UNIVERSIDAD DE LA REPÚBLICA

FACULTAD DE VETERINARIA

Programa de Posgrados

# ANTECEDENTES PREVENCIÓN Y CONTROL DE *Rhipicephalus microplus* Y DE LA TRISTEZA PARASITARIA EN URUGUAY

Mónica Cecilia Miraballes Ferrer

TESIS DE DOCTORADO EN SALUD ANIMAL

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# RESUMEN

En Uruguay, la garrapata *Rhipicephalus microplus* y la tristeza parasitaria causan pérdidas económicas anuales estimadas en 32.7 millones de dólares. El 44.6% de estas son costos por tratamientos que, muchas veces, no son realizados en los momentos adecuados o que, debido a la resistencia de *R. microplus* a los acaricidas, no son totalmente efectivos. Consecuentemente, se aumenta la dosis y el número de tratamientos, habiendo productores que realizan hasta 15 tratamientos por año, solo para el control, sin posibilidades de eliminación. El alto número de tratamientos puede llevar a la presencia de residuos en carne y poner en riesgo la salud pública. El objetivo de esta tesis fue aportar información sobre la adopción de medidas de control más eficientes que disminuyan la utilización de fármacos. Se realizó una revisión bibliográfica sobre los antecedentes de *R. microplus* y de la tristeza parasitaria en Uruguay para determinar los factores que influyeron en la dispersión de la garrapata y poder plantear alternativas de control. Debido a que Uruguay está situado en un área marginal para el desarrollo de la garrapata con el consecuente desequilibrio enzoótico, los brotes de tristeza parasitaria son frecuentes. Para evaluar y estimular el uso de las hemovacunas preventivas de tristeza parasitaria se realizó un ensayo que comparó la eficacia de dos vacunas actualmente disponibles. Ambas vacunas fueron eficientes e indujeron la producción de anticuerpos en entre el 93% y 98,3% de los bovinos para cada uno de los tres agentes. Se desarrolló un modelo de análisis de riesgo que determina, con 92% de precisión, la probabilidad de reintroducción de la garrapata a los establecimientos. Para el desarrollo de este modelo se consideraron variables epidemiológicas y de bioseguridad, estimándose que las más relevantes para el aumento de la probabilidad de introducción fueron el tipo de producción cría, los vecinos infestados y el mal estado de alambrados. Este modelo puede ser utilizado para decidir si es conveniente erradicar o controlar la garrapata en cada establecimiento, dependiendo de las características epidemiológicas y de las medidas de bioseguridad.

Palabras claves: *Rhipicephalus microplus,* Tristeza Parasitaria, Control, Uruguay

# SUMMARY

In Uruguay, the cattle tick, *Rhipicephalus microplus,* and the tick fever causes economic losses estimated on 32.7 million dollars annually being 44.6% of those due to treatment, which in many occasions, are not performed in the appropriate time or, due to resistance of *R. microplus* to the acaricides, are not completely effective. Consequently, the doses and the number of treatments is increased, and some farmers perform up to 15 treatments per year, just for control, without possibilities of elimination. The high number of treatments could lead to the presence of residues on meat putting public health at risk. The objective of this thesis was to provide information regarding the adoption of efficient control measures to decreases the use of chemicals. A review of the history of *R. microplus* and tick fever in Uruguay was developed to determine the factors that influence the dispersion of the cattle tick and to create control alternatives. Since Uruguay is in a marginal area for the development of the cattle tick, with the consequent enzootic instability to the tick fever, the outbreaks of these diseases are frequent. To evaluate and stimulate the use of hemovaccines to prevent tick fever, a trial that compares the efficacy of the two vaccines currently available in the country was performed. Both vaccines were efficient and induce the production of antibodies in between 93% and 98.3% of the bovines for each of the tree agents. A risk assessment model was developed to determine, with 92% of accuracy, the probability of *R. microplus* introduction into farms. For the development of this model epidemiological and of biosecurity variables were considered, estimating that the most relevant for the increase of the probability of introduction were the production type breeding, the neighbors infested and the bad status of the boundary fences. This model can be used to evaluate if it is more convenient eradicate or control the cattle tick in each farm, depending on the epidemiological characteristic and on the biosecurity measures.

Key words: *Rhipicephalus microplus,* Tick Fever, Control, Uruguay

# 1. INTRODUCCIÓN

Se presume que con la introducción de la ganadería a Uruguay por Hernandarias desde el Paraguay en 1611 y posteriormente por los Jesuitas, desde Rio Grande del Sur, Brasil en 1634 ingresó la garrapata *Rhipicephalus microplus,* antes conocida como *Margaropus annulatus australis* (Hooker, 1909).

*Rhipicephalus microplus* es una garrapata de un solo hospedador (bovinos). El ciclo parasitario (mudas y copulación) se realiza enteramente sobre un solo animal. Luego de que la hembra ingurgitada (teleógina) se desprende del bovino lleva a cabo la puesta de los huevos en el ambiente y muere. De los huevos emergen larvas que buscarán un nuevo hospedador (Nuñez *et al*., 1986). Desde la década del 70 se han realizado numerosos estudios en Uruguay que han determinado la epidemiología de la garrapata (Nari *et al*., 1979; Cardozo *et al*., 1984; Sanchis *et al*., 2008, Cuore, 2017). Se definió que las garrapatas pueden producir 3 generaciones anuales, con un rango de 2 a 3,5 dependiendo de la región y del ambiente. Generalmente, la primera generación va desde julio/agosto a setiembre/octubre, la segunda de noviembre/diciembre a enero/febrero y la tercera ocurre durante el otoño. En áreas de monte, se pueden producir 3,5 generaciones, mientras que en áreas de serranías como las del departamento de Lavalleja ocurren 2 generaciones. Durante el invierno se produce una interrupción del ciclo en el que las formas no parasitarias (huevos y larvas), que pueden sobrevivir en el ambiente por 8 a 10 meses, son responsables de la primera generación en la primavera siguiente (Cuore *et al.,* 2013).

En el Uruguay el control de la garrapata se inició por la Policía Sanitaria en 1910 con la ley 3606; posteriormente, en 1940 (Ley 9.965) se declaró obligatoria la eliminación de la garrapata en todo el país, comenzando por la zona sur con miras a proseguir con el saneamiento en la zona norte. Desde el 2008 rige una ley (N° 18.268) que divide al País en zonas libres, de control y erradicación y califica el riesgo epidemiológico (Errico *et al.,* 2009; Cuore *et al.,* 2012). A pesar de haber resultados de investigación y existir el marco legal para el control de la garrapata, en el Uruguay, las campañas sanitarias han tenido resultados variables y actualmente la situación de la dispersión de la garrapata es muy semejante a la del año 1917 (Cardozo, 1987; Silva *et al.,* 1987; Miraballes & Riet-Correa, 2018). Esto puede ser debido a diversos factores, incluyendo la discontinuidad de las campañas sanitarias, recursos financieros variables para las mismas, cambios en las características de los movimientos de ganado, fallas en la divulgación de las herramientas para el control, cambio climático, alteraciones ambientales como la forestación y resistencia de *R. microplus* a los acaricidas. En 1922 fueron introducidos al Uruguay los baños arsenicales, que se usaron de manera continua por 28 años, hasta que poblaciones, posiblemente resistentes, fueron controladas con Lindano (Bertino & Tajam, 2002; Cuore *et al.,* 2013). A mediados de la década de los 60, los organoclorados (Lindano y Dieldrhin) fueron remplazados por los organofosforados (OP). La primera población resistente a los OP fue diagnosticada en 1978 y, en 1982, la resistencia se encontraba ampliamente dispersa en el país (Nari, 1984; Castro-Janer *et al.,* 2015). Los Piretroides Sintéticos y mezclas con OP comenzaron a utilizarse en los 80s, siendo la resistencia detectada por primera vez en 1994 (Cardozo, 1995). El Amitraz se registró en 1977 y la primera población resistente se diagnosticó en 2009 (Cuore *et al.,* 2012). El Fipronil se registra en 1997 y en el 2006 se encontró la primera población resistente (Cuore *et al.,* 2007). En algunos casos la resistencia al Fipronil se evidenciaba después de 3 a 7 aplicaciones. Debido a que el Fipronil actúa en el mismo sitio de acción que el Lindano, se cree que esto pueda deberse a resistencia cruzada (Castro-Janer *et al.,* 2015). El diagnóstico de la primera población de garrapatas resistentes a tres acaricidas (multirresistentes) se realizó en el 2009 (Cuore y Solari, 2014; Cuore *et al.,* 2015) y en el 2010 se realiza el primer diagnóstico de una población resistente a Ivermectina (Cuore *et al.,* 2015). Hasta mediados de 2016 se registraron 14 establecimientos con diagnóstico de resistencia a 4 acaricidas y 13 establecimientos con resistencia a 5 acaricidas (Cuore, *et al.,* 2017; Cuore, 2017). El Fluazurón, que se encuentra disponible en Uruguay desde 1996, hasta la fecha no ha tenido de sospechas de resistencia (Cuore y Solari, 2014). Cabe destacar que la técnica de diagnóstico *in vitro* para este principio activo todavía no se utiliza. No obstante, en Río Grande del Sur ya se encontraron poblaciones de *R. microplus* resistentes a Fluazurón (Reck *et al.,* 2014).

Actualmente, se estima que la garrapata común del ganado, *R. microplus*, causa pérdidas económicas estimadas en 32,7 millones de dólares anuales, debidas a lesiones en los cueros, gastos por tratamientos, enfermedades transmitidas por la garrapata y gastos en las campañas de control (Avila, 1998). A esto se agregan las pérdidas por contaminación de los alimentos y probables pérdidas de mercados para las carnes uruguayas. Estas últimas han tenido un destaque reciente en consecuencia del rechazo de varios contenedores exportados a Estados Unidos con residuos de

Ethión, lo que determinó que el Ministerio de Ganadería Agricultura y Pesca (MGAP) suspendiese temporalmente la venta y registros de este producto en el país (Aguerre, 2016).

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# 2. ANTECEDENTES ESPECÍFICOS

El conocimiento generado en la epidemiología de la garrapata ha llevado a que se desarrollen métodos eficientes de control o eliminación, que incluyen los conceptos de control integrado (Nari *et al.,* 2013) y del tratamiento generacional (Cuore *et al.,* 2008). El control integrado de parásitos se define por la FAO como "un sistema de manejo de pestes, que utiliza todas las técnicas y métodos apropiados para combatir una o más pestes, interfiriendo lo menos posible en el medio ambiente y manteniéndolas a un nivel que no produzcan daño" (Nari *et al.,* 2013). El tratamiento generacional, ha sido utilizado con éxito en Uruguay para eliminar poblaciones resistentes de *R. microplus* y como forma de retardar la aparición de resistencia. Este consiste en tratar cada generación de garrapatas con un principio activo diferente, con distintos mecanismos de acción, en forma estratégica (Cuore *et al.* 2012; 2015).

A pesar de todos esos avances en el conocimiento y en las estrategias de control, la garrapata parasita el ganado en grandes áreas de Uruguay, incluyendo 6 departamentos al sur del Río Negro, y la tristeza parasitaria es una de las enfermedades más prevalentes del litoral-oeste (Errico *et al.,* 2009; Buroni, 2014). Además, la detección de insecticidas en carnes uruguayas exportadas a Estados Unidos es una alerta para posibles trabas sanitarias a la exportación de carne (Aguerre, 2016).

Otro problema que puede estar agravando esta situación es la utilización de insecticidas para el control de *Haematobia irritans* (mosca de los cuernos) ya que el Ethión, detectado en carnes uruguayas puede proceder tanto de tratamientos acaricidas como de tratamientos para el control de la mosca de los cuernos. En Uruguay, la mosca de los cuernos fue detectada por primera vez en el año 1991 (Carballo & Martinez, 1992) diseminándose rápidamente por todo el país. A los cinco años de su ingreso se detectó resistencia a la cipermetrina, la cual se encuentra ampliamente dispersa (Márques *et al*., 1997, Cuore *et al.,* 2013). A pesar de que en Uruguay no se han demostrado pérdidas significativas de producción por la presencia de mosca de los cuernos (Castro *et al.,* 2001, 2002, 2008), la utilización de insecticidas para el control de *H. irritans* es un importante riesgo, ya que puede significar la presencia de residuos en la carne. Asimismo, la mayoría de los insecticidas interfieren en la población de garrapatas pudiendo generar presión para la selección de resistencia y de esta manera agravar la situación ya existente (Nari *et al.,* 2013).

