Epidemiological profile of rat leptospirosis in Butembo city

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1 ABSTRACT

Leptospirosis is a cosmopolitan bacterial anthropozoonosis reported worldwide. It has a high incidence in tropical regions, where transmission conditions are favorable for the development of leptospires. Animals constitute the reservoir for the bacteria, particularly the rat(Azfar *et al.,* n.d.). poor knowledge of the disease can promote its maintenance in an environment. In this study, a cross-sectional survey in the city of Butembo, in the Nord-Kivu province/DRC, between January and May 2020 was conducted, with the aim of determining the prevalence of leptospirosis in rats, as well as the degree of knowledge of the disease among public health staff. A total of 150 urine samples from four municipalities in the city were collected from the rats. These samples were analyzed by conventional polymerase chain reaction (PCR) to detect the presence of DNA of leptospires. Simultaneously, the degree of knowledge of leptospirosis among 150 people working in the city's health services was assessed. A total of 55 doctors, 42 veterinary doctors, 23 pharmacists, 20 nurses, 5 laboratory technicians as well as 5 health workers were involved in this study. Their age ranged from 20 to 63 years with a median of 45 years. It appears, from this study, that a significant part of the staff (65.3%) had heard about leptospirosis especially from the internet (39.1%) and as part of their training (25.5%) and, to a lesser extent through books (17.6%) and seminars (9.8%). Among those who had heard of leptospirosis, more than half demonstrated a good knowledge of the symptoms, its zoonotic nature and its transmission source. This molecular analysis of 150 urine samples did not reveal the presence of leptospires in rats in Butembo. However, the absence of leptospirose in rats in Butembo does not exclude the presence of disease environment, hence the need to extend this study to other animal species as well a to human because in 1958 there was report

2 INTRODUCTION

The concept of "One Health" has sparked global action towards policy, practices aimed at strengthening the mutual links between human and animal health and their interaction with the environment. The increasing interpenetration of living environments between humans and other animals, domestic and wild, leads to health problems, most of which are zoonoses (Capek *et al.,* 2006). The Zoonoses continue to grow with climate change, human behavioral change, demographic growth, progress in research and the evolution of medical knowledge(Karpagam & Ganesh, 2020). Leptospirosis is one of these zoonoses and precisely an anthropozoonosis with worldwide distribution, frequently found in rural and urban areas in tropical climates. This disease affects many species of mammals, including humans. The disease manifests itself by high fever, muscle pain, chills, redness of the mucous membrane of the eye, abdominal pain, jaundice, skin and mucous membrane hemorrhages, as well as meningitis (Schneider *et al.,* 2018). For other animals, leptospirosis causes acute kidney failure, abortions and reduced production. It is of comparable or even greater importance than other neglected tropical diseases, such as dengue or leishmaniasis (Mahendra, 2015). Leptospirosis is caused by leptospires which are Gram-negative, strictly anaerobic bacteria, grouped in the species *Leptospira interrogans* (Mahendra, 2015) and is difficult to diagnose. Rats constitute, in the majority of countries, the main reservoir of leptospires; where they play a preponderant disseminating role. The bacteria sit in the renal tubules of the host. Once excreted into the external environment through urine, leptospires have a great capacity to survive in the environment, lasting up to several months in fresh water; which promotes contamination (Benacer *et al.,* 2013) for humans and domestic animals which constitute accidental hosts. Leptospirosis represents a risk for humans in permanent contact with water prone to contamination (sewers, swamps, wastewater). International Leptospirosis Society estimates that the incidence of human leptospirosis is 350,000 to 500,000 cases per year (Vries *et al.,* 2014); but this may be an underestimate due to lack of reporting. The World Health Organization (WHO) has put the global number of annual cases at more than 800,000, including 48,000 deaths in 2013. In its 2004 report, the WHO described an epidemic of leptospirosis which occurred in two schools in the western part of Kenya, involving more than 141 suspected cases and 8 deaths while a study conducted in Rwanda in 2011 by (Poster *et al.,* 2011)demonstrated a very high prevalence of the disease (40.1%) in asymptomatic adults in this country compared to neighboring countries like Kenya (7%) and Somalia (37%). In another study, exposure to *Leptospira interrogans* was particularly high among farmers, breeders and slaughterhouse workers and the presence of rats was significantly associated with the disease (Etienne *et al.,* 2021). In the Democratic Republic of the Congo (DRC), data on leptospirosis are very rare. In 1958, a researcher detected cases of leptospirosis among white workers in the eastern part of the country (Weiner M, 1996). Similarly, Van Riel demonstrated leptospirosis in white workers and dogs in the Belgian Congo in 1946 (Riel, 1955). These data, although very limited, clearly show that leptospirosis is present in the country, but further investigations are necessary in order to better understand its spread as well as the factors which militate in favor of its maintenance. It is in this context that this study has been initiated in Butembo city with the aim of determining the epidemiological profile of this disease in the potential reservoirs that are rats and evaluate the level of knowledge of the disease among personnel of both human and animal health sectors.

