

Epidemiological Situation of Canine Leishmaniosis in Kabylia

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Abstract

In order to evaluate the epidemiological situation of canine Leishmaniasis in the Kabylia of Djurdjura, one of the most active focus of human visceral Leishmaniasis, indirect immunofluorescence serological screening was carried out on two batches of dogs: the first batch was consisting of 43 animals from veterinary practices in the city of Tizi Ouzou, the second batch consists of 559 dogs randomly selected through the various ecological strata that form this region.

Overall, 10.13% of the animals showed specific antibodies, with a titre equal to or greater than 1/80. The dogs in the first batch were positive in 18.6% of cases while those in the second group had a prevalence of 9.48%. Among the dogs of the second batch, the seroprevalence varies according to the site where the animals reside: whereas it is 0% at the level of the littoral, it reaches 17.12% in the depression of Dra El Mizan; the dogs living in the Sébaou Valley and in the Massif ancien had an equivalent seroprevalence, around 7%.

By considering the seroprevalence according to the age of the animals, we noticed that the older ones were the most affected compared to the young people. The difference is statistically significant ($p < 0.05$), but the sex does not seem to have an impact on the affection.

With regard to the breed of dogs, German shepherds had the highest seroprevalence.

Among the clinical signs observed, it is the skin lesions that come back most often, whereas 67.92% of seropositive dogs were asymptomatic.

We carried out on a random sample of 90 animals the search for the parasite DNA by the so-called "classical" PCR. The rate of PCR-positive animals is 74.44% while the same sample detected by the IFI has a seropositivity of 16.66%. Both diagnostic approaches seem totally discordant (Mc Nemar test ($p < 0.0001$)).

On 30 samples inoculated on NNN medium and rabbit serum (20 from haemocultures and 10 from ganglionic juice), only one strain could evolve normally and be identified as belonging to the MON-1 zymodeme. The reference for the isolated strain is: MCAN/DZ/2007/LIPA 5207.

Keywords: Canine Leishmaniasis; Dogs; IFI Diagnosis; PCR; Kabylie; Algeria

Introduction

Canine Leishmaniasis is an infectious and zoonotic protozoosis due to the pathogenic action and multiplication in cells of the mononuclear phagocyte system (PMS) of *Leishmania infantum*. The transmission of the infection is mainly done by the bite of sandflies (Diptera, Psychodidae) which plays the role of biological vector in the cycle of the parasite.

In humans, Leishmaniasis represent a clinical spectrum ranging from a simple self-resolving skin lesion to the lethal visceral form without treatment. In addition, the latter is considered a full-fledged opportunistic disease given its frequency and severity during HIV infection [36].

The outbreak of visceral Leishmaniasis in Kabylia has been known for many years as one of the most active in the western Mediterranean. Indeed Addadi, *et al.* [2] reported that 253 cases out of 497 reported in Algeria during the period 1965 - 1974 originated from this region, i.e. more than 50% of the cases recorded. During the next decade (1975 - 1984), Belazzoug, *et al.* observed a prevalence of 26.4% in this same region [7]. Finally, Harrat, *et al.* 285 cases (25.4%) were collected during the period 1985 - 1990, with a relatively high mortality rate (6%) [20,24].

Previous studies undertaken in this large focus by Dedet, *et al.* in 1977 then by Harrat, *et al.* in 1992, showed that the spatial distribution of Leishmaniasis was uneven with areas of predilection for the transmission of the disease [15].

In Algeria, since the work of the Sergent brothers in 1910 [40], we admit that the dog is the main reservoir of visceral Leishmaniasis. Later, Dedet, *et al.* in 1977, showed that 11.4% of dogs in Kabylia were affected. This role of reservoir was only admitted by deduction, and this is the work of Belazzoug, *et al.* (1984 - 1985 and 1987) who confirmed the role played by this animal and correlated the focus of canine Leishmaniasis with human visceral Leishmaniasis [5].

