A lethal model of *Leptospira* infection in hamster nasal mucosa

Jiaqi Wang\(^1\*\), Wenlong Zhang\(^{1,2}\*\), Zhao Jin\(^1\), Yue Ding\(^1\), Shilei Zhang\(^1\), Dianjun Wu\(^1\)*, Yongguo Cao\(^{1,2}\*)

\(^1\) Department of Clinical Veterinary Medicine, College of Veterinary Medicine, Jilin University, Changchun, People’s Republic of China, \(^2\) Key Laboratory for Zoonosis Research, Ministry of Education, College of Veterinary Medicine, Jilin University, Changchun, People’s Republic of China

* These authors contributed equally to this work.

\* wdjok@jlu.edu.cn (DW); ygcao82@jlu.edu.cn (YC)

Abstract

Leptospirosis is a fatal zoonosis caused by contact between skin or a mucosal surface and contaminated soil or water. Hamsters were infected by intraperitoneal injection to establish experimental leptospirosis, which is not a natural route of infection. There are no reports of nasal mucosal infection in hamsters. In this study, infection of the nasal mucosa was performed to establish a model of natural infection. Both methods of infection can cause lethal models with similar symptoms in the later stages of infection, such as weight loss, blood concentration, increased neutrophils (GRAN), and decreased lymphocytes (LYM) in the blood, severe organ damage and liver function obstruction. The burden of *Leptospira* in the organs and blood was lower in the mucosal inoculation groups at 1 day after infection. However, mucosal infection induced a higher *Leptospira* burden in urine than intraperitoneal infection in the late stages of infection. After nasal mucosal infection, antibody levels were higher and lasted longer. These results indicated that the route of nasal mucosal infection is a good choice for studying leptospirosis in hamsters.

Author summary

The establishment of a leptospirosis experimental model is still key to elucidating the pathogenesis of leptospirosis. Hamsters were infected by intraperitoneal injection to establish experimental leptospirosis, although this is not a natural route of infection. The transmission characteristics of *Leptospira* and the disease progression in hamsters infected by a natural transmission route (e.g. through mucosal surfaces) had not been explored. In this study, we compared the dynamics of *Leptospira* infection in hamsters inoculated via the nasal mucosa or by intraperitoneal inoculation, and compared the burden of *Leptospira* and the level of antibodies produced with disease progression, such as body weight, serology, haematological changes and histopathological changes. Our data suggested that there are significant differences in the dynamics of infection between intraperitoneal and mucosal infection pathways. Although the result was the same in the later stage of infection, the course of mucosal infection was slower, which may better recapitulate the natural
Introduction

Leptospirosis is a worldwide zoonotic disease. It is estimated that there are 1.03 million cases of this disease and 60,000 deaths each year [1,2]. In recent years, the number of outbreaks has increased with increases flooding and urbanization, as well as the deterioration of living conditions of slums [3]. Leptospirosis occurs in humans through contact with infected urine from animals in daily activities, or by being in contact with contaminated soil or water [4–6]. Human leptospirosis ranges from a mild form to a severe infection called Weil’s disease, which is characterized by jaundice, renal failure and haemorrhage with a fatality rate of 5–15% [7]. In animals, *Leptospira* infection also causes reproductive failure and acute febrile disease with renal and liver failure [8,9]. The establishment of a leptospirosis experimental model remains key to elucidating the pathogenesis of leptospirosis. Hamsters or guinea pigs have been reported as animal models in most studies exploring the pathogenicity of *Leptospira*, because infection with virulent *Leptospira* can lead to fatal acute diseases in these species, similar to severe human leptospirosis [2,10].

In animal models of leptospirosis, including hamsters and guinea pigs,subjects were typically infected by intraperitoneal (IP) injection which, because it is not a natural route of infection, ignores natural mucosal and skin defence mechanisms, with the result that this infection pathway may overestimate transmission time and pathogen load during transmission. Other routes of infection, such as contact between contaminated soil or water and epidermal, conjunctival, subcutaneous, intradermal, oral, intracardiac, and intracranial surfaces have also been reported [11,12], while detailed infection processes have been described only for intraperitoneal and subcutaneous intradermal routes of infection [12,13]. However, the assessment of leptospirosis progression in hamsters through the nasal mucosal infection route, which is an important natural transmissionpathway, remains elusive. The development of animal models for replication of natural transmission pathways is essential for understanding host pathogen interactions especially in the early stages of *Leptospira* infection.

The transmission characteristics of *Leptospira* and the disease progression of hamsters infected by natural transmission routes (e.g., through the mucosal surface) remain to be explored. Our purpose was to analyse similarities and differences between *Leptospira* transmission through the nasal mucosa and standard intraperitoneal transmission. In this study, we compared the dynamics of *Leptospira* infection in hamsters infected via the nasal mucosa and intraperitoneal routes, and compared the burden of *Leptospira* and the level of antibodies produced with disease progression, in addition to changed in body weight, serology, haematology changes and histopathology.

