Insights for brucellosis eradication in Italy through a model-based spread evaluation in grazing livestock - Sicily case study

Lara Savini, Luca Candeloro, Paolo Calistri, Alessio Di Lorenzo*, Margherita Perilli,
Armando Giovannini and Fabrizio De Massis

[1]*OIE Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy.*

**Corresponding author at: OIE Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy.*
E-mail: a.dilorenzo@izs.it.

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Summary

Brucellosis is one of the world's major zoonotic pathogens and is responsible for enormous economic losses as well as considerable human morbidity in endemic areas. Definitive control of human brucellosis requires control of brucellosis in livestock through practical solutions that can be easily applied to the field. In Italy, brucellosis remains endemic in several southern provinces, particularly in Sicily Region.

The purpose of this paper is to describe the developed brucellosis model and its applications, trying to reproduce as faithfully as possible the complex transmission process of brucellosis accounting for the mixing of grazing animals. The model focuses on the contaminated environment rather than on the infected animal, uses real data from the main grazing areas of the Sicily Region, and aims to identify the best control options for minimizing the spread (and the prevalence) and to reach the eradication within the concerned areas. Simulation results confirmed the efficacy of an earlier application of the controls, showed the control should take place 30 days after going to pasture, and the culling time being negligible. Moreover, results highlighted the importance of the timing of both births and grazing pastures (and their interaction) more than other factors.

As these factors are region-specific, the study encourages the adoption of different and new eradication tools, tuned on the grazing and commercial behavior of each region. This study will be further extended to improve the model's adaptability to the real world, with the purpose of making the model an operational tool able to help decision makers in accelerating brucellosis eradication in Italy.

Introduction

Brucellosis is an important zoonotic disease caused by infection with bacteria of the genus *Brucella*. The disease may affect several animal species, such as cattle, buffaloes, sheep, goats, pigs, and humans. Having a worldwide distribution (Pappas *et al*. 2006), it is one of the most important zoonoses in the Mediterranean and Middle East regions. In the Mediterranean area, bovine brucellosis is typically caused by *B. abortus* while ovine and caprine brucellosis are mainly caused by *B. melitensis*, although cross-species infections may occur (De Massis *et al*. 2019). The typical clinical sign of the infection in affected animals is the occurrence of abortion (although this depends on whether the infection is recent or has been chronically present) as well as low fertility and

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milk production. However, the disease can be present in an animal for several years without clinical signs (Akakpo *et al.* 1987). Humans can contract the disease by contact with infected animals or their products, with unpasteurized milk being the most common source of brucellosis in urban populations (Godfroid *et al.* 2005, Moreno 2014). *Brucella melitensis* is the most frequent agent of brucellosis in humans. It leads to the most severe manifestation of the disease (Corbel 2006), with the occurrence of infection highly related to the consumption of contaminated dairy products from sheep and goats (De Massis *et al.* 2005).

Human brucellosis is a systemic infectious disease with varying clinical manifestations. Patients often develop fever of unknown origin with an insidious clinical on-set. The disease is often difficult to diagnose because of its similarities with other febrile diseases, such as malaria or other undulating fevers, and it occurs as a subacute or chronic illness that is generally not lethal (Pappas *et al.* 2006). In the European Union, 619 cases of human brucellosis were reported in 2008 (EFSA 2008), and this figure decreased to 437 cases in 2015 (EFSA 2016). The highest incidence was recorded in some member states still not officially free from bovine and sheep and goat brucellosis (Italy, Portugal, Greece, and Spain).

Due to the high public health and economic burden of brucellosis, European countries have applied surveillance, control, and eradication programs for many years, and most of them have acquired the Officially *Brucella melitensis*-Free (OBF) status. The disease, however, persists in several countries in the Mediterranean area. In Italy, despite implementation of the eradication program for over 50 years, brucellosis remains endemic in several southern areas, particularly in Sicily. The current brucellosis surveillance system in Italy involves annual serological testing and slaughtering of the positive animals from which a bacteriological isolation is performed for confirmation of the diagnosis (Italian Ministry of Health 1992, 1994, 2015). Control testing is performed twice a year in non-OBF Regions, where the main aim is eradication of brucellosis, and once a year (or less frequently according to the epidemiological situation) in the OBF regions, where the goal is to control the reintroduction of the disease (Garafolo *et al.* 2013). The articulated Italian National Eradication Program for bovine brucellosis is summarized in **Supporting information (S1 Figure)**.

In Italy, while central and northern provinces are declared officially brucellosis free, with limited numbers of cases reported annually (EU Commission Decision 1992, 2003), bovine brucellosis is endemic in the southern part of the country, as well as sheep and goat brucellosis (Graziani *et al.* 2013).

Susceptibility of cattle to brucellosis infection is influenced by age, sex and reproductive status of the individual animal. Susceptibility increases as stage of gestation increases (Pérez-Sancho *et al.* 2015, Diaz-Aparicio *et al.* 2015). The spread of the disease from one herd to another (inter-herd spread), and from one area to another, is primarily due to animal contacts at pastures or through animal movements between herds (Calistri *et al.* 2013). In the event of an abortion, or at the time of parturition, an infected cow may excrete large quantities of bacteria through the fetus and lochia (uterine fluids) (Nicoletti 1980). Male cattle can sometimes become infertile when infected and may discharge bacteria in their semen (Crawford *et al.* 1990). The long and variable incubation period (Nicoletti 1980) and the occurrence of latent infection in heifers born to infected dams that show no serum reaction upon future infection (Wilesmith 1978), also may lead to difficulties in eradicating brucellosis. The control of brucellosis is based on the identification through active and passive surveillance of the infected herds. As soon as an infected herd has been detected, all animal movements from and to this herd are blocked and the infected animals within the herd are culled. Meanwhile, all herds that had contacts (through animal movements, pasture contacts, indirect contacts through exchange of materials or personnel, etc.) are identified and checked for the presence of infection. The tracing and checking of herds in contact is a cumbersome activity that may require long time to be carried out. During this time, the infection can further spread (Savini *et al.* 2017).

Studies have demonstrated that mathematical modelling can aid the veterinary services in developing brucellosis control strategies (Savini *et al.* 2017). The majority of these mathematical models are based on a compartmental model in which the individuals are grouped according to their disease status and some of them are embedded in animal movement networks (Roy *et al.* 2011, Aïnseba *et al.* 2010, Dubie *et al.* 2014, Zinsstag *et al.* 2005).

Our objective was to study possible ways to prioritize the herd in-contact to limit the possible spread of the disease during this phase. Therefore, modelling the spread of brucellosis requires knowledge about where and when animals move into and out respective herds, to be able to trace potentially infected animals.

Aim of this model has been to develop a tool that could be useful for field application in order that brucellosis transmission can be reduced and the eradication process facilitated.

