Quantitative assessment of the probability of introducing bovine brucellosis into English cattle herds by imported live cattle

Running title: Probability of introducing bovine brucellosis into English cattle herds by imported cattle

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Abstract

A stochastic simulation model was developed to estimate the quarterly probability (*PIntro*) of introducing bovine brucellosis into English cattle herds, by at least one imported live cattle (potential carrier of *Brucella abortus*). The probability of spread after introduction was not included and imports from several countries were considered. Information used to parameterise model´s inputs was obtained from the literature, legislation and analysis of several national datasets (2013 to 2016), which contained information on imported cattle and testing schemes used in the English cattle population. Exporting countries were divided according to official brucellosis status *"J"* into: Officially Brucellosis Free (OBF), Non-Officially Brucellosis Free (Non-OBF) and in OBF-Validation (during the first five years of OBF status). The entire English cattle population was divided into eight strata *"S"* by combination of laboratory testing data and herd type. The only risk mitigation measure considered was the testing for antibodies on animals older than one year and imported from Non-OBF countries. Probabilities of introduction at herd and stratum level were combined into the overall national *PIntro*. Two scenarios were run. In the baseline scenario, the between herds prevalence *BHP(OBF)* in OBF countries was set according to information from EFSA and from the EU legislation. In the alternative scenario only the former was used and *BHP(OBF)* was set very low/negligible. For Non-OBF and OBF-Validation countries, the *BHP* was set with distributions based on the literature. In the baseline scenario, between 2013 and 2016, the quarterly median *PIntro* ranged from 1.3% to 5.5%. For the last year considered, the median of the quarterly medians *PIntro* was 2.8% (median of 5th percentiles = 0.4%; median of 95th percentiles =10.7%). Therefore, on average, at least one introduction could be expected each (approximated) 36 surveillance periods (9, 281), so each ≈ 9 years (2; 70). According to the alternative scenario, the *PIntro* was very low and on average at least one introduction could be expected each ≈ 125 years.

Key words: Bovine brucellosis, imported cattle, quantitative assessment, probability of introduction

Introduction

England, as part of Great Britain (GB)[1], has been Officially Brucellosis Free (OBF) since 1985 (Hesterberg et al., 2009), although two re-introductions of bovine brucellosis occurred by imported cattle in 1993 and 2003, while in 2004 a third outbreak of unknown origin occurred in Cornwall.

Bovine brucellosis is mainly caused by the bacterium *Brucella abortus,* less frequently by *B. melitensis* and *B. suis* (OIE, 2009). For the latter, the extent to which this species can cause disease in bovines is less clear. From a general point of view, abortion, retained placenta, orchitis, and infertility are the main symptoms of cattle infected with *B. abortus*. Large numbers of organisms are excreted in abortion and birth material in the uterine discharges. Organisms can also be shed in milk. Transmission among cattle can occur by contact with contaminated material, venereal (not common in cattle other than by artificial insemination) and oral route (e.g. due to ingestion of milk from infected cows, or feed/water contaminated with birth products or excreta from infected animals) (Akhtar and Mirza, 1995; CFSPH, 2009). Moreover, brucellosis is a notifiable zoonotic disease, which can be transmitted from cattle to humans through abraded skin and by oral (e.g. unpasteurized milk), respiratory or conjunctival pathways, causing the debilitating disease known as "undulant fever" (CFSPH, 2009; OIE, 2009).

The epidemiology of brucellosis in cattle can be affected by several factors such as: the environment (e.g. temperature, humidity, etc.), the herd structure, the herd type and management (Bercovich, 1998; CFSPH, 2009, Matope et al., 2010; Godfroid et al., 2011; Makita et al., 2011; Mai et al., 2012; Assenga et al., 2015). Uncertainty persists on the actual epidemiological significance of the potential pathways of disease spread within and between herds. For instance, it is known that infected animals can remain latent carriers (Dolan, 1980; Priyantha, 2011) and in some review papers it is mentioned that, as well as in aborted material and milk, *B. abortus* can also be found in urine and semen (CFSPH, 2009). Some other authors have argued that calves fed with colostrum from infected dams could constitute a risk to *Brucella* free herds and that those calves may be responsible for the spread of infection to other calves (within the same pen) (Akhtar

[1] N.B. A list of abbreviations is provided at the end of this article in the Appendix (Table 1).

and Mirza, 1995; Bercovich, 1998). Regarding the spread of disease by infected bulls, it is usually assumed that this depends on how those animals are used. Makita et al. (2011) found that, the use of bulls was not a significant risk factor for brucellosis at the herd level. The herd prevalence was similar between herds using artificial insemination and herds with natural mating, but animal prevalence was higher using bull/s (5.5%) than using artificial insemination (3.2%). Bercovich (1998) stated that "if the bull is used for natural service, it *may fail* to spread the infection since the infected semen is not deposited in the uterus". In contrast, in a more recent study, the prevalence of brucellosis was found higher in naturally inseminated cattle and buffalos than in artificially inseminated animals, though such a difference was not statistically significant (Basit et al., 2015). Thus, if infected young animals and/or infected bulls can not be excluded (a priori) as potential shedders of *B. abortus*, then movement of infected cattle (including latent carriers of any age and sex) could be considered as a pontential source of disease spread between countries.

In GB, between 2004 and 2009, three studies were carried out to evaluate: i) the risk of disease reintroduction from abroad (Jones et al., 2004), ii) the rate of disease spread under a variety of testing regimes (England et al., 2004), and iii) the performance of the local surveillance system (Hesterberg et al., 2009). Jones et al. (2004) estimated that on average brucellosis-infected cattle could be imported into GB every 2.63 years from Northern Ireland (NI) and every 3.23 years from the Republic of Ireland (ROI). England et al. (2004) suggested that abortions notifications are an important mean of surveillance to limit the probability of between-herds disease spread before detection. Hesterberg et al. (2009) found that the surveillance system sensitivity (*SSe*) and the confidence in OBF status (*PFree*) (Martin et al., 2007a-b) could be maintained as high (> 95%) if monthly bulk tank milk (BTM) testing was used in dairy herds, and if abortion notifications were continued to be used, especially in beef herds.

During recent years, the status of cattle herds in NI and ROI has changed. Currently (beginning-2020) ROI is OBF, while NI is in the "OBF-Validation" [2] period (since October 2015 until the same month of 2020).

[2] For the purpose of this paper, countries that have been OBF for more than five years (as ROI since August 2014) were referred to as OBF, while those that had been OBF for less than five years were referred to as OBF- Validation. See sections below about assumptions and between-herds prevalence.

Furthermore, since April 2011 BTM is tested quarterly in all three GB countries: England, Scotland and Wales. Additionally, during the last decade (2010 onwards), the number of statutory submissions of samples from aborted cows has steadily decreased (unpublished data, personal communication by Dr. Martyn Blissitt, Veterinary Adviser - Notifiable Diseases - Animal Health and Welfare Division, Agriculture and Rural Economy Directorate, Scottish Government).

Due to the changes in the health status of the main trading partners of GB (NI and ROI) and due to the changes in the local testing frequency and coverage, a review of the three national GB surveillance systems was considered necessary by the Department for Environment Food and Rural Affairs (Defra), the Scottish and the Welsh Governments. Such a review, had two objectives for each GB country: I) assessing the probability of disease introduction (*PIntro*) from abroad and II) assessing the *SSe* and the *PFree* at the between herds design prevalence (*Ph* ≤ 0.2%) set in the EU legislation (Council Directive, 64/432/EEC).

The main aim of this article was to estimate the *PIntro* (objective I above). England was used as example GB country, because according to the four years data (2013 to 2016) used for this study, approximately 66-68 % of the GB herds are located in this country.

Materials and methods

A stochastic simulation model was developed in @Risk-6 (Palisade Corporation). Model´s inputs, assumptions and structure were based on information from the literature, legislation and analysis of national data.

The model was run with 20,000 iterations and Latin Hypercube sampling. For deciding how many iterations to use, the convergence of the model was checked every 100 iterations, on any combination of mean, standard deviation, and percentile for the main output (*PIntro*). The convergence tolerance was specified at 3% and the confidence level at 95% (Palisade Corporation, 2019).

