

## Determination of the seroprevalence of *Leptospira* spp. and the main serovars circulating in cattle in the province of Manabí, Ecuador

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## Summary

Leptospirosis is a transmissible zoonotic disease caused by pathogenic strains of the genus *Leptospira*. Clinical signs in cattle are fever, haematuria, haemoglobinuria, meningitis, abortion, birth of weak calves and infertility and, in the most severe cases, it can cause death to the animal. The few studies conducted in Ecuador, and in particular the province of Manabí, have revealed varying prevalence rates, ranging from 35.8% to 75%. The objective of this study is to determine the seroprevalence of leptospirosis in cattle and to ascertain the main serovars circulating in the province of Manabí. A cross-sectional epidemiological study was conducted from November 2015 to March 2016, for which seven cantons were selected at random and a total of 854 animals from 67 herds were investigated. The samples were processed in the laboratories of the Animal Diagnostics Directorate of the Ecuadorian Agency for Agriculture Quality Assurance (AGROCALIDAD) in Tumbaco, using the microscopic agglutination test (MAT). The sera were analysed to check whether they contained any of the eight serovars of *Leptospira interrogans* circulating most frequently in the country: Canicola, Hardjo, Pomona, Icterohaemorrhagiae, Grippotyphosa, Wolffi, Bratislava and Copenhageni. Overall seroprevalence at herd level was 97.01%, with the most common serovars being Pomona, Icterohaemorrhagiae, Grippotyphosa, Bratislava and Canicola. It was concluded that there is high seroprevalence at herd level in the province of Manabí.

## Keywords

Cattle – Ecuador – *Leptospira* spp. – Seroprevalence – Serovar – Zoonosis.

## Introduction

Leptospirosis is one of the most widely distributed diseases in the world (1). It is a zoonosis that causes public health problems and affects both developing and developed countries (2). It is caused by pathogenic strains of the genus *Leptospira* (3), which are transmitted directly or indirectly from animals to humans (4).

The epidemiology of leptospirosis has varied as a result of changes in animal husbandry, climate and human behaviour (5). It affects the majority of animals and causes economic losses in the livestock sector (6). In cattle, it causes reproductive and production problems characterised by abortion, perinatal mortality, birth of weak offspring, infertility and a decrease in milk yield (7, 8).

Studies conducted in Ecuador's coastal region show an increase in the seroprevalence of human leptospirosis, rising from 56 confirmed cases in 2005 to 645 in 2012, which highlights the importance of studying the disease and its associated factors (9).

Between 2010 and 2012, the health authorities of Portoviejo (Manabí Province) reported more than 2,000 serologically confirmed human cases of febrile leptospirosis (10).

Studies conducted in rural areas of Ecuador suggest that cattle and pigs may be the main reservoirs for human transmission of the disease, owing to the high prevalence of pathogenic species of the genus *Leptospira* in these animals (11).

In Ecuador, the few studies carried out on cattle have reported varying rates of *Leptospira* spp. seroprevalence, ranging from 35.8% (11) to 75% (12).

Control of leptospirosis requires active epidemiological surveillance and changes to public policies regarding the disease (13). This study therefore sets out to determine the seroprevalence of leptospirosis in cattle and to ascertain the main serovars circulating in this animal species in the province of Manabí.

## **Materials and methods**

### **Study area**

The study area is in the province of Manabí and spans 18,893.7 km<sup>2</sup> (or 7.36% of Ecuador's total area); its altitude ranges from 0 metres to 500 metres above sea level (14). This province has the largest cattle population in the country: 977,140 head (15).

The study was conducted in the cantons of Bolívar, Chone, El Carmen, Flavio Alfaro, Jama, Junín and Tosagua.

### **Study design**

A cross-sectional epidemiological study was carried out in the province of Manabí from November 2015 to March 2016, for which seven cantons were selected and a total of 67 herds were investigated. The study included dairy, beef and dual-purpose herds containing at least five animals.

### **Sample size**

The number of samples to be taken was determined by the formula proposed by Lwanga and Lemeshow (16):

$$n = Z^2 * p * (1 - p) / d^2.$$

Where:

$Z = 1.96$  (confidence level of 95%)

$p = 0.60$  (anticipated proportion of the population affected)

$1 - p = 0.40$

$d = 0.04$  (absolute precision on either side of the proportion).