The model will use real data from the field, in order to identify the best control options possible to accelerate the eradication process in the specific areas concerned.

A SEIR stochastic model was developed by the authors in the past after a comparison of different approaches in modelling bovine brucellosis (Savini *et al.* 2017). However, in some situations the model did not predict the occurrence of brucellosis outbreaks in certain geographical areas, and, in particular, for some areas of Sicily, when pasture mixing effect is present.

The model here described will consider the effect of mixing animals at pastures on Brucella transmission taking into account the data from Sicily, in particular from the Messina, Enna and Catania Provinces (main grazing areas of the Region). The structure of the model has been made enough flexible, to be possible to use it also on sheep and goat brucellosis (just changing the input values).

The aim has been to predict correctly the situation that is observed in the field, and to be a real and useful tool for planning the control and eradication activities. This has been possible also thanks to the fact that in Italy, since 2006-2007, National Control Authorities have in place an electronic database containing all the checks carried out in the framework of the National Brucellosis Eradication Program.

The conceptual framework of the new model is synthesized in Figure 1.

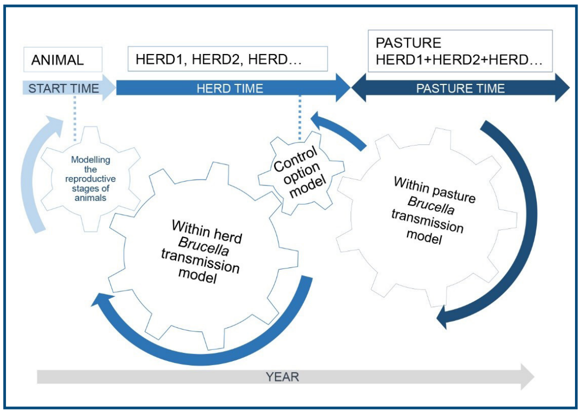

Figure 1. *Conceptual framework. Conceptual framework describing the working of the developed Brucellosis transmission and control model.*

The model has been structured in three different modular models:

1) individual based model to assess the reproductive stage of each cow;
2) brucellosis transmission SEIR model;
3) control option model.

The first model simulates the reproductive stage of susceptible animals, at single animal level. The second model, interconnected with the first, is a classical Susceptible - Exposed – Infected - Recovered (SEIR) model. Finally, a third model interconnected with the second has been conceptualized considering different possibilities of control options to evaluate the risk reduction and the efficacy of the actions taken for control and eradication.

Materials and methods

Demographic and reproductive data description and analysis

Demographic and reproductive data were calculated for each single cow considering the data stored in the National Database for Animal Identification and Registration (NDB) for Messina, Enna and Catania provinces of Sicily Region, Italy (**Figure 2)**.
A five-year dataset, from 2014 to 2018, was used to reduce possible distortions due to variability among years. The aim was to use real data, in order to model the reproductive stages of cows in the area based on the real situation.

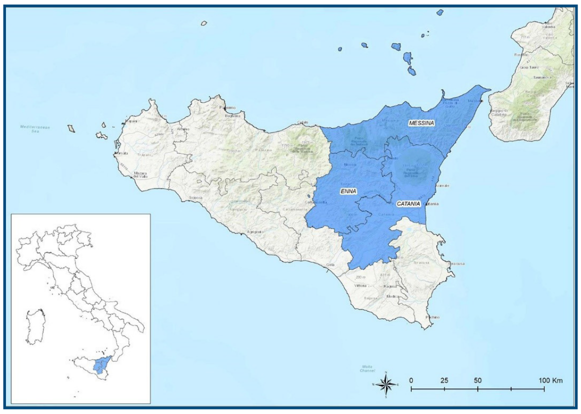

Figure 2. *Study area. Messina, Enna and Catania provinces of Sicily Region.*

Herd size

The first aspect investigated has been to identify the average herd size of cattle herds, in terms of number of heads per herd. The average herd size in the area under study is around 30 heads (standard deviation equal to 37), the 5% of herds having more than 98 heads. The herd size (N) (considering only females) has been sampled from the best fitting distribution for the observed data, rounded to the closest integer value, an exponential shifted distribution (29.339, 0.99302).

Age distribution

The second aspect investigated has been to identify how those herds are structured in terms of age of the animals.

The distribution of females according to the age classes is shown in the following **Table I (b)**. Although around 18% of female cattle kept in the three provinces aged more than 8 years, these animals have not been included in the model, because not considered relevant for further brucellosis transmission (England *et al.* 2004). Another reason was to try to compensate errors in entering the ages of the animals in the NDB which generally can lead to an overestimated number of older animals. From the zootechnical point of view, this would mean that, in optimal condition, these animals might have as much as 5 parturitions in life. The 16.7% of animals less than 1 year of age has been calculated excluding the animals slaughtered for meat consumption during the first year of life and not intended to be kept for reproduction. However, according to common agricultural practice, young slaughtered animals are replaced with purchased ones, in order to compensate the loss of adult animals and to increase the breeding performances of the herd.

Temporal distribution of new born animals

Another important aspect to consider is the distribution of births, given that is one of the major risk factors in the transmission of brucellosis. The parturitions, and therefore, the number of new-born animals, are not homogeneously distributed during the year, but a given seasonal pattern can be observed (**Table I (a)**). This phenomenon has been already observed in sheep and goats (De Massis *et al.* 2005), but is less common in cattle. However, this is common in Sicily in the area under study, and actually is a common practice in all areas of the Mediterranean basin in which animals are sent to pastures in given periods of the year (spring and summer) to facilitate births during the best climatic period of the year. Natural and/or Artificial Insemination are organized in order to reach this objective. Data from NDB show that in the study area cattle are going to pastures in the warmest period of the year, i.e. from April-May until the first half of November. Moreover, the type of husbandry is much more devoted to meat production rather than milk production, and this implies that mating and parturitions are programmed in a way to have more calves at disposal in the period of highest demand by the consumers.

For estimating the annual fertility rate for single cow, the proportion of new-born animals (males and females) over the adult cows (> 24 months of age) present each year in each farm has been calculated. The median value of the distribution obtained was equal to 0.65 and this value has been used in the model.

For estimating the annual turnover of adult cows, the number of the new-born females remaining in the same farm of birth for at least 24 months, natural deaths, slaughtered cows older than two years, the sold and culled cows, has been considered. The median value of the distribution obtained was equal to 0.20 and this value has been used in the model.

