

Clinical and seroepidemiological study of Leishmaniasis in Northern Morocco

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(Reçu le 19/06/2000 ; Révisé le 17/04/2001 ; Accepté le 01/06/2001)

دراسة وبائية لداء ليشمانيا الكلب في شمال المغرب

في دراسة سريرية وطبيلية مصلية شملت ثلاث محافظات وحوالي 17 منطقة في شمال المغرب وذلك في الفترة ما بين 1993 حتى 1996 والتي أوضحت نسبة عالية للإصابة بليشمانيا الكلاب. في هذه الدراسة تم فحص حوالي 1362 كلب وتم أخذ أمصالها وتم اختبار هذه الأمصال لمعرفة نشاط الأجسام المضادة لطفيل ليشمانيا أنفانتوم وذلك باستخدام إختبار إليزا ELISA. النتائج المحصل عليها من خلال هذا المسح المصلي في هذه الدراسة تتراوح نسبة الإصابة ما بين 3,77 إلى غاية 35,7%. تم دراسة وتحديد ستة أنواع (عَتَرَات) في هذه الدراسة خمسة منها تم تصنيفهم كليشمانيا أنفانتوم في محافظتي الخميسات وتازة. أما النوع الأخير تم تصنيفه كليشمانيا تروبيكا وهي أخذت في حالة تحتوي على الليشمانيا الأحشائية في محافظة تاونات. أغلب الكلاب المصابة سريريا تظهر عليها تقرحات جلدية، فقدان الوزن وتضخم الغدد اللمفاوية. أما العمى والإسهالات فقد لوحظت فقط في منطقة الخميسات.

الكلمات المفتاحية : ليشمانيا كلبية- ليشمانيا أنفانتوم- ليشمانيا تروبيكا - مسح وبائي- المغرب

Étude séro-épidémiologique de la leishmaniose canine dans le nord du Maroc

Une étude clinique, parasitologique et sérologique entreprise de 1993 à 1996 dans trois régions (17 localités) du nord du Maroc a révélé une fréquence élevée de la leishmaniose canine. 1362 chiens ont été examinés. Les sérums ont été collectés et testés selon la technique ELISA utilisant la souche locale *Leishmania infantum* comme antigène. Les résultats obtenus ont révélé une séroprévalence (quand elle n'est pas nulle) allant de 3,77% à 35,7%. Six isolats ont été caractérisés : *Leishmania infantum* ont été identifiés dans les provinces de Khémisset et Taza et une souche *Leishmania tropica* isolé d'un cas leishmanien dans la province de Taounate. La majorité des chiens cliniquement suspects ont montré des lésions ulcératives de la peau, amaigrissement, onychogriphose et lymphadenopathie. L'alopecie, la cécité et la diarrhée ont été observés uniquement dans la province de Khémisset.

Mots clés : Leishmaniose canine *Leishmania infantum* - *Leishmania tropica* - Séro-épidémiologie - Maroc

Clinical and seroepidemiological study of Leishmaniasis in Northern Morocco

A clinical, parasitological and serological survey carried out from 1993 till 1996 in three regions including 17 localities of Morocco, revealed a high prevalence of canine leishmaniasis. A total of 1,362 dogs were examined, sera were collected and tested for antibody activity against *Leishmania infantum* antigens using the enzyme linked immunosorbent assay (Elisa). The data obtained revealed a seroprevalence (when not equal to zero) ranging from 3.77 to 35.7%. Six isolates were characterised: 5 were typed as *Leishmania infantum* in the Khemisset and Taza regions and one stock as *Leishmania tropica* from a visceralized leishmaniasis case in the Taounate area. Most of clinically suspicious dogs showed ulcerative skin lesions, weight loss, Onychogryphosis and lymphadenopathy. Alopecia, blindness and diarrhoea were observed only in the Khemisset province.

Key words : Canine Leishmaniasis - *Leishmania infantum* - *Leishmania tropica* - Seroepidemiology - Morocco

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INTRODUCTION

Leishmaniasis is a severe, devastating and often fatal disease which develops commonly in human beings and dogs in the Mediterranean basin where dogs are the appropriate disease reservoirs (Peters & Killick-Kendrick, 1987). In many Mediterranean countries, this disease is a zoonosis and the incidence of canine infection can be as high as 40% in endemic areas (Flemmings *et al.*, 1984). In dogs, visceral leishmaniasis caused by *Leishmania donovani* is the most common leishmanial infection worldwide (Pearsen *et al.*, 1983).