The stochasticity of the model was set by using probability distributions in some of the inputs e.g. for the within (*WHP*) and between-herds (*BHP*) prevalence abroad, for the test sensitivity (*Se*) and for the number of animals (*N_anim*) imported per consignment into English herds.

General assumptions

The probability of disease spread and consequences after eventual introduction of at least one infected animal from abroad were not considered, and thus, the term "probability of introduction or *PIntro"* (Martin et al., 2007a-b) is used throughout the paper.

B. abortus was considered as the main disease agent of bovine brucellosis. The probability of introduction of other types of *Brucella* (e.g. *melitensis*) was not investigated. Therefore, the main (potential) source of disease introduction from abroad was considered to be the imports of cattle of any sex and age, which could be carriers of *B. abortus* (Dolan 1980, Akhtar and Mirza, 1995, CFSPH, 2009, Priyantha, 2011; Basit et al., 2015). In GB, if an animal is found to be seropositive it is put under restrictions. Thus, from a technical point of view, antibody positive cattle would be considered as positive carriers by the risk mitigation measures and in the local surveillance system.

Imports of embryos and semen were considered during the literature review, but were not included in the model, because their actual role for disease introduction was assumed marginal due to the high biosecurity measures that need to be implemented before international trade (Council Directive, 89/556/EEC; Council Directive 2003/43/EC; Council Directive, 64/432/EEC).

Countries from which animals were imported were divided into OBF, Non-OBF and in OBF-Validation. European Member States may be declared OBF if no case of abortion due to *Brucella* infection and no isolation of *B. abortus,* has been recorded for at least three years and if at least 99,8% of herds have achieved OBF status each year for five consecutive years (Council Directive 64/432/EEC, Annex A, art. 7a). Thereafter, the OBF status can be retained if "every year for the first five years after attaining status, all bovine animals over 24 months of age in not less than 20 % of herds have been tested and have reacted negatively to a serological test" (Council Directive 64/432/EEC, Annex A, art. 8b). After five years of OBF status, the level of surveillance could be reduced. For the purpose of this paper, countries that had been OBF for more than five years were referred to as OBF, while those that had been OBF for less than five years were referred to as OBF- Validation.

This distinction was used because according to the Operational Manual (OM) of APHA (not published), animals older than one year and imported from Non-OBF countries, should be tested at arrival and (eventually) at their first post import calving (PIC) in GB, with an antibody indirect Enzyme-Linked Immunosorbent Assay (iELISA) (McGiven et al., 2003; McGiven et al., 2008a-b; Thomson et al., 2009). If applicable, animals from OBF-Validation countries are subjected to PIC testing, while animals from OBF countries are not required to be tested. The PIC testing was considered as a surveillance component of the local cattle population and was disregarded as risk mitigation measure here in this model, because animals could calve several months after import.

Data used

Data were extracted from different national databases and were combined by the investigated surveillance period (Q) and by the County Parish Holding (CPH) number, which is the herd identification number. The data files were handled and analysed using the free statistical software R (R Core Team 2013-04-03, R-3.0.0). Results of data analysis were used to inform the model and are reported in the Appendix (Tables 2 to 4, Figures A-B).

The overall population of English cattle herds considered for each Q-period was defined according to monthly data obtained from the Cattle Tracing System (CTS) and from the Rapid Analysis and Detection of Animal Related Risks (RADAR). All datasets were extracted for years 2013 to 2016 (corresponding to 16 quarterly surveillance periods), because the SAM database, which was used to complete the list of milking herds (see below), was stabilized in 2013. Herds with CPHs of more than 10 digits (including separation bars) were considered as the population of interest (production herds), while herds with four digits were considered as non-producing herds (e.g. slaughterhouses).

Data on imported live cattle (from CTS) included: the ear tag of the imported animal, its country of origin, the CPH of arrival in England, and the date of arrival.

According to surveillance testing data from the Laboratory Information Management System (LIMS), all tested herds and animals were classified as negative. Datasets on abortion and PIC testing contained information on: CPH, date of testing, and test/s used per cow.

Datasets on quarterly BTM testing (LIMS) contained records for England and Wales. Information was available on: the CPH of the tested herds (for approximately 75% of the data lines, depending on year), their address, the date when the BTM sample was received by the National Milk Laboratories (NML) and the date of testing at the APHA laboratories. For records where the CPH was not entered, this was found in other databases such as CTS and SAM (which contains records of testing for bovine tuberculosis) by matching the postcode of the herd.

Population stratification

The model was structured in a way that, probabilities of disease introduction from abroad could be related to herd type and local surveillance components. In this way, the model could be used in other studies to inform surveillance system evaluation, as shown in Foddai et al. (2020). Thus, Q-periods of three months each were considered because, as explained above, BTM is tested quarterly.

For each Q-period, herds were classified as “M” (for milk) if tested at least in the BTM, while all the others were classified as “B” (for beef, non-dairy). Each combination of herd type (M or B) and local surveillance component represented an independent population stratum.

Accordingly, dairy herds were divided into four strata: herds BTM tested only (M), herds tested in BTM and abortion/s (M-Abo), herds tested in BTM and PIC (M-PIC), and herds tested in BTM, abortion/s and PIC (M-A-PIC) on the same Q-period.

Beef herds (B) were divided as: herds tested in abortion/s (B-Abo), herds tested in PIC (B-PIC), herds tested in abortion/s and PIC (B-A-PIC), and herds not tested at all at the APHA laboratories (B-NoTest) during the investigated Q-period. The latter were still considered as a stratum, though not tested at the APHA, because they could still import animals and introduce disease.

Between-herds prevalence in OBF countries.

In the baseline simulation scenario, for the OBF countries, the annual between-herds prevalence *BHP(OBF)* was set as a Pert distribution ranging from 0 (no infected herds in the sending country) to 0.2%, which is the design prevalence (*Ph*) from the EU legislation (Council Directive 64/432/EEC). This represented a large value because, apart from Belgium, OBF countries sending cattle to England (Appendix, Table 2) did not report any case during the investigated years (2013-2016). Thus, the *BHP(OBF)* distribution represented the uncertainty on the potential "true" *BHP* value for OBF herds, at which disease could be present abroad before detection.

The most likely value was set as uniform distribution ranging from 0% to [Beta (1+1, 1,375,934 – 1+1)]. The latter was a Beta distribution with $\alpha = n+1$ and $\beta = N - n+1$, where n = number infected herds out of the total population of herds (N) considered. According to EFSA (2015), only one herd out of 1,375,934 OBF cattle herds (from 16 OBF Member States) was classified positive in Belgium, during 2013. Hence, in line with official reporting from OBF Member States (EFSA, 2015), the most likely annual *BHP(OBF)* was assumed ranging from 0% to negligible, if a cut-off = 10^{-4} was considered (EFSA, 2012; WHO and FAO, 2009).

Moreover, the annual reported herds prevalence was divided by four, to obtain an average incidence estimate per quarter of a year (Q-1 to Q-4). Thus, the eventual herd incidence and herd prevalence were assumed to be similar (maximum a few infected herds per Q-period).

Between-herds prevalence in Non-OBF countries

The quarterly between-herds prevalence for Non-OBF countries *BHP(Non-OBF)* was also set as a Pert distribution (0.0%, ≈ 0.07%; 0.4%). In that case, the minimum was 0.0% (no infected herds present in the Non-OBF country) while the maximum was the *BHP* from Italy (EFSA, 2016). The most likely value (≈ 0.07%) was the median simulated from all *BHP* inputs assigned to the single Non-OBF countries, which sent animals to England.

The quarterly *BHP* inputs for Italy and Spain were calculated as (598/40,025)/4 = 0.4% and (47/120,329)/4 = 0.01%, respectively (EFSA, 2016).