Table I (a). *Percentage of new births and (b) of female cattle per age in the study area.*

a		b	
Month	**Percent of births**	**Age (years)**	**Percent of females**
Jan	9.4	<1	16.7
Feb	11.0	1-2	12.7
Mar	13.5	2-3	11.8
Apr	13.6	3-4	9.8
May	11.6	4-5	8.8
Jun	10.3	5-6	7.8
Jul	7.1	6-7	7.8
Aug	5.0	7-8	6.9
Sep	3.9	8-9	5.9
Oct	4.2	9-10	3.9
Nov	5.3	10-11	2.9
Dec	5.1	11-12	2.9
		> 12	2.0

Demographic categories of epidemiological interest

The age at the first parturition and the numbers of days between following parturitions from the NDB were used to obtain the description of the different reproductive stages of female cattle population.

The length of pregnancy has been considered fixed and equal to 285 days (Nogalsky and Piwczyński 2012). This fixed value has been adapted from literature taking into consideration that in the type of husbandry and breed present in the study area, beef cattle have pregnancies little longer than dairy cattle. Another fixed value is sexual maturity, which has been set at 484 days (Schefers and Weigel 2012). This value derives from the consideration that the average time (in days) before a sexually mature cow becomes pregnant is different for a primiparous vs multiparous cow (119 days longer for primiparous, NDB data). These data have been used in the model to obtain the number of cows in each demographic category of epidemiological interest (d): not sexually mature (NM), sexually mature (M), and pregnant per month (p1-p10).

Simulator framework

The simulator described in **Figure 3** consists of three integrated models:

1) the Individual-based model (IBM) to assess the reproductive stage of each cow;
2) the compartmental SEIR model describing the brucellosis dynamics in each herd and at the pasture;
3) the control option model.

The IBM determines the demographic categories of epidemiological interest (d) for each cow, considering a turnover of 20%, and a time window of 15 years to create the input data for the com-partmental epidemiological model.

The compartmental SEIR (Susceptible, Exposed or Latent infectious, Infectious events, and Recovered) model simulates the infection transmission in each herd and at the pasture for 8 consecutive years. "I" represents the number of infectious events (*Brucella* shed). It is linked to places rather than infectious animals and thus, it is shared between herds during the pasture period, and between animals within the farm.

A detailed description of compartmental flows among each demographic category of epidemiological interest has been reported in **Supporting information** (**S2A Figure**).

The control option model simulates the control carried out on all animals of the fertile population in each herd in a certain period of the year (Tc: Control Time) and subsequently the culling of test-positive animals (Ta: elapsed time between control and culling).

The model redistributes, in Tc + Ta, the animals in the relative SER compartments and demographic categories of epidemiological interest based on the test results.

The Simulator simulates the demographic categories of epidemiological interest for each cow by IBM model. Then, for each temporal step (one day), the transmission and control (in the time step = Tc) of brucellosis in the population composed of five cattle herds in the pasture period (according to the different scenarios that will be considered by the model), and in the five cattle herds separately in the remaining of the year.

The Simulator was implemented and analyzed using R software (R Core Team 2015) and the simulation algorithm has been summarized in **Supporting information (S2B)**.

Scenario

The number of herds in the same pasture has been chosen considering the data stored in the NDB for Sicily.

It has been noted that in the same pasture it is possible to have up to five herds at the same time, so the model was set on this scenario in order to simulate the worst condition possible.

It is assumed that only one (the first) herd of the five herds at the pasture is already infected with a number of initial exposed cows = 2. Twenty pastures were generated, and each one simulated 50 times for a period of 8 years. It is also assumed that the pasture carrying capacity and then the density of grazing animals remain constant throughout the years.

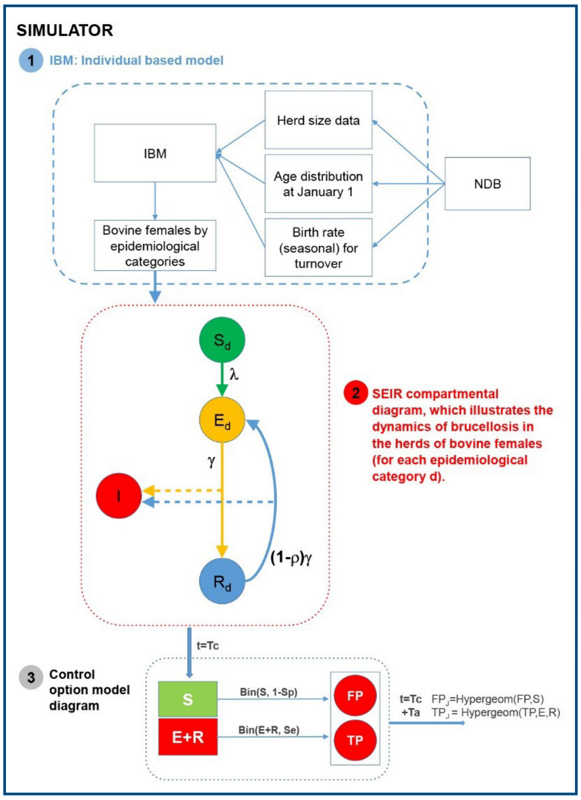

Figure 3. *Simulator framework. 1 IBM model, 2 The compartmental SEIR model, and 3 The control option model.*

The pasture period where the populations are perfectly mixed is from the mid of April to the mid of November and was estimated using the pasture movements data extracted from the NDB. **Table II** reports the analysis results aimed at defining the time spent grazing in days which was estimated at 214 days.

Only horizontal transmission of brucellosis has been considered in the model. In particular, only the transmission due to abortion/parturition followed by *Brucella* shed has been taken into account, given that this represents the main route of transmission.
Even if some authors consider them potentially dangerous for the transmission of the disease (Godfroid *et al.* 2005), the possible contribution of infected bulls by natural mating has been considered negligible for the purposes of the model (Farina and Scatozza 1998), while not sexually mature females are not considered susceptible to brucellosis infection (Godfroid *et al.* 2003).
In relation to brucellosis transmission and considering the reproductive stages two exposure conditions have been taken into account:

a) infection during non-pregnant stages in sexually mature animals: In this case, the abortion and dissemination of *Brucella* is considered to occur at the following calving after the exposure with a probability of abortion sampled from a triangular distribution as reported in **Table III**

b) infection during pregnancy: In this case, the abortion and dissemination of Brucella is considered to occur with a probability depending on the month of pregnancy during which the infection took place (**Table III**).

It is known that cows that aborted once normally would not abort again in following pregnancies. However, there is a given probability that, following a first abortion, at the next calving there may be a chance that *Brucella* can be eliminated, even with the birth of a normal, live, and viable calf (Farina and Scatozza 1998). Therefore, in all cases, the model considers a probability equal to 20% of having the shed of *Brucella* at the following parturition after a first post-exposure abortion and 0% for those still following.

In any case, the elimination of *Brucella* from the genital tract ends at the end of the uterine post-partum involution period (Fraser *et al.* 1991), which normally identified with the Voluntary Waiting Period (VWP), which ranges between 21 and 74 days (Chang *et al.* 2007).

