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Original article

# Probability of *Rhipicephalus microplus* introduction into farms by cattle movement using a Bayesian Belief Network



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## ABSTRACT

Attempts to eliminate Rhipicephalus microplus from Uruguay have been unsuccessful, and, currently, the country is divided into two areas: a tick-free area and a tick-infested area. In the tick-infested area, different farms face different situations. Some farms are in regions where, due to environmental conditions or a lack of infrastructure, it is difficult to eliminate R. microplus, and the only option is to control it. In contrast, other farms can attempt complete removal. Before deciding whether a farmer should attempt to eliminate R. microplus, the probability of reintroduction must be evaluated. The objective of this study was to develop a probabilistic model based on a Bayesian Belief Network (BBN) to assess the likelihood of a farm becoming infested with R. microplus via the introduction of tick-infested cattle. Only the tick-infested area was considered in the development of this model. Nine variables related to environmental conditions and biosecurity measures, with a focus on cattle movement, were considered. Three different sources of data were used to populate the BBN model: data from the literature; a representative national survey from 2016; and a survey developed to identify biosecurity practices on farms. Model sensitivity and specificity were assessed, and an overall accuracy of 92% was obtained. The model was applied to 33 farms located in the tick-infested area. For one farm, the probability of introduction of R. microplus was 1%; for three farms, the probability was between 21% and 34%; for seven farms, it was between 66% and 76%; and for 22 farms, the probability was greater than 83%. This model was useful for estimating the probability of the introduction of R. microplus into farms, making it possible to assess the impact that the evaluated biosecurity measures have on the probability of introduction and, thus, guiding more objective decision making about the control or elimination of R. microplus from farms.

#### 1. Introduction

Because ticks are obligate parasites, they can easily spread via the movements of their hosts. The cattle tick *Rhipicephalus microplus* was introduced into South America presumably from Asia and/or Africa (Gonzales et al., 2013), and through the movement of cattle, it has spread to different areas (Barré and Uilenberg, 2010). The first references to its appearance in Brazil, Argentina and Uruguay were in 1835 (Gonzales et al., 2013), 1838 (Lombardero, 1983) and 1901 (Hooker, 1909), respectively.

In Uruguay, R. microplus causes economic losses estimated at 32.7 million dollars per year, with 89% of these costs directly affecting

farmers due to treatments, deaths and weight losses due to tick fever, among other factors (Avila, 1998). Additionally, the use of acaricides for tick control can produce residues in animal products if the withdrawal periods are not respected, which may lead to market restrictions, increasing these losses (Aguerre, 2016).

The population dynamics of cattle ticks have been studied in Uruguay, and it was determined that the number of generations per year depends on both the region and the environment (Nari et al., 1979). In native forest areas, 3.5 generations can be produced per year, while only two generations can occur in highland areas (Cardozo et al., 1984; Sanchis et al., 2008). During winter, the cycle is interrupted, and eggs and larvae, which may survive in the environment for 8 to 10

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Fig. 1. Map of Uruguay showing the regions according to the prevalence of cattle ticks on farms. In Region A, the presence of cattle ticks has been continuous over the last 100 years. In Region B, the presence of cattle ticks has been variable over the years, and in the free area, outbreaks are sporadic, and tick infestations are legally required to be eliminated.

months, make up the first generation the following spring (Cuore et al., 2008, 2013). Generally, the first generation of the year occurs from July/August to September/October, the second from November/December to January/February and the third from March to June (Nari et al., 1979).

Although Uruguay is in a marginal area for the development of the cattle tick (Cardozo et al., 1994), attempts to eliminate R. microplus throughout the country (MGA, 1956) have been unsuccessful and, at present, Uruguay is divided into two areas: a tick-free area and a tickinfested area. The tick-free area is naturally devoid of a tick population, although sporadic outbreaks may occur. If an outbreak occurs in the tick-free area, elimination is mandatory (Errico et al., 2009). A farm is considered to have achieved elimination after having carried out one year of suppressive treatments followed by one year without treatment and with no visible presence of the parasite (DGSG, 2011a). To maintain the tick-free area, the Ministry of Livestock, Agriculture and Fisheries (MGAP) developed a regulation for cattle movement; before bovines from the tick-infested area are transported to the tick-free area, they must receive a mandatory precautionary treatment against the cattle tick prior to leaving the farm, and they cannot travel with visible parasites (DGSG, 2011b).

In the tick-infested area, any farm may be infested with *R. microplus*. Although the presence of the cattle tick is allowed in this area, continuous control treatment to maintain a small tick population is required. One control measure that has been suggested for the tick-infested area is the application of a generational treatment, in which each generation is treated with a different acaricide. The basis for generational treatment is that, within the same generation, the ticks that are found in cattle differ temporally from their offspring, which are responsible for forming the next generation. Therefore, genetic resistance

to a particular active principle should not be selected if the use of each acaricide is restricted exclusively to one tick generation time in field conditions (Cuore et al., 2008). This control method aims to reduce resistance pressure and to use the smallest number of treatments possible, because both these variables are essential for the emergence of resistance (George et al., 2004; Thullner et al., 2007; Jonsson et al., 2010), and adequate control can be achieved with only five treatments per year (Cuore et al., 2008).

In the tick-infested area, different farms face different epidemiological situations. In some regions, due to environmental conditions (large areas of native or natural forests) or lack of infrastructure (suitable fences or trained personnel), it is difficult to eliminate the cattle tick, and the only option is to control the parasite. In contrast, farms located in regions that are environmentally favorable for tick control (lowlands, without large areas of forest) and that have adequate infrastructure and personnel can attempt elimination, which is likely to be more economical in the long term (unpublished data, 2018). Nevertheless, before deciding whether a farmer should attempt to control or eliminate the cattle tick, the possibility of reintroduction of *R. microplus* must be evaluated to reduce the subjectivity of the decision-making process.