Materials and methods

Ethics statement

All animals were maintained on standard rodent chow with water supplied ad libitum and with a 12/12h light/dark cycle during the experimental period. All animal experiments followed the regulations for the Administration of Affairs Concerning Experimental Animals in China. The protocol was approved by the Institutional Animal Care and Use Committee of Jilin University (20170318).
**Leptospira and animals**

*Leptospira interrogans Lai type Lai (56601) and Java type (56602) are gifts from Professor Guo Xiaokui. Australian (56607) type and Wolffel (56635) type strains are cultivated in our laboratory all year round. Leptospira was grown in liquid Ellinghausen-McCullough-Johnson-Harris (EMJH) medium at 29˚C. The virulence of the Leptospira was maintained by passage in hamsters. Leptospira was passaged *in vitro* less than three times in liquid EMJH for all infection studies. Before infection, the concentration of Leptospira was determined using a Petroff-Hauser counting chamber and a dark-field microscope. Syrian golden hamsters (*Mesocricetus auratus*) were provided by the Liaoning chang sheng biotechnology co. LTD.

**Hamster infection and sample collection**

Intraperitoneal infection was performed as described previously [14]. Female hamsters obtained from the Animal Center of Jilin University, 5 to 6 weeks of age, were inoculated (1 mL) with $10^6/10^7/10^8/10^9$ leptospires on day 0. *Leptospira* was counted by using a Petroff-Hauser chamber under a dark field microscope and confirmed by quantitative PCR (qPCR). For nasal mucosa (NM) infection, a maximum of 40 μl of sterile saline containing $\sim 10^6/10^7/10^8/10^9$ spirochetes was deposited as small drops into one nostril of anesthetized hamsters, synchronized with inhalation. Body weights were monitored daily. After challenge with leptospires, all hamsters were observed no less than 3 times per day for a period of 21 days, during which serious sickness mouse appeared moribund was observed and then was humanely euthanized by CO$_2$. Regarding sample collection, three hamsters in each group were humanely euthanized every day for the first five days, and then three hamsters in each group were humanely euthanized on 7/9/10/15 days. At every point in time, urine, blood and organs of hamster were collected. Blood was collected for routine blood test and chemical analyses (Jilin University Animal Hospital). Organs were collected for hematoxylin and eosin (H&E) staining and DNA extraction. Urine was collected for DNA extraction. Surviving hamsters were humanely euthanized after 21 days using CO$_2$.

**Histopathology**

Organs were collected and fixed with 4% paraformaldehyde for 24 hours at room temperature and then embedded in paraffin and sectioned at a thickness of 4 μm. Pathological changes of organ slices were examined by hematoxylin and eosin (H&E) staining, and the organ injury index (the injured area/total organ area x100%) was calculated for each slice. The severity of leptospire induced lesions was graded as previous description [14,15].

**Leptospiral load and qPCR assay**

The leptosiral burden in organs, blood and urine of hamsters was determined by quantitative PCR (qPCR). The DNA extraction was followed as previously described [16]. The DNA concentration was measured by spectrometry. The primers used were specific for the lipL32 gene as described previously [17]. The qPCR assays were performed using previously published primers, optimized reaction mixtures and cycling parameters. A standard curve analyzed by serial dilutions ($10^9$–$10^2$) of DNA was established to calculate the number of *Leptospira* in the organ. Leptospiral load was expressed as the amount of genome equivalents per μg tissue DNA [14].

**Anti-leptospira ELISA**

*Leptospira* was collected by centrifuging at 12,000 g for 5 min, after which *Leptospira* was resuspended in PBS and coated overnight at 4˚C in 96-wells plates at a density of $10^7$. Then the
plate was blocked with PBS-5% BSA at room temperature for 1 hour, washed, and a 15-fold dilution of hamster serum was added to the plate and kept at room temperature for 2 hours. After washing, HRP-conjugated goat anti-hamster IgG, IgG1, IgG2/3 and IgM were added for 1h at room temperature. The plates were washed and peroxidase activity was revealed by TMB substrate (Sigma, USA), and stopped by H$_2$SO$_4$. Reading was performed at 450 nm. The titres of the sera were expressed as the dilution corresponding to twice the background level of the ELISA [18]. Alternatively, same dilutions of the different sera were also compared at 450 nm.

**Serum Microscopic Agglutination test (MAT)**

All sera were tested by MAT. Serum water bath (56°C, 30min) was used to remove complement, then 15-fold dilution, and then continuous dilution of 8 times. The serum was mixed with an equal amount of $10^7$ 56601/56602/56607/56635. The mixture was incubated at 37°C for 2 hours and observe the agglutination under a dark field microscope. The antibody titers were recorded as the maximum dilution of serum with 50% agglutination or 50% reduction of *Leptospira* [19].