For Canada, Jersey, Guernsey and Hungary the quarterly *BHP* was set as described above for the OBF countries, because those four Non-OBF countries were supposed to have a similar *BHP* to OBF Member States. In fact, according to Pare' et al. (2012) Canada had no infected cattle herds (< 0.02% with 95% confidence), while Jersey, Guernsey and Hungary (EFSA, 2007) should also have had herd prevalence around 0%.

Only four animals were imported from Greece into English cattle herds during the investigated period (Appendix, Table 2). Thus, a *BHP* value for this country was not included to calculate the (median) most likely *BHP(Non-OBF)*, which was considered as sufficiently represented by the other mentioned Non-OBF countries.

Between-herds prevalence in OBF-Validation countries

ROI and NI changed their brucellosis status during the four considered years. Those changes were taken into account in the model. For instance, ROI was simulated in OBF-Validation until the end of 2014, but the *BHP* for this country was set equal to that of OBF countries, because no cases have been reported during the investigated years.

NI was Non-OBF between 2013 and the last quarter of 2015 (Q-4-15) when it started the validation period. The *BHP* used for this country, was based on values reported by the Department of Agriculture, Environment and Rural Affairs (DAERA; 2016) during years 2013-2016, when the overall annual herd incidence was 0.13%, 0.04%, 0.00% and 0.03% in 2013, 2014, 2015 and 2016; respectively.

Moreover, because the incidence ranged between 0.0% and 0.17% (Armagh in 2013) depending on regions, the *BHP* of NI was set as a Pert distribution, where the most likely value was the annual herd incidence mentioned above. The minimum was 0.0% while the maximum was the value reported in the region with the highest incidence in the country during the investigated year. For example, in 2013, the annual herd incidence was 0.13% and ranged from 0.0% in Londonderry to 0.17% in Armagh. Accordingly, for each surveillance period (Q-1, 2, 3 and 4) of that year, the average quarterly *BHP(OBF-Validation)* was set as: RiskPert (0.0%, 0.13%, 0.17%) / 4.

Within-herd prevalence abroad (WHP).

The uncertainty about within herd prevalence (*WHP*) in infected herds of countries exporting cattle to England (before their detection), was described by a Uniform distribution (from 1% to 10%), and using estimates by Pehlivanoğlu et al. (2011) from Turkey (country with endemic disease status at the time of that study). This distribution was used for all the countries exporting cattle to England, due to lack of data on the main trading partners, where the disease has been eradicated for a long time, or is present in a few regions at low prevalence (Commission Decision 2003/467/EC; EFSA, 2016).

Moreover, Pehlivanoğlu et al. (2011), quoted another study by Çelebi and Otlu (2011) (in Turkish), where a similar prevalence of cows carrying *B. abortus* in milk and uterine infections was observed. Therefore, the *WHP* used in this assessment could be considered representative of antibody positive cows, but also of potential carriers and shedders of *Brucella abortus.*

Furthermore, a similar *WHP* input has been used to evaluate the surveillance system in France (Hénaux and Calavas, 2017), which is an example of OBF countries exporting cattle to England during 2013-2016 (Appendix, Table 2).

Test sensitivity

For Non-OBF consignments, it was assumed that the (eventually) infected, imported and tested animal could have resulted false negative to the test, with probability 1-*Se*. The *Se* was the sensitivity of the serum antibody iELISA used on animals older than one year and imported from Non-OBF countries. Uncertainty around this input was set after consultation of experts from the APHA laboratories (Weybridge, UK) and according to literature.

Firstly, a sensitivity input was set for each estimate from each considered study (McGiven et al., 2003; McGiven et al., 2008a-b; Thomson et al., 2009). When a range or a confidence interval was available, a Pert distribution was used to represent uncertainty within study. For instance, in McGiven et al. (2003) the mean sensitivity was estimated to 97.2% (95% confidence interval: 94.6%; 99.9%) and this input was set as Pert (94.6%; 97.2%, 99.9%). For McGiven et al. (2008b) and for Thomson et al. (2009) the sensitivity distributions were set as Pert (89.2%; 96.3%, 100%) and (89.1%; 100%, 100%), respectively.

Secondly, to represent uncertainty between studies, an overall Pert distribution was set for the final *Se* input used. Then, *Se* = Pert distribution ranging from 86.7% (McGiven et al., 2008a) to 100% (McGiven et al., 2008b; Thomson et al., 2009), whereas the most likely value was 95.5% (overall median across 20,000 iterations, by using all individual sensitivity distributions).

Probability that the consignment imported from an infected herd was infected

A consignment was defined as the delivery to a specific English herd during a single day of one or more animals from a single country status "*J*". It was assumed that an imported consignment arrived from a single herd abroad.

Furthermore, since animals arrived into England during the investigated periods (Appendix, Tables 2 to 4) it was assumed that their herd of origin abroad was classified as OBF and was not under restriction on the day of export (Council Directive 64/432/EEC).

Then, if the herd abroad was infected and was undetected (so exported); the probability *PInf_Con(J)* that the imported consignment from country status *"J"* included at least one infected animal was calculated. For consignments arriving from Non-OBF countries the *PInf_Con(J)* was calculated as:

$$PInf_{Con(Non\text{-}OBF)} = 1 - [1 - (WHP * Prop_{Test} * (1 - Se) + WHP * (1 - Prop_{Test}))]^{Nanim} \quad \text{(Eq. 1)}$$

In Figure 1 are shown the inputs and the conditional steps used within Eq. 1 as: P1 = *WHP* * *Prop_Test* * (*1- Se*) and P2 = *WHP* * (1- *Prop_Test*). For consignments arriving from OBF or OBF-Validation countries, Eq. 1 was modified and only *WHP* was kept within the squared brackets, because testing was not needed.

Prop_Test was the probability that the animal imported from a Non-OBF country was older than 12 months and was tested. From the data analysis it was known that between 0.0% (e.g. in Q-4-16) and 27.8% (Q-2-14) of the animals imported quarterly from Non-OBF trading partners were older than one year. Thus, *Prop_Test* was set according to actual data for each surveillance period.

N_anim was the number of animals present in the consignment. Variability on this input was set as Pert distribution according to results of data analysis (Appendix, Tables 3 and 4).

Probability that the importing herd became infected

A local English herd was considered as infected if it received at least one infected consignment containing at least one positive animal (potential carrier) which entered in the herd because: I) it was missed at testing if older than one year and arriving from a Non-OBF country, or II) it was not tested.

The probability *PInf_Herd(S,J)* that an importing English herd located within a specific stratum "*S*" became infected by one or more consignments from country status *"J"* , during period Q, was estimated as:

$$PInf_{Herd(S,J)} = 1 - [1- BHP_{(J)} * PInf_{Con(J)}]^{Ncon(S,J)} \quad \text{(Eq. 2)}$$

Where, *N_con(S,J)* was the quarterly median number of consignments from country status *"J"* received per English herd located within stratum "*S*". According to data analysis the median *N_con(S,J)* was = 1 in 14 out of 16 Q-periods, while in Q-2-15 and Q-4-16 the median *N_cons(S,J)* was = 2. Those values were used in Eq. 2, to simulate *N_con(S,J)* in the respective Q-periods.

Probability of infection per population stratum and at national level

A stratum *"S"* was considered infected, if at least one of its importing herds introduced at least one infected animal/consignment from any country status *"J".* Then the probability of disease introduction per stratum *PInf(S,J)* was calculated as:

$$PInf_{(S,J)} = 1- [1- PInf_{Herd(S,J)}]^{N_{herds(S,J)}} \quad \text{(Eq. 3)}$$

In this case, *PInf(S,J)* was the quarterly probability that at least one of the herds *N_herds(S,J)* within the stratum "*S*" (M, or M-Abo, or M-PIC, or M-A-PIC, or B-Abo, or B-PIC, or B-A-PIC, or B-NoTest) which imported consignment(s) from country status “*J*” (OBF, or Non-OBF or OBF-Validation) became infected. Therefore, *PInf(S,J)* was calculated in each Q-period, for 24 combinations resulting from eight strata *“S”* times three country statuses *“J”*.

