

Behavior of Leptospirosis According to Equine and Human Positive Reactors during a Decennium Previous COVID-19

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Summary

The proposal had the goal of evaluating the relationship between human and equine reactors to *Leptospira* spp. in the province of Camagüey, Cuba. A data of 5.768 equine sera from the province collected during a decade were investigated. Their reactivity was established by microscopic agglutination (MAT). From the same period and territory, data corresponding to 141 human patients positive to indirect hemoagglutination were used. The Shapiro-Wilk test was used to establish the distribution of the investigated variables. The relationship between equine and human reactors was determined by Spearman's nonparametric correlation. Of the total number of equine sera, 2.617 (45.37%) were positive. The ratio of equine reactors/samples worked had a stable behavior from the third year onwards, when for every two samples analyzed, an average value close to one reactor was obtained. The ratio of equine reactors to human reactors was high (approximately 14:1), with a correlation $Rho=0.85$ ($P=0.002$). The great number of equines, their use for transporting passengers in urban areas and the inadequate keeping conditions to which they are frequently subjected, make them potential candidates in the transmission of leptospirosis in Camagüey. They require close collaboration between human and animal health surveillance systems.

Keywords: Equines; Leptospirosis; *Leptospira*; Reservoirs; Zoonosis

Introduction

In the midst of a pandemic such as COVID-19, it could be utopian to warn of other threats to human existence, especially when some of them have a millenary history (Ehrenberg *et al.*, 2020). How to assume that, in the shadows of this virosis that marks a turning point in the destiny of humanity, multiple zoonoses are spreading silently, gaining ground day by day. Leptospirosis is an eloquent example in this regard (Barreto *et al.*, 2020a). It persists in the 21st

century as a reemerging disease. Its evolution affects industrialized and developing countries indistinctly (Ghazaei, 2018).

Between 1940 and 1950, leptospirosis in domestic animal species was the priority disease in veterinary and public health diagnostics. Then, inexplicably, its attention declined. Of course, significantly in developing countries. Perhaps due to: 1) the reduced number of reported cases in humans and animals; 2) the existence of effective

options for its prevention and therapy; 3) the confidence of having a correct epidemiological control of this zoonosis (Wasiński and Dutkiewicz, 2013).

Were these regions really the ones who determined the decision? Given the subsequent repercussions there is no point in looking for culprits. Suffice it to emphasize that overconfidence has taken a heavy toll. So far this millennium it has become the most widespread zoonosis on the planet. Annually, it affects 1.03 million people, 58,900 of whom die (Barreto *et al.*, 2020a). Values biased by inaccuracies in reporting in the poorest regions of the planet, the most affected; also those lacking the required epidemiological surveillance systems (Barreto *et al.*, 2019, 2020a).

Numerous domestic animal species act as reservoirs and play a prominent role in the chain of transmission of pathogenic serovars to humans. Unfortunately, some of them are not given due attention. In this regard, sheep, goats and horses are generally underestimated as intermediate hosts and sources of transmission of leptospirosis (Barreto *et al.*, 2020a).

In Camagüey, there are 84.314 equines, 70.714 of which correspond to the private sector. They are mostly used as traction animals for transporting people in urban areas and its immediacies (Rodríguez *et al.*, 2017). This research had the goal of evaluating the relationship between human and equine reactors to *Leptospira* spp. in the province of Camagüey, Cuba.

Materials and Methods

Equine data

The results corresponding to 5.768 equine sera investigated for leptospirosis by means of micro agglutination (MAT) during a decade were taken from the Record Book of the Laboratory of Animal Health of Camagüey. The positive reactors to *Leptospira* spp. were established against a battery integrated by serovars Icterohaemorrhagiae, Canicola, Ballum, Australis, Pomona, Tarassovi, Hebdomadis and Sejroe as antigens (Puentes *et al.*, 2009).

Human data

At the same stage, the data recorded in the Record Book of the Provincial Center of Hygiene, Epidemiology and Microbiology (PCHEM) of Camagüey, concerning 141 people seropositive to *Leptospira* spp. in indirect hemagglutination assays (Pedro Kourí Institute of Tropical Medicine -IPK, 2008) were collected.

Statistical processing

Descriptive statistics tests and the Shapiro-Wilk test were developed to establish the type of distribution of the variables under study: equine samples worked, equine reactors, human reactors, equine reactors/worked samples and equine reactors/human reactors. The relationship between equine and human reactors in the decade was established using Spearman's non-parametric correlation. The IBM* SPSS* Statistics Vertion 24 (2016) package was used in all cases.

Results and Discussion

Of the total number of equine sera tested in the decade 2.617 (45.37%,) were positive. The ratio of equine reactors/samples worked had a stable behavior from the third year onwards, at which point, for every two samples analyzed, an average value equivalent to one positive reactor was obtained. The equine reactor/human reactor ratio, although variable, was high (approximately 14:1), and its correlation, $\rho=0.85$ ($P=0.002$) (Table 1).

