COMPANION OR PET ANIMALS

Case of clinical canine leptospirosis in Uganda

Lordrick Alinaitwe,[1] Steven Kakooza,[1] Wilfred Eneku,[1] Anou Dreyfus,[2] Sabrina Rodriguez-Campos[3]

[1]College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University, Kampala, Uganda
[2]Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[3]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Correspondence to
Dr Sabrina Rodriguez-Campos, Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland; sabrina.rodriguez@vetsuisse.unibe.ch

Received 28 April 2017
Revised 5 October 2017
Accepted 17 October 2017

SUMMARY

In this report, we present the first confirmed case of clinical leptospirosis in a dog in Uganda. A five -year-old entire male German shepherd dog from a kennel of 25 guard dogs was admitted to the animal clinic and presented hypothermia, lethargy and jaundice of the mucous membranes. The body temperature remained low during the three days post admission until death. The postmortem examination and histological findings led to suspicion of acute leptospirosis. The diagnosis was confirmed by demonstration of pathogenic *Leptospira* spp DNA in homogenates of the kidney, liver and lungs by real-time PCR. This case highlights that accurate diagnostic methods are needed to clarify if clinical leptospirosis is to date underestimated in Uganda and if it has an impact on public health. Awareness should be raised among veterinarians to consider leptospirosis more often as a differential diagnosis as a consequence of the non-specific signs observed in the presented case.

BACKGROUND

Leptospirosis has been reported in human beings and many animal species all over the world.[1–4] In many African countries including Uganda, the disease is usually not taken into account as a differential diagnosis, not even in cases of febrile illnesses that might indicate an early stage of leptospirosis. While a number of recent serological studies in Uganda have indicated the presence of antileptospiral antibodies in human beings,[5] dogs[6] and wildlife,[7] none of these studies was aimed at proving clinical disease, and as a result the burden of leptospirosis in Uganda has remained speculative. The lack of awareness as well as of diagnostic methods leads to poor prognosis of canine leptospirosis that may course with a mortality of up to 70 per cent.[8] This case also raises the question of the frequency of underdiagnosis and under-reporting of the disease in Uganda. In recent years the pet population has rapidly grown, especially in urban Uganda, increasing the likelihood of animal–human transmission. Many homesteads in rural Uganda keep dogs together with cattle, goats and sheep, facilitating interspecies transmission and shedding of *Leptospira* spp into the environment.[1] We believe that reporting this case will raise veterinarians' awareness of leptospirosis and motivate prompt testing and will trigger further epidemiological studies in Uganda. This will promote understanding of the circulating serovars, detect possible host species and identify risk factors in order to establish the best preventive control measures in terms of animal and public health.

CASE PRESENTATION

Here we describe a case of a five-year-old entire male German shepherd dog from a kennel of 25 guard dogs that was presented with hypothermia (36°C; reference range: 37.5°C–39.0°C), dullness, polydipsia and jaundice of the mucous membranes upon admission to a private clinic. The dog had a body condition score of 3/5. Unfortunately, detailed clinical records were not available. Previous to this case, a dog from the same holding died under similar circumstances, and the pathological findings pointed to a possible case of leptospirosis, although this remained unconfirmed due to lack of diagnostic methods at that time. All other dogs from the kennel were asymptomatic; the dogs were individually caged but shared training grounds. The dogs had only received rabies vaccination.

INVESTIGATIONS

The clinic was not equipped for further laboratory analyses, including haematology, clinical biochemistry and urinalysis. Following postmortem examination, leptospirosis was suspected; therefore a visit was made to the training grounds and kennel of the dog for further investigation. This revealed several potential risk factors: (1) the caretakers reported seeing rodents around the kennels and in the training grounds; (2) the dogs were kept individually, but with a central drainage point where waste was collected; (3) the training grounds were in a swampy area that received surface run-off from uphill slums; and (4) the month before the admission of the German shepherd dog was very rainy, indicating a possible involvement of the before-mentioned surface run-off. Following this investigation, the rest of the animals in the holding were suspected to have been exposed to *Leptospira* and were thus administered with doses of ampicillin (20 mg/kg, orally, twice a day for 2 weeks) and no further incidences were reported. Further scrutiny of animals from the kennel by serology to detect a possible circulating serovar or real-time PCR of urine to detect shedding of leptospires was not possible at this time due to lack of laboratory capacity.