The overall quarterly national *PIntro* was estimated combining the quarterly probabilities of introduction of the eight strata altogether as:

$$PIntro = 1 - \prod_{S,J=1}^{24} [1 - PInf_{(S,J)}] \quad \text{(Eq. 4)}$$

Sensitivity analysis

The sensitivity analysis was carried out consulting in @Risk the tornado graph of the Spearman Rank correlation coefficients. The last quarter of 2016 was used as reference surveillance Q-period, because it was the most recent considered in the study and because the brucellosis status of the trading partners (Appendix, Table 2) was similar at the date of writing.

Alternative scenario analysis

According to the output of the sensitivity analysis (see results) an alternative scenario was investigated. For this purpose the model was run after reducing the herd prevalence of OBF countries (namely *BHP(OBF))*. A Uniform distribution ranging from 0 to [Beta (1+1; 1,375,934 – 1+1)] was set to represent uncertainty on *BHP(OBF).* Accordingly, the large value (0.2% from Council Directive 64/432/EEC) used in the initial *BHP(OBF)*, was disregarded.

Results

English cattle population and general import patterns

From a general point of view, during the four investigated years, the overall number of English cattle herds reduced from 48,733 (Q-1-13) to 47,193 (Q-1-16).

In each surveillance period, the dairy herds tested at least in the BTM represented 12-14% of the English cattle population, while the remaining 86-88% were beef herds (B, non-dairy herds).

Considering the four years altogether, most of the live animals imported into production cattle herds arrived from: countries which were always OBF (The Netherlands 17.6%; Germany 16.5%, Denmark 8.8%, Isle Of Man 7.6%, and France 6.5%), from ROI (23.0%), and from NI (16.7%). The latter two changed their status during the four years. Then, a very small percentage of animals arrived from other countries (Appendix, Table 2).

Number of importing herds and number of imported consignments

The quarterly median number of herds importing animals was higher for the M and for the B-NoTest strata, which received imports mainly from OBF countries. Whereas the quarterly median number of dairy and beef herds located in other population strata and importing from other country statuses (Non-OBF and OBF-Validation) was very low (Appendix, Tables 3-4; Figures, A-B).

Regarding the total quarterly number of imported consignments, it was higher from OBF countries than from Non-OBF or OBF-Validation countries, especially in the M stratum within the dairy sector (Appendix, Fig. A) and in the B-NoTest stratum within the beef sector (Appendix, Fig. B).

The peak of imports from OBF countries in the M stratum was registered in Q-3-14, and in the following surveillance period for the B-NoTest herds (Appendix, Fig. A-B). The latter and beef herds in general, imported also a higher total number of consignments from Non-OBF and OBF-Validation countries. Those consignments arrived mainly from NI. In fact, the consignments from NI were counted as Non-OBF before the last quarter of 2015 (Q-4-15) and as consignments from OBF-Validation countries thereafter.

The number of animals per consignment used to set the *N_anim* distribution in Eq. 1, is shown in Tables 3 and 4 of the Appendix, for each stratum and Q-period.

Probability of disease introduction at national level. Baseline scenario

The quarterly median baseline *PIntro* ranged from 1.3% (5^{th} percentile = 0.2%; 95^{th} percentile = 4.9%) in the first quarter of 2016, to 5.5% (0.6%; 20.1%) in the third quarter of 2014 (Fig. 2). During the 16 surveillance periods, the median of the medians *PIntro* was 3.5% (median of 5^{th} p.= 0.5%; median of 95^{th} p. =13.5%).

In the four surveillance periods of 2016, the median of the medians *PIntro* was ≈ 2.8% (median of 5^{th} p.= 0.4%; median of 95^{th} p.=10.7%).

Therefore, on average, at least one introduction could be expected each (approximated) 36 surveillance periods (9, 281), so each 9 years (2; 70).

Output of sensitivity analysis.

In the sensitivity analysis, the ranking of inputs was checked according to the tornado graph of the Spearman Rank correlation coefficients.

The five most important inputs were in the order: 1) the *BHP(OBF)* , 2) the *WHP*, 3) the *BHP(Non-OBF)*, 4) the *N_anim* imported from OBF countries into B-NoTest herds, and 5) the *N_anim* imported from OBF countries into M herds. In the tornado graph, those inputs showed Spearman Rank correlation coefficients (bars) up to 90%, 27%, 26%, 20% and 9%, respectively. Other inputs had coefficients ≤ 5%.

Output of alternative scenario analysis.

In the alternative scenario, the quarterly median *PIntro* reduced remarkably compared to estimates from the baseline scenario, and ranged from 0.002% (5^{th} p. = 0.0001%; 95^{th} p. = 0.008%) in Q-4-15 to 0.3% (0.02%; 1.6%) in Q-1-13.

Considering periods Q-1 to Q-4-16, the median of the medians *PIntro* was 0.2% (median of 5^{th} p. = 0.03%; median of 95^{th} p.= 0.7%).

Therefore, according to the alternative scenario and the last considered year (2016), on average at least one introduction could be expected each ≈ 500 surveillance periods, so each ≈ 125 years.

Discussion

The output of this study represents important information for policy makers and risk managers, because it gives an insight into the probability of introducing bovine brucellosis into the English cattle population (*PIntro*) by imported live cattle.

The relationship between import patterns and surveillance components across herd types was included in the model, and it can be considered when the national surveillance system for bovine brucellosis is evaluated (Foddai et al., 2020).

Furthermore, the simulation model is very flexible and can be used in the future if the brucellosis status of trading partners changes. It combines the information from literature and from national/international legislation, with information from real data collection and descriptive data analysis. In this way, the assessment could be carried out from the single herd level to the population stratum level, and then to the national level (*PIntro*). Thus, although with some simplifications, uncertainty on the inputs as well as variability between surveillance periods, farms, sectors, and population strata were taken into account altogether for the simulation process.

Main results: Information from the quantitative assessment

According to the baseline scenario, at least an introduction could be expected on average each 9 years (2; 70). Nevertheless, in the alternative scenario where the *BHP(OBF)* was reduced, the *PIntro* reduced as well to at least an average introduction each ≈ 125 years. In this case, the impact of OBF consignments was lower, and the *PIntro* depended more by Non-OBF and OBF-Validation countries, which anyway contributed with fewer deliveries than OBF countries (Appendix, Tables 2 to 4, Fig. A-B).

According to the data analysis, most of the imported consignments arrived from OBF countries into herds tested in milk only (M stratum) or to beef herds which were not tested at the APHA in the same surveillance period (B-NoTest stratum) (Appendix, Table 3-4, Fig. A-B). This information is important to consider because, eventual disease introduction due to imports of animals into the latter stratum could be more difficult to detect compared to dairy herds that are actively tested quarterly at least on BTM (Foddai et al., 2020).

Especially in Q-1 and Q-2-16, the *PIntro* of the baseline scenario lowered compared to previous Q-periods, because total imports reduced and because none of the most frequent trading partners (ROI and NI) was Non-OBF (Appendix, Table 2, Fig. A-B). The increased *PIntro* observed in Q-3 and Q-4-16, compared to Q-1 and Q-2 of the same year (Fig. 2), was mainly related to the increased imports from OBF countries into both sectors (Appendix, Fig. A-B).

The baseline scenario could be considered as more conservative (risk averse), but allowed incorporating uncertainty relating to OBF countries. In those countries the level of surveillance could be relaxed after the first five years of OBF status, though new outbreaks can still occur in OBF countries (Fretin et al., 2015; EFSA, 2015). For example, OBF countries could rely completely on voluntary abortion submission and, as observed by Bronner et al. (2014), farmers could be reluctant or not aware to submit abortion samples for surveillance of bovine brucellosis. Accordingly, the passive surveillance components could take some time before new disease introductions are detected, especially in beef herds where active BTM testing is not carried out. Meanwhile, infected animals could be exported during the high risk period, between infection and detection in the source country. For these reasons, the baseline scenario was considered as the main scenario.

