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Clinical Evidence of Lyme Disease in Dogs and Disease Awareness Among Students and Veterinarians in Veracruz, Mexico

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ABSTRACT

Background: Lyme disease is a relatively new and zoonotic canine pathology mostly unknown by people involved in the management and care of dogs. Objective: to assess the knowledge about Lyme disease by veterinarians and veterinary students in Veracruz, Mexico. Methodology: three questionnaires were designed and applied to 290 individuals (40 small animal veterinarians, 50 in other professional practice and 200 veterinary students). Results and discussion: in general, the three groups were unaware about the disease, although there is a high interest in learning about this problem. Graduates from seven universities included in this study stated that Lyme disease was not part of the veterinary curriculum in their schools. Five clinical cases suggestive of Lyme disease occurring in dogs in the area were detected at the time of this research. Conclusion: although the presence of the agent causing Lyme disease has not been demonstrated in the studied area due to the lack of laboratory support and the apparent absence of the vector, it cannot be ruled out; furthermore, it is important that veterinary practitioners and student alike be aware of the potential presence of Lyme disease, paying particular attention to differential diagnosis when resembling clinical signs are observed.

1. Introduction

Lyme disease is a common zoonosis in the United States and Europe, where it can represent up to 90% of infections transmitted by ticks [1-4]. The disease is caused by *Borrelia burgdorferi*, which is transmitted by the bite of ticks that are usually parasite different animal species. The disease causes different signs affecting skin, joints, cardiovascular and central nervous system [5].

Mexico has identified the vector *Ixodes* in Baja California, the Yucatan Peninsula, the Gulf of Mexico and states in the northeast of the country. Out of 26 *Ixodes* species

found in Mexico, only six have been identified in the state of Veracruz, without any proven act as vector of *Borrelia burgdorferi* [6]. It is possible that migratory birds infested with *Ixodes* carrying this germ can spread the problem [7-8], also, dogs that participate in national and international exhibitions can be a vehicle. In addition, although the presence of *Ixodes dammini*, the main vector of transmission demonstrated *B. burgdorferi*, has not be identified in the state of Veracruz, it is possible that other species of *Ixodes* or even other vectors may play a role in the spread of disease [6].

Some cases suggestive of Lyme borreliosis were de-

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scribed in the early 1990s in the Mexican states of Sinaloa and Nuevo Leon, but aetiological confirmation was not achieved [9]. In recent years, several positive people living in Mexico City, and in northern states such as Nuevo Leon, Tamaulipas and Coahuila were identified achieved [10]. Also, a 8.2% seroprevalence to Lyme disease was reported in dogs from Mexicali, along the border with the United States of America [11], and in Monterrey, Nuevo Leon, a seroprevalence of 1% in dogs was recognized by Meléndez Salinas *et al.* (2011) [12].

Lyme disease is considered a foreign animal disease in Mexico and there is no documented evidence of clinical cases in the state of Veracruz, because no investigation has been conducted. Therefore, the aim of this study was to determine the knowledge about Lyme disease that practitioners and students of Veterinary Medicine possess, as well as the possible existence of clinical cases suggestive of Lyme borreliosis in dogs of the Veracruz metropolitan area in Mexico.

2. Methodology

2.1 Location

The city of Veracruz is located along the coast in the central part of the state of Veracruz, Mexico and with the neighboring municipality of Boca del Río has over 600,000 inhabitants. The climate is tropical warm, with an average annual temperature of 26 °C and an average annual rainfall of 1500 mm. Most vegetation is of the rainforest type.

2.2 Study Design

To obtain the necessary data for this study, three different types of stakeholders who could provide information were considered, namely veterinary undergraduate students, small animal practitioners, and veterinarians in other professional fields. Private veterinary clinics were visited to inquire about clinical cases suggestive of Lyme disease. Also secondary information about the disease was acquired through literature review.