Dentro de las pérdidas económicas causadas por la garrapata están las ocasionadas por la ocurrencia del complejo llamado tristeza parasitaria, que comprende dos enfermedades: babesiosis y anaplasmosis. La primera es causada por los protozoarios *Babesia bovis* y *Babesia bigemina*, mientras que el agente etiológico de la segunda es una rickettsia, *Anaplasma marginale* (Rubino, 1946). Si bien estas dos enfermedades (babesiosis y anaplasmosis) se engloban dentro de dicho complejo, tienen un comportamiento epidemiológico diferente. Tanto *B. bigemina* como *B. bovis* están estrictamente ligadas a la epidemiología de su vector biológico: *R. microplus.* Por el contrario, *A. marginale* no solo es transmitida por *R. microplus,* sino que puede valerse de insectos hematófagos para su propagación (Solari, 2006). A su vez, puede ser transmitida de bovino a bovino por otras vías que incluyen la iatrogenia y la infección vertical (transplacentaria) (Mangol & Mastropaolo, 2013). Los brotes de tristeza parasitaria causados por *Babesia* spp. se presentan generalmente de forma aguda, ocurriendo en aproximadamente 10 días, con hasta 50% de morbilidad y 25% de mortalidad del rodeo afectado (Solari *et al.,* 2013). En el caso de la anaplasmosis, las muertes se presentan en goteo ya que el período de prepatencia puede variar dependiendo del inóculo entre 20 a más de 60 días (Solari, 2006). Sin embargo, la tristeza parasitaria está subdiagnosticada ya que, a campo, muchas veces el veterinario realiza el diagnóstico en base a la sintomatología y la anamnesis, sin llegar a conocer con certeza su agente etiológico.

Aun estando subdiagnosticada, en el DILAVE de Paysandú, en el período de 1993-2003 fueron diagnosticados 159 focos de tristeza parasitaria, siendo la enfermedad más frecuente. En el período 2004-2013 se detectaron 141 focos, siendo la segunda enfermedad en frecuencia, después de la mastitis (Buroni, 2014). En total, sumando todos los diagnósticos realizados desde el año 1993 hasta abril de 2016 fueron diagnosticados 410 focos, de los cuales: 16 correspondieron a infecciones mixtas de *Babesia* spp. y *A. marginale* y 104 y 290 fueron infecciones simples de *A. marginale* y *Babesia* spp., respectivamente (Solari et al., 2013). En la región Este, el número de focos diagnosticados por el DILAVE Treinta y Tres superó los 100 en el período 1988-2011, siendo más frecuente la babesiosis por *B. bovis* (Solari *et al.,* 2013). A nivel de establecimientos, se estima que en el norte del país la tristeza parasitaria causa pérdidas de 7,3 dólares por animal por año, considerando solamente las pérdidas por muertes y el costo del tratamiento, sin tomar en cuenta las pérdidas de peso y los abortos (Solari *et al.,* 2013).

La alta frecuencia de tristeza parasitaria se debe, principalmente, a que Uruguay está situado en una zona considerada de inestabilidad enzoótica, con alto número de establecimientos con un 20% a un 80% de bovinos con anticuerpos para *Babesia* spp. y *A. marginale*, lo que determina el riesgo de ocurrencia de brotes todos los años (Solari *et al.,* 2013). El conocimiento de la epidemiología de la tristeza parasitaria y el diagnóstico diferencial de sus agentes es esencial para delinear medidas de control eficaces.

Las técnicas disponibles actualmente para el diagnóstico de tristeza parasitaria se pueden subdividir en técnicas para el diagnóstico del brote y técnicas para la determinación de la situación de riesgo (serología). Las técnicas más usadas para el diagnóstico de brote son aquellas que detectan la presencia del parásito, entre las que encontramos al frotis sanguíneo que puede ser fino, teniendo la ventaja de poder observar los detalles de los agentes causales, o grueso, generando mayores posibilidades de encontrar al agente causal. También se utiliza la reacción en cadena polimerasa (PCR) (Solorio-Rivera & Rodriguez-Vivas, 1997; Gayo *et al.,* 2003). Actualmente, en Uruguay, se está desarrollando una PCR multiplex basándose en el protocolo de Figueroa et al. (1993), para diagnosticar los tres parásitos de forma simultánea, lo que disminuye sustancialmente los costos.

En cuanto a la serología, permite determinar cómo queda el rodeo desde el punto de vista inmunitario después de un brote, determinando el riesgo futuro, y permite analizar por primera vez la situación de un rodeo (Solari, 2006). Entre las técnicas más usadas para determinar la situación de riesgo se encuentran la inmunofluorescencia indirecta (IFI) y el ensayo inmunoenzimatico (ELISA) que determinan presencia de inmunoglobulina G en los sueros problema. La técnica de IFI ha mostrado sensibilidad y especificidad de 98%, mientras que el ELISA obtuvo una sensibilidad de 97% y una especificidad de 93%. Aunque ambas técnicas resultan eficientes para la realización de estudios epidemiológicos, la técnica de ELISA tiene mayor ventaja sobre la IFI, debido a que se pueden analizar un mayor número de muestras por persona/día y se obtienen resultados que no dependen de la subjetividad de la interpretación ya que están automatizados (Dominguez-Alpizar, *et al*., 1995). Sin embargo, hasta la fecha, en Uruguay, se cuenta solo con la técnica de IFI para el diagnóstico de *B. bovis* y *B. bigemina.*

Una alternativa para la prevención de la tristeza parasitaria en establecimientos con inestabilidad enzoótica es la utilización de vacunas. A pesar de que la DILAVE Miguel C. Rubino ha producido esa vacuna desde 1941 y la sigue produciendo y existe otra vacuna congelada producida por un laboratorio comercial, la cobertura de vacunación es muy baja. El número de vacunas comercializadas anualmente entre los años de 2008 y 2016 fue alrededor de 22.000 dosis, lo que representa un número extremamente reducido si consideramos que en el área se producen más de 2.000.000 de terneros por año (Miaraballes & Riet-Correa, 2018).

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## 3. PLANTEAMIENTO DEL PROBLEMA

La garrapata, *Rhipicephalus microplus*, es el principal parásito causante de pérdidas económicas en bovinos del Uruguay. Se producen pérdidas por los efectos de este parásito sobre los bovinos como causa de baja calidad de los cueros. Otras importantes pérdidas se deben a que *R. microplus* transmite la tristeza parasitaria, causada por 3 agentes: *Babesia bigemina, Babesia bovis* y *Anaplasma marginale*. Estas enfermedades causan perjuicios económicos debido a la muerte de los animales y a pérdidas productivas. También ocurren pérdidas por los costos en tratamientos y control de la parasitosis y de la tristeza parasitaria. A estas numerosas pérdidas, estimadas en 32.7 millones de dólares anuales, se suma el problema de resistencia desarrollada por *R. microplus* a los acaricidas, por lo que en algunas situaciones se aumenta la frecuencia de tratamientos y la cantidad de principio activo. Esto contribuye al aumento de la contaminación ambiental y de los residuos en productos de origen animal, poniendo en riesgo la salud animal, la salud pública, y el acceso a mercados. Uno de los principales problemas para el control eficiente de la garrapata y de la tristeza parasitaria es la carencia de diagnóstico de la situación en cada establecimiento, que permitiría utilizar el conocimiento generado para el diseño de planes de control o erradicación eficientes adecuados a cada caso.

Seguramente se podrían disminuir significativamente las pérdidas causadas por garrapata y tristeza parasitaria, la selección de poblaciones multiresistentes y los riesgos de contaminación de los productos de origen animal con acaricidas aplicando tecnologías ya conocidas en Uruguay, incluyendo el tratamiento generacional y la vacunación contra hemoparásitos. Por qué los productores no aplican esas tecnologías es una pregunta que debería ser respondida en un corto plazo para que sea posible revertir esa situación.

Por lo expuesto anteriormente, es necesario determinar las causas de la no adopción por parte de los productores de tecnologías adecuadas para el control de la garrapata y las enfermedades transmitidas.

# 4. HIPÓTESIS

Los programas sanitarios para el control o eliminación de *Rhipicephalus microplus* en establecimientos comerciales serán eficientes en la medida de que se tome en cuenta la situación de la investigación y del control de la garrapata y la tristeza parasitaria en Uruguay, adicionándole herramientas que colaboren con la toma de decisiones con menor subjetividad en cada establecimiento.

# 5. OBJETIVOS

## 5.1. Objetivo general

Aportar información para sensibilizar a los productores sobre la adopción de medidas apropiadas de control que disminuyan la utilización de garrapaticidas.

## 5.2. Objetivos específicos

- Revisar los antecedentes de la garrapata y la tristeza parasitaria en Uruguay
- Evaluar la presencia de anticuerpos en animales inmunizados con las vacunas comerciales disponibles contra de tristeza parasitaria.
- Realizar una evaluación de riesgo de introducción de *R. microplus* a los establecimientos.

# 6. INVESTIGACIÓN

La presente tesis consta de tres artículos que fueron realizados con el fin de cumplir con los objetivos planteados anteriormente. En el artículo uno se realizó una revisión de la situación de la garrapata y la tristeza en el Uruguay, desde el año 1909, con el objetivo de estudiar retrospectivamente la situación de estas parasitosis y plantear alternativas para su control. Este artículo fue publicado en la revista Experimental and Applied Acarology. El articulo dos, consta de la evaluación de la presencia de anticuerpos en animales inmunizados con las vacunas que se encuentran disponibles en el mercado para prevenir la tristeza parasitaria. Tuvo por objetivo evaluar la presencia de anticuerpos impartida por una vacuna congelada y otra refrigerada, y difundir esta tecnología en Uruguay por lo que fue publicado en la revista Veterinaria Uruguay. Para el tercer artículo se realizó una evaluación de riesgo utilizando un modelo de análisis Bayesiano con el objetivo de evaluar la probabilidad de introducción de la garrapata a establecimientos comerciales. Ese análisis fue aplicado a 33 establecimientos comerciales ubicados en la zona control de garrapata, ayudando a los veterinarios de libre ejercicio encargados de estos predios y a los productores en la toma de decisiones en cuanto a controlar o eliminar la garrapata. Este artículo fue publicado en la revista Tick and Tick Borne Diseases.

## 6.1. ARTÍCULO 1

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# A review of the history of research and control of *Rhipicephalus* (*Boophilus*) *microplus*, babesiosis and anaplasmosis in Uruguay

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## Abstract

In Uruguay, control of *Rhipicephalus microplus* began in 1910. In 1941 the eradication of *R. micoplus* throughout the country was declared mandatory, although this attempt was unsuccessful. Since 2008 the country was divided into two regions: the south-western region, which is free of ticks; and a region of tick control that includes all departments to the north of the Rio Negro and five departments in the eastern region. In Uruguay, investigations on *R. microplus*, babesiosis and anaplasmosis started in 1921, and in the 1970s, studies of the epidemiology of *R. microplus* determined that from 2 to 3.5 generations can be produced annually and that the country is in an area of enzootic instability for babesiosis and anaplasmosis. Knowledge of tick epidemiology and of tick resistance to different acaricides led to the development of efficient methods of control or eradication, including integrated control and generational treatment. Although research results have led to a legal framework regarding *R. microplus* control, these measures have had variable results. This can be attributed to several factors, such as the discontinuation of the control measures, variable financial resources, changes in the dynamics of livestock movement, failure to adopt available technology for tick control by farmers, climate change, environmental alterations such as forestation and the increasing resistance of ticks to acaricides, which led to the development of multiresistant ticks. This paper reviews the history of *R. microplus*, babesiosis and anaplasmosis in Uruguay and proposes alternatives for their control.

Keywords Uruguay · Cattle tick · Babesiosis · Anaplasmosis · Control

## Introduction

Cattle was introduced into Uruguay between 1611 and 1634 from Paraguay and Brazil (Sala de Touron et al. 1968). The first reference of *R. microplus* (cattle tick), babesiosis and anaplasmosis in South America appeared in Brazil at the beginning of the nineteenth century (Gonzales et al. 2013). In Argentina, the first reference appeared in 1838 (Lombardero 1983), and in Uruguay, in 1901 (Hooker 1909).

In Uruguay, in 1910, cattle tick, babesiosis and anaplasmosis were included in Law 3606 of the Animal Health Police, which determined that reporting outbreaks of cattle tick, babesiosis and anaplasmosis was mandatory. Uruguay is divided into 19 departments. It is located between latitudes 30°–35°S and longitudes 53°–58°W, has a temperate climate, and is considered marginal for the development of cattle tick (Nari 1995). From 1917 to 2017 the area north of the Rio Negro was considered tick infested, and the southern area had variable infestations. The objective of this article is to review the history of *R. microplus*, babesiosis and anaplasmosis in Uruguay since 1910, including its importance, the control measures used, the variations in the geographic distribution of ticks over time, and the factors associated with this distribution. Based on this historic analysis, control alternatives are proposed.

## Economic losses caused by *Rhipicephalus microplus*, babesiosis and anaplasmosis

In Uruguay, it is estimated that *R. microplus* causes economic losses of 32.7 million dollars annually (Avila 1998), mostly due to treatment costs, weight gain–loss and mortality due to babesiosis and anaplasmosis (Solari et al. 2013). Additionally, there are losses due to acaricide residues in food and, therefore, probable restrictions of the markets for Uruguayan meat and milk products. Such situation occurred recently due to the rejection of containers of meat exported to the United States of America (USA) that contained ethion residues (Aguerre 2016).