The Individual Based Model

The IBM (**Figure 3(1)**) simulates the reproductive stages of each single female bovine from birth to 8 years of age.

For each cow and with 1-day time-step (for 15 years) the following data are simulated: age, timing of parturition (for sexually mature cows, from which the state of pregnancy is obtained in the previous 285 days).

The turnover rate is assumed to be constant during the year while the birth rate is seasonally based.

This implies the population may decrease during periods of low birth rates. In any case, the accumulated losses are recovered as soon as the new births become available. In this way, the model tries to restore the initial population size during the year.

To check the consistency of IBM (in terms of age distribution, the temporal distribution of new-borns, leaving the system animals, and overall population), the model's simulation results were compared with NDB data and reported in **Supporting information S3**. The IBM variables and parameters calculation are reported in **Table IV**.

Table II. *Quantiles of the herd time spent grazing (in days).*

Min	Max	Pasture Period (average)	P 0.05	P 0.25	P 0.5	P 0.75	P 0.95
1	361	214	22	107	164	290	326

Table III. *Model variables and parameters definition.*

Variable/ Parameter	Definition	Reference
S	Number of susceptible animals at each time step t belonging to the demographic categories (not sexually mature animals are not susceptible).	
E	Number of exposed or infected animal at each time step t.	
I	Number of *Brucella* shedding events at each time step t.	
R	Number of recovered animals at each time step t. Recovered means that animals may be still infected but no more infectious.	
π	The probability a sexually mature cow will be impregnated on a specific day of the year is πday-285 i.e. πday shifted by 285 days (see **Table 4**).	
π1	see **Table 4**	
μ	see **Table 4**	
λ	Force of infection rate.	
β	The monthly infectious contact rate, calculated starting from an abortion or birth event with *Brucella* shedding: P(month) = [0.95, 0.9, 0.8, 0.6, 0.5, 0.5, 0.3,0.2, 0.1,0.1, 0.1, 0.1]	[28, 36]
γi	The probability of having an abortion and shed of *Brucella* at the following parturition is calculated considering the reproductive stage: Non-pregnant animals: P= Triang(0.7, 0.8, 1) for the following calving; Pregnant animals considering the month of pregnancy: P=[0.001, 0.001, 0.001, 0.1, 0.75, 0.8, 0.75, 0.5, 1]	Modified from England et al., and Yamamoto *et al*. [28, 36]
ρ	Recovery rate: 0.8 for the first parturition following the abortion; 1 for the following parturitions.	Modified from Farina & Scatozza and Saegerman *et al*. [32, 37]

The brucellosis compartmental SEIR model

The bovine female population was divided into the demographic categories of interest (d) ac-cording to their ability to spread the infection. The population within all demographic categories but the not sexually mature (NM) was subdivided into three compartments Susceptible (S), Exposed (E), and Recovered (R), accordingly to the Infectious events (I) as shown in the flow chart of **Figure 3 (2)**.

The SEIR stochastic model for each demographic category involved in *Brucella* spread is de-scribed by the following ODEs . For the sake of simplicity, the compartment E also includes cows that do not recover after the first exposure, and the female population is closed to the turnover:

$$\begin{cases} \frac{dS_d}{dt} = -Binomial(S_{dt}, \lambda) \\ \frac{dE_d}{dt} = Binomial(S_{dt}, \lambda) - Binomial(Binomial(E_{dt}, \gamma_i), \rho) \\ \frac{dI}{dt} = Binomial(Binomial(E_{dt}, \gamma_i), 2 - \rho) \\ \frac{dR_d}{dt} = Binomial(Binomial(E_{dt}, \gamma_i), \rho) \end{cases} \tag{1}$$

where:

- d is the demography category;
- λ is the force of infection rate and depends on the abortions that have occurred in the past 12 months: -

$$\lambda = \frac{1 - \prod_{i=1}^{I}(1 - \beta_i)}{N}; \tag{2}$$

- β is the contact rate and is variable (decreasing) from the moment when an exposed animal aborts;
- γ_i is the inverse of the duration of the incubation period with i the month of pregnancy;
- ρ is the recovery rate.

The SEIR model variables and parameters values are reported in **Table III**. The probability of an animal becoming infected with brucellosis is not constant over time, as it is in many other diseases. Similarly, the duration of the incubation period is not constant over time. These time variations are because the transmission of brucellosis occurs at the moment of parturition or abortion and the amount of *Brucella* eliminated by an infected animal decreases with the time elapsing from the moment of parturition or abortion. Therefore, the force of infection (λ) depends on a contact parameter (β), which on turn is variable (decreasing) from the moment when an infected animal delivers or aborts within the herd. The value of β by month after the parturition or abortion of the infected animal is shown in **Table III**.

The elimination of *Brucella* by an infected animal and, thus, the infectivity period starts when an animal delivers or aborts. Therefore, the spread of brucellosis is closely related to the incidence of abortion, or parturition after infection. The occurrence of abortion depends on when the cow becomes infected. In general, abortion caused by brucellosis infection occurs between the fifth and eighth month of pregnancy. The probabilities of abortion in each month of pregnancy (γ) are reported in **Table III**.

Table IV. *IBM variables and parameters calculation.*

Definition	Name	Calculation	
Animal ages	Age Class	$AgeClassSize^{t0}$=Multinomial(N,p)	N=population size; p = the relative frequency of the age classes
	Cow Age	$AgeClassSize^{t0}_{i,j}$=Uniform(l_i,u_i)	*j*=1:N; li and µi the lower and upper bound of Age class (*i*). The age class >12 years has been manipulated to exponentially decay
Turnover rate assumed as the animals leaving the system at each time step	µ	$Turnover^{day}$= $Turnover^{Y}$ /365 = µ	$Turnover^{Y}$= 20% [38] and $Turnover^{day}$ its daily rescaled value.
The probability a sexually mature cow will give a birth on a specific day of the year	π	π^{day} = $Productivity^{y}$ * pBM /(dd^{M} * pFt)	$Productivity^{y}$ = 65% (Percentage of adult females in a herd which give birth to a calf in one year. NDB data); pB_M = the proportion of births according to the month (**Table 1 (a)**); dd^M = number of days of the month; pFt = the ratio between not pregnant cows with age greater than 484 days (π1) and less than 8 years and the population.
New born cows become sexually mature with an average time	π1	π1=484 days	

The control option model

Given the current epidemiological situation for brucellosis in Italy, the National Competent Authority, the Ministry of Health, has chosen a program of eradication of the disease in cattle, buffaloes, sheep and goats.