For each stakeholder type, a specific questionnaire was designed to obtain the necessary data. The questionnaire aimed to veterinarians tried to identify how well the veterinary profession was informed about Lyme disease and its biological cycle, years of clinical experience, the existence of clinical cases and its diagnosis and treatment, among other variables. The questionnaire aimed to the student community included variables such as: year of admission, species on which it has a predilection, definition of zoonosis, knowledge about Lyme disease as well as of the causative agent and vectors, clinical manifestations, affected species, treatment and interest about the disease.

2.3 Sampling

A convenience sampling was undertaken to collect information from veterinarians working in the Veracruz – Boca del Río metropolitan area. For veterinary undergraduate students, the local veterinary school was visited and students willing to participate in the study were selected. The veterinary medicine program encompasses five years of education and every year 150 new students are admitted representing about 750 in total.

Each questionnaire was validated with a small number of members from each group before being applied to the whole sample

2.4 Data Analysis

Data were collected using a standardized form and were tabulated into a Microsoft Excel spreadsheet. Descriptive statistics were used to summarize information.

3. Results and Discussion

The study aimed to differentiate the knowledge and awareness existing about Lyme disease among various interest groups, namely undergraduates in veterinary medicine, veterinarians enrolled in small animal practices and veterinarians devoted to other species and professional activities.

3.1 Students Survey

Out of 200 students surveyed, 98 were women and 102 men. Table 1 condenses information about them. The percentage of students aware of Lyme disease was calculated based on the number of students per class, not on the total respondents. Therefore, students who recently entered the university first had less awareness about Lyme disease than those who entered it later. This little awareness in the student community might result from the fact that Lyme disease is not a part of veterinary curriculum, so those who reported having some knowledge about the disease, have obtained it as a result of their personal curiosity.

Table 1. Sample composition of veterinary medicine students surveyed and their awareness about Lyme disease in Veracruz, Mexico

Class	Students, No. (%)	Students aware of Lyme disease, No. (%)
First year	18(9)	4 (4.44)
Second year	41(20.5)	5(24.4)
Third year	54(27)	5 (18.5)
Fourth year	45(22.5)	3(13.3)
Fifth year	42(21)	3(14.3)

As in all academic activity, theoretical knowledge is the primary basis of students education. Out of 200 students only 20 (10%) are aware of Lyme disease. However, even if Lyme disease is considered an "exotic" disease which is not a public health problem today in Mexico, it is important to consider the risks that such zoonoses represent and take into consideration the potentially affected species, clinical signs, and the way of transmission, among other variables.

The value of information to the veterinary student community should be highlighted, since *Borrelia burgdorferi* is an agent which is important to identify, prevent and treat by the future veterinary professionals. Out of the 20 students who said they had information about Lyme disease and its characteristics, 16 people were right in terms of the causal agent, while the other four said that the agent is a rickettsia. In fact, *Borrelia* is a gram-negative bacterium, obligate anaerobe with periplasmic flagella that produce a spinning movement which allows the whole bacterium to move forward like a corkscrew^[13]. Instead, Rickettsiae are, gram-negative and immobile anaerobes germs. Of this students, 16 (80%) are aware of the role of Ixodes tick as transmitting vector, while four students (20%) ignored it. The bacterium that causes Lyme disease, *Borrelia burgdorferi*, is present in other types of ticks, but it is only transmitted to humans and animals by Ixodes ticks. Vector of transmission in most of United States is *Ixodes scapularis*, the black-legged tick, while on the west coast of this country is *Ixodes pacificus*^[14, 15]. In Europe the vector is *Ixodes ricinus*^[16], in Australia *Ixodes holocyclus*^[17], and in Asia *Ixodes persulcatus*^[18].

Students were asked to mention at least four clinical manifestations of Lyme disease, whether in humans, dogs or other animal species which may be attacked by the causative agent. This question was only answered by 20 students with knowledge of Lyme borreliosis. Of these, eight (40%) mentioned the four clinical manifestations, five students (25%) cited only three events, five students (25%) cited only two, and two students (10%) only one.

