0.1–3.4) in countries where the notification is mandatory after a series of abortions (N = 4), and 0.2% of herds (IQI 0.1–0.3) in countries where the notification was not mandatory (N = 3).

Among countries with mandatory notification of all suspect cases (i.e. all abortions, abortions within the last trimester, or abortions suspected of being due to brucellosis), 22 countries required the investigation of all notified abortions. Other countries required investigations to be conducted on the foetuses (Bosnia and Herzegovina), within 28 days following the abortion (Czech Republic) or when the proportion of abortions during the current or previous year exceeded 5% in the herd (Finland).

Insert Table V here

Screening tests

Both serological and bacteriological first-line tests are performed following an abortion, with a total of ten different methods reported among the countries (Tables IV and V). The most common tests were culture from an aborted foetus, and RBT, iELISA and CFT from the dam's serum. One test only was used in 16 countries, two tests in ten countries and three to five tests in eight countries. In addition, in Finland, serological screening of bulk bovine milk (by iELISA) was

reported to be conducted in dairy herds with an increased number of abortions.

The four countries (Belgium, Denmark, Sweden and Slovenia) using culture on an aborted foetus as the only first-line screening test considered the result as definitive. In other countries, the most common confirmatory tests included CFT, iELISA, RBT, cELISA, culture on post-mortem samples, PCR on tissue samples and culture on vaginal swabs. The number of possible confirmatory tests varied among countries, ranging from one to six alternative methods (one: six countries, two: eight countries, three: nine countries, four or more: seven countries).

In all responding countries except Estonia, a positive diagnostic test confirming brucellosis infection in the aborted cow was necessary to consider the herd as infected. In Estonia, a herd was considered as infected following abortion notification.

Movement surveillance

Conditions for testing

Surveillance of movements (trade) between herds consisted of pre-movement tests in 12 countries and post-movement tests in nine countries (Table VI). The conditions for testing varied with the country status. Among the 12 countries performing pre-movement tests, seven countries (including some with non-OBF status or with OBF regions) tested any bovine systematically before movement; in Spain, these controls were applied in non-OBF regions only. In other countries, pre-movement testing was conducted in specific cases: export certification (three countries), bovine of unknown status (i.e. if there were no data to prove the animal health status) (one country), or when a risk assessment deemed that testing was necessary (one country). Post-movement tests were conducted systematically (one country), when transport duration was longer than six days (one country), in the absence of a pre-movement test (three countries) or for imported bovines (four countries, including one country where tests were decided on a risk-based assessment). In addition to pre- or

post-movement controls, four countries performed controls of movements in transhumant herds.

Insert Table VI here

Screening tests

The most common methods of brucellosis testing for movement controls were the RBT, iELISA on individual bovine serum, CFT, SAT and iELISA (Tables IV and VI). Six countries used only one test method, three countries used two alternative tests and two countries used three or four tests. In Great Britain, the test depended on the type of movement: iELISA for bull hire and SAT for artificial insemination.

Other surveillance measures

Five OBF countries conducted brucellosis testing at the slaughterhouse, all by serology. Three countries tested less than 25% of culled bovines, by sampling animals at random (Lithuania and Sweden) or by selecting all bovine animals over 12 months including males for fattening (Malta). In Northern Ireland, testing was targeted at animals over 24 months (which represented 25–50% of culled bovines) and in Ireland the surveillance goal was to test at least 80% of culled cows.

Routine testing at bull stations was in force in nine countries: Denmark, Finland, France, Great Britain, Latvia, Northern Ireland, the Netherlands, Poland and Slovakia. Screening is usually conducted on certified breeding bulls at the semen collection centre. In Finland, which does not conduct routine surveillance of cattle herds, the herds that breed bulls for artificial insemination are tested annually.

Discussion

This overview highlighted great diversity in regulations regarding bovine brucellosis surveillance in countries across the European continent, related to the epidemiological situation, time since the acquisition of OBF status, and characteristics of bovine livestock.

These results are discussed with regard to the EU Council Directive (which states the framework for surveillance in EU Member States) and are compared with surveillance components in other countries, including two OBF countries (Norway and Switzerland) and five non-OBF countries (Albania, Bosnia and Herzegovina, FYROM, Montenegro and Serbia). It is noted that, among non-OBF countries, Portugal implemented vaccination in a limited number of epidemiological units, with no consequence for the national surveillance scheme.