Information from sensitivity analysis

In the sensitivity analysis the between-herds prevalence used for the OBF countries (namely *BHP(OBF)*) appeared as the most important input affecting the *PIntro.* This was due to two main reasons: i) most of the consignments arrived from OBF countries (Appendix, Fig. A-B), and thus, this input was sampled for most of the deliveries; and ii) in the baseline scenario a relatively large range was used in the Pert distribution, which caused most of the uncertainty associated with the *PIntro*.

Moreover, the most likely annual *BHP(OBF)*, was set in a complex way, as a Uniform distribution from 0 to [Beta (1+1, 1,375,934 – 1+1)], for different reasons. If it had been set as single value (1/1,375,934), it would have disregarded that in 2014-2015 and 2016 no cases were reported in OBF countries (EFSA, 2015). In contrast, if it had been fixed to 0%, it would have assumed 100% confidence in freedom for OBF countries, but absolute proof of freedom cannot be reached (Cameron et al., 2014). Therefore, the most likely value was set in a way, which was considered as the best compromise between the two options.

It could be argued that in theory Beta distributions can range between 0 and 100%, and that in some iteration the most likely value could have exceeded 0.2% (which was set as maximum in the Pert *BHP(OBF)*). This was excluded, at least for most of the iterations, because the simulated *BHP(OBF)* had median 0.03% and 99th percentile = 0.12%. Therefore, as expected, the simulated *BHP(OBF)* was unlikely to exceed the design prevalence (0.2%) set in the EU legislation (Council Directive, 64/432/EEC).

The *BHP* of Non-OBF countries had a large range (0.0% to 0.4%) as well, but it appeared in 3rd position according to the tornado graph. Hence, the relatively “lower” importance of the *BHP(Non-OBF)* was captured in the sensitivity analysis. During the last investigated year, only 0.5% of the imported animals arrived from Non-OBF countries (Appendix, Table 2). Hence, those kind of consignments contributed less to the variation on *PIntro* of the baseline scenario compared to OBF consignments.

The within herd prevalence (*WHP*) used for infected herds abroad, was the second most important parameter. During the literature review, it was difficult to find estimates of *WHP* for all the trading partners from where animals were imported into England, because most of those countries (Appendix, Table 2) achieved the OBF status since a long time and/or because even in most of the Non-OBF countries the between herds prevalence was reported to be around 0%. Therefore, the *WHP* was set according to a single study (Pehlivanoğlu et al., 2011) from Turkey and some uncertainty remains on the used distribution. The *WHP* is related to the cattle management and to the herd structure (Mai et al., 2012), which can vary remarkably between countries, continents and their respective environmental factors. Thus, *WHP* is rather unpredictable, and it depends from the time elapsed between disease introduction in the herd of origin and day of export. This uncertainty was flagged in the sensitivity analysis. The maximum *WHP* used in this study was set as relatively low (10%), because if the export from the herd was allowed, the disease could have been present at undetectable level only (low *WHP*). For instance, if there was suspicion that the disease was present in the herd due to high abortion rates, then the export was unlikely to occur.

The number of animals per consignment (*N_anim* in Eq. 1) imported from OBF countries into B-NoTest or into M herds were the other most important inputs. Those were based on the results of the data analysis (Appendix, Tables 3-4) and represented the variability of imported animals per consignment. Other inputs played a less important role.

Considerations on model´s assumptions, structure, and limitations

During the data handling and analysis, the overall number of herds was calculated as the sum of all CPHs appearing in at least one of the datasets. The CPH was not found for 5.8 (2016) to 6.8% (2013) of the data lines from the original BTM list of England and Wales (LIMS data). Therefore, part of the dairy herds could have been misclassified as B-NoTest herds when testing of bulk milk had in fact occurred, but the CPH was available only on the CTS/RADAR data and not in the BTM list. The impact of this misclassification was likely to have been marginal, because some of those records were from Wales (not used here), and some herds could appear in more data lines during the same period. For example, if more than one milk tank was tested in large herds. These would have contributed more than once to the 5.8-6.8%.

Regarding the mathematical formulas, it must be noted that the combination of Equations 1 and 2 allowed accounting for the clustering effect within the consignments imported from the same country status and from the same sending herd. Unfortunately, detailed information on the source herd abroad about structure, size and type of management, was not available for this study. Thus, it could not be checked if all animals in the consignment arrived from a single farm. Nevertheless, it seems a reasonable assumption because, according to the median *N_anim* per consignment (Appendix, Tables 3 and 4), it could be the case that all animals of the consignment arrived in 1-2 trucks from the same herd.

Moreover, Equations 1 to 3 were based on the binomial distribution. Hence, it was assumed that the sampled units represented a small proportion (e.g. <10% Martin et al., 2007b; Cameron 2014) of the population from which they were taken. For Equations 1 and 2, this assumption could not be checked because there was not data about the overall denominators abroad. Anyway, it seems possible that less than 10% of the animals present in the exporting herd contributed to the consignment (*N_anim*) and that less than 10% of the consignments abroad were imported into a single English herd. For Equation 3 the assumption was valid in most of the situations according to the data analysis. The only exceptions happened when strata such as M-A-PIC and B-A-PIC, were composed by a very small number of herds, and thus between these, a percentage higher than 10% could import cattle. The probability of introduction posed by these herds contributed marginally to *PIntro*, because very few of these properties (or none) imported cattle. In future studies the M-A-PIC and B-A-PIC herds could be included within the M-PIC and B-PIC strata, also because no herds belonged to those strata in most of the surveillance periods (Appendix, Tables 3-4).

Considering Equation 4, the two main assumptions were: i) independence between strata and ii) independence between import events. These were both confirmed in the data analysis. Herds appearing in one stratum did not appear in the other strata, and during a single surveillance period, it was assumed that a herd could import animals from OBF, or from Non-OBF, or from Validation countries, but not from different country statuses at the same time. Usually, herds imported a single (median) consignment per surveillance period and if more consignments arrived, they were mostly from the same country status. In fact, from previous analysis (data 2011 to 2016) we knew that at GB level, when the sending countries were divided into the three statuses (OBF, Non-OBF and Validation) the number of consignments was 13,520. Whereas the number of consignments counted per single country name was 13,815. Therefore, only for 2.1%

(295/13,815) of the GB consignments, a single herd could import animals from more than one country on the same date, and we generalized those as consignments from the same country status.

Uncertainty and variability were both captured in the used inputs and affected the final *PIntro*. It is usually preferred to separate the two kinds of variation of the inputs, but from a pragmatic point of view, the policy makers also need an overall picture output (still stochastic). Hence, a simplification was used to avoid complex answers by splitting uncertainty and variability into several separated inputs/outputs. For example the model's ability of reflecting the variability between importing herds of the same stratum (during the same Q-period), was simplified. On one hand, simulating each single herd and consignment within each stratum could have made the model more precise from a technical point of view. On the other hand, simplifying to 24 combinations of stratum "*S*" and status *"J"* of country of origin, allowed a fast running time and several surveillance periods could be investigated. The model structure was discussed through meetings and workshops during the study, within an ad-hoc steering committee. The committee was composed of several professional figures: cattle experts, veterinarians, risk assessors, risk managers and data providers. It was agreed that the distributions used for the number of animals imported per herd (*N_anim*) within each stratum were representative enough, because based on actual data and because they related to relatively small periods of three months each. Additionally, 20,000 iterations were used and in each iteration a different input was taken from the defined distributions of *BHP*, *WHP* and *N_anim*. Therefore, the structure of the model was considered informative enough for the purposes of the study. Moreover, with the simplification used, the model could be adapted easily for other countries with similar data, e.g. Scotland and Wales (Foddai et al., 2018)

In GB, some consignments from traders or farmers considered at risk (e.g. for previous non compliances) could also be tested according to the APHA Operational Manual (OM). Data regarding these situations were not included and those eventual further tests were disregarded in this study. Hence, from that point of view, the actual *PIntro* could be lower than the estimates reported here.

Furthermore, in this assessment, all animals imported from Italy and Spain were considered as Non-OBF despite some regions are recognised as OBF. Unfortunately, information on the region of origin was not available and the impact of those imports on *PIntro* could have been overestimated. Nevertheless, such overestimation should be marginal, because only a very small proportion of the animals arrived from those countries during the investigated periods (Appendix, Table 2). This assumption was confirmed in the alternative scenario, where the *PIntro* was very low and the contribution of Non-OBF and OBF-Validation countries became clearer than in the baseline scenario.