DIFFERENTIAL DIAGNOSIS

Jaundice can be prehepatic, hepatic or posthepatic. Prehepatic jaundice, for example, as caused by babesiosis, relies on the presence of haemolytic anaemia, which was not recorded. The differentiation between hepatic and posthepatic jaundice commonly relies on advanced

To cite: Alinaitwe L, Kakooza S, Eneku W, *et al*. *Vet Rec Case Rep* Published Online First: [*please include* Day Month Year]. doi:10.1136/vetreccr-2017-000484

imaging modalities such as abdominal ultrasound, which is not commonly at disposition in veterinary clinics in Uganda. Posthepatic jaundice could be caused by pancreatitis or gall bladder disease. Liver injury could be consistent with leptospirosis, bacterial cholangiohepatitis, acute canine hepatitis caused by an adenovirus, babesiosis caused by a parasite, or toxic hepatopathies. Polydipsia while having a large differential diagnosis can be linked to renal dysfunction. A wide range of signs have been described for clinical leptospirosis in dogs, with the predominant clinical signs being acute kidney injury and liver impairment.[8 9] On the pathological examination, jaundice in combination with disseminated multifocal haemorrhages in the lungs was consistent with severe pulmonary leptospirosis despite no obvious clinical signs of respiratory compromise.

TREATMENT

Upon admission to the private clinic, the dog received fluid therapy once intravenously with 500 ml of 5 per cent dextrose , to which oxytetracycline (5 mg/kg) was added. Dexamethasone (0.5 mg/kg) was administered intramuscularly. On the second day oxytetracycline (10 mg/kg) and a 2 ml multivitamin supplement were administered once intramuscularly.

OUTCOME AND FOLLOW-UP

On the second day of admission, the animal first showed signs of recovery, becoming more alert and attempting to walk sluggishly, but later on showed haematemesis and could only consume water. On the third day the dog was referred to the Small Animal Clinic, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University (Kampala, Uganda), where it died upon arrival from cardiopulmonary arrest. The temperature did not rise above 36°C during the three days post admission until death.

On postmortem examination the carcase had a body condition score of 3/5, and discharge of blood was observed in the mouth and perianal area. Macroscopically the carcase showed severe icterus of the conjunctivae and buccal mucosa, as well as generalised icterus of subcutaneous tissue and viscera. The urinary bladder was distended, with urine that showed deep yellow to brownish pigmenturia. Disseminated multifocal haemorrhages were observed in the lungs, kidneys and heart. The stomach wall and intestines had severe diffuse mucosal haemorrhages and oedema. The liver was moderately enlarged and friable.

Histological examination revealed multifocal to diffuse interstitial infiltration of mononuclear inflammatory cells in the renal cortex and medulla of the kidney (Fig 1A) with tubular necrosis. In addition, some tubules showed sloughed epithelium and collecting ducts haemoglobin in their lumen. The spleen was haemorrhagic with increased deposits of haemosiderin, and moderate follicular lymphoid hyperplasia and extramedullary haematopoiesis (with multiple foci of megakaryocytes) were detected. In the liver mild hepatic cord atrophy, random mild necrotic foci and periportal mononuclear cell infiltrations were detected. Additionally, the hepatocytes were detached into discrete cells with eosinophilic granular cytoplasm and hyperchromic small nuclei. The lungs were oedematous and diffusely congested with multifocal to coalescent collection of free erythrocytes in the alveolar spaces (haemorrhages) (Fig 1B). The macroscopical and histological findings, in particular those of the lung and kidney, are consistent with leptospirosis.[10 11]

An attempt to culture the causative agent from the kidney tissue resulted in the detection of live motile spirochaetes by dark-field microscopy after 14 days, yet subsequent bacterial contamination prevented the strain isolation.

DNA was extracted from homogenates of the kidney, lung and liver using guanidinium thiocyanate as described by Pitcher and others.[12] Real-time PCR using primers and probe targeting the gene *lipL32*[13] encoding a major outer membrane protein present only in pathogenic *Leptospira* spp yielded positive results for all samples. The molecular testing was carried out at the Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern (Switzerland) as part of a laboratory training of one of the authors (LA), and the methods have now been established at the Central Diagnostic Laboratory at the Makerere University (Kampala, Uganda).