In Morocco, the biogeographical distribution of human visceral leishmaniasis (VL) is related to regions in the north of the country (Dedet, 1979 ; Mahjour *et al.*, 1992). From 1996, an epidemiological survey was undertaken on the prevalence of the disease in dogs. Several canine leishmaniasis foci were identified. The current study reports on the clinical and parasitological features and the seroprevalence of canine leishmaniasis (CL) in three provinces of Northern Morocco.

MATERIALS AND METHODS

1. Surveyed areas

Our three regions (Khemisset, Taounate and Taza) were selected on the basis of previous work done on human leishmaniasis by the Rabat paediatrics' hospital (Agoumi *et al.*, 1991 ; Thimou *et al.*, 1996).

- Khemisset is located in north of Morocco, 80 km from the country capital (Rabat). The climate is typically Mediterranean (sub humid to sub arid), with an altitude of 145m and a mean annual rainfall of 700 mm. The temperature varies between 5°C (January) and 37°C (June).
- Taounate is a vast region made up of two distinct physiographic areas. The northern part is mountainous and the southern part is a valley. The climate is continental and sub humid. Temperatures reach 45°C in Summer and drops below 1°C in Winter. The mean annual precipitation vary from 600-800 mm in the northern part to 400-500 mm in the south.
- The Taza region has a sub arid climate with an altitude of 944 m above sea level. The annual precipitations vary from 800-900 mm.

2. Animals

The surveys were carried out in 3 north Morocco provinces and consisted in clinical, serological and parasitological examinations on 1,362 dogs.

In Khemisset province, the survey was carried out in 1993-1994 in Sid El Ghandour hamlet (around a canine leishmaniasis case) and five other rural localities (Jbel, Tafoudait, Ait Iskou Hirra ou Ait Azzouz ou Ali and Fouaizen ait Abbou) belonging to the khemisset province on 323 dogs identified by their owners and blood sampled.

In Taounate, the survey was carried out in 1994-1995 in the Ouled Hssain village (around a human visceral leishmaniasis case) and four surrounding hamlets (Bouda, Kalaa, Hmirou and Imaghdan on 219 dogs examined and sampled for sera.

In Taza (Known as a focus of human visceral leishmaniasis), a total of 820 dogs were examined in 1995-1996 and sampled for sera in Taza city and five rural localities (Traiba, Beni-Ftah, Gzenaya-janoubia, Ajdir, Zrarda and Taza city).

3. Clinical examination

A total of 1,362 dogs were clinically examined for disease symptoms; notes were taken on the general status of the dogs as well as on the condition of the skin, nails and lymph nodes.

4. Parasitological examinations

Eight sick dogs were brought back from field to Veterinary clinic (6 from Khemisset, 1 from Taounate and 1 from Taza). Parasitological exams were carried out on popliteal lymph nodes tissue and spleen biopsy guided by echography and the material obtained was partly smeared on slides for Giemsa staining (Lanotte *et al.*, 1974). While other part was inoculated on NNN medium for parasites isolation. For the clinically suspected field dogs, the parasitological examination was only done on popliteal lymph nodes tissue.

The 6 isolated stocks were typed by Unit of Research on leishmaniasis, Laboratory of Parasitology and Mycology, Faculty of Medicine, Casablanca; by multilocus enzyme electrophoresis (10 loci) on cellulose (Kreutzer & Christensen, 1980).

5. Serological examination

Assessment of antibodies was carried out by ELISA using promastigotes from a local *Leishmania infantum* (LRG) stock isolated from a sick dog from Sid El Ghandour hamlet (Khemisset province) which isoenzyme pattern is indistinguishable from the WHO reference strain MHOM/TN/80/IPT1 zymodeme MON-1. The Elisa test used is a modified procedure used in humans by Voller *et al.* (1980). Promastigotes were washed 3 times in PBS, sonicated and then adjusted to 100 µg/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6. The protein content of antigens was determined using the method of Bradford (1976) and then adsorbed on Elisa plates (Dynatech Immulon) and allowed to adhere overnight at 4°C. The plates were blocked for 2 hr in PBS/2%BSA (Bovine Serum Albumin), washed 3 times in PBS/Tween 0.05% (washing solution) and stored at -30°C until use. Sera were tested by incubating the plates with 100 µl of diluted serum (1/100 in PBS) at 37°C for 1 hr, then the wells were washed 3 times. The conjugate (Protein A-horseradish peroxidase) was diluted to 1/8000 in PBS and 1 µl added to each well. The plates were incubated for 1 hr at 37°C, and then washed 3 times. Finally, 100 µl/well of substrate 2,2'-azino-bis (3-ethylbenzthiazoline sulfonate) (ABTS) was used and the absorbance read at 405 nm after 20 minutes. Positive and negative controls were used in each experiment. Positive values were defined as those which optical density (OD) is >0.3.