Regarding the impact of follow up tests on suspicious imports, it must be said that further serial testing on positives could reduce the overall sensitivity if the used tests have *Se* < 100% and the last test/s override/s the result/s of the previous one/s. If in reality several follow ups are carried out, the *PIntro* would be underestimated.

Finally, as a conservative approach, it was assumed that all (potential) "truly" positive animals of any sex and age could be potential sources of introduction, because it is unclear to what extent antibody positives could spread the pathogen through their excreta (e.g. in what amount). In the data used, information on the pregnancy status of the imported animals was not available. Neither was it known if the imported males could go to artificial insemination centres producing semen for commercial sale, where all bulls undergo frequent testing and are kept in high biosecurity. Thus, due to these uncertainties, the conservative approach was preferred and the *PIntro* could have been overestimated. Estimates could become more precise in the future, if studies are carried out to investigate the epidemiological importance of age, sex, faeces, urines and natural mating, for disease spread.

Conclusion

- In the baseline scenario, which assumed a more conservative approach towards OBF consignments, it appeared that on average at least one introduction could occur each 9 years (2; 70).
- When the between-herds prevalence in OBF countries was reduced, the probability of introduction at national level also reduced remarkably for the most recent considered year (2016) due to the large number of imported animals from those countries.

- Future studies on the role of latent carriers of different age and sex and on the presence of *Brucella abortus* in excreta from antibody positive animals, could reduce the uncertainty on the *PIntro* estimates.

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Appendix: Table 1. List of Abbreviations

Abbreviation	Meaning
APHA	Animal and Plant Health Agency
B-Abo	Beef herds (non-dairy) tested in abortion/s in the investigated surveillance period
B-A-PIC	Beef herds (non-dairy) tested in abortion/s and PIC in the investigated surveillance period
BHP(J)	Probability that the herd abroad (from where the consignment/s was/were picked up in country status *"J")* was infected.
BHP(OBF)	Probability that the exporting herd located in an OBF country was infected
BHP(Non-OBF)	Probability that the exporting herd located in a Non-OBF country was infected
BHP(OBF-Validation)	Probability that the exporting herd located in a Validation country was infected
B-NoTest	Beef herds not tested at the APHA laboratories in the investigated surveillance period
B-PIC	Beef herds (non-dairy) tested in Post Import Calving/s in the investigated surveillance period
BTM	Bulk tank milk
CPH	County Parish Holding number (herd ID in GB)
CTS	Cattle Tracing System database
Defra	Department for Environment, Food and Rural Affairs (United Kingdom)
DES	Department of Epidemiological Sciences (APHA, Weybridge)
EFSA	European Food Safety Authority
EU	European Union
GB	Great Britain
iELISA	Serum indirect Enzyme-Linked Immunosorbent Assay
LIMS	Laboratory Information Management System
M	Dairy herds only tested in the bulk tank milk in the investigated surveillance period
M-Abo	Dairy herds only tested in the bulk tank milk and abortion/s in the investigated surveillance period
M-A-PIC	Dairy herds tested in milk, abortion/s and post import calved cows in the investigated surveillance period
M-PIC	Dairy herds only tested in the bulk tank milk and post import calved cows in the investigated surveillance period
N_anim	Number of animals per imported consignment
N_con(S,J)	Median number of consignments from country status "*J*" received per English herd within stratum "*S*" in the investigated surveillance period.
N_herds(S,J)	Number of herds within stratum "*S*" importing cattle from country status "*J*" in the investigated surveillance period
NI	Northern Ireland
NML	National Milk Laboratories
Non-OBF	Non-Officially Brucellosis Free country status
OBF	Official Brucellosis Free country status

OBF-Validation	Official Brucellosis Free country status during the first five years after attaining the status and when serology is still applied according to Council Directive 64/432/EEC (called as validation period in this study)
OM	APHA Operational Manual
Ph	Design herd prevalence from the European Legislation (Council Directive 64/432/EEC), below which (after five consecutive years) European countries can be recognised OBF
PFree	Confidence in freedom from disease based on the epidemiological concept of negative predictive value given test-negative surveillance results from a given surveillance period
PIC	Post Import Calving testing
$PInf_{Con(Non\text{-}OBF)}$	Probability that the consignment imported from a Non-OBF country contained and introduced at least one infected animal
PInf_Con(S,J)	Probability that the consignment imported from an infected herd from country status "*J*" contained at least one infected animal
PInf_Herd(S,J)	Probability that an importing English herd located within a specific stratum "*S*", became infected by one or more consignments from country status "*J*" (during surveillance period Q)
PInf(S,J)	Probability that at least one of the herds of the stratum *S* (M, or M-Abo, or M-PIC, or M-AboPIC, or B-Abo, or B-PIC, or B-A-PIC, or B-NoTest), which imported consignment(s) from country status "*J*" (OBF, or Non-OBF or Validation), became infected.
PIntro	National level probability of disease introduction into the investigated cattle population during a surveillance period Q
Prop_Test	Probability that the animal imported from a Non-OBF country was older than 12 months and was tested.
RADAR	Rapid Analysis and Detection of Animal-Related Risks database
RiskPert	Pert distribution set in @Risk
ROI	Republic of Ireland
Se	Sensitivity of the serum antibody indirect ELISA (iELISA) used on animals (older than 12 months) imported from Non-OBF countries
SSe	Surveillance system sensitivity
WHP	Within-herd prevalence abroad

Table, 2. Number of animals imported by English production cattle herds, per sending country (period 2013-2016).

Country	Animals 2013	Percentage	Animals 2014	Percentage	Animals 2015	Percentage	Animals 2016	Percentage
AUSTRIA	27	0.1%	15	0.0%	12	0.0%	83	0.4%
BELGIUM	114	0.4%	280	0.7%	242	0.9%	442	1.9%
CZECH REPUBLIC	0	0.0%	2	0.0%	166	0.6%	0	0.0%
DENMARK	2059	6.6%	4095	10.6%	3061	10.8%	1415	6.2%
FINLAND	0	0.0%	0	0.0%	0	0.0%	4	0.0%
FRANCE	1503	4.9%	1244	3.2%	2398	8.5%	2630	11.6%
GERMANY	4880	15.8%	7254	18.9%	4035	14.3%	3703	16.3%
IRELAND [a]	6993	22.6%	9993	26.0%	6675	23.7%	4084	18.0%
ISLE OF MAN	1702	5.5%	2201	5.7%	1934	6.9%	3283	14.4%
LUXEMBOURG	0	0.0%	587	1.5%	687	2.4%	408	1.8%
NETHERLANDS	6481	20.9%	7602	19.8%	3981	14.1%	3108	13.7%
NORWAY	0	0.0%	0	0.0%	2	0.0%	3	0.0%
POLAND	0	0.0%	5	0.0%	2	0.0%	3	0.0%
ROMANIA	1	0.0%	0	0.0%	0	0.0%	0	0.0%
SLOVAKIA	0	0.0%	48	0.1%	0	0.0%	0	0.0%
SWEDEN	0	0.0%	3	0.0%	0	0.0%	0	0.0%
SWITZERLAND	0	0.0%	0	0.0%	1	0.0%	17	0.1%
NORTHERN IRELAND [b]	7087	22.9%	4816	12.5%	4798	17.0%	3434	15.1%
CANADA [c]	0	0.0%	10	0.0%	0	0.0%	0	0.0%
GUERNSEY [c]	0	0.0%	0	0.0%	0	0.0%	0	0.0%
HUNGARY [c]	14	0.0%	143	0.4%	100	0.4%	0	0.0%
ITALY [c]	33	0.1%	128	0.3%	20	0.1%	21	0.1%
JERSEY [c]	74	0.2%	47	0.1%	93	0.3%	93	0.4%
SPAIN [c]	0	0.0%	2	0.0%	9	0.0%	6	0.0%
GREECE [c]	4	0.0%	0	0.0%	0	0.0%	0	0.0%
Overall total	30,972	100.0%	38,475	100.0%	28,216	100.0%	22,737	100.0%

[a] From OBF-Validation to OBF in August 2014. [b] From Non-OBF to OBF-Validation in October 2015. [c] Always Non-OBF during the considered period.