It is reported that most of the infections caused by *Leptospira* spp. in horses are unapparent, reason why it is perhaps underestimated as a source of transmission of zoonosis. Although, there are reports that testify the high seroconversion against the serovars Icterohaemorrhagiae, Ballum and Pomona (Felt *et al.*, 2011), Icterohaemorrhagiae and Australis (Roqueplo *et al.*, 2013), as well as to Ballum, Canicola, Icterohaemorrhagiae and Australis, the latter results obtained in previous research carried out in the province of Camagüey (Barreto *et al.*, 2017; Rodríguez *et al.*, 2017). Studies in which, it was demonstrated that equines had a similar participation to swine, canines and bovines as reservoirs of pathogenic serovars to the human species.

For its part, the high proportion in the equine reactor/human reactor ratio supports the previous result regarding the potential risk posed by these animals to humans. A very real risk when the following arguments are taken into account: a) Most of the affected animals behave as asymptomatic carriers (Rodríguez *et al.*, 2017). b) The disease presents a symptomatology very similar to other diseases, even to some of viral etiology such as dengue, which is endemic in Latin America (Mattar *et al.*, 2017). c) A high percentage of horses are used for urban passenger transport (Fig. 1), as well as in surrounding areas (Rodríguez, 2019). d) Veterinary-epidemiological control of these animals is sometimes insufficient (Rodríguez *et al.*, 2017).

Years	Equine serum samples	Equine reactors (HR)	Human reactors (HR)	a	b
1	112	18	10	0.16	1.80
2	117	26	10	0.22	2.60
3	75	35	4	0.47	8.75
4	111	54	8	0.49	6.75
5	15	6	1	0.40	6.00
6	1024	496	33	0.48	15.03
7	1948	804	27	0.41	29.78
8	1583	803	21	0.51	38.24
9	605	300	13	0.50	23.08
10	178	75	14	0.42	5.36
$\bar{x} \pm SE$	-	-	14.10 \pm 3.19	-	13.71 \pm 3.97
Median	147.50	64.50	-	0.45	-
Sig. (Shapiro-Wilk test)	0.008	0.005	0.529	0.011	0.069

Table 1: Ratio of equine reactors/total of samples (a) and equine reactors/human reactors (b) for the assessment period.



Figure 1: Horses used as traction animals for passenger transportation in urban areas of Camaguey.

To the above threats, it is worth adding that these spirochetes remain viable in the urine of horses for 14 weeks. Such prolonged viability is due to the weakly basic character (pH = 7.2-7.4) of this fluid in the equine species; precisely the optimal pH range of *Leptospira*. Although they are rapidly eliminated from most tissues in infected animals, could survive and multiply in the brain, kidneys and eyes, from where are expelled through urination, contaminating the streets and parking lots during the day (Rodríguez, 2019).

After the exhausting workday, many of these animals, in their rest, are exposed to environments where sanitary conditions and infrastructures are inadequate. Sometimes close to garbage dumps,

sewage dumps, sewage systems, etc. Usually in contact with animals of other species, including wild animals (Rodríguez, 2019).

The Hatibonico River crosses the city of Camaguey from north to south. Sometimes domestic waste are erroneously discharged into it. It is a place where horse owners are frequently seen bathing horses or leaving them to graze freely and quench their thirst (Fig. 2). It is an environment where the risk of infection, via contact with contaminated soil or water, is high. They depend on the adaptability of *Leptospira* spp. to survive in both environments. They also depend on the presence of susceptible hosts to infect (Barragán et al., 2017).



Figure 2: a) Waste accumulates on the bank of the Hatibonico River in Camaguey. b) Risk of domestic sewage. c) Horse downstream in search of water after hours of sun exposure.

In recent years, there has been an increase in research aimed at evaluating the primary role of contaminated soil and water in the transmission of leptospirosis (Picardeau, 2017, Guernier *et al.*, 2018; Vincent *et al.* 2019). Rainfall, floods and other extreme weather events usually become the antechamber of a good part of the outbreaks reported worldwide (Mwachui *et al.*, 2015). In this regard, the ability of *Leptospira* to form biofilms and cell aggregates stands out. This strategy allows spirochetes to circumvent the dispersive effect of urine in large bodies of water, thus maintaining sufficient concentrations (quorum) that enable infection (Yamaguchi *et al.*, 2018; Sato *et al.*, 2019).

In an investigation aimed at evaluating the participation of swine species in the swine reactor/human reactor ratio, the ratio ranged between three and four. A highly significant ratio ($P < 0.0001$). Result aggravated by the high number of pigs in the so-called backyard farms. These are artisanal forms of production in which hygienic and sanitary management conditions are generally deficient (Barreto *et al.*, 2020b).

The results previously discussed show the potential of the equine species as a source of leptospirosis transmission to humans. This risk increases in cities such as Camagüey, due to the high number of equines and their use as traction animals for passenger transportation in urban areas. This situation will worsen, like other bacterial zoonosis, in the face of an emergency such as COVID-19, which demands the priority attention of all epidemiological surveillance and control systems in the country.

Conclusions

The equine species, due to: being asymptomatic carriers of *Leptospira* spp. serovars, their high number, being sometimes subjected to inadequate holding conditions and their systematic transit through urban areas for passenger transportation, constitutes a high risk in the transmission of leptospirosis to humans in Camagüey. This situation demands a close collaboration between public health surveillance systems and their counterparts in the veterinary field.

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