A specific survey carried out in 1992 by the team of the Pasteur Institute of Algeria, in the locality of Dra El Mizan (Kabylia) showed that 25% of the dogs sampled had specific antibodies [20]. A 2006 study of 105 dogs in the same locality revealed that 45 animals had an antibody level higher than the 1/80 threshold at IFI (Indirect Immunofluorescence), i.e. a seroprevalence of 42.8% [22].

In 2011, *Leishmania infantum* was isolated by the team of the Pasteur Institute of Algeria on a jackal (*Canis aureus*) [8].

Human visceral Leishmaniasis remains a cause for concern in this region of Algeria, particularly among children. Despite the few ad hoc studies carried out, this remains very insufficient; better knowledge of the canine reservoir would allow more effective management of the disease.

In this contribution, we wanted to give an estimate of the prevalence of canine Leishmaniasis in Kabylia across the different geographic entities of the region, its distribution by age, sex and breed of animals. We also compared two diagnostic approaches: indirect immunofluorescence serology (IFI) and molecular biology (PCR) in order to see the concordance between the two techniques.

Presentation of the study region

Kabylia is a mountainous region located in the north of Algeria. It is a geographic and sociolinguistic entity with no real political-administrative existence, it is spread over 7 departments: Tizi Ouzou, Bejaia, Bouira, Boumerdes, Bordj Bou Arreridj, Sétif and Jijel.

Our study only concerns the region of Tizi Ouzou, formerly known as the department of Grande Kabylie.

The department of Tizi Ouzou, also called the Kabylie of Djurdjura is located between 36° 45 and 36° 89 north latitude and 3° 89 and 4° 70 east longitude, on the Mediterranean 100 km east of Algiers. Its orographic system is mainly divided into three mountain ranges: the coastal range, the Old Kabyle Massif and the Djurdjura range which narrow from west to east and meet in the eastern part of the study area [32]. The most important mountain range is Djurdjura, the north slope of which is stepped and covered by numerous foothills forming the Old Kabyle Massif, it is also bordered, to the north, in its western part by the depression of Dra El Mizan-Boghni. Between the old Massif and the littoral chain, flows the main river of the Djurdjuréenne Kabylie: the Sébaou which takes its source in the south-east in the region of Azazga and winds towards the north-west to throw itself in the Mediterranean Sea around Dellys [32].

This river determines a more or less extensive valley where Tizi Ouzou is located, the main city of this region.

Djurdjuran Kabylia is located in the area of contact and struggle between the polar and tropical air masses. From October-November to March-April, arctic air masses generally prevail and determine a cold and wet season. During the other months of the year, tropical air masses rise and create heat and dryness.

The humidity is due to polar front depressions that sweep the mountains and cause rain and snow. The average rainfall is between 600 and 1000 mm of water per year. Precipitation can vary considerably from one year to another and snow can be abundant on the Djurdjura and the eastern end of the old massif. Some nuances due to the altitude sometimes correct the general pattern: Presence of many micro-climates [25].

Animals

A sample of 602 dogs was investigated. In order to avoid sampling biases, we have distinguished two batches of animals: dogs which come to the veterinarian for consultation and therefore potentially sick (Leishmaniosis or others) form the first batch; the others, more numerous, are chosen without prejudging the clinical aspect, form the second batch and constitute, from our point of view, a representative sample for the estimation of the prevalence of canine Leishmaniosis in the region.

The ideal would have been to know the exact number of dogs living in the region, as well as their distribution across the different ecological systems, in order to take a representative sample. In the absence of a census of the canine population in our region, we have adopted a theoretical model inspired by studies carried out by WHO as part of the promotion of vaccination campaigns for dogs against rabies [9]. These studies show that the ratios applied to populations vary very little depending on the regions of the world, in particular the ratio of dogs to men in American and European countries. The studies of Matter (1987) and Artois (1986), cited by Seghaier, *et al.* [39] on the socio-ecological aspects of the canine population in Tunisia can constitute by extrapolation, an interesting basis for the estimation of the numbers of this population in our study region.