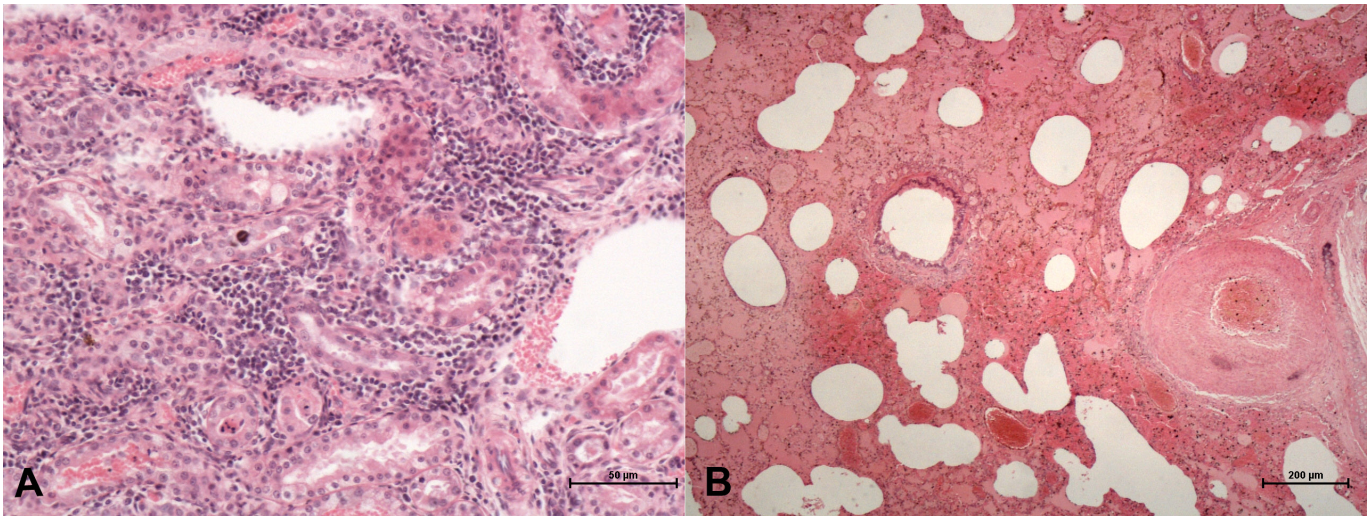

FIG 1: Histopathology of the kidney and the lung of the German shepherd dog that was presented with mild to moderate lymphohistiocytic and plasmacytic interstitial nephritis (haematoxylin and eosin (HE), bar 50 µm) (A), and diffuse congestion, oedema and haemorrhages of the lung (HE, bar 200 µm) (B).

Following the incident, the management considered transferring the dogs to a low-risk area, and now the conditions at the training grounds are improved to prevent surface run-offs and formation of puddles. In the year following the diagnosis and the subsequent treatment of the remaining dogs at risk of exposure, no further incidences of the disease have been reported in the kennel.

DISCUSSION

Information on the incidence, circulating serogroups and serovars in different animal species, important host species and transmission pathways of leptospirosis from tropical Africa including Uganda is scarce. To our knowledge, there have been no reports of clinical leptospirosis in Uganda. Previous studies focused on seropositivity in dogs,[6] buffaloes,[7] and more recently in human beings.[5] The location of the dog's training grounds in a swampy area with surface run-offs is a potential risk factor considering the environmental habitat of leptospires and the significant correlation between the occurrence of leptospirosis and periods of high rainfall as previously reported, that is, in the USA, Canada and Switzerland.[14–16] Dogs living in kennels or shelters have also been shown to have a higher prevalence of antibodies to *Leptospira* spp[17] and a higher prevalence of shedding leptospires via the urine.[18] Moreover, male dogs of working and herding breeds were found to be at greater risk.[15 16]

In the present case the presentation of the dog with polydipsia hinted at possible kidney injury; however, azotaemia could not be confirmed due to the lack of access to laboratory analyses. It is difficult to assess whether the observed polydipsia was due to dehydration in this case. The observed hypothermia in the present case could be associated to the kidney injury or even circulatory shock due to the advanced stage of the disease. Although leptospirosis is included among the febrile diseases, fever is usually more frequent in the early course of the disease.[9]