3 MATERIALS AND METHODS

3.1 Study area: The study was carried out in the city of Butembo (Figure 1) in the province of North Kivu, in the East of the DRC. This town was select because in 1958 leptospirosis was reported. The urban district is located between 0°05" and 0°10" north latitude and 29°17" and 29°18" east longitude. With an area of approximately 190 km^2, the city is located close to the territories of Lubero and Beni. Populated by approximately a million inhabitants, Butembo is subdivided into four communes: Bulengera, Mususa, Kimemi and Vulamba. It is located approximately 20 km north of the equator line, 54 km south of Beni and 280 km north of Goma with an average altitude of 1750 m. The average annual precipitation is estimated at 1365 mm while the average annual temperature is around 18°C with low thermal amplitudes (Sahani, 2011).

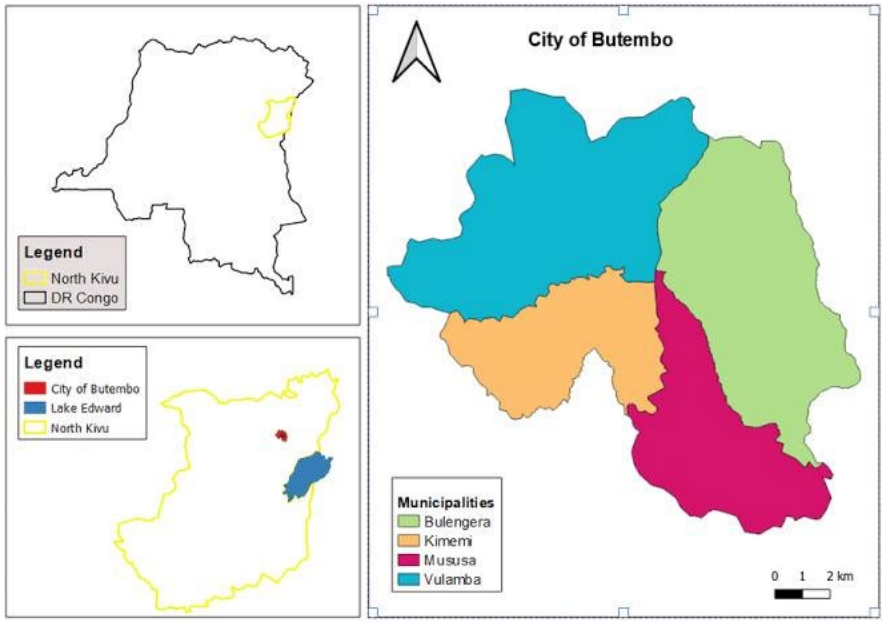

Figure 1. Administrative map of the City of Butembo

3.2 Study design: A cross-sectional descriptive study was used. Our study was composed of two parts: the first related to a survey which allowed the collection of data on the knowledge of people working in the health sector about leptospirosis while the second focused on the prevalence of the leptospirosis in rats in the city of Butembo. A randon sampling approach was used for the data collection.to calculate the sample size we found it based on prevalence found in other country in Tanzania(20%).