Among regionalised or non-OBF countries, six provided results about the presence of infected herds, with an estimated prevalence (calculated as the number of infected herds divided by the number of herds) ranging between 0.02% and 0.51%.

Abortion surveillance

Clinical surveillance was implemented in all responding countries but with varying notification rules. While the Council Directive requires the mandatory notification and investigation of abortions suspected of being due to brucellosis, no definition of a brucellosis-suspect abortion is provided. Our analysis indicated that, in most countries, an abortion was characterised by the expulsion of a foetus; in some cases, only those events occurring during a specific period of gestation were included. In other countries, the loss of pregnancy was considered as an abortive event, which is a broader definition than the expulsion of a foetus. Only Switzerland considered neither the expulsion of a foetus nor the loss of pregnancy as abortive events. Half of the countries included premature birth, stillbirth or the death of a calf within 12, 24 or 48 h following pregnancy among abortions. This is consistent with the fact that bovine brucellosis infection may cause the (premature) birth of weak calves, although these cases are rare (14).

While most countries required the notification and investigation of all abortions, few countries opted for the investigation of series of abortions. This latter policy aims at targeting primarily *Brucella* strains that cause abortion in a large proportion of infected females (8, 15, 16). Yet, in some cases, abortion may be less frequent, in

particular with *B. melitensis* (17), and may go undetected in infected herds (18). However, *B. melitensis* may be as virulent as *B. abortus* (19), with potential zoonotic consequences. For example, in 2012, an outbreak of *B. melitensis* in a dairy herd in France resulted in two human infections due to consumption of raw milk cheese (4).

Surveillance protocols requiring the notification of all abortions aim at maximising the sensitivity of the systems, thus leading to an earlier identification of brucellosis outbreaks. However, such systems suffer from a high level of abortion under-reporting (11, 20) for multiple reasons related to risk perception, health and economic factors, practical difficulties, and integration in socio-technical networks (21). Accordingly, the observed low proportion of herds notifying abortions (between 0.1 and 10.1% for countries with mandatory notifications of all abortions) underlines a widespread under-reporting of abortive events in European countries. This issue limits the ability of an abortion-based surveillance system to achieve early detection of any introduction of bovine brucellosis (as well as other diseases causing abortion, such as Rift Valley fever). Clinical surveillance requires the active participation of both farmers and veterinarians, and taking into account their perceptions would help improving the credibility of the system and thus their adherence (21). Therefore, targeting series of abortions instead of sporadic abortive events, whose cause is often presumed and excludes brucellosis infection, would be more relevant for field actors. Besides, the abortion aetiology is currently identified in only 25 to 60% of events (22). Providing technical support to veterinarians by implementing a differential diagnosis protocol (23) is expected to benefit farmers (via improved herd health and animal welfare, farm profitability, etc.), who would therefore be more likely to participate in the system.

Programmed serological surveillance

Routine herd surveillance aims to evaluate the prevalence of brucellosis-infected herds in a country (or region) or to demonstrate the absence of the disease in order to obtain or retain OBF status. Bovine brucellosis is a reproductive disease, transmitted primarily

through contact with the vaginal discharge of an infected cow, an aborted foetus or products of abortion (17), from infected breeding bulls or semen at the time of service (24), or through vertical transmission (24, 25). Therefore, while herds with reproductive bovines may be exposed to *Brucella*, fattening herds (veal-calf or bull) are not considered to be at risk and may be excluded from bovine brucellosis surveillance (5). However, relatively few countries apply this derogation: about one-third of OBF countries and one-third of non-OBF or regionalised countries. In other countries, fattening herds may be partly exempt from surveillance or subject to specific surveillance components. For instance, in Malta, males for fattening were exempted from routine testing but were tested at slaughter.