RESULTS

1. Clinical pattern of clinically suspected dogs

Detailed clinical observations are presented in Table 1. Clinical manifestations such as Onychogryphosis and lymphadenopathy are a common finding in the three regions. In Khemisset province, certain signs are more marked: loss of weight and emaciation. Dandruff production, dermatitis and ulcers are more marked in dogs from Taounate. For all dogs examined in Taounate and Taza provinces, we noted the absence of alopecia, blindness and diarrhoea signs and no nervous signs in Taza region were recorded. Among 219 dogs examined in Taounate, only 5 (2.28%) dogs exhibited some clinical signs and out of 820 dogs examined in Taza, only 55 (6.7%) were clinically suspect against 35 (10.83%) in Khemisset.

Table 1. Clinical pattern of clinically suspected dogs

Clinical signs	Khemisset (35 dogs) (%)	Taounate (5 dogs) (%)	Taza (55 dogs) (%)
General status			
Thinness	33(94.28)	3(60)	20(36.36)
Illness	15(42.85)	1(20)	2(3.63)
Emaciation	17(48.57)	1(20)	20(36.36)
Skin lesions			
Alopecia	8(22.85)	0(0)	0(0)
Dermatitis	15(42.85)	3(60)	4(7.27)
Dandruff production	14(40)	4(80)	0(0)
Ulcers	8(22.85)	3(60)	4(7.27)
Onychogryphosis	35(100)	5(100)	52(94.54)
Ocular lesions			
Conjunctivitis	15(42.85)	1(20)	4(7.27)
Blue keratitis	7(20)	0(0)	2(3.63)
Blindness	7(20)	0(0)	0(0)
Digestive signs			
Diarrhoea	2(5.71)	0(0)	0(0)
Visceral signs			
Lymphadenopathy	35(100)	2(40)	48(87.27)
Splenohepatomegaly	6(17.14)	1(20)	3(5.45)
Nervous signs			
Paralysis of rear limbs	1(2.85)	1(20)	0(0)

2. Parasitological examination

The 5 stocks isolated from Khemisset and Taza provinces were all typed as *Leishmania infantum*: 4 from Khemisset (MCAN/MA/94/LRG, MCAN/MA/94/KH4, MCAN/MA/95/KH5, MCAN/MA/95/KH6) and one from Taza (MCAN/MA/95/RF). The only stock isolated from Taounate was as *Leishmania tropica* (MCAN/MA/95/T10).

The direct observation of the parasite (amastigotes) in spleen or lymph node aspiration smears is the most reliable method for confirming the disease.

3. Seroprevalence of canine leishmaniasis

The data referring to the prevalence of dogs with leishmaniasis in the three surveyed areas are reported in Tables 2, 3 and 4. The Elisa test revealed a seroprevalence rate of 16.71% in Khemisset province, 15.98% in Taounate and 16.09% in Taza. 85.71% of seropositive dogs in Taounate and 58.33% in Taza did not show any typical sign of the disease, against only 35.18% in Khemisset province. The highest frequency of Elisa positive sera was found at Oulad Hssain locality in the Taounate province (35.7%).

Table 2. Seroprevalence of canine leishmaniasis (CL) in the Khemisset Province

Localities	Sera tested	Dogs c.s.	Cases cfp (%)	Elisa positive dogs (%)
Jbel	65	8/65 (12.30)	ND	13/65 (20.00)
Tafoudait	70	7/70 (10.00)	ND	11/70 (15.71)
Ait IskouHira	62	9/62 (14.51)	ND	9/62 (14.51)
Ait azzouz ou Ali	35	2/35 (5.71)	ND	8/35 (22.85)
Fouaizen ait abou	36	0/36 (0.00)	ND	0/36 (0.00)
Sid ElGhandour	55	9/55 (16.36)	6/55 (10.90)	13/55 (23.63)
Total	323	35/323 (10.83)	6/323 (1.85)	54/323 (16.71)

Dogs c.s. : Dogs clinically suspected ; Cases cfp : Cases confirmed by finding parasites

Table 3. Seroprevalence of canine leishmaniasis in Taounate Province

Localities	Sera tested	Dogs c.s.	Cases cfp (%)	Elisa positive dogs (%)
Oulad Hssain	70	3/70(4.28)	1	25/70 (35.71)
Bouda	58	2/58(3.44)	ND	5/58(8.62)
Kalaa	25	0/25(0.00)	ND	2/25(8)
Hmirou	22	0/22(0.00)	ND	3/22 (13.63)
Imaghdane	44	0/44(0.00)	ND	0/44 (0.00)
Total	219	5/219(2.28)	1/219 (0.45)	35/219(15.98)