The objective of this study was to develop a probabilistic model using a Bayesian Belief Network (BBN) to assess the likelihood of a farm becoming infested with *R. microplus* and to evaluate the effectiveness of present biosecurity practices on the reduction of this probability. This model was built with a focus on livestock movement because *R. microplus* is a one-host tick, and the main way it is spread is through its primary bovine host.

#### 2. Materials and methods

Only the tick-infested area was included in the development of this probabilistic model. This area was divided into two regions based on the distribution of the cattle tick over the last 100 years: Region A (departments of Artigas, Salto, Rivera, Tacuarembó, Cerro Largo, Rio Negro and Paysandú) is the region that has been continuously infested over the last 100 years, and Region B (departments of Rocha, Maldonado, Lavalleja and Treinta y Tres) is considered an endemic area with a variable presence of ticks over the years (Miraballes and Riet-Correa, 2018) (Fig. 1).

#### 2.1. Bayesian Belief Network construction

A Bayesian Belief Network (BBN) is a mathematical model that has the advantage of combining data from different sources (literature, field and expert opinion) and is used to update the knowledge of a parameter of interest (e.g., probability of introduction). In addition, a BBN allows the transfer of knowledge from the outcome to the inputs (for instance, if we need the probability of introduction in one region with a certain production type, then we can predict the probability of having biosecurity measures such as good fences or no cattle on the roads) (see video 1). A BBN is built by connecting the variables to be studied with arrows, following the Bayes theorem of conditional probabilities. The relationships among the nodes (variables) are determined by the arrows and by the conditional probability tables (see Table 1).

A BBN was developed to describe the direct dependencies among a

set of variables, which are represented by nodes and connected with arrows that represent directed causal relations. The BBN calculates the probability that a farm will become infested according to a set of environmental conditions and biosecurity measures. Although causality flows only in the direction of the arrows, information can flow in either direction (Fenton and Neil, 2013). From now on, we will use capital letters to refer to the variables represented by the nodes (e.g., Region). The BBN model was constructed using the freely available software GeNIe 2.3 (https://www.bayesfusion.com, accessed on 12/15/2018). The following approach was used to develop the BBN.

#### 2.1.1. Identification of relevant variables

The variables considered in this model accounted for environmental conditions that might predominantly influence the prevalence of cattle ticks and a farm's failure to adopt biosecurity measures, which could increase the rate of introduction of tick-infested cattle into a farm. Based on the available literature, different variables related to environmental conditions (infestation by region, seasonality of the cattle tick and infestation according to seasonality) were assessed. In addition, a survey was conducted to obtain information about biosecurity measures (Survey 2018). The survey was sent by email, and 157 farmers answered questions regarding the variables that the authors, due to their experience of working on farms, considered relevant. A final question was added to evaluate additional variables that may not have been considered. In total, the research team considered nine variables (Table 1).

#### Table 1

| Marginal and conditional | probabilities for the ten | nodes used in the BBN t | o estimate the probabilit             | v of R. microplu                        | s introduction into farms. |
|--------------------------|---------------------------|-------------------------|---------------------------------------|---|----------------------------|
|                          |                           |                         | · · · · · · · · · · · · · · · · · · · | / · · · · · · · · · · · · · · · · · · · |                            |

| Node                      | Parent Parent State |                    |             | Probability* | Data Source** |
|---------------------------|---------------------|--------------------|-------------|--------------|---------------|
| 1) Region                 | -                   | -                  | A           | 0.67         | 2016 Survey   |
|                           |                     |                    | В           | 0.33         |               |
| 2) Production type        | -                   | Region A           | Cow-Calf    | 0.60         | 2016 Survey   |
|                           |                     |                    | Complete    | 0.18         |               |
|                           |                     |                    | Fattening   | 0.22         |               |
|                           |                     | Region B           | Cow-Calf    | 0.69         |               |
|                           |                     |                    | Complete    | 0.08         |               |
|                           |                     |                    | Fattening   | 0.23         |               |
| 3) Farm prevalence        | -                   | Region A           | Low         | 0.09         | 2016 Survey   |
|                           |                     |                    | Medium      | 0.34         |               |
|                           |                     |                    | High        | 0.57         |               |
|                           |                     | Region B           | Low         | 0.35         |               |
|                           |                     |                    | Medium      | 0.53         |               |
|                           |                     |                    | High        | 0.12         |               |
| 4) Season                 | -                   | -                  | Unfavorable | 0.33         | Literature    |
|                           |                     |                    | Favorable   | 0.67         |               |
| 5) Infestation            | Infestation         | Season Unfavorable | No          | 0.90         | Literature    |
|                           | No                  |                    | Yes         | 0.10         |               |
|                           |                     | Season Favorable   | No          | 0.50         | Assumption    |
|                           |                     |                    | Yes         | 0.50         |               |
|                           | Infestation         | Season Unfavorable | No          | 0.50         |               |
|                           | Yes                 |                    | Yes         | 0.50         |               |
|                           |                     | Season Favorable   | No          | 0.10         | Literature    |
|                           |                     |                    | Yes         | 0.90         |               |
| 6) Walk-trough pathway    |                     | No                 | No          | 0.62         | 2018 Survey   |
|                           |                     |                    | Yes         | 0.38         |               |
|                           |                     | Yes                | No          | 0.73         |               |
|                           |                     |                    | Yes         | 0.27         |               |
| 7) Neighbors infested     |                     | No                 | No          | 0.65         | 2018 Survey   |
|                           |                     |                    | Yes         | 0.35         |               |
|                           |                     | Yes                | No          | 0.01         |               |
|                           |                     |                    | Yes         | 0.99         |               |
| 8) Boundary fences status |                     | No                 | Bad         | 0.01         | 2018 Survey   |
|                           |                     |                    | Good        | 0.99         |               |
|                           |                     | Yes                | Bad         | 0.20         |               |
|                           |                     |                    | Good        | 0.80         |               |