This implies the prohibition of vaccination, allowing only a policy of testing and slaughter of positives animals (Italian Ministry of Health 1992, 1994, 2015). Therefore, the latter control option has been considered in the model. According to the Italian National Book of Rules, the serological tests to be used in the context of the Brucellosis National Eradication plan are the Rose Bengal Test (RBT) and the Complement Fixation Test (CFT). The sensitivity and specificity estimates of these tests are high [EFSA 2006, Greiner *et al.* 2009], and are shown in **Table V**.

The control option model simulates a simplified version of the control system by performing the RBT test on all fertile animals and the CFT test for confirmation on RBT positive animals (**Figure 3 (3)**). It is assumed that the control takes place every year and involves the five grazing herds on Tc day and the tested-positive animals (TP + FP) are culled after a Ta time and added to animals leaving the system.

The tested-positive animals at Tc time are extracted from the following distributions:

The tested animals will then be redistributed at Tc

$$TP \sim Binomial(E + R\ , Se_{RBT}Se_{CFT}) \quad (3)$$
$$FP \sim Binomial(S, 1 - Sp_{RBT}Sp_{CFT}) \quad (4)$$

+ Ta time in the various epidemiological catego-ries into the specific S, E, R compartments using the multivariate hypergeometric distributions as fol-lowing: where:

$$TP_j = Hypergeom(TP, E, R) \quad (5)$$
$$FP_j = Hypergeom(FP, S) \quad (6)$$

- Se and Sp are the sensitivity and specificity of tests (**Table V**);
- S is the susceptible population at Tc time involving all demographic categories;
- E+R is the exposed and recovered susceptible population at Tc time involving all demographic categories;
- j is the specific demographic category;
- FP and TP are false and true test positive animals respectively.

The control system was simulated considering ten simulation scenarios that allow analyzing the effectiveness of the different control strategies adopted through the evidence of the different Brucellosis diffusion curves obtained in terms of the probability that at least one herd (non-seed) become infected at a given time.

The parameters differentiating the scenarios are:

- culling time (Ta) reflecting three range of efficiency: maximum, medium and according to the current Italian legislation;
- control time (Tc): considering that the control could takes place before, during, and in the middle time of the grazing period (assuming that the yearly grazing period starts on 104th day);
- control starting year: considering that the control may start the first year of the simulated period or with a delay of two years (i.e. starting at the beginning of the third year);
- grazing period: considering the variability of the period spent in grazing, as reported in **Table II**.

Table V. *IBM variables and parameters calculation.*

Test	Se	Sp
RBT	0.981	0.998
CFT	0.96	0.998

Results

The Brucellosis compartmental SEIR model

SEIR model has simulated the transmission of brucellosis in twenty pastures for a period of 8 years and 50 iterations. For each pasture it was considered a population composed by five cattle herds, one of which infected at t0 with 2 exposed animals, Turnover = 20% and a Productivity = 65%.

Figure 4 shows the temporal trend of the SER compartments for each of the five herds of the first simulated pasture (with 0.25, 0.50, and 0.75 CI) and the median values of the 50 simulations.

The vertical dotted lines (grey) outline the pasture period from the 104th to the 318th day of the year. The in-fection reaches all healthy herds during the second year of simulation and does not die out in any herd. Brucellosis becomes endemic if no control measures are applied.

The first graph (at the top) represents the seed farm. You may notice that the red line is already present at the beginning of the time.

The other herds will be then infected after, for having had contact in the same pasture. Therefore, the number of exposed (red line) and the number of recovered (green line) is raising over time.

Then, eventually, the infection will reach the other four herds and, in absence of control measures, it would stabilize overtime at a given prevalence (in other words that means the disease be-came endemic or R0 is equal to one). **Figure 5** shows the distribution of probability that one of the four herds (not exposed at t0) has at least one exposure over time (with 0.5 and 0.95 CI and the median values of the 50 simulations).

The vertical dotted lines (red) outline the pasture period from the 104th to the 318th day of the year. The probability with which the infection reaches a new herd on pasture increases progressively over the years.

The probability of infection tends to increase when animals are in pasture, while it is descending or stable in other periods, and so on. In other words, if you are mixing herds at pasture without controls for brucellosis, at the end of the second year there will be a probability of 50% that at least another herd would be infected.

This has been defined as Scenario 0 (no control measures applied over time).

Discussion

The model has considered various combinations of control schemes and frequencies to identify the best ones able to reduce, as much as possible, the likelihood of moving infected animals to pastures and/or increasing the probability of detecting the infected animals on pastures. Actually, the major point for policy-makers would be to avoid having infected (and infectious) animals on pastures, because this is the place where brucellosis may better escape from control.

When we consider the transmission on pastures, the probability for a healthy herd to be infected is increasing progressively over the years. If the infection is not controlled, the probability that a further herd would be infected in the same pasture will remain stable from the 5th year onwards, with a value around a 70% (**Figure 5**). This is consistent with what is generally known about the epidemiology of brucellosis, when the infection chronically spreads in herds or flocks that are not subject to disease control. Three scenarios regarding the elapsed times between the detection of positive animals and their culling (Culling time) have been considered in the model considered (day 0, day 7, or day 15). Each of those seems not to have an impact in the infection trend with respect to the others (Figure 6). This finding could be related to the different magnitude of the variable Ta for the three scenarios, as well as to the time with which an infected animal generates new ones (the inverse of contact rate).