The calculations showed that the minimum number ( $n$ ) of samples required was 576.

### **Blood sample collection**

Cattle blood samples were taken from the jugular vein or caudal artery at a volume of 5 ml of blood per sample; no anticoagulant was used. All the animal owners signed an informed consent form.

A commercial system of vacuum tubes and disposable needles was used to collect the blood samples, which were then kept at a temperature of approximately 20°C. After clot formation, the serum was extracted by centrifugation of the blood at 3,000 revolutions per minute (rpm) for

10 minutes. The serum was divided into 0.5 ml aliquots and frozen at –20°C until the time of processing (17).

### **Diagnostic methodology**

The samples were analysed in the laboratories of the Animal Diagnostics Directorate of the Ecuadorian Agency for Agriculture Quality Assurance (AGROCALIDAD) in Tumbaco, using the microscopic agglutination test (MAT), which is considered to be the gold-standard test for the serological diagnosis of this disease (18); titres of  $\geq 1:100$  were considered positive. As recommended by the OIE, the antigens selected for use in the MAT included representative strains of the serogroups known to exist in the region, all the sera were analysed to check whether they contained any of the eight serovars of *Leptospira interrogans* circulating most frequently in the Ecuador (i.e. Canicola, Hardjo, Pomona, Icterohaemorrhagiae, Grippotyphosa, Wolffi, Bratislava and Copenhageni) (18). The strains used in the diagnosis had been certified by the National Veterinary Services Laboratories of the United States Department of Agriculture.

### **Data processing and statistical analysis**

The study data were recorded in a Microsoft Excel spreadsheet and used to calculate individual and herd seroprevalence in the different cantons studied. A comparison of proportions was made to determine whether there were differences depending on cantons or circulating serovars.

For the statistical analysis, XLSTAT software (version 7.5), with a confidence level of 95%, was used and ArcGIS 9.3.1. software was used to map prevalence in the study regions on a canton by canton basis (19).