There is currently no strong evidence to support the use of immunosuppressive therapy, for example, dexamethasone, in the treatment of acute leptospirosis especially considering the risk of complications, such as gastrointestinal symptoms.[8] It has to be noted that 5 per cent dextrose is not an appropriate fluid for rehydration or volume expansion. The patient should have received an electrolyte containing infusion. The authors consider the preventive treatment of the other dogs of the same holding critical since prophylactic administration of antibiotics in dogs should generally be avoided to prevent emergence of resistance. While penicillin derivatives such as ampicillin are often used for the treatment of leptospiral bacteraemia, especially in patients with gastrointestinal signs, the prophylactic treatment of choice would have been doxycycline for 14 days.[8] Although doxycycline is available for intravenous administration in some countries, phlebitis is a common side effect and the drug may be cost prohibitive. Due to the latter, doxycycline was not available for the treatment of the dogs at risk of exposure in this particular case.

Vaccination with a polyvalent vaccine may help prevent infections with pathogenic *Leptospira* spp.[19 20] However, available vaccines only cover for specific serogroups and do not provide cross-protection against other serogroups. To date serogroups known to be circulating in Uganda include Australis, Canicola, Grippotyphosa, Icterohaemorrhagiae and Tarassovi in dogs,[6] Pyrogenes in dogs and human beings,[5 6] and Sejroe in cattle and buffaloes.[7] It is noteworthy that these studies were geographically restricted, and nationwide information on circulating serovars, and hence serogroups, in animals and human beings is still not clearly identified in Uganda. A novel tetravalent vaccine recently evaluated by Spiri and others[21] would potentially cover the most prevalent serogroups (Australis, Canicola, Grippotyphosa, Icterohaemorrhagiae) reported in dogs.

Serological studies are necessary to gain insight into circulating leptospiral serovars and are useful from an epidemiological point of view. Unfortunately, the attempt of isolating the causative strain remained unsuccessful, because for ideal performance of serological studies the use of local strains is recommended.[22] Clinical cases should be diagnosed as early as possible after infection and require modern detection methods such as real-time PCR. Molecular detection of pathogenic *Leptospira* spp in blood can be successful in the first seven days postinfection and in urine from ten days postinfection on. Furthermore, it is useful for postmortem detection in organs[23] and may give information on shedding of the pathogens by carrier animals, for example, via the urine, or the presence in environmental sources of infection. Serological detection of antileptospiral antibodies, in contrast, is possible 10 days postinfection and serodiagnosis of acute leptospirosis needs confirmation through seroconversion or a fourfold titre rise that implies testing of a follow-up sample after one to two weeks.[22] This may impede timely onset of treatment of the patient.

This case report highlights the importance of appropriate diagnosis of leptospirosis, including a basic clinical diagnostic work-up. The access to diagnostic services and up-to-date methods such as real-time PCR is a step forward in the control of important zoonotic diseases such as leptospirosis in Uganda and other developing countries.

Acknowledgements The authors would like to acknowledge Management and staff of the Small Animal Clinic,Dr. Wampande Eddie and the management of the Central diagnostic laboratory, CoVAB,Makerere University. The authors are thankful for the support of Dr. Mutebi Francis,Dr. Afayoa Mathias and Isabelle Brodard.

Contributors AD, LA and SRC conceived the study. LA, AD and SRC were responsible for the study coordination and the data and sample collection. LA, SK and WE were responsible for the pathological and histopathological analyses and bacteriological sampling. LA visited the kennels and was responsible for the follow-up of the case. LA and SRC were responsible for the DNA extraction and real-time PCR. LA, SK, WE, AD and SRC drafted the manuscript. SRC edited the manuscript. All of the authors read and approved the final manuscript.

Funding This study was possible thanks to financial support from the North-South Cooperation at the University of Zurich, the University of Bern and the Swiss Federal Food Safety and Veterinary Office.

Competing interests None declared.

Provenance and peer review Externally peer reviewed.

Data sharing statement More data can be obtained from the authors upon request.