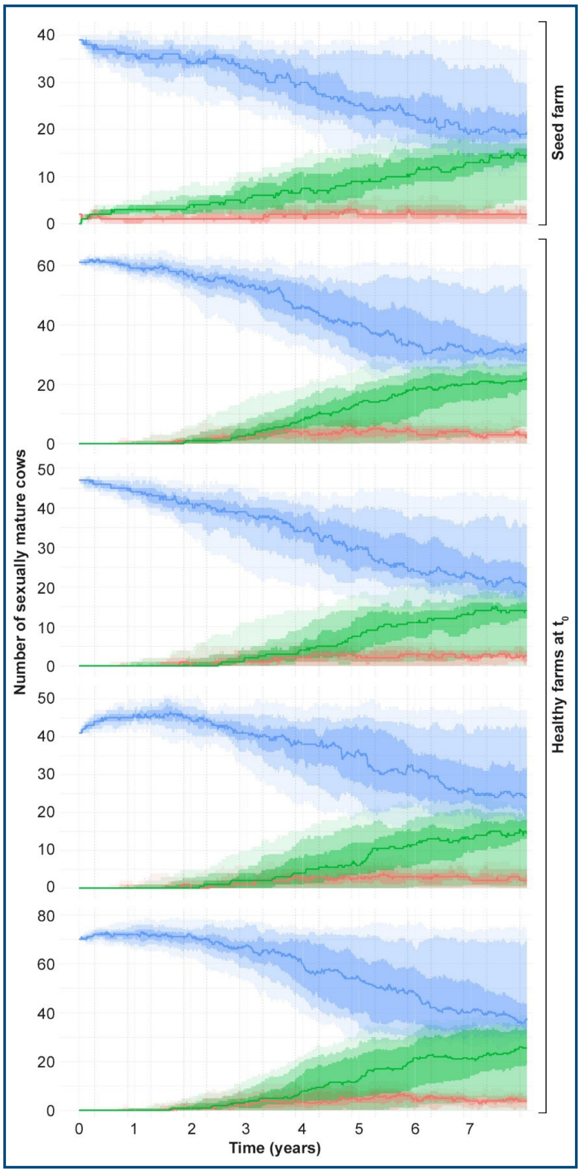

Figure 4. *The temporal trend of the SER compartments (no control measures adopted). S (susceptive animals) in blue, E (exposed animals) in red, and R (recovered animals) in green lines respectively, for each of the 5 herds of the first simulated pasture. The max value reported on the y-axes indicates the size of each herd involved. The vertical dotted lines outline the pasture period from the 104th to the 318th day of the year.*

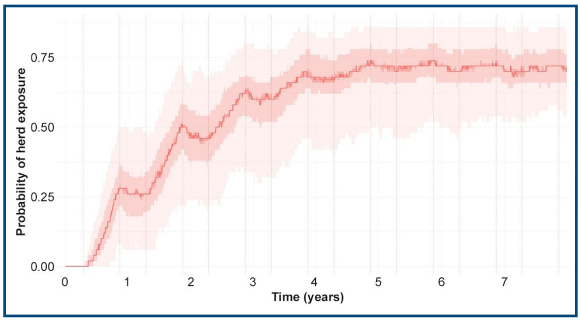

Figure 5. *Scenario 0. The distribution of probability of herd exposure over time (red line). The vertical dotted lines (red) outline the pasture period from the 104th to the 318th day of the year.*

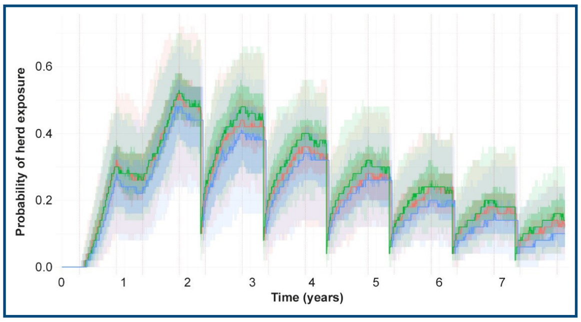

Figure 6. *Shows the simulation results of three scenarios representing the control strategies as the culling time (Ta) varies in maximum, and medium efficiency, and according to the current legislation. The control time is the same in each scenario (i.e. Tc = 74 days, then, 30 days before going to the pasture) and the control is starting in the third year..*

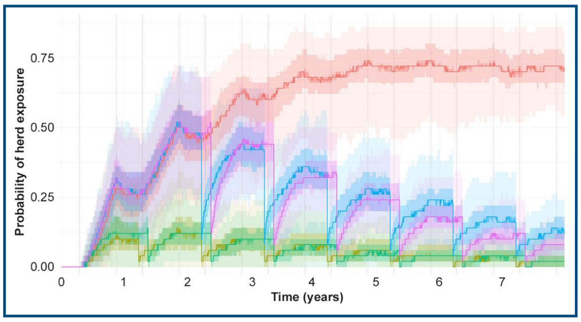

Figure 7. *Shows the simulation results of four scenarios representing the control strategies as the control time (Tc) and the year of control starting varies in 30 days before or 30 days after going to the pasture and starting in the first and third year, respectively. The culling time is the same in each scenario (i.e. Ta = 7 days).*

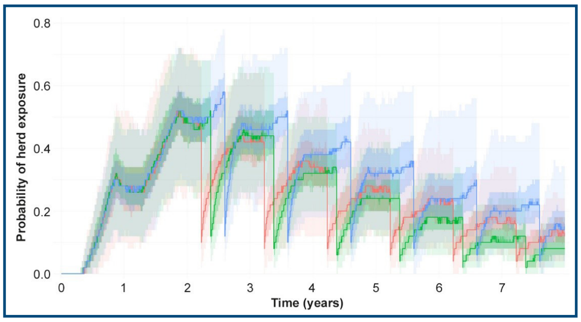

Figure 8. *Shows the simulation results of the scenario representing the control strategy in the control time oc-curring in the middle of the grazing period Tc=211th day, compared with scenarios in which Tc=74th day and Tc=134th day, respectively. The control starts in the third year and the culling time is the same Ta = 7 days in each scenario.*

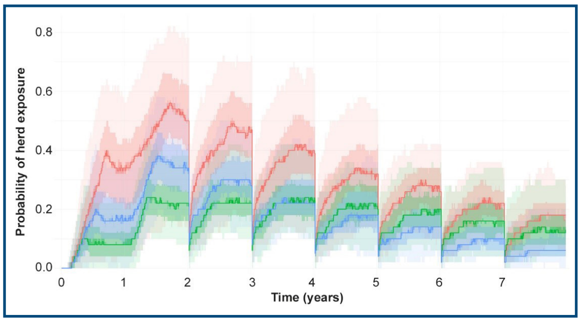

Figure 9. *Shows the simulation results of three scenarios representing the control strategies as the grazing period varies from 100 days, 164 days, and 290 days, respectively. The control time is the same Tc = 74th day, then, 30 days before going to the pasture, and starts in the third year.*

This in the context of pre-movement test and for a grazing period of 214 days, and could be related to the fact that the model considers the movement of false negative animals, thus considering the sensitivity of the tests concerned. *Brucella* dissemination at pasture and the related environmental contamination are in all cases under control.

They determine a decrease of the prevalence over time, but not to a level of disease eradication. The increase in culling time (i.e. to 30 days) is not expected to im-prove the situation, however, this scenario has not been considered because outside the requirements of current legislation (Italian Ministry of Health 2015).When the different control options are considered in terms of time on which the control begins, the results highlight the evidence that an earlier application of the controls (year 1 vs year 3) would determine a faster and more marked containment of the infection (**Figure 7**). This is consistent with the current surveillance program in force in Italy, where cattle should be tested for brucellosis at least once a year, and it is what should be recommended to competent authorities. However, the legislation does not state the exact time on which the control should take place (i.e. if it should take place before, during or after summer grazing) (Italian Ministry of Health 1992, 1994, 2015). If we consider the options of performing the control 30 days before going to pasture (day 74) or after (in pasture at different control timing, i.e. day 134 or 211), little or no difference is observed (**Figure 7** and **Figure 8**).