Table 3. Quarterly median (minimum and maximum) number of herds (*N_herds(S,J)*) within each population stratum "*S*"; and importing from the different country statuses "*J*" (OBF, Non-OBF, and OBF-Validation). In columns Q-1-2013 to Q-4-2014, is the quarterly median number (*N_anim*) of imported animals (minimum and maximum) per consignment, during years 2013 and 2014.

Stratum "*S*" (Status "*J*")	**N. Herds**	**Q-1-2013**	**Q-2-2013**	**Q-3-2013**	**Q-4-2013**	**Q-1-2014**	**Q-2-2014**	**Q-3-2014**	**Q-4-2014**
M (OBF)	81 (31; 163)	16 (1, 88)	19 (1, 66)	15 (1, 105)	16 (1, 69)	16 (1, 61)	12 (1, 170)	12 (1, 82)	14 (1, 99)
M-Abo (OBF)	7 (0, 24)	17 (14, 20)	24 (1, 38)	34 (6, 68)	22 (2, 35)	17 (6, 36)	21 (1, 89)	30 (3, 141)	35 (2, 71)
M-PIC (OBF)	3 (0,8)	12 (2, 24)	12 (5, 36)	6 (1, 37)	34 (14, 36)	8 (6, 10)	4 (1, 10)	36 (5, 38)	20 (1, 37)
M-A-PIC (OBF)	0 (0, 1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	17 (17, 17)	0 (0, 0)
M (OBF-Validation)	3 (0, 30)	18 (1, 46)	24 (3, 55)	20 (1, 61)	18 (2, 44)	20 (4, 46)	16 (1, 41)	17 (5, 35)	0 (0, 0)
M-Abo (OBF-Validation)	0 (0, 6)	0 (0, 0)	8 (6, 34)	17 (12, 22)	23 (9, 45)	0 (0, 0)	9 (4, 35)	20 (16, 24)	0 (0, 0)
M-PIC (OBF-Validation)	1 (0, 13)	15 (10, 36)	20 (1, 68)	31 (1, 39)	20 (5, 39)	36 (27, 37)	8 (1, 39)	19 (19, 35)	0 (0, 0)
M-A-PIC (OBF-Validation)	0 (0, 1)	0 (0, 0)	46 (46, 46)	0 (0, 0)	40 (40, 40)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
M (Non-OBF)	4 (0, 11)	9 (1, 17)	6 (1, 29)	11 (1, 49)	2 (1, 18)	0 (0, 0)	1 (1, 35)	14 (1, 62)	10 (1, 46)
M-Abo (Non-OBF)	0 (0, 1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	12 (12, 12)	0 (0, 0)	15 (15, 15)	0 (0, 0)	0 (0, 0)
M-PIC (Non-OBF)	0 (0, 1)	0 (0, 0)	19 (19, 19)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	20 (8, 32)	0 (0, 0)
M-A-PIC (Non-OBF)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
B-NoTest (OBF)	94 (59, 144)	7 (1, 96)	13 (1, 70)	11 (1, 59)	11 (1, 80)	10 (1, 101)	10 (1, 70)	10 (1, 80)	17 (1, 86)
B-Abo (OBF)	2 (0, 7)	28 (20, 35)	26 (6, 36)	4 (4, 4)	8 (2, 15)	30 (2, 35)	15 (15, 15)	20 (6, 69)	23 (1, 70)
B-PIC (OBF)	2 (0, 4)	12 (12, 12)	34 (34, 68)	66 (1, 131)	2 (1, 4)	1 (1, 1)	36 (36, 36)	46 (2, 106)	6 (1, 34)
B-A-PIC (OBF)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
B-NoTest (OBF-Validation)	23 (0, 47)	12 (1, 39)	24 (1, 147)	8 (1, 71)	11 (1, 103)	12 (1, 102)	31 (1, 88)	14 (1, 75)	0 (0, 0)
B-Abo (OBF-Validation)	0 (0, 1)	0 (0, 0)	38 (38, 38)	0 (0, 0)	20 (16, 40)	6 (6, 6)	0 (0, 0)	0 (0, 0)	0 (0, 0)
B-PIC (OBF-Validation)	2 (0,4)	32 (12, 37)	20 (3, 38)	8 (1, 20)	7 (6, 12)	20 (9, 44)	24 (1, 74)	53 (17, 54)	0 (0, 0)
B-A-PIC (OBF-Validation)	0 (0, 1)	45 (45, 45)	0 (0, 0)	42 (42, 42)	0 (0, 0)	40 (21, 52)	0 (0, 0)	40 (40, 40)	0 (0, 0)
B-NoTest (Non-OBF)	30 (0, 52)	18 (1, 107)	19 (1, 59)	20 (1, 68)	3 (1, 62)	6 (1, 108)	1 (1, 67)	37 (1, 104)	2 (1, 81)
B-Abo (Non-OBF)	0 (0, 1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	1 (1, 1)
B-PIC (Non-OBF)	1 (0, 3)	22 (22, 22)	0 (0, 0)	1 (1, 1)	0 (0, 0)	1 (1, 1)	0 (0, 0)	5 (1, 18)	40 (40, 40)
B-A-PIC (Non-OBF)	0 (0,0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)

Table 4. Table 3 continued. Quarterly median number (*N_anim*) of imported animals (minimum and maximum) per consignment, during years 2015 and 2016; and according to stratum "*S*" of importing herd and status "*J*" of country of origin.

Stratum "*S*" (Status "*J*")	**Q-1-2015**	**Q-2-2015**	**Q-3-2015**	**Q-4-2015**	**Q-1-2016**	**Q-2-2016**	**Q-3-2016**	**Q-4-2016**
M (OBF)	10 (1, 69)	16 (1, 178)	24 (1, 80)	18 (1, 70)	22 (1, 48)	34 (1, 70)	22 (1, 78)	15 (1, 70)
M-Abo (OBF)	29 (1, 35)	25 (2, 65)	20 (5, 70)	24 (10, 73)	0 (0, 0)	30 (19, 36)	30 (1, 34)	36 (11, 70)
M-PIC (OBF)	16 (3, 35)	20 (2, 70)	0 (0, 0)	12 (12, 12)	0 (0, 0)	0 (0, 0)	12 (12, 12)	33 (33, 33)
M-A-PIC (OBF)	0 (0, 0)	0 (0, 0)	0 (0, 0)	26 (16, 37)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
M (OBF-Validation)	0 (0, 0)	0 (0, 0)	0 (0, 0)	1 (1, 1)	1 (1, 1)	1 (1, 1)	0 (0, 0)	1 (1, 1)
M-Abo (OBF-Validation)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	1 (1, 1)
M-PIC (OBF-Validation)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	11 (11, 11)	0 (0, 0)	0 (0, 0)	0 (0, 0)
M-A-PIC (OBF-Validation)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
M (Non-OBF)	1 (1, 1)	1 (1, 40)	3 (1, 40)	0 (0, 0)	0 (0, 0)	0 (0, 0)	2 (1, 17)	0 (0, 0)
M-Abo (Non-OBF)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	50 (50, 50)	0 (0, 0)
M-PIC (Non-OBF)	0 (0, 0)	1 (1, 1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
M-A-PIC (Non-OBF)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
B-NoTest (OBF)	10 (1, 80)	15 (1, 106)	15 (1, 70)	14 (1, 107)	4 (1, 55)	7 (1, 82)	17 (1, 129)	6 (1, 160)
B-Abo (OBF)	35 (3, 35)	32 (2, 35)	35 (17, 35)	2 (1, 15)	0 (0, 0)	0 (0, 0)	25 (15, 35)	35 (15, 35)
B-PIC (OBF)	26 (26, 27)	21 (3, 39)	14 (1, 36)	16 (3, 21)	2 (2, 2)	0 (0, 0)	0 (0, 0)	45 (21, 49)
B-A-PIC (OBF)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
B-NoTest (OBF-Validation)	0 (0, 0)	0 (0, 0)	0 (0, 0)	4 (1, 57)	2 (1, 98)	1 (1, 99)	30 (1, 75)	1 (1, 103)
B-Abo (OBF- Validation)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	2 (2, 2)	0 (0, 0)
B-PIC (OBF-Validation)	0 (0, 0)	0 (0, 0)	0 (0, 0)	32 (5, 51)	2 (1, 31)	1 (1, 1)	0 (0, 0)	0 (0, 0)
B-A-PIC (OBF-Validation)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	53 (53, 53)	0 (0, 0)	0 (0, 0)
B-NoTest (Non-OBF)	3 (1, 55)	3 (1, 95)	37 (1, 60)	0 (0, 0)	0 (0, 0)	0 (0, 0)	2 (1, 5)	2 (2, 16)
B-Abo (Non-OBF)	0 (0, 0)	0 (0, 0)	2 (2, 2)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
B-PIC (Non-OBF)	32 (9, 68)	32 (1, 38)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	2 (2, 2)	0 (0, 0)
B-A-PIC (Non-OBF)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)