(continued on next page)

#### Table 1 (continued)

| Node                            | Parent Parent State |           |        | Probability | Data Source** |
|---------------------------------|---------------------|-----------|--------|-------------|---------------|
| 9) Cattle on the roads          |                     | No        | No     | 0.12        | 2018 Survey   |
|                                 |                     |           | Yes    | 0.88        |               |
|                                 |                     | Yes       | No     | 0.04        |               |
|                                 |                     |           | Yes    | 0.96        |               |
| 10) Probability of introduction | No                  | Cow-Calf  | Low    | 0.73        |               |
|                                 |                     |           | Medium | 0.43        |               |
|                                 |                     |           | High   | 0.22        |               |
|                                 |                     | Complete  | Low    | 0.99        |               |
|                                 |                     |           | Medium | 0.67        |               |
|                                 |                     |           | High   | 0.15        |               |
|                                 |                     | Fattening | Low    | 0.75        |               |
|                                 |                     |           | Medium | 0.57        | Survey 2016   |
|                                 |                     |           | High   | 0.26        |               |
|                                 | Yes                 | Cow-Calf  | Low    | 0.27        |               |
|                                 |                     |           | Medium | 0.57        |               |
|                                 |                     |           | High   | 0.78        |               |
|                                 |                     | Complete  | Low    | 0.01        |               |
|                                 |                     |           | Medium | 0.33        |               |
|                                 |                     |           | High   | 0.85        |               |
|                                 |                     | Fattening | Low    | 0.25        |               |
|                                 |                     |           | Medium | 0.43        |               |
|                                 |                     |           | High   | 0.74        |               |

\* See point 2.1.4 for the explanation of how these values were obtained.

\*\* See point 2.1.3 for the explanation of how these variables were used.

#### 2.1.2. Creation of the BBN structure

A structured BBN model was built and is presented in Fig. 2. Bayesian belief networks models use a graphical framework to describe networks of causes and effects. The information included in one node depends on the information in its predecessor nodes. The outputs of these models provide clear communication of results with rigorous quantification of risks (Fenton and Neil, 2013). Each node, with its state and source of information, is presented in Table 1.

The conditional probability tables (CPT) for each node were developed with a maximum of two parents per node, as suggested by Fenton and Neil (2013).

## 2.1.3. Sources of information

Three different sources of information were used.

- 1 Data from the literature: A literature review carried out previously by the authors (Miraballes and Riet-Correa, 2018) was used for the construction of this network, focusing on the seasonality of *R. microplus* with an emphasis on Argentina and Uruguay. The specific studies used were Nari et al., 1979; Cardozo et al., 1984; OPS, 1998; Sanchis et al., 2008 and Canevari et al., 2017.
- 2 Survey 2016: In Uruguay, an annual survey is conducted to determine the status of foot and mouth disease and brucellosis in the country. This survey uses a representative sample of all the cattle and sheep farms and, in 2016, was issued to 650 cattle farms. In this year, questions about the status of the cattle tick and tick fever (babesiosis and anaplasmosis) were also included. The following information from this survey was used in the BBN model: 1) farm location; 2) presence of cattle ticks (had, have or never had); and 3) number and category of bovines on the farm.
- 3 Survey 2018: An epidemiologic survey about the cattle tick was issued to farmers located in the tick-infested area (Regions A and B). To identify the frequency of use of four biosecurity measures, to ensure that all important variables have been considered and to assess the validity of the model, the following information was requested: 1) farm location; 2) presence of cattle ticks in the last 3 years (yes or no); 3) number of tick control treatments per year; 4) status of boundary fences (bad or good); 5) presence of cattle ticks in neighboring farms (yes or no); 6) presence of a mandatory walkthrough pathway on the farm (yes or no); 7) presence of cattle in the

neighboring rural roads (yes or no); and 8) other factors considered relevant for the introduction of ticks into the farm. The survey was sent by email to 822 farmers, of whom 157 responded. For this model, any farms responding "no" to the presence of ticks and using less than four treatments per year were designated as tick-free farms ("true negatives") (n = 27). Any farms responding "yes" to the presence of ticks and applying more than three treatments per year were designated as tick-infested farms ("true positives") (n = 95). In total, 122 farms were considered: 77% located in Region A and 23% in Region B.

#### 2.1.4. Marginal and conditional probabilities

The following describes each of the ten nodes used for the BBN. All the probability values are presented in Table 1.

- 1 **Region**: The probability that a farm was located in either Region A or B was established based on the number of farms included in each region in the 2016 survey.
- 2 **Production type**: The CPT for this node was calculated based on the steer/cow ratio in the Region as reported in the 2016 survey. If this relationship was greater than one, the herd was considered a fattening herd; if it was between 0.50 and one, it was considered a complete cycle herd (e.g., cow-calf and fattening); and if the ratio was less than 0.50, it was considered a cow-calf herd (OPS, 1998).
- 3 Farm prevalence: The probability of a farm being infested depended on the region. The CPT for this node was calculated considering the probability that a farm was infected in Region A or B according to the 2016 survey. Prevalence was considered to be low when 25% or fewer of the farms were infested, medium when 26%–59% of the farms were infested, and high when 60% or more were infested.
- 4 **Season**: The probability of a farm being in a favorable or unfavorable season for the development of the cattle tick was calculated using information from epidemiological studies carried out in Uruguay (Nari et al., 1979; Cardozo et al., 1984; Sanchis et al., 2008). The period from October to May that corresponded to the second and third generations of the cattle tick was considered to be the favorable season (shorter cycles and higher numbers of cattle ticks per animal), while the unfavorable season was from June to September, corresponding to the first generation.



Fig. 2. Bayesian Belief Network (BBN) and steady state for the ten nodes.