3.3 Rat capture and sample collection: Rats were captured using traps in slaughterhouses, markets, garbage bins and within selected households. Each animal was assigned a zone based on the municipality of capture. The traps were baited with dried fish, roasted peanuts and dried meat, placed on the ground, in corners of plots, near garbage bins and in fairly dark areas of markets and slaughterhouses. To optimize the chances of capture, the traps were placed around 6 p.m. and checked the next morning between 5 a.m. and 6 a.m. Traps containing rodents were transported to a treatment site and placed in another location the same day after rebaiting. Trapping was successful for a week, after which no further rat captures were observed. The captured rodents were euthanized by an injection of 0.1 ml of xylazine after which each animal underwent a necropsy to collect both kidneys and a urine

sample (1 ml) collected intravesically with a mounted insulin syringe and placed in a sterile dry tube. Kidneys and urine samples were collected in a sterile manner and then stored in freezer at 0°C to -10°C while awaiting transportation to the central veterinary laboratory in Kinshasa for molecular analyses. Rat species identification was carried out based on morphometric data.

3.4 Data collection on knowledge of leptospirosis: The assessment of the population's level of knowledge regarding leptospirosis was carried out among 150 people working in the health field. These people came from hospitals, clinics and laboratories. Variables used in this study are shown in Table 1.

Table 1: The variables used in this study

Variables	Désignation	Modalities
Independent	Age	Years
	Commune	Bulengera Mususa Kimemi Vulamba
	Sex	Male and female
	Occupation	Doctor Laboratory assistant Veterinarian Male nurse Health worker
Dependent	Have heard of leptospirosis	Yes and no
	Signs of illness	Jaundice Fever Jaundice and fever
	Knowledge channel	University Seminar Internet
	Type of disease	Zoonotic Animal Human
	Presence of leptospirosis in rats	Yes and no

3.5 Molecular analyses : A standard PCR was carried out at the Laboratory of the Pluridisciplinary Unit of Molecular Biology of the National Institute of Biomedical Research (INRB) in Kinshasa. DNA was extracted from urine samples with Zymo Quick DNA Kit following the manufacturer's instructions. The targeted Leptospira specific gene sequences were simultaneously amplified with three protocols used in parallel (Table 2) according to (Aderonke & Sansi, 2012) , (Erh & Mosallam T.E, 2014) and (Salehi, 2014)

Table 2 : Primers sequences

Primers	Sequences	References
LP1	ATA CAA CTT AGGAAG AGC	Aderonke& Sansi 2012
LP2	GCT TCT TTG ATATAG ATC AA	
LIG1	TCAATC AAAAACAAGG GGCT	Erth,2014
LIG2	ACTTGC ATTGGA AAT TGAGAG	
RAV2 F	CAAGTCAAGCGGAGTAGCA A	Salehi, 2014
RAV2 R	CTTAACCTGCTGCCTCCCGTA	

The final volume of the master mix was 20 µl made up of 12.5 µl One Taq 2x MM mixed with 6.5 µl of water free nuclease with 0.5 µl of each primer. The PCR parameters for amplification, notably the number of cycles and the annealing temperature, were different for each protocol. Thus, 40, 29 and 35 cycles were used for LP, LIG and RAV2 primers respectively with an annealing temperature of 50°C, 63°C and 57°C for 45 seconds for each pair of primers, respectively. For all the three primer sets, initial denaturation was 94°C for 5 minutes, cycle denaturation was 94°C for 45 seconds, cycle extension was 72°C for 45 seconds while final extension was initiated at 72°C for 5 minutes. The amplification was carried out in a Biometra T3000 thermal cycler. Thus, three PCR tests were performed on each sample according to the three different protocols mentioned above.

3.6 Data processing and analysis: Data obtained in this study were entered into an Excel database and statistical tests were carried out using SPSS.20 software. The statistical analysis essentially consisted of establishing the correlation between the variables and the modalities recorded in Table 3. Thus, we used a binary model of the LOGIT type in which the degree (measured by the probability that an individual having knowledge) of leptospirosis is explained by one's age (AGE), sex (SEX) and profession broken down into several binary variables according to the number of modalities present in this variable: doctor, pharmacist, nurse, veterinarian, agent public health or laboratory technician.