**Inhibition of Serum on growth of *Leptospira***

The serum were collected 15 day after infection, diluted 15 times and mixed with $10^7$ *Leptospira* 56601 in EMJH. Then the mixtures were incubated at 37°C for 2 hours. The growth of *Leptospira* was observed by microscopy daily.

**Statistical analysis**

Survival differences between the study groups were compared by using the Kaplan-Meier log-rank test. Comparisons between groups were performed by using t-test. Differences were considered significant at P $< 0.05$.

**Results**

**Nasal mucosal infection causes hamsters death**

Both methods of infection can establish lethal models. The median lethal dose (LD$_{50}$) for intra-abdominal infection was $1.375 \times 10^6$, and the LD$_{50}$ for nasal mucosal infection was $1.29 \times 10^7$ (Fig 1A). Representative photographs of hamster livers, kidneys and lungs were selected during the dying period (Fig 1B). Pathological changes in the livers of the three groups were tight junction defects and areas of necrosis and inflammatory infiltration (Fig 1Ba and 1Bd). Dramatic lesions with haemorrhage were found in the renal tissues (Fig 1Bb and 1Be). Haemorrhage was also observed in pulmonary tissues. Generalized interstitial pneumonia was noted in the lungs, alveolar congestion and infiltration of mononuclear cells (Fig 1Bc and 1Bf). The histopathological scores of the two groups were similar (Fig 1C).

**The late stage of nasal mucosal infection can simulate leptospirosis in sensitive animals, similar to intraperitoneal injection infection**

The indicators of the two groups of hamsters infected with *Leptospira* $10^8$ were monitored. Infected hamsters continued to gain weight until the day before death. The weight of the hamsters in the IP group was reduced during the dying period by more than 5% compared with the previous day, and all of them died at 4 d.p.i. At 9 d.p.i., 75% of the hamsters with NM challenge had lost $\geq 5\%$, and these hamsters only survived to 9 d.p.i. The total protein content in
the serum increased with the severity of the disease. The data show that the two infection routes had the same trend (Fig 2A).

After infection with *Leptospira*, both alkaline phosphatase (ALKP) and glutamic pyruvic transaminase (GPT) continued to increase. The intraperitoneal group reached its peak on the third day. The NM group peaked at 9 dpi (Fig 2B). During the late stage of infection, the percentage of neutrophils in the blood of hamsters of the both routes of infection increased significantly, from approximately 30% to approximately 60%, while the percentage of lymphocytes decreased significantly, from approximately 70% to 20% (Fig 2C).

*Leptospira* burden in the organs and blood of hamsters was detected at 1 d p.i. in different groups. The route of intraperitoneal infection allowed *Leptospira* to invade the hamster’s
organs and blood faster (Fig 3A–3D). No Leptospira was detected in urine on the first day of infection (Fig 3E). During the dying period, large numbers of leptospires were found in organs and blood and the difference was not significant among the three groups (Fig 3A–3D). Interestingly, the Leptospira burden in the urine of the intraperitoneal group was much lower than that in the mucosal group. The average amount of Leptospira in urine of the intraperitoneal group was 52, while the average amount of Leptospira in urine of the mucosal group was more than $10^5$ (Fig 3E).

Results for other infectious doses are in S1–S6 Figs.

**Nasal mucosal infections produce stronger and effective antibodies to fight the infection**

The antibody levels of two groups of golden hamsters infected with Leptospira $10^6$ in the peritoneum and nasal mucosa were detected. The results showed that the serum IgG level after
nasal mucosal infection was slightly higher than that of intraperitoneal infection, and IgG\textsubscript{1} was significantly higher than that of intraperitoneal infection after 10 days of infection. IgM produced via nasal mucosal infection was higher than that produced by intraperitoneal infection, and IgM from intraperitoneal infection was significantly reduced after 10 days, while IgM produced by nasal mucosal infection continued to increase within 15 days (Fig 4A).

Fig 3. Leptospiral burdens in hamsters infected with 10\textsuperscript{8} Leptospira. Leptospiral burdens in the livers (A), kidneys (B), lungs (C), blood (D) and urine (E) of hamsters in the IP group (n = 3), the NM group (n = 3) at 1 d.p.i, and the day that they appeared moribund (AM) as determined by qPCR. Samples were collected on the 1st day after Leptospira infection and the day the hamsters appeared moribund. The results are presented as the number of genomic equivalents per microgram of tissue DNA, and the differences were compared by one-way ANOVA. *, P < 0.05.

https://doi.org/10.1371/journal.pntd.0010191.g003
The MAT results with 56601/56602/56607/56635 showed that the antibody titre produced by nasal mucosal infection was higher, and the binding ability to the four kinds of Leptospira was stronger (Fig 4B).