Sensitivity analysis for the Infestation node (node 5).

| Infestation                      | Evidence: No |           |             |           | Evidence: Yes |           |             |           |
|----------------------------------|--------------|-----------|-------------|-----------|---------------|-----------|-------------|-----------|
| Probability of Introduction      | tion No Yes  |           |             | No        |               | Yes       |             |           |
| Season                           | Unfavorable  | Favorable | Unfavorable | Favorable | Unfavorable   | Favorable | Unfavorable | Favorable |
| A. Unknown                       |              |           |             |           |               |           |             |           |
| Infestation: No                  | 0.9          | 0.5       | 0.5         | 0.1       | 0.9           | 0.5       | 0.5         | 0.1       |
| Infestation: Yes                 | 0.1          | 0.5       | 0.5         | 0.9       | 0.1           | 0.5       | 0.5         | 0.9       |
| Probability of introduction: Yes | 0.33         |           |             |           | 0.74          |           |             |           |
| B. Assumption                    |              |           |             |           |               |           |             |           |
| Infestation: No                  | 0.9          | 0.8       | 0.2         | 0.1       | 0.9           | 0.8       | 0.2         | 0.1       |
| Infestation: Yes                 | 0.1          | 0.2       | 0.8         | 0.9       | 0.1           | 0.2       | 0.8         | 0.9       |
| Probability of introduction: Yes | 0.18         |           |             |           | 0.88          |           |             |           |

#### Table 3

Confusion matrix of the BBN model for assessing the risk of cattle tick introduction into a farm, using the information from 122 farms from the survey of 2018.

|                   |                    | Predict<br>farms | ed infested | Total<br>observed |     |
|-------------------|--------------------|------------------|-------------|-------------------|-----|
|                   |                    | No               | Yes         |                   |     |
| Observed infested | No                 | 17               | 9           | 26                |     |
| farms             | Yes                | 1                | 95          | 96                |     |
|                   | Total<br>predicted | 18               | 104         | 122               |     |
|                   | Specificity        |                  |             |                   | 65% |
|                   | Sensitivity        |                  |             |                   | 99% |
|                   | Total accuracy     |                  |             |                   | 92% |

5 **Infestation**: This node was created to capture the influence of the season on the probability of introduction of *R. microplus* into a farm. The CPT for this node estimated the probability of an area being infested given the season (favorable or unfavorable) and the probability of introduction (yes or no). The probability that animals were infested, given the season, was estimated according to Canevari et al. (2017). In the unfavorable season (Jun-Sept), 10% of the animals were infested, and in the favorable season (Oct-May), between 80% and 100% of the animals were infested. Based on these estimates, we assumed that if an area (and a farm) was not infested during the unfavorable season, it was more likely to be a cattle-tick-free farm. If a farm was classified as "free" (e.g., Probability of Introduction = "No") during the unfavorable season, then the probability of the area not being infested was set to 0.90 (and that of its



Fig. 3. Receiver Operating Characteristic (ROC) curve obtained from the BBN used to estimate the risk of introduction of cattle ticks into farms in Uruguay (y-axis and x-axis represent the sensitivity and 1-specificity of the BBN, respectively).

being infested to 0.10). Furthermore, when a farm was infested in the favorable season, the probability of the area not being infested was set to 0.10 (and of being infested to 0.90) (Table 1). However, it was more difficult to estimate the probability of an area being uninfested during the favorable season or of being infested during the unfavorable season; thus, in these cases, we assumed a probability of 0.50. These values were then assessed according to a sensitivity analysis.

- 6 Walk-through pathway: In Uruguay, some farms have a mandatory walk-through pathway that allows the transit of cattle from other farms that lack access to rural roads. The probability of a farm having a walk-through pathway was estimated based on the 2018 survey.
- 7 **Neighbors infested**: In areas where the cattle tick is endemic, it is very common for several adjacent farms to be infested. This phenomenon was assessed using the information provided in the 2018 survey.
- 8 **Boundary fence status**: The probability of having good or bad boundary fences was assessed with the information provided in the 2018 survey. A boundary fence was considered to be in a good state when the neighbor's cattle were typically excluded. Conversely, a boundary fence that was frequently breached by neighboring cattle was considered to be in a bad state.
- 9 **Cattle on the roads**: The presence of cattle on the rural roads neighboring a farm, although forbidden by law in the country, is common practice. The CPT for this node was estimated using the information provided in the 2018 survey.
- 10 **Probability of introduction**: The CPT for this node was estimated using the 2016 survey. A farm was considered to be free of cattle ticks (Probability of Introduction = No) if the answer to the question regarding tick infestation was "had" or "never had", while those that replied "have" to this question were considered infested (Probability of Introduction = Yes).

#### 2.1.5. Sensitivity analysis

As there was no information available regarding Probability of Introduction = No in the favorable season or of Probability of

Introduction = Yes in the unfavorable season, a probability of 0.50 was used (node 5) (Table 2– A). To assess the impact of using this value, we substituted 0.50 with 0.80 and 0.20 for the probability of an area not being infested in the favorable season and being infested in the unfavorable season, respectively (Table 2– B).

#### 2.1.6. Model validation

The model goodness of fit was assessed by computing the confusion matrix between the observed (true negative and true positive) and the predicted Probability of Introduction (Yes or No) of the cattle tick with the following components (calculated using the 2018 survey information from the 122 responding farms): specificity (Sp), sensitivity (Se) and accuracy (percentage of correctly classified outcomes). The Sp was estimated based on the relation between the number of farms predicted to be free of infestation and the number of observed tick-free farms. The Se was calculated based on the relation between the number of farms predicted to have infestations and the number of infested farms observed. The accuracy was estimated based on the ratio between the true positives plus the true negatives and the total number of observed farms. Accuracy was calculated based on the equation:  $(TP + TN)/T_{obs}$ . For the Probability of Introduction node, a Receiver Operating Characteristic (ROC) curve was determined to assess the Area Under the Curve (AUC).