4 RESULTS

4.1 Knowledge of leptospirosis according to sex: The results of the level of knowledge of leptospirosis according to the sex of the respondents are recorded in table 3. In view of these results, it appears that a large proportion of the men surveyed had a good knowledge of leptospirosis, compared to women with a significant difference ($p<0.006$).

Table 3: Degree in knowledge of leptospirosis according to sex

Degree of knowledge	Male (%)	Female (%)	Total (%)
Have Heard of leptospirosis	93 (69.4)	5 (31.2)	98 (65.3)
Have never heard of leptospirosis	41 (30.6)	11 (68.8)	52 (34.7)
Total (%)	134 (100)	16 (100)	150 (100)

4.2 Knowledge of leptospirosis according to the age of the respondent: The results recorded in Table 4 present the level of knowledge of leptospirosis according to age groups. Given these results, it appears that the proportion of respondents with good knowledge of leptospirosis increases in increasing order of age groups.

Table 4. Degree of knowledge of leptospirosis according to age group

Range age (in years)	**Good knowledge (%)**	**No knowledge (%)**	**Total (%)**
[<35]	13 (17.7)	47 (78.3)	60 (100)
[35-44]	19 (38.8)	30 (61,2)	49 (100)
[45-54]	12 (50)	12 (50)	24 (100)
[>55]	8 (66.7)	4 (33,3)	12 (100)
Total	**52 (35.9)**	**93 (64,1)**	**100)**

4.3 Knowledge of leptospirosis according to professional occupation: The results of the degree of knowledge of leptospirosis according to the type of professional occupation of the respondents working in the health field in Butembo City are shown in Figure 2. These results indicate a small proportion of pharmacists, nurses and veterinarians surveyed having a good knowledge of leptospirosis while more than half of doctors, public health workers and laboratory technicians reported having heard of leptospirosis.

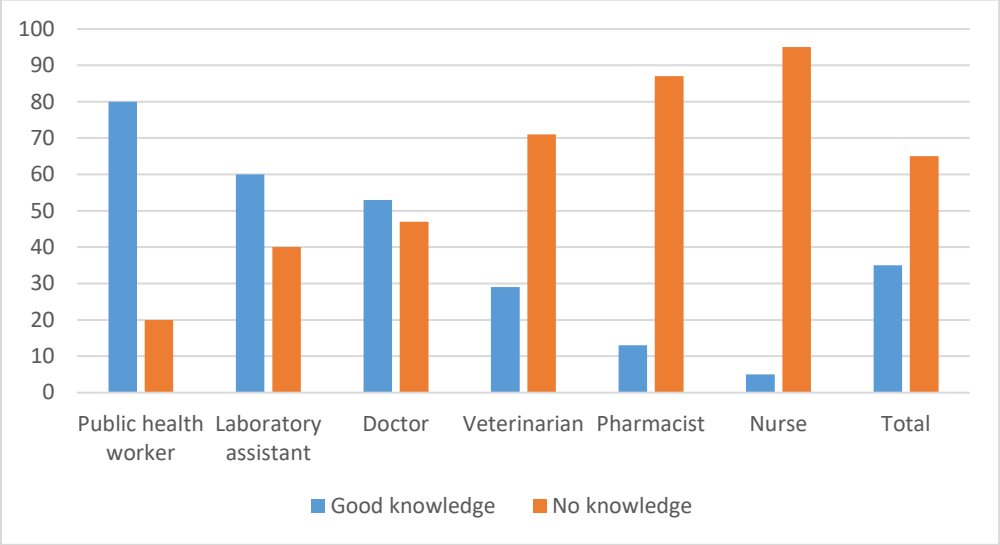

Figure 2. Knowledge of leptospirosis according to employment

4.4 Knowledge acquisition channel: Table 6 reveals that our respondents with knowledge of leptospirosis mostly acquired this through documentary research by consulting websites, followed by university education ~~benches~~, reading books and lastly by participating in seminars.

