

# Estimating the probability of freedom from bovine brucellosis in the Galapagos Islands

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## Short Paper

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### Abstract

Bovine brucellosis is a worldwide zoonotic disease that still burdens several countries in the Mediterranean, Asia, Africa and Latin America. Although the disease is present in Ecuador, the Galapagos Islands seem to be free from the disease based on a survey conducted in 1997 where all tested animals showed negative results. This study aimed at estimating the probability of freedom from brucellosis in this Ecuadorian province in 2014. A survey was implemented on the three main cattle-producing islands of the province: Santa Cruz, Isabela and San Cristóbal. Thirty-three cattle farms and 410 cattle were tested for brucellosis using the Rose Bengal test and indirect ELISA. All animals showed negative results for both tests. Probability of freedom was estimated at 98%, 91% and 88% for Santa Cruz, Isabela and San Cristóbal, respectively, considering a herd-level design seroprevalence of 20% and animal-level design seroprevalence of 15%, and assuming a perfect specificity of the survey. The negative results found in 1997 and present surveys suggest that the Galapagos Islands are free from bovine brucellosis.

Brucellosis is a worldwide zoonotic disease that has been classified by the World Health Organization (WHO) as a neglected zoonosis [1]. Bovine brucellosis, mainly caused by *Brucella abortus*, is generally associated with abortions, reduced fertility and reduced milk production in cattle. Humans may contract the disease through contact with infected animals and consumption of contaminated unpasteurised milk or dairy products [1]. Although a handful of countries are considered free of brucellosis, several nations in the Mediterranean region, Africa, Asia and Latin America are still burdened by this disease which impacts their public health and economy [1].

The Galapagos Islands are a set of insular territories 960 km off the coast of Ecuador and an Ecuadorian province. Only 3% of its territory is colonised while the remaining 97% belongs to the Galapagos National Park. The introduction of livestock, mainly cattle, into the islands dates back from the beginning of the 19th century. Today, cattle production is present among the four colonised islands: Santa Cruz, Isabela, San Cristóbal and Floreana. Cattle importation from mainland Ecuador was banned in 2003. In mainland Ecuador, bovine brucellosis is spread throughout the country: the seroprevalence in dairy and mixed cattle has been estimated at 17% [2]. In the northwestern part of Ecuador, humans have been found to be infected with *B. abortus* [3, 4]. Considering the movement of animals between mainland Ecuador and the Galapagos Islands, the likelihood of the introduction of bovine brucellosis into the islands could not be excluded. In 1997, a serological survey was performed in 114 farms in the Galapagos Islands and all the 507 serum samples collected were found to be negative in the Rose Bengal test [5]. Based on these results, the Ecuadorian Ministry of Agriculture considered the region to be free from brucellosis and suggested other surveys be performed to further support this status [5]. This study aimed at estimating the probability of freedom from bovine brucellosis in the Galapagos Islands after the implementation of a new survey.

In 2014, the Galapagos Islands had a total of approximately 10 000 cattle distributed among the islands of Santa Cruz (~6500 cattle), Isabela (~1500 cattle), San Cristóbal (~1500 cattle) and Floreana (~100 cattle). Santa Cruz holds the majority of cattle farms and is the main milk producer of the region, while Floreana has only a small number of cattle farms that are mostly located within the Galapagos National Park area. In 2013, the number of farms for the three islands with the largest cattle population was estimated at 111 for Santa Cruz, 65 for San Cristóbal and 38 for Isabela, with an average number of cattle per herd of 62, 23 and 40, respectively. All farms use extensive cattle raising systems.

As a limited number of animals could be sampled considering costs and practical implementation of the survey, it was only implemented in the islands with the largest cattle

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population (i.e. Santa Cruz, Isabela and San Cristóbal). To estimate the probability of freedom from bovine brucellosis, a between-herd design seroprevalence of 20%, prescribed by the EU Commission for Member States to retain their official status of brucellosis free [6], was used. Based on within-herd seroprevalence values reported in infected holdings in Argentina (from 4.3% to 40%) [7], a within-herd design seroprevalence of 15% was chosen. For each island the sample size was calculated to detect those seroprevalence values with imperfect tests and a 95% level of confidence using EpiTools [8]. This yielded herd sample sizes of 11 in San Cristóbal, 11 in Isabela and 17 in Santa Cruz with 28 animals per herd in the largest herds and 14 in the smallest herds. It was decided to randomly select 15 animals over 6 months of age in each herd. In herds with less than 15 animals, all animals were tested.

