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"Estudio epidemiológico de *Borrelia burgdorferi* transmitida por *Rhipicephalus sanguineus* en perros de Mexicali, Baja California, México"

T E S I S

PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS

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RESUMEN

La enfermedad de Lyme es causada por *Borrelia burgdorferi* y trasmitida por garrapatas, principalmente *Ixodes scapularis* e *I. pacificus*. Está caracterizada por desórdenes polisistémicos y es zoonótica. En México la enfermedad de Lyme ha sido reportada en animales y en humanos. El objetivo de este estudio fue estimar la seroprevalencia de *B. burgdorferi*, los factores de riesgo asociados y demostrar su transmisión a través de la garrapata *Rhipicephalus sanguineus* en perros de la ciudad de Mexicali. Se seleccionaron aleatoriamente 384 muestras sanguíneas de perros atendidos en clínicas veterinarias y 384 de perros capturados por los centros de control animal. Los sueros fueron analizados con el kit *B. burgdorferi* ELISA® Helica Biosystems, Inc. La seroprevalencia fue de 6.8% (95% IC 3.5-8.9%) en perros de clínicas veterinarias y de 12% (95% IC 7.5-14.3%) en perros de los dos centros de control animal. La seroprevalencia de ambos grupos fue de 9.3% (95% IC 6.3-10.6). La prevalencia en este estudio fue baja comparada con la de Monterrey (16%) en donde el principal vector ha sido *I. scapularis*, y en Sao Paulo, Brazil (15.6%) donde el principal vector ha sido *Amblyomma cajennense*. Los factores de riesgo evaluados resultaron relacionados con la edad, y la ausencia de un programa preventivo. Se demostró que los perros ≥ 1 año de edad tiene mayor riesgo que los menores con OR= 2.7 (95% IC 1.2-6.1) y los que no tuvieron acceso a un programa preventivo, el cual consiste de al menos 2 tratamientos desparasitantes, 2 baños garrapaticidas y 2 fumigaciones en el hogar al año, con OR= 4.9 (95% IC 1.4-16.8). No se comprobó la transmisión de *B. burgdorferi* por garrapatas adultas de *R. sanguineus* en perros utilizando el protocolo experimental para *I. scapularis*; pero se encontraron evidencias moleculares de *B. burgdorferi* en perros y en garrapatas *R. sanguineus*.

Palabras clave: *Borrelia burgdorferi*, garrapata, enfermedad de Lyme disease, borreliosis

ABSTRACT

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi* and transmitted by ticks, primarily from *Ixodes scapularis* and *I. pacificus*. It is characterized by polisystemic disorders. In Mexico, Lyme disease has been reported in humans and animals. In dogs *B. burgdorferi* infection has been also reported in several areas of the country. The aim of this study was to estimate the seroprevalence of *B. burgdorferi*, associated risk factors and the transmission by tick *Rhipicephalus sanguineus* in dogs of the city of Mexicali. Randomly blood samples of dogs were selected of 384 dogs attended in private veterinary clinics and in 384 dogs captured by the animal control centers. The sera were analized by the kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc. An adjusted prevalence of 6.8% (95% CI 3.5-8.9%) was obtained in dogs from veterinary clinics and of 12% (95% IC 7.5-14.3%) in dogs from animal control centers. The seroprevalence of both groups was 9.3% (95% CI 6.3-10.6). The seroprevalence in this study was lower compared to those in Monterrey (16%) where the principal vector was *I. scapularis*, and in Sao Paulo, Brazil (15.6%) where the principal vector was *Amblyomma cajennense*. Risk factors associated with *B. burgdorferi* seropositivity were age, OR= 2.7 (95% CI 1.2-6.1), and the absence of a preventive program, OR= 4.9, (95% CI 1.4-16.8). The transmission of *B. burgdorferi* was not demonstrated by adult ticks of *R. sanguineus* in dogs using the experimental protocol for *I. scapularis*; but found molecular evidences of *B. burgdorferi* in dogs and ticks *R. sanguineus*.

Keywords**:** *Borrelia burgdorferi*, tick, Lyme disease, borreliosis.

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ANTECEDENTES

Definición y etiología de borreliosis

La borreliosis conocida como enfermedad de Lyme es una zoonosis producida por los diversos genotipos del complejo *Borrelia burgdorferi* sensu lato, es de distribución mundial y transmitida por garrapatas (Liveris *et al*., 1999; Escudero *et al*., 2000; Thomas *et al*., 2001; Qiu *et al*., 2002; Yoshinari *et al*., 2003). La mayoría de estas espiroquetas miden de 20 a 30µ de largo por 0.2 a 0.3µ de ancho y tiene de 7 a 11 flagelos (Barbour and Hayes, 1986; Greene *et al*., 2000).

Borrelia burgdorferi es capaz de infectar a roedores, ciervos, perros, gatos, vacas, caballos, reptiles, aves y a varias especies de garrapatas, además de afectar al humano (Faul *et al*., 1999; Straubinger, 2000). Se ha demostrado que el perro es el más importante reservorio de esta espiroqueta en el ambiente domiciliario (Straubinger, 2000; De Lacerda *et al*., 2004).

La borreliosis se describió por primera vez en el continente americano en la comunidad de Lyme, Connecticut, E.U.A, en 1972 (Steere *et al*., 1977) y el agente etiológico fue aislado por primera vez por Willy Burgdorfer en 1982 (Burgdorfer, 1984; Dunlop and Williams, 1996; Manley, 1996). Hay antecedentes en Europa que datan desde 1910 por el dermatólogo sueco Arvid Afzelius (Afzelius, 1910) y 12 años después 2 médicos franceses C. Garin y R. Bujadoux reportan una enfermedad rara que afectaba la piel y otros órganos, causaba parálisis y al parecer era provocada por picaduras de garrapatas (Garin and Bujadoux, 1922).

En 1983 Johnson demostró que este microorganismo era una nueva especie de espiroqueta del género *Borrelia* y propone el nombre de *Borrelia burgdorferi* en honor a su descubridor Willy Burgdorfer (Johnson *et al*., 1984).

El complejo *B.burgdoferi* sensu lato comprende genoespecies que están estrechamente relacionadas, pero genéticamente son diferentes. En Estados Unidos de América la enfermedad de Lyme es producida por *B. burgdorferi* sensu stricto. En Europa el complejo *B. burgdorferi* sensu lato es representada por 5 genoespecies distintas: *B. burgdorferi* sensu stricto*, B. afzelli, B. garinii, B. valaisiana* y *B. lusitaniae*. Cada una provoca manifestaciones clínicas distintas en el humano (Berglund *et al*., 1995; Escudero *et al*., 2000; Derdakova *et al*., 2003). En Japón el complejo está representado por *B. japonica* (Kawabata *et al*., 1993).

Se han encontrado coinfecciones con diferentes genoespecies de *B. burgdorferi* y con especies de los géneros *Ehrlichia*, *Bartonella* (Varde *et al*., 1998; Schouls *et al*., 1999) y *Babesia* (Yoshinari *et al*., 2003).

Han sido descritas 6 diferentes proteínas de la superfice externa (Osp) de *B. burgdorferi*, OspA, OspB, OspC, OspD, OspE y OspF. Cuando no se alimenta la garrapata infectada, las espiroquetas producen en su mayoría OspA y rara vez OspC. Una vez que la garrapata empieza a alimentarse muchas de estas espiroquetas dejan de producir OspA y producen gran cantidad de OspC, que es más inmunogénica (Wilske *et al*., 1993; Wang *et al*., 1999a; Wang *et al*., 1999b; Schwan and Piesman, 2000). Está demostrado que estas proteínas determinan la

inmunopatogénesis de *B. burgdorferi* (Salinas-Meléndez *et al*., 1995; Dickinson-Meneses and Batlle-Almodóvar, 1997; de Silva *et al*., 1999; Piesman *et al*., 2001).

Transmisión

La transmisión ocurre cuando la espiroqueta *B. burgdorferi* es inoculada durante la alimentación de sangre del huésped por ciertos géneros y especies de garrapatas infectadas, especialmente *Ixodes scapularis* (Steere *et al*., 1983; Burgdorfer, 1984; Burgdorfer *et al*., 1985; Varde *et al*., 1998; Liveris *et al*., 1999; Escudero *et al*., 2000; Thomas *et al*., 2001; Qiu *et al*., 2002; Yoshinari *et al*., 2003), hasta en un 90% de los casos, aunque se ha demostrado que también puede ser transmitida por *I. pacificus* (Burgdorfer, 1984; de Silva *et al*., 1999)*, I. ricinus* (Ramamoorthy and Philipp, 1998), *I. neotomae* (Lane and Pascocello, 1989), *Amblyomma americanum* y *Dermacentor variabilis, D. occidentalis* (Spach *et al*., 1993); pero actualmente no se ha encontrado a *Rhipicephalus sanguineus* como vector de esta espiroqueta (Filippova, 1990; Miserez *et al*., 1990; Luckhart *et al*., 1991; Kurtti *et al*., 1993; Munderloh *et al*., 1993; Bernasconi *et al*., 1997; Walker *et al*., 1998; Bouattour *et al*., 1999; Costa *et al*., 2002; Sanogo *et al*., 2003; Baptista *et al*., 2004; Nebreda Mayoral *et al*., 2004; Merino *et al*., 2005).

Las garrapatas son vectores de gran importancia en el mundo, en la transmisión de enfermedades a los animales y al hombre. Son parásitos obligados que requieren succionar sangre de huéspedes para subsistir. Pueden transmitir con una sola picadura, diversos patógenos tales como: bacterias, espiroquetas, riquetsias, protozoarios, virus, nematodos y toxinas. Dentro de las enfermedades más comunes transmitidas por la mordedura de las garrapatas, son la enfermedad de Lyme, erliquiosis monocítica, granulocítica y trombocítica, babesiosis, fiebre de las montañas rocosas, fiebre de la garrapata de Colorado, tularemia, fiebre Q, parálisis por garrapata, fiebre botonosa y encefalitis por garrapata. Además las garrapatas pueden dar lugar a infecciones secundarias y reacciones alérgicas a la proteína de la saliva que producen (Spach *et al*., 1993; Edlow, 1999).

En condiciones naturales, las ninfas de *I. scapularis* son los principales vectores de *B. burgdorferi* sensu stricto en Norteamérica (Piesman, 1991; Piesman *et al*., 2001); pero experimentalmente en perros los estadios adultos de la misma especie son más eficientes en la transmisión que las ninfas (Korshus *et al*., 2004). Cabe mencionar que no todas las garrapatas tienen la misma facultad de transmitir esta espiroqueta, se demostró que *I. scapularis* es 3.6 veces más eficiente que *I. pacificus* en transmitir *B. burgdorferi* y en mantener la infección (Piesman, 1993).

La enfermedad de Lyme es trasmitida por garrapatas, aunque sólo en el 25% en los casos en humanos ha habido historia de mordedura de garrapata (Klempner *et al*., 2001).

La prevalencia más alta de garrapatas en perros ocurre entre los meses de mayo y julio, sin embargo puede variar según la región (Raghavan *et al*., 2007); en Cuernavaca, Morelos, México, la prevalencia más alta de *R. sanguineus* ocurre en primavera, verano y otoño (Cruz-Vazquez and Garcia-Vazquez, 1999).

Las garrapatas *I. scapularis* adquieren la infección cuando la larva o ninfa se alimenta de un huésped infectado. Los patógenos ingeridos colonizan el intestino y persisten hasta la siguiente muda, cuando las garrapatas se alimentan otra vez éstos se multiplican en el intestino, después algunos escapan del intestino a la hemolinfa e invaden a las glándulas salivales para entrar a la epidermis del huésped a través de la mordida. El riesgo de transmitir los patógenos succionando sangre aumenta significativamente a las 72 o más horas (de Silva and Fikrig, 1997; de Silva *et al*., 1999; Nadelman *et al*., 2001).

Para capturar a las garrapatas que se encuentran libres en el medio ambiente, para ser identificadas taxonómicamente, se puede lograr mediante las trampas de cebo con CO^2 (Oliveira *et al*., 2000).

La garrapata *R. sanguineus* se encuentra ampliamente distribuida en el mundo. Puede establecerse en las paredes y muebles de hogares, y en perreras. Está implicada como vector de varios patógenos, incluyendo *Rickettsia rickettsii* y *Ehrlichia canis*. (Marquez-Jimenez *et al*., 2005; Merino *et al*., 2005; Quintero *et al*, 2004).

El ciclo de *R. sanguineus*, puede requerir de 63 a 236 días para ser completado, desde la formación del huevo, la larva, ninfa y el estadio adulto. Cada etapa requiere alimentarse de sangre de algún huésped para la morfogénesis. Durante la alimentación las garrapatas permanecen fijas en el hospedador por horas o días (Jacobs *et al*., 2004).

Los adultos de *R. sanguineus* son encontrados en su mayoría en orejas y en los espacios interdigitales de los perros. La larva y ninfa se adhiere frecuentemente en el pelo largo en la espalda o en el cuello. La hembra deposita sus huevos en grietas cerca de los lugares donde descansa el perro. El macho puede ser encontrado copulando con las hembras mientras se alimentan. Esta garrapata, como la mayoría, tiene gran tendencia a escalar, a menudo se esconde en los marcos de las ventanas o en las cortinas. El ciclo corresponde al de 3 huéspedes, es decir, los tres estadios de la garrapata (larva, ninfa y adulto) deben alimentarse en el perro antes de dejarse caer al suelo y realizar la muda al siguiente estadio (Lord, 2001).

La hembra adulta se alimenta en el huésped aproximadamente por una semana, luego cae al suelo y busca algún lugar apartado para el desarrollo de los huevos. Poco después empieza a ovopositar durante 4 días, después de 4 días de haberse alimentado y de caer del huésped. Esta etapa de pre-ovoposición puede extenderse hasta 21 días. Cuando ovoposita, pasa sus huevos sobre sus áreas porosas de la parte caudal de la base del gnatosoma, cubriéndolos con sus secreciones para mantenerlos con humedad. Después de que termina su ovoposición muere. La producción máxima de huevos puede ser de 3,200 por cada hembra. La incubación varía de 19-72 días. El período de premuda de la larva alimentada es de 9.5-36.5 días y de la ninfa 15-44.5 días (Jacobs *et al*., 2004).

Después de cada etapa empiezan a buscar a un huésped. Todos los estadios de esta garrapata prefieren perros, sin embargo pueden alimentarse de otros animales y del humano. La larva se alimenta por 3-7 días, luego toma

alrededor de 2 semanas para desarrollarse a la fase de ninfa. La ninfa entonces se alimenta por 5-10 días y otra vez toma alrededor de 2 semanas para desarrollarse como adulto. Los adultos, tanto machos como hembras, se fijan a los huéspedes para alimentarse, sin embargo los machos sólo se alimentan por cortos periodos. El ciclo completo puede llevarse a cabo en 2 meses, pero frecuentemente tarda más si hay pocos huéspedes disponibles o en climas fríos. Las garrapatas son notoriamente longevas y pueden vivir hasta 3-5 meses en cada estadio sin alimentarse. Los adultos se alimentan de sangre por 6-50 días, pueden sobrevivir por 18 meses sin alimentarse. A 29°C el ciclo puede ser completado en 63 días. Cada hembra ovoposita entre 1,000 a 3,000 huevos. Bajo condiciones ambientales normales y disponibilidad de huéspedes, puede haber hasta 4 generaciones al año (Lord, 2001). El incremento del número de casos de parasitismo en humanos por *R. sanguineus* reportados en la literatura indican que la interacción entre humanos y esta garrapata puede ser más común de lo que actualmente es reconocida (Dantas-Torres, 2008).

Patogenia

Algunas características de *B. burgdorferi* tienen una función crucial en la patogenia de la enfermedad de Lyme, que resulta ser un proceso complejo resultante de inflamación, liberación de citocinas, diseminación y adherencia del microorganismo a los diferentes tejidos. *B. burgdorferi* estimula a diversas citocinas inflamatorias (interleucina 1 y 6) y al factor de necrosis tumoral alfa (FNT-alfa) que pudieran desempeñar alguna función en la reacción inflamatoria que acompaña a la enfermedad. La diseminación del microorganismo se facilita por la alta permeabilidad de los vasos sanguíneos y la activa penetración de la bacteria a través de las membranas endoteliales. La invasión de los diferentes tipos de tejidos se produce como resultado de la adherencia del germen a distintos tipos de células (fibroblastos y células endoteliales) y estructuras ampliamente distribuidas en el huésped humano (Dickinson-Meneses and Batlle-Almodóvar, 1997; Craig-Mylius *et al*., 2005).

La respuesta inmunitaria, normalmente protectora, no resulta eficaz para erradicar los microorganismos y puede contribuir a la enfermedad al desarrollar un proceso autorreactivo. Esta reacción está basada en la reactividad cruzada antigénica entre epítopos comunes al agente y al huésped, especialmente localizados en las llamadas "proteínas de estrés" o "proteínas de choque térmico" de las cuales en *B. burgdorferi* se han detectado hasta 7. Estas proteínas protegen a la bacteria del daño producido por componentes bactericidas (como el calor, los radicales reactivos de oxígeno y otros). Una de ellas, la chaperona denominada GroEl, que se cree está presente en todas las células vivientes y son las responsables de la respuesta al estrés, tiene una importante función en la supervivencia de la espiroqueta en el huésped durante la transición del vector (con baja temperatura corporal) a los animales de sangre caliente (Dickinson-Meneses and Batlle-Almodóvar, 1997; Craig-Mylius *et al*., 2005).

El éxito de la invasión de un organismo patógeno depende en gran medida de varios factores citoplasmáticos, asociados a la superficie del germen y

secretados. Las dificultades técnicas de manipulación de las espiroquetas hacen difícil el reconocimiento de los factores específicos implicados en la entrada y larga supervivencia de este microorganismo en el huésped. Evidencias indirectas sugieren que la producción de GroEL modifica la capacidad de la espiroqueta de sobrevivir en el medio hostil del huésped infectado (Dickinson-Meneses and Batlle-Almodóvar, 1997).

Estudios realizados sobre las proteínas de la superficie externa (Osp) sugieren que son muy importantes también para la interacción parásito-huésped. Esto ha sido sustentado por estudios de un anticuerpo monoclonal (9B3D) contra OspA que es capaz de bloquear el acoplamiento de *B. burgdorferi* a la célula. Parece ser que la disminución de la infectividad por el germen está relacionada con la pérdida de proteínas y plásmidos específicos. El gen específico de una de esas proteínas ha sido identificado y es una lipoproteína (Lap 30) especificada por un plásmido de 38 kilodaltones (Dickinson-Meneses and Batlle-Almodóvar, 1997).

Se ha demostrado otra proteína de la membrana externa de *B. burgdorferi*, denominada p66 (Oms66), la cual funciona como una porina y una adhesina, lo que sugiere que éstas participan en la patogénesis. Las proteínas de la superfie externa (Osp) son extremadamente inmunogénicas y los anticuerpos a estas proteínas dominan en una respuesta inmune. La ausencia de estas lipoproteínas en la superficie de un organismo lo desprotegen y lo hacen susceptible a la actividad destructiva anti-Osp del suero inmune (Exner *et al*., 2000).