According to these studies, the density of dogs (including stray dogs) varies with the type of habitat and the cultural and socio-economic levels of the human population:

- In rural areas: More than 80% of households have at least one dog. The density is one dog per 3.0 to 6.8 inhabitants.
- In urban areas, the density is around one dog per 16 inhabitants.
- In a semi-urban area, it would be one dog per 46 inhabitants.

In addition, ownerless dogs represent between 7 and 8% of the total canine population.

In our study area, apart from Tizi Ouzou center, which can be assimilated to an urban area, all the other localities that we prospected can be considered as semi-urban areas.

In addition, even the animals sampled in the commune of Tizi Ouzou, actually come from the outskirts of the city, an area which can also be assimilated to a semi-urban locality.

We can then estimate a dog for 46 inhabitants, including for the "Tizi Ouzou" zone (Table 1).

Materials and Methods

For animals in the first batch, the samples were taken by the veterinarian, after a full clinical examination and the collection of information on cards prepared for this purpose. For the dogs in the second batch, we moved from village to village, accompanied each time by the head of the local hygiene office. We have taken great care to reach all the sites representing the different geosystems that make up our

Locality	CN Preleves	Inhabitants	Population Estimated Canine	% Dogs Collected
Ain Zaouia	24	17320	377	6,37
Amechras	4	12683	276	1,45
Aym	57	20426	444	12,84
Azeffoun	16	16647	362	4,42
Boghni	24	31263	680	3,53
Bouzguene	132	24311	529	24,95
Dem	8	38886	845	0,95
Freha	23	24228	527	4,36
Frikat	11	12791	278	3,96
Lni	6	29376	639	0,94
Mathkas	51	32121	698	7,31
Ouadhias	98	15771	343	28,57
Sidi Naman	15	10688	232	6,47
Tadmait	1	22838	496	0,20
Tigzirt	3	11962	260	1,15
Tizi Gheniff	86	29409	639	13,46
Tizi Ouzou	43	135088	2937	1,46
Total	602	485808	10562	5,70

Table 1: Theoretical estimate of the dog population in the Tizi Ouzou region.

study region, namely: the coast, the Sebaou valley, the Dra El Mizan depression and finally the Massif ancien. The animals sampled were chosen at random, however favoring the most docile. It is always the owner of the dog who ensures the restraint.

This work took place in two phases:

- The first consists of collecting samples and epidemiological and clinical data on information sheets designed for this purpose. The information sheet includes: the identification number and possibly the name of the animal, its sex, its age and the clinical signs observed during the examination, and finally the name and address of the owner of the animal.
- The blood is collected in dry tubes and citrated tube (10%), the serum collected from the dry tube is intended for serological examination; samples from citrated tubes are kept for additional tests (molecular biology PCR test).
- In addition, when the animal expresses clinical signs evoking Leishmaniasis, ganglionic juice is taken sterile from the popliteal ganglia, then transferred sterile into eppendorf tubes for direct examination, looking for parasitic DNA by PCR and the culture of the parasite.
- The second phase consists in the serological analysis of the sera collected and possibly the cultivation on NNN medium from the leukocyte layers or lymph node punctures.
- All the laboratory analysis part is carried out at the National Reference Center for *Leishmania* of the Pasteur Institute of Algeria.

Diagnostic tests

All sera have been tested for specific antibodies by indirect immunofluorescence.

A sample of 90 samples (lymph node juice or buffy coat from blood samples) were processed using the PCR technique to search for parasite DNA.

The purpose of this double examination is, on the one hand to compare these two techniques in terms of sensitivity, specificity and on the other hand to estimate the rate of dogs carrying the parasite or at least its DNA.

