EQUINE LEPTOSPIROSIS AND RODENT INFECTION: Their importance as components of this zoonosis reservoir, regarding the epidemiology and prevention of human and animal infection.

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Introduction

Bacteria of *Leptospira* genus infect annually about 15/100000 people in Uruguay, mainly young male rural workers, and also a significant proportion of national production animals, domestic mammals as dogs, and wild species, from which they are transmitted to humans.

Bovine and human infections have been extensively studied, but there is still much to know about other components of the animal reservoir of this zoonosis.

Objectives

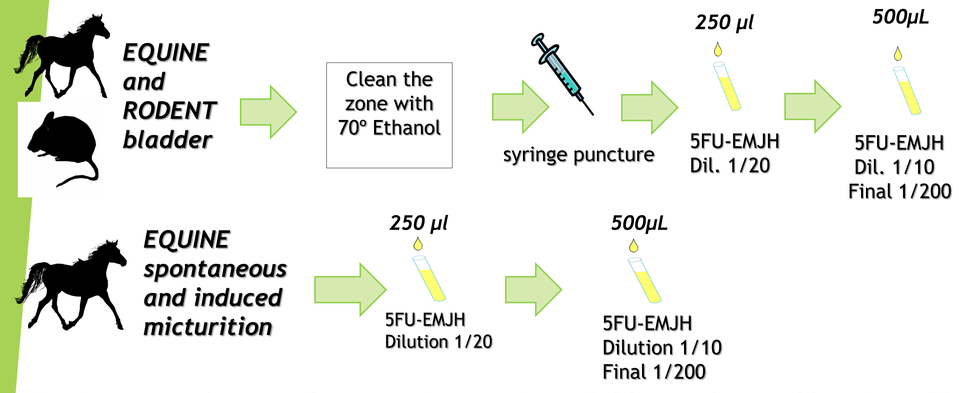
The general objective of this work was to produce useful knowledge for contributing to the control of the health and economic damages of leptospirosis
The specific objectives were:

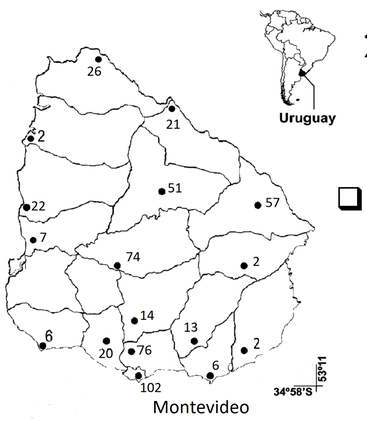
- ❑ To study the prevalence and characteristics of *Leptospira* infections in equine and rodent populations, as part of the microbial reservoir;
- ❑ To sustain the diagnosis and to extend the epidemiologic knowledge about Leptospirosis in human groups exposed to components of the animal reservoir, in urban and rural areas;
- ❑ To identify animal and environmental *Leptospira* isolates, for guiding the development of protective immunogens from circulating strains.

Methods

- ❑ During 2018-19, urban and rural Montevideo rats (n=64) were captured with Sherman and Tomahawk traps. Antibody seroprevalence was studied through microagglutination technique MAT; when obtained after euthanasia, the urine of rodents was cultured in EMJH liquid medium with 200 ug/ml 5-Fluorouracil.
- ❑ Sera and urine of equines (n=520) from all the country were studied through 2017-19. A few bovine samples were also included.
- ❑ Samples were taken from stud farms (70), rural work establishments (109), race or raid groups (127), army veterinary services (137) and meat processing plants (77).

1. Cultures





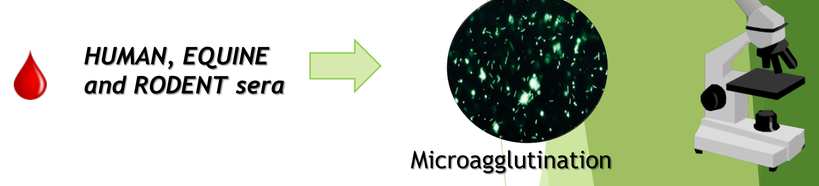
Distribution of equines in the sample

- ❑ Environmental water from equine settings: 0.22 membrane filtration; 500 µl inoculation in Fletcher and EMJH media

- ❑ Seroprevalence of anti-*Leptospira* antibodies was also evaluated with MAT in human workers (n=138) linked to studied horses.

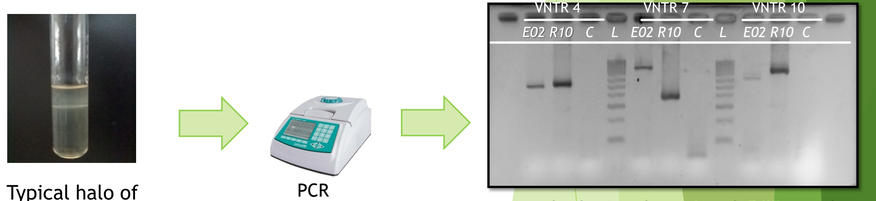
- ❑ Animal sera were considered reactive when titers were ≥ 100. Human sera with titers 50 for two or more serogroups, or ≥100 for at least one serogroup were recorded as reactive, revealing past or recent infection.