Signología

La enfermedad de Lyme es multisistémica e inflamatoria, en la forma temprana se manifiesta por erupción cutánea, eritema migrante, fatiga, artralgia, mialgia, cefalea, fiebre, adenopatía, rigidez del cuello por neuritis craneal y meningitis linfocítica. También puede producir varios grados de bloqueo atrioventricular y complicaciones respiratorias. En la forma crónica se presenta artritis crónica, afección crónica del sistema nervioso, dermatitis crónica, queratitis y encefalopatías que pueden persistir por más de 10 años (Burgess, 1986; Magnarelli *et al*., 1987b; Spach *et al*., 1993; Berglund *et al*., 1995; Edlow, 1999; Faul *et al*., 1999; Smith *et al*., 2002). También puede presentar signos renales como azotemia, proteinuria, cilindruria, piuria y hematuria (Grauer *et al*., 1988).

El período de incubación de borreliosis en perros es de 2-5 meses (Appel *et al*., 1993).

Epidemiología

En la mayoría de los países de Europa se han reportado seroprevalencias de borreliosis, particularmente en la República de Eslovaquia, donde mediante ELISA, se detectó en 40% en perros de cacería (Stefancikova *et al*., 1996), 11.8% en perros de servicio y 29.4% en perros de compañía (Stefancikova *et al*., 1998).

En Holanda (Goossens *et al*., 2001) se reportó una seroprevalencia por ELISA de *B. burgdorferi* del 18% en perros de cacería y del 17% en perros de compañía.

En España se han reportado prevalencias de borreliosis en perros de 11-21%, obtenidas mediante inmunofluorescencia (Merino *et al*., 2000).

En Estados Unidos de América se han reportado las siguientes seroprevalencias: 66.5% en Connecticut por ELISA (Magnarelli *et al*., 1987b), 52.0% en Rhode Island por ELISA (Hinrichsen *et al*., 2001), 5.5% en Texas por IFA (Cohen *et al*., 1990) y 2.3% en San Diego, California por ELISA (Olson *et al*., 2000).

En San Pablo, Brasil, la prevalencia estimada fue del 9.7% (23/237) en perros domésticos mediante ELISA y confirmados por Western blot (Joppert *et al*., 2001).

En Río de Janeiro, Brasil, un estudio mediante ELISA indirecta para detectar anticuerpos homólogos clase IgG contra *B. burgdorferi* sensu lato, demostró una seroprevalencia en perros de 48.2% (De Lacerda *et al*., 2004).

En México, en perros del área metropolitana de Monterrey, Nuevo León, se reportó una seroprevalencia de 16% con la prueba de inmunofluorescencia indirecta para detectar anticuerpos a *B. burgdorferi* (Salinas-Meléndez *et al*., 1999).

Hubo evidencia molecular de *B. burgdorferi* en perros con artritis en Monterrey, Nuevo León, México, lo que sugiere la presencia de enfermedad de Lyme en esa región. La ampliación de las secuencias de ADN fueron tomadas a partir de muestras de líquido sinovial (Salinas-Meléndez *et al*., 1995). En el mismo lugar regiomontano se encontró una seroprevalencia de 3% en ciervos (n= 350), en donde este rumiante (*Odocoileus virginianus texanus*) se considera importante en el mantenimiento del vector *I. scapularis* de la región (Martinez *et al*., 1999).

En la ciudad de Mexicali, Baja California, un estudio piloto demostró una prevalencia de 6.6% (2/30) de *B. burgdorferi* en perros con historia de epistaxis, diagnosticados mediante ELISA con el kit INDX Canine Multi-Test Dip-S-Ticks® PanBio, con una sensibilidad de 94.8% y especificidad del 100% (Romano *et al*., 1998).

En la zona urbana de Mexicali, Baja California, un estudio piloto realizado en el otoño de 2003, demostró que el 59.6% (56/94) de los perros estaban infestados con garrapatas, y el 100% de las garrapatas fueron *R. sanguineus* (Tinoco-Gracia *et al*, *en prensa*).

Colateral a este estudio, se determinó la seroprevalencia a esta espiroqueta con *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc., resultando 8.2% (95% I.C. 1.5-13.3%) en 94 perros analizados durante el otoño (septiembre, octubre y noviembre), con una sensibilidad de 96% y una especificidad de 95% (Tinoco-Gracia *et al*., 2007).

Debido a la susceptibilidad de los perros a borreliosis y al riesgo zoonótico, han sido utilizados estos animales como centinelas para la detección del riesgo de *B. burgdorferi* en humanos (Goossens *et al*., 2001; Guerra *et al*., 2001; Duncan *et al*., 2005).

En el Centro para Control y Prevención de Enfermedades (CDC) de Atlanta, Georgia se han reportado 157,410 casos de borreliosis en humanos en Estados Unidos desde 1990 a 2001 En el año 2002 se reportaron 23,763 casos en los

Estados Unidos (CDC, 2004). Más del 90% de los casos ocurrieron en los estados de la costa noreste y sobre el oeste medio donde se encontró el vector *I. scapularis;* y en el norte de California donde se encontró *I. pacificus* (Spach *et al*., 1993; Gordillo-Pérez *et al*., 2003).

En Europa la incidencia anual es de 70/100,000 de la población, donde el vector es *I. ricinus*. En Suiza la prevalencia varía de 10-30% (Berglund *et al*., 1995; Faul *et al*., 1999). El 20.5% de la garrapatas (*I. ricinus*) analizadas por PCR en la República Checa fueron positivas a *B. burgdorferi* (Derdakova *et al*., 2003). En Holanda el 13% (Schouls *et al*., 1999) y en Turquía el 4% (Guner *et al*., 2003); otros estudios han demostrado un rango del 10-35% (Berglund *et al*., 1995; Schouls *et al*., 1999).

En el Distrito Federal y el noreste de la República Mexicana, un estudio de 2,346 sueros sanguíneos de humanos analizados por ELISA y confirmados con Western blot para el diagnóstico de *B. burgdorferi*, encontraron una prevalencia del 3.4 y 6.2% respectivamente (Gordillo-Pérez *et al*., 2003). Además 4 pacientes residentes del Distrito Federal que fueron mordidos por garrapatas al visitar parques forestales (3 en la Ciudad de México y uno en Quintana Roo), resultaron positivos por PCR de biopsias de piel a *B. burgdorferi* utilizando iniciadores del gen de flagelina. Uno de los cuales resultó también positivo por secuenciación al gen OspA (Gordillo-Pérez *et al*., 2007).

Diagnóstico

La prueba de diagnóstico más específica para la enfermedad de Lyme en perros es el cultivo de *B. burgdorferi* del eritema migrante en piel, de líquido sinovial y de la vejiga urinaria, pero este procedimiento requiere un medio especial que no es rápido, ni fácilmente disponible. Las pruebas más utilizadas son ELISA e inmunofluorescencia, detectando anticuerpos contra esta espiroqueta desde los 21 días postinoculación (Callister *et al*., 2000) y son detectables hasta por 2 años posinfección (Appel *et al*., 1993); recientemente para esclarecer resultados de ELISA se recurre a Western blot, que tiene menor sensibilidad, pero mayor especificidad (Guerra *et al*., 2000) y las pruebas que incluyen la reacción en cadena de la polimerasa (PCR,) (Faul *et al*., 1999; Schouls *et al*., 1999; Piesman *et al*., 2001; Derdakova *et al*., 2003; Hanincova *et al*., 2003). Sin embargo, puede haber reacción cruzada con treponemas, leptospiras o enfermedades inflamatorias (Fikrig *et al*., 1993; Shin *et al*., 1993; Sugiyama *et al*., 1993). Aunque la reacción cruzada con leptospiras es discutida (Magnarelli *et al*., 1987a; Schulze *et al*., 1987; Joppert *et al*., 2001).

El cultivo de *B. burgdorferi* puede hacerse a partir de garrapatas infectadas con esta espiroqueta. Las garrapatas son desinfectadas con varios pasajes de 2 minutos en 2-propanol y 70% de etanol, seriadamente bañadas en fosfato amortiguado salino y el medio Barbour-Stoenner-Kelly II (BSK-II), y puestas en BSK-II fresco, donde los especímenes son abiertos con agujas. La suspensión se filtra con un filtro de jeringa (µStar 0.45-µm Corning Inc., Corning, N.Y.) y se agrega a un tubo de cultivo de 5 ml conteniendo 4.5 ml de BSK suplementado con 6% suero de conejo (BSK-RS) o directamente agregarlo sin filtración al tubo BSK-

RS suplementado con 0.4 µg de ciprofloxacina por ml y 40 µg de rifampicina por ml (BSK-CR). El segundo medio a utilizar, con la suspensión no filtrada del compuesto de BSK-RS suplementado con 8 µg de kanamicina por ml y 230 µg de 5-fluorouracil por ml (BSK-K5). Se hacen pasajes ciegos hechos siempre a 24-48 horas de inoculación para evitar una posible toxicidad de detritos de garrapata y para prevenir algún efecto adverso de los antibióticos en el crecimiento de las espiroquetas. Los cultivos se examinan mediante microscopía de campo obscuro cada semana en el primer mes y dos veces al mes en el segundo mes después de la inoculación. Las espiroquetas de los cultivos positivos se pueden congelar a -70°C en BSK suplementado con 10% dimetil sulfóxido (Stoenner, 1974; Abel *et al*., 2000; Escudero *et al*., 2000; Straubinger, 2000; von Lackum and Stevenson, 2005).

Para analizar las garrapatas para la detección y cuantificación (densidad) de *B. burgdorferi* a través de PCR, se extrae el DNA del intestino y glándulas salivales usando el kit comercial Qiagen, Inc. (Dickinson-Meneses and Batlle-Almodóvar, 1997; Straubinger, 2000; Piesman *et al*., 2001).

La transmisión experimental de *B. burgdorferi* por larvas de garrapatas *Ixodes scapularis* se puede lograr por inmersión en 0.5 ml de suspensión de la espiroqueta a partir del cultivo BSK-II en un tubo de microcetrifugación de 1.5 ml, a una temperatura de 32°C durante 45 minutos. Los tubos deben agitarse con el vórtice cada 10 minutos para redistribuir las larvas en solución. Las larvas infectadas experimentalmente se colocan en el ratón *Peromyscus leucopus*. Al final se comprueba la infección en los ratones mediante seroconversión y por cultivo de su vejiga urinaria (Policastro and Schwan, 2003).

Por otra parte, la transmisión experimental de *B. burgdorferi* por ninfas y estadios adultos de garrapatas *I. scapularis* se puede lograr por alimentación por capilaridad de medio BSK-H con *B. burgdorferi* con tubos de capilaridad colocados en el hipostoma en una cámara a 34°C (Korshus *et al*., 2004).

Tratamiento

Los antibióticos más efectivos para tratar la forma aguda de enfermedad de Lyme son doxiciclina, amoxicilina, cefuroxina y azitromicina prescritos por 3 semanas vía oral. Para la enfermedad crónica se han utilizado por vía intravenosa cefriaxona o penicilina por 3 semanas (Spach *et al*., 1993; Dattwyler *et al*., 1997).

Control y prevención

La primera línea de defensa contra la infección es evadir a las garrapatas infectadas y al hábitat de las garrapatas, además el uso de repelentes y ropa protectora cuando la exposición no puede ser evitada (Rahn, 2001).

Para lograr un satisfactorio control de esta garrapata se requiere un programa de 3 pasos que consiste en: 1) sanidad, 2) control de garrapatas con acaricidas en el medio ambiente, y 3) control de garrapatas en el perro. Las casas o perreras deben ser completamente limpiadas para eliminar lo más posible las garrapatas. Los lugares de descanso del perro deben tener especial atención. Los

perros infestados deben ser tratados por un médico veterinario al mismo tiempo que el lugar es tratado. Los lugares ocupados por el perro pueden ser tratados con aspersiones residuales o polvos. Algunos pesticidas para el control de garrapatas son aletrin, bendiocarb, carbaryl, clorpirifos, esfenvalerato, y permetrin u otras piretrinas (Monmouth, 1999).

Importancia zoonótica

La enfermedad de Lyme puede ser transmitida al humano por la picadura de garrapatas infectadas con *B. burgdorferi* (Burgdorfer, 1984; Anderson *et al*., 1996), las cuales actúan como vectores a partir principalmente de roedores o de perros (Mather *et al*., 1994; Duncan *et al*., 2004).

La borreliosis se manifiesta en humanos inicialmente con un eritema migrante en el sitio de la picadura por la garrapata y puede diseminarse vía sanguínea a la piel, corazón, sistema nervioso, articulaciones y otros órganos, resultando en miocarditis, síndromes artríticos y neurológicos (Edlow, 1999).

La seroprevalencia de borreliosis en perros es similar a la obtenida en seres humanos en una población. Este hallazgo da evidencia de una asociación estrecha entre la seroprevalencia del humano y del perro (Merino *et al*., 2000; Olson *et al*., 2000; Guerra *et al*., 2001).

Se ha demostrado que la incidencia de *B. burgdorferi* en perros registrada en una localidad está correlacionada con la enfermedad de Lyme en humanos y con la abundancia de la garrapata vector *Ixodes scapularis* (Guerra *et al*., 2001).

En el Estado de California, E.U., la garrapata *I. pacificus* es el vector primario en humanos de *B. burgdorferi* (Brown and Lane, 1992).

Objetivo general

Estimar la prevalencia de *Borrelia burgdorferi* y demostrar su transmisión a través de la garrapata *Rhipicephalus sanguineus* en perros de Mexicali, Baja California, México.

Objetivos específicos

1. Estimar la prevalencia de *B. burgdorferi* a través de ELISA, confirmada por PCR y secuenciación de ADN, en perros atendidos en clínicas veterinarias y en perros capturados por los centros de control animal; y en caso de las garrapatas a través de PCR y secuenciación de ADN.

2. Evaluar factores de riesgo asociados a *B. burgdorferi* en perros de la zona urbana de Mexicali, Baja California.

3. Demostrar la transmisión experimental de *B. burgdorferi* a través de la garrapata *R. sanguineus* en perros.

Hipótesis general

La garrapata *R. sanguineus* es vector de *B. burgdorferi* en perros en la zona urbana de Mexicali, Baja California, México.

Hipótesis específicas

Las garrapatas *R. sanguineus* colectadas de perros de la zona urbana de Mexicali transmiten *B. burgdorferi*.

La espiroqueta *B. burgdorferi* está presente en perros y en garrapatas *R. sanguineus* de la zona urbana de Mexicali.

Los factores de riesgo intrínsecos (edad, sexo, raza, talla y pelaje) y extrínsecos (hábitat, presencia de garrapatas y manejo zootécnico del perro) están asociados a la presencia de *B. burgdorferi* en perros de Mexicali.

2.- MATERIAL Y MÉTODOS

El trabajo de investigación comprendió las siguientes:

A) Monitoreo,
B) Aislamiento, y
C) Estudio experimental de transmisión de *B. burgdorferi*.

A) Monitoreo de *B. burgdorferi*

Marco muestral del área de estudio

Esta fase del trabajo, clasificado como un estudio descriptivo seccional cruzado, se llevó a cabo en la zona urbana de Mexicali, Baja California, México; incluyó 39 establecimientos de servicios médico-veterinarios (clínicas veterinarias), el Centro Antirrábico Veterinario y el Centro Municipal de Control Animal de Mexicali (centros de control animal).

Mexicali es una ciudad fronteriza del noroeste de México, que colinda con el Estado de California, Estados Unidos. Mexicali es la ciudad más al norte de América Latina, está localizada en 32°40′0″N, 115°28′0″O y cuenta con 855,962 habitantes (Wikipedia, 2006). El clima es extremoso tipo desértico con un promedio de precipitación pluvial de 0.63 ± 0.43 cm. Las condiciones climatológicas fueron obtenidas por *National Weather Service* de *National Oceanic and Atmospheric Administration* de los Estados Unidos (http://www.nws.noaa.gov/).

Los sujetos muestreados incluyeron a: *i*) perros atendidos en clínicas veterinarias, *ii*) perros capturados por personal de los dos centros de control animal de la ciudad y *iii*) garrapatas colectadas de los animales muestreados.

La duración del estudio epidemiológico fue de 24 meses, a partir del 10 de agosto de 2005. Los análisis de las muestras se realizaron en el Laboratorio de Biología Molecular del Instituto de Investigaciones en Ciencias Veterinarias de la Universidad Autónoma de Baja California; México y se confirmaron en el Laboratorio del Departamento de Pato-Biología y Medicina Diagnóstica del Colegio de Medicina Veterinaria de la Universidad de Kansas State, E.U.A.

Criterios de inclusión

Fueron incluidos en el estudio los perros de al menos un mes de edad, de cualquier sexo o raza atendidos en cualquiera de las clínicas veterinarias participantes en el estudio, así como los perros capturados por el personal de los centros control animal; además las garrapatas colectadas de los animales muestreados.

Criterios de exclusión

Se excluyeron las muestras sanguíneas hemolizadas e insuficientes.

Muestreo

Los muestreos de sangre y garrapatas en conjunto con la aplicación de los cuestionarios epidemiológicos, se realizó sin reemplazo al azar de individuos por cada grupo para estimar la estacionalidad de la seroprevalencia y para evaluar los factores de riesgo.

La presencia de *B. burgdorferi* en perros fue estimada a partir de los resultados obtenidos por ELISA de los sueros obtenidos; y los que resultaron positivos se comprobó mediante PCR analizando el coágulo de la muestra sanguínea. En garrapatas fue a través de PCR de sus tejidos digeridos con proteasa K (Belperron and Bockenstedt, 2001; Piesman *et al*., 2001).

Las muestras de sangre fueron obtenidas por personal calificado. A cada individuo se le colectaron 3 ml de sangre por punción de la vena cefálica después de la asepsia del área con alcohol isopropílico y colocados en tubos Vacutainer® de 6 ml. Después de identificadas todas la muestras fueron centrifugadas a 3500 RPM por 10 minutos para separar el suero del coágulo. El suero fue transferido a viales de plástico de 1.5 ml con tapa, identificados y almacenados a –20°C hasta el momento de realizar la prueba serológica.

Para estimar la seroprevalencia de la enfermedad se utilizó el kit semicuantitativo de *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc.

La colecta de las garrapatas se realizó siguiendo los procedimientos previamente descritos (Farley, 1996; Lyon and Restifo, 2000). Los tubos recolectores estériles de 15 ml con tapadera hermética, conteniendo etanol al 70% conteniendo a las garrapatas fueron transportados al laboratorio de Biología Molecular del Instituto de Investigaciones en Ciencias Veterinarias de la Universidad Autónoma de Baja California. Las garrapatas colectadas de los perros muestreados se conservaron en los tubos de ensayo con etanol hasta realizar su identificación taxonómica bajo observación estereoscópica (Quiroz, 1999) y el diagnóstico de *B. burgdorferi*, el cual se llevó a cabo aplicando la técnica de PCR (Thomas *et al*., 2001), evitando procesar garrapatas mayores de 4mm que pudieran inhibir el PCR (Beichel *et al*., 1996).