Part of the lymph node punctures is examined directly under the microscope, after staining with May Grunwald Giemsa for the search for the parasite, a second fraction is seeded on the NNN culture medium (Nicolle, Neal, Mc Novy) in order to type the parasite by the method iso enzymes and finally a third aliquot and used for research of parasitic DNA.

Indirect immunofluorescence (IFI)

It is a reference technique widely used in laboratories. The antigen consists of promastigote forms attached to the slides. This a fairly specific method, with very good sensitivity. It also makes it possible to follow the evolution of the antibodies after treatment.

Handling lasts an average of two hours. Cross-reactions (false positives) have been reported with other parasitic diseases such as trypanosomiasis (sleeping sickness). In our study we followed the operating protocol described by Lanotte [28].

A volume of 20 µl of an antigen solution, containing 5.106 promastigotes/ml, is placed in each well of a Teflon coated slide. The antigen is then fixed by immersion in an acetone bath. After incubation of the diluted sera, a dog antiserum labeled with fluorescein, diluted 1/50, is applied. The sera are first tested at the 1/20 dilution and, in the case of sera found positive for this reason, the dilution is pushed by geometric progression of reason 2 up to 1/640. Sera with a titer greater than or equal to 1/80 are considered positive (threshold set by the Pasteur Institute of Algeria) [22].

Demonstration of the parasite's DNA by the PCR technique (Polymerase Chain Reaction)

This technique is much more sensitive than direct parasite research and serology. It makes it possible to highlight, from various samples, very small quantities of DNA from Leishmanias. As far as we are concerned, we used as a sample, either the buffy coat removed after hemoconcentration, or the puncture products from the lymph nodes, or finally, the scraping products from skin lesions.

PCR is the technique of choice for the diagnosis of parasitaemia. It is useful in the detection of asymptomatic dogs, but also in the monitoring of an animal during treatment because it makes it possible to follow the evolution of the parasitic load in the animal [11].

For each PCR there is a very specific protocol which varies according to the type of primer and the reagents used. In our work, the PCR technique applied to DNA samples from the *Leishmania* parasite, targets ribosomal DNA sequences. We used the R221/R332 primer pair.

The DNA samples were prepared according to the protocol described by Lachaud., *et al* [27].

Identification of strains

The isolates were identified by the technique of isoenzyme electrophoresis on thick starch gel according to the technique described by Rioux., *et al* [38]. Protein extracts for electrophoresis are obtained after mass culture on the Heart Brain Rabbit Blood medium, supplemented with RPMI 1640, enriched with 10% in decomplexed fetal calf serum and added with antibiotics (penicillin 100.000 IU/ml streptomycin 50 µg/ml).

Fifteen enzymatic systems were tested. The iso-enzymatic typing of canine strains was carried out compared to reference strains (controls) coming from the WHO collaborating center for Leishmania, the Laboratory of Medical Ecology of Montpellier (LEM), Les marker strains used are:

- MHOM/FR/78/LEM75: *Leishmania infantum* zymodème MON-1
- MHOM/FR/80/LEM189: *Leishmania infantum* zymodème MON-11
- MHOM/DZ/82/LEM417: *Leishmania infantum* zymodème MON-24
- MCAN/ES/83/LEM935: *Leishmania infantum* zymodème MON-77
- MHOM/DZ/83/LEM425: *Leishmania infantum* zymodème MON-80.