The rules regarding testing regimes and animals' age at testing are variable, depending on the brucellosis status, duration of OBF status, proportion of OBF herds, and herd-level prevalence. Programmed serological surveillance was implemented in four-fifths of the countries studied; the other fifth included only countries that had been OBF for more than five years, for which no rules about programmed surveillance are stipulated in the Council Directive. For the first five years after attaining OBF status at region or country scale, the Council Directive requires annual testing in not less than 20% of herds, on all animals over 24 months old. Most OBF countries (70%) and the regionalised countries (for their OBF regions) aligned with this rule or were more restrictive, with a higher proportion of herds tested and/or a lower minimum age at sampling. In other OBF countries, blood serology occurred either annually in 20% of animals over 24 months old in all herds (France) or at a lower frequency but combined with several milk tests per year in dairy herds (Northern Ireland and Great Britain), or with a lower minimum age at sampling (six months, Sweden) or a sampling of all bovines (Germany). These countries, except Northern Ireland, had been OBF for more than five years in 2015. In accordance with the EU regulation, non-OBF countries or regions implemented serological testing in more than 75% of herds, except Albania and Bosnia and Herzegovina, which are not EU Member States. Serological tests were conducted annually or twice a year in all bovines over 12 months old (which is requested for

countries with less than 1% of infected herds) or 24 months old (allowed in non-OBF countries/regions with more than 99.8% of OBF herds). Sampling is required for bovines over 12 months old because the available tests do not allow confident identification of exposed or infected prepubertal individuals (17). However, up to 10% of calves born to infected females may develop a latent infection, which remains in most cases serologically latent from birth to an age between nine months and the first pregnancy (26). Sampling at an earlier age (six months, as in Sweden) may allow the detection of some latent carriers before they develop clinical disease.

The Council Directive does not provide any sampling protocol to determine the herds to be tested. Risk-based surveillance is expected to yield both higher sensitivity and higher positive predictive value than surveillance conducted randomly across the population, provided that high-risk groups can be predicted from risk factors (27). Trading and movements of live cattle have been widely considered as the main pathways for brucellosis introduction into *Brucella*-free cattle herds (28, 29). Transmission from wildlife has also been demonstrated, with different reservoir species (30). Therefore, surveillance efficacy may be increased or the required sample size reduced by targeting herds that introduce a large number of bovines, have contact with other herds on pasture, or have potential contact with a wildlife reservoir.

Movement controls

Given the high risk of introduction of bovine brucellosis through bovine movements (28, 29), the Council Directive requires that a bovine entering a herd comes from a OBF herd and has been tested during the 30 days prior to or after the date of its introduction into the herd. However, the test is not required in countries/regions where the herd-level prevalence of infection has not exceeded 0.2% for at least two years. Overall, half of all countries conducted tests for cattle movements, either before departure from the herd of origin and/or after arrival in the new herd, including half of the non-OBF and regionalised countries, and one-third of OBF countries. The reasons for testing varied among countries depending on their status. Except

for two OBF countries that conducted systematic pre- and/or post-movement controls, other OBF countries restricted testing to export and import movements (sometimes only for bovines coming from a non-OBF country) or when the risk was increased. Among non-OBF and regionalised countries, systematic controls occurred before movement, completed by post-movement controls if no pre-movement tests had been conducted. Therefore, in these non-OBF and regionalised countries all movements were tested, except in Bosnia and Herzegovina where only bovines with unknown health status were tested. It is noteworthy that some non-OBF countries did not mention movement controls although the number of infected herds exceeded 0.2% in 2015.

Other surveillance measures

The use of infected semen in artificial insemination could be an important issue (24) that may be strictly controlled by regularly testing bulls used for artificial insemination. This is required by the Council Directive of 14 June 1988 laying down the animal health requirements applicable to intra-community trade in and imports of deep-frozen semen of domestic animals of the bovine species (31). In addition, all bovine animals kept at an approved semen collection centre must be subjected once a year to a serological test carried out in accordance with the procedure described in Directive 64/432/EEC with negative results. However, only a quarter of countries mentioned practising this surveillance measure. This unexpected result may be explained by a lack of clarity in the survey between measures applied in herds versus semen collection centres.

Slaughterhouse surveillance was used as a main surveillance stream in one country (with sampling of 80% of culled cows) or as a complement to routine herd screening in four countries (with sampling of either less than 25% or 25–50% of culled bovines). Since those countries were all OBF, this surveillance programme aims at documenting disease freedom and detecting re-emergence.