Although students considered themselves to possess knowledge about the disease, some of them named clinical signs unrelated to Lyme disease pathology such as: acute attack to intestines, blood system blockage, spots on the skin throughout the body, "bone pain", epistaxis, jaundice and pyrexia, which shows that there is consistency between the opinion of the group of students who think they know about Lyme disease and have actual knowledge thereof. By the same token, when asked to name three species affected, out of 20 students who considered to be familiar with the disease, the affected species named were: dogs, cats, cattle, sheep, horses; bovine got the highest

number of mentions. According to them, the numbers of affected species were: one species (10%), two species, 5 (25%), three species, 13 (65%).

In endemic areas of Lyme disease and surrounding areas, it has been noted that several species of domestic animals (dogs, horses and bovine cattle) are infected with *B. burgdorferi*^[19, 20, 21]. In Nuevo Leon State, Mexico Lyme disease has been found in humans, dogs and deers^[22, 23, 24], however the disease can be developed in other animal species because the infection with *B. burgdorferi* has been recognized in several other species all over the world.

Like in any other disease, in the case of Lyme disease is not only important to possess a general knowledge about the disease and its vector, but also about the treatments that could be administered early. This is critical because an early treatment allows for a complete cure while if treatment is not started right away, chances of total healing reduce. It is relevant to be informed of proper drugs to be used for each disease stage and the species concerned. Out of the 20 students who expressed to be aware of Lyme disease, 14 (70%) ignored treatment and only 6 (30%) reported to know about it. Of the latter, some students rightly mentioned some antibiotics such as doxycycline, penicillin, amoxicillin and ceftriaxone^[25], but equally some respondents wrongly believe that the use of antiemetics and cefalexinas help cure the disease. Indeed, some students mentioned tick control or euthanasia as a treatment, even though this is not considered a proper treatment, but techniques to prevent the disease spreading.

As mentioned before, it is vital that after concluding their studies the new veterinary professional have the necessary knowledge on the different subjects related to veterinary medicine practice. From the perspective of the surveyed students, only 5% of them all believe that it is not necessary to acquire knowledge about Lyme disease, while most do.

3.2 Survey to Mixed Practice Veterinarians

Out of 50 veterinary doctors enrolled in mixed practice or dealing with animal species other than dogs and cats that participated in the present study, 72% (36) were men. The highest proportion of participants corresponded to male individuals in the range of 20 to 30 years. When considering the percentage of knowledge in relation to gender and age, the aforementioned group exhibited less awareness about Lyme disease than veterinarians located in the 41 to 50 years bracket, who showed that they are more aware about the problem (66%). Female participants, despite representing a smaller proportion of the sample, showed a higher proportion of Lyme disease awareness (Table 2). Overall, of 36 men surveyed, only nine (25%) said they

were aware about Lyme disease and of 14 women surveyed, 5 (36%) have notions on this condition. Regarding the professional experience, the majority of participants were in a range between 20-30 years (40%), although this age group showed less awareness than others; only three (15%) out of 20 exhibited understanding about Lyme borreliosis. In general, groups between 31-40 years and 51-60 years acknowledged to ignore everything about the disease. On the other hand, people in the 41-50 years and 61-70 years brackets said they had more information about the disease. As a result, although those with less knowledge of the subject are among the youngest age groups, the understanding of Lyme disease is not related to years of professional experience.

Table 2. Veterinarians enrolled in mixed practice or in other professional fields participating in the survey about awareness of Lyme disease in Veracruz, Mexico

Age, years	Professional experience, years	Sample composition			Awareness of Lyme disease	
		Male	Female	Total	Male	Female
20-30	0-6	12	8	20	0	3
31-40	0-15	3	3	6	1	1
41-50	0-25	6	0	6	4	0
51-60	0-30	9	3	12	1	1
61-70	15-46	6	0	6	3	0
Total		36	14	50	9	5

Most veterinary doctors participating in this survey were professors teaching diverse subjects in the local veterinary school, as well as some veterinary graduate students. Most respondents (45, 90%) were graduates from the University of Veracruz of which 12 (26%) were aware about Lyme disease; three (6%) were graduates of the Mexico's National Autonomous University, of which two were aware of the subject (66%). There was also one graduate from the Mesoamerican University and one from the Higher School of Veterinary Medicine and Animal Science; only the veterinarian graduated from the later school had information on Lyme disease.