The mixture of serum and 56601 was incubated for 2 hours in EMJH. Daily microscopic examination recorded the growth of Leptospira. After incubation with serum produced by the nasal mucosal infection route, Leptospira grew, and reproduction was significantly suppressed (Fig 4C).

Discussion

Hamsters are currently one of the most widely used animal models of acute leptospirosis because of their reproducibility and sensitivity to multiple pathogenic Leptospira strains [20–22]. A feasible lethal model that is closer to the natural infection mode is more helpful to research on leptospirosis. There have been a few reports on this aspect of the disease. Reports on mucosal infection pathways have used mice or rats as experimental animals [23,24]. The Albert I Ko team used hamsters to compare changes in the amount of Leptospira after infection of the conjunctiva and the abdominal cavity [25], but did not study the route of infection of the nasal mucosa and did not describe the mortality rate. However, nasal mucosal infection has been the main method of inducing the disease and cannot be ignored. Studies have shown that oral mucosal infection of Leptospira does not cause hamster death. [26] Therefore, in this study, we think that inoculation via the nasal mucosa will be an effective and comprehensive method for modelling the disease. Our ultimate goal was to assess whether hamsters infected via the nasal mucosa could reproduce disease markers, histopathology, and other diseases previously caused by intraperitoneal infection.
Infection with different concentrations of *Leptospira* led to the death of hamsters, and the damage to the organs during the dying period was similar. However, at the same dose, mucosal infection had a slower course and a lower fatality rate than abdominal infection. The LD50 for intra-abdominal infection was 1.375 \times 10^6, and the LD50 for nasal mucosal infection is 1.29 \times 10^7. (Fig 1) This difference might be caused by a decrease in the number of Leptospires that cross the natural mucosal barrier or the rapid activation of mucosal immunity to clear a large number of leptospires present in the inoculum [27–29]. The epithelial layer consists of columnar ciliated and secretory cells that line the airway surface and help it serve as an environmental barrier for inhalation. These cells cover smaller basal cells, which have the characteristics of progenitor cells. They not only show the ability of self-renewal and clonal expansion in the homeostasis and epithelial repair process after injury, but also cause basal, ciliary and secretory lineages [29]. Secreted goblet cells combine with the submucosal gland to produce mucus, which contains hydrated gelatine and many host defence and cytoprotective molecules such as antimicrobial molecules, proteases and antioxidants [30].

However, this step may be absent in the route of intra-abdominal infection, which may explain why *Leptospira* strains deficient in genes that were shown to have important functions in vitro remain infectious during intraperitoneal inoculation of a living host [31–33]. The results from this study, which show differences in infection kinetics, suggest that it may be crucial to study the infectivity contribution of those genes using more physiologically relevant models, such as the mucosal route of infection.

Analysing the sample data of 10×LD50 infection, we found that when the weight is reduced by more than 5% from the previous day, death will result cause death (Fig 2). This finding may be used to predict or avoid death. Weight loss and total protein increased simultaneously, suggesting that these changes may be due to dehydration and blood concentration caused by leptospirosis, which may be due to reduced fluid intake and impaired sodium reabsorption in proximal renal tubules [34]. In addition, the identified histological changes were initially related to the glomeruli and interstitium, while the renal tubules were only affected in the late stage, which was the reason why the total protein increased rapidly in the late stage [35]. Elevated TP may have indicated inflammation in this study as TP is documented to increase due to hyperfibrinogenemia [36].

In the late stage of leptospirosis, the results of the three infection routes were similar. There were many leptospires in the liver and kidney, and pathological damage was severe (Fig 1). Jaundice was found when the abdominal cavity was opened, and serological examination also showed severe liver damage (Fig 2). Hamsters died quickly due to infection of the abdominal cavity. *Leptospira* was not detected in the urine, while the number of *Leptospira* in the urine of mucosal infections reached \(10^4\) (Fig 3). This is consistent with the previous study by Nair et al. in mice [23]. Although *Leptospira* can also be detected in urine at the later stage of abdominal cavity infection, hamsters died prematurely from abdominal infection, and hamsters survived for a longer period with mucosal infection. *Leptospira* colonize the renal tubules of hamsters in the late stage of mucosal infection. Combined with pathological sections, kidney damage was serious. This is consistent with the rapid development of a hamster model similar to human leptospirosis, which directly leads to severe liver and kidney dysfunction [37,38]. A large number of leptospires could be detected in the blood, and haematological analysis found that the percentage of neutrophils increased and the percentage of lymphocytes decreased, which was a manifestation of severe sepsis [39,40].