#### 2.1.7. Scenario analysis

To investigate the impact that the factors used in the BBN had on the Probability of Introduction, a series of scenarios were constructed according to two influence types. 1) The influence of the environmental conditions was assessed with five scenarios by setting evidence on these nodes. The scenarios were as follows: **Scenario 1**. Evidence on: Farm Prevalence = Low; Production Type = Complete; Season = Favorable; Infestation = No. **Scenario 2**. Evidence on: Farm Prevalence = Medium; Production Type = Fattening; Season = Unfavorable; Infestation = No. **Scenario 3**. Evidence on: Farm Prevalence = Medium; Production Type = Cow-Calf; Season = Unfavorable; Infestation = No. **Scenario 4**. Evidence on: Farm Prevalence = High; Production Type = Cow-Calf; Season = Unfavorable; Infestation = No. **Scenario 5**. Evidence on: Farm

Description of the nodes; estimated probabilities (%) and relative risks under different scenarios compared to the steady state by setting evidence on the environmental conditions.

| Node                        | State       | Steady    | Scenarios  |           | Evidences             |             |           |
|-----------------------------|-------------|-----------|------------|-----------|-----------------------|-------------|-----------|
|                             |             | state (%) | 1          | 2         | 3                     | 4           | 5         |
| Farm prevalence             | Low         | 18        | 100        | 0         | 0                     | 0           | 0         |
|                             | Medium      | 40        | 0          | 100       | 100                   | 0           | 0         |
|                             | High        | 42        | 0          | 0         | 0                     | 100         | 100       |
| Production type             | Cow-Calf    | 63        | 0          | 0         | 100                   | 100         | 0         |
|                             | Complete    | 15        | 100        | 0         | 0                     | 0           | 100       |
|                             | Fattening   | 22        | 0          | 100       | 0                     | 0           | 0         |
| Season                      | Unfavorable | 33        | 0          | 100       | 100                   | 100         | 100       |
|                             | Favorable   | 67        | 100        | 0         | 0                     | 0           | 0         |
| Infestation                 | No          | 40        | 100        | 100       | 100                   | 100         | 0         |
|                             | Yes         | 60        | 0          | 0         | 0                     | 0           | 100       |
|                             |             |           |            | Estimated | probability (%) (Rela | ative Risk) |           |
| Probability of introduction | No          | 42        | 100 (2.38) | 70 (1.66) | 58 (1.38)             | 34 (0.80)   | 3 (0.07)  |
|                             | Yes         | 58        | 0 (0)      | 30 (0.51) | 42 (0.72)             | 66 (1.13)   | 97 (1.67) |
| Walk-through pathway        | No          | 68        | 62 (0.91)  | 65 (0.95) | 67 (0.98)             | 69 (1.01)   | 73 (1.07) |
|                             | Yes         | 32        | 38 (1.18)  | 35 (1.09) | 33 (1.03)             | 31 (0.96)   | 27 (0.84) |
| Neighbors infested          | No          | 28        | 65 (2.32)  | 46 (1.64) | 38 (1.35)             | 23 (0.82)   | 3 (0.10)  |
| -                           | Yes         | 72        | 35 (0.48)  | 54 (0.75) | 62 (0.86)             | 77 (1.07)   | 97 (1.34) |
| Boundary fences status      | Good        | 88        | 99 (1.12)  | 93 (1.06) | 91 (1.03)             | 86 (0.97)   | 81 (0.92) |
|                             | Bad         | 12        | 1 (0.08)   | 7 (0.58)  | 9 (0.75)              | 14 (1.16)   | 19 (1.58) |
|                             | No          | 7         | 12 (1.71)  | 10 (1.42) | 9 (1.28)              | 7 (1.00)    | 4 (0.57)  |
| Cattle on the roads         | Yes         | 93        | 88 (0.94)  | 90 (0.96) | 91 (0.97)             | 93 (1.00)   | 96 (1.03) |

Type = Complete; Prevalence = High; Production Season =Unfavorable; Infestation = Yes. Changes in the probability values for the Probability of Introduction and biosecurity measures were then compared to the steady state (e.g., the probability values of all the states, for each node, with no evidence). 2) The influence of the biosecurity measures was evaluated with another six scenarios by setting evidence on these nodes and comparing the changes in the environmental conditions and Probability of Introduction with the steady state. The scenarios were as follows: **Scenario 1**. Evidence on: Walk-Through Pathway = No; Neighbors Infested = No; Boundary Fence Status = Good; Cattle on the Roads = No. Scenario 2. Evidence on: Walk-Through Pathway = No; Neighbors Infested = No; Boundary Fence Status = Good; Cattle on the Roads = Yes. Scenario 3. Evidence on: Walk-Through Pathway = Yes; Neighbors Infested = No; Boundary Fence Status = Good; Cattle on the Roads = Yes. Scenario 4. Evidence on: Walk-Through Pathway = No; Neighbors Infested = No; Boundary Fence Status = Bad; Cattle on the Roads = Yes. Scenario 5. Evidence on: Walk-Through Pathway = No; Neighbors Infested = Yes; Boundary Fence Status = Good; Cattle on the Roads = Yes. Scenario 6. Evidence on: Walk-Through Pathway = No; Neighbors Infested = Yes; Boundary Fence Status = Bad; Cattle on the Roads = Yes. The relative risk (RR) was calculated on these nodes by dividing the new value of each scenario's node by the steady-state value.

#### 2.1.8. Application of the BBN

The model was applied to 33 farms that are part of a current project that aims at implementing control or elimination measures according to the probability of introduction of the cattle tick into the farm by the movement of infested cattle. The information related to the following nodes was available for each of these farms: 1) Region; 2) Production Type; 3) Season; 4) Infestation; 5) Mandatory Walk-Through Pathway; 6) Neighbors Infested; 7) Boundary Fence Status; and 8) Cattle on the Roads.