Programmed surveillance of wildlife was mentioned in Denmark only. The ongoing system is targeted at penned boars in close contact with

free-ranging wild boars and is aimed at demonstrating the absence of *B. suis* brucellosis from the country.

Diagnostic tests

All the serological tests used are approved by EU regulation, and the RBT, CFT, SAT, milk ring test and iELISA should be standardised according to international guidelines (5, 32). The inventory of laboratory tests performed by each country underlined the preferential use of a few tests (RBT as first-line test on serum samples, followed by CFT as confirmatory test for programmed, clinical and movement surveillance and also iELISA as a first-line test on bulk milk samples for programmed surveillance). The wide use of RBT as a screening test is explained by its high sensitivity, low cost and easy implementation (33, 34, 35). The iELISA on individual serum is also largely used as first-line assay: its performance is similar to that of the RBT, but it can be automated to allow testing of a high number of samples. The CFT shows a higher specificity than RBT, allowing its use as a confirmatory test (34). The iELISA on bulk milk is the only available method for detecting antibodies in milk that is suitable for high-throughput analyses. Despite its controversial performance (34, 36, 37), the cELISA is used as a confirmatory assay in nine countries. However, a large range of tests were reported as being used across countries, underlining that no single serological test is appropriate in all epidemiological situations and that a confirmatory strategy relies on a range of complementary tests, conducted separately or in parallel (7, 32). This variety of methods also raises the question of harmonisation within the EU, which is partially addressed by the existing reagent standardisation requirements in EU regulations and EU proficiency tests organised by the EURL. Recent results of EU proficiency tests showed a high level of performance and consistency of serological results (92% of satisfactory results) on a well-characterised panel of sera (EURL unpublished data).

Perspectives

Europe co-funded a portion of the bovine brucellosis surveillance and control measures in five Member States (Croatia, Portugal, Spain,

Italy and Northern Ireland), with a contribution of €10.9 million in 2015, representing about 5.2% of the total planned EU veterinary programmes that year (38). For many years, this programme (39) has contributed to the reduction in prevalence and eradication of bovine brucellosis and to the improvement of both animal and human health within the EU. The EU financial contribution represents a small part of the total cost incurred by countries to cover eradication programmes, and significant savings from the reduction of control costs can be made when a region gains the certification of freedom (40). In addition, maintaining a high level of surveillance to enable the early detection of brucellosis introduction in regions or countries free of the disease has a substantial impact on both state budgets and the agricultural profession. For example, the annual cost of surveillance was estimated to be about €17 million in France (41), €10.5 million in Spain (42), €1.9 million in Croatia (43), and €620,000 in the United Kingdom (44). The implementation of risk-based surveillance, which aims at detecting disease rather than providing representative prevalence estimates, may be more cost-effective in OBF countries (27, 45). Part of those savings could be re-injected into the system to improve the participation of field actors in clinical surveillance by providing support, enhancing risk communication, developing adequate diagnostic tools or implementing a national protocol for differential diagnosis of abortions (23). This study provides an inventory of possible surveillance strategies (according to country status). The evaluation and comparison of current system effectiveness and efficiency (effectiveness related to cost) with alternative surveillance measures, through modelling approaches (46, 47, 48), may help policy-makers to improve surveillance and resource allocation.

The participation of farmers and veterinarians is essential for the good functioning of clinical surveillance but incomplete abortion detection and reporting will remain the major limitations of such systems. Complementary syndromic surveillance systems may contribute to overcoming these issues to some extent (49). With regard to diseases causing abortion, recent studies have underlined the potential of different indicators (abortion incidence rate, calving interval, and birth

rate of live calves) calculated from routinely collected reproductive data (artificial inseminations or calving dates) to identify any increase in bovine abortions (50, 51, 52). However, these herds will be identified at best several months after the occurrence of abortions owing to the time needed for the aborting cows to be mated (or inseminated) and to calve (51). Although this surveillance approach cannot be used to detect new brucellosis outbreaks, such an approach may identify farmers with unreported abortion issues and may thus encourage them to participate in the system. As syndromic surveillance is non-specific, such an approach needs to be considered for the benefit of not only brucellosis but a wide range of abortive diseases.