Con el propósito de conocer la afinidad de las garrapatas hacia alguna región corporal del perro y su asociación con los factores de riesgo, se incluyó al cuestionario un esquema en donde se registró su localización en las distintas regiones corporales del perro:

1.- Cara.
2.- Orejas.
3.- Cuello.
4.- Dorso.
5.- Extremidades.
6.- Abdomen.

Durante los muestreos, se aplicó el cuestionario a los propietarios de los perros atendidos en clínicas veterinarias. Para el caso en los perros de los centros de control animal se incluyó únicamente información sobre sexo (macho, hembra),

edad (≤ 1 año, > 2 años), talla (chico, mediano, grande) y pelaje (corto, mediando, largo). En el cuestionario aplicado para perros atendidos en clínicas veterinarias, se incluyó información sobre la presencia y grado de infestación de garrapatas en el perro (ligera de 1 a 10, moderada de 11 a 30, severa > 30), programa ectoparasiticida (compuesto químico, periodicidad y vía de administración), el desplazamiento del perro de casa a la calle, el traslado del perro de la ciudad al campo, información sobre el programa de fumigación en el hogar y estación de muestreo, primavera (marzo, abril y mayo), verano (junio, julio y agosto), otoño (septiembre, octubre y noviembre) e invierno (diciembre, enero y febrero). También se incluyó la siguiente información sobre la familia: colonia de residencia, número de integrantes, número de mascotas en el hogar, ocupación, escolaridad, ingreso, características del espacio físico donde habita la mascota.

B) Aislamiento de *B. burgdorferi*

Para el aislamiento de *B. burgdorferi* se utilizó el medio de cultivo BSK-H® SIGMA (Barbour-Stoenner-Kelly-H® SIGMA) para cultivar muestras de tejidos y garrapatas de perros seropositivos; además de borrelias recibidas de la Universidad de Kansas State, cortesía del Dr. Manuel Moro y Dr. Javier Vinasco.

En el caso de perros atendidos en clínicas veterinarias, con autorización de su propietario, se les colectaron muestras de líquido sinovial, bajo anestesia por inhalación con isofluorano, en condiciones asépticas, en cantidad de 0.2 a 0.5 ml, por punción de la articulación de ambos hombros, utilizando una aguja calibre 22 x 32 mm y una jeringa de 3 ml.

En perros de los centros de control de animal, bajo necropsia, se le tomaron muestras de tejidos que tienen mayor probabilidad de alojar a *B. burgdorferi*, como lo son líquido sinovial de hombro (Straubinger *et al*., 1997), ganglio linfático axilar (Harter *et al*., 1999), hígado, bazo, riñón, vejiga y médula ósea (Schwan *et al*., 1988; Dorward *et al*., 1991; Hovius *et al*., 1999; Straubinger, 2000; Exner and Lewinski, 2003).

Para el cultivo de garrapatas, se colectaron 10 garrapatas adultas, 5 hembras y 5 machos por cada uno de 5 perros seropositivos; se lavaron bajo condiciones estériles por vórtice durante 3 minutos en sucesivas soluciones de peróxido de hidrógeno al 3%, alcohol al 95%, hipoclorito de sodio al 0.1% y salina fosfato amortiguada (PBS; pH 7.2). Después de lavadas, las garrapatas se colocaron en cajas de Petri estériles y luego diseccionadas individualmente con un bisturí con navaja #11. El instrumental utilizado fue previamente esterilizado para cada grupo de garrapatas.

Inmediatamente después de haber sido colectadas las muestras de tejido y de procesadas las garrapatas, además de las borrelias enviados de la Universidad de Kansas State, en la cámara de seguridad se efectuó la siembra en tubos estériles de 10 ml con tapón con 6 ml de medio BSK-H® SIGMA. Enseguida se colocaron en una incubadora a 34°C hasta un máximo de 180 días.

Después de 24 horas se confirmó que no haya evidencia de contaminación. Los tubos de cultivo fueron centrifugados a 75 x *g* por 5 minutos. Después 4 ml del

sobrenadante fue removido, el sedimento se resuspendió y se adicionó medio BSK-H hasta completar los 6 ml. Los cultivos fueron alimentados cada 3 días con BSK-H hasta el momento de su congelación (Varela *et al*., 2004; Moyer *et al*., 2006).

Los cultivos se examinaron mediante microscopía de campo obscuro cada semana después de la siembra (Korshus *et al*., 2004). Una vez logrado el cultivo, las espiroquetas en el mismo medio de cultivo se congelaron a -80°C con el kit CryoCare Bacterial Preservers® Key Scientific Products (www.keyscientific.com), hasta el momento de ser utilizadas para el desarrollo del estudio experimental de transmisión de *B. burgdorferi* por *R. sanguineus*.

C) Estudio de la transmisión experimental de *B. burgdorferi* por *R. sanguineus*

Área experimental y animales

Para demostrar la transmisión de *B. burgdorferi* por *R. sanguineus,* se utilizaron 3 perros de la misma camada de 6 meses de edad, de talla mediana, de pelaje corto, sin importar sexo ni raza, descendientes de una perra sin evidencia serológica ni molecular de esta espiroqueta. Los individuos seleccionados fueron clínicamente sanos, sin signos compatibles con borreliosis, como claudicación, fiebre, depresión, signos respiratorios, hepáticos o renales; también resultaron negativos a *B. burgdorferi* diagnosticados por ELISA y por PCR de muestras sanguíneas. La madre de los cachorros se obtuvo del Centro Municipal de Control Animal de Mexicali desde 2 semanas antes de parto y se alojó en el área experimental hasta que los cachorros alcancen las 6 semanas de edad. Los cachorros se vacunaron y desparasitaron con los protocolos de la región. Se distribuyeron individualmente en jaulas dentro del área experimental, 2 de ellos fueron expuestos a garrapatas hembras adultas sin alimentar *R. sanguineus* infectadas con *B. Burgdorferi* en las jaulas 1 y 2, y en la jaula 3 el cachorro que fue expuesto a garrapatas no infectadas por *B. burgdorferi*, el cual fue el control.

El área experimental midió 3 m de ancho, 3 m de largo y 2.6 m de altura, con 2 de las paredes, el techo y el piso de concreto con material impermeable y lavable, y las otras 2 paredes de vidrio armado con aluminio. Las jaulas individuales fueron de acero inoxidable de 61 cm de ancho, 61 cm de altura y 72 cm de profundo, contaron con una canaleta al frente de cada jaula para efecto de drenaje a un centro común para procesamiento de excretas, el aislamiento, así como la ventilación, cumplió con las normas de bioseguridad y de cuidado animal, según el protocolo del Acta de bienestar animal (Animal Welfare Act) del gobierno de Estados Unidos de la Universidad de Kansas State para animales de experimentación; y aprobado por el Subcomité Institucional para el Cuidado de Animales en Experimentación de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de México (ANEXOS).

Incubación y desarrollo de *R. sanguineus*

Para esta parte del estudio, se colectaron 10 hembras adultas repletas *R. sanguineus* del medio ambiente, específicamente del piso del Centro Municipal de Control Animal de Mexicali. La colección fue individual en tubos de ensayo de 15 ml de capacidad con tapón de algodón, para ser llevados al Laboratorio de Parasitología del IICV de la UABC. Al llegar al laboratorio las garrapatas después de ser lavadas con agua y detergente, se colocaron individualmente en cajas de Petri de 6 cm de diámetro, en una incubadora a 30°C con 80% de humedad. Después de 4 días, cada conjunto de huevos producidos por cada garrapata se pasó a un frasco de vidrio con boca ancha con capacidad de 30 ml con tapón de algodón. Los huevos de *R. sanguineus* se incubaron a 30°C con 80% de humedad (Jacobs *et al*., 2004; Szabo *et al*., 2005). Una vez eclosionadas las larvas, lo que sucedió en 11 días, se dejaron en la misma incubadora 3 días más sin alimentar y luego se colocaron en los perros de experimentación durante 4 días (Korshus *et al*., 2004). Las larvas ya alimentadas se pasaron a la incubadora para mudar a ninfa, en 9 días. Después de 3 días de ayuno las ninfas se colocaron en los perros durante 4 días para que se alimenten y entonces a la incubadora a 30°C con 80% de humedad durante 11 días hasta que ocurrió la muda al estadio adulto (Jacobs *et al*., 2004; Szabo *et al*., 2005) para posteriormente realizar el inóculo de cultivo BSK-H con *B. burgdorferi* después de 2 días de ayuno y luego fueron colocadas en los perros de experimentación durante 7 días (Korshus *et al*., 2004).

Inoculación de *B. burgdorferi* en *R. sanguineus*

La inoculación de *B. burgdorferi* se realizó en los estadios adultos hembras (5 hembras/perro) de *R. sanguineus* por capilaridad suministrándoles una suspensión de cultivo BSK-H con *B. burgdorferi*, a una concentración de 1×10^8 espiroquetas/ml. Las garrapatas fueron inmovilizadas en cajas de Petri sobre su dorso usando una cinta de doble lado adhesivo. Se utilizaron tubos capilares de microhematócrito de 20 µL, para alimentarlas de la suspensión del cultivo colocándolos cuidadosamente bajo observación estereoscópica sobre su hipostoma, en una cámara húmeda a 34°C por 24 horas, después de que ocurra la distensión abdominal de las garrapatas y evidencia de excreción anal del medio BSK-H. Estas garrapatas fueron utilizadas para demostar la transmisión de borrelias en los perros de experimentación (Korshus *et al*., 2004; Matsumoto *et al*., 2005).

Exposición experimental en perros a *R. sanguineus* infectadas con *B. burgdorferi*

Para la exposición experimental se utilizaron 5 machos y 5 hembras de garrapatas adultas *R. sanguineus* por cada uno de los perros. Las garrapatas seleccionadas de la incubadora aleatoriamente fueron colocadas con guantes de látex estériles en cajas de Petri de 6 cm de diámetro también estériles. Las cajas de Petri fueron adheridas en un área rasurada de la zona dorsal anterior del tórax de cada perro. Las cajas de Petri se aseguraron colocando una malla de tela que abarque todo el tórax y parte del abdomen. Las garrapatas adultas fueron

colocadas en el perro para que se alimenten de sangre por 7 días hasta que éstas se repleten, tomando en cuenta que se requieren cuando menos 3 días para que el vector transmita *B. burgdorferi* (Korshus *et al*., 2004; Szabo *et al*., 2005). Se utilizó uno de los 3 perros de experimentación como control, al cual se le colocaron la misma cantidad de garrapatas que a los demás perros, pero libres de borrelias.

ELISA

En el monitoreo epidemiológico, para estimar la seroprevalencia de la enfermedad se utilizó el kit semicuantitativo de *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc., que detecta IgG canino con una sensibilidad y especificidad de 95.8 y 94.7%, respectivamente; esta prueba utiliza como antígenos extractos de la bacteria entera de tres diferentes especies, *B. burgdorferi, B. afzelli y B. garinii*. La densidad óptica (DO) fue determinada a 450 nm. Las muestras fueron analizadas en un lector de ELISA utilizando un filtro de 450 nm. Se consideraron como casos positivos a los perros con muestras serológicas con DO ≥ 0.3, de acuerdo al protocolo del fabricante.

A los animales de experimentación se les realizó la prueba serológica los días 0, 7, 14, 21 y 28 después de la exposición a garrapatas infectadas con *B. burgdorferi* (Moyer *et al*., 2006).

PCR

El diagnóstico molecular de *B. burgdorferi* se hizo por PCR de la siguiente manera:

1) En los estadios adultos de *R. sanguineus* se realizó, a partir de los tejidos de las garrapatas, seleccionando al azar la cantidad equivalente al 10% de los individuos que se utilizaron en la exposición experimental a perros.
2) En los cachorros de experimentación se hizo a partir de muestras de piel (4mm de diámetro de la periferia del área eritematosa de la picadura por la garrapata) y de sangre, los días 0, 7, 14, 21 y 28 pos-exposición a garrapatas (Straubinger, 2000).

Se utilizaron como blanco un fragmento del gen codificante de la flagelina (FLA) y otro de la Proteína A de la superficie externa (OspA) de la bacteria. El producto de PCR de (FLA) con un tamaño de 256 pares de bases (pb), con los siguientes iniciadores:

FLA 107 (5'TTA ATC GAG CTT CTG ATG ATG CTG C3')
FLA 335 (5'ATT TCG TCT GTA AGT TGC TCT ATT TCA A3')

El inicio de PCR fue con un calentamiento de 94°C por 4 min, seguido por 45 ciclos de desnaturalización de 94°C por 45 s, hibridación de 52°C por 45 s y extensión de 72°C por 1 min. Después de los 45 ciclos, se hizo una extensión adicional de 72°C por 7 min. Los productos de amplificación de PCR fueron

visualizados en geles de agarosa al 1% en solución amortiguadora TBE 0.5 conteniendo bromuro de etidio.

Los productos de PCR para OspA tendrán un tamaño de 345 pb con los siguientes iniciadores:

OspAEF (5'-AAA AAA TAT TTA TTG GGA ATA GG-3')
OspAER (5'-GTT TTT TTG CTG TTT ACA CTA ATT GTT AA-3')
OspAIF (5'-GGA GTA CTT GAA GGC G-3')
OspAIR (5'-GCT TAA AGT AAC AGT TCC-3')

La primera amplificación para el fragmento del gen OspA fue corrido por 20 ciclos de 94°C por 30 s, 37°C por 30 s, y 72°C por 2 min. La segunda, amplificación interna procedió por 10 ciclos de 94°C por 30 s, 60°C por 30 s, y 72°C por 1 min; 10 ciclos de 94°C por 30 s, 55°C por 30 s, y 72°C por 1 min; y finalmente, 10 ciclos de 94°C por 30 s, 50°C por 30 s, y 72°C por 1 min. (Guttman *et al*., 1996; Norris *et al*., 1997).

Para prevenir contaminación las preparaciones, la extracción del ADN, la amplificación y detección de productos de PCR fueron realizados en diferentes áreas de laboratorio (Chang *et al*., 2000).

Manejo de datos

Se diseñó una base de datos en un programa de computadora EXCEL (Microsoft) para la captura y manejo de información generada en este proyecto. La base de datos fue integrado por 4 tablas con la siguiente información: 1) resultados de ELISA de los perros, 2) resultados de PCR de tejidos de los perros; 3) resultados de los cuestionarios epidemiológicos; y 4) resultados de PCR de las garrapatas.

Análisis estadístico

Con la finalidad de estimar la seroprevalencia de *B. burgdorferi*, se tomó al azar una muestra de 384 perros atendidos en clínicas veterinarias y de 384 perros capturados por los centros de control de animal, considerando un estimador de *P* = 0.5, con un 95% de confianza y un 5% de precisión (Scheafer *et al*., 1987), El valor del estimador *P* fue basado en la varianza máxima, 50%.

La seroprevalencia fue calculada dividiendo el número de sueros positivos entre el total de muestras analizadas. La prevalencia ajustada y su 95% intervalo de confianza fue obtenida usando el estimador Rogan-Gladen (Greiner and Gardner, 2000).

Se construyeron tablas de frecuencias para la descripción estacional, asociadas al estado serológico, el cual se expresó en forma dicotómica (negativo o positivo).

Se estimó la magnitud de asociación mediante la razón de probabilidades (Odds ratio, OR) y χ^2 de Mantel-Haenszel para evaluar los factores de riesgo en su asociación con la seropositividad a *B. burgdorferi* (Walker, 1997a).

En el estudio experimental se medió la seropositividad y la presencia por PCR de *B. burgdorferi*, utilizando el análisis estadístico de Kaplan-Meier.

Para los análisis estadísticos se utilizó el paquete SAS (Statistical Analysis System) versión 9.1.3 para Windows (SAS, 2004).

Infraestructura

El laboratorio de Biología Molecular completamente equipados de la Unidad de Laboratorios de Diagnóstico del Instituto de Investigaciones en Ciencias Veterinarias de la Universidad Autónoma de Baja California.

Laboratorio del Departamento de Pato-biología y Medicina Diagnóstica del Colegio de Medicina Veterinaria de la Universidad de Kansas State.

39 clínicas veterinarias de la ciudad de Mexicali.

El Centro Antirrábico Veterinario y el Centro Municipal de Control Animal de Mexicali.

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3.- RESULTADOS

A) Monitoreo de *B. burgdorferi*

La seroprevalencia de *B. burgdorferi* fue de 6.8% (95% IC 3.5-8.9%) en perros atendidos en clínicas veterinarias de Mexicali y de 12% (95% IC 7.5-14.3%) en perros de los dos centros de control animal de la ciudad, ambas calculadas con el estimador Rogan-Gladen (Greiner and Gardner, 2000). La seroprevalencia de ambos grupos fue de 9.3% (95% IC 6.3-10.6).

En el caso de los perros atendidos en clínicas veterinarias, los factores de riesgo que se encontraron relacionados con la seropositividad a *B. burgdorferi* fueron la edad de los mismos (Mantel-Haenszel χ^2, $P = 0.02$), y la ausencia de un programa preventivo (Mantel-Haenszel χ^2, $P = 0.005$). Los resultados indican que los perros con mayor riesgo fueron ≥ 1 año de edad que los menores con OR= 2.7 (95% IC 1.2-6.1), así como los que no tuvieron acceso a una programa preventivo que consiste en al menos dos tratamientos desparasitantes, dos baños garrapaticidas y dos fumigaciones del hogar al año con OR= 4.9 (95% IC 1.4-16.8).

En el caso de los perros capturados por los centros de control animal, ninguno de los factores de riesgo evaluados en ese estudio, como la edad, sexo, talla y pelaje de los perros capturados por los centros de control animal, presentaron asociación a la seropositividad a *B. burgdorferi*.