#### 3. Results

#### 3.1. Bayesian belief network

Fig. 2 depicts the steady state of the BBN according to the values presented in Table 1. As an example, it shows that any farm located in

the tick-infested area of Uruguay has a 67% likelihood of being in Region A and a 33% likelihood of being in Region B (Table 1). The probability of a farm having the cow-calf production type is 63%, and the probability of the introduction of cattle ticks to any farm located in the tick-infested region is 58%.

#### 3.2. Sensitivity analysis

Table 2 shows the changes in the distribution of the Probability of Introduction when the information on the CPT of the Infestation node was changed. The sensitivity was analyzed according to two different assumptions. The first assumption was that, if there was no infestation in the Favorable Season, then the probability of a farm (or an area) not being infested was 0.80, and that of its being infested was 0.20, because in the favorable season, it is less likely that an infestation could go undetected. If there was infestation in the Unfavorable Season, then the probability of a farm (or an area) being infested was assumed to be 0.80. With these assumptions, the Probability of Introduction of the cattle tick was estimated at 0.18 if there was no infestation according to the season and 0.88 if there was infestation according to the season. Although the results did not change much with these new assumptions (Table 2-B), the value of 0.50 was used for the ultimate analysis because it increased the probability of introduction and decreased the probability of no introduction when the evidence was established (Table 2-A).

### 3.3. Model validation

Table 3 shows the confusion matrix and the accuracy of the model in predicting true infested and true uninfested farms. The overall accuracy was 92%. The model provided acceptable performance for predicting farms that will become infested with the cattle tick (Se = 99%) but was not as good at predicting farms that will not become infested (Sp = 65%). The ROC curve showed an AUC of 86.9% (95%CI: 78.5%–95.5%) (Fig. 3, which indicates that the model has good discrimination power (Dohoo et al., 2009).

Description of the nodes; estimated probabilities (%) and relative risks under different scenarios compared to the steady state by setting evidence on the biosecurity measures.

| Node                        | State       | Steady    | Scenarios | Estimated probability (%) (Relative Risk) |           |           |           |           |
|-----------------------------|-------------|-----------|-----------|---|-----------|-----------|-----------|-----------|
|                             |             | state (%) | 1         | 2   | 3         | 4         | 5         | 6         |
| Region                      | А           | 67        | 58 (0.86) | 58 (0.86)                                 | 58 (0.86) | 63 (0.94) | 71 (1.05) | 74 (1.27) |
|                             | В           | 33        | 42 (1.27) | 42 (1.27)                                 | 42 (1.27) | 37 (1.12) | 29 (0.87) | 26 (0.78) |
| Farm prevalence             | Low         | 18        | 31(1.72)  | 31(1.72)                                  | 31(1.72)  | 23 (1.2)  | 12 (0.66) | 7 (0.38)  |
|                             | Medium      | 40        | 47 (1.17) | 47 (1.17)                                 | 47 (1.17) | 43 (1.07) | 38 (0.95) | 36 (0.90) |
|                             | High        | 42        | 22 (0.52) | 22(0.52)                                  | 22(0.52)  | 34 (0.80) | 50 (2.27) | 57 (1.36) |
| Production type             | Cow-Calf    | 63        | 59 (0.93) | 59 (0.93)                                 | 59 (0.93) | 62 (0.98) | 64 (1.01) | 66 (1.04) |
|                             | Complete    | 15        | 16 (1.06) | 16 (1.06)                                 | 16 (1.06) | 15 (1.00) | 14 (0.87) | 14 (0.93) |
|                             | Fattening   | 22        | 25 (1.13) | 25 (1.13)                                 | 25 (1.13) | 23 (1.04) | 21 (0.95) | 21 (0.95) |
| Season                      | Unfavorable | 33        | 33 (1.00) | 33 (1.00)                                 | 33 (1.00) | 33 (1.00) | 33 (1.00) | 33 (1.00) |
|                             | Favorable   | 67        | 67 (1.00) | 67 (1.00)                                 | 67 (1.00) | 67 (1.00) | 67 (1.00) | 67 (1.00) |
| Infestation                 | No          | 43        | 63 (1.46) | 62 (1.44)                                 | 63 (1.46) | 49 (1.14) | 31 (0.72) | 24 (0.56) |
|                             | Yes         | 57        | 37 (0.64) | 38 (0.66)                                 | 37 (0.64) | 51 (0.89) | 69 (1.21) | 76 (1.33) |
| Probability of introduction | No          | 42        | 99 (2.35) | 98 (2.33)                                 | 98 (2.35) | 65 (1.54) | 20 (0.47) | 1 (0.02)  |
|                             | Yes         | 58        | 1 (0.02)  | 2 (0.03)                                  | 1 (0.02)  | 35 (0.60) | 80 (1.37) | 99 (1.70) |
|                             |             |           |           |   | Evidence  |           |           |           |
| Walk-through pathway        | No          | 68        | 100       | 100                                       | 0         | 100       | 100       | 100       |
|                             | Yes         | 32        | 0         | 0   | 100       | 0         | 0         | 0         |
| Neighbors infested          | No          | 28        | 100       | 100                                       | 100       | 100       | 0         | 0         |
|                             | Yes         | 72        | 0         | 0   | 0         | 0         | 100       | 100       |
| Boundary fences status      | Good        | 88        | 100       | 100                                       | 100       | 0         | 100       | 0         |
|                             | Bad         | 12        | 0         | 0   | 0         | 100       | 0         | 100       |
| Cattle on the roads         | No          | 7         | 100       | 0   | 0         | 0         | 0         | 0         |
|                             | Yes         | 93        | 0         | 100                                       | 100       | 100       | 100       | 100       |