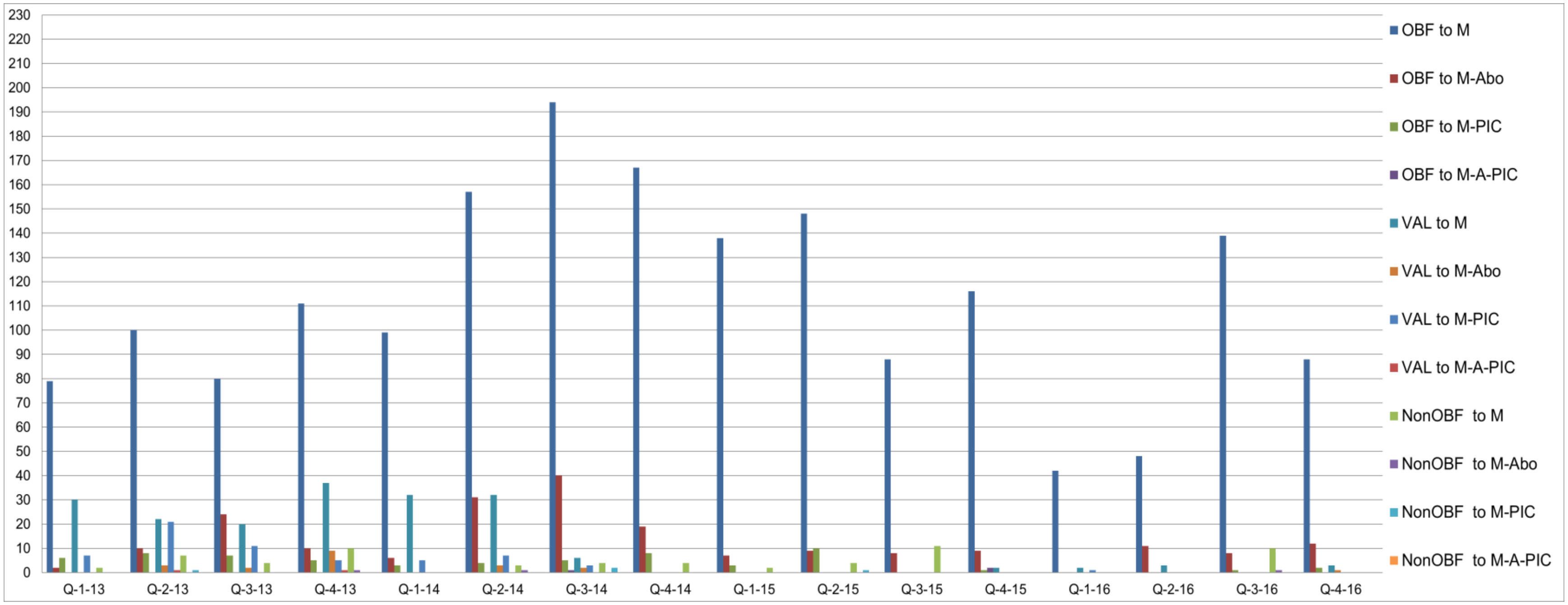

Figure A. Number of imported consignments received within the dairy sector, within each stratum (M = milk herds only tested in bulk tank milk (BTM); M-Abo = milk herds tested in BTM and abortion/s; M-PIC = milk herds tested in BTM and post import calving; M-A-PIC = milk herds tested in BTM, abortion/s and at post import calving), according to status of country of origin (OBF = Officially Brucellosis Free, Non-OBF = Non Officially Brucellosis Free, and VAL = OBF-Validation) and per surveillance period (Q-1 to Q-4) during years 2013 (13) to 2016 (16).

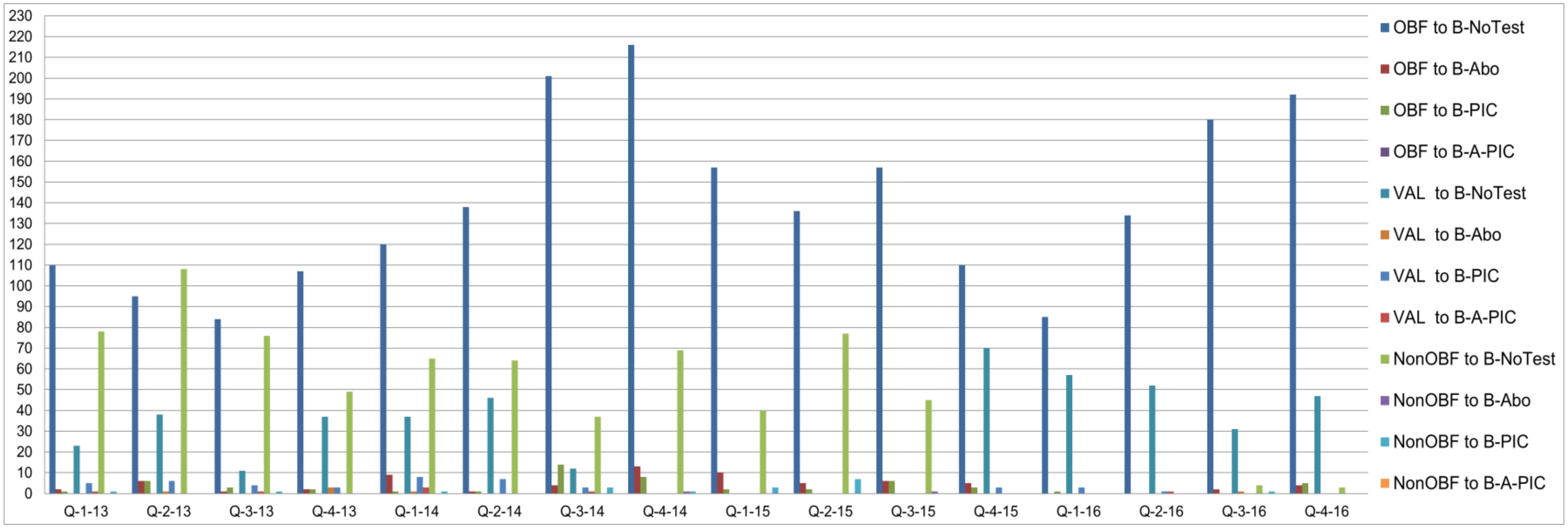

Figure B. Number of imported consignments received within the beef (B, non-dairy) sector, within each stratum (B-Abo = beef herds tested in abortion/s only; B-PIC = beef herds tested in post import calving only; B-A-PIC = beef herds tested in abortion/s and post import calving), according to status of country of origin (OBF = Officially Brucellosis Free, Non-OBF = Non Officially Brucellosis Free, and VAL = OBF-Validation) and per surveillance period (Q-1 to Q-4), during years 2013 (13) to 2016 (16).