Detection of serum antibodies in golden hamsters infected with \(10^6\) *Leptospira* showed that nasal mucosal infections produced higher levels of antibodies than individuals with abdominal infections, especially after ten days, and the levels of IgM and IgG1 were significantly higher than those with intraperitoneal infections. Through MAT detection of the antibody titres that
bind to the four serotypes of *Leptospira*, it was found that the antibodies produced by nasal mucosal infections have stronger binding ability to different serotypes of *Leptospira*, so the antibodies produced by nasal mucosal infection may have stronger capability (Fig 4).

The experimental results on hamsters are similar to those on rats and mice [11,23,24]. Oral mucosal infection does not cause infection, while infections through the conjunctiva and nasal mucosa can kill sensitive animals. In tolerance animal models, *Leptospira* can enter the blood and colonize the kidneys. This may be related to secretions in the oral cavity that differ from those in the nasal cavity and conjunctiva.

In summary, our data suggested that there are significant differences in the dynamics of infection between intraperitoneal and mucosal disease pathways. More leptospires may be required to overcome mucosal defence and transit through tissue in sufficient numbers before systemic spread can occur. Although the result was the same in the later stage of infection, the course of mucosal infection was slower, which that may better recapitulate the natural history of the disease, thereby better assisting evaluations of kidney disease caused by *Leptospira*, and providing an excellent animal model for the study of leptospirosis mucosal immunity. There were differences in the immune pathways activated by the two paths of infection, which may yield different efficacy outcomes in testing of vaccines and preventive drugs in these different hamster models of infection.

**Supporting information**

**S1 Fig. Hamster symptoms after 10<sup>9</sup> *Leptospira* infections initiated via intraperitoneal and mucosal infection paths.** (A) The weight of hamsters after infection. Animals were weighed at the time of IP and NM challenge (Day 0) and daily thereafter. The figure also shows the mean change in weight relative to the original weight and the amount of total protein in the serum. (B) Kinetics of inflammatory markers and liver enzymes. Alkaline Phosphatase (ALKP) and Glutamic Pyruvic Transaminase (GPT). (C) Neutrophils% and lymphocytes%. Blood samples were collected at the time of IP and NM challenge (Day 0) and daily thereafter.

**S2 Fig. Leptospiral burdens in hamsters infected with 10<sup>9</sup> *Leptospira*.** Leptospiral burdens in the livers (A), kidneys (B), lungs (C), blood (D) and urine (E) of hamsters in the IP group (n = 3), the NM group (n = 3) at 1 d.p.i., and the day that they appeared moribund (AM) as determined by qPCR. Samples were collected on the 1st day after infected *Leptospira* infection and the day the hamsters appeared moribund. The results are presented as the number of genomic equivalents per microgram of tissue DNA, and the differences were compared by one-way ANOVA. *, P < 0.05.

**S3 Fig. Hamster symptoms after 10<sup>7</sup> *Leptospira* infections initiated via intraperitoneal and mucosal infection paths.** (A) The weight of hamsters after infection. Animals were weighed at the time of IP and NM challenge (Day 0) and daily thereafter. The figure also shows the mean change in weight relative to the original weight and the amount of total protein in the serum. (B) Kinetics of inflammatory markers and liver enzymes. Alkaline Phosphatase (ALKP) and Glutamic Pyruvic Transaminase (GPT). (C) Neutrophils% and lymphocytes%. Blood samples were collected at the time of IP and NM challenge (Day 0) and daily thereafter.

**S4 Fig. Leptospiral burdens in hamsters infected with 10<sup>7</sup> *Leptospira*.** Leptospiral burdens in the livers (A), kidneys (B), lungs (C), blood (D) and urine (E) of hamsters in the IP group (n = 3), the NM group (n = 3) at 1 d.p.i., and the day that they appeared moribund (AM) as
determined by qPCR. Samples were collected on the 1st day after infected *Leptospira* infection and the day the hamsters appeared moribund. The results are presented as the number of genomic equivalents per microgram of tissue DNA, and the differences were compared by one-way ANOVA. *, P < 0.05.

S5 Fig. Hamster symptoms after 10^6 *Leptospira* infections initiated via intraperitoneal and mucosal infection paths. (A) The weight of hamsters after infection. Animals were weighed at the time of IP and NM challenge (Day 0) and daily thereafter. The figure also shows the mean change in weight relative to the original weight and the amount of total protein in the serum. (B) Kinetics of inflammatory markers and liver enzymes. Alkaline Phosphatase (ALKP) and Glutamic Pyruvic Transaminase (GPT). (C) Neutrophils% and lymphocytes%. Blood samples were collected at the time of IP and NM challenge (Day 0) and daily thereafter.