Moreover, if the control is performed in pasture at day 134 (even having less efficacy in the first two years with respect of the control performed before going to pasture), it seems to have a higher efficacy over time after the fourth year of controls onwards. This may suggest that the control in pasture may have a protective effect, however, this effect is not present if the control is per-formed in the middle of grazing period (at day 211, **Figure 8**).

This could be related a mixed effect between the reproductive stage of animals on pasture and the related (and subsequent) environmental contamination. Indeed, this approach should also be evaluated in the light of the real reproductive cy-cles and the calving seasons of the animal population in the area concerned. This is correct for cattle farmed in the area under study, which demonstrated a marked seasonality in their reproductive pat-tern, and is even more real for sheep and goats, where driving the seasonality in parturitions is a common husbandry practice in Italy (De Massis *et al*. 2005). Given that the pasture represents a physical place in which different herds come in connection to-gether, a longer grazing period should lead to a higher probability of infection for healthy herds. This is what happens in absence of control measures (as shown in **Figure 9**, looking at first two years).

How-ever, the results of the model show that a shorter grazing period retains a greater ability to spread the disease than a medium term grazing period (**Figure 9**). This apparently unexpected result is probably due to three circumstances:

1. the majority of infectious events (abortions) occur in the first months of grazing, then a short grazing period represents the worst period in terms of probability of an infectious event;
2. in practice, as in the real world, the transmission of the disease occurs much faster within the farm (given the concentration of animals) than on pasture, and the relationship between time spent on pasture and time spent on farm determines unfavorable outcomes in terms of prob-ability of infection for the shorter grazing period;
3. the time spent on the farm between control and the start of grazing (1 month) is sufficient to infect new animals even after the test-and-slaughter within the farm because the longer peri-od on the farm has produced a high number of infectious events, keeping consequently the in-fection pressure in the first period of grazing.

Taking into consideration the link to the place the infectious events rather than to the infected animals could allow a reduction of the infection pressure through the years by changing the pasture place or dividing the pasture area into more areas for alternate use. This study will extend further to improve the model's adaptability to reality. The overcoming of its limits and conditions of applicability will let to share the model through a web application.

The model could possibly integrate the use of biosecurity measures within the farm, and could be extended by using real data on animal movements. In this case it could take into account the different times and the different periods on which farms are sending animals to pastures, as well the time of pasture sharing in relation to the actual births seasonality. In this way, it will be possible to further evaluate how the different variables would influence the spread of brucellosis, to provide precise and specific indications for each territorial reality to identify the most effective control time. In light of the results, it is very important to start biosecurity measures before going to pasture.

Conclusions

Overall, and regardless of the strategies implemented, it is evident that a considerable reduction of the infection is obtained within the 8-years period considered, without reaching disease eradication. This is consistent with the limits of diagnostic tests in terms of sensitivity and specificity [39], and demonstrates once again that serological testing cannot lead to brucellosis eradication alone. Instead, at the end of the eradication program, it is necessary to implement a more sophisticated and interactive system of diagnostic and epidemiological investigations, aimed to the detection of the residual sources of infection.

References

Aïnseba B, Benosman C, Magal P 2010. A model for ovine brucellosis incorporating direct and indirect transmission. J Biol Dyn. 2010; 4: 2±11. https://doi.org/10.1080/17513750903171688 PMID: 22881067.

Akakpo AJ, Bornarel P.1987. Epidémiologie des brucelloses animales en Afrique tropicale: enquêtes clinique, sérologique et bacté riologique. Rev Sci Tech Off Int Epiz 6: 981–1027. https://doi.org/10.20506/rst.6.4.313.

38. Amicabile S, 2018. L'utile lordo di stalla. In: Economia agraria e dello sviluppo territoriale. Pp XII-252 ISBN 978-88-203-8323-7 http://www.amicabile.net/archivio/cepa2/sfogliacepa.pdf.

Calistri P, Iannetti S, Atzeni M, Di Bella C, Schembri P, Giovannini A. Risk factors for the persistence of bovine brucellosis in Sicily from 2008 to 2010. Prev Vet Med. 2013; 110: 329±334. https://doi.org/10.1016/j.prevetmed.2012.12.008 PMID: 23287716.

Chang Y M, González-Recio O, Weigel K A, Fricke P M, 2007. Genetic Analysis of the Twenty-One-Day Pregnancy Rate in US Holsteins Using an Ordinal Censored Threshold Model with Unknown Voluntary Waiting Period. J. Dairy Sci. 90: 1987–1997 https://doi:10.3168/jds.2006-359.

Corbel M. 2006. Brucellosis in humans and animals, WHO/CDS/EPR/2006.7.World Health Organization in collaboration with the Food andAgriculture Organization of the United Nations and World Organization for Animal Health. WHO Press, Geneva, Switzerland

Crawford RP, Huber JD, Adams BS, "Epidemiology and surveillance" in: Animal Brucellosis, K Nielsen and J R Duncan, Eds., CRC Press, Boca Raton, Fla, USA, 1990.

De Massis F, Di Girolamo A, Petrini A, Pizzigallo E, Giovannini A, 2005. Correlation between animal and human bru-cellosis in Italy during the 1997-2002 period. Clinical Microbiology and Infection. 11: (8) 632-636. https://doi.org/10.1111/j.1469-0691.2005.01204.x.

De Massis F, Zilli K, Di Donato G, Nuvoloni R, Pelini S, Sacchini L, D'Alterio N, Di Giannatale E, 2019. Distribution of Brucella field strains isolated from livestock, wildlife populations, and humans in Italy from 2007 to 2015. PLoS ONE 14(3): e0213689. https://doi.org/10.1371/journal.pone.0213689.

Diaz-Aparicio E, 2013. Epidemiology of brucellosis in domestic animals caused by Brucella melitensis, Brucella suis and Brucella abortus. Rev Sci Tech Int Epiz 2013 32 1 53-60.

Dubie T, Adugna M, Sisay T, Mukitar Y 2014. The economic and public health significance of brucellosis. Global Research Journal of Public Health and Epidemiology. ISSN-2360-7920: Vol. 1: (7): 54-64.

EFSA, European Food Safety Authority, 2006. Annex to Scientific Opinion on performances of Brucellosis diagnostic methods for bovines, sheep, and goats (adopted on 11 December 2006 and including the Scientific Report as Annex). EFSA J. 432, 1–44.

EFSA, European Food Safety Authority, 2008. The community summary report on trends and sources of zoonoses and zo-onotic agents and food-borne outbreaks in the European Union in 2008. EFSA J (2010) 8: 1496.

EFSA, European Food Safety Authority, 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA J (2016) 14: 4634.

England T, Kelly L, Jones RD, MacMillan A, Wooldridge M, 2004. A simulation model of brucellosis spread in British cattle under several testing regimes Preventive Veterinary Medicine 63: 63–73. https://doi.org/10.1016/j.prevetmed.2004.01.009.