*Rhipicephalus microplus* is the only tick species with economic significance to the livestock industry (Venzal et al. 2003). In farms in the northern region, it is estimated that an outbreak of babesiosis and/or anaplasmosis can cause a loss of $7.30 (USD) per bovine when only deaths and treatment costs are considered (Solari et al. 2013). Excluding mastitis, babesiosis and anaplasmosis were the most frequent diseases diagnosed by the Direction of Veterinary Laboratories (DILAVE) in Paysandú between 1993 and 2003, representing 10.3% of all diagnosed cases (Buroni 2014).

## Legislation

In 1910, the Sanitary Animal Police Law 3606 was created, determining the mandatory communication regarding cattle tick, babesiosis and anaplasmosis by farmers and veterinary practitioners and the interdiction (mandatory tick control and control of cattle movement) of the affected farms (MAP 1910). In 1917, a decree dividing the country into three areas was created: an area free of ticks, south of the Santa Lucia River (excluding the department of Montevideo, which was considered permanently tick-infested); a tick-infested area to the north of the Rio Negro, that was considered endemic for cattle tick; and an intermediate area where outbreaks were sporadic and should be eradicated, between the Santa Lucia River and the Rio Negro (Fig. 1). The transit of animals with ticks through the tick-free area was forbidden.

In 1923, a regulation called "Regulation for the Removal of the Tick" was created, designating the eradication of cattle ticks from the entire country. To achieve this objective, the country was divided into three areas: (1) the south area included the departments of Canelones, Maldonado and Rocha; (2) the intermediate area included the departments of Soriano, Colonia, San José, Flores, Florida, Durazno, Treinta y Tres, Minas and Cerro Largo; and (3) the north area included the departments of Artigas, Salto, Paysandú, Rio Negro, Rivera and Tacuarembó (Fig. 1). As Montevideo remained tick-infested, it was considered separately. In the farms located in the south and intermediate areas, the farmers were required to report the presence of ticks and cases of babesiosis and anaplasmosis to the Animal Health Authorities. Tick-infested farms were interdicted, and treatment of the cattle every two weeks became mandatory. In such cases, removing cattle from the farm was authorized only after a "tick-free" certificate. This interdiction remained until it could be demonstrated that the farm was free of ticks. Since the north area was considered endemic for the presence of ticks, the decree only mentioned the need to report deaths due to babesiosis and anaplasmosis. The interdictions of farms in the north area was determined only if the Animal Health Authorities found that the degree of tick infestation was due to negligence or if it constituted a danger to the area or to neighboring farms. In the north area, the traffic of animals with ticks was prohibited along roads that crossed or bordered farms that were free of ticks. The transit of animals with ticks from the north area to the *La Tablada* market in Montevideo was allowed only by railway (Ministerio de Industrias 1923).

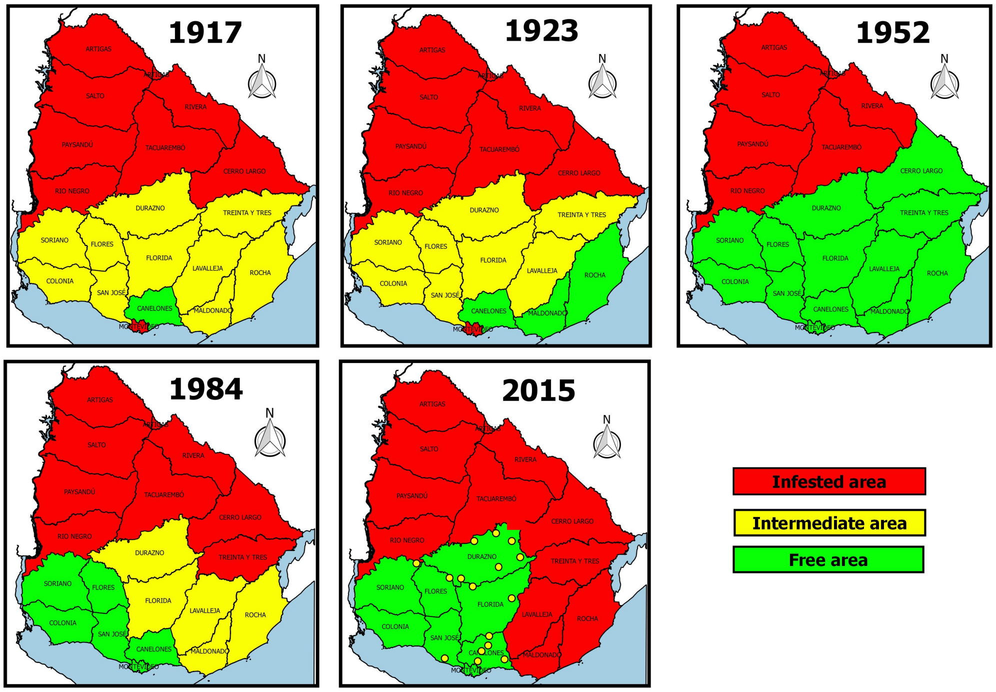

Fig. 1 Maps of the distribution of the cattle tick in Uruguay from 1917 to 2015. Green represents the areas free of the cattle tick, red represents the tick-infested areas, and yellow represents the intermediate area where outbreaks were sporadic and should be eradicated. For 2015, the yellow spots within the green area represent 16 farms interdicted for the presence of ticks that are under control. The following sources have been consulted for the construction of the maps: 1917 (Bertino and Tajam 2000), 1923 (Ministerio de Industrias 1923); 1952 (MGA 1956); 1984 (Cardozo 1987); 2015 (MGAP 2008; DIGESEGA 2015).

A law (N° 9.965) declaring compulsory tick eradication in the whole country, with emphasis in the south area, was approved on November 5, 1940. The country was divided into two areas: (1) the north area included the departments to the north of the Rio Negro and the department of Cerro Largo; and (2) the south area included all the departments south of the Rio Negro except Cerro Largo. This law prohibited the transit of animals with ticks in the south area. It determined that, in the north area cattle leaving farms should be free of ticks (MGA 1941).

In 1953, outbreaks in the south area were eradicated, and those in the eastern area were almost eradicated. Consequently, another law (N° 12.293) declared the tick as a national pest and proposed the eradication of the parasite throughout the whole country. Regional committees for tick control were formed, financial support was available, and sanctions were set for violations of the law (MGA 1956; Cardozo 1987). Unfortunately, this law did not have the expected success, probably because of the economic crisis that occurred in Uruguay in the 1960s.

In 2003, different institutions began to work on the current cattle tick control law (Law 18.268), which was approved in 2008. In this law, the country was divided into an area free of ticks, including the departments of Montevideo, Canelones, San Jose, Florida, Flores, Colonia, Soriano and most of Durazno (excluding the 7th police district of this department), and a tick-control area, including Artigas, Salto, Paysandú, Rio Negro, Rivera, Tacuarembó, Cerro Largo, Treinta y Tres, Lavalleja, Maldonado, Rocha, and the 7th pólice district of Durazno (Fig. 1) (MGAP 2008). The creation of a third area (in eradication) where tick eradication would be the goal, was also planned; however, this creation has not been implemented yet. This law also created divisions in the country according to the epidemiological risk for the presence of cattle ticks. The area along the northern coast of the Uruguay river and the deep basalt region were considered to have greater risks (Errico et al. 2009). In addition, the General Direction of Livestock Services (DGSG) proposed the interdiction of farms considered as high risk (DGSG 2011a). The potential reasons for interdiction of a farm were: (a) if the farm's situation caused damage to another farm; (b) if the owner travelled with animals parasitized repeatedly to other farms or slaughterhouses; (c) if ticks were found on a farm located in an area free of ticks; (d) if the farm was considered high risk because of their production characteristics; (e) if the tick population was resistant to an acaricide with no previous history of resistance in the country or in the area; (f) if the farm had outbreaks of babesiosis and/or anaplasmosis; and (g) if the farm had more than 1 engorged tick per cow between June and October, 5 ticks per cow between November and January or 10 ticks per cow between February and May in more than 20% of the cattle (DGSG 2011a). Interdicted farms, besides having controlled movements, must follow a control plan proposed and executed by private veterinarians accredited by the Animal Health Authority (DGSG 2011b). The accreditation of private veterinarians to carry out the control programs started in 2017.

In 2016, the USA prohibited the registration of products containing ethion in their formulation, and the maximum residue limit (MRL) for this drug was reduced to zero. Consequently, several containers with meat from Uruguay were rejected by American authorities because of ethion residues. As a result, the Ministry of Livestock, Agriculture and Fisheries (MGAP) withdrew all products with ethion from the market and prohibited its use until new tests for residues were performed on tissue samples and the results were in accordance with the American MRL. At the same time, the MGAP increased requirements for farmers to properly use the acaricides, mainly respecting waiting times (Aguerre 2016). Although the decree N° 9/010 in 2010 allowed the transportation of cattle with ticks to slaughterhouses; until 2016, this authorization was considered an exception. After the detection of ethion residues in Uruguayan meats, the MGAP began to encourage the shipment of cattle with ticks from tick-infested farms to slaughterhouses, without penalty. Finally, in October 2017, the MGAP once again authorized the use of ethion by spraying or immersion for tick control under the supervision of an authorized veterinarian. Additionally, the waiting period before slaughter was extended from 15 to 130 days (DGSG 2017).

## Geographic distribution

The distribution of *R. microplus* in Uruguay from 1917 until 2017 underwent several changes that were characterized by a constant presence in the area north of the Rio Negro and a variable presence to the south of this river (Fig. 1). The best control occurred in the 1950s with the decision to eradicate the cattle tick. However, despite human and financial resources and the support of the farmers, it was not possible to advance the eradication of the parasite north of Rio Negro (Cardozo 1987) and there was a continuous increase in the dispersion of the parasite. During this time, only in the 1950s was it possible to restrict cattle tick to the north of the Rio Negro. The main reason for this situation was probably the economic crisis in Uruguay in the 1960s, which resulted in a sharp decline in health campaign resources (Millot and Bertino 1996). When Law 18,268 was created in 2008, it was determined that it was impossible to eradicate the cattle tick from the country. In addition to all the departments north of Rio Negro, four departments in the south-east region (Cerro Largo, Rocha, Maldonado and Lavalleja) were included in the tick-infested area with no possibility of eradication (Errico et al. 2009). In 2015, in the area considered free of ticks, there were at least 16 interdicted farms (Fig. 1) (DIGESEGA 2015). Several factors could be responsible for this situation:

### Resources for the health campaign

Economic, financial and human resources have been variable over time, partly explaining the variation in the geographic distribution of ticks over the years. By 1948, 650 officers of the Animal Health Police were working to control the cattle tick, scabies, tuberculosis and foot-and-mouth disease, among other diseases (Bertino and Tajam 2000). At the end of 1960 in Uruguay, a political, economic and social crisis led to the withdrawal of resources from health campaigns, resulting in the consequent increase in cattle tick in the north and in the number of outbreaks in the south. Uruguay started its economic recovery after the 2002 crisis, which was mainly due to the introduction of foot and mouth disease. Since then, there have been no significant investments in the campaign against cattle tick. Currently, only 205 veterinarians and animal health officers are working in the Animal Health Authority for the control of all diseases. However, with the current law, there has been a change in the tick control policy, giving more responsibility to farmers and private veterinarians who are replacing the many field officers used previously (Errico et al. 2009).

### Livestock movement dynamics

An important factor in the dispersion of the cattle tick has been the transit of animals, which has been consistently changing over the last century. Until the end of the nineteenth century, livestock were transported by herders and in wagons. At the end of the nineteenth century and the beginning of the twentieth century, because of the installation of the first slaughterhouses in Montevideo, trains became the most important means for cattle transportation in Uruguay. In 1876, in Montevideo, *La Tablada*, the main commercial livestock center until the middle of the twentieth century, was created.

In 1891, 150,000 animals were transported by train, and in 1907 the number increased to 1,430,000 (Millot and Bertino 1996). In 1923, the transit of cattle from tick-infested areas to *La Tablada* was only allowed by train. Rigorous criteria were established for the transportation of animals from *La Tablada* to other regions and for the transport of cattle within tick-infested areas and between the tick-infested and tick-free area. From 1969, *La Tablada* lost importance because slaughterhouses increased their purchase of livestock directly from the farmers and other forms of trade. Currently, there are 31 companies that export meat distributed in several departments (INAC 2017).

Since the 1950s, Uruguay began using trucks for cattle transportation, increasing the interdepartmental transport in several directions. Despite the obligation to certify that the animals transported were free of ticks, first by animal health officers and, after the approval of Law 18,268 in 2008, by private veterinarians, this type of transport probably contributed to spread the cattle tick.