S6 Fig. Leptospiral burdens in hamsters infected with 10^6 *Leptospira*. Leptospiral burdens in the livers (A), kidneys (B), lungs (C), blood (D) and urine (E) of hamsters in the IP group (n = 3), the NM group (n = 3) at 1 d.p.i, and the that they day appeared moribund (AM) as determined by qPCR. Samples were collected on the 1st day after infected *Leptospira* infection and the day the hamsters appeared moribund. The results are presented as the number of genomic equivalents per microgram of tissue DNA, and the differences were compared by one-way ANOVA. *, P < 0.05.

Acknowledgments
We thank Dr. Xiaokui Guo (Shanghai Jiao Tong University, Shanghai, China) for providing *Leptospira* interrogans serovar Lai strain Lai (56601).

Author Contributions
Formal analysis: Jiaqi Wang, Wenlong Zhang.
Investigation: Yue Ding, Shilei Zhang.
Resources: Yue Ding, Shilei Zhang.
Validation: Zhao Jin, Dianjun Wu, Yongguo Cao.
Writing – original draft: Jiaqi Wang.
Writing – review & editing: Jiaqi Wang, Wenlong Zhang, Zhao Jin, Yue Ding, Shilei Zhang, Dianjun Wu, Yongguo Cao.

References
1. Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, et al. Global Morbidity and Mortality of Leptospirosis: A Systematic Review. PLoS neglected tropical diseases. 2015; 9(9): e0003898. https://doi.org/10.1371/journal.pntd.0003898 PMID: 26379143; PubMed Central PMCID: PMC4574773.
2. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, et al. Leptospirosis: a zoonotic disease of global importance. The Lancet Infectious diseases. 2003; 3(12):757–71. https://doi.org/10.1016/s1473-3099(03)00830-2 PMID: 14652202.
3. Picardeau M. Leptospira and Leptospirosis. Methods in molecular biology. 2020; 2134:271–5. https://doi.org/10.1007/978-1-0716-0459-5_24 PMID: 32632877.
4. Caldas EM, Sampaio MB. Leptospirosis in the city of Salvador, Bahia, Brazil: a case-control seroepidemiologic study. International journal of zoonoses. 1979; 6(2):85–96. PMID: 356125.

5. Monahan AM, Miller IS, Nally JE. Leptospirosis: risks during recreational activities. Journal of applied microbiology. 2009; 107(3):707–16. https://doi.org/10.1111/j.1365-2672.2009.04220.x PMID: 19302325.

6. Lehmann JS, Matthias MA, Vinetz JM, Fouts DE. Leptospiral pathogenomics. Pathogens. 2014; 3(2):280–308. https://doi.org/10.3390/pathogens3020280 PMID: 25437801; PubMed Central PMCID: PMC4243447.

7. Vijayachari P, Sugunan AP, Shiriram AN. Leptospirosis: an emerging global public health problem. Journal of biosciences. 2008; 33(4):557–69. https://doi.org/10.1007/s12038-008-0074-z PMID: 19208981.

8. Adler B, de la Pena Moctezuma A. Leptospira and leptospirosis. Veterinary microbiology. 2010; 140(3–4):287–96. https://doi.org/10.1016/j.vetmic.2009.03.012 PMID: 19345023.

9. Schuller S, Francyte Y, Hartmann K, Hugonnard M, Kohn B, Nally JE, et al. European consensus statement on leptospirosis in dogs and cats. The Journal of small animal practice. 2015; 56(3):159–79. https://doi.org/10.1111/jasp.12328 PMID: 25754092.

10. Leveti PN. Leptospirosis. Clin Microbiol Rev. 2001; 14(2):326–296. https://doi.org/10.1128/CMR.14.2.296-326.2001 PMID: 11292640; PubMed Central PMCID: PMC88975.

11. Lourdault K, Aviat F, Picardeau M. Use of quantitative real-time PCR for studying the dissemination of Leptospira interrogans in the guinea pig infection model of leptospirosis. J Med Microbiol. 2009; 58(5):648–55. https://doi.org/10.1099/jmm.0.008169-0 WOS:000266018900016. PMID: 19369528

12. Zhang Y, Lou XL, Yang HL, Guo XK, Zhang XY, He P, et al. Establishment of a leptospirosis model in guinea pigs using an epicutaneous inoculations route. BMC infectious diseases. 2012; 12. Arnl 2010.1186/1471-2334-12-20. WOS:000302947700001. https://doi.org/10.1186/1471-2334-12-20 PMID: 22273178

13. Coutinho ML, Matsunaga J, Wang LC, de la Pena Moctezuma A, Lewis MS, Babbitt JT, et al. Kinetics of Leptospira interrogans infection in hamsters after intradermal and subcutaneous challenge. PLoS neglected tropical diseases. 2014; 8(11):e3307. https://doi.org/10.1371/journal.pntd.0003307 PMID: 25411782; PubMed Central PMCID: PMC4239013.