Table 6. Channel of transmission of knowledge of leptospirosis by respondents

	Number	Percentage
Internet	24	46.1
University	13	25.1
Books	9	17.3
Seminar	6	11.5
Total	52	100

4.5 Knowledge of the type of disease: The results recorded in Table 7 show that only almost half (51.9%) of our respondents recognize leptospirosis as being a zoonotic disease; a small number associate it either with humans (17.3%) or with animals (18.9%). Only 6 respondents out of 52 (11.5%) recognized that it is a contagious disease.

Table 7. Knowledge of the type of disease by respondents

Disease	Number	Percentage
Zoonotic	27	51.9%
Animal	10	19.2%
Human	9	17.3%
Contagious	6	11.5%
Total	52	100%

4.6 Knowledge of the clinical signs of the disease: The results shown in Table 8 indicate that jaundice and/or fever are the symptoms of leptospirosis best known by the majority (75%) of those interviewed who mentioned having heard of leptospirosis; nevertheless, a quarter of them do not acknowledge any symptoms of this disease.

Table 8. Knowledge of clinical signs of the disease by respondents

Signs	**Number**	**Percentage**
Jaundice	5	9.6
Fever	13	25
Jaundice + Fever	21	40.4
Do not know	13	25
Total	52	100

4.7 Knowledge of the disease reservoir: The results recorded in Table 9 show that respondents with knowledge of leptospirosis do not share the same opinion on leptospirosis reservoirs; a third affirms that it is wild animals which are reservoirs of this disease without knowing the species of animals responsible. However, rats were specifically mentioned by a quarter of respondents, the environment was mentioned by about 15% of respondents while dogs by around 14%. Nearly 12% of respondents said they did not know the leptospirosis reservoir.

Table 9. Knowledge of the leptospirosis reservoir by respondents

Type of Animals	**Number**	**Percentage**
Wild Animals	19	36.5
Rat	12	23.1
Dog	7	13.5
Environment	8	15.4
Do not know	6	11.5
Total	52	100

4.8 Distribution of rats captured by species and municipality: The distribution of rats captured by species and by municipality are shown in table 10. The Kimemi commune had the greatest number of rats captured unlike Vulamba where a smaller proportion of rats was collected. The species *Rattus norvergicus* and *Rattus. rattus* were the most abundant of the three

rat species captured with a proportion of 44 and 43%, respectively. The *Steatomys cuppedius* species was the least abundant with a prevalence of 13%.

Table 10: Rat samples collected by species and location

	KIMEMI (n = 51)	BULENGERA (n = 46)	MUSUSA (n=32)	VULAMBA (n = 21)	Total (n = 150)
Rattus norvergicus	22 (43.1%)	23 (50%)	13 (40.6%)	8 (38.1%)	66 (44%)
Rattus rattus	21 (41.1%)	21 (45.7%)	14 (43.8%)	8 (38.1%)	64 (43%)
Steatomys cuppedius	8 (15.6%)	2 (4.3%)	5 (15.6%)	5 (23.8%)	20 (13%)

4.9 Detection of leptospires using the PCR method: After analyzes carried out in the laboratory of INRB for leptospires, no sample was found to be positive although three pairs of primers (LP, LIG and RAV2) were used in this study for each individual sample.