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Table I

Surveillance of brucellosis in cattle in European countries during 2015, by official country status

Surveillance components	OBF countries/regions (total no.) names [a]	Regionalised countries	Non-OBF countries (total no.) names
Abortion	(3) **Finland**, Norway, Switzerland		
Abortion and herd-screening	(12) Austria, Belgium, Bulgaria, Czech Republic, Germany, **Latvia**, **Malta**, **Poland**, Romania, **Slovakia**, Slovenia, **Sweden**		(5) Albania, Croatia, FYROM, Montenegro, Serbia
Abortion and movement	(3) **Denmark**, **Ireland**, the **Netherlands**		
Abortion, herd screening and movement	(5) Estonia, **France**, **Great Britain**, **Lithuania**, **Northern Ireland**	(2) Italy, Spain	(4) Bosnia and Herzegovina, Cyprus, Hungary, Portugal

FYROM: former Yugoslav Republic of Macedonia

OBF: officially brucellosis free

a) Countries/regions that implemented additional surveillance components are indicated in bold

Table II

Countries in which a portion of the bovine population was exempted from surveillance during 2015, by official country status

Surveillance derogations	OBF countries (total no.) names	Regionalised countries	Non-OBF countries (total no.) names
Dairy cows			(1) Bosnia and Herzegovina
Fattening herds	(5) France, Ireland, Malta, Poland, Romania	(1) Spain [(a)]	(3) Croatia, Cyprus, Serbia

OBF: officially brucellosis free

a) In Spain, fattening herds are exempt from surveillance only if they do not have females older than 17 months of age among their number

Table III

Description of routine surveillance in herds, by official country status

Routine surveillance characteristics	OBF countries/regions (total no.) names	Regionalised countries	Non-OBF countries (total no.) names
Frequency of screening			
Monthly	(1) Northern Ireland (milk)		
Four times yearly	(1) Great Britain (milk)		(1) Cyprus (milk)
Twice yearly		(2) Italy (non-OBF regions), Spain (regions with low prevalence)	(3) Bosnia and Herzegovina, Cyprus (blood), Portugal (milk)
Annual	(13) Austria, Belgium, Bulgaria, Czech Republic, Estonia, France, Latvia, Lithuania, Malta, Poland, Romania, Slovakia, Slovenia	(2) Italy (OBF regions), Spain (regions with nil prevalence)	(7) Albania, Croatia, FYROM, Hungary, Montenegro, Portugal (blood), Serbia
Every two years	(1) Northern Ireland (blood)		
Every three years	(2) Germany, Sweden		
Proportion of herds sampled			
(0–25%)	(9) Austria, Czech Republic, Estonia, Latvia, Lithuania, Poland, Slovakia, Slovenia, Sweden		(1) Albania
(25–50%)			(1) Bosnia and Herzegovina
(50–75%)			
(75–100%)	(5) Bulgaria, France, Great Britain, Malta, Romania	(2) Italy, Spain	(7) Croatia, Cyprus, FYROM, Hungary, Montenegro, Portugal, Serbia
Cattle age (blood serology)			
>6 months	(1) Sweden		(1) Portugal (non-OBF herds)
>12 months	(2) Great Britain, Malta	(2) Italy, Spain	(7) Albania, Bosnia and Herzegovina, Croatia, Cyprus, FYROM, Montenegro, Serbia
>24 months	(14) Austria, Belgium, Bulgaria, Czech Republic, Estonia, France, Germany, Latvia, Lithuania, Northern Ireland, Poland, Romania, Slovakia, Slovenia		(2) Hungary, Portugal (OBF herds)
First-line tests			
Rose Bengal test	(9) Bulgaria, Czech Republic, France, Great Britain, Latvia, Malta, Poland, Romania, Slovenia	(2) Italy, Spain	(8) Albania, Bosnia and Herzegovina, Croatia, Cyprus, FYROM, Montenegro, Portugal, Serbia
iELISA pooled sera	(6) Bulgaria, Czech Republic, Estonia, Germany, France, Lithuania		
iELISA individual serum	(8) Austria, Bulgaria, Czech Republic, Germany, Great Britain, Lithuania, Northern Ireland (high-risk samples), Sweden	(1) Spain	(1) Hungary