Además de evidencias serológicas de *B. burgdorferi* en perros, también hubo evidencias moleculares de flagelina de esta espiroqueta en sangre, membrana y líquido sinovial y vejiga. En una muestra de sangre se secuenció su ADN resultando el 100% de identidad a la proteína externa de su superficie A de *B. burgdorferi* (Figura 1). También se pudo demostrar por PCR flagelina de esta espiroqueta en garrapatas *R sanguineus* de los perros muestreados. Estos resultados moleculares están en proceso para publicación

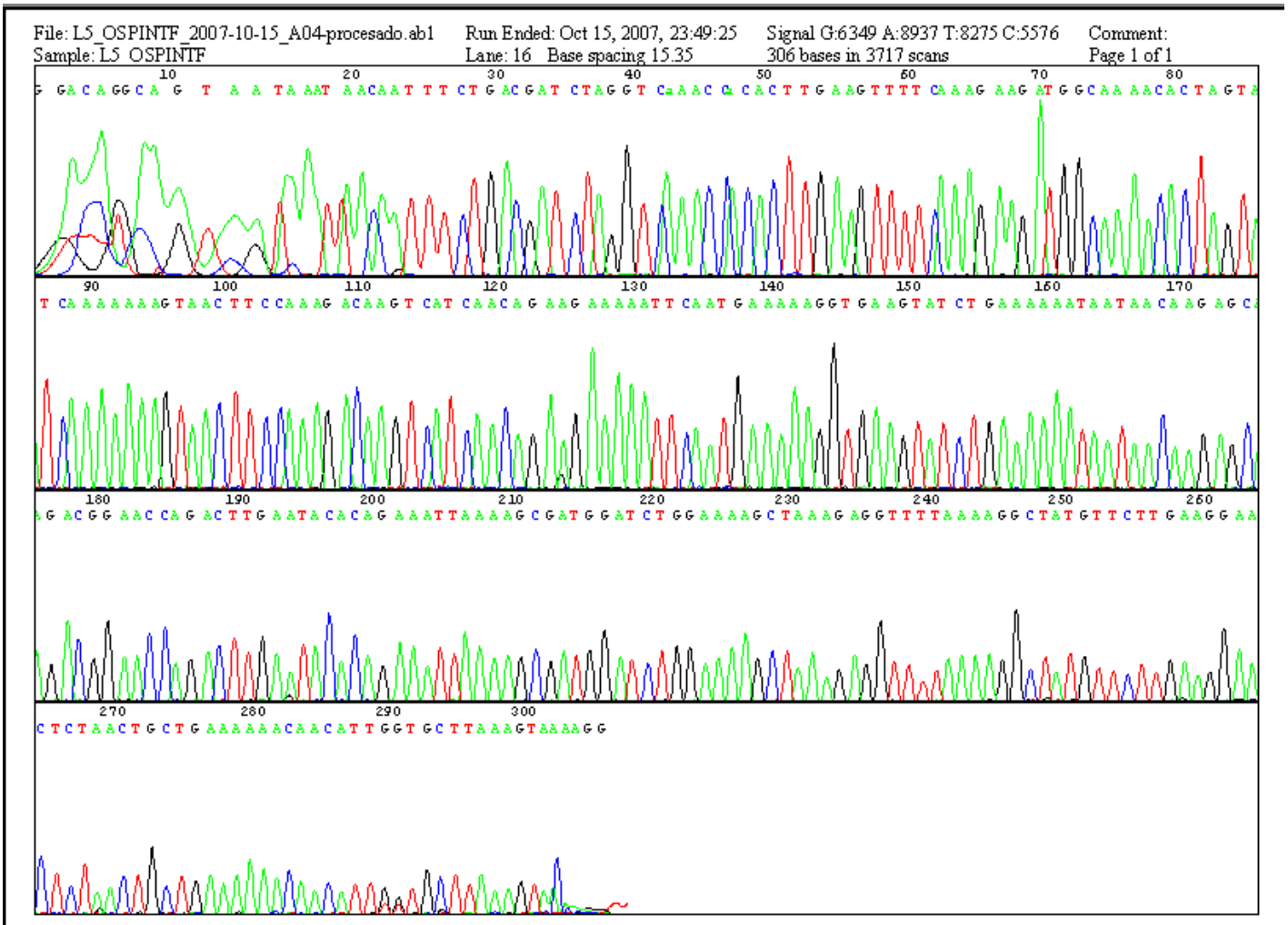

Figura 1.- Cromatograma de la secuenciación de OspA de *B. burgdorferi* de un perro de la ciudad de Mexicali, B.C., México. El análisis fue a partir de una muestra de coágulo sanguíneo conservada en papel filtro. Se muestra 100% de identidad con OspA de *Borrelia burgdorferi* con la clave gb|DQ867085.1|.

B) Aislamiento de *B. burgdorferi*

Se tomaron 45 muestras de sangre, tejido y de garrapatas de perros seropositivos a *B. burgdorferi*, de las cuales no se logró el cultivo de *B. burgdorferi* con el medio BSK-H a 34°C.

Es importante considerar que algunos investigadores han documentado que con el medio BSK no ha habido crecimiento de una espiroqueta con identidad filogénica de *B. borgdorferi*, y que el hallazgo diagnóstico se ha logrado por métodos de biología molecular, como PCR. Como en el caso de la espiroqueta *B. lonestari* y transmitida sólo por *Amblyomma americanum* que requiere para su crecimiento de células embrionarias de garrapatas alimentadas con BSK (Barbour *et al*., 1996; Armstrong *et al*., 2001; James *et al*., 2001; Varela *et al*., 2004; Moyer *et al*., 2006).

El día 24 de diciembre de 2007, se cultivaron en BSK-H con gelatina unos especímenes de *B. burgdorferi*, cortesía del Dr. Manuel Moro y Dr. Javier Vinasco de la Universidad de Kansas State. Después de 5 días de incubación a 34°C se procedió a congelarlas a -20°C con 12 perlas del kit CryoCare Bacterial Preservers® Key Scientific Products, para luego resembrarlas con la finalidad de utilizarlas para la parte experimental de la tesis.

Para comprobar la eficacia de la resiembra, el día 6 de enero de 2008 se utilizó una de las perlas con borrelias congeladas colocándola en medio BSK-H con gelatina, suero de conejo y sin antibióticos en incubación a 34°C. Se observó crecimiento abundante de borrelias después de 8 días de incubación. Otra comprobación de resiembra poscongelación con el mismo protocolo anterior, fue el día 3 de mayo de 2008, y la observación de abundante borrelias ocurrió después de 10 días de incubación

C) Transmisión experimental de *B. burgdorferi por R. sanguineus*

Área experimental y animales

Los 3 cachorros de experimentación cumplieron 8 meses de edad. Como trabajo previo a la transmisión experimental, los perros 2 y 3 fueron utilizados para alimentar 100 larvas cada uno, las cuales mudaron a ninfas (80 en cada uno); estas ninfas se incubaron para mudar a estadios adultos.

Incubación y desarrollo de *R. sanguineus*

Después de 4 días de incubación, las 4 garrapatas hembras repletas colectadas del suelo del Centro de Control Animal, inician la ovoposición y la teriman cinco días después. Después de 14 días de incubación inicia la eclosión de las larvas. Se colocaron aproximadamente 100 larvas de *R. sanguineus* a cada una de las 2 perras de experimentación. Se colectaron aproximadamente 80 larvas alimentadas de *R. sanguineus* de cada una de las 2 perras de experimentación. Mudaron las larvas de *R. sanguineus* a ninfas, en total fueron 120 ninfas mudadas. Se colocaron las ninfas de *R. sanguineus* en 2 de las perras de experimentación. En total se colectaron 60 ninfas alimentadas de *R.*

sanguineus de 2 de las perras de experimentación y se colocaron en incubación. Las ninfas *R sanguineus* mudan a adultas después de 11 días de incubación. En total mudaron 26 hembras adultas y 24 machos adultos

Inoculación de *B. burgdorferi* en *R. sanguineus*

Después de haber inoculado a 10 hembras adultas de *R. sanguineus* con borrelias, se logró colocar 5 garrapatas hembras y 5 machos a cada uno de los 3 perros de experimentación. En la perra 1 se colectó sólo una garrapata viva de las 5 hembras, también se colectaron vivos los 5 machos colocados. En la perra 2 se colectaron vivos sólo los 5 machos, las hembras murieron. En el perro 3 (control) se colectaron vivos las 5 hembras y los 5 machos que fueron colocados

Respuesta serológica y molecular

Después de analizar por ELISA las muestras sanguíneas de los perros de experimentación, se demostró que no hubo diferencias serológicas entre los expuestos a garrapatas “infectadas” y a las “no infectadas”. Tampoco hubo evidencia molecular de flagelina de *B. burgdorferi* por PCR en la piel del sitio de la picadura de la garrapata de los perros expuestos. Lo que demuestra que no hubo transmisión de *B. burgdorferi* por garrapatas adultas de *R. sanguineus* en perros utilizando 5 hembras inoculadas con esta espiroqueta a una dosis de 20 µL una concentración de 1×10^{8} espiroquetas/ml, siguiendo el protocolo utilizado para demostrar la transmisión con *Ixodes scapularis*.

4.- DISCUSIÓN Y CONCLUSIONES

En la zona urbana de Mexicali, B.C., México, hubo una seroprevalencia de 6.8% (95% IC 3.5-8.9%) a *B. burgdorferi* en perros atendidos en clínicas veterinarias y de 12% (95% IC 7.5-14.3%) en perros capturados por los centros de control animal, siendo una región donde sólo se han identificado garrapatas *R. sanguineus* en perros. Los factores de riesgo que resultaron asociados a borreliosis en perros atendidos en clínicas veterinarias en este estudio fueron la edad de los perros, y la ausencia de un programa de medicina preventiva. Se demostró que los perros con más riesgo son ≥ 1 año de edad con OR= 2.7 (95% IC 1.2-6.1), así como los que no tuvieron acceso a un programa preventivo, con OR= 4.9 (95% IC 1.4-16.8). Para el caso de los perros atendidos por los centros de control animal, ninguno de los factores de riesgo evaluados en ese estudio, como la edad, sexo, talla y pelaje, presentaron asociación a la seropositividad a *B. burgdorferi*.

Aunque la prevalencia en este estudio fue baja comparada con la de Monterrey, N.L., con una prevalencia de 16% (136/850) en perros analizados por inmunofluorescencia (Salinas-Meléndez *et al*., 1999). La baja seroprevalencia a *B. burgdorferi* encontrada en este estudio puede deberse a que no se han encontrado vectores ya conocidos para esta enfermedad, como *I. scapularis*, *I. pacificus*, *Dermacentor variabilis* o *Amblyomma americanum* (Magnarelli and Anderson, 1988; Lane, 1996; Adelson *et al*., 2004). La única garrapata encontrada en Mexicali ha sido *R. sanguineus* (*en prensa*), la cual no ha sido considerada como vector de borreliosis en otras regiones del mundo.

La alta prevalencia en perros ≥ 1 año de edad, similar a otros estudios (Merino *et al*., 2000; Goossens *et al*., 2001; De Lacerda *et al*., 2004), está justificada por la naturaleza de la borreliosis, la cual es una enfermedad crónica que muestra los síntomas más notorios después de meses de haberse adquirido, como la claudicación de una o varias extremidades (Magnarelli *et al*., 1987b).

El sexo de los perros no mostró asociación a la seropositividad a *B. burgdorferi*, similar a otros estudios (Merino *et al*., 2000; Guerra *et al*., 2001; De Lacerda *et al*., 2004), lo que indica que aparentemente las feromonas sexuales del perro no contribuyen a la atracción del vector por el huésped, como sucede con otros artrópodos.

La talla y el pelaje del perro tampoco mostraron asociación a borreliosis, comparado con otro estudio de España (Merino *et al*., 2000), donde el pelaje fue importante relacionado con esta seropositividad, mientras que la talla no fue relevante, aunque se podría suponer que los perros de mayor talla y pelaje más largo, más garrapatas tendrían, debido a que sería mayor el área donde se alojen las citadas garrapatas transmisoras, y el pelaje largo las protegería de ser eliminadas por el huésped, pero no hay evidencia de esta asociación ya que una sola mordida por el vector es necesaria para transmitir la enfermedad de Lyme (Baumgarten *et al*., 1999).

Aunque se pensó que la prevalencia a borreliosis estuviera relacionada con el número de perros en el hogar, con la falta de piso de concreto de la residencia, y el tránsito de perros entre la casa y la calle, como sucedió en otros estudios (Guerra *et al*., 2001), así como el grado de infestación de garrapatas (Merino *et al*., 2000; De Lacerda *et al*., 2004), en este estudio no mostró esta asociación, probablemente por la misma razón de que una sola mordida del vector es suficiente para transmitir borreliosis (Baumgarten *et al*., 1999), además que las garrapatas pueden provenir de otro perro de la misma casa o de los vecinos.

La falta de un programa preventivo para el control de garrapatas también fue asociado a *B. burgdorferi*, como en otros estudios (Merino *et al*., 2000; Guerra *et al*., 2001), lo que significa que la seroprevalencia puede reducirse siguiendo un programa que incluya al menos dos tratamientos desparasitantes, dos baños garrapaticidas y dos fumigaciones del hogar al año para interrumpir el ciclo biológico del vector. Aunque la seroprevalencia parece ser baja en este estudio, la borreliosis es una enfermedad zoonótica y puede reducirse siguiendo un programa preventivo mínimo.

En el caso de los perros capturados por los centros de control anima, se supone que tienen la misma probabilidad de contraer borreliosis, a pesar de las razones por las cuales se estudiaron, como en el caso de la edad, por tratarse de una enfermedad crónica.

Las evidencias confirman la existencia de *B. burgdorferi* en perros y garrapatas en una área donde sólo se han registrado garrapatas *R. sanguineus*, aunque no han sido consideradas un importante vector para *B. burgdorferi*.

Aunque no se haya comprobado la transmisión de *B. burgdorferi* por garrapatas adultas de *R. sanguineus* en perros utilizando el protocolo para demostrar la transmisión con *Ixodes scapularis*; es necesario realizar más estudios, debido a las evidencias serológicas y su confirmación molecular por PCR y secuenciación de ADN obtenidos de muestras sanguíneas de perro de la ciudad de Mexicali y que el 100% de las garrapatas colectadas de los perros muestreados fueron *R. sanguineus*, además del hallazgo de ADN por PCR de la espiroqueta en esta garrapata.

Es importante hacer notar que a las 24 horas pos-colocación de garrapatas en los perros 1 y 2, murieron 9 de las 10 hembras inoculadas con espiroquetas, las cuales consumieron 15-20 µL cada una, y sobrevivió la garrapata que consumió 7 µL; esto sugiere que la dosis utilizada no pudo ser soportada por *R. sanguineus* como lo hace *I. scapularis*.

Es necesario continuar con más estudios para tratar de demostrar la transmisión de esta espiroqueta por *R. sanguineus* en perros, tomando en cuenta los resultados positivos de seroprevalencia de borreliosis.

Las expectativas para demostrar la transmisión de *B. burgdorferi* a través de *R. sanguineus* son:

a) Utilizar 5-7 µL de medio BSK-H con 1×10^8 espiroquetas/ml como inóculo en lugar de 20 µL.

b) Realizar la inoculación de borrelias en la fase de ninfa en vez de la adulta.
c) Inocular con borrelias a 30 garrapatas hembras en lugar de 5 por cada perro de experimentación.

5.- ARTICULOS ACEPTADOS PARA PUBLICACION

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Prevalence of *Rhipicephalus sanguineus* ticks on dogs in an urban region on the Mexico-USA border

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TICKS are the second most important group of arthropod vectors of diseases transmissible to animals and human beings. They are obligate parasites, and while feeding on a host may transmit a range of pathogens, such as bacteria, spirochaetes, rickettsiae, protozoa, viruses and nematodes, and toxins. The tickborne diseases most commonly transmitted to human beings include Lyme disease, ehrlichiosis, babesiosis, Rocky Mountain spotted fever, Colorado tick fever, tularaemia, Q fever, tick paralysis, spotted fever and tick encephalitis. Ticks may also facilitate secondary infections, and allergic reactions may develop to proteins in their saliva (Spach and others 1993, Fellow 1999). Ticks can be a zoonotic risk because, as well as being encountered outdoors, they may be found in homes, where they can come in contact with human beings while searching for favourable environmental conditions to subsist. (Gaviola and others 1997, Cruz-Vazquez and Garcia-Vazquez 1999, Quintero and others 2004).

The tick *Rhipicephalus sanguineus* is distributed worldwide and has been implicated as a vector of several pathogens including *Rickettsia rickettsii* and *Ehrlichia canis* (Marquez-Jimenez and others 2005, Merino and others 2005). The life cycle of *R sanguineus*, comprising egg, larval, nymph and adult stages, may require up to two years to be completed, pathogens may be transmitted at any of these stages (Spach and others 1993). Adult *R sanguineus* are most frequently found in the ears and interdigital spaces of dogs; larvae and nymphs adhere easily to long hair on the back or neck. The larval, nymph and adult stages must all feed on a host (Quiroz 1999).

The objective of this pilot study was to estimate the prevalence of ticks on dogs in the urban area of Mexicali, in Baja California, an area on the border between Mexico and the USA, and assess the association between risk factors and the detection of ticks.

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The study was carried out between September 1 and November 30, 2003, its purpose was to determine the feasibility of performing a larger study and to define the appropriate sample size.

Mexicali, the capital of the State of Baja California, Mexico, is a city in the north-west of the country at 32° 40' N latitude, 115° 28' W longitude. It has an extreme, desert-type climate, with mean summer maximum and minimum temperatures of 40·3 and 23·7°C, and mean winter maximum and minimum temperatures of 23·7 and 7·2°C.

The average annual rainfall is 0·63 (0·43) cm. Data on the climatic condition were obtained from the United States National Weather Service of the National Oceanic and Atmospheric Administration (www.nws.noaa.gov/).

Ninety-four dogs were studied: 54 were recruited from any of the 10 private veterinary clinics in the city, having been taken there for any reason, and 40 had been captured by personnel of the Veterinary Center for Rabies Control. To be included in the study, dogs had to be at least one month old, but could be of any breed, size or sex. Dogs were randomly selected from each group. All the selected dogs were examined for ticks on the face, ears, neck, back and limbs (interdigital spaces), and the number of ticks per region were recorded. Dogs on which at least one tick was found were considered positive. The degree of infestation (Table 1) was determined based on the tick count on each dog, irrespective of the area of the body where they were found.

Ticks were collected by a veterinary surgeon following the procedures described by Farley (1996) and Lyon and Restifo (2000). Samples were sent to the Molecular Biology Laboratory of the Veterinary Science Research Institute, Autonomous University of Baja California, in 70 per cent ethanol in sterile 15 ml collector tubes with a sealing cap. Taxonomic identification was performed by stereoscopic observation (Quiroz 1999).

The prevalence by study factor (tick species, sex, age and size of dog, dog's group of origin, body region where ticks were found and degree of infestation) and total prevalence were estimated. Prevalence values within each factor were compared by chi-squared tests. Likewise, other variables, such as sex, age, size and group, were evaluated as risk factors for a dog being tick positive by using odds ratio analysis in 2 × 2 tables (Walker 1997). All statistical analysis were performed using the SAS statistical package version 9.1 (SAS 2004).

At least one tick was found on 56 of the 94 dogs sampled: there was no significant difference between the prevalence in the two groups of dogs. All of the ticks collected from dogs were identified morphologically as *R sanguineus*. Most of the ticks were found on the ears (29 dogs), followed by the interdigital spaces (27 dogs) and the dorsal region (23 dogs), they were found on the neck of 11 dogs and on the face of six dogs. Forty-four of the 56 tick-positive dogs were graded as having a slight infestation, nine had a medium infestation and three had a severe infestation.

Dogs up to one year old had a significantly ($P<0·05$) higher tick prevalence (73·6 per cent) than the estimated prevalence for older dogs (50 per cent). Dogs up to one year old are 2·8 times more likely to be infested with ticks than older dogs (Table 2). There were no significant differences between the prevalence of ticks in dogs of different sexes and sizes.