EU, European Union 2003. Commission Decision 2003/467/EC of 23 June 2003 establishing the official tuberculosis, brucel-losis, and enzootic-bovine-leukosis-free status of certain Member States and regions of Member States as regards bovine herds. OJ L 156, 25.6.2003, p 74-78. http://data.europa.eu/eli/dec/2003/467/2020-04-22.

EU, European Union, 1992. Commission Decision of 21 December 1992 recording the compliance by certain Member States or regions with the requirements relating to brucellosis (B. melitensis) and according them the status of a Member State or re-gion officially free of the disease. OJ L 13, 21.1.1993, p. 14–15. http://data.europa.eu/eli/dec/1993/52/2019-11-28.

Farina R, Scatozza F, 1998.Trattato di malattie infettive degli animali. Seconda Edizione. UTET, Torino. ISBN 88-02-05315-4.

Fraser CM, Bergeron JA, Mays A,Aiello SE, 1991. The Merck Veterinary Manual. Merck & Co., Inc. Rahway, N.J., USA.

Garofolo G, Di Giannatale E, De Massis F, Zilli K, Ancora M, Camma C, Calistri P, Foster JT. 2013. Investigating genetic di-versity of Brucella abortus and Brucella melitensis in Italy with MLVA-16. Infect Genet Evol 19:59 –70. https://doi.org/10.1016/j.meegid.2013.06.021.

Godfroid J, Al-Mariri A, Walravens K, Letesson J-J, 2003. Brucellosis bovine. In: Lefevre, P.C. (ed.); fre; Blancou, J. (ed.); Chermette, R. (ed.); Principales maladies infectieuses et parasitaires du betail: Europe et regions chaudes. Editions TEC & DOC - Lavoisier Paris (France). ISBN 2-7430-0495-9.

Godfroid J, Cloeckaert A, Liautard JP, Kohler S, Fretin D, Walravens K, Garin-Bastuji B, Letesson J-J. 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet Res 36:313–326. https://doi.org/10.1051/vetres:2005003.

Graziani C, Mancini F R, Adone R, Marianelli C, Pasquali P, Rizzo C, Bella A, De Massis F, Danzetta ML, Calistri P, Primavera A, Ruocco L, Busani L, 2013. La brucellosi in Italia dal 1998 al 2011. Roma: Istituto Superiore di Sanità (Rapporti ISTISAN 13/45), 75 pp.

Greiner M, Verloo D, De Massis F 2009. Meta-analytical equivalence studies on diagnostic tests for bovine brucellosis al-lowing assessment of a test against a group of comparative tests. Preventive Veterinary Medicine 92: 373–381. https://doi.org/10.1016/j.prevetmed.

Italian Ministry of Health, 1992: Decree n.651 of 2 July 1992, Regolamento concernente il piano nazionale per la eradicazione della brucellosi negli allevamenti ovini e caprini. Gazzetta Ufficiale della Repubblica Italiana n. 276 of 23/11/1992.

Italian Ministry of Health, 1994: Decree n.651 of 27 August 1994, Regolamento concernente il piano nazionale per la eradi-cazione della brucellosi negli allevamenti bovini. Gazzetta Ufficiale della Repubblica Italiana n. 277 of 26/11/1994.

Italian Ministry of Health, 2015: Order of 28 May 2015, Misure straordinarie di polizia veterinaria in materia di tubercolosi, brucellosi bovina e bufalina, brucellosi ovi-caprina, leucosi bovina enzootica. Gazzetta Ufficiale della Repubblica Italiana n. 144 of 24/16/2015.

Moreno E. 2014. Retrospective and prospective perspectives on zoonotic brucellosis. Front Microbiol 5:213. https://doi.org/10.3389/fmicb.2014.00213.

Nicoletti P, 1980. The epidemiology of bovine brucellosis. Adv Vet Sci Comp Med. 1980; 24:69-98.

Nogalski Z and Piwczyński D, 2012. Association of Length of Pregnancy with Other Reproductive Traits in Dairy Cattle. Asian-Aust. J. Anim. Sci. 25: (1) 22-27 DOI: https://doi.org/10.5713/ajas.2011.11084.

Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV, 2006. The new global map of human brucellosis. Lancet Infect Dis 6: 91–99. https://doi.org/10.1016/S1473-3099(06)70382-6 PMID: 16439329.

Pérez-Sancho M, García-Seco T, Domínguez L, Álvarez J, 2015 Control of Animal Brucellosis - The Most Effective Tool to Prevent Human Brucellosis. "Updates on Brucelloses" book chapter. http://cdn.intechopen.com/pdfs-wm/49083.pdf http://dx.doi.org/10.5772/61222.

R Core Team. http://www.R-project.org/[Internet]. 2015.

Roy S, McElwain TF, Wan Y, 2011. A network control theory approach to modeling and optimal control of zoonoses: case study of brucellosis transmission in sub-Saharan Africa. PLoS Negl Trop Dis. 5: e1259. https://doi.org/10.1371/journal.pntd.0001259 PMID: 22022621.

Saegerman C, Berkvens D, Godfroid J, Walravens K. Bovine brucellosis. in: Lefèvre P.-C., Blancou J., Chermette R., Uilenberg G. (Eds.) Infectious and parasitic diseases of livestock. Editions TEC & DOC, Paris, France. 2010.

Savini L, Candeloro L, Conte A, De Massis F, Giovannini A, 2017. Development of a forecasting model for brucellosis spreading in the Italian cattle trade network aimed to prioritise the field interventions. PLoS ONE 12(6): e0177313. https://doi.org/10.1371/journal.pone.0177313.

Schefers M and Weigel KA, 2012. Genomic selection in dairy cattle: Integration of DNA testing into breeding programs. Animal Frontiers 2: (1) 4-9. https://doi.org/10.2527/af.2011-0032.

Wilesmith JW. 1978. The persistence of Brucella abortus infection in calves: a retrospective study of heavily infected herds. Vet Rec 103(8):149-53. https://doi.org/10.1136/vr.103.8.149.

Yamamoto T, Tsutsui T, Nishiguchi A, Kobayashi S, 2008. Evaluation of surveillance strategies for bovine brucellosis in Japan using a simulation model. Prev Vet Med 86: 57-74. https://doi.org/10.1016/j.prevetmed.2008.03.004 PMID: 18440660.

Zinsstag J, Roth F, Orkhon D, Chimed-Ochir G, Nansalmaa M, Kolar J, Vounatsou P, 2005. A model of animal-human brucellosis transmission in Mongolia. Prev Vet Med., 69: (1) 77-95. https://doi.org/10.1016/j.prevetmed.2005.01.017 PMID: 15899298.