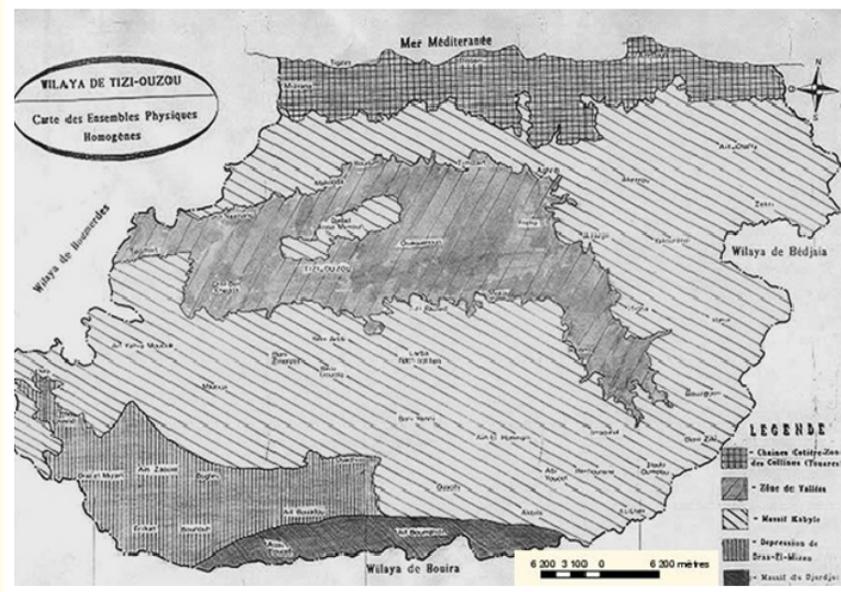


Figure 1: Department of Tizi Ouzou, map of physical complexes. <http://www.tiziouzou-dz.com>.

Statistical tests

The data capture and statistical analysis was done using Excel 2013 and Xlstat French version software. The χ^2 test was used to compare the percentages and the proportional distributions.

Results

Out of a total of 602 sera from the two batches of animals examined by the IFI serological technique, 61 were positive with a titer equal to or greater than 1/80, which gives us a rate of 10.13% and 109 had a fluorescence at 1/20 or 1/40 dilutions, i.e. 18.1% of doubtful cases (Table 2).

The distribution of the sera tested by the IFI technique, as a function of the antibody titer, follows a bi-modal type distribution (Figure 2).

Distribution of seroprevalence by age of animals

The average age of the dogs in the first batch is 45.8 weeks, the animals in the second batch are slightly younger since the average age here is 39.8 weeks.

Antibody Title	Number of animals	Rate %
Negative	432	71,76
1/20	82	13,62
1/40	28	4,65
1/80	57	9,30
1/160	4	0,66
Total	602	100

Table 2: Distribution of anti-Leishmania antibody titres of the sera examined.

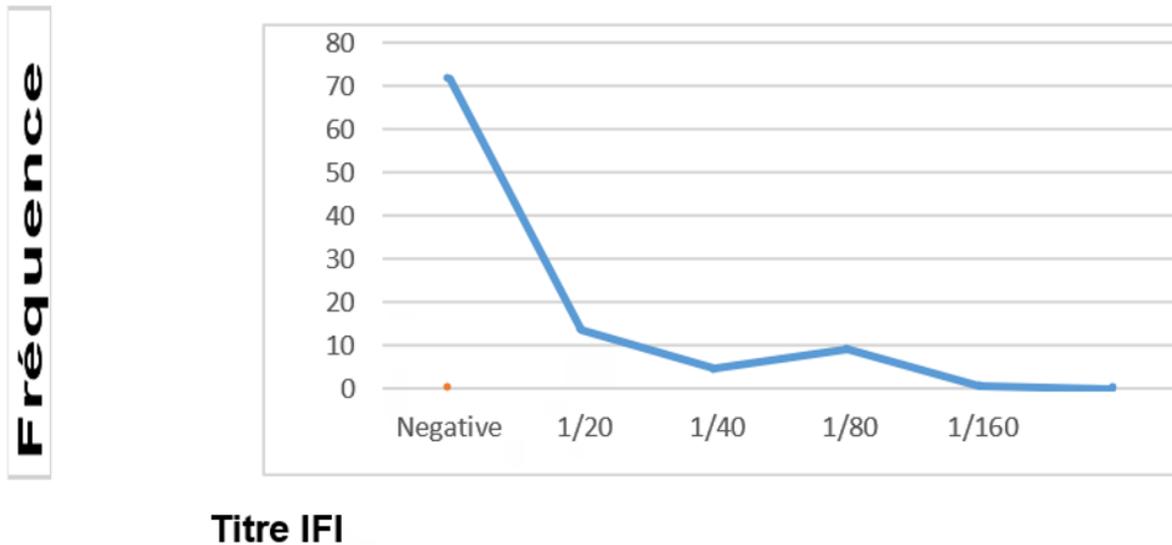


Figure 2: Distribution of antibody titres in IFI of the examined dog population.