### **Results**

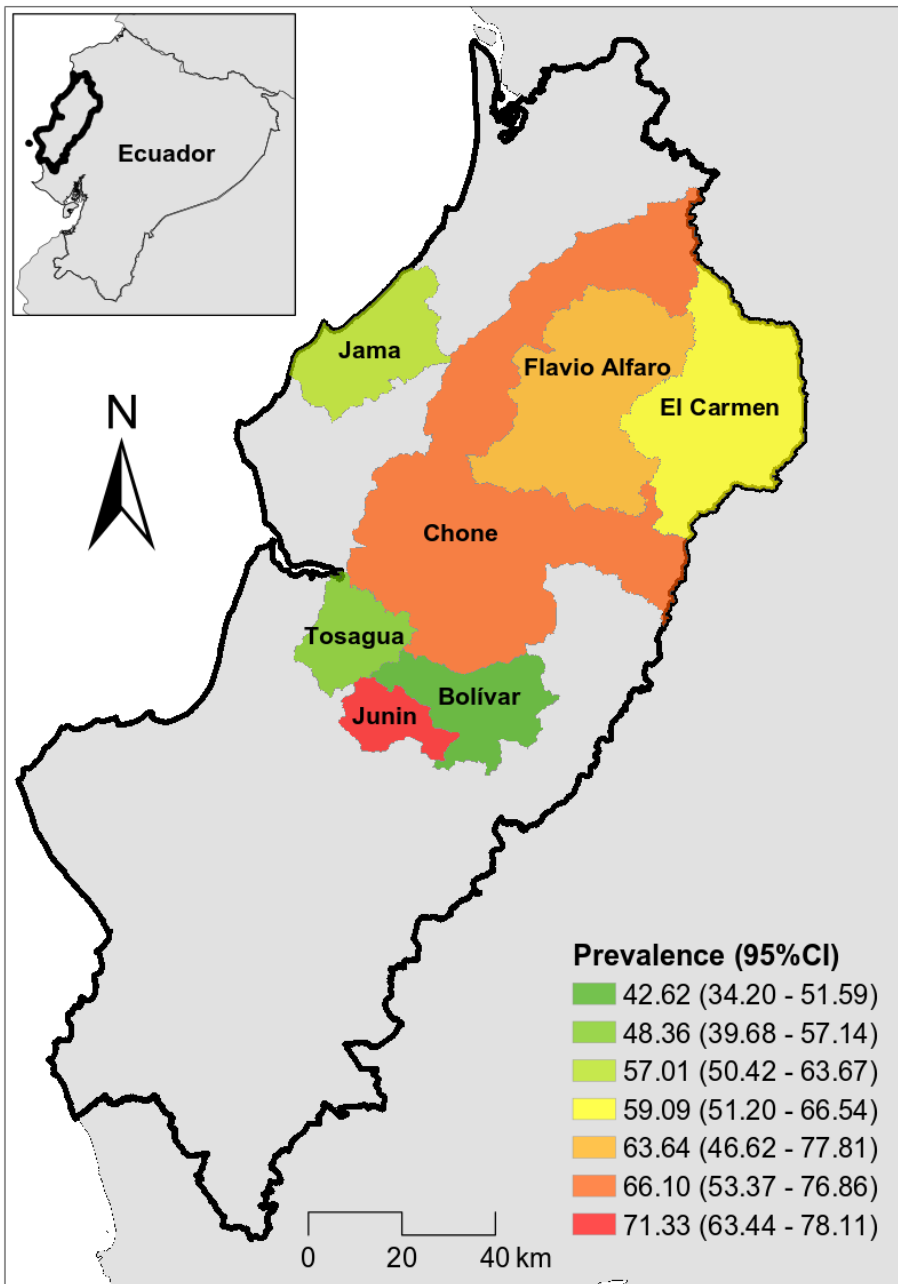
Overall seroprevalence in animals was 57.38% (Table I) and the highest rates ( $p \leq 0.05$ ) were found in the cantons of Junín, Chone, Flavio Alfaro and El Carmen (Fig. 1).

**Table I**  
**Individual-level seroprevalence of *Leptospira* spp., by canton**

Canton	No. of animals investigated	No. of positive animals	Seroprevalence (%)	CI 95%
Junín	143	102	71.33 <sup>a</sup>	0.63–0.78
Chone	59	39	66.10 <sup>ab</sup>	0.53–0.57
Flavio Alfaro	33	21	63.64 <sup>abc</sup>	0.47–0.78
El Carmen	154	91	59.09 <sup>abcd</sup>	0.51–0.67
Jama	221	126	57.01 <sup>bcd</sup>	0.50–0.63
Tosagua	122	59	48.36 <sup>cd</sup>	0.40–0.57
Bolívar	122	52	42.62 <sup>d</sup>	0.34–0.51
<b>Total</b>	<b>854</b>	<b>490</b>	<b>57.38</b>	<b>0.54–0.61</b>

In each column, the values followed by different superscript letters are significantly different from each other ( $p \leq 0.05$ )

CI: confidence interval



CI: confidence interval

**Fig. 1**

**Prevalence of *Leptospira* spp. by canton, province of Manabí**

A total of 67 herds were sampled and 97% of these herds were seropositive (Table II).

**Table II**  
**Herd-level seroprevalence *Leptospira* spp., by canton**

Canton	No of herds investigated	No. of positive herds	Seroprevalence (%)	CI 95%
Bolívar	13	13	71.33	0.63–0.78
El Carmen	12	12	66.10	0.53–0.57
Jama	12	12	63.64	0.47–0.78
Junín	11	11	59.09	0.51–0.67
Flavio Alfaro	4	4	57.01	0.50–0.63
Tosagua	9	8	48.36	0.40–0.57
Chone	6	5	42.62	0.34–0.51
<b>Total</b>	<b>67</b>	<b>65</b>	<b>57.38</b>	<b>0.54–0.61</b>

CI: confidence interval

Seropositivity was detected for all eight serovars studied and the serovar most frequently identified was Pomona ( $p \leq 0.05$ ) (Table III).