The results of this small pilot study, which was carried

out in the autumn in Mexicali, northern Mexico, indicate that there was a high prevalence of ticks (59·6 per cent) in dogs in the city – greater than the prevalence reported for *Rhipicephalus sanguineus* found in two other places in Mexico, Culiacán, Sinaloa (46 per cent) (Gaxiola and others 1997), and Cuernavaca, Morelos (20 per cent) (Cruz-Vazquez and Garcia-Vazquez 1999), as well as in Israel (16 to 34 per cent) (Mumcuoglu and others 1993), Brazil (27 per cent) (Szabo and others 2001), Nigeria (19·5 per cent) (Ugochukwu and Nnadozie 1985), Japan (4·8%) (Shimada and others 2003) and Italy (19.7%) (Tringali and others 1986).

All of the ticks collected from the dogs were identified as *R sanguineus*; similar results have been reported in Brazil (Szabo and others 2001), Mexico (Cruz-Vazquez and Garcia-Vazquez 1999) and also from another study carried out in Mexicali (Quintero and others 2004). *R sanguineus* is potentially capable of biting human beings and transmitting zoonotic diseases such as ehrlichiosis and rickettsiosis (Carpenter and others 1990, Quintero and others 2004, Marquez-Jimenez and others 2005, Merino and others 2005, Dantas-Torres and others 2006). A study in Italy reported approximately 500 human tick bites per 100,000 people; and 10 per cent of the bites were attributed to *R. sanguineus* (Manfredi and others 1999). One study in Mexico reported a general seroprevalence of *E canis* in dogs of 33·1 per cent while for the state of Baja California the seroprevalence was 70·2 per cent (Reference ???). In another study in Mexicali, 28 of 30 dogs (93.3 per cent) were seropositive for *E canis* and five of 30 (16·7 per cent) were seropositive for *R rickettsii* (Romano and others 1998). Another study in the same city indicated an adjusted seroprevalence of *E canis* of 49·3 per cent (95 per cent confidence interval [CI] 30·8 to 54·1 per cent) (Tinoco-Gracia and others 2007a).

In the present study, young dogs were found to have a higher prevalence of tick infestation than older dogs; however, as age increases, the cumulative number of exposures to ticks will increase. Older dogs may develop resistance to reinfestations with *R sanguineus* (Inokuma and others 1997).

Another observation in this study was the preference of ticks for certain locations on the dogs' body – the ears, interdigital spaces and back – which is in agreement with previous published studies (Papazahariadou and others 2003); in another study, the ears and the abdomen were found to be preferred sites (Mumcuoglu and others 1993). It is likely that these sites are preferred because they are less accessible to the dog to remove ticks with its paws, as compared with locations such as the neck or face.

Slight tick infestations were most commonly found in the dogs in this study; medium and severe infestations were much less frequent. The dogs were sampled during the autumn; previous studies have shown that the presence of ticks on dogs is reduced in the autumn, to protect the ticks from unfavourable environmental conditions (Quiroz 1999) such as low temperatures during the autumn and winter; during this period, ticks move to places where they are more sheltered, such as crevices in floors and walls, and shutters (Quintero and others 2004).

Other important data that should be taken into account relate to the serological evidence that has been published on *Borrelia burgdorferi* in dogs living in the Mexicali area; in two previous studies, prevalences of 6·6 per cent (Romano and others 1998) and 8·2 per cent (95 per cent CI 1·5 to 13·3

per cent) (Tinoco-Gracia and others 2007b) were reported. Although no vector has been proven, *R sanguineus* could be responsible for the transmission *of B burgdorferi* in this area.

The present results, and the implications of transmission of pathogens by ticks, suggest a need for further studies in order to understand comprehensively factors such as the definitive diagnosis, culture and molecular biology of the relevant zoonotic diseases, as well as the epidemiology (frequency, distribution and risk factors) of these diseases in the region, in order to design preventive medicine programme. Campaigns of fumigation to kill ticks, regular treatment of dogs with dewormers and acaricides, and public education, related to the risks of zoonotic transmission and tick prevention strategies, are needed; such programmes will require the participation of veterinarians epidemiologists, doctors in the public and private health services, as well as the collaboration of the general public.

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TABLE 1: Definition of the degree of tick infestation on each dog

Degree of infestation	Number of ticks
Light	1 to 10
Medium	11 to 30
Severe	>30

TABLE 2: Prevalence of *Rhipicephalus sanguineus* ticks on dogs of different age groups in the city of Mexicali, Baja California, Mexico

Age group	Number of dogs	Number positive	Prevalence (%)
≤ 1 year	38	28	73.6[a]
>1 year	56	28	50.0[b]
Total	94	56	59.6

[ab] Different letters indicate a significant difference ($P \leq 0.05$)

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Seroprevalence of *Borrelia burgdorferi* in Dogs From a Mexico-U.S. Border Desert Region: Pilot Study

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Abstract: The aim of this pilot study was to estimate the seroprevalence to *Borrelia burgdorferi* in dogs from a Mexico-U.S. border region. A total of 94 dogs were tested by *Borrelia burgdorferi* ELISA kit. Borreliosis or Lyme desease is a worldwide zoonotic tick-borne disease caused by the espirochete *Borrelia burgdorferi* This disease is characterized by arthritis, lameness, erythema migrans, fatigue, anorexia, general malaise, muscle pain, stiff neck, fever, heart block, kidney failure and neurological changes such as seizures, aggression. In some cases it is cause of death. The resullts show an adjusted prevalence to *Borrelia burgdorferi* of 8.2% (95% I.C. 1.5-13.3%), obtained using Rogan-Gladen estimator. Since *B. burgdorferi* is transmitted by ticks, preventive and control measures to erradicate ticks have to be established in order of minimize the risk of infection.

Key words: Seroprevalence of *Borrelia burgdorferi*, border desert, pilot study, ELISA

INTRODUCTION

Borreliosis or Lyme disease is a worldwide zoonotic disease caused by the espirochete *Borrelia burgdorferi* transmitted by tick bite, primarily *Ixodes scapularis* and *I. pacificus*. This spirochete lenght 8-30 μm and width 0.2-0.3 (Barbour and Hayes, 1986; Greene *et al*., 2000). It is the most frequent tick-borne disease in Europa and the United States in animals and human beings. This disease is characterized by arthritis, lameness, erythema migrans, fatigue, anorexia, general malaise, muscle pain, stiff neck, fever, heart block, kidney failure and neurological changes such as seizures, aggression. In some cases it is cause of death (Burgess, 1986; Faul *et al*., 1999; Straubinger, 2000).

The aim of this pilot study was to estimate the seroprevalence to *Borrelia burgdorferi* in dogs in the urban area of Mexicali Baja California, a Mexico-U.S. border desert region.

MATERIALS AND METHODS

Study design and characteristics of the population: A cross-seccional descriptive study was conduced where 10 veterinary clinics and the Animal Control Center participated. The duration of the study was from September to November 2003. A total of 94 dog serum samples were randomly taken, 54 from veterinary clinics and 40 from the Animal Control Center. Mexicali city is situated along the state's northern border with the U.S. state of California and is the northernmost city in Latin America, located at 32°40'0"N, 115°28'0"W, with 855,962 inhabitants (Wikipedia, 2006). Climate is extreme, desert type and the average annual rainfall is 0.63±.43 cm. Climatic conditions data was collected from the United States National Weather Service of the National Oceanic and Atmospheric Administration (http://www.nws.noaa.gov/).

Data collection: A questionnaire was designed to collect information of the tested dogs and included: General information of dog: gender (female, male), age (≤1 year, >2 years), size (small, medium, big) and Intensity of tick infestation: None, low (1-10 ticks), moderate (11-30 ticks), intense (>30 ticks). The outcomes of most of the questions were dichotomous.

Blood collection: Blood samples were collected by certified personal. Briefly, 3 mL of blood were collected by punction of the cephalic vein after proper antisepsis of

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the area with isopropyl alcohol and placed in tubes Vacutainer®. Each sample was properly labeled and centrifuged at 3500 RPM for 10 min to separate the serum. The serum was transferred into 1mL vials, labeled and stored at-20°C until testing.

Serology: Antibodies against *Borrelia burgdorferi* were measured with the kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc. for the detection and semi quantification of canine IgG class which guarantees a 95.8% sensibility and a 94.7% specifity. The Optical Density (OD) at 450 nm was registred, where an OD<1.0 was considered negative and OD = 1.0 as positive, according to the manufacturer.

Statistical analysis: Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed. The adjusted prevalence and its 95% CI were obtained using Rogan-Gladen estimator (Greiner and Gardner, 2000). The significance of the class variables (gender, age, size and intensity of tick infestation) was determined by Chi-squared test (Walker, 1997). All statistical analysis were performed using the Statistical Analysis System for Windows version 9.1.3 (SAS, 2004).

RESULTS AND DISCUSSION

The results of this study indicate an adjusted seroprevalence to *Borrelia burgdorferi* of 8.2% (95% C.I. 1.5-13.3%) in dogs from Mexicali, calculated by Rogan-Gladen estimator (Greiner and Gardner, 2000). The adjusted prevalence obtained in dogs from veterinary clinics and from the Animal Control Center is presented in Table 1.

The seroprevalence obtained in this study was similar than that from 1988 (6.6%) in the same city (Mexicali). Nevertheless were tested 30 dogs with epistaxis (Romano *et al*., 1998). The prevalence of this study was lower than found in another study done in other region of the Mexican Republic as Monterrey, Nuevo León, where found a prevalencia of 16% (160/850) in dogs tested by an indirect immunofluorescent assay (Salinas-Melendez *et al*., 1999). A possible cause of low seroprevalence to *B. burgdorferi* can be that have not been found the known vectors of this disease in this region as *Ixodes scapularis, I. pacificus, Dermacentor variabilis* and *Amblyomma americanum* (Magnarelli and Anderson, 1988; Lane, 1996; Adelson *et al*., 2004). The unique tick found it in Mexicali has been *R. sanguineus* (in press),which is not considered as vector of borreliosis in other regions of the world. However, in Sao Paulo, Brasil, where *Ixodes loricatus, I. didelphidis* and *Amblyomma cajennense* were found, the prevalence was of 9.7% (23/237) in dogs by ELISA and confirmed by Western blot (Joppert *et al*., 2001).

Table 2 shows the unadjusted prevalence values stratified by origin, gender, age, size and intensity of tick infestation. In general, not differences (p>0.05) were observed in prevalence values according to gender, age and intensity of tick infestation between dogs from veterinary clinics and those from the Animal Control Center. However, dogs of big size from Animal Control Center showed higher (p<0.05) seroprevalence than those dogs of small and medium size.

Although the prevalence found in this pilot study was low, it is necessary to perform a complete study that includes an appropriate sample size, serum sampling all year long and the evaluation of risk factors so that the appropriate preventive and control measures are established. Also, since borreliosis is a zoonotic desease that may require expensive hospitalization and may cause death it is imperative to know the prevalence in humans, particularly in places like Mexicali, where a serologic evidence in dogs has been observed.

Table 1: Adjusted prevalence* to *Borrelia burgdorferi* of dogs from Mexicali, an urban area of Mexico-U.S. border desert region

Origin	n	Positives	Adjusted prevalence* (%)	95% I.C. (%)
General	94	7	8.2	1.5-13.3
Veterinary clinics	54	4	8.1	1.5-13.2
Animal control center	40	3	8.2	1.6-13.3

*Rogan-Gladen estimator (Greiner and Gardner, 2000)

Table 2: Seroprevalence* to *Borrelia burgdorferi* in dogs from Mexicali, stratified by origin, gender, age, size and intensity of infestation

	Origin					
	Veterinary clinics			Animal control center		
Class variable[1]	n	Positive	(%)	n	Positive	(%)
Gender						
Male	30	3	10.0[a]	15	2	13.3[a]
Female	24	1	4.1[a]	25	1	4.0[a]
Age						
<1 year old	16	2	12.5[a]	22	1	4.5[a]
>1 year old	38	2	5.2[a]	18	2	11.1[a]
Size						
Small	10	2	20.0[a]	9	0	0.0
Medium	28	1	3.5[a]	23	0	0.0
Big	16	1	6.2[a]	8	3	37.5[a]
Intensity of tick infestation						
None	21	1	4.7[a]	17	0	0.0
Low	26	3	5.5[a]	18	2	11.1[a]
Moderate	5	0	0.0	4	1	25.0[a]
Intense	2	0	0.0	1	0	0.0

* Unadjusted values [1]Equal letters by class variable within origin, indicate no differences (p>0.05)

CONCLUSION

A low seroprevalence of 8.2% (95% I.C. 1.5-13.3%) to *Borrelia burdorgferi* was observed in this study and because borreliosis is a zoonotic disease, a complete study has to be done, where both dogs and humans are included with an appropriate sample size and the evaluation of risk factors. Since *Borrelia burgdorferi* is transmitted by ticks, preventive and control measures to erradicate ticks have to be established in order of minimize the risk of infection.

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----- Mensaje original ----
De: Kelli Howell <khowell@jarcet.com>
Para: tinoco.luis@yahoo.com
Enviado: miércoles, 8 de octubre, 2008 16:31:58
Asunto: Journal of Applied Research
Dear Dr Tinoco:

It is my pleasure to inform you, on behalf of The Journal of Applied Research, that your article, **"Prevalence and risk factors for *Borrelia burgdorferi* infection in Mexicali, Baja California, a Mexico-US border city"** has been accepted for publication, and is scheduled for the November 2008 issue.

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Prevalence and risk factors for *Borrelia burgdorferi* infection in Mexicali, Baja California, a Mexico-US border city

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1. Abstract

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *B. burgdorferi* which is transmitted by a tick bite, primarily from *Ixodes scapularis* and *I. pacificus*. It is characterized by polisystemic disorders. In Mexico, native Lyme disease has been recently reported in humans. In dogs *B. burgdorferi* infection has been also reported in several areas of the country. In Monterrey, Nuevo Leon, Mexico, a seroprevalence of 16% was observed in dogs (136/850) and molecular evidence was found in dog synovial fluid. Moreover, a preliminar study performed in 2003 in Mexicali, Baja California, Mexico showed a prevalence of 7.4% (7/94) in dogs infested only by the tick *Rhipicephalus sanguineus*. The aim of this study was to estimate the seroprevalence of *B. burgdorferi* in canine patients that were brougth into private veterinary clinics in Mexicali. From out of a total of 98 active private clinics in Mexicali, 39 (40%) agreed to participate. Blood samples of 384 dogs were randomly selected from February 2005 to December 2006, and their sera were analized by the semiquantitative kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc., with 96% sensibility and 95% specificity. An adjusted prevalence of 6.8% (95% CI 3.5-8.9%) was obtained using the Rogan-Gladen estimator. The seroprevalence obtained in this study was lower compared to those in Monterrey (16%) where the principal vector was *Ixodes scapularis*, and in Sao Paulo, Brazil (15.6%) where the principal vector was *Amblyomma cajennense*. Risk factors associated with *B. burgdorferi* seropositivity were age (Mantel-Haenszel χ^2, P = 0.02, OR= 2.7 [95% CI 1.2-6.1]), and the absence of a preventive program (Mantel-Haenszel χ^2, P = 0.005, OR= 4.9, [95% CI 1.4-16.8]). This study confirms the existence of *B. burgdorferi* past/present infection in dogs in an area where the only identified tick is *R. sanguineus.*

Keywords: *Borrelia burgdorferi*, tick, Lyme disease, borreliosis.

2. Introduction

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi*, which is transmitted by tick bite, primarily *Ixodes scapularis* and *I. pacificus* in North America (Barbour and Hayes, 1986; Magnarelli *et al*., 1987b; Greene *et al*., 2000). It is the most frequent tick-borne disease in Europe and the United States in human beings.

The disease is characterized by arthritis, lameness, erythema migrans, fatigue, anorexia, general malaise, muscle pain, stiff neck, fever, heart block, kidney failure, and neurological changes such as seizures and aggressive behavior (Burgess, 1986; Magnarelli *et al*., 1987b; Faul *et al*., 1999; Straubinger, 2000).

Several animal species can be infected by the *B. burgdorferi* spirochete, including rodents, deer, dogs, cats, cows, horses, reptiles, birds and some other tick species (Faul *et al*., 1999; Straubinger, 2000). Dogs are considered the most important reservoir for ticks in the home environment (Straubinger, 2000; De Lacerda *et al*., 2004).

In the city of Monterrey, Mexico, an indirect immunofluorescent assay (IFA) was used to detect antibodies against *B. burgdorferi* in dogs; with a resulting seroprevalence of 16% (Salinas-Melendez *et al*., 1999). Molecular evidence of *B. burgdorferi* was also found by amplification and DNA selected sequences from synovial fluid samples from dogs with arthritis, which suggests the presence of Lyme disease in the area (Salinas-Melendez *et al*., 1995).

In a pilot study in the city of Mexicali, Mexico, seroprevalence to this spirochete was determined using *B. burgdorferi* ELISA® Helica Biosystems, Inc., which resulted in 8.2% (95% CI 1.5-13.3%) out of the 94 dogs that were tested in autumn (September-November, with a sensitivity of 96% and a specifity of 95% (Tinoco-Gracia *et al*., 2007).

In Distrito Federal and the northeast of Mexico, a study of 2,346 human sera analyzed with ELISA and confirmed by Western blot found a prevalence of 3.43 and 6.2% respectively (Gordillo-Pérez *et al*., 2003). Also, 4 patients who reside in Distrito Federal and were bitten by ticks while visiting forestal parks (3 in Mexico City and 1 in Quintana Roo) were positive to *B. burgdorferi* when their skin biopsies were tested by PCR using primers for the *fla* gene; one of the patients was also positive to OspA gene by sequencing. (Gordillo-Pérez *et al*., 2007).

The aim of this study was to estimate the seroprevalence to *B. burgdorferi* and associated risk factors in canine patients at veterinary clinics in the urban area of Mexicali, Baja California, a Mexico-U.S. border desert region.

3. Materials and methods

3.1. Study design and characteristics of the population

A descriptive study was designed, and 39 veterinary clinics agreed to participate. The data and blood collection started on February 2005 and ended on December 2006. A total of 384 serum samples were randomly taken from canine patients at veterinary clinics in the urban area of Mexicali. This city is situated along the state's northern border with California, and is the northernmost city in Latin America; it is located at 32°40′0″N, 115°28′0″W, with 855,962 inhabitants (Wikipedia, 2006). Climate is extreme, desert type and the average annual rainfall is 0.63 ± .43 cm. Climatic conditions data was collected from the United States *National Weather Service* of the *National Oceanic and Atmospheric Administration* (http://www.nws.noaa.gov/).

3.2. Data collection

A questionnaire was designed to collect information of the tested dogs and it included: 1) General information of dog: gender (female, male), age (< 1 year, ≥ 1 year),

size (small, medium, large), coat (short, medium, large); 2) dog handling: number of dogs in the house, antiparasitic treatments, dog mobilization between house and street, intensity of tick infestation: none, low (1-10 ticks), moderate (11-30 ticks), intense (>30 ticks); and 3) living conditions of dogs: type of surface (ground or grass, or concrete), indoor/outdoor status of dog, origin of dog, and history of taking the dog outside the city limits. The outcomes of most of the questions were dichotomous. Questionnaires were administered by trained personnel at the clinics.