It can be noted that the prevalence increases with age: it goes from 5.94% in puppies under two years of age to 19.13% in old dogs over 5 years of age, passing by a seroprevalence of 10.95% in animals between 2 and 5 years of age (Table 3).

Age	Dogs collected	IFI +	Rate%
< 2 years	286	17	5,94
2 - 5 years	201	22	10,95
> 5 years	115	22	19,13
Total	602	61	10,13

Table 3: Number and rate of seropositive dogs by age.

The difference in proportions by age is statistically significant ($p < 0.05$).

Breakdown by geo-ecological entity

It can be observed in table 4 that the seroprevalence in dogs in the first batch (Tizi Ouzou) is 18.6% while it is only 9.48% in those in the second batch. The comparison of these two percentages gives us an X^2 lower than the threshold value (3.84). The difference is therefore not significant ($p > 0.05$).

Sites	Number of dogs removed	Number IFI+	%IFI+
Depression	146	25	17,12
Littoral	19	0	0,00
Sebaou	39	3	7,69
Massive	355	25	7,04
Tizi Ouzou	43	8	18,60
Total	602	61	10,13

Table 4: Seropositive dogs divided according to geo-ecological entities.

The prevalence in dogs of the second batch is very heterogeneous, since we go from 0% seropositive, at the littoral level to 17.12% in the depression of Dra El Mizan, the difference the difference is significant ($p < 0,05$).

One cannot, however, consider the region of the littoral completely free from canine Leishmaniosis. The sample size (19 dogs) is relatively small.

In the Sébaou valley and the Massif ancien, the seroprevalences are roughly identical around 7% of dogs with HIV ($p > 0.05$).

Presence of clinical signs

The 43 dogs in the first batch all showed clinical signs. Among the symptoms observed, skin lesions, onychogryphosis, epistaxis and cachexia are the most present.

Eight dogs in this group were seropositive with a titer equal to or greater than 1/80, for the 35 seronegative animals, their symptoms are probably due to other ailments.

For dogs in the second batch, out of 559 animals, only 120 showed signs which could suggest canine Leishmaniosis.

Overall, 10 clinical signs of uneven frequency were observed. Skin signs, onychogryphosis and cachexia were the most observed (Table 5). The other signs were uncommon such as the sign of "glasses" or lymphadenopathy. Finally, other symptoms such as respiratory signs are not suggestive of the disease.

Of the 53 dogs infected with the second batch, 36 showed no symptoms, representing a rate of 67.92% of healthy carriers.

Gender-related seroprevalence

For all of the two batches, we sampled 509 males for 93 females, i.e. a sex ratio of 5.5.

Females had slightly lower seroprevalence than males.

This difference is not statistically significant ($p > 0.5$) (Table 6).

Symptoms	Name	%
No sign	439	78,53
Skin signs	67	11,99
Onychogryphosis	34	6,08
Cachexia	28	5,01
Lymphadenopathy	15	2,68
Epistaxis	12	2,15
Nervous signs	11	1,97
Respiratory signs	6	1,07
Eye signs	6	1,07
Sign glasses	6	1,07
All the signs	4	0,72
Diarrhea vomiting	2	0,36

Table 5: Main clinical signs observed.

Serology	Mâles %	Females %	Total %
IFI+	10,22	9,68	10,13
IFI-	89,78	90,32	89,87
Total	100	100	100

Table 6: Seroprevalence by gender.