Complement fixation test	(1) Slovakia	(1) Spain	(1) Bosnia and Herzegovina
Serum agglutination test	(2) Belgium, Northern Ireland (low- and high-risk samples)		
iELISA bulk milk	(10) Austria, Belgium, France, Germany, Great Britain, Latvia, Lithuania, Northern Ireland, Romania, Sweden	(2) Italy, Spain	(2) Cyprus, Portugal
iELISA individual milk	(2) Germany, Lithuania		
iELISA pooled milk [(a)]	(1) Estonia		
Ring test	(1) Malta		
Culture on post-mortem samples	(4) Belgium, Great Britain, Poland, Slovakia		
Culture on vaginal swabs	(1) Great Britain		
Confirmatory tests			
Rose Bengal test	(8) Austria, Estonia, France, Germany, Latvia, Lithuania, Poland, Slovakia		(2) Cyprus, Hungary
iELISA individual serum	(11) Belgium, Bulgaria, Croatia, Estonia, Germany, Great Britain, Latvia, Malta, Northern Ireland, Poland, Romania	(1) Spain	(3) Bosnia and Herzegovina, Montenegro, Serbia
Complement fixation test	(17) Austria, Bulgaria, Czech Republic, Croatia, Estonia, France, Germany, Great Britain, Latvia, Lithuania, Malta, Northern Ireland, Poland, Romania, Slovakia, Slovenia, Sweden	(2) Italy, Spain	(8) Albania, Bosnia and Herzegovina, Cyprus, Hungary, FYROM, Montenegro, Portugal, Serbia
Serum agglutination test	(4) Latvia, Northern Ireland, Poland, Slovakia		(1) Hungary
cELISA	(6) Bulgaria, Croatia, Estonia, Great Britain, Romania, Slovakia	(1) Spain	(3) Bosnia and Herzegovina, Montenegro, Serbia
Brucellin skin test	(1) Belgium		
iELISA bulk milk	(2) France, Northern Ireland		
iELISA individual milk	(3) Bulgaria, Germany, Northern Ireland		
Ring test	(1) France		
Culture on milk samples	(1) Poland		(1) Cyprus
Culture on post-mortem samples	(3) Belgium, Poland, Romania	(1) Spain	(1) Cyprus
Culture on vaginal swabs	(2) Estonia, Lithuania	(1) Spain	
PCR on tissue samples	(2) Lithuania, Poland		
Final confirmatory tests			
Culture	(17) Austria, Belgium, Croatia, Czech Republic, Estonia, France, Germany, Great Britain, Latvia, Lithuania, Malta, Northern Ireland, Poland, Romania, Slovenia, Slovakia, Sweden	(1) Spain	(5) Albania, Cyprus, FYROM, Hungary, Portugal
PCR	(10) Bulgaria, Croatia, Czech Republic, Estonia, Germany, Lithuania, Malta, Romania,	(1) Spain	(3) Bosnia and Herzegovina, FYROM, Hungary

	Slovenia, Slovakia	
Serological tests (iELISA, cELISA, CFT, milk ring test)	(2) France, Germany	(2) Bosnia and Herzegovina, Montenegro

cELISA: competitive enzyme-linked immunosorbent assay

FYROM: former Yugoslav Republic of Macedonia

iELISA: indirect enzyme-linked immunosorbent assay

OBF: officially brucellosis free

PCR: polymerase chain reaction

a) Indicated as 'Other test' in the survey

Table IV

Proportion of countries using each bovine brucellosis screening method according to the surveillance component