From the beginning of the twentieth century, livestock was mostly marketed at local fairs. Considering the existence of several fairs in each department, the animals did not travel great distances when they were traded between farmers. Even though the selling of animals with ticks at local fairs was always prohibited and the treatment of tick infested animals was mandatory, this system probably contributed to the spread of ticks, mainly within each department. In 1999, a virtual sale modality called sale by screen was established. This system is based on filming the animals that are sold by a set of rural trade agencies that allow the farmers to acquire cattle from different regions without having to travel. With this system, livestock are transferred greater distances than those sold at local fairs, and although they are required to be free of ticks, the risks of the dispersion of this parasite are greater.

Currently, 4.5–4.7 million cattle are sold in Uruguay each year. Of that number, from 2000 to 2017, 46% went to slaughter, and 54% were cattle bought for replacement (Bruno Lanfranco, National Institute for Agricultural Research (INIA), Las Brujas, personal communication, 2017). An important system adopted in Uruguay in 2006 allows all cattle to be tracked, which enables the National Livestock Information System (SNIG) to know the owner of each animal and its date and place of birth, sex, breed, movements and place of slaughter or death. This traceability may allow the adoption of more effective measures for the surveillance and control of cattle tick and the detection of the origin of outbreaks (SNIG 2017).

The various marketing systems and transportation between the different regions of the country surely participated in the dispersion of ticks. Establishing correct forms of control and actively engaging veterinarians and farmers in this task is essential to avoid tick dispersal.

## Evolution of resistance of ticks to acaricides

In 1922, arsenical dips were introduced for tick control in Uruguay. Arsenicals were used continuously for 28 years, and strains possibly resistant to arsenic were controlled with lindane (Cuore et al. 2013). In the mid-1960s, organochlorines (lindane and dieldrin) were replaced by organophosphates. The first strain resistant to organophosphates was diagnosed in 1978, and in 1982, resistance to this drug was widely dispersed in the country (Nari et al. 1984; Castro-Janer et al. 2015). Synthetic pyrethroids and mixtures with organophosphates began to be used in the 1980s, and resistance to these drugs was detected in 1994 (Cardozo 1996). Amitraz was registered in 1977, and the first tick-resistant population was found in 2009 (Cuore et al. 2012). Fipronil was registered in 1997, and in 2006, the first resistant strain was found (Cuore et al. 2007). In some cases, resistance to fipronil appeared after 3–7 applications. Because fipronil acts at the same site of action as lindane, the rapid onset of resistance to this molecule may have been due to cross-resistance with this organochlorine (Castro-Janer et al. 2015). In 2010, the first detection of resistance to ivermectin was made (Cuore et al. 2015). The first multiresistant strain was diagnosed in 2009, and since that date, multiresistant strains for at least three acaricides have been detected in Uruguay (Cuore and Solari 2014; Cuore et al. 2015). Until the middle of 2016, there were 14 farms with ticks showing resistance to 4 acaricides and 13 with ticks resistant to 5 acaricides (Cuore et al. 2017). Currently, of 116 samples to carry out tests for resistance, evaluated between 2015 and July of 2016, 13 samples were simultaneously resistant to pyrethroids, organophosphates, amitraz, fipronil and macrocyclic lactones. The most prevalent resistance was to pyrethroids (91%), and the least prevalent was to macrocyclic lactones (33%) (Cuore et al. 2017). From 62 resistant samples evaluated from 2016 until 2017, the level of resistance was low (< 20% of the larvae survive) to ivermectin, low-median (between 20 and 50% of the larvae survive) to fipronil and amitraz and high (more than 50% of the larvae survive) to ethion and cypermethrin (Cuore et al. 2017). The techniques available for the detection of resistance are the adult immersion test (AIT) (Drummond et al. 1973) and the larval package test (LPT) (Stone and Haydook 1962). Fluazuron has been available in Uruguay since 1996, but there is no indication of resistance to this drug in the country (Cuore and Solari 2014), although the techniques to detect tick resistance to this acaricide are not yet available. However, the resistance of ticks to fluazuron has been diagnosed in Rio Grande do Sul, a state sharing a border with Uruguay (Reck et al. 2014).

Another problem associated with tick resistance is the contamination of animal products with insecticides. This situation is possibly the most worrying and requires immediate and efficient action. For an efficient tick control and to avoid acaricide residues in animal products it is necessary that resistance test to the acaricides, including fluazuron, becomes available to all farmers. Without such information, it will be impossible to implement adequate control measures in the farms, including generational treatment, with the use of fewer acaricides and greater chances of success (Miraballes et al. 2018a).

### No adoption of available technology for tick control

Undoubtedly, the lack of adopting available techniques has been one of the main factors in the failure to control ticks, babesiosis and anaplasmosis and has also contributed to the selection of tick strains resistant to acaricides. Recommendations, such as generational treatment, are not used and are not generally known by the farmers, nor do treatments begin in August (at the beginning of the first generation), which has been proposed since the first research results on the epidemiology of the cattle tick became available (Nari et al. 1979; Cardozo 1987). Currently, 85% of the farms use nine or more treatments per year for cattle tick control (Miraballes et al. 2018a), while adequate control could be achieved with four or five treatments (Nari et al. 2013). Also, there are a small number of farms with a known resistance profile of ticks to acaricides (Cuore et al. 2015, 2017) and only a few babesiosis and anaplasmosis vaccine doses are sold in Uruguay. In the tick-infested areas there are nearly two million calves that need to be vaccinated annually. Nearly 10,000 doses of refrigerated vaccine were sold annually until 2008 and, between 2008 and 2016, with the presence in the market of a frozen vaccine, approximately 22,000 doses of both vaccines have been sold annually (Miraballes et al. 2018b). Due to the non-adoption of technology, a unified message was created for an extension campaign carried out since July 2016. The detection of tick resistance to acaricides, generational treatment for tick control or eradication, serological diagnoses to determine stability or instability for babesiosis and anaplasmosis, and vaccination of at-risk calves were recommended.

**Environmental changes**

Table 1 shows that, in recent years in Uruguay, the hectares dedicated to forestation and agriculture have increased, decreasing the hectares dedicated to a larger number of cattle, creating more difficulties for tick control (MGAP 2015). In forestation areas, the grazing of livestock is recommended to control the risk of fire, and usually owners rent these areas to several farmers at the same time. This favors the mixing of animals, increasing the difficulties of tick control and the risk of outbreaks of babesiosis and anaplasmosis, due to the different degrees of susceptibility to hemoparasites (Solari et al. 2013). Additionally, with the installation of a second pulp mill in Uruguay, the area planted with eucalyptus will increase in the short term. Adequate control measures in those areas should be defined. The results of a project that is currently being developed by the DILAVE entitled "Sustainable Control of Parasites in Silvopastoral Conditions with Emphasis on the tick *Rhipicephalus (Boophilus) microplus* and Hemoparasites" will help to define an adequate control policy for these areas (Cuore 2017).

Due to the current environmental policies carried out by Uruguay, there has been an increase in the native forest area, which currently occupies approximately 4% of the country (MGAP 2015) and should be considered as an environmentally favorable area.

**Table 1 Variation in the area (ha) dedicated to different activities and the number of livestock in Uruguay based on censuses in 1990 and 2011 (MGAP 2015)**

| Hectares dedicated to: | 1990 census* | 2011 census* |
|---|---|---|
| Forestation | 186,000 | 1,071,000 |
| Agriculture | 693,000 | 1,604,000 |
| Livestock | 14,589,000 | 13,595,000 |
| **Livestock population** | | |
| Cattle | 8.7 million | 11.1 million |
| Sheep | 26 million | 7.5 million |

Currently, in Uruguay, there are 18.5 million fewer sheep than in 1990 (Table 1), lowering the probability of using alternative grazing for tick control (Nari et al. 2013) and increasing the rate at which ticks encounter cattle (DGSG 2011a).

## Climate change

In Uruguay, there has been a 0.5 °C increase in both the minimum and maximum average annual temperatures over the last 43 years (Fig. 2). In addition, a study carried out by Giménez et al. (2009) on data collected between 1931 and 2000 determined that there has been a decrease in the number of days with frost in winter (between 13 and 26 days fewer than in 1931) and that the minimum temperature in winter has increased 1.9 °C degrees during that period. The increase in soil temperature improves the habitat suitability and the possibility of survival of the non-parasitic stage of cattle ticks as well as the permanence in areas that were previously inadequate for tick development (Estrada-Peña et al. 2005).

As occurs in other countries, this may be an important factor for the dispersion of ticks in Uruguay. However, the temperature increases in Uruguay over more than 40 years (0.5 °C) is not as marked as the increase observed in tropical countries, where the temperature over 30 years increased by more than 1 °C (Benavides et al. 2016).

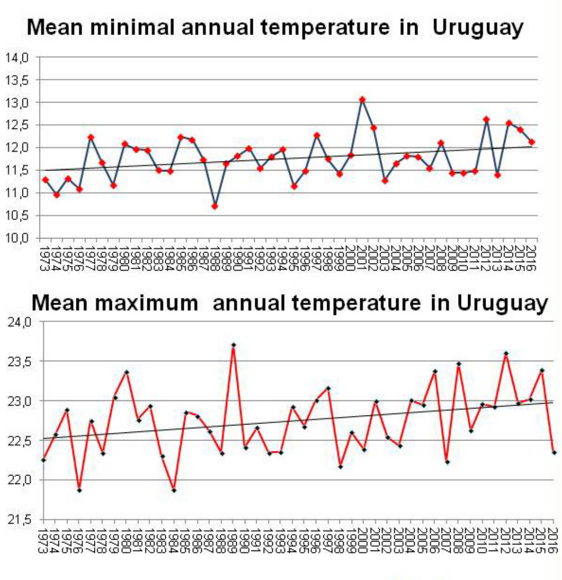

Fig. 2 Variation in the maximal and minimal temperatures in Uruguay from 1973 to 2016.

## Research and control on *Rhipicephalus microplus*

In Uruguay, early research on cattle tick biology and transmissibility of disease agents between species and the differential diagnosis of babesiosis and anaplasmosis started in 1920 and based on the experience of the USA using arsenical dips every 14 days for 8 months, the eradication of ticks was proposed (Rubino 1946). In 1936, an experimental station for the approval of veterinary products was created (Bertino and Tajam 2000). A parasitology research group created in 1972 studied the population dynamics of the cattle tick determining that 2–3.5 generations can be produced annually, depending on the region and the environment. The first generation occurs from July/August to September/October, the second from November/December to January/February and the third during autumn (March/May) (Nari et al. 1979). In native forest areas, 3.5 generations can be produced, while only 2 generations occur in highland areas (Cardozo et al. 1984). During the winter, the cycle is interrupted, and non-parasitic forms (eggs and larvae), which may survive in the environment for 8–10 months, are responsible for the first generation the following spring (Cuore et al. 2007, 2013).

Knowledge of *R. microplus* epidemiology has led to the development of efficient methods for control or eradication, including the concepts of integrated control (Nari et al. 2013) and generational treatment (Cuore et al. 2008). Integrated control was successfully applied in Uruguay to control cattle tick, babesiosis and anaplasmosis, horn flies, gastrointestinal nematodiasis and fasciolosis in cattle and sheep (Nari et al. 2013). Generational treatment, which has been successfully used in Uruguay to eradicate resistant strains of *R. microplus* and as a way of avoiding the emergence of resistance, consists of strategically treating each generation of ticks with a different acaricide (Cuore and Solari 2014; Cuore et al. 2015). Based on tick susceptibility, residual effects and waiting periods of the acaricides, and their mode of action, route of application and cost, the veterinarian will choose the three chemicals to be used (Cuore et al. 2008).

One proposal for generational treatment is to perform the first treatment at the beginning of the first generation (end of July or start of August), two treatments with another acaricide during the second generation (November/December to January/February), and two treatments with a third acaricide during the third generation (March to May) (Cuore et al. 2008). Another alternative is to perform the first treatment at the beginning of the first generation; in the second generation, treat animals with another acaricide when there are two partially engorged ticks (on one side) of 20% of a group of sentinel cattle (Cuore et al. 2008; DGSG 2011a); and in the third generation, apply three suppressive treatments with a third chemical to reduce the tick load for the following season (Cuore et al. 2008).

For eradication proposes suppressive treatments should be applied during the whole year, using different acaricides in each generation. Depending on the residual effect of each acaricide, the period between treatments will be approximately 21 days for amitraz, organophosphates, mixtures of organophosphates and synthetic pyrethroids, and Ivermectin 1%, 35 days for fluazuron and fipronil, and 45 days for ivermectin 3.15%, but this might vary between brands (Cuore et al. 2008). Once 12 months of suppressive treatments have been completed, and there are no visible ticks found during the third generation, treatments should be stopped, and the animals should be inspected every 21 days for a year, to demonstrate the success of the eradication program (DGSG 2011a).