14. Xin X, Zhang W, Ding Z, Wang H, Wu D, Xie X, et al. Efficacy of the Rabbit Polyclonal Anti-leptospira Antibody against Homotype or Heterotype Leptospira Infection in Hamster. PLoS neglected tropical diseases. 2016; 10(12):e0005191. https://doi.org/10.1371/journal.pntd.0005191 PMID: 28027297; PubMed Central PMCID: PMC5189943.

15. Cao Y, Faisal SM, Yan W, Chang YC, McDonough SP, Zhang N, et al. Evaluation of novel fusion proteins derived from extracellular matrix binding domains of LigB as vaccine candidates against leptospirosis in a hamster model. Vaccine. 2011; 29(43):7379–86. https://doi.org/10.1016/j.vaccine.2011.07.070 PMID: 21803087.

16. Zhang W, Zhang N, Wang W, Wang F, Gong Y, Jiang H, et al. Efficacy of cefepime, ertapenem and norfloxacin against leptospirosis and for the clearance of pathogens in a hamster model. Microbial pathogenesis. 2014; 77:78–83. https://doi.org/10.1016/j.micpath.2014.11.006 PMID: 25450882.

17. Rojas P, Monahan AM, Schuller S, Miller IS, Markey BK, Nally JE. Detection and quantification of leptospires in urine of dogs: a maintenance host for the zoonotic disease leptospirosis. European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology. 2010; 29(10):1305–9. https://doi.org/10.1007/s10096-010-0991-2 PMID: 20559675.

18. Chassin C, Picardeau M, Goujon JM, Bourhy P, Quellard N, Darche S, et al. TLR4- and TLR2-mediated B cell responses control the clearance of the bacterial pathogen, Leptospira interrogans. Anticancer research. 2011; 31(9A):3611–7. https://doi.org/10.21873/anticanres.2332; PubMed Central PMCID: PMC3260679.

19. Lizer J, Velineni S, Weber A, Kreic M, Meeus P. Evaluation of 3 Serological Tests for Early Detection Of Leptospira-specific Antibodies in Experimentally Infected Dogs. Journal of veterinary internal medicine. 2018; 32(1):201–7. https://doi.org/10.1111/jvim.14865 PMID: 2931400; PubMed Central PMCID: PMC5787205.

20. van den Ingh TS, Hartman EG. Pathology of acute Leptospira interrogans serotype icterohaemorrhagiae infection in the Syrian hamster. Veterinary microbiology. 1986; 12(4):367–76. https://doi.org/10.1016/0378-1135(86)90086-6 PMID: 3530643.

21. Arean VM. Studies on the pathogenesis of leptospirosis. II. A clinico-pathologic evaluation of hepatic and renal function in experimental leptospiral infections. Laboratory investigation; a journal of technical methods and pathology. 1962; 11:273–88. PMID: 13862140.

22. Ferguson LC, Hamd y AH. Virulence of Leptospira pomona in hamsters and cattle. American journal of veterinary research. 1957; 18(66):35–42. PMID: 13394814.
23. Nair N, Guedes MS, Werts C, Gomes-Solecki M. The route of infection with Leptospira interrogans serovar Copenhageni affects the kinetics of bacterial dissemination and kidney colonization. PLoS neglected tropical diseases. 2020; 14(1):e0007950. https://doi.org/10.1371/journal.pntd.0007950 PMID: 31905198; PubMed Central PMCID: PMC6964914 the following competing interests: MSG and MGS are or were employed in part by a commercial company, Immuno Technologies, Inc. MGS holds more than 5% financial interest in Immuno Technologies, Inc. NN and CW declare no conflicts.

24. Zilber AL, Belli P, Grezdel D, Antois M, Kodjo A, Djelouadi Z. Comparison of Mucosal, Subcutaneous and Intraperitoneal Routes of Rat Leptospirosis Infection. PLoS neglected tropical diseases. 2016; 10(3): e0004569. https://doi.org/10.1371/journal.pntd.0004569 PMID: 27031867; PubMed Central PMCID: PMC4816568.

25. Wunder EA Jr., Figueira CP, Santos GR, Lourdault K, Matthias MA, Vinetz JM, et al. Real-Time PCR Reveals Rapid Dissemination of Leptospira interrogans after Intraperitoneal and Conjunctival Inoculation of Hamsters. Infection and immunity. 2016; 84(7):2105–15. https://doi.org/10.1128/IAI.00994-16 PMID: 27141082; PubMed Central PMCID: PMC4936353.

26. Asoh T, Saito M, Villanueva SY, Kanemaru T, Gloriani N, Yoshida S. Natural defense by saliva and mucosa against oral infection by Leptospira. Canadian journal of microbiology. 2014; 60(6):383–9. https://doi.org/10.1139/cjm-2014-0016 PMID: 24861456.