Blood samples were collected in August 2014 and sera were tested using rose bengal test (RBT) (Pourquier Rose Bengale Ag, IDEXX Montpellier, France) and indirect ELISA (I-ELISA) (IDEXX Brucellosis Serum Ab Test, IDEXX Montpellier, France) according to the OIE prescriptions as well as manufacturer's instructions. All reagents used for this study were previously controlled as fulfilling EU and OIE standardisation requirements. The sensitivities of the RBT and I-ELISA were combined as parallel tests to increase survey sensitivity using the following formula:

$$Se_{\text{Comb}} = 1 - (1 - Se_1) \times (1 - Se_2)$$

where  $Se_{\text{Comb}}$  is the combined sensitivity of both tests and  $Se_1$  and  $Se_2$  are the sensitivities of each test.

Survey sensitivity (Sse) was calculated based on methods described by Martin *et al.* (2007) [9]. The Sse is the probability that at least one seropositive animal will be detected by the survey given the infection is present above the specified design prevalence. To take into account that brucellosis, if present in the population, would be clustered in herds, the sensitivity of detection was calculated separately for each herd sampled using a hypergeometric probability formula. Herd sensitivity (SeH) was calculated as:

$$SeH = 1 - \left(1 - SeA \times \frac{n}{N}\right)^{P_{\text{WH}} \times N}$$

where  $n$  is the number of tested animals,  $N$  is the total number of animals in the herd and  $P_{\text{WH}}$  is the within-herd design seroprevalence. The sensitivity of detection for each animal (SeA) is the sensitivity of the combined diagnostic tests used ( $Se_{\text{Comb}}$ ). To take into account the variability in the test sensitivities, we assigned a Pert distribution to SeA. The reported means and the minimum and maximum values of the confidence intervals of the rose bengal test (97.7%; (95.9–99.3)<sub>95% CI</sub>) and indirect ELISA (95.7%; (93.4–98.0)<sub>95% CI</sub>) [10] were used as the most likely value, minimum and maximum values of the Pert distribution.

Sse for each island was calculated using the following formula:

$$Sse = 1 - \left(1 - SeH_{\text{avg}} \times \frac{n_H}{N_H}\right)^{P_{\text{BH}} \times N_H}$$

where  $SeH_{\text{avg}}$  is the mean of the herd sensitivities,  $n_H$  is the number of tested herds,  $N_H$  is the total number of herds in the island and  $P_{\text{BH}}$  is the between-herd design seroprevalence.

Freedom from brucellosis can be defined as a certain level of confidence that the true seroprevalence is below the specified between-herd and within-herd levels design seroprevalence.

To calculate the probability of freedom, we used the Bayesian approach proposed by Martin *et al.* (2007) [7]. We considered that the conditions affecting the probability of freedom from a disease are the sensitivity of the survey and the prior probability of freedom (PriorPFree) assuming a perfect specificity of the survey. The probability of freedom (PFree) for each island was calculated as

$$PFree = \frac{\text{PriorPFree}}{1 - Sse \times (1 - \text{PriorPFree})}$$

By convention, the PriorPFree is set to 0.5. However, as the survey conducted in 1997 in the Galapagos Islands yielded negative results, we chose a most likely value of 0.7, a minimum value of 0.5 and a maximum value of 0.8 for the Pert distribution assigned to this variable.

Formulas were implemented in Excel 2010 with the add-in Poptools [11] to estimate sensitivities and probability of freedom from brucellosis per island. A stochastic process was generated using 1000 iterations to take into account the variability of the test sensitivities and the uncertainty of the prior probability of freedom. The spreadsheet can be obtained upon request from the corresponding author.