3.3. Blood collection

Blood samples were collected by trained personnel. Briefly, 3 ml of blood were collected by punction of the cephalic vein after proper antisepsis of the area with isopropyl alcohol, and placed in Vacutainer® tubes. Each sample was properly labeled and centrifuged at 3500 RPM for 10 min to separate the serum. The serum was transferred into 1ml vials, labeled and stored at –20°C until testing.

3.4. Serology

Antibodies against *B. burgdorferi* were measured with the semiquantitative kit *B. burgdorferi* ELISA® Helica Biosystems, Inc. for the detection of canine IgG class which guarantees a 95.8% sensitivity and a 94.7% specificity. The optical density (OD) at 450 nm was recorded, where an OD < 0.3 was considered negative and OD ≥ 0.3 as positive, according to the manufacturer.

3.5. Statistical analysis

Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed (24/384). The adjusted prevalence and its 95% CI (confidence interval) were obtained using the Rogan-Gladen estimator (Greiner and Gardner, 2000).

A Mantel-Haenszel χ^2 test and Odds Ratio (OR) were used to determine differences in seroprevalence to *B. burgdorferi* by groups and the association of risk factors. Statistical significance was considered at a *P*-value of < 0.05. Exact binomial confidence intervals were calculated individually for each proportion (Walker, 1997b).

All statistical analysis were performed using the Statistical Analysis System for Windows version 9.1.3 (SAS, 2004).

4. Results and discussion

The results of this study indicated an adjusted seroprevalence to *B. burgdorferi* of 6.8% (95% CI 3.5-8.9%) in canine patients from veterinary clinics of Mexicali. Since there is no vaccination against Lyme disease included in current immunization programs for dogs in Mexicali, the results of this research will not be affected by the presence of post-vaccination antibodies.

Furthermore, the prevalence in this study was lower to that found in Monterrey, Mexico, with a prevalence value of 16% (136/850) in dogs tested by an indirect immunofluorescent assay (Salinas-Melendez *et al*., 1999). The lower seroprevalence to *B. burgdorferi* found in this study may be due to the fact that the known vectors for this

spirochete, *I. scapularis*, *I. pacificus*, *Dermacentor variabilis* and *Amblyomma americanum* in North America (Magnarelli and Anderson, 1988; Lane, 1996; Adelson *et al*., 2004), have not been found in this region. The only species of tick found in Mexicali has been *R. sanguineus* (*in press*), which has not been considered a vector of borreliosis in other regions of the world. Meanwhile, in Sao Paulo, Brasil, the prevalence in dogs was of 15.6% (31/199) and the main tick vector was *Amblyomma cajennense* (O'Dwyer *et al*., 2004).

Risk factors found to be associated with *B. burgdorferi* infection were age (Mantel-Haenszel χ^2, $P = 0.02$), and to the absence of a preventive plan (Mantel-Haenszel χ^2, $P = 0.005$). The study showed that the dogs that were more at risk were those of ≥ 1 year of age with an OR= 2.7 (95% CI 1.2-6.1), as well as the ones who did not have access to a preventive program which consisted of at least 2 antiparasitic treatments (endo and ectoparasites), 2 tick treatment baths and 2 home fumigations a year, with an OR= 4.9 (95% CI 1.4-16.8).

The higher prevalence in dogs of ≥ 1 year of age, similar to others studies (Merino *et al*., 2000; Goossens *et al*., 2001; De Lacerda *et al*., 2004), is justified by the nature of borreliosis, which is a chronic disease that may take several months to show its most notorious sign in dogs, lameness of one or more limbs (Magnarelli *et al*., 1987b).

Gender in tested dogs showed no relation to seropositivity to *B. burgdorferi*, similar to others studies (Merino *et al*., 2000; Guerra *et al*., 2001; De Lacerda *et al*., 2004), which indicates that apparently sexual pheromones specific to each sex do not contribute to vector attraction, as it happens to other arthropod animals.

Size and coat did not show an association to borreliosis either, compared with another study from Spain (Merino *et al*., 2000), where coat turned out to be important but size was not relevant, although it was believed that the larger the body and the longer the hair, the more ticks the animal would get, since there would be broader body areas to be infested, and the longer hair would make it harder on the dog to shed the ticks, but there is no evidence of any association since a bite of the vector is enough to start the Lyme infection (Baumgarten *et al*., 1999).

Although it was intially thought that the prevalence to borreliosis may be associated with the number of dogs in a house, the lack of concrete flooring throughout the residence, and to the transit of dogs into and out of the house, as it happened in the study conducted in Wisconsin and Illinois (Guerra *et al*., 2001), or tick infestation level (Merino *et al*., 2000; De Lacerda *et al*., 2004), this study did not show any statistical association with those factors.

The lack of a tick control system also was found to be associated to seropositivity to *B. burgdorferi*, as it resulted in other studies (Merino *et al*., 2000; Guerra *et al*., 2001), which means that seroprevalence could be reduced just by following a preventive plan, as previosly mentioned, since it would interrupt the biological cycle of the vector.

Although the results of this study may suggest a relatively low prevalence for *B. burgdorferi* infection in dogs, borreliosis is a zoonotic disease and it can be reduced following minimum preventive measures, like those analyzed in this study. Furthermore, it is necessary to determine the vector involved in the transmission of borreliosis in this area, considering that the only tick that has been observed in Mexicali dogs is *R. sanguineus*. There were no statistical differences in the seroprevalence for dogs that have been kept

within the city limits all of their lives and dogs that were either born in other geographic areas or taken outside the city limits where they might have been exposed to *Ixodes* ticks. It will be important to determine borreliosis prevalence in humans, specifically in places like Mexicali, where exposure to this agent is being demonstrated in the dog population and dogs may act as sentinels for *B. burgdorferi* infection in humans (Goossens *et al*., 2001; Guerra *et al*., 2001; Duncan *et al*., 2005).

This study confirms the existence of *B. burgdorferi* in dogs in an area where *R. sanguineus* is the only tick that has been identified so far. Although this tick has not been considered an important vector for *B. burgdorferi*, it can be brought into the homes and feed on humans (Beichel *et al*., 1996; Cruz-Vazquez and Garcia-Vazquez, 1999), considering that it only takes one bite from the vector to spread borreliosis (Baumgarten *et al*., 1999). Molecular evidence of *B. burgdorferi* in dogs and ticks is currently being searched for, as well as the transmission mechanism is being determined.

5. Conclusions

A seroprevalence value of 6.8% (95% CI 3.5-8.9%) to *B. burgdorferi* was observed in dogs in Mexicali, B.C., an area where the only specie of tick that has been observed in dogs is *R. sanguineus*.

The evaluated risk factors were found to be related both to age in the dogs that were sampled (Mantel-Haenszel χ^2, $P = 0.02$), and to the absence of a preventive plan (Mantel-Haenszel χ^2, $P = 0.005$). The study showed that the dogs that were more at risk were those of ≥ 1 year of age with an OR= 2.7 (95% CI 1.2-6.1), as well as the ones who did not have access to a preventive program.

As well as the ones who did not have access to a preventive program, which should include at least 2 desparasite treatments (endo and ectoparasites), 2 tick treatment baths and 2 home fumigations a year to help reduce seroprevalence to Lyme disease.

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Prevalence and risk factors for *Borrelia burgdorferi* infection in dogs of Animal Control Centers from Mexicali, Baja California, a Mexico-US border city

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ABSTRACT

Lyme borreliosis, a worldwide zoonotic disease and characterized by polisystemic disorders, is caused by the spirochete *B. burgdorferi*, which is transmitted by a tick bite, primarily from *Ixodes scapularis* and *I. pacificus*. In Mexico, native Lyme disease has been recently reported in humans, while canine *B. burgdorferi* infection has been also reported in several areas of the country. In Monterrey, Mexico, a seroprevalence of 16% was observed in dogs (136/850) and molecular evidence was found in canine synovial fluid. Moreover, a preliminar study performed in 2003 in Mexicali, Mexico showed a prevalence of 7.4% (7/94) in dogs infested only by the tick *Rhipicephalus sanguineus*. The aim of this study was to estimate the seroprevalence of *B. burgdorferi* in dogs captured by personnel from the animal control centers in the city of Mexicali. Blood samples from 384 dogs were randomly selected from February 2005 to December 2006, and their sera were analized by the semiquantitative kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc., with 96% sensitivity and 95% specificity. An adjusted prevalence of 12% (95% IC 7.5-14.3%) was obtained using the Rogan-Gladen estimator. The seroprevalence obtained in this study was lower compared to those in Monterrey (16%), where the principal vector was *Ixodes scapularis*; and in Sao Paulo, Brazil (15.6%), where the main vector was *Amblyomma cajennense*. No risk factors were associated with *B. burgdorferi* seropositivity. This study confirms the existence of *B. burgdorferi* past/present infection in dogs in an area where the only identified tick is *R. sanguineus.*

Keywords: *Borrelia burgdorferi*, tick, Lyme disease, borreliosis.

INTRODUCTION

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi*, which is transmitted by tick bite, primarily *Ixodes scapularis* and *I. pacificus* in North America (Barbour and Hayes, 1986; Magnarelli *et al*., 1987b; Greene *et al*., 2000). In Europe and the United States, it is the most frequent tick-borne disease in human beings. Among its sypmtoms: arthritis, lameness, erythema migrans, fatigue, anorexia, general

malaise, muscle pain, stiff neck, fever, heart block, kidney failure, and neurological changes such as seizures and aggressive behavior (Burgess, 1986; Magnarelli *et al*., 1987b; Faul *et al*., 1999; Straubinger, 2000).

Several animal species can be infected by the *B. burgdorferi* spirochete, including rodents, deer, dogs, cats, cows, horses, reptiles, birds and some other tick species (Faul *et al*., 1999; Straubinger, 2000). Dogs are considered the most important reservoir for ticks in the home environment (Straubinger, 2000; De Lacerda *et al*., 2004).

An indirect immunofluorescent assay (IFA) was used to detect antibodies against *B. burgdorferi* in dogs in Monterrey, Mexico, with a resulting seroprevalence of 16% (Salinas-Melendez *et al*., 1999). Also, molecular evidence of *B. burgdorferi* was found by amplification and DNA selected sequences from canine synovial fluid samples from dogs with arthritis, which suggests the presence of Lyme disease in the area (Salinas-Melendez *et al*., 1995).

In a pilot study in the city of Mexicali, Mexico, seroprevalence to this spirochete was determined using *B. burgdorferi* ELISA® Helica Biosystems, Inc., which resulted in 8.2% (95% CI 1.5-13.3%) out of the 94 dogs that were tested in autumn (September-November), with a sensitivity of 96% and a specifity of 95% (Tinoco-Gracia *et al*., 2007).

In Distrito Federal and the northeast of Mexico, a study of 2,346 human sera, analyzed with ELISA and confirmed by Western, blot found a prevalence of 3.43 and 6.2% respectively (Gordillo-Pérez *et al*., 2003). Also, 4 patients who reside in Distrito Federal and were bitten by ticks while visiting forestal parks (3 in Mexico City and 1 in Quintana Roo) were positive to *B. burgdorferi* when their skin biopsies were tested by PCR using primers for the *fla* gene; one of the patients was also positive to OspA gene by sequencing. (Gordillo-Pérez *et al*., 2007).

The purpose of this study was to estimate the seroprevalence to *B. burgdorferi* and associated risk factors in canines captured by personnel from both animal control centers in Mexicali, Mexico, a Mexico-U.S. border desert region.

MATERIALS AND METHODS

Study design and characteristics of the population

A descriptive study was designed in both animal control centers in Mexicali, Mexico, *Centro Antirrábico Veterinario de Mexicali* and *Centro Municipal de Control Animal de Mexicali*. The data and blood collection started on February 2005 and ended on December 2006. A total of 384 serum samples were randomly taken. Mexicali is situated along the state's northern border with California, and is the northernmost city in Latin America; it is located at 32°40′0″N, 115°28′0″W, with 855,962 inhabitants (Wikipedia, 2006). Its climate is extreme, desert type and the average annual rainfall is 0.63 ± .43 cm. Climatic conditions data was collected from the United States *National Weather Service* of the *National Oceanic and Atmospheric Administration* (http://www.nws.noaa.gov/).

Data collection

A questionnaire was designed to collect information of the tested dogs and it included: general information of dog, such as gender (female, male), age (< 1 year, ≥ 1 year), size (small, medium, large), and coat (short, medium, large); and intensity of tick

infestation: none, low (1-10 ticks), moderate (11-30 ticks), intense (>30 ticks). The outcomes of most of the questions were dichotomous.

Blood collection

Blood samples were collected by trained personnel. 3 ml of blood were collected by punction of the cephalic vein after proper antisepsis of the area with isopropyl alcohol, and placed in Vacutainer® tubes. Each sample was properly labeled and centrifuged at 3500 RPM for 10 min to separate the serum. The serum was transferred into 1 ml vials, labeled and stored at –20°C until testing.

Serology

Antibodies against *B. burgdorferi* were measured with the semiquantitative kit *B. burgdorferi* ELISA® Helica Biosystems, Inc. for the detection of canine IgG class which guarantees a sensitivity of 95.8% and a specificity of 94.7%. The optical density (OD) at 450 nm was recorded, where an OD < 0.3 was considered negative and OD ≥ 0.3 as positive, according to the manufacturer.

Statistical analysis

Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed (24/384). The adjusted prevalence and its 95% CI (confidence interval) were obtained using the Rogan-Gladen estimator (Greiner and Gardner, 2000).

A Mantel-Haenszel χ^2 test and Odds Ratio (OR) were used to determine differences in seroprevalence to *B. burgdorferi* by groups and the association of risk factors. Statistical significance was considered at a *P*-value of < 0.05. Exact binomial confidence intervals were calculated individually for each proportion (Walker, 1997b).

All statistical analysis were performed using the Statistical Analysis System for Windows, version 9.1.3 (SAS, 2004).

RESULTS AND DISCUSSION

The results of this study indicated an adjusted seroprevalence to *B. burgdorferi* of 12% (95% CI 7.5-14.3%) in dogs from both animal control centers in Mexicali, Mexico (Greiner and Gardner, 2000), and it was slightly higher than the one obtained from a pilot study carried out in the same city, 8.2% (95% CI 1.5-13.3%) (Tinoco-Gracia *et al*., 2007). Also, the prevalence in this study was lower to that found in Monterrey, Mexico, with a prevalence value of 16% (136/850) in canines tested by an indirect immunofluorescent assay (Salinas-Melendez *et al*., 1999). The lower seroprevalence to *B. burgdorferi* found in this study may be due to the fact that the known vectors for this spirochete, *I. scapularis*, *I. pacificus*, *Dermacentor variabilis* and *Amblyomma americanum* in North America (Magnarelli and Anderson, 1988; Lane, 1996; Adelson *et al*., 2004), have not been found in this region. The only specie of tick found in Mexicali has been *R. sanguineus* (*in press*), which has not been considered a vector of borreliosis in other regions of the world.

Meanwhile, in Sao Paulo, Brasil, the prevalence in dogs was of 15.6% (31/199) and the main tick vector was *Amblyomma cajennense* (O'Dwyer *et al*., 2004).

None of the risk factors considered for this study—age, gender, size and coat of the canines in the animal control centers—seemed to be related to *B. burgdorferi* seropositivity. This could mean that these dogs have the same chances to get borreliosis, despite the reasons they were considered for this study: age, since borreliosis is a chronic disease; gender, in case there could be some affinity of the infected ticks to sexual hormones in the host; size, since the larger the host, the larger the area available for the vectors, even though it has been demonstrated that it only takes one bite from an infected tick for the host to get infected with Lyme disease; and coat, since the seroprevalence could be closely related to the length of the hair of the host, because long hair would protect the infected ticks. An important finding was that the only tick reported so far in Mexicali was *R. sanguineus* (*in press*), which is not considered as vector for borreliosis. It is also necessary to determine the vector involved in the transmission of *B. burgdorferi* in this area. Also, since borreliosis is a zoonotic disease, it is imperative to know the prevalence in humans, particularly in places like Mexicali, where serological evidence has been obtained.

CONCLUSIONS

A seroprevalence value of 12% (95% CI 7.5-14.3%) to *Borrelia burgdorferi* was observed in dogs captured by personnel from the animal control centers in Mexicali, Mexico, a region where the only tick that has been taxonomically identified in canines is *Rhipicephalus sanguineus*. And none of the factors considered for this study showed any association to seropositivity to *B. burgdorferi*.

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5.- ARTICULOS ACEPTADOS PARA PUBLICACION

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Prevalence of *Rhipicephalus sanguineus* ticks on dogs in an urban region on the Mexico-USA border

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TICKS are the second most important group of arthropod vectors of diseases transmissible to animals and human beings. They are obligate parasites, and while feeding on a host may transmit a range of pathogens, such as bacteria, spirochaetes, rickettsiae, protozoa, viruses and nematodes, and toxins. The tickborne diseases most commonly transmitted to human beings include Lyme disease, ehrlichiosis, babesiosis, Rocky Mountain spotted fever, Colorado tick fever, tularaemia, Q fever, tick paralysis, spotted fever and tick encephalitis. Ticks may also facilitate secondary infections, and allergic reactions may develop to proteins in their saliva (Spach and others 1993, Fellow 1999). Ticks can be a zoonotic risk because, as well as being encountered outdoors, they may be found in homes, where they can come in contact with human beings while searching for favourable environmental conditions to subsist. (Gaviola and others 1997, Cruz-Vazquez and Garcia-Vazquez 1999, Quintero and others 2004).

The tick *Rhipicephalus sanguineus* is distributed worldwide and has been implicated as a vector of several pathogens including *Rickettsia rickettsii* and *Ehrlichia canis* (Marquez-Jimenez and others 2005, Merino and others 2005). The life cycle of *R sanguineus*, comprising egg, larval, nymph and adult stages, may require up to two years to be completed, pathogens may be transmitted at any of these stages (Spach and others 1993). Adult *R sanguineus* are most frequently found in the ears and interdigital spaces of dogs; larvae and nymphs adhere easily to long hair on the back or neck. The larval, nymph and adult stages must all feed on a host (Quiroz 1999).

The objective of this pilot study was to estimate the prevalence of ticks on dogs in the urban area of Mexicali, in Baja California, an area on the border between Mexico and the USA, and assess the association between risk factors and the detection of ticks.

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The study was carried out between September 1 and November 30, 2003, its purpose was to determine the feasibility of performing a larger study and to define the appropriate sample size.

Mexicali, the capital of the State of Baja California, Mexico, is a city in the north-west of the country at 32° 40' N latitude, 115° 28' W longitude. It has an extreme, desert-type climate, with mean summer maximum and minimum temperatures of 40·3 and 23·7°C, and mean winter maximum and minimum temperatures of 23·7 and 7·2°C.

The average annual rainfall is 0·63 (0·43) cm. Data on the climatic condition were obtained from the United States National Weather Service of the National Oceanic and Atmospheric Administration (www.nws.noaa.gov/).