	Programmed surveillance [a]		Abortion surveillance		Movement surveillance
Methods	**First-line**	**Confirmation**	**First-line**	**Confirmation**	
Serum samples					
Rose Bengal test	68% (19/28)	36% (10/28)	47% (16/34)	39% (12/31)	64% (7/11)
iELISA on pooled bovine sera	21% (6/28)	–	–	–	18% (2/11)
iELISA on individual bovine serum	36% (10/28)	54% (15/28)	26% (9/34)	45% (14/31)	36% (4/11)
Complement fixation test	11% (3/28)	96% (27/28)	26% (9/34)	77% (24/31)	18% (2/11)
Serum agglutination test	7% (2/28)	18% (5/28)	6% (2/34)	16% (5/31)	18% (2/11)
cELISA	–	36% (10/28)	3% (1/34)	29% (9/31)	–
Brucellin skin test	–	4% (1/28)	–	–	–
Milk samples			–	–	
iELISA on bulk milk	88% (14/16)	33% (2/6)	–	–	9% (1/11)
iELISA on individual milk	13% (2/16)	50% (3/6)	–	–	–
Ring test	6% (1/16)	17% (1/6)	–	–	–
iELISA on pooled bovine milk [a]	6% (1/16)	–	–	–	–
Culture on milk samples	–	33% (2/6)	9% (3/34)	6% (2/31)	–
Other samples					
Culture on post-mortem samples	100% (4/4)	71% (5/7)	–	19% (6/31)	–
Culture on vaginal swabs	25% (1/4)	43% (3/7)	9% (3/34)	13% (4/31)	–
Culture from aborted foetus	–	–	50% (17/34)	–	–

PCR on tissue samples	–	14% (1/7)	–	16% (5/31)	–
PCR on aborted foetuses [(b)]	–	–	6% (2/34)	–	–
Stamp's modification of the Ziehl–Neelsen stain [(b)]	–	–	3% (1/34)	–	–

cELISA: competitive enzyme-linked immunosorbent assay

iELISA: indirect enzyme-linked immunosorbent assay

PCR: polymerase chain reaction

(a) Following the first-line screening and the confirmatory test, an additional test (PCR, culture or serology) is conducted in 26 countries to definitely confirm the infection – see text for details

(b) Indicated as 'Other test' in the survey

Table V
Description of abortion surveillance in herds, by official country status

Abortion surveillance characteristics	OBF countries/regions (total no.) names	Regionalised countries	Non-OBF countries (total no.) names
Abortion definition			
Loss of pregnancy	(10) Belgium, Czech Republic, Finland, Germany, Ireland, Latvia, Northern Ireland, Romania, Slovakia, Slovenia		(6) Albania, Bosnia and Herzegovina, Cyprus, Montenegro, Hungary, Serbia
Expulsion of foetus	(18) Austria, Belgium, Bulgaria, Denmark, Estonia, France, Germany, Great Britain, Latvia, Lithuania, Malta, Northern Ireland, Norway, Poland, Romania, Slovakia, Slovenia, the Netherlands	(2) Italy, Spain	(8) Albania, Bosnia and Herzegovina, Cyprus, FYROM, Hungary, Montenegro, Portugal, Serbia
Stillbirth	(11) Belgium, Finland, France, Germany, Great Britain, Hungary, Latvia, Malta, Northern Ireland, Norway, Slovakia	(1) Spain	(4) Albania, Bosnia and Herzegovina, Cyprus, Montenegro
Death within 12 hours of birth	(1) Great Britain		
Death within 24 hours of birth	(2) Belgium, Northern Ireland		
Death within 48 hours of birth	(2) Finland, France		(1) Albania
Premature birth	(3) Finland, Norway, Switzerland	(1) Spain	(3) Albania, Bosnia and Herzegovina, Cyprus
Abortion notification			
Not mandatory	(3) Denmark, Romania, Sweden		
Mandatory after one abortion	(17) Austria, Belgium, Czech Republic, Estonia, France, Finland, Germany, Great Britain, Ireland, Latvia, Lithuania, Malta, Northern Ireland, Poland, Slovakia, Slovenia, the Netherlands	(1) Italy	(8) Albania, Bosnia and Herzegovina, Croatia, Cyprus, Hungary, Montenegro, Portugal, Serbia
Mandatory after a series of abortions	(2) Bulgaria, Norway		(1) FYROM
Variable (see text for details)	(1) Switzerland (depends on herd type)	(1) Spain (depends on geographical areas)	
First-line tests following abortion			
Rose Bengal test	(9) Czech Republic, Estonia, Finland, France, Great Britain, Latvia, Malta, Poland, Romania	(2) Italy, Spain	(5) Bosnia and Herzegovina, Croatia, Cyprus, Montenegro, Serbia
iELISA individual serum	(8) Austria, France, Ireland, Lithuania, Malta, Northern Ireland, Norway, Switzerland		(1) Hungary
Complement fixation test	(6) Bulgaria, Czech Republic, Ireland, Latvia, Romania, Slovakia	(2) Italy, Spain	(1) Cyprus
Serum agglutination test	(2) Northern Ireland, the Netherlands		