**Table III**  
***Leptospira interrogans* serovars identified**

Serovar	No of animals investigated	No. of seropositive animals	%	CI 95%
Pomona	854	239	27.99 <sup>a</sup>	0.25–0.31
Icterohaemorrhagiae	854	184	21.55 <sup>b</sup>	0.19–0.24
Grippityphosa	854	158	18.50 <sup>bc</sup>	0.16–0.21
Bratislava	854	147	17.21 <sup>c</sup>	0.15–0.20
Canicola	854	141	16.51 <sup>c</sup>	0.14–0.19
Wolffi	854	79	9.25 <sup>d</sup>	0.07–0.11
Copenhageni	854	13	1.52 <sup>e</sup>	0.01–0.03
Hardjo	854	12	1.40 <sup>e</sup>	0.00–0.02

In each column, the values followed by different superscript letters are significantly different from each other ( $p \leq 0.05$ )

CI: confidence interval

A total of 56.73% of the positive animals had antibody titres for more than one serovar and as many as seven serovars were detected in some cases. Antibody titres in positive animals ranged from 1:100 to 1:6,400 and, in cases with titres over 1:100, the most frequent serovar was Pomona.

## Discussion

This study found 97.01% of herds to be affected, a rate similar to the more than 90% prevalence reported in affected herds in Brazil and Ireland (20, 21).

Of all the animals sampled, 57.38% were seropositive, close to the 54.2% seroprevalence reported in Colombia (22). Other authors found higher seroprevalence in Ecuador (23), of approximately 75%, and in Colombia, where it was found to be 74.4% (24). However, the 49.8% seroprevalence detected in slaughterhouses in Ecuador (25) was lower than that found in the current study.

Cattle are considered the natural reservoir of serovars Hardjo, Pomona and Grippotyphosa (26). This study detected seropositivity for all the serovars studied, with Pomona being the most common (27.99%), followed by Icterohaemorrhagiae (21.55%), Grippotyphosa (18.50%), Bratislava (17.21%), Canicola (16.51%) and Hardjo (1.40%).

These results agree with those of cattle studies by other authors (22, 27, 28), according to which Pomona is the commonest serovar in cattle. In some countries, such as New Zealand (29), Colombia (30) and Mexico (31), it is also the most common cause of *Leptospira* infection in humans.

The seroprevalence of serovar Hardjo was low, in contrast with the rate cited in other cattle studies, which report it to be one of the commonest serovars (32, 33, 34).

A total of 56.73% of positive animals had titres for more than one serovar, with Pomona–Icterohaemorrhagiae being the most common combination. Similar results were obtained in studies in Thailand and Uganda (35, 36), where more than 40% of the animals that tested

positive had antibody titres for more than one serovar. Future studies should investigate further to determine whether this is due to infection with several serovars or to cross-reactions among them, as reported by other authors (35, 36).

Between 70% and 100% of positive animals had antibody titres of 1:100 to 1:200, which may point to chronic or new infections. Future studies should therefore use paired sera to ascertain the dynamics of antibody production (20).

The results of this study indicate that leptospirosis may pose a risk to humans in the region studied, as other researchers point out (23), especially considering that cattle are natural reservoirs of the causative agents of this disease (37).

These results are similar to those reported in previous studies of Ecuador's coastal region, where it was found that exposure to domestic animals, mainly cattle and pigs, may pose the biggest risk for human infection (11).

The high seroprevalence of *Leptospira* spp. in apparently healthy cattle may pose a risk to farmers, livestock workers and animal owners (27), as this agent may be shed in urine for a long period of time and infect humans. The results should therefore be a cause for concern for the province's health authorities and serve as motivation for the development of effective strategies for prevention and control of the disease, such as the establishment of vaccination programmes for animals and people at risk or training for all people involved in the cattle supply chain.

## Conclusions

Individual seroprevalence was 57.38% and herd seroprevalence was 97.01%.

Positive cases were detected for all the serovars studied and the highest seroprevalence rates were for serovar Pomona, followed by Icterohaemorrhagiae, Bratislava and Grippotyphosa.

The highest seroprevalence rates were found in the cantons of Junín, Chone, Flavio Alfaro and El Carmen.

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