The number of species to which each veterinarian is dedicated seems to be related to the knowledge about the disease, because those who work with a greater number of species seem to have a better understanding of Lyme disease than those more specialized (Table 3). In relation to education, most respondents (36%, 18/50) have opted for a master's degree to support their professional work; 28% (14/50) have done so by continuous education courses, 18% (9/50) through two or more different training, while 14% (7/50) hold a doctorate. Professionals with specialization studies represented the smallest amount among the

respondents, 4% (2/50).

Table 3. Animal species to which veterinarians enrolled in mixed practice are devoted in the Lyme disease survey in Veracruz, Mexico

Specie(s)	Number of veterinarians	Number aware of Lyme disease, No. (%)
Bovine	8	1(12.5)
Swine	4	1(25)
Poultry	2	0
Wildlife	2	0
Dogs and cats	8	37
Bovine and equine	3	66
Sheep and goats	4	0
Bovine and bees	1	0
Bovine, sheep and goats	4	25
Bovine, dogs and cats	3	33
Equine, sheep and goats	1	0
Equine, dogs and cats	1	0
More than four animal species	4	50
More than five animal species	5	60

Out of the 50 veterinarians enrolled in mixed practice or in other professional fields who collaborated in the study, 14 (28%) said they were aware Borreliosis, although four of them said they did not remember the name of the causative agent, while 72% (36/50) admitted they had no knowledge about it. As mentioned earlier, even though Borreliosis still does not represent a public health problem in Mexico, Lyme disease is considered as an endemic disease in many parts of the world. Gordillo-Pérez *et al.* (2003) investigated in 2346 sera the presence of *Borrelia burgdorferi* in Mexico City and northeast Mexico, detected by ELISA IgG antibodies against *Borrelia burgdorferi* and confirmed them by Western blotting. They found a seroprevalence of 3.43% in Mexico City and 6.2% in the northeast area of the country, being Tamaulipas the state with the highest seroprevalence ^[10].

As for the knowledge about the borreliosis transmission vector, out of 14 people who claim to possess knowledge about the disease, only nine have the notion that the genus Ixodes tick acts as a disease carrier. Unlike what was observed in the students survey, all veterinarians who responded knowing about the disease noted down the four clinical manifestations that were required in the survey about their knowledge on Borreliosis; For the purposes of such questioning, all veterinary doctors understand the clinical manifestations of the disease, however and in the same way as with the students, some clinical signs unrelated to the pathology were mentioned such as: jaundice, ascites, respiratory distress and pale mucous membranes.

As for the questioning on the three species affected by the spirochete *Borrelia burgdorferi*, they were mentioned: human, deer and rat, except for an answer which wrongly included the bovine.

Regarding the adequate treatment of the disease, seven doctors correctly commented on tetracycline, while two others advised doxycycline. The same problem seen with students was repeated, since some people commented that as a part of treatment the vector should be controlled, while this is a preventive measure and not recovery one. Two other veterinary doctors are unaware of the treatment, while vaguely, one respondent commented that antibiotics should be used, to end the recommendation of use an “antiprotozoal such as sulfonamides or praziquantel” (sic).

Ninety-four percent (47/50) of the surveyed veterinarians were interested in knowing more about the disease and its importance for the veterinary community, while the remaining 6% (3/50) believe that this would not provide any benefit due that this disease is unrelated to the animal species they work with, and that it would only be important if the pathogen attacked their animal species of interest.

3.3 Survey Carried Out in Small Animal Veterinary Clinics

Out of 40 veterinary doctors dedicated to small animal clinics that participated in the survey, the majority (60%) were men (Table 4). Overall, a greater proportion of men (62.5%, 15/24) than women (56.25%, 9/16) said they were familiar with the disease. The largest number of respondents aware of Lyme disease were in the range between 41 and 50 years (89% 8/9) in the case of men, and in the category between 31 and 41 years (56 %, 5/9) in the case of women. In relation to professional experience, as age and experience of participants increased it was observed a greater proportion of participants aware of Lyme disease.