## 3.4. Scenario analysis

The results of the scenario analysis are depicted in Tables 4 and 5. Table 4 depicts the changes in biosecurity measures when the evidence is set on the environmental conditions. The probability of introduction increased from 0% to 97% when the different scenarios were applied. The greatest changes within the biosecurity measures occurred with bad boundary fences (RR of 0.08 to 1.58) and with no infested neighbors (RR of 0.10 to 2.32). Table 4 is presented without Region evidence because this node depended on the Farm Prevalence and the Production Type. In Evidence 5 (Table 4), it can be observed that, when the Probability of Introduction was high (97%), the neighboring farms were 1.34 times more likely than in the steady state to be infested with cattle ticks; the state of the boundary fences was 1.58 times more likely to be bad, and there were cattle present on a farm's neighboring rural roads (RR 1.03). These conditions are completely opposite to those of Evidence 1, which had a Probability of Introduction of 0%; there, the evidence was set on a low farm prevalence, the production type was a complete cycle, and there was no infestation in the favorable season. Table 5 shows the different scenarios when the evidence was set on the biosecurity measures. The Probability of Introduction increased from 1% to 99% when the different scenarios were applied. As an example, when boundary fences were poor, and cattle were on the farm's neighboring rural roads, but no infestation was present on the neighboring farms, the Probability of Introduction (Yes) was 35% (Table 5 -Evidence 4). With the same conditions but with neighboring farms infested, this probability increased to 99% (Table 5 - Evidence 6). Scenario 4, in which the evidence was set on the neighboring farms infested, good boundary fences, and the presence of cattle on the farm's neighboring rural roads, was common in the tick-infested area (Table 5 - Evidence 5). By keeping cattle off these roads, the Probability of Introduction was decreased by 25% (e.g., from 80% to 55%), and by also removing infestation from the neighboring farms, the Probability of Introduction was decreased by an additional 54% (e.g., from 55% to 1%).

#### 3.5. Application of the BBN

The description of the environmental conditions and biosecurity measures from each of the 33 farms and their predicted probabilities of introduction are presented in Table 6. The probability of introduction = Yes was 1% for one farm, 21% to 34% for three farms, 66% to 76% for seven farms, and 84% to 100% for 22 farms. Among the biosecurity measures, 97% of the farms had neighbors infested, 60% had frequent presence of cattle on the neighboring rural roads, and 33% had poor boundary fences.

#### 4. Discussion

Uruguay is in a marginal area for the development of *R. microplus*, with no possibility for the elimination of this parasite throughout the country (Errico et al., 2009), as was planned in Law N° 12.293 in 1953. However, there are farms in the tick-infested area that, because they are in a favorable environment and have adequate infrastructure, can achieve elimination. It is also possible to create tick-free areas within the tick-infested area; this process has been planned but has not yet been implemented by the current Law N° 18,268, which regulates the control of *R. microplus* (MGAP, 2008). As an example of the possibility of creating areas free of cattle ticks within the infested area, in the Tacuarembo department, which is divided into 16 police districts, the MGAP verified that all farms in two police districts were naturally free of ticks and that, in five police districts, between 70% and 92% of the farms were also free of ticks (Miraballes and Riet-Correa, 2018).

The BBN model developed in this study provides farmers and veterinarians with a less subjective decision support tool for the elimination or control of cattle ticks in the region. At the same time, it is

Conditions of the 33 farms under the project of control or eradication of R. microplus and probability of introduction of the cattle tick into a farm by cattle movement.

| N farms | Conditio | ons             |             |             |                         |                       |                           |                    | Probability of<br>- introduction |
|---------|----------|-----------------|-------------|-------------|-------------------------|-----------------------|---------------------------|--------------------|----------------------------------|
|         | Region   | Production type | Season      | Infestation | Walk through<br>pathway | Neighbors<br>infested | Boundary fences<br>status | Cattle on the road |                                  |
| 1       | А        | Fattening       | Favorable   | Yes         | No                      | No                    | Good                      | No                 | 1%                               |
| 1       | Α        | Fattening       | Favorable   | No          | No                      | Yes                   | Good                      | No                 | 21%                              |
| 1       | Α        | Cow-Calf        | Favorable   | No          | No                      | Yes                   | Good                      | No                 | 26%                              |
| 1       | Α        | Complete        | Favorable   | No          | Yes                     | Yes                   | Good                      | Yes                | 34%                              |
| 1       | Α        | Cow-Calf        | Favorable   | Yes         | Yes                     | Yes                   | Good                      | No                 | 66%                              |
| 1       | Α        | Fattening       | Favorable   | Yes         | No                      | Yes                   | Good                      | No                 | 70%                              |
| 2       | Α        | Complete        | Favorable   | Yes         | No                      | Yes                   | Good                      | No                 | 71%                              |
| 3       | Α        | Cow-Calf        | Favorable   | Yes         | No                      | Yes                   | Good                      | No                 | 76%                              |
| 1       | В        | Cow-Calf        | Favorable   | Yes         | No                      | Yes                   | Good                      | Yes                | 84%                              |
| 3       | Α        | Cow-Calf        | Favorable   | Yes         | Yes                     | Yes                   | Good                      | Yes                | 86%                              |
| 1       | Α        | Fattening       | Favorable   | Yes         | No                      | Yes                   | Good                      | Yes                | 88%                              |
| 4       | В        | Cow-Calf        | Favorable   | Yes         | No                      | Yes                   | Good                      | Yes                | 91%                              |
| 1       | Α        | Complete        | Unfavorable | Yes         | Yes                     | Yes                   | Good                      | Yes                | 93%                              |
| 1       | Α        | Cow-Calf        | Unfavorable | Yes         | Yes                     | Yes                   | Good                      | Yes                | 95%                              |
| 1       | Α        | Cow-Calf        | Favorable   | Yes         | Yes                     | Yes                   | Bad                       | No                 | 98%                              |
| 1       | Α        | Complete        | Favorable   | Yes         | No                      | Yes                   | Bad                       | No                 | 98%                              |
| 1       | Α        | Cow-Calf        | Unfavorable | No          | Yes                     | Yes                   | Bad                       | Yes                | 98%                              |
| 1       | Α        | Cow-Calf        | Favorable   | Yes         | Yes                     | Yes                   | Bad                       | Yes                | 99%                              |
| 1       | Α        | Complete        | Favorable   | Yes         | No                      | Yes                   | Bad                       | Yes                | 99%                              |
| 1       | В        | Cow-Calf        | Favorable   | Yes         | Yes                     | Yes                   | Bad                       | Yes                | 99%                              |
| 1       | Α        | Cow-Calf        | Favorable   | Yes         | Yes                     | Yes                   | Bad                       | Yes                | 99%                              |
| 1       | Α        | Cow-Calf        | Unfavorable | Yes         | No                      | Yes                   | Bad                       | No                 | 100%                             |
| 1       | А        | Cow-Calf        | Favorable   | Yes         | No                      | Yes                   | Bad                       | Yes                | 100%                             |
| 2       | Α        | Cow-Calf        | Favorable   | Yes         | No                      | Yes                   | Bad                       | Yes                | 100%                             |