In tick-infested areas, different farms are in different situations. In some areas, due to the environmental conditions (areas of forestation or native forests) or infrastructure (lack of suitable fences or trained personnel), it is difficult to eradicate the cattle tick, and the only option is to control the parasite to reduce economic losses. In contrast, in farms located in areas that are environmentally favorable for tick control and have adequate infrastructure and personnel, farmers should attempt eradication, which is likely to be more economical in the long term. In any case, before deciding if a farmer should attempt to control or eradicate the cattle tick, the possibility of eradication, the risk of reintroduction or the parasite, the cost of control or eradication, the necessary biosecurity measures, and the expenses in the event of reintroduction must be evaluated. A very important point for the control of ticks will be to establish control or eradication programs in the largest possible number of farms (Miraballes et al. 2018a). Within tick-infested areas, it is also important to establish eradication areas as provided by law. In that regard, the Animal Health Authority of the department of Tacuarembó reviewed 374 farms belonging to 15 police sections and verified that 2 police sections were free of ticks and that in 5 police sections, between 70 and 92% of the farms were free of ticks (Cortez; Dirección de Sanidad Animal, Tacuarembó, unpublished data, 2017).

The environmental impact of acaricides was also studied in Uruguay, showing that there were 5282 dip baths for cattle in 2000, and 76% of those discharged a mean of

10,000 L of bath contents directly into the field without any inactivating treatment (Nari et al. 2011). Currently, based on a survey conducted in 2016, 22.5% of farmers use dip baths to treat cattle (Araoz, INIA La Estanzuela, unpublished data, 2017). In 2017, a pharmacovigilance system was created in which shops selling veterinary products must keep a register of farmers who buy acaricides. This register is entered in the SNIG database to help control the dates of acaricide purchase, as well as the treatment of animals and their shipment to slaughter (SNIG 2017).

Due to the high cost of using acaricides, the increasing resistance of ticks, the risk of residues in animal products, and the fact that the development of new acaricides for tick control could take some 10 years and 100 million dollars, it is critical to develop alternative technologies to control ticks that decrease the use of acaricides (Pérez de León et al. 2017). In Uruguay, cattle tick vaccines based on the Bm86 gene antigen have been tested with diverse results, possibly due to the high degree of polymorphism of the Bm86 gene.

Currently, a research group is investigating the possibility of using the Bm86 gene present in Uruguayan ticks, called Bm86uy, and tick saliva proteins to produce recombinant vaccines that would be efficient in this country (Benavidez 2014). Additionally, there is an integrated tick control project that includes vaccination with a Colombian vaccine (Tick Vac) based on a polyprotein antigen (Cuore 2017).

There is also a project underway called "Biological Control of *Rhipicephalus* (*Boophilus*) *microplus* Ticks with Native Isolation of the Pathogenic Fungus *Lecanicillium lecanii*" that uses a fungus isolated from commercial farms for tick control. This project has provided good technical and economic results, but the fungus is not yet commercially available (Alda Rodriguez, Laboratorio Bio Uruguay, personal communication, 2017).

In Uruguay, most of the cattle population is composed of Hereford (48%), Angus (31%), Holstein (4%) and other *Bos taurus* breeds (13%), which are highly susceptible to tick infestation. Zebus, crossbred breeds, including Bradford (3/8 Brahman and 5/8 Hereford) and Brangus (3/8 Brahman and 5/8 Angus), represent only 3 and 1% of the population, respectively (SNIG 2015).

There is no information about the possible importance of wild animals in the epidemiology of *R. microplus* in Uruguay. Currently, there are three species of deer in the country; two are autochthonous, *Ozotoceros bezoarticus* and *Mazama gouazoubira*, and one is introduced, *Axis axis*. The presence of *R. microplus* has been reported on *O. bezoarticus* (Venzal et al. 2003), but it is not known if this or other species are able to act as a primary host for *R. microplus*.

## Babesiosis and anaplasmosis

The first studies of babesiosis and anaplasmosis in the South American region were published by Lignières in 1899 in Argentina, who confirmed the role of the cattle tick in the transmission of *Babesia* spp. (Signiorini and Mattos 1987). In Uruguay, the first studies in 1920 determined that babesiosis was produced by *B. bigemina* and *B. bovis* and anaplasmosis by *Anaplasma marginale*. As early as 1941, the natural resistance of young cattle to babesiosis and anaplasmosis was studied, and immunization with a live refrigerated vaccine was recommended for 3–6 months-old calves (Rubino 1946).

In Uruguay, babesiosis and anaplasmosis affect mainly adult cattle that have not been exposed to these hemoparasites during the first 9 months of life. In general outbreaks of babesiosis, which are transmitted only by ticks, occur from March to May, when the tick load is higher. Anaplasmosis, which has a more prolonged prepatent period and may be transmitted by other bloodsucking insects and by iatrogenic transmission, is more temporally dispersed and may occur during the whole year (Solari et al. 2013). Outbreaks of babesiosis normally occur over a short period of time (nearly 10 days); while outbreaks of anaplasmosis occur over a period of 20–60 days (Solari et al. 2013). Of the 400 outbreaks reported by three diagnostic laboratories since 1980–2013, 49.3% were caused by *B. bovis*, 12.9% by *B. bigemina* and 37.8% by *A. marginale* (Solari et al. 2013). The mean morbidity for anaplasmosis or babesiosis in 300 of those outbreaks was 10.1 ± 14.4%, with a mortality of 4.5 ± 5.9% and a lethality of 51.9 ± 30.3% (Solari et al. 2013). From January 1993 to April 2016, 410 outbreaks were diagnosed in the DILAVE in Paysandú, of which 16 were mixed infections of babesiosis and anaplasmosis and 104 and 290 were simple infections by *A. marginale* and *Babesia* spp., respectively (Buroni 2014).

The high frequency of babesiosis and anaplasmosis is mainly because Uruguay is in an area marginal for the development of *R. microplus*, with a high number of farms in areas of enzootic instability, in which 20–79% of bovines have antibodies to *Babesia* spp. and *A. marginale* (Smith et al. 2000; Solari et al. 2013). In this country, serological diagnoses of babesiosis and anaplasmosis are made by indirect immunofluorescence (IFI) for *B. bovis* and *B. bigemina* and by the card agglutination test for *A. marginale*. It is important to improve the serologic diagnosis of babesiosis and anaplasmosis, mainly using ELISA, which is not available in the country. Although IFI has shown sensitivity and specificity of 98% and ELISA has a sensitivity of 97% and a specificity of 93%, using ELISA enables the analysis of a greater number of samples and provides results that do not depend on the subjectivity of the observer (Cardozo et al. 1992; Domínguez-Alpizar et al. 1995). Serologic studies performed from 1972 to 1992 in 169 farms located between latitudes 32° and 33°5′S found that 65 and 74% of cattle had antibodies against *Babesia* spp. and *A. marginale*, respectively (Berdie et al. 1979; Cardozo et al. 1992; Solari and Quintana 1994). In 2017, in 31 farms located between 30°54′S and 32°22′S, in the control region, 48% of the farms were in enzootic instability for *B. bovis*, 65% for *B. bigemina* and, 48% for *A. marginale*. Only two farms were in enzootic stability for the three parasites. Twenty of the 31 farms reported outbreaks of tick fever and only 4 of them used the vaccine (Miraballes, INIA Tacuarembó, unpublished data, 2017). In the department of Lavalleja (34° south latitude) from a population of 125 farms, the dispersion of *Babesia bovis* was estimated in 70.5% of the farms (Solari et al. 1992; Cardozo et al. 1994).

As mentioned before, few cattle in Uruguay are currently vaccinated against babesiosis and anaplasmosis, and vaccination is an alternative to prevent these diseases in farms with enzootic instability (Miraballes et al. 2018b). In Uruguay, there is a refrigerated vaccine that has been produced by DILAVE since 1941. In 1996, a private company started the production of a frozen vaccine. Both vaccines are currently available in the market, however, the reasons why farmers do not use them may be related to problems with the proper handling, including transport and preservation. Identifying and solving these problems should improve the use of vaccines.

## Conclusions

*Rhipicephalus microplus*, babesiosis and anaplasmosis have been a major problem in Uruguay for at least 100 years. This country has the legislation and technical knowledge to control *R. microplus*, babesiosis and anaplasmosis, but the current geographic distribution of the cattle tick is similar to what it was 100 years ago. It is necessary to improve control measures, including the systematic detection of cattle tick resistance to acaricides and the use of generational treatment. It is also important to create free areas within the tick-control area, to increase surveillance in cattle movement and to improve the surveillance and control of outbreaks within tick-free areas. To prevent babesiosis and anaplasmosis it is necessary to increase the use of vaccines in calves within the infested area.

## Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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## 6.2. ARTÍCULO 2

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# Eficacia de dos vacunas, congelada y refrigerada, contra la tristeza parasitaria bovina

Efficacy of frozen and refrigerated vaccines against bovine tick fever

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## Resumen

La babesiosis y la anaplasmosis, son endémicas en Uruguay y producen pérdidas económicas de aproximadamente 14 millones de dólares anuales. Para prevenir estas enfermedades existen vacunas disponibles comercialmente en forma refrigerada o congelada. A pesar de la disponibilidad de vacunas, la cobertura de vacunación es muy baja. Las causas del bajo número de vacunas utilizadas pueden ser varias: falta de información por parte de los productores y veterinarios; dificultades en el manejo y conservación de las vacunas; y costos por dosis y aplicación. Con el objetivo de comparar la eficacia de la vacuna refrigerada con la congelada contra *Babesia bovis*, *Babesia bigemina* y *Anaplasma marginale* fue realizado un ensayo en un establecimiento comercial ubicado en el departamento de Tacuarembó, donde 57 terneras raza Brangus fueron vacunadas con la vacuna refrigerada y 58 con la vacuna congelada. Para determinar la eficacia de ambas vacunas se realizaron las pruebas de inmunofluorescencia indirecta para *B. bovis* y *B. bigemina* y aglutinación en tarjeta para *Anaplasma* spp. al día 62 postvacunación. No existieron diferencias significativas entre la protección ofrecida por ambas vacunas (p> 0,05), encontrándose entre el 93% y 98,3% de los animales positivos para cada uno de los tres agentes. Este trabajo demuestra que ambas vacunas son eficientes para la profilaxis de la enfermedad y deben ser utilizadas para la protección de bovinos en áreas de inestabilidad enzoótica. Es necesario continuar con las tareas de extensión para aumentar la cobertura de vacunación a nivel nacional e investigar posibles fallas en la vacunación.

**Palabras claves:** Vacunas, Tristeza Parasitaria, Eficacia

## Summary

Babesiosis and anaplasmosis, are endemic in Uruguay and produce economic losses of approximately 14 million dollars per year. To prevent these diseases, refrigerated or frozen vaccines are commercially available. Despite the availability of vaccines, vaccination coverage is very low probably due to several causes: the lack of information of farmers and veterinarians; difficulties in the management and conservation of the vaccines; and costs per dose and application. In order to compare the efficacy of the refrigerated vaccine with a frozen vaccine against *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale* we conducted a trial in a commercial farm located in the department of Tacuarembó. Fifty-seven Brangus calves were vaccinated with the refrigerated vaccine and 58 with the frozen vaccine. The indirect immunofluorescence tests for *Babesia bovis* and *Babesia bigemina* and the card test for *Anaplasma* spp. were performed to determine the efficacy of both vaccines at day 62 post vaccination. There were no significant differences between the protection offered by both vaccines ($p > 0.05$), being between 93% and 98.3% of the animals positive for each of the three agents. These results show that both vaccines are efficient for the prophylaxis of the disease and should be used for the protection of bovines in areas of enzootic instability. It is necessary to continue with the extension tasks to increase the national coverage of vaccinated animals and to investigate possible vaccine failure.

**Key words:** Vaccination, Tick fever, Efficacy

## Introducción

Las enfermedades transmitidas por la garrapata *Rhipicephalus* (*Boophilus*) *microplus*, babesiosis, causadas por *Babesia bovis* y *Babesia bigemina,* y anaplasmosis causada por *Anaplasma marginale*, son endémicas en Uruguay (Solari, 2006). Aunque la babesiosis es ocasionada por protozoarios y la anaplasmosis por una rickettsia, causan signos clínicos semejantes, formando parte de un complejo denominado tristeza parasitaria bovina (Rubino, 1946). Tanto *B. bovis* y *B. bigemina* son transmitidas únicamente por *R. microplus*, mientras que *A. marginale* puede ser transmitida tanto por este parásito como por vía iatrogénica y por otros vectores (tábanos) (Alonso y col., 1992; Hornok y col., 2008). En Uruguay, generalmente, los brotes de babesiosis y/o anaplasmosis no ocurren cuando más del 80% de los bovinos presentan anticuerpos por haber sido infectados durante los primeros 10 meses de vida. Esta situación es definida como de estabilidad enzoótica, la cual se logra cuando existe una elevada tasa de transmisión del parásito entre el vector y el hospedero. Cuando la tasa de inoculación no es lo suficientemente elevada para asegurar una transmisión continua, se encuentran anticuerpos en el 20% a 79% de los bovinos pudiendo ocurrir brotes, situación que se define como de inestabilidad enzoótica. Cuando menos del 20% de los bovinos tienen anticuerpos se considera que el rodeo está en estabilidad enzoótica y no ocurren brotes (Solari y col., 2013). Los brotes afectan principalmente a los bovinos adultos, causando elevadas pérdidas económicas, estimadas en 14 millones de dólares anuales, ocasionadas por costos de tratamientos, pérdidas de peso y muertes (Ávila, 1998). Aunque la aparición de los brotes depende directamente de la edad en que el animal es infectado por primera vez, es importante considerar que el manejo, el estado nutricional del hospedero, la presencia de enfermedades intercurrentes y las condiciones del medio ambiente pueden contribuir a la ocurrencia de tristeza parasitaria (Guglielmone, 1995; Bock y De Vos, 2001).