Culture on vaginal swabs	(1) Estonia	(1) Spain	(1) Albania
Culture on aborted foetus	(12) Austria, Belgium, Denmark, Estonia, Finland, Germany, Ireland, Latvia, Romania, Slovakia, Slovenia, Sweden	(2) Italy, Spain	(4) Albania, Cyprus, Hungary, Portugal
Culture on milk sample		(2) Italy, Spain	(1) Albania
cELISA	(1) Bulgaria		
PCR on aborted foetus			(1) FYROM
Stamp's modification of the Ziehl–Neelsen stain [(a)]	(1) Switzerland		
Confirmatory tests following abortion			
Rose Bengal test	(10) Austria, Czech Republic, Germany, Latvia, Lithuania, Romania, Slovakia, Slovenia, Switzerland, the Netherlands		(3) Cyprus, Hungary, Portugal
iELISA individual serum	(10) Belgium, Bulgaria, Germany, Great Britain, Ireland, Latvia, Northern Ireland, Norway, Romania, the Netherlands	(1) Spain	(3) Bosnia and Herzegovina, Croatia, Montenegro
Complement fixation test	(17) Austria, Bulgaria, Czech Republic, Estonia, Finland, France, Germany, Ireland, Latvia, Lithuania, Norway, Poland, Romania, Slovakia, Slovenia, Switzerland, the Netherlands	(2) Italy, Spain	(6) Bosnia and Herzegovina, Croatia, Cyprus, Hungary, Montenegro, Serbia
Serum agglutination test	(4) Latvia, Slovakia, Northern Ireland, Poland		(1) Hungary
Culture on vaginal swabs	(3) France, Lithuania, Norway		(1) Albania
Culture on milk sample			(2) Albania, Cyprus
cELISA	(4) Estonia, Malta, Romania, Slovakia		(4) Bosnia and Herzegovina, Croatia, Montenegro, Serbia
Culture on post-mortem tissues	(4) Austria, Estonia, Latvia, the Netherlands		(2) Albania, Cyprus
PCR on tissue sample	(5) Latvia, Lithuania, Norway, Slovakia, the Netherlands		

cELISA: competitive enzyme-linked immunosorbent assay

FYROM: former Yugoslav Republic of Macedonia

iELISA: indirect enzyme-linked immunosorbent assay

OBF: officially brucellosis free

PCR: polymerase chain reaction

a) Indicated as 'Other test' in the survey

Table VI
Description of movement surveillance in herds, by official country status

Movement surveillance characteristics	OBF countries/regions (total no.) names	Regionalised countries	Non-OBF countries (total no.) names
Conditions for pre-movement tests			
Systematic control	(2) Estonia, Lithuania	(2) Italy, Spain (non-OBF regions only)	(3) Cyprus, Hungary, Portugal
Export/import certification	(3) Denmark, Great Britain, the Netherlands		
Control required by risk assessment	(1) Northern Ireland		
Bovine with unknown health status			(1) Bosnia and Herzegovina
Conditions for post-movement tests			
Systematic control	(1) Lithuania		
Export/import certification	(4) Belgium, Denmark, Great Britain, the Netherlands		
If no pre-movement test			(3) Cyprus, Portugal, Spain
Transport duration longer than 6 days	(1) France		
Other types of movements with controls			
Animal transhumance	(1) Lithuania	(2) Italy, Spain	(1) Portugal
First-intention tests			
Rose Bengal test	(2) Estonia, France	(2) Italy, Spain	(3) Bosnia and Herzegovina, Cyprus, Portugal
iELISA individual serum	(3) France, Great Britain, Lithuania		(1) Hungary
iELISA pooled sera	(2) France, Lithuania		
Complement fixation test		(1) Spain	(1) Portugal
Serum agglutination test	(2) Great Britain, Northern Ireland		
iELISA individual milk	(1) Lithuania		
iELISA bulk milk	(1) Lithuania		

FYROM: former Yugoslav Republic of Macedonia

iELISA: indirect enzyme-linked immunosorbent assay

OBF: officially brucellosis free

PCR: polymerase chain reaction