Dog-related seroprevalence

We have classified the dogs into six ethnic groups (Table 7). German shepherds seem to be the most susceptible to canine Leishmaniosis with a prevalence of 17.78%, followed by dogs of local breed with 10.15% of seropositive. Hunting dogs and cross-breed dogs seem to be the least susceptible to the disease. This difference in rate is statistically significant ($p < 0.05$).

Race	Number of samples	IFI+	Taux %
German shepherds	45	8	17,78
Type Berger	69	4	5,80
Hunting dogs	35	1	2,86
Cross Race	29	0	0
Watch dogs	20	7	35
Local Race	404	41	10,15
Total	602	61	10,13

Table 7: Seropositive dogs by race.

Comparison of the two diagnostic methods IFI and PCR

We performed parasite DNA testing by PCR on ninety dogs taken at random. All these animals come from the second batch.

Sixty-seven samples were carrying the *Leishmania* genetic material, which gives us a positivity of 74.44%. The sera from the same sample, tested by the IFI serological technique, were positive in 15 cases (titer > 1/80), i.e. a rate of 16.66%.

Table 8 shows that there are 28 concordant cases (11 PCR +/IFI + and 17 PCR-/IFI-) but in 48 cases the two types of diagnosis are discordant. The Mc Nemar test allows to compare two paired variables. In our case, the results obtained by PCR and those obtained by IFI are statistically different (p-value < 0.0001).

PCR/IFI	IFI+	IFI-
PCR+	11	44
PCR-	4	17
Total	15	61

Table 8: Contingency table between PCR results and IFI results.

However, of 14 dogs considered questionable in serology, 12 were positive for PCR.

Result of parasite identification

Out of 30 samples seeded on NNN medium and rabbit serum (20 from blood cultures and 10 from ganglionic juice), only one strain was able to evolve normally and be identified as belonging to the MON-1 zymodem.

The reference of the isolated strain is: MCAN/DZ/2007/LIPA 5207.

The other samples were soiled during the collection or during subcultures.

The identified strain comes from a 2 year old male Rottweiler breed from the Boghni region. The animal showed a positive IFI at 1/80, the PCR is positive and clinically it showed signs of thinning of hair loss from skin lesions and onychogryphosis.

Discussion

In Leishmaniasis, the surveillance of canine foci (extension or appearance) by the veterinarian is a public health necessity, there is a close relationship between the incidence of canine cases and the importance of human cases [12]. Canine Leishmaniasis is far more important than human visceral Leishmaniasis [20] and is an interesting indicator of the activity of the latter.

Since the notification of the first case of canine Leishmaniasis in Algeria by the Sergeant brothers, several serological studies have been carried out in order to assess the frequency and the course of the disease over time. The results obtained were different depending on the period of investigation and the sample of dogs analyzed. The prevalence of the benzootia varied between 3 and 36.5% [22].

In our survey, out of 602 dogs, coming from the two batches, we recorded a rate of 10.13% of seropositive dogs with an IFI titer equal to or higher than 1/80. The curve representing the distribution of the antibody titers is bimodal in appearance (Figure 2), it is a characteristic of the endemic areas of canine Leishmaniasis. This type of distribution has been obtained by other authors [1,29,37]:

- The two modes which successively express non-specific and specific reactions overlap at the level of the title 1/80 (Figure 2) [37]. This result is fairly close to the 11.4% obtained by Dedet., *et al.* in 1977 during a study on canine Leishmaniasis made in the 1970s, in Greater Kabylia [15]. On the other hand, other authors obtained higher results: Belazzoug., *et al.* in 1985 found that 37.5% of dogs with HIV in the Azazga region (Massif ancien in Grande Kabylie) [6]; Harrat in 2006 recorded an even higher rate, 42.6% in the Dra El Mizan - Boghni - Dra Ben Khedda region [22]. This difference can be the consequence of several possibilities.