5 DISCUSSION

This work reports on the epidemiological profile of leptospirosis of rats in Butembo City and the level of knowledge of this disease among health professional workers. To our knowledge, studies exploring health professionals' knowledge of leptospirosis have not yet been carried out in the DRC. However, several works devoted to leptospirosis in the DRC in certain studies reported by (Riel, 1953) and (Vanriel J, 1956)cited by (Kurilung *et al.,* 2017) showed the existence of an outbreak of leptospirosis in Kivu and the circulation of 21 *Leptospira* spp strains in the country. Following our survey, it was noted that the respondents had, in general, limited knowledge of leptospirosis, which corroborates a study carried out in India among medical students (Desvars *et al.,* 2013) in which the same conclusions about the limited knowledge regarding this disease were drawn. The fact that health workers have limited knowledge could be explained by ignorance of zoonotic diseases them. Indeed, only 34.7% of our surveyed subjects had good knowledge of leptospirosis. In Nigeria, on the other hand, the proportion of respondents who had good knowledge of leptospirosis was higher than in our study at 52.8% (Alhaji *et al.,* 2019). Nevertheless, our results showed that 52.7% of doctors, 80% of public health workers and 60% of laboratory technicians had good knowledge of the disease, which is positive for disease surveillance in the community and its laboratory diagnosis. Regarding the source of information, 47.1% of respondents reported having obtained information about the disease from the internet. This could be attribute to online coverage of leptospirosis cases, one of the most widely used information media in the intellectual community. The seminars contributed to the knowledge of leptospirosis at 9.8%; several factors may contribute to this, as most health workers participate to seminars regarding health under the new One Health vision. Similarly, the internet was mentioned as a source for acquiring knowledge of leptospirosis in a study conducted by (Mayfield *et al.,* 2018) in Fiji. Most respondents with good knowledge about leptospirosis are confused about the signs and the reservoir. Indeed, most health workers do not have sufficient knowledge of the symptoms of leptospirosis and only 36.5% of personnel working in the health field know leptospirosis as a zoonosis. The results of this study sufficiently show that the level of knowledge about leptospirosis among personnel working in the health sector is low. Van Riel reported in a study, a positive case of leptospirosis in dog sera collected in the city of Butembo (RIEL, 1955). The detection of leptospires in rats has been reported in other neighboring countries such as Rwanda (Poster *et al.,* n.d.), Uganda, Tanzania and the Republic of Congo (Mbete, 2012) with

prevalences ranging from 5% to 20%. However, another study carried out in Tanzania revealed zero prevalence in 384 rodents (including 320 rats) as in our study, notable finding given that the rodents were sampled in areas known to have a high incidence of human leptospirosis (Mirambo *et al.,* 2018). The findings of the study conducted in Tanzania indicated that peridomestic rodents in this region are not a significant source of human infection (Mirambo *et al.,* 2018). Despite several attempts, we failed to detect leptospires in rats in the city of Butembo. Like the Tanzanian study, the rat samples analyzed in our study were collected in the urban area of Butembo City. These zero prevalences observed both in Tanzania and in the DRC, in our study, may be due either to the absence of circulation of leptospires in the study areas or to the absence of bacteria at the time of sampling or finally to their presence below the detection threshold. Other studies have noted changing climatic conditions that may influence the epidemiology of leptospirosis. The work carried out in Canada (Hazart *et al.,* 2010), in Mayotte and in the Seroe Islands (Desvars, 2012) highlighted climate change as having an influence on the transmission of leptospirosis and the maintenance of adequate rat density. In the City of Butembo and its surroundings, climatic hazards largely affected by geographical features such as mountains, forests and lakes can thus have a positive or negative influence on diseases, especially infectious ones. The recent Ebola epidemics which occurred twice in a space of two years in a geographical area previously considered outside the disease's influence may well explain this phenomenon. More in-depth investigations targeting other animal species also considered as reservoirs of leptospirosis such as dogs are necessary to clarify the epidemiology of this disease in this region(de Vries *et al.,* 2014).

6 CONCLUSION

This study was carried out to determine the epidemiological profile of leptospirosis in Butembo city and the knowledge of leptospirosis among people working in the health field. Although our investigation could not detect the presence of leptospirosis in rats, it made it possible to determine the level of knowledge of health personnel about this disease, which turns out to be very low among the different professional categories surveyed. Despite the absence of positive cases in rats in Butembo city, this study constitutes a first step towards unravelling the epidemiology of the disease. Further investigations in other areas are needed as well as the inclusion of other animal species including humans. Such subsequent studies will make it possible to refine this first observation, which may then be compared with data from other parts of the DRC.

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