possible to evaluate changes in the probability of introduction once biosecurity measures are improved, which may allow farmers to make better decisions under different conditions. Additionally, the model can be accessed through a computer website, facilitating interactive use by farmers and veterinarians.

Although the model showed low specificity, its sensitivity was high, and the overall accuracy was acceptable. In this case, a model with high sensitivity is preferred to reduce the possibility of recommending cattle tick elimination from farms that could easily experience reintroduction of this parasite, since the cost of elimination in one year is higher than the cost for control in the same period due to the higher number of treatments required for elimination. This model also provides a flexible and friendly framework that can be modified as new data are obtained (Gustafson et al., 2010). As long as new studies are being conducted, new information can be included to continuously improve the BBN.

When different scenarios among the environmental conditions were tested, it was demonstrated that the presence of a walk-through pathway was not a risk factor for the introduction of cattle ticks, as it was considered at the beginning of the study. This finding could be because farmers are aware of this risk and take measures to prevent the entry of cattle infested with ticks, such as requiring a precautionary treatment of the neighbors' livestock before entering. However, this finding could also be attributed to a low return rate since only 36 of the 122 farmers reported having a mandatory walk-through pathway in their farms.

Among the evaluated biosecurity measures, an infested neighboring farm was the greatest risk factor for a farm becoming infested. Farms with a Probability of Introduction greater than 42% have infested neighbors. In the future, it will be important to collect data about the number of infected neighbors, since these results could vary depending on whether a farm has one or several infected neighbors. In addition, it is likely that multiple neighboring farms are infested with the cattle tick in endemic areas, and thus, the status of boundary fences is a critical factor associated with cattle tick introduction. Moreover, in some cases, both conditions exist (the boundary fences are in poor condition and tick-infested cattle are present on the neighboring rural roads), and the risk of cattle tick introduction to a farm may therefore be exacerbated. Although the interactions between fence status and livestock on the roads and between fence status and neighboring infestation were not considered, it is likely that the effect is the same when considering these variables separately or together.

Although farms of the fattening production type were previously thought to have a greater risk of becoming infested, the analysis showed that this was not the case. This finding could be because the application of preventive treatments is a regular practice before cattle enter the farm, or because these farms are mostly located in areas that are good for fattening (e.g., grasslands), which are unlikely to be favorable for the development of the cattle tick. In the future, it will be important to collect information regarding the biosecurity measures that farmers usually take when buying livestock, e.g., treating the animals before entering the farm or making purchases from the tick-free area. Cow-calf farms presented the highest risk of being infested. These farms are more likely to be in areas that are more favorable for the development of the cattle tick (e.g., areas with superficial basal soil).

When this model was applied to the 33 farms that are currently part of a project for the control of the cattle tick, only one farm had a Probability of Introduction of 1%, while 22 farms had probabilities greater than 83%. Notably, these farmers showed interest in participating in this control project because they were having problems controlling or eliminating the cattle tick. Of these 22 farms, 100% had infested neighbors; 86% mentioned the frequent presence of cattle on the neighboring rural roads; and 50% indicated that the state of their boundary fences was poor. Among these measures, the only one that the farmers could implement without the help of the MGAP is to improve their boundary fences. As mentioned above, there is legislation available for the creation of tick-free areas within the tick-infested area, as well as for the prohibition of the presence of livestock on neighboring rural roads; thus, it is important to increase compliance with this legislation to improve control measures for cattle ticks.

For the construction of this BBN, we took into consideration the factors that could favor the introduction of R. microplus-infested cattle to farms, related either to the environmental conditions or to biosecurity measures (Madder et al., 2011; Miller et al., 2012; Miraballes and Riet-Correa, 2018). Although it is known that several species of deer (Ozotoceros bezoarticus, Odocoileus virginianus, Mazama gouazoubira, and Cervus elaphus, among others) (Cançado et al., 2009; Pound et al., 2010; da Silveira et al., 2011; Rodriguez-Vivas et al., 2013) can act as primary hosts for R. microplus, this factor was not considered in the model as a source for the probability of infestation. In Uruguay, there are three species of deer: O. bezoarticus, M. gouazoubira and Axis axis. The presence of R. microplus has been reported on O. bezoarticus (Venzal et al., 2003), but this species is not common in farms because they are in danger of extinction, with only 1000 individuals remaining in the whole country (Vazquez et al., 2018). Other forms of introduction of R. microplus into farms, such as streams, although possible, are not considered epidemiologically important (Cuore et al., 2013).

#### 5. Conclusion

The Bayesian Belief Network developed in this research is a useful tool for quantifying the probability of introduction of *R. microplus* into farms by the movement of cattle and for identifying the important factors and scenarios that could help farmers and veterinarians make decisions regarding control or elimination strategies for the cattle tick on farms in Uruguay.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.04.009.

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