Table 4. Veterinarians enrolled in small animal practice participating in the survey about awareness of Lyme disease in Veracruz, Mexico

Age, years	Professional experience, years	Sample composition			Awareness of Lyme disease	
		Male	Female	Total	Male	Female
20-30	1-5	8	2	10	3	2
31-40	3-18	3	9	12	2	5
41-50	15-27	9	5	14	8	2
51-60	25-35	4	0	4	2	0
Total		24	16	40	15	9

Participants in the survey were veterinary professionals graduated from six different universities, of which the ma-

majority (85%, 34/40) came from the University of Veracruz. Among these, the proportion of those who were aware of Lyme disease was 55.9% (19/34) (Table 5).

Table 5. Lyme disease awareness of veterinarians enrolled in small animal practice in Veracruz, Mexico based on their university of graduation

University of graduation	Graduated veterinarians, No.	Awareness of Lyme disease, No.
Benemeritous Autonomous University of Puebla	1	1
Mesoamerican University, Campus Puebla	1	0
Metropolitan Autonomous University	1	1
Zacatecas Autonomous University	1	1
Mexico's National Autonomous University	2	2
University of Veracruz	34	19
Total	40	24

In relation to the professional update of veterinarians specializing in small species, most resort to continuous education courses (62.5%, 25/40), and to a lesser degree to diplomate programs (7.5%, 3/40), specialty training (2.5%, 1/40) or doctorate (2.5%, 1/40); while there is a good proportion (25%, 10/40) that opt for two or more of the above options. The lowest level of knowledge about Lyme disease understanding was found among veterinarians who opted only for the continuous education courses, despite representing the highest proportion of respondents.

Considering differential diagnosis of Lyme disease, small animal specialists mentioned: dilofiriasis, erlichiosis, anaplasmosis, among others. As a result, five doctors expressed seeing neurological alterations, uveitis and dermatitis in a single patient, and 14 selected three or more options, which included: severe lameness, neurological abnormalities, dermatitis, acute joint inflammation, uveitis and nephropathies; four clinicians reported myocarditis with arrhythmia, neurological disorders, dermatitis and uveitis, choosing two to three manifestations per patient. Consecutively to these manifestations, veterinary doctors determined as diagnosis in a higher percentage erlichiosis (18, 45%). Among the diseases not included in the questionnaire, the clinical doctors added that the manifestations were due to conditions such as: ligament injury (this in an animal which not only had severe lameness but also neurological alterations), mycosis, intervertebral discs disease, pyoderma, atopy, flea bite dermatitis, septicemia due to surgery, but especially conditions related to old-age. Canine anaplasmosis (5, 13%) was related to conditions such as neurological disorders, uveitis, dermatitis, acute joint inflammation and severe lameness. It is noteworthy that of the five veterinarians who made such a diagnosis,

four said they knew about Borreliosis, four felt that their diagnosis was *dilophilariasis*, and three of them related the observed clinical signs to acute joint inflammation. There was no relationship between the conditions mentioned and Lyme disease was not considered in any of the patients, even in those clinics where it was said to have had Lyme disease cases in the past. Lyme disease manifests itself as a multi-inflammatory disease; that is, it affects the skin and spreads to the joints, causes abnormalities in the cardiac and nervous systems, and in its most advanced stage can affect other organs^[26].

As mentioned before, out of 40 participants dedicated to small animal practice, 24 clinicians say they were aware of the disease (60%), but their knowledge was not applied in any diagnosis. Indeed, it is difficult to establish an early diagnosis of Lyme disease in animals. The characteristic skin lesion and chronic migratory erythema do not occur in dogs^[5]; in addition, Lyme disease is not considered as an endemic disease in the study region, and thus is not usually considered in differential diagnosis, even though some clinical signs are similar to other conditions, and the fact that the disease in canines can go for a long time without showing any signs.