La forma más adecuada de prevenir la tristeza parasitaria es erradicar la garrapata de los establecimientos y, en el caso de anaplasmosis, evitar también la transmisión iatrogénica (Suárez y Noh, 2011). Sin embargo, la erradicación del vector muchas veces es inviable y los tratamientos quimioterápicos para la tristeza parasitaria por si solos no evitan las pérdidas productivas causadas por esta enfermedad (Guglielmone, 1995). En estas condiciones, la única alternativa disponible para prevenir brotes de tristeza parasitaria, en establecimientos con inestabilidad enzoótica, es el uso de la vacuna con organismos vivos atenuados de *Babesia* spp. y con *Anaplasma centrale* (heterólogo de *A. marginale*), que proveen inmunidad durante toda la vida de los bovinos (Solari y Quintana, 1994; Bock y De Vos, 2001). Además de Uruguay, estas vacunas han sido utilizadas también en Sudáfrica, Brasil, Israel y Argentina (de Castro, 1997; Bock y De Vos, 2001).

La División de Laboratorios Veterinarios Miguel C. Rubino (DILAVE) ha producido esta vacuna desde 1941 y la continua produciendo (Rubino, 1946). En 1996, el Laboratorio BioSur inició la producción de otra vacuna refrigerada y una vacuna congelada (Etchebarne, comunicación personal, 2017). Actualmente están disponibles en el mercado la vacuna refrigerada de DILAVE y la vacuna congelada de BioSur, ambas producidas en terneros esplenectomizados. A pesar de la disponibilidad de vacunas, la cobertura de vacunación es muy baja. En la actualidad hay aproximadamente dos millones de terneros en el área de control de garrapata (DICOSE-SNIG, 2017). Sin embargo, hasta 2008 se vendían en promedio 10.000 dosis anuales de la vacuna refrigerada y entre 2008 y 2016, con la presencia de una vacuna congelada en el mercado, el número aumentó a 22.000 dosis anuales aproximadamente (Miraballes y Riet-Correa, 2018). Las causas del bajo número de vacunas utilizadas pueden ser varias: falta de información por parte de los productores y veterinarios; dificultades en el manejo y conservación; y costos por dosis y aplicación. Hay productores que relatan casos o brotes de tristeza en animales vacunados, por lo que es necesario determinar si las posibles fallas de vacunación son debidas a errores en la conservación y aplicación de vacuna, al estado inadecuado de los animales después de vacunados por estrés, problemas nutricionales o enfermedades concomitantes o a fallas de eficiencia de las vacunas. Por otro lado, no hay publicaciones sobre la eficiencia comparada de las dos vacunas disponibles en nuestro país contra tristeza parasitaria. El objetivo de este ensayo experimental fue comparar la eficacia de la vacuna refrigerada con la vacuna congelada contra *B. bovis*, *B. bigemina* y *A. marginale* en Uruguay.

## Materiales y métodos

El experimento fue realizado en un establecimiento comercial ubicado en la localidad de Cañas, en el departamento de Tacuarembó, Uruguay. Este establecimiento se caracteriza por convivir con la garrapata *R. microplus,* controlándola mediante el uso de tratamiento generacional (Cuore y col., 2008). Se realizó la aplicación de ivermectina al 3.15% en todo el rodeo el 01/08/2017, tratamiento correspondiente a la primera generación de garrapatas. Para la realización de este ensayo fueron utilizadas 115 terneras hembras de 10 meses de edad de raza Brangus. Al día 0 (16/8/2017) se separaron de forma aleatoria las 115 terneras en 2 grupos. En ese mismo día se tomaron muestras de sangre de 30 terneras de cada grupo para realizar serología de *B. bovis, B. bigemina* y *A. marginale*. Las muestras de sangre fueron extraídas de la vena coxígea con tubos vacutainer, sin anticoagulante, utilizando una aguja por animal.

El Grupo 1 (VR), con 57 terneras, fue vacunado con la vacuna refrigerada producida por DILAVE. El Grupo 2 (VC), con 58 terneras, fue vacunado con la vacuna congelada producida por el laboratorio BioSur. La aplicación de ambas vacunas se realizó siguiendo las recomendaciones de los fabricantes (Solari y Quintana, 1994; BioSur, 2017).

Al día 62 post vacunación (18/10/2017) se procedió nuevamente a la toma de muestras de sangre de las 115 terneras para medir la protección inducida por ambos tipos de vacunas mediante serología.

Los procedimientos de extracción de sangre de los animales participantes del ensayo fueron aprobados por el Comité de Bioética del Instituto Nacional de Investigación Agropecuaria (INIA).

Para el análisis serológico se realizaron las técnicas de aglutinación en tarjeta para la detección de anticuerpos contra *Anaplasma* spp. e inmunofluorescencia indirecta (IFI) para la detección de anticuerpos contra *B. bovis* y *B. bigemina* siguiendo el procedimiento descripto en el manual del IICA (1987). Se determinó cada suero problema como positivo para *A. marginale* cuando se observaban conglomerados macromoleculares visibles directamente, confrontados con un control positivo y un control negativo (Solari, 2006). Como criterio de positividad para la IFI se consideró cada suero problema como positivo cuando se observaba fluorescencia evidente sobre los glóbulos rojos parasitados, en el título de 1/25 para *B. bigemina* y 1/50 para *B. bovis,* confrontados con un control positivo y un control negativo para cada hemoparásito en el mismo título de positividad previamente definido (Ríos-Osorio y col., 2010).

Durante los 45 días posteriores a la vacunación, los animales fueron observados diariamente por el productor para constatar evidencias de signos clínicos: decaimiento, separación del rodeo, presencia de orina con sangre, agresividad o cualquier otra indicación de enfermedad. En el caso de la presencia de alguno de estos signos el productor debía tomar la temperatura rectal y tratar los animales con aceturato de diminazeno a la dosis de 1,2 mg/kg de peso vivo.

Para determinar si existían diferencias significativas ($p < 0,05$) entre ambos grupos se realizó una prueba exacta de Fisher al día 0 y otra al día 62, utilizando el programa estadístico STATA (2015).

## Resultados

En ambos grupos ningún bovino manifestó signos clínicos por lo que no fue necesario registrar la temperatura ni realizar el tratamiento de los mismos durante el período de 45 días de observación.

Cuadro 1. Número y porcentaje de bovinos con anticuerpos para *B. bovis*, *B. bigemina* y *Anaplasma* spp. al día 0 y 62 postvacunación

| **Día** | **Grupo** | **n** | ***B. bovis*** | **P** | ***B. bigemina*** | **p** | ***Anaplasma* spp.** | **p** |
|---|---|---|---|---|---|---|---|---|
| 0 | 1(VR)* | 30 | 3 (10%) | 0.500 | 8 (26,7%) | 0.139 | 9 (30%) | 0.105 |
|  | 2(VC)* | 30 | 2 (6,7%) |  | 13 (43,3%) |  | 4 (13,3%) |  |
| 62 | 1(VR) | 57 | 56 (98,2%) | 0.748 | 53 (93%) | 0.331 | 55 (96,5%) | 0.348 |
|  | 2(VC) | 58 | 57 (98,3%) |  | 56 (96,5%) |  | 54 (93,1%) |  |

*VR Vacuna Refrigerada; VC Vacuna Congelada

Como se puede observar en el Cuadro 1 ambas vacunas confirieron inmunidad igual o mayor al 93% para los tres hemoparásitos, no observándose diferencias significativas entre las mismas.

## Discusión

Este trabajo demuestra que ambas vacunas son eficientes para la profilaxis de la tristeza parasitaria y pueden ser utilizadas para la protección de bovinos en áreas de inestabilidad enzoótica. Uruguay está ubicado en un área marginal para el desarrollo de *R. microplus*, lo que lleva a que la mayoría de los establecimientos ubicados en la zona de control se encuentren en inestabilidad enzoótica debido a la tasa irregular de infestaciones, a menos que el establecimiento sea libre de garrapatas (Solari y col., 2013). En este caso, es necesario la adopción o mantención de estrictas medidas de bioseguridad para evitar la introducción del parásito. Otra alternativa viable para la prevención de la tristeza parasitaria podría ser controlar las poblaciones de garrapatas hasta mantener baja la tasa de inoculación, de forma tal que menos del 20% de los animales presenten anticuerpos (Nari, 1995). Si se decide por esta opción, hay que tener en cuenta que cualquier factor que pueda llevar a un aumento en la población de garrapatas (fallas de tratamientos por resistencia, factores climáticos, o que no se consiga tratar la totalidad del rodeo) puede llevar a brotes por hemoparásitos, con alta morbilidad y mortalidad. En establecimientos que por tener alta carga de *R. microplus* se encuentren en estabilidad enzoótica y el productor decida aplicar medidas de control para bajar esta carga, es recomendable comenzar con un plan de vacunación (Callow y col., 1997). Es conveniente determinar si el rodeo se encuentra en estabilidad o inestabilidad enzoótica previo a la vacunación mediante la realización de las técnicas serológicas (Solari, 2006). Según un estudio realizado por Solari y col. (1992) existen diferencias significativas en la ganancia de peso entre animales naturalmente infectados y libres de hemoparásitos, por lo que incluso en casos de establecimientos con estabilidad enzoótica la vacunación de los terneros puede ser económicamente rentable.

A pesar de que se ha comprobado que una sola dosis de esta vacuna induce inmunidad eficiente que dura toda la vida productiva de los animales (Bock y De Vos, 2001), pueden existir fallas en la vacunación debidas a problemas de inmunogenicidad de la vacuna, insuficiente respuesta inmunitaria de los animales o a variaciones en el genotipo de las cepas de campo en relación con las cepas vacinales (Bock y De Vos, 2001; Palmer y Mcelwain, 1995). En Uruguay no se han investigado las posibles fallas de vacunación, ni las variaciones genotípicas de *Anaplasma marginale* y *Babesia* spp. Ambos aspectos deberán ser estudiados en el caso de que se registren sospechas de tristeza parasitaria en animales vacunados.

Para el uso correcto de ambas vacunas es necesario que los veterinarios responsables de la vacunación cumplan con las recomendaciones de manejo y conservación, teniendo en cuenta que las vacunas refrigeradas deben ser aplicadas hasta 72 hs después del envío, manteniéndolas en todo momento refrigeradas (Solari y Quintana, 1994). En el caso de la vacuna congelada se debe conservar en nitrógeno líquido y descongelar en baño maría entre 37 °C y 38 °C teniendo en cuenta que una vez descongelada, la validez de uso es por 6 hs, debiendo mantenerse refrigerada durante la inoculación y no se pueden volver a congelar (BioSur, 2017). El mayor problema para la aplicación de la vacuna congelada por parte de los veterinarios es el manejo en nitrógeno líquido. Actualmente, los comercios veterinarios que venden esta vacuna ofrecen el servicio de vender la vacuna ya preparada para aplicar. También algunas veterinarias alquilan los termos con nitrógeno líquido para facilitar la adopción de esta medida de prevención por parte de los veterinarios (Taño, 2017). Es importante considerar que la vacuna debe ser empleada en invierno, cuando las poblaciones de garrapatas son bajas, o utilizando potreros seguros, para no ocurra una sobreinfección por cepas de campo antes del día 60 (Solari y Quintana, 1994).

Sin duda que la baja cobertura de vacunación es una de las principales causas de las altas pérdidas económicas causadas por la tristeza parasitaria en el Uruguay. Una tarea fundamental para controlar estas pérdidas es aumentar la tasa de vacunación, continuando con las tareas de extensión iniciadas recientemente por la Comisión interinstitucional de extensión para el control de la garrapata y tristeza parasitaria (IICA, 2017). A modo de ejemplo, en Australia, con una población similar de bovinos en el área enzoótica (7 millones), se comercializan 800.000 dosis anuales (Bock y De Vos, 2001). Como ambas vacunas son producidas en animales esplenectomizados sería conveniente comenzar a producir estas vacunas en cultivos celulares como forma de evitar posibles transmisiones de otras enfermedades y considerar el bienestar animal (Callow y col., 1997).

## Conclusiones

Se concluye que las vacunas para tristeza parasitaria disponibles en Uruguay son eficaces e inocuas en terneros y que es necesario ampliar la cobertura de vacunación en el área enzoótica para disminuir las pérdidas económicas causadas por esta enfermedad. En casos de que ocurran fallas en la vacunación, es necesario invesar las causas.