REFERENCES

1 Levett PN. Leptospirosis. *Clin Microbiol Rev* 2001;14:296–326.
2 WHO. *Report of the Second Meeting of the Leptospirosis Burden Epidemiology Reference Group*. Geneva, Switzerland: WHO Press, World Health Organization, 2011. http://apps.who.int/iris/bitstream/10665/44588/1/9789241501521_eng.pdf (accessed 7 Mar 2017).
3 ILRI. *Mapping of poverty and likely zoonoses hotspots. Zoonoses Project 4, Report to Department for International Development*. UK Nairobi, Kenya: International Livestock Research Institute, 2012:119. Available at. https://assets.publishing.service.gov.uk/media/57a08a63ed915d622c0006fd/ZooMapDFIDreport18June2012FINALsm.pdf
4 OIE. *Chapter 2.1.12. Leptospirosis OIE Terrestrial Manual*, 2014:1–15.

5 Dreyfus A, Dyal JW, Pearson R, *et al*. *Leptospira* Seroprevalence and risk factors in health centre patients in hoima district, Western Uganda. *PLoS Negl Trop Dis* 2016;10:e0004858.
6 Millán J, Chirife AD, Kalema-Zikusoka G, *et al*. Serosurvey of dogs for human, livestock, and wildlife pathogens, Uganda. *Emerg Infect Dis* 2013;19:680–2.
7 Atherstone C, Picozzi K, Kalema-Zikusoka G. Seroprevalence of *Leptospira hardjo* in cattle and African buffalos in southwestern Uganda. *Am J Trop Med Hyg* 2014;90:288–90.
8 Schuller S, Francey T, Hartmann K, *et al*. European consensus statement on leptospirosis in dogs and cats. *J Small Anim Pract* 2015;56:159–79.
9 Sykes JE, Hartmann K, Lunn KF, *et al*. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. *J Vet Intern Med* 2011;25:1–13.
10 Mcgavin MD, Zachary JF. *Pathologic Basis of Veterinary Disease*. 4th edn: Mosby Elsevier, 2007.
11 Greene CE, Sykes JE, Moore GE, *et al*. Chapter 42 Leptospirosis. In Greene CE, ed. *Infectious Diseases of the Dog and Cat*. 4th edn, 2012:431–47.
12 Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* 1989;8:151–6.
13 Villumsen S, Pedersen R, Borre MB, *et al*. Novel TaqMan® PCR for detection of *Leptospira* species in urine and blood: pit-falls of in silico validation. *J Microbiol Methods* 2012;91:184–90.
14 Ward MP. Seasonality of canine leptospirosis in the United States and Canada and its association with rainfall. *Prev Vet Med* 2002;56:203–13.
15 Ward MP, Glickman LT, Guptill LE. Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970-1998). *J Am Vet Med Assoc* 2002;220:53–8.
16 Major A, Schweighauser A, Francey T. Increasing incidence of canine leptospirosis in Switzerland. *Int J Environ Res Public Health* 2014;11:7242–60.
17 Scanziani E, Origgi F, Giusti AM, *et al*. Serological survey of leptospiral infection in kennelled dogs in Italy. *J Small Anim Pract* 2002;43:154–7.
18 Rojas P, Monahan AM, Schuller S, *et al*. Detection and quantification of leptospires in urine of dogs: a maintenance host for the zoonotic disease leptospirosis. *Eur J Clin Microbiol Infect Dis* 2010;29:1305–9.
19 Goldstein RE, Lin RC, Langston CE, *et al*. Influence of infecting serogroup on clinical features of leptospirosis in dogs. *J Vet Intern Med* 2006;20:489–94.
20 Klaasen HL, van der Veen M, Molkenboer MJ, *et al*. A novel tetravalent *Leptospira* bacterin protects against infection and shedding following challenge in dogs. *Vet Rec* 2013;172:181.
21 Spiri AM, Rodriguez-Campos S, Matos JM, *et al*. Clinical, serological and echocardiographic examination of healthy field dogs before and after vaccination with a commercial tetravalent leptospirosis vaccine. *BMC Vet Res* 2017;13:138.
22 Goris MG, Hartskeerl RA. Leptospirosis serodiagnosis by the microscopic agglutination test. *Curr Protoc Microbiol* 2014;32:Unit 12E.5.
23 Stoddard RA. Detection of pathogenic Leptospira spp. through real-time PCR (qPCR) targeting the LipL32 gene. *Methods Mol Biol* 2013;943:257–66.

Case of clinical canine leptospirosis in Uganda

Lordrick Alinaitwe, Steven Kakooza, Wilfred Eneku, Anou Dreyfus and Sabrina Rodriguez-Campos

Vet Rec Case Rep 2017 5:
doi: 10.1136/vetreccr-2017-000484