About the vector, four veterinarians commented correctly about *Ixodes* tick; however, there is no certainty that *Ixodes dammini*, *Ixodes ricinus* and *Ixodes scapularis* exist in Mexico^[6]. Montiel *et al.* (2007) reported the presence of a male, seven larvae and 15 nymphs of *Ixodes cookei* in the state of Veracruz in a Natural Protected Area at a height of 2420 masl, and in Nuevo León in the feces of *Urocyon cinereoargenteus* and *Peromyscus* sp^[27].

Out of the 40 participating veterinary doctors, five clinical cases suspected of Lyme disease were detected in two veterinary clinics, one located in the municipality of Veracruz which provided a case, and another clinic located in the neighbor municipality of Boca del Rio, which provided four additional clinical cases. Lyme disease clinically suspected cases were tested by a commercial diagnostic kit. One of the most confusing aspects in the Lyme disease diagnosis has been testing, since a positive result by itself does not constitute a diagnosis of Lyme disease; moreover, the commercial kit is based on the ELISA serological technique, which provides the veterinarian with data regarding three more vector-borne diseases that affect dogs: canine *erlichiosis*, *dirofilariasis* and canine *anaplasmosis*^[28]. The history of infection with ticks or contact with ticks, along with tick biting and suggestive clinical signs is of great importance to make a presumptive diagnosis of Lyme disease^[29,30]. Apparently, it is easier to isolate the agent from skin lesions than from blood. Indirect immunofluorescence tests with sera conjugated

with IgM and IgG are widely used^[31]. Patients with late disease manifestations (arthritis, cardiac or neurological abnormalities) show elevated IgG titers^[32]. Indirect ELISA test was more sensitive and specific than the immunofluorescence test^[33]. In Mexico, the presence of *Ixodes* ticks has not been established yet and laboratory tests for Lyme disease such as Western blotting are not extensively available in veterinary clinics, as a consequence it cannot be determined with total certainty that suspected clinical cases are positive, since with available diagnostic tests a positive result may be due to cross reactions.

All veterinarians enrolled in small animal practice in Veracruz, Mexico want to know more about Lyme disease and consider more information about this topic should be shared with the veterinary community. In study conducted in British Columbia to determine physicians' level of awareness and knowledge of Lyme disease in a low-prevalence area, Henry *et al.* (2012) found that physicians were knowledgeable about the clinical signs and symptoms of Lyme disease and aware of the risk of the disease despite being in a low-endemic area and suggested that raising awareness among physicians that Lyme disease is reportable might improve reporting of future cases^[34]. As Cripps (2000) states, this situation may be exacerbated where there is inadequate communication between veterinarians, the various health care professionals and public health organisations. Undergraduate and postgraduate training courses must promote a greater understanding of the importance of zoonoses and of how to investigate and control them. A huge area of opportunity is perceived to increase awareness amongst qualified veterinary personnel and human health care workers and to facilitate inter-disciplinary discussions and collaborative ventures^[35]. The re-emergence of zoonoses, together with other issues such as bioterrorism, pollution incidents, antimicrobial resistance, xenotransplantation, among other factors, make urgent a collaborative interprofessional approach to veterinary public health^[36].

In conclusión, awareness of Lyme disease is almost null among the veterinary student community and veterinarians enrolled in mixed practice or in other professional fields, but they are open to be educated on Lyme disease. All veterinarians enrolled in small animal practice showed interest in learning more about Lyme disease and stressed the importance of managing adequate information within the veterinary profession, but none included it in their differential diagnosis of suspected patients, even when signology in some sick animals is consistent with Lyme disease.

In the region of Veracruz, Mexico there are not official reports on presence of Lyme disease and its vector; thus, this disease is not included in any subject of the current

curriculum at the local School of Veterinary Medicine. Since five cases suggestive of borreliosis in dogs were determined based on clinical examination and ELISA testing, it is suggested to carry out more studies to confirm the presence of Lyme disease and its vector, to educate veterinary students and veterinarians alike on the disease, and to review the curriculum of the local Veterinary Medicine School to increase awareness.

Conflict of Interest

The authors declare that they have not any conflict of interest.

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