Ninety-four dogs were studied: 54 were recruited from any of the 10 private veterinary clinics in the city, having been taken there for any reason, and 40 had been captured by personnel of the Veterinary Center for Rabies Control. To be included in the study, dogs had to be at least one month old, but could be of any breed, size or sex. Dogs were randomly selected from each group. All the selected dogs were examined for ticks on the face, ears, neck, back and limbs (interdigital spaces), and the number of ticks per region were recorded. Dogs on which at least one tick was found were considered positive. The degree of infestation (Table 1) was determined based on the tick count on each dog, irrespective of the area of the body where they were found.

Ticks were collected by a veterinary surgeon following the procedures described by Farley (1996) and Lyon and Restifo (2000). Samples were sent to the Molecular Biology Laboratory of the Veterinary Science Research Institute, Autonomous University of Baja California, in 70 per cent ethanol in sterile 15 ml collector tubes with a sealing cap. Taxonomic identification was performed by stereoscopic observation (Quiroz 1999).

The prevalence by study factor (tick species, sex, age and size of dog, dog's group of origin, body region where ticks were found and degree of infestation) and total prevalence were estimated. Prevalence values within each factor were compared by chi-squared tests. Likewise, other variables, such as sex, age, size and group, were evaluated as risk factors for a dog being tick positive by using odds ratio analysis in 2 × 2 tables (Walker 1997). All statistical analysis were performed using the SAS statistical package version 9.1 (SAS 2004).

At least one tick was found on 56 of the 94 dogs sampled: there was no significant difference between the prevalence in the two groups of dogs. All of the ticks collected from dogs were identified morphologically as *R sanguineus*. Most of the ticks were found on the ears (29 dogs), followed by the interdigital spaces (27 dogs) and the dorsal region (23 dogs), they were found on the neck of 11 dogs and on the face of six dogs. Forty-four of the 56 tick-positive dogs were graded as having a slight infestation, nine had a medium infestation and three had a severe infestation.

Dogs up to one year old had a significantly ($P<0·05$) higher tick prevalence (73·6 per cent) than the estimated prevalence for older dogs (50 per cent). Dogs up to one year old are 2·8 times more likely to be infested with ticks than older dogs (Table 2). There were no significant differences between the prevalence of ticks in dogs of different sexes and sizes.

The results of this small pilot study, which was carried

out in the autumn in Mexicali, northern Mexico, indicate that there was a high prevalence of ticks (59·6 per cent) in dogs in the city – greater than the prevalence reported for *Rhipicephalus sanguineus* found in two other places in Mexico, Culiacán, Sinaloa (46 per cent) (Gaxiola and others 1997), and Cuernavaca, Morelos (20 per cent) (Cruz-Vazquez and Garcia-Vazquez 1999), as well as in Israel (16 to 34 per cent) (Mumcuoglu and others 1993), Brazil (27 per cent) (Szabo and others 2001), Nigeria (19·5 per cent) (Ugochukwu and Nnadozie 1985), Japan (4·8%) (Shimada and others 2003) and Italy (19.7%) (Tringali and others 1986).

All of the ticks collected from the dogs were identified as *R sanguineus*; similar results have been reported in Brazil (Szabo and others 2001), Mexico (Cruz-Vazquez and Garcia-Vazquez 1999) and also from another study carried out in Mexicali (Quintero and others 2004). *R sanguineus* is potentially capable of biting human beings and transmitting zoonotic diseases such as ehrlichiosis and rickettsiosis (Carpenter and others 1990, Quintero and others 2004, Marquez-Jimenez and others 2005, Merino and others 2005, Dantas-Torres and others 2006). A study in Italy reported approximately 500 human tick bites per 100,000 people; and 10 per cent of the bites were attributed to *R. sanguineus* (Manfredi and others 1999). One study in Mexico reported a general seroprevalence of *E canis* in dogs of 33·1 per cent while for the state of Baja California the seroprevalence was 70·2 per cent (Reference ???). In another study in Mexicali, 28 of 30 dogs (93.3 per cent) were seropositive for *E canis* and five of 30 (16·7 per cent) were seropositive for *R rickettsii* (Romano and others 1998). Another study in the same city indicated an adjusted seroprevalence of *E canis* of 49·3 per cent (95 per cent confidence interval [CI] 30·8 to 54·1 per cent) (Tinoco-Gracia and others 2007a).

In the present study, young dogs were found to have a higher prevalence of tick infestation than older dogs; however, as age increases, the cumulative number of exposures to ticks will increase. Older dogs may develop resistance to reinfestations with *R sanguineus* (Inokuma and others 1997).

Another observation in this study was the preference of ticks for certain locations on the dogs' body – the ears, interdigital spaces and back – which is in agreement with previous published studies (Papazahariadou and others 2003); in another study, the ears and the abdomen were found to be preferred sites (Mumcuoglu and others 1993). It is likely that these sites are preferred because they are less accessible to the dog to remove ticks with its paws, as compared with locations such as the neck or face.

Slight tick infestations were most commonly found in the dogs in this study; medium and severe infestations were much less frequent. The dogs were sampled during the autumn; previous studies have shown that the presence of ticks on dogs is reduced in the autumn, to protect the ticks from unfavourable environmental conditions (Quiroz 1999) such as low temperatures during the autumn and winter; during this period, ticks move to places where they are more sheltered, such as crevices in floors and walls, and shutters (Quintero and others 2004).

Other important data that should be taken into account relate to the serological evidence that has been published on *Borrelia burgdorferi* in dogs living in the Mexicali area; in two previous studies, prevalences of 6·6 per cent (Romano and others 1998) and 8·2 per cent (95 per cent CI 1·5 to 13·3

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per cent) (Tinoco-Gracia and others 2007b) were reported. Although no vector has been proven, *R sanguineus* could be responsible for the transmission of *B burgdorferi* in this area.

The present results, and the implications of transmission of pathogens by ticks, suggest a need for further studies in order to understand comprehensively factors such as the definitive diagnosis, culture and molecular biology of the relevant zoonotic diseases, as well as the epidemiology (frequency, distribution and risk factors) of these diseases in the region, in order to design preventive medicine programme. Campaigns of fumigation to kill ticks, regular treatment of dogs with dewormers and acaricides, and public education, related to the risks of zoonotic transmission and tick prevention strategies, are needed: such programmes will require the participation of veterinarians epidemiologists, doctors in the public and private health services, as well as the collaboration of the general public.

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TABLE 1: Definition of the degree of tick infestation on each dog

Degree of infestation	Number of ticks
Light	1 to 10
Medium	11 to 30
Severe	>30

TABLE 2: Prevalence of *Rhipicephalus sanguineus* ticks on dogs of different age groups in the city of Mexicali, Baja California, Mexico

Age group	Number of dogs	Number positive	Prevalence (%)
≤ 1 year	38	28	73.6[a]
>1 year	56	28	50.0[b]
Total	94	56	59.6

[ab] Different letters indicate a significant difference ($P \leq 0.05$)

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Seroprevalence of *Borrelia burgdorferi* in Dogs From a Mexico-U.S. Border Desert Region: Pilot Study

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Abstract: The aim of this pilot study was to estimate the seroprevalence to *Borrelia burgdorferi* in dogs from a Mexico-U.S. border region. A total of 94 dogs were tested by *Borrelia burgdorferi* ELISA kit. Borreliosis or Lyme desease is a worldwide zoonotic tick-borne disease caused by the espirochete *Borrelia burgdorferi* This disease is characterized by arthritis, lameness, erythema migrans, fatigue, anorexia, general malaise, muscle pain, stiff neck, fever, heart block, kidney failure and neurological changes such as seizures, aggression. In some cases it is cause of death. The resullts show an adjusted prevalence to *Borrelia burgdorferi* of 8.2% (95% I.C. 1.5-13.3%), obtained using Rogan-Gladen estimator. Since *B. burgdorferi* is transmitted by ticks, preventive and control measures to erradicate ticks have to be established in order of minimize the risk of infection.

Key words: Seroprevalence of *Borrelia burgdorferi*, border desert, pilot study, ELISA

INTRODUCTION

Borreliosis or Lyme disease is a worldwide zoonotic disease caused by the espirochete *Borrelia burgdorferi* transmitted by tick bite, primarily *Ixodes scapularis* and *I. pacificus*. This spirochete lenght 8-30 μm and width 0.2-0.3 (Barbour and Hayes, 1986; Greene *et al*., 2000). It is the most frequent tick-borne disease in Europa and the United States in animals and human beings. This disease is characterized by arthritis, lameness, erythema migrans, fatigue, anorexia, general malaise, muscle pain, stiff neck, fever, heart block, kidney failure and neurological changes such as seizures, aggression. In some cases it is cause of death (Burgess, 1986; Faul *et al*., 1999; Straubinger, 2000).

The aim of this pilot study was to estimate the seroprevalence to *Borrelia burgdorferi* in dogs in the urban area of Mexicali Baja California, a Mexico-U.S. border desert region.

MATERIALS AND METHODS

Study design and characteristics of the population: A cross-seccional descriptive study was conduced where 10 veterinary clinics and the Animal Control Center participated. The duration of the study was from September to November 2003. A total of 94 dog serum samples were randomly taken, 54 from veterinary clinics and 40 from the Animal Control Center. Mexicali city is situated along the state's northern border with the U.S. state of California and is the northernmost city in Latin America, located at 32°40'0"N, 115°28'0"W, with 855,962 inhabitants (Wikipedia, 2006). Climate is extreme, desert type and the average annual rainfall is 0.63±.43 cm. Climatic conditions data was collected from the United States National Weather Service of the National Oceanic and Atmospheric Administration (http://www.nws.noaa.gov/).

Data collection: A questionnaire was designed to collect information of the tested dogs and included: General information of dog: gender (female, male), age (≤1 year, >2 years), size (small, medium, big) and Intensity of tick infestation: None, low (1-10 ticks), moderate (11-30 ticks), intense (>30 ticks). The outcomes of most of the questions were dichotomous.

Blood collection: Blood samples were collected by certified personal. Briefly, 3 mL of blood were collected by punction of the cephalic vein after proper antisepsis of

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the area with isopropyl alcohol and placed in tubes Vacutainer®. Each sample was properly labeled and centrifuged at 3500 RPM for 10 min to separate the serum. The serum was transferred into 1mL vials, labeled and stored at-20°C until testing.

Serology: Antibodies against *Borrelia burgdorferi* were measured with the kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc. for the detection and semi quantification of canine IgG class which guarantees a 95.8% sensibility and a 94.7% specifity. The Optical Density (OD) at 450 nm was registred, where an OD<1.0 was considered negative and OD = 1.0 as positive, according to the manufacturer.

Statistical analysis: Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed. The adjusted prevalence and its 95% CI were obtained using Rogan-Gladen estimator (Greiner and Gardner, 2000). The significance of the class variables (gender, age, size and intensity of tick infestation) was determined by Chi-squared test (Walker, 1997). All statistical analysis were performed using the Statistical Analysis System for Windows version 9.1.3 (SAS, 2004).

RESULTS AND DISCUSSION

The results of this study indicate an adjusted seroprevalence to *Borrelia burgdorferi* of 8.2% (95% C.I. 1.5-13.3%) in dogs from Mexicali, calculated by Rogan-Gladen estimator (Greiner and Gardner, 2000). The adjusted prevalence obtained in dogs from veterinary clinics and from the Animal Control Center is presented in Table 1.

The seroprevalence obtained in this study was similar than that from 1988 (6.6%) in the same city (Mexicali). Nevertheless were tested 30 dogs with epistaxis (Romano *et al*., 1998). The prevalence of this study was lower than found in another study done in other region of the Mexican Republic as Monterrey, Nuevo León, where found a prevalencia of 16% (160/850) in dogs tested by an indirect immunofluorescent assay (Salinas-Melendez *et al*., 1999). A possible cause of low seroprevalence to *B. burgdorferi* can be that have not been found the known vectors of this disease in this region as *Ixodes scapularis, I. pacificus, Dermacentor variabilis* and *Amblyomma americanum* (Magnarelli and Anderson, 1988; Lane, 1996; Adelson *et al*., 2004). The unique tick found it in Mexicali has been *R. sanguineus* (in press),which is not considered as vector of borreliosis in other regions of the world. However, in Sao Paulo, Brasil, where *Ixodes loricatus, I. didelphidis* and *Amblyomma cajennense* were found, the prevalence was of 9.7% (23/237) in dogs by ELISA and confirmed by Western blot (Joppert *et al*., 2001).

Table 2 shows the unadjusted prevalence values stratified by origin, gender, age, size and intensity of tick infestation. In general, not differences (p>0.05) were observed in prevalence values according to gender, age and intensity of tick infestation between dogs from veterinary clinics and those from the Animal Control Center. However, dogs of big size from Animal Control Center showed higher (p<0.05) seroprevalence than those dogs of small and medium size.

Although the prevalence found in this pilot study was low, it is necessary to perform a complete study that includes an appropriate sample size, serum sampling all year long and the evaluation of risk factors so that the appropriate preventive and control measures are established. Also, since borreliosis is a zoonotic desease that may require expensive hospitalization and may cause death it is imperative to know the prevalence in humans, particularly in places like Mexicali, where a serologic evidence in dogs has been observed.

Table 1: Adjusted prevalence* to *Borrelia burgdorferi* of dogs from Mexicali, an urban area of Mexico-U.S. border desert region

Origin	n	Positives	Adjusted prevalence* (%)	95% I.C. (%)
General	94	7	8.2	1.5-13.3
Veterinary clinics	54	4	8.1	1.5-13.2
Animal control center	40	3	8.2	1.6-13.3

*Rogan-Gladen estimator (Greiner and Gardner, 2000)

Table 2: Seroprevalence* to *Borrelia burgdorferi* in dogs from Mexicali, stratified by origin, gender, age, size and intensity of infestation

	Origin					
	Veterinary clinics			Animal control center		
Class variable[1]	n	Positive	(%)	n	Positive	(%)
Gender						
Male	30	3	10.0[a]	15	2	13.3[a]
Female	24	1	4.1[a]	25	1	4.0[a]
Age						
<1 year old	16	2	12.5[a]	22	1	4.5[a]
>1 year old	38	2	5.2[a]	18	2	11.1[a]
Size						
Small	10	2	20.0[a]	9	0	0.0
Medium	28	1	3.5[a]	23	0	0.0
Big	16	1	6.2[a]	8	3	37.5[a]
Intensity of tick infestation						
None	21	1	4.7[a]	17	0	0.0
Low	26	3	5.5[a]	18	2	11.1[a]
Moderate	5	0	0.0	4	1	25.0[a]
Intense	2	0	0.0	1	0	0.0

* Unadjusted values [1]Equal letters by class variable within origin, indicate no differences (p>0.05)

CONCLUSION

A low seroprevalence of 8.2% (95% I.C. 1.5-13.3%) to *Borrelia burdorgferi* was observed in this study and because borreliosis is a zoonotic disease, a complete study has to be done, where both dogs and humans are included with an appropriate sample size and the evaluation of risk factors. Since *Borrelia burgdorferi* is transmitted by ticks, preventive and control measures to erradicate ticks have to be established in order of minimize the risk of infection.

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----- Mensaje original ----
De: Kelli Howell <khowell@jarcet.com>
Para: tinoco.luis@yahoo.com
Enviado: miércoles, 8 de octubre, 2008 16:31:58
Asunto: Journal of Applied Research
Dear Dr Tinoco:

It is my pleasure to inform you, on behalf of The Journal of Applied Research, that your article, **"Prevalence and risk factors for *Borrelia burgdorferi* infection in Mexicali, Baja California, a Mexico-US border city"** has been accepted for publication, and is scheduled for the November 2008 issue.

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Prevalence and risk factors for *Borrelia burgdorferi* infection in Mexicali, Baja California, a Mexico-US border city

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1. Abstract

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *B. burgdorferi* which is transmitted by a tick bite, primarily from *Ixodes scapularis* and *I. pacificus*. It is characterized by polisystemic disorders. In Mexico, native Lyme disease has been recently reported in humans. In dogs *B. burgdorferi* infection has been also reported in several areas of the country. In Monterrey, Nuevo Leon, Mexico, a seroprevalence of 16% was observed in dogs (136/850) and molecular evidence was found in dog synovial fluid. Moreover, a preliminar study performed in 2003 in Mexicali, Baja California, Mexico showed a prevalence of 7.4% (7/94) in dogs infested only by the tick *Rhipicephalus sanguineus*. The aim of this study was to estimate the seroprevalence of *B. burgdorferi* in canine patients that were brougth into private veterinary clinics in Mexicali. From out of a total of 98 active private clinics in Mexicali, 39 (40%) agreed to participate. Blood samples of 384 dogs were randomly selected from February 2005 to December 2006, and their sera were analized by the semiquantitative kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc., with 96% sensibility and 95% specificity. An adjusted prevalence of 6.8% (95% CI 3.5-8.9%) was obtained using the Rogan-Gladen estimator. The seroprevalence obtained in this study was lower compared to those in Monterrey (16%) where the principal vector was *Ixodes scapularis*, and in Sao Paulo, Brazil (15.6%) where the principal vector was *Amblyomma cajennense*. Risk factors associated with *B. burgdorferi* seropositivity were age (Mantel-Haenszel χ^2, P = 0.02, OR= 2.7 [95% CI 1.2-6.1]), and the absence of a preventive program (Mantel-Haenszel χ^2, P = 0.005, OR= 4.9, [95% CI 1.4-16.8]). This study confirms the existence of *B. burgdorferi* past/present infection in dogs in an area where the only identified tick is *R. sanguineus.*

Keywords: *Borrelia burgdorferi*, tick, Lyme disease, borreliosis.

2. Introduction

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi*, which is transmitted by tick bite, primarily *Ixodes scapularis* and *I. pacificus* in North America (Barbour and Hayes, 1986; Magnarelli *et al*., 1987b; Greene *et al*., 2000). It is the most frequent tick-borne disease in Europe and the United States in human beings.

The disease is characterized by arthritis, lameness, erythema migrans, fatigue, anorexia, general malaise, muscle pain, stiff neck, fever, heart block, kidney failure, and neurological changes such as seizures and aggressive behavior (Burgess, 1986; Magnarelli *et al*., 1987b; Faul *et al*., 1999; Straubinger, 2000).

Several animal species can be infected by the *B. burgdorferi* spirochete, including rodents, deer, dogs, cats, cows, horses, reptiles, birds and some other tick species (Faul *et al*., 1999; Straubinger, 2000). Dogs are considered the most important reservoir for ticks in the home environment (Straubinger, 2000; De Lacerda *et al*., 2004).

In the city of Monterrey, Mexico, an indirect immunofluorescent assay (IFA) was used to detect antibodies against *B. burgdorferi* in dogs; with a resulting seroprevalence of 16% (Salinas-Melendez *et al*., 1999). Molecular evidence of *B. burgdorferi* was also found by amplification and DNA selected sequences from synovial fluid samples from dogs with arthritis, which suggests the presence of Lyme disease in the area (Salinas-Melendez *et al*., 1995).