2. Serology (MAT)



- ❑ Animal urine and environmental water cultures were examined with dark field microscope; identification of isolates was performed with 16S and LipL32PCR techniques. Positive LipL32 PCR cultures were studied through MLVA (Multi-Locus Variable Number Tandem Repeat Analysis) and partial 16S sequencing.

3. PCR technique



Typical halo of *Leptospira* culture in Fletcher media

PCR technique

Agarose gel electrophoresis of PCR products obtained with MVLA primers (4-7-10). *E02: equine urine, R10: rat urine, C: negative control, L: ladder*

Results.

- ❑ EQUINE
  - Seroprevalence 46.2%
  - No difference between sexes

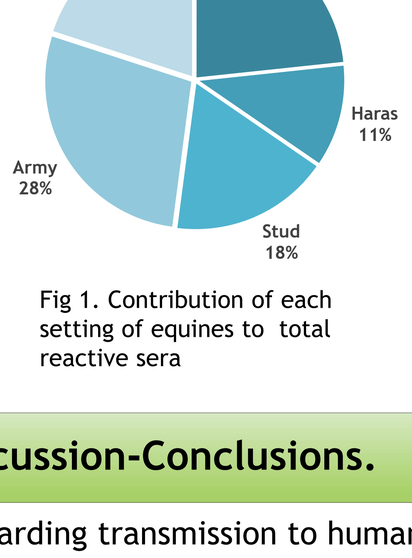


Fig 1. Contribution of each setting of equines to total reactive sera

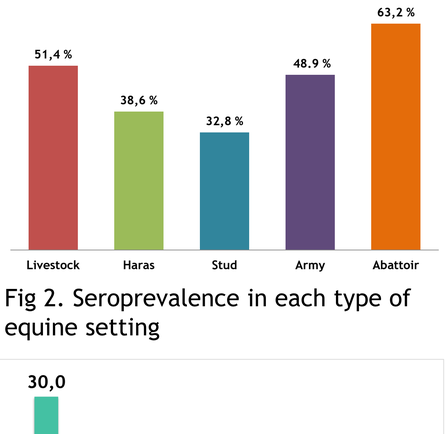


Fig 2. Seroprevalence in each type of equine setting

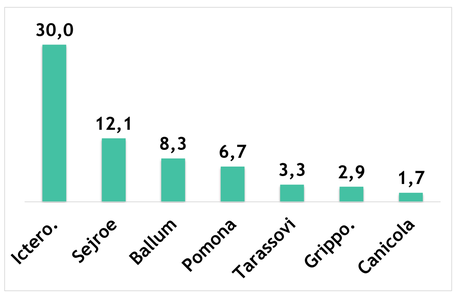


Fig 3. Percent reactivity in equine sera of *Leptospira* serogroups by MAT

- ❑ *Rattus rattus* and *Rattus norvegicus*
  - Seroprevalence 11,1% (7pos/64)
  - 5 additional sera reacted 1/50
  - 6 of 7 reactive sera were from male rats
  - No difference between species

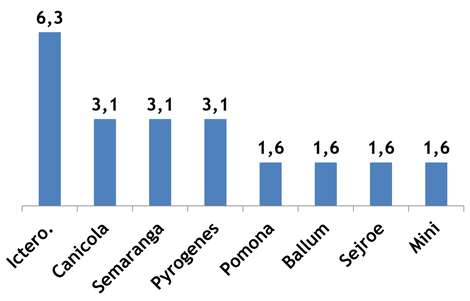


Fig 4. Percent reactivity in rodent sera of *Leptospira* serogroups by MAT

- ❑ Human reactive sera: only 2 in 138 workers linked to equines

- ❑ URINE AND ENVIRONMENTAL ISOLATES
  Presumptive identification

Qty	Origin	Source	Sequencing	Serogroup
2	environment	water	*L. biflexa*	
			L. meyeri	
2	bovine	urine	*L. borgpetersenii*	Pending ID
2	rats	urine	*L. interrogans*	Icterohaemorrhagiae
1	equine	urine	*L. interrogans*	louisiana

Discussion-Conclusions.

Seroprevalence in equines was high and similar to that previously determined in bovines. Equine infection does not look relevant regarding transmission to humans. Frequency of rat infection was low, but considering their population, they cannot be though discarded as important components of the leptospirosis reservoir. Icterohaemorrhagic serogroup linked to equine and rodent infection has not been locally isolated from humans. More *Leptospira* isolates are needed, for comparative identification that may improve the epidemiologic knowledge and guide prevention of leptospirosis.

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