Serum samples were collected from 410 cattle in 33 farms in the three sampled Galapagos Islands (Table 1). For practical reasons, only eight herds in Isabela and eight in San Cristóbal could be sampled. All cattle showed negative results in both serological tests (RBT and I-ELISA). Table 1 shows the survey sensitivity and probability of freedom results found for each island. The highest values were obtained for Santa Cruz (survey sensitivity: 95.8% (95.7–95.9)<sub>95% CI</sub>; probability of freedom: 98.0% (96.9–98.8)<sub>95% CI</sub>) and the lowest for San Cristóbal (survey sensitivity: 71.0% (70.9–71.1)<sub>95% CI</sub>; probability of freedom: 88.0% (82.1–92.2)<sub>95% CI</sub>).

All of the cattle samples in this study were negative for *Brucella* spp., in line with the 1997 serological survey conducted in cattle of the Galapagos Islands. Considering a between-herd prevalence of 20% and within-herd prevalence of 15%, we obtained an optimal survey sensitivity and probability of freedom values for Santa Cruz Island, while they were lower for Isabela and San Cristóbal. This difference may be explained by the sampling of less herds and hence less animals in Isabela and San Cristóbal.

According to OIE standards, a zone may only be declared free from bovine brucellosis if regular testing for the past three years shows brucellosis is not present in 99.8% of all herds representing at least 99.9% of all bovines in the zone [12]. Therefore, neither the survey implemented by the Ecuadorian government in 1997, nor our survey meet this OIE requirement since not all herds have been tested. The design between-herd prevalence of 20% that were used would be in accordance with the one prescribed by the EU Commission for Member States to retain their official status of brucellosis free [11]. However, other factors should be taken into account to support the brucellosis-free status. First, if the disease were present, it would be widespread in the Galapagos Islands considering that animals can move freely between neighbouring farms since there are no physical delimitations between herds. Moreover, no vaccination against brucellosis has ever been implemented in the Galapagos Islands. In regions with a

**Table 1.** Survey sensitivity and probability of freedom from bovine brucellosis for the three main cattle-producing Galapagos Islands

Island	No. of herds sampled	No. of cattle sampled	Survey sensitivity (%) (95% CI)	Probability of freedom (%) (95% CI)
Santa Cruz	17	236	95.8 (95.7–95.9)	98.0 (96.9–98.8)
Isabela	8	87	79.4 (79.3–79.5)	91.1 (86.1–94.4)
San Cristóbal	8	87	71.0 (70.9–71.1)	88.0 (82.1–92.2)

similar situation, the disease is widespread. This is the case of the Kafr El Sheikh Governorate in the Nile Delta region of Egypt, where the high density of ruminants along with the free movement of small ruminant herds allowed frequent contact between animals of different households and villages [13]. These factors combined with the absence of vaccination led to a high seroprevalence of brucellosis in the region. Second, despite the presence of brucellosis in Ecuador, the insular isolation of the Galapagos Islands, especially after the ban on animal importations in 2003, has protected these islands from the introduction of infectious diseases such as brucellosis. A similar situation occurs in the insular region of Argentina named Tierra del Fuego considered as free from bovine brucellosis since 2011. This declaration was based on the negative results obtained in a survey performed on all cattle farms in the region, the absence of brucellosis reports in both the human and cattle population, the insular isolation of the province and the few well-controlled animal movements into the region and among farms [14]. Third, there have not been reports of clinical cases in humans or cattle.

The Ministry of Agriculture and Livestock from Ecuador is willing to improve the management and the control of livestock animal diseases in the Galapagos Islands. This study, conducted with the approval of the Galapagos Biosecurity Agency (ABG), was launched in that framework. A second step that could help to substantiate freedom from brucellosis would be to set up an early detection system that includes, at least, the submission of abortion samples to laboratory testing and the diagnostic capacity for performing brucellosis diagnosis. As the implementation of a survey that would allow testing all farms is not feasible, we could further suggest using collected cattle blood samples in future surveys to detect other diseases for brucellosis testing as well. The results of all surveys including ours could be combined, incorporating the probability of introduction as suggested by Martin *et al.* (2007) [7], to estimate the probability of freedom. In this way, it is possible that lower design prevalence values could be taken into account for the estimation of the probability of freedom.

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**Conflict of interest.** None.

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