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## 6.3. ARTÍCULO 3

Tick and Tick-Borne Diseases

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

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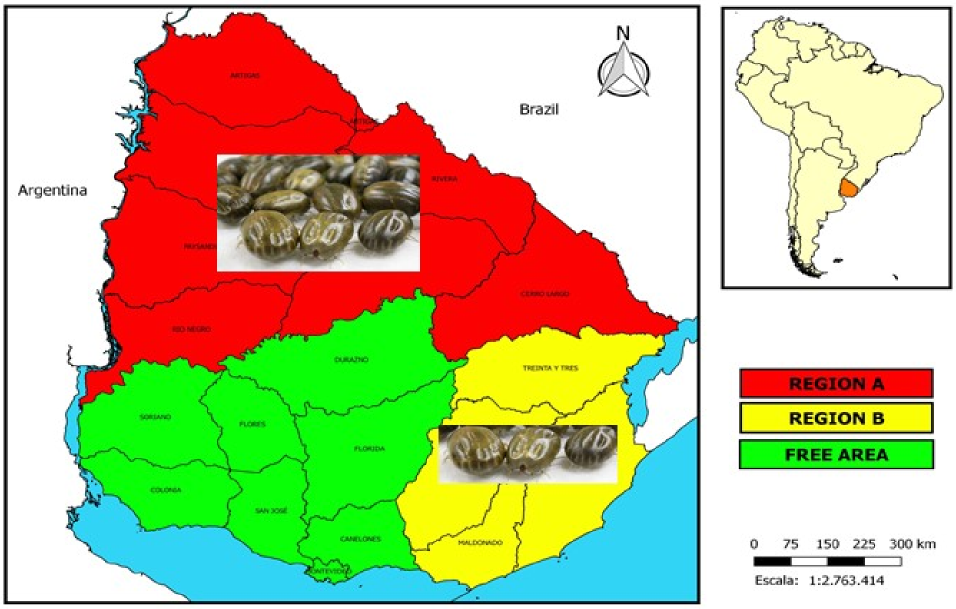
## Graphical abstract



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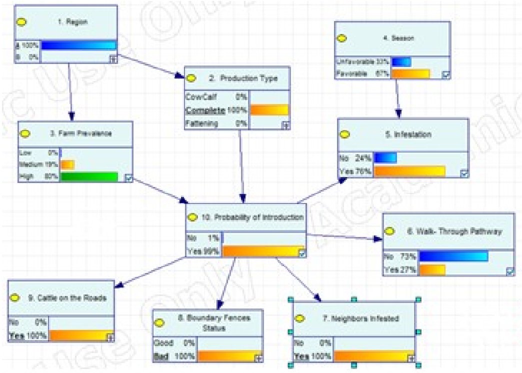

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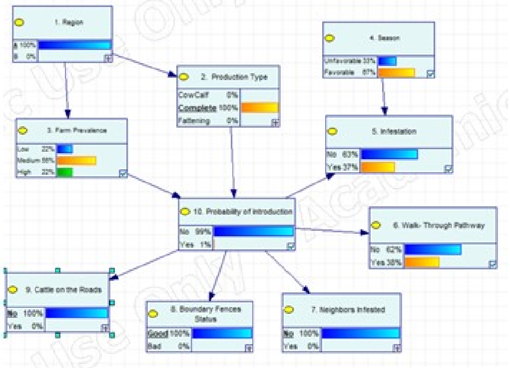

## Abstract

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.

**Key words:** Risk assessment; Cattle tick elimination; Bayesian Belief Network; *Rhipicephalus microplus*

## 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, 2009). 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 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, 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 tick-infested 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 (Erico 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), considered an endemic area with a variable presence of ticks over the years (Miraballes and Riet-Correa, 2018) (Fig. 1).

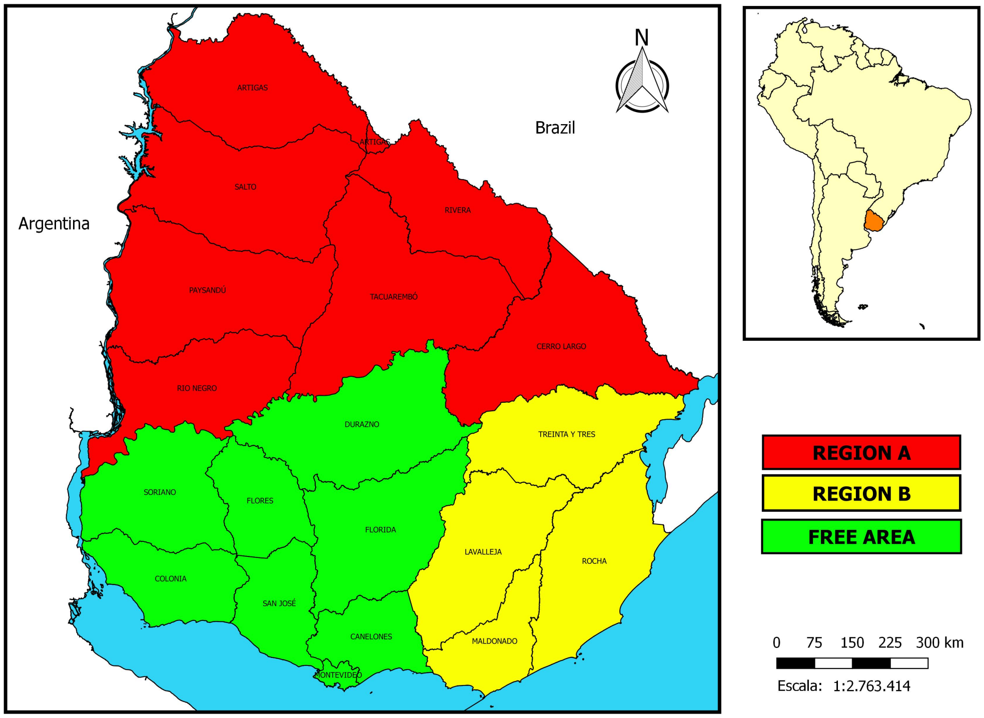

Fig. 1 Map of Uruguay showing the regions according to the prevalence of cattle ticks on farms. In Region A, the presence of cattle ticks has been continuous over the last 100 years. In Region B, the presence of cattle ticks has been variable over the years, and in the free area, outbreaks are sporadic,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

### 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).

### 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).

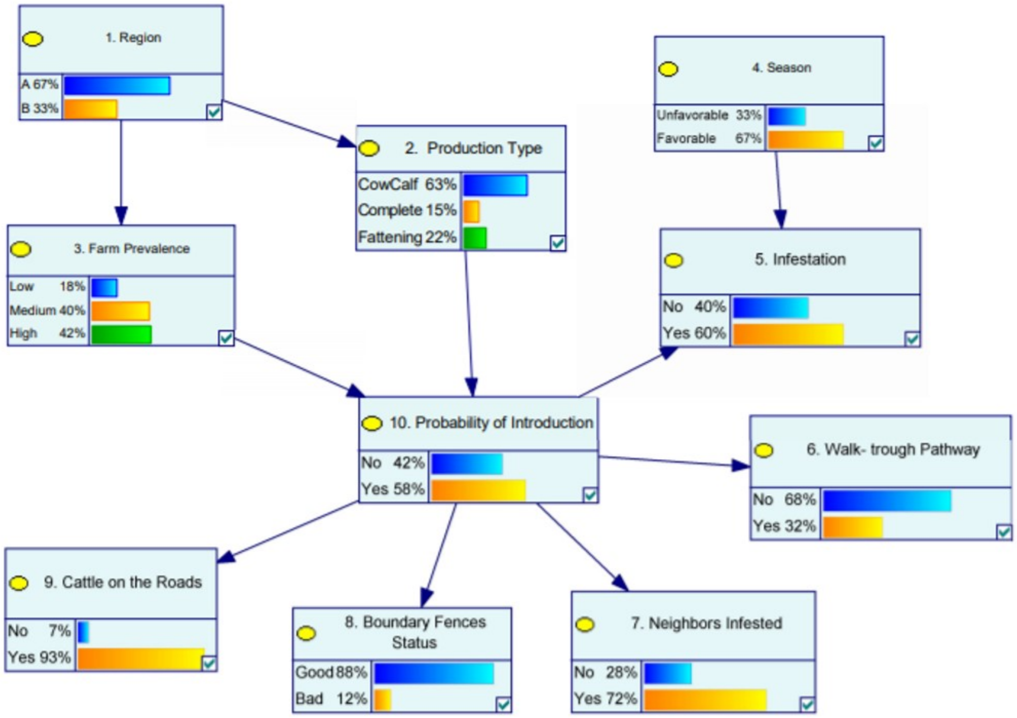

Fig 2. Bayesian Belief Network and steady state for the ten nodes.

**Table 1. Marginal and conditional probabilities for the ten nodes used in the BBN to estimate the probability of *R. microplus* introduction into farms**

| Node | Parent | Parent | State | Probability* | Data Source** |
|---|---|---|---|---|---|
| 1) Region | - | - | A | 0.67 | 2016 Survey |
| | | | B | 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 | |

Continue

**Table 1 (continuation). Marginal and conditional probabilities for the ten nodes used in the BBN to estimate the probability of *R. microplus* introduction into farms**

| | | | | | |
|---|---|---|---|---|---|
| 5) Infestation | Infestation No | Season Unfavorable | No | 0.90 | Literature |
| | | | Yes | 0.10 | |
| | | Season Favorable | No | 0.50 | Assumption |
| | | | Yes | 0.50 | |
| | Infestation Yes | Season Unfavorable | No | 0.50 | |
| | | | 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 | |
| 9) Cattle on the Roads | | No | No | 0.12 | 2018 Survey |
| | | | Yes | 0.88 | |
| | | Yes | No | 0.04 | |
| | | | Yes | 0.96 | |

Continue

**Table 1 (continuation). Marginal and conditional probabilities for the ten nodes used in the BBN to estimate the probability of *R. microplus* introduction into farms**

| | | | | |
|---|---|---|---|---|
| 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 |
| | | | 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.

### 2.1.3 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 & 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 walk-through 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, 1988).

**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% to 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.

**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 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).

Table 2. Sensitivity analysis for the Infestation node (node 5)

| Infestation | Evidence: No | | | | Evidence: Yes | | | |
|---|---|---|---|---|---|---|---|---|
| Probability of Introduction | 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** | | | |

### 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)/ Tobs. 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 Prevalence= High; Production Type= Complete; 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) for the Probability of Introduction node, which indicates that the model has good discrimination power (Dohoo et al., 2009).

**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**

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

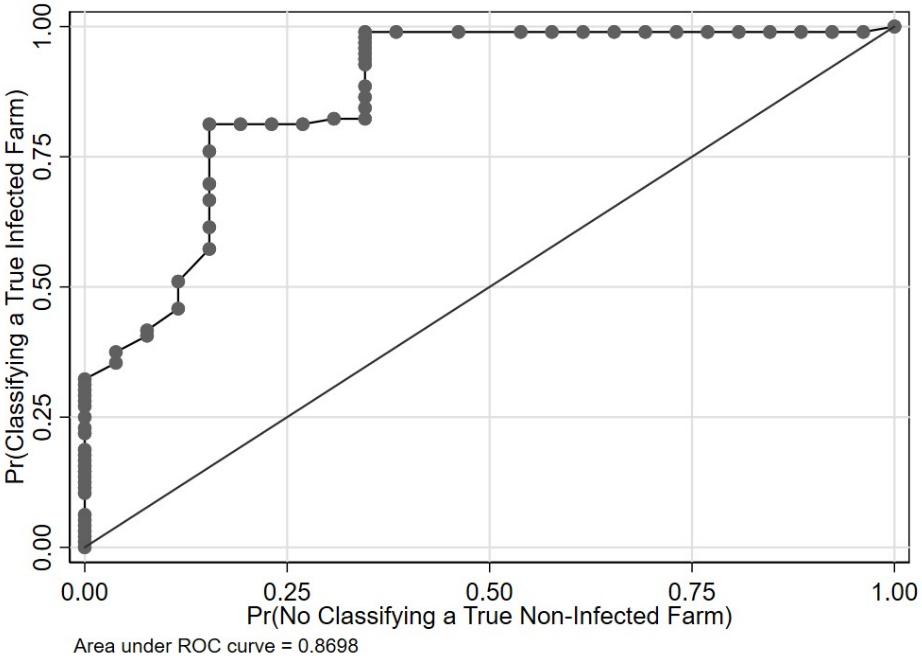

Fig. 3 Receiver Operating Characteristic (ROC) curve obtained from the BBN used to estimate the risk of introduction of cattle ticks into farms in Uruguay (y-axis and x-axis represent the sensitivity and 1-specificity of the BBN, respectively).