27. Iwasaki A. Mucosal dendritic cells. Annu Rev Immunol. 2007; 25:381–418. https://doi.org/10.1146/annurev.immunol.25.022106.141634 PMID: 17378762.

28. Flajnik MF. Advances in immunology. BioEssays: news and reviews in molecular, cellular and developmental biology. 1994; 16(9):671–5. https://doi.org/10.1002/bies.950160913 PMID: 7990494.

29. Davies DE. Epithelial barrier function and immunity in asthma. Annals of the American Thoracic Society. 2014; 11 Suppl 5:S244–51. https://doi.org/10.1513/AnnalsATS.201407-304AW PMID: 25525727.

30. Swindle EJ, Collins JE, Davies DE. Breakdown in epithelial barrier function in patients with asthma: identification of novel therapeutic approaches. The Journal of allergy and clinical immunology. 2009; 124(1):23–34; quiz 5–6. https://doi.org/10.1016/j.jaci.2008.12.006 PMID: 19560576.

31. Murray GL, Srikram A, Hoke DE, Wunder EA, Henry R, Lo M, et al. Major Surface Protein LipL32 Is Not Required for Either Acute or Chronic Infection with Leptospira interrogans. Infection and immunity. 2013; 81(9):2768–76. https://doi.org/10.1128/IAI.00531-13 PMID: 23690405; PubMed Central PMCID: PMC3719587.

32. Croda J, Figueira CP, Wunder EA Jr., Santos CS, Reis MG, Ko AI, et al. Targeted mutagenesis in pathogenic Leptospira species: disruption of the ligB gene does not affect virulence in animal models of leptospirosis. Infection and immunity. 2008; 76(12):5826–33. https://doi.org/10.1128/IAI.00999-08 PMID: 18809657; PubMed Central PMCID: PMC2583667.

33. King AM, Bartpho T, Sermswan RW, Bufal DM, Eshghi A, Picardeau M, et al. Leptospiral outer membrane protein LipL41 is not essential for acute leptospirosis but requires a small chaperone protein, lep, for stable expression. Infection and immunity. 2013; 81(8):2768–76. https://doi.org/10.1128/IAI.00531-13 PMID: 23690405; PubMed Central PMCID: PMC3719587.

34. Andrade L, Rodrigues AC Jr., Sanches TR, Souza RB, Seguro AC. Leptospirosis leads to dysregulation of sodium transporters in the kidney and lung. American journal of physiology Renal physiology. 2007; 292(2):F586–92. https://doi.org/10.1152/ajprenal.00102.2006 PMID: 16940563.

35. Sitprija V, Pipatanagul V, Mertowidjjo K, Boonpunknavig V, Boonpunknavig S. Pathogenesis of renal disease in leptospirosis: Clinical and experimental studies. Kidney international. 1980; 12E 2. https://doi.org/10.1002/9780471729259.mc12e02s02 PMID: 18770517; PubMed Central PMCID: PMC4442676.

36. Erdogan HM, Karapehli van M, Citil M, Atakisi O, Uzlu E, Unver A. Serum sialic acid and oxidative stress parameters changes in cattle with leptospirosis. Veterinary research communications. 2007; 32(4):333–9. https://doi.org/10.1007/s11259-008-9036-y PMID: 18247150.

37. Haake DA, Levett PN. Leptospirosis in humans. Current topics in microbiology and immunology. 2015; 387:65–97. https://doi.org/10.1007/978-3-662-45059-8_5 PMID: 25388133; PubMed Central PMCID: PMC4442676.

38. Haake DA. Hamster model of leptospirosis. Current protocols in microbiology. 2006; Chapter 12:Unit 12E 2. https://doi.org/10.1002/9780471729259.mc12e02s02 PMID: 18770576; PubMed Central PMCID: PMC2667198.

39. Al-Harbi NO, Nadeem A, Ahmad SF, Alansari MM, Aldossari AA, Alasmari F. Amelioration of sepsis-induced acute kidney injury through inhibition of inflammatory cytokines and oxidative stress in dendritic cells and neutrophils respectively in mice: Role of spleen tyrosine kinase signaling. Biochimie. 2019; 158:102–10. https://doi.org/10.1016/j.biochi.2018.12.014 PMID: 30599182.

40. Jiang J, Du H, Su Y, Li X, Zhang J, Chen M, et al. Nonviral infection-related lymphocytopenia for the prediction of adult sepsis and its persistence indicates a higher mortality. Medicine. 2019; 98(29):e16535. https://doi.org/10.1097/MD.00000000000016535 PMID: 31335735; PubMed Central PMCID: PMC6708870.