In a pilot study in the city of Mexicali, Mexico, seroprevalence to this spirochete was determined using *B. burgdorferi* ELISA® Helica Biosystems, Inc., which resulted in 8.2% (95% CI 1.5-13.3%) out of the 94 dogs that were tested in autumn (September-November, with a sensitivity of 96% and a specifity of 95% (Tinoco-Gracia *et al*., 2007).

In Distrito Federal and the northeast of Mexico, a study of 2,346 human sera analyzed with ELISA and confirmed by Western blot found a prevalence of 3.43 and 6.2% respectively (Gordillo-Pérez *et al*., 2003). Also, 4 patients who reside in Distrito Federal and were bitten by ticks while visiting forestal parks (3 in Mexico City and 1 in Quintana Roo) were positive to *B. burgdorferi* when their skin biopsies were tested by PCR using primers for the *fla* gene; one of the patients was also positive to OspA gene by sequencing. (Gordillo-Pérez *et al*., 2007).

The aim of this study was to estimate the seroprevalence to *B. burgdorferi* and associated risk factors in canine patients at veterinary clinics in the urban area of Mexicali, Baja California, a Mexico-U.S. border desert region.

3. Materials and methods

3.1. Study design and characteristics of the population

A descriptive study was designed, and 39 veterinary clinics agreed to participate. The data and blood collection started on February 2005 and ended on December 2006. A total of 384 serum samples were randomly taken from canine patients at veterinary clinics in the urban area of Mexicali. This city is situated along the state's northern border with California, and is the northernmost city in Latin America; it is located at 32°40′0″N, 115°28′0″W, with 855,962 inhabitants (Wikipedia, 2006). Climate is extreme, desert type and the average annual rainfall is 0.63 ± .43 cm. Climatic conditions data was collected from the United States *National Weather Service* of the *National Oceanic and Atmospheric Administration* (http://www.nws.noaa.gov/).

3.2. Data collection

A questionnaire was designed to collect information of the tested dogs and it included: 1) General information of dog: gender (female, male), age (< 1 year, ≥ 1 year),

size (small, medium, large), coat (short, medium, large); 2) dog handling: number of dogs in the house, antiparasitic treatments, dog mobilization between house and street, intensity of tick infestation: none, low (1-10 ticks), moderate (11-30 ticks), intense (>30 ticks); and 3) living conditions of dogs: type of surface (ground or grass, or concrete), indoor/outdoor status of dog, origin of dog, and history of taking the dog outside the city limits. The outcomes of most of the questions were dichotomous. Questionnaires were administered by trained personnel at the clinics.

3.3. Blood collection

Blood samples were collected by trained personnel. Briefly, 3 ml of blood were collected by punction of the cephalic vein after proper antisepsis of the area with isopropyl alcohol, and placed in Vacutainer® tubes. Each sample was properly labeled and centrifuged at 3500 RPM for 10 min to separate the serum. The serum was transferred into 1ml vials, labeled and stored at –20°C until testing.

3.4. Serology

Antibodies against *B. burgdorferi* were measured with the semiquantitative kit *B. burgdorferi* ELISA® Helica Biosystems, Inc. for the detection of canine IgG class which guarantees a 95.8% sensitivity and a 94.7% specificity. The optical density (OD) at 450 nm was recorded, where an OD < 0.3 was considered negative and OD ≥ 0.3 as positive, according to the manufacturer.

3.5. Statistical analysis

Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed (24/384). The adjusted prevalence and its 95% CI (confidence interval) were obtained using the Rogan-Gladen estimator (Greiner and Gardner, 2000).

A Mantel-Haenszel χ^2 test and Odds Ratio (OR) were used to determine differences in seroprevalence to *B. burgdorferi* by groups and the association of risk factors. Statistical significance was considered at a *P*-value of < 0.05. Exact binomial confidence intervals were calculated individually for each proportion (Walker, 1997b).

All statistical analysis were performed using the Statistical Analysis System for Windows version 9.1.3 (SAS, 2004).

4. Results and discussion

The results of this study indicated an adjusted seroprevalence to *B. burgdorferi* of 6.8% (95% CI 3.5-8.9%) in canine patients from veterinary clinics of Mexicali. Since there is no vaccination against Lyme disease included in current immunization programs for dogs in Mexicali, the results of this research will not be affected by the presence of post-vaccination antibodies.

Furthermore, the prevalence in this study was lower to that found in Monterrey, Mexico, with a prevalence value of 16% (136/850) in dogs tested by an indirect immunofluorescent assay (Salinas-Melendez *et al*., 1999). The lower seroprevalence to *B. burgdorferi* found in this study may be due to the fact that the known vectors for this

spirochete, *I. scapularis*, *I. pacificus*, *Dermacentor variabilis* and *Amblyomma americanum* in North America (Magnarelli and Anderson, 1988; Lane, 1996; Adelson *et al*., 2004), have not been found in this region. The only species of tick found in Mexicali has been *R. sanguineus* (*in press*), which has not been considered a vector of borreliosis in other regions of the world. Meanwhile, in Sao Paulo, Brasil, the prevalence in dogs was of 15.6% (31/199) and the main tick vector was *Amblyomma cajennense* (O'Dwyer *et al*., 2004).

Risk factors found to be associated with *B. burgdorferi* infection were age (Mantel-Haenszel χ^2, $P = 0.02$), and to the absence of a preventive plan (Mantel-Haenszel χ^2, $P = 0.005$). The study showed that the dogs that were more at risk were those of ≥ 1 year of age with an OR= 2.7 (95% CI 1.2-6.1), as well as the ones who did not have access to a preventive program which consisted of at least 2 antiparasitic treatments (endo and ectoparasites), 2 tick treatment baths and 2 home fumigations a year, with an OR= 4.9 (95% CI 1.4-16.8).

The higher prevalence in dogs of ≥ 1 year of age, similar to others studies (Merino *et al*., 2000; Goossens *et al*., 2001; De Lacerda *et al*., 2004), is justified by the nature of borreliosis, which is a chronic disease that may take several months to show its most notorious sign in dogs, lameness of one or more limbs (Magnarelli *et al*., 1987b).

Gender in tested dogs showed no relation to seropositivity to *B. burgdorferi*, similar to others studies (Merino *et al*., 2000; Guerra *et al*., 2001; De Lacerda *et al*., 2004), which indicates that apparently sexual pheromones specific to each sex do not contribute to vector attraction, as it happens to other arthropod animals.

Size and coat did not show an association to borreliosis either, compared with another study from Spain (Merino *et al*., 2000), where coat turned out to be important but size was not relevant, although it was believed that the larger the body and the longer the hair, the more ticks the animal would get, since there would be broader body areas to be infested, and the longer hair would make it harder on the dog to shed the ticks, but there is no evidence of any association since a bite of the vector is enough to start the Lyme infection (Baumgarten *et al*., 1999).

Although it was intially thought that the prevalence to borreliosis may be associated with the number of dogs in a house, the lack of concrete flooring throughout the residence, and to the transit of dogs into and out of the house, as it happened in the study conducted in Wisconsin and Illinois (Guerra *et al*., 2001), or tick infestation level (Merino *et al*., 2000; De Lacerda *et al*., 2004), this study did not show any statistical association with those factors.

The lack of a tick control system also was found to be associated to seropositivity to *B. burgdorferi*, as it resulted in other studies (Merino *et al*., 2000; Guerra *et al*., 2001), which means that seroprevalence could be reduced just by following a preventive plan, as previosly mentioned, since it would interrupt the biological cycle of the vector.

Although the results of this study may suggest a relatively low prevalence for *B. burgdorferi* infection in dogs, borreliosis is a zoonotic disease and it can be reduced following minimum preventive measures, like those analyzed in this study. Furthermore, it is necessary to determine the vector involved in the transmission of borreliosis in this area, considering that the only tick that has been observed in Mexicali dogs is *R. sanguineus*. There were no statistical differences in the seroprevalence for dogs that have been kept

within the city limits all of their lives and dogs that were either born in other geographic areas or taken outside the city limits where they might have been exposed to *Ixodes* ticks. It will be important to determine borreliosis prevalence in humans, specifically in places like Mexicali, where exposure to this agent is being demonstrated in the dog population and dogs may act as sentinels for *B. burgdorferi* infection in humans (Goossens *et al*., 2001; Guerra *et al*., 2001; Duncan *et al*., 2005).

This study confirms the existence of *B. burgdorferi* in dogs in an area where *R. sanguineus* is the only tick that has been identified so far. Although this tick has not been considered an important vector for *B. burgdorferi*, it can be brought into the homes and feed on humans (Beichel *et al*., 1996; Cruz-Vazquez and Garcia-Vazquez, 1999), considering that it only takes one bite from the vector to spread borreliosis (Baumgarten *et al*., 1999). Molecular evidence of *B. burgdorferi* in dogs and ticks is currently being searched for, as well as the transmission mechanism is being determined.

5. Conclusions

A seroprevalence value of 6.8% (95% CI 3.5-8.9%) to *B. burgdorferi* was observed in dogs in Mexicali, B.C., an area where the only specie of tick that has been observed in dogs is *R. sanguineus*.

The evaluated risk factors were found to be related both to age in the dogs that were sampled (Mantel-Haenszel χ^2, $P = 0.02$), and to the absence of a preventive plan (Mantel-Haenszel χ^2, $P = 0.005$). The study showed that the dogs that were more at risk were those of ≥ 1 year of age with an OR= 2.7 (95% CI 1.2-6.1), as well as the ones who did not have access to a preventive program.

As well as the ones who did not have access to a preventive program, which should include at least 2 desparasite treatments (endo and ectoparasites), 2 tick treatment baths and 2 home fumigations a year to help reduce seroprevalence to Lyme disease.

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Prevalence and risk factors for *Borrelia burgdorferi* infection in dogs of Animal Control Centers from Mexicali, Baja California, a Mexico-US border city

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Prevalence and risk factors for *Borrelia burgdorferi* infection in dogs of Animal Control Centers from Mexicali, Baja California, a Mexico-US border city

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ABSTRACT

Lyme borreliosis, a worldwide zoonotic disease and characterized by polisystemic disorders, is caused by the spirochete *B. burgdorferi*, which is transmitted by a tick bite, primarily from *Ixodes scapularis* and *I. pacificus*. In Mexico, native Lyme disease has been recently reported in humans, while canine *B. burgdorferi* infection has been also reported in several areas of the country. In Monterrey, Mexico, a seroprevalence of 16% was observed in dogs (136/850) and molecular evidence was found in canine synovial fluid. Moreover, a preliminar study performed in 2003 in Mexicali, Mexico showed a prevalence of 7.4% (7/94) in dogs infested only by the tick *Rhipicephalus sanguineus*. The aim of this study was to estimate the seroprevalence of *B. burgdorferi* in dogs captured by personnel from the animal control centers in the city of Mexicali. Blood samples from 384 dogs were randomly selected from February 2005 to December 2006, and their sera were analized by the semiquantitative kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc., with 96% sensitivity and 95% specificity. An adjusted prevalence of 12% (95% IC 7.5-14.3%) was obtained using the Rogan-Gladen estimator. The seroprevalence obtained in this study was lower compared to those in Monterrey (16%), where the principal vector was *Ixodes scapularis*; and in Sao Paulo, Brazil (15.6%), where the main vector was *Amblyomma cajennense*. No risk factors were associated with *B. burgdorferi* seropositivity. This study confirms the existence of *B. burgdorferi* past/present infection in dogs in an area where the only identified tick is *R. sanguineus.*

Keywords: *Borrelia burgdorferi*, tick, Lyme disease, borreliosis.

INTRODUCTION

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi*, which is transmitted by tick bite, primarily *Ixodes scapularis* and *I. pacificus* in North America (Barbour and Hayes, 1986; Magnarelli *et al*., 1987b; Greene *et al*., 2000). In Europe and the United States, it is the most frequent tick-borne disease in human beings. Among its sypmtoms: arthritis, lameness, erythema migrans, fatigue, anorexia, general

malaise, muscle pain, stiff neck, fever, heart block, kidney failure, and neurological changes such as seizures and aggressive behavior (Burgess, 1986; Magnarelli *et al*., 1987b; Faul *et al*., 1999; Straubinger, 2000).

Several animal species can be infected by the *B. burgdorferi* spirochete, including rodents, deer, dogs, cats, cows, horses, reptiles, birds and some other tick species (Faul *et al*., 1999; Straubinger, 2000). Dogs are considered the most important reservoir for ticks in the home environment (Straubinger, 2000; De Lacerda *et al*., 2004).

An indirect immunofluorescent assay (IFA) was used to detect antibodies against *B. burgdorferi* in dogs in Monterrey, Mexico, with a resulting seroprevalence of 16% (Salinas-Melendez *et al*., 1999). Also, molecular evidence of *B. burgdorferi* was found by amplification and DNA selected sequences from canine synovial fluid samples from dogs with arthritis, which suggests the presence of Lyme disease in the area (Salinas-Melendez *et al*., 1995).

In a pilot study in the city of Mexicali, Mexico, seroprevalence to this spirochete was determined using *B. burgdorferi* ELISA® Helica Biosystems, Inc., which resulted in 8.2% (95% CI 1.5-13.3%) out of the 94 dogs that were tested in autumn (September-November), with a sensitivity of 96% and a specifity of 95% (Tinoco-Gracia *et al*., 2007).

In Distrito Federal and the northeast of Mexico, a study of 2,346 human sera, analyzed with ELISA and confirmed by Western, blot found a prevalence of 3.43 and 6.2% respectively (Gordillo-Pérez *et al*., 2003). Also, 4 patients who reside in Distrito Federal and were bitten by ticks while visiting forestal parks (3 in Mexico City and 1 in Quintana Roo) were positive to *B. burgdorferi* when their skin biopsies were tested by PCR using primers for the *fla* gene; one of the patients was also positive to OspA gene by sequencing. (Gordillo-Pérez *et al*., 2007).

The purpose of this study was to estimate the seroprevalence to *B. burgdorferi* and associated risk factors in canines captured by personnel from both animal control centers in Mexicali, Mexico, a Mexico-U.S. border desert region.

MATERIALS AND METHODS

Study design and characteristics of the population

A descriptive study was designed in both animal control centers in Mexicali, Mexico, *Centro Antirrábico Veterinario de Mexicali* and *Centro Municipal de Control Animal de Mexicali*. The data and blood collection started on February 2005 and ended on December 2006. A total of 384 serum samples were randomly taken. Mexicali is situated along the state's northern border with California, and is the northernmost city in Latin America; it is located at 32°40′0″N, 115°28′0″W, with 855,962 inhabitants (Wikipedia, 2006). Its climate is extreme, desert type and the average annual rainfall is 0.63 ± .43 cm. Climatic conditions data was collected from the United States *National Weather Service* of the *National Oceanic and Atmospheric Administration* (http://www.nws.noaa.gov/).

Data collection

A questionnaire was designed to collect information of the tested dogs and it included: general information of dog, such as gender (female, male), age (< 1 year, ≥ 1 year), size (small, medium, large), and coat (short, medium, large); and intensity of tick

infestation: none, low (1-10 ticks), moderate (11-30 ticks), intense (>30 ticks). The outcomes of most of the questions were dichotomous.

Blood collection

Blood samples were collected by trained personnel. 3 ml of blood were collected by punction of the cephalic vein after proper antisepsis of the area with isopropyl alcohol, and placed in Vacutainer® tubes. Each sample was properly labeled and centrifuged at 3500 RPM for 10 min to separate the serum. The serum was transferred into 1 ml vials, labeled and stored at –20°C until testing.

Serology

Antibodies against *B. burgdorferi* were measured with the semiquantitative kit *B. burgdorferi* ELISA® Helica Biosystems, Inc. for the detection of canine IgG class which guarantees a sensitivity of 95.8% and a specificity of 94.7%. The optical density (OD) at 450 nm was recorded, where an OD < 0.3 was considered negative and OD ≥ 0.3 as positive, according to the manufacturer.

Statistical analysis

Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed (24/384). The adjusted prevalence and its 95% CI (confidence interval) were obtained using the Rogan-Gladen estimator (Greiner and Gardner, 2000).

A Mantel-Haenszel χ^2 test and Odds Ratio (OR) were used to determine differences in seroprevalence to *B. burgdorferi* by groups and the association of risk factors. Statistical significance was considered at a *P*-value of < 0.05. Exact binomial confidence intervals were calculated individually for each proportion (Walker, 1997b).

All statistical analysis were performed using the Statistical Analysis System for Windows, version 9.1.3 (SAS, 2004).

RESULTS AND DISCUSSION

The results of this study indicated an adjusted seroprevalence to *B. burgdorferi* of 12% (95% CI 7.5-14.3%) in dogs from both animal control centers in Mexicali, Mexico (Greiner and Gardner, 2000), and it was slightly higher than the one obtained from a pilot study carried out in the same city, 8.2% (95% CI 1.5-13.3%) (Tinoco-Gracia *et al*., 2007). Also, the prevalence in this study was lower to that found in Monterrey, Mexico, with a prevalence value of 16% (136/850) in canines tested by an indirect immunofluorescent assay (Salinas-Melendez *et al*., 1999). The lower seroprevalence to *B. burgdorferi* found in this study may be due to the fact that the known vectors for this spirochete, *I. scapularis*, *I. pacificus*, *Dermacentor variabilis* and *Amblyomma americanum* in North America (Magnarelli and Anderson, 1988; Lane, 1996; Adelson *et al*., 2004), have not been found in this region. The only specie of tick found in Mexicali has been *R. sanguineus* (*in press*), which has not been considered a vector of borreliosis in other regions of the world.

Meanwhile, in Sao Paulo, Brasil, the prevalence in dogs was of 15.6% (31/199) and the main tick vector was *Amblyomma cajennense* (O'Dwyer *et al*., 2004).

None of the risk factors considered for this study—age, gender, size and coat of the canines in the animal control centers—seemed to be related to *B. burgdorferi* seropositivity. This could mean that these dogs have the same chances to get borreliosis, despite the reasons they were considered for this study: age, since borreliosis is a chronic disease; gender, in case there could be some affinity of the infected ticks to sexual hormones in the host; size, since the larger the host, the larger the area available for the vectors, even though it has been demonstrated that it only takes one bite from an infected tick for the host to get infected with Lyme disease; and coat, since the seroprevalence could be closely related to the length of the hair of the host, because long hair would protect the infected ticks. An important finding was that the only tick reported so far in Mexicali was *R. sanguineus* (*in press*), which is not considered as vector for borreliosis. It is also necessary to determine the vector involved in the transmission of *B. burgdorferi* in this area. Also, since borreliosis is a zoonotic disease, it is imperative to know the prevalence in humans, particularly in places like Mexicali, where serological evidence has been obtained.

CONCLUSIONS

A seroprevalence value of 12% (95% CI 7.5-14.3%) to *Borrelia burgdorferi* was observed in dogs captured by personnel from the animal control centers in Mexicali, Mexico, a region where the only tick that has been taxonomically identified in canines is *Rhipicephalus sanguineus*. And none of the factors considered for this study showed any association to seropositivity to *B. burgdorferi*.

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