### 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%).

**Table 4. 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 state (%)** | **Scenarios** | | **Evidences*** | | |
|---|---|---|---|---|---|---|---|
| | | | 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 (%) (Relative 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) |

**Table 5. 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 state (%)** | **Scenarios** | | **Estimated probability (%) (Relative Risk)** | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 |
| Region | A | 67 | 58 (0.86) | 58 (0.86) | 58 (0.86) | 63 (0.94) | 71 (1.05) | 74 (1.27) |
| | B | 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* |

### 3.5 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.

**Table 6. 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 | Conditions | | | | | | | | Probability of Introduction |
|---|---|---|---|---|---|---|---|---|---|
| | Region | Production Type | Season | Infestation | Walk Through Pathway | Neighbors Infested | Boundary Fences Status | Cattle on the Road | |
| 1 | A | Fattening | Favorable | Yes | No | No | Good | No | 1% |
| 1 | A | Fattening | Favorable | No | No | Yes | Good | No | 21% |
| 1 | A | Cow-Calf | Favorable | No | No | Yes | Good | No | 26% |
| 1 | A | Complete | Favorable | No | Yes | Yes | Good | Yes | 34% |
| 1 | A | Cow-Calf | Unfavorable | Yes | Yes | Yes | Good | No | 66% |
| 1 | A | Fattening | Favorable | Yes | No | Yes | Good | No | 70% |
| 2 | A | Complete | Favorable | Yes | No | Yes | Good | No | 71% |
| 3 | A | Cow-Calf | Favorable | Yes | No | Yes | Good | No | 76% |
| 1 | B | Cow-Calf | Favorable | Yes | No | Yes | Good | Yes | 84% |
| 3 | A | Cow-Calf | Favorable | Yes | Yes | Yes | Good | Yes | 86% |
| 1 | A | Fattening | Favorable | Yes | No | Yes | Good | Yes | 88% |
| 4 | B | Cow-Calf | Favorable | Yes | No | Yes | Good | Yes | 91% |
| 1 | A | Complete | Unfavorable | Yes | Yes | Yes | Good | Yes | 93% |
| 1 | A | Cow-Calf | Unfavorable | Yes | Yes | Yes | Good | Yes | 95% |
| 1 | A | Cow-Calf | Favorable | Yes | Yes | Yes | Bad | No | 98% |
| 1 | A | Complete | Favorable | Yes | No | Yes | Bad | No | 98% |

Continue.

**Table 6 (continuation). 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**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | A | Cow-Calf | Unfavorable | No | Yes | Yes | Bad | Yes | 98% |
| 1 | A | Cow-Calf | Favorable | Yes | Yes | Yes | Bad | Yes | 99% |
| 1 | A | Complete | Favorable | Yes | No | Yes | Bad | Yes | 99% |
| 1 | B | Cow-Calf | Favorable | Yes | Yes | Yes | Bad | Yes | 99% |
| 1 | A | Cow-Calf | Favorable | Yes | Yes | Yes | Bad | Yes | 99% |
| 1 | A | Cow-Calf | Unfavorable | Yes | No | Yes | Bad | No | 100% |
| 1 | A | Cow-Calf | Favorable | Yes | No | Yes | Bad | Yes | 100% |
| 2 | A | Cow-Calf | Favorable | Yes | No | Yes | Bad | Yes | 100% |

## 3. 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° 18268, 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 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).

## 4. 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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# 7. CONSIDERACIONES FINALES

Estos resultados forman parte de una línea de investigación que tiene por objetivo conocer la situación epidemiológica de la garrapata y la tristeza parasitaria y su importancia económica en establecimientos comerciales. Con la información generada será posible aplicar medidas de control para disminuir la prevalencia y las pérdidas ocasionadas por estos agentes en Uruguay, minimizando los riesgos de la presencia de residuos de insecticidas en productos de origen animal.

Además de los datos publicados que forman parte de esta tesis, fueron establecidos planes de control o eliminación de *R. microplus* en 30 establecimientos comerciales (datos no publicados, 2018). La base de la elaboración de estos planes consistió en el diagnóstico de situación inicial de cada establecimiento y en la evaluación mensual de los avances de estos planes por parte de los productores. Este diagnóstico de situación inicial se realizó mediante una entrevista personal con los productores o asesores encargados de cada predio donde se recabaron datos generales del establecimiento, de su manejo productivo y el manejo de *R. microplus* y de la tristeza parasitaria. Otra herramienta utilizada para este diagnóstico de situación fue el análisis serológico de tristeza parasitaria mediante IFI y Card Test, para determinar la situación de estabilidad o inestabilidad enzoótica al inicio del proyecto y al año de ejecución para determinar la evolución de los planes. Además, en aquellos establecimientos que contaban con poblaciones suficientes de garrapatas teleologías (n>100) se realizó una prueba de resistencia a los acaricidas (Saporiti, datos no publicados 2018). Con esos datos se crearon planes específicos para cada establecimiento en base al tratamiento generacional para el control de *R. microplus,* a la vacunación contra hematozoarios y a la mejora de las medidas de bioseguridad. Hasta la fecha, de los 30 establecimientos, cinco lograron eliminar la garrapata, cuatro están en vías de su eliminación, uno la eliminó y tuvo reintroducción de garrapatas post sequía, situación que se pudo controlar rápidamente debido a su detección precoz. Ocho establecimientos están realizando control utilizando entre cinco y seis tratamientos por año, en 10 se está trabajando para disminuir el número de tratamientos y dos tuvieron que entregar los campos arrendados por brotes con numerosas muertes de animales por tristeza. Los establecimientos con riesgo de brotes de tristeza parasitaria que prosiguieron con los planes de control comenzaron a utilizar la hemovacuna como medida preventiva, ya sea en terneros o en terneros y vaquillonas. El diagnóstico diferencial de la tristeza parasitaria en los casos de sospecha de brotes permitió disminuir costos por tratamientos innecesarios y tomar las medidas apropiadas para el control de cada brote. Un establecimiento con diagnóstico presuntivo de tristeza parasitaria y tratamientos contra esta enfermedad tuvo diagnóstico definitivo de *Senecio braziliensis* y la solución fue cambiar a los animales de potrero y comenzar el control de la seneciosis con ovinos. En otro caso la causa del brote fue *Lantana cámara* y todos los animales que se encontraron afectados murieron, a pesar de los tratamientos repetidos contra tristeza. Cuando se llegó al diagnóstico se interrumpieron los tratamientos innecesarios. Esto fue posible gracias al Núcleo de Salud Animal (NUSAT) en Tacuarembó, quienes realizan los diagnósticos de muertes de especies productivas por necropsia e histopatología que ocurren en el norte del país y tienen contacto fluido con los productores.

El trabajo realizado con los productores permite definir que, si se pretende controlar la garrapata y convivir con ella, deberá plantearse la realización del menor número de tratamientos posibles por año y, en la mayor parte de los casos, utilizar la hemovacuna para prevenir muertes por tristeza. Además, se recomienda mantener un potrero libre de garrapatas para poder dejar a los animales tratados y sin desafíos de larvas previo a la venta. De esta manera, se evitarán los gastos generados por los repetidos despachos de tropa, si los animales no están libres de garrapata, y los gastos extras por fletes que ocurren cuando los animales son obligados a retornar al establecimiento de origen por presencia de garrapatas cuando son comercializados con destino a ferias u otros establecimientos. Para los establecimientos en los que se decida eliminar la garrapata, el uso de la hemovacuna dependerá del histórico de la tristeza en cada establecimiento. Además, se plantearán lograr la eliminación en un año haciendo tratamientos supresivos y seguir monitoreando la ausencia de la parasitosis por un año más, sin utilizar tratamientos. Se incluyen dentro de las recomendaciones para la eliminación, evaluar las medidas de bioseguridad que tenga cada establecimiento para la prevención de introducción de ganado con garrapatas. Estas incluyen el mantenimiento del correcto estado de los alambrados y la utilización de un potrero de cuarentena para mantener, por lo menos, 15 días, animales comprados que provengan de la zona de control.

Estos datos recabados sumado a cuatro encuestas que han sido llevadas a cabo entre 2016 y 2018, hace posible conocer la situación actual y, en consecuencia, detectar puntos críticos donde se deberá realizar investigación, diagnóstico y extensión. A modo de ejemplo, durante la ultima encuesta llevada a cabo por el INIA Tacuarembó se pudo evaluar que, más del 43% de los establecimientos que contestaron que tenían presencia de garrapatas en su campo, realizan más de 6 tratamientos al año. De los establecimientos que dicen no tener garrapatas, el 31% realiza más de 3 tratamientos por año. Evidentemente que queda mucho camino por recorrer en cuanto a la difusión de los planes de control o eliminación en establecimientos.

El conocimiento de la historia del control de la garrapata y la tristeza parasitaria en Uruguay permite evaluar los factores que influyeron en la dispersión de *R. microplus* y de la tristeza parasitaria hasta la fecha, y, con ese conocimiento, generar medidas de control apropiadas, estableciéndose que la mayor carencia se debe a la falta de difusión del conocimiento a los propietarios y asesores de establecimientos para realizar planes más efectivos. Sin dudas que el articulo de la evaluación de las vacunas permite aumentar la difusión de esta tecnología, con el consiguiente aumento de la cobertura vacinal en el país. Este aumento deberá ser evaluado periódicamente para determinar posibles retrocesos. En el ensayo publicado en el marco de esta tesis fueron vacunadas todas las terneras presentes en el establecimiento, ya que el productor se dedicaba a la cría y recría de hembras y el uso de la hemovacuna era una práctica corriente en el establecimiento. Debido a esto, no fue posible incluir un grupo control, ya que el predio tenía alto riesgo de tener brotes de tristeza parasitaria en caso de dejar animales sin vacunar.

En el marco de este proyecto se desarrolló un análisis de riesgo para determinar la probabilidad de reintroducción de *R. microplus* a los establecimientos. Si bien este modelo es efectivo y permite colaborar en la toma de decisiones para controlar o eliminar la garrapata en cada establecimiento, es necesario complementarlo con el desarrollo de otro modelo que sirva para determinar cuál es la probabilidad de lograr la eliminación de *R. microplus*, sumándole un análisis de costo-beneficio por controlar o eliminar. Para realizar este segundo modelo, se requieren datos de un alto número de establecimientos que hayan podido eliminar la garrapata, y de otros que hayan intentado la eliminación sin éxito. Estos modelos podrán ser utilizados por veterinarios y productores por medio de una página web para colaborar con el diagnóstico de situación del predio. El uso de estos modelos, sumado al diagnóstico de resistencia de las garrapatas a los garrapaticidas y al diagnóstico de situación de la tristeza parasitaria, mediante estudios serológicos, permitirá tomar decisiones de eliminación o control de la garrapata en cada caso, y evaluar las distintas medidas de manejo de la tristeza parasitaria.

Por otra parte, es importante considerar la posibilidad de generar zonas libres dentro de la zona control, ya que luego de un estudio realizado por el Dr. Marcelo Cortes del departamento de Sanidad Animal del MGAP en Tacuarembó, se evidenció que dos seccionales policiales estaban libres de garrapatas y que, en otras tres, entre el 88% y el 92.5% de los establecimientos no tenían garrapatas. Sin dudas, comenzar a crear áreas libres protegidas llevará a una disminución de las pérdidas por garrapata y tristeza parasitaria en todo el país.

**Perspectivas a futuro**

El trabajo en conjunto con los productores nos ha dado la visión de la realidad, para continuar con nuevos proyectos de investigación. Podemos definir que es necesario evaluar las pérdidas económicas debidas a la disminución de la ganancia de peso en animales en crecimiento por la tristeza parasitaria. Sin dudas que determinar si los animales que contraen esta enfermedad con cepas de campo, en su etapa de resistencia fisiológica, pierden peso, será otra herramienta para difundir esta tecnología. Además, es necesario buscar soluciones para el control de la garrapata en lechería de la zona norte y este del país, donde el uso de acaricidas es limitado por la posible presencia de residuos en leche. También es necesario generar herramientas que puedan disminuir la trasmisión de *A. marginale* por vectores mecánicos. Con este fin se propone la continuidad de esta línea de investigación mediante proyectos que contemplen posibles métodos de control físico o biológico de *R. micropulus* en establecimientos lecheros y distintas herramientas para la disminución de la prevalencia y brotes de enfermedades trasmitidas mecánicamente. A su vez, las revisiones bibliográficas realizadas han resaltado la necesidad de generar información que no se encuentra actualmente disponible en Uruguay, como por ejemplo determinar el potencial de los tábanos presentes en nuestro país para trasmitir *A. marginale* y la prevalencia de *R. microplus* en animales a lo largo del año. Con el objetivo de generar estos conocimientos, están en marcha dos proyectos, uno de ellos, pretende determinar los géneros y especies de tábanos presentes en Uruguay, la presencia de *A. marginale* en esos insectos y el potencial de trasmitirlo. En el otro proyecto, se está determinando la prevalencia de *R. microplus* en establecimientos comerciales bajo distintos planes de control. Estos resultados generarán datos que podrán mejorar el análisis de riesgo desarrollado en esta tesis y a su vez, se podrán comenzar líneas de investigación sobre la eficacia del tratamiento selectivo de animales para el control de *R. microplus*, ya que cualquier medida con posibilidades de disminuir el uso de químicos innecesarios en el ganado debe ser evaluada.