Table 4. Seroprevalence of Canine Leishmaniasis in Taza Province

Localities	Sera tested	Dogs c.s. cfp (%)	Cases dogs (%)	Elisa positive
Traiba	122	14/122 (11.47)	ND	19/122(15.57)
Beni-Ftah	53	0/53(0.00)	0/4(0.00)	2/53(3.77)
Gzenaya- Janoubia	86	4/86(4.65)	0/1(0.00)	11/86(12.79)
Ajdir	37	3/37(8.10)	0/4(0.00)	7/37(18.91)
Zrarda	367	26/367(7.08)	1/9(11.11)	78/367(21.25)
Taza city	155	8/155(5.16)	0/1(0.00)	15/155(9.67)
Total	820	55/820(6.70)	1/19(5.26)	132/820(16.09)

DISCUSSION

Several canine leishmaniasis foci were identified by many authors in the majority of countries around the Mediterranean basin (Adler *et al.*, 1938; Dedet & Belazzoug, 1985). But to date, no study on canine leishmaniasis in the North of Morocco has been carried out. Our diagnosis was based on clinical appearance of the lesions, the presence of Leishmaniasis. Clinical records

consisted of a large variability of signs including visceral and cutaneous changes. 85.71% of dogs in Taounate and 58.33% of dogs in Taza were asymptomatic against 35.18% at Khemisset province. Our results confirmed the findings of other investigators, Adler & Theodor (1932) and by Abranches *et al.* (1991) who have verified that CL has a prolonged period without clinical signs. The majority of infected dogs in these areas showed signs as has been observed in other endemic areas (Vasilios *et al.*, 1993).

Our survey has showed a high prevalence (35.7%) of CL in Oulad Hssain locality in Taounate province compared to the figures reported in the Mediterranean area by other authors who have also used serological techniques, e.g. 1 to 42% by Ashford & Bettini (1987). The higher prevalence of dogs with leishmaniasis out of the 17 localities has been found in Sid El Ghandour locality in Khemisset (23.6%), Oulad Hssain (35.7%) in Taounate and Zrarda (21.25%) in Taza. From these three localities we have isolated our strains (4 strains from Sid El Ghandour; 1 from Oulad Hssain and 1 from Zrarda). We have also noted that in these localities farmers use waste water for irrigation and their dogs are chained overnight to guard the produce so more accessible to Phlebotomies. We must also mention that during this study, there was no history of travel outside the province by any of the dogs tested. We can conclude that Sid El Ghandour, Oulad Hssain and Zrarda are potential foci of canine leishmaniasis in this part of Morocco.

At Khemisset province, canine leishmaniasis does not sustain human leishmaniasis because no human VL case was notified in this region during the whole study period which suggests that it is a specific canine VL focus. The absence of human leishmaniasis should not be surprising. There are several CanL foci where no human VL cases have been reported (e.g. in Italy, Monte Argentario (Tuscany) and in the island of Ustica, Sicily cited by Bettini & Gradoni (1986) and by Abranches *et al.* (1983) in Portugal).

In Taounate province, the only parasitologically confirmed CanL showed *L. tropica*. This infected dog found at Oulad Hssain locality exhibited several lymph nodes from which the parasite was isolated and other signs like hepato-splenomegaly. No cutaneous lesions were noted which confirm visceral form. In the same locality and precisely at the neighbouring house of the owner of this dog, a

human VL case (infant) was confirmed and the stock typed as *L. infantum* (Guessous-Idrissi *et al.*, 1997a). Dogs are known as reservoirs of *L. infantum* in the Mediterranean area.

In Morocco *L. tropica* canine cutaneous infections in dogs has been demonstrated by Dereure *et al.* (1991), with no evolution towards visceral form. This new *L. tropica* canine case is furthermore striking as for the first time in Morocco a canine viscearalizing *L. tropica* case is described (Guessous-Idrissi *et al.*, 1997b). Bettini & Gradoni (1986) noted that it is still unknown whether some of canine isolates, which were believed to VL if transmitted to man, would have been in effect strains with dermatropic characteristics.

Results from the present study have produced evidence that dogs are an important reservoir host of leishmaniasis in our country. Our surveys constitute an essential step for understanding of the epidemiology of canine leishmaniasis in Morocco.

ACKNOWLEDGEMENT

We are grateful to Professor N. Guessous-Idrissi (Laboratory of Parasitology and Mycology, Faculty of Medicine, Casablanca, Maroc) for the typing of our canine strains isolated.

We are also grateful to Professor A. Hmidouch (Department of PMC of Institut Agronomique et Vétérinaire Hassan II, Rabat) for his the isolation of our strains. This works was supported by USAID/NIAID-NIH, USA project

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