- Samples are taken from restricted sites (Azazga for the first and Dra El Mizan for the second), dogs are exposed to the same conditions, in particular exposure to infected sandflies.
- On the other hand, many authors have observed that canine Leishmaniosis, which follows human visceral Leishmaniosis, experiences a cyclical, oscillating, long-term development, spread over 8 to 10 years, with regular epidemic outbreaks [2]. The period of the investigation may therefore explain the difference.
- And finally, the sampled dogs are probably chosen from among those who are clinically suspect and will therefore have a higher seroprevalence than in the case of random sampling.

We saw this difference in our study. Indeed, the seroprevalence of dogs in the first batch, clinically suspect, is 18.6% while it is only 9.48% for dogs in the second batch, sampled randomly, even if this difference is not significant ($p > 0.05$).

If we consider the seroprevalence according to the geographic origin of the animals, there is a significant variability, it goes from 17.12% in the depression of Dra El Mizan to 0% at the coast. In the Sébaou valley and in the old Massif, it is respectively 7.69% and 7.04%. Canine Leishmaniosis is called “vector-borne disease”, it is the distribution of the vector (*Phlebotomus perniciosus*, *P. longicuspis* and probably *P. perfiliewi*) that determines the distribution of the prevalence of the disease. An entomological study carried out in this same region shows that the density of sandflies captured in the Dra El Mizan depression is 115.5 specimens per m², while it is only 10.6 sandflies per m² at the coast, at the two other sites an intermediate density was recorded (around 51 ph/m²) [34]. This distribution corresponds entirely to the distribution of cases of dogs with HIV.

The age of dogs seems to influence the seroprevalence since it goes from 5.9% in dogs under two years old to 19.13% in animals over 5 years old. This difference is statistically significant ($p < 0.05$). This difference is even clearer in a survey conducted in Algeria in 2006, only 26 dogs under 2 years of a total of 437 are found positive (3.42%) while in old dogs 66 out of 68 were positive (97.06%) [22]. Indeed, canine Leishmaniosis being an achronic disease whose evolution is rarely spontaneously regressive, the probability for a dog to be infected increases with the duration of its exposure during the periods of sandfly activity and therefore with age.

In our survey, the seroprevalence also varies according to the breed of dogs. German shepherds seem to be the most susceptible to the disease, followed by dogs of the local breed. Hunting dogs and cross-breed dogs seem to be the least susceptible to the disease. This difference in rate is statistically significant ($p < 0.05$). These observations agree with those found by Harrat, *et al.* in 2002 in the Algerians, who note that 80% of positive animals are German shepherds, on the other hand, dogs of the common breed are the least affected which they attribute to a difference in receptivity [21]. Crotti, *et al.* in 2007 in Italy found that German shepherds were the most affected by canine Leishmaniosis [14].

The high prevalence of Leishmaniosis in the German shepherd used in particular as a guard dog, seems linked to its activity, outside the homes, which exposes it during the night to the bites of sand flies, this assumption is also valid for dogs of breed local people who are living on day parole.

On the other hand, sex does not seem to influence Leishmanian infection in dogs, 10.22% of males with HIV vs 9.68% for females. This difference is not statistically significant. In the Rif (Morocco) the same observation was made by Rami, *et al.* in 2003 [37].

Regarding the clinical signs, all the dogs in the first batch showed symptoms, but not always specific or suggestive of the disease. But today, thanks to increasingly refined means of diagnosis, atypical forms of canine Leishmaniosis are reported such as respiratory or cardiovascular disorders, as well as musculoskeletal disorders [18]. In our study, of the 43 sera in the first batch, only 8 dogs were seropositive, the others, despite the simultaneous presence in certain animals of several clinical signs, had a doubtful or negative serology, this is probably related to an impairment of the immune response seen at an advanced stage of the disease.

For the second batch, out of 559 dogs examined, 120 expressed one or more clinical signs. Skin lesions are the most encountered (11.9%), followed by lengthening of the claws (6.08%) and weight loss (5.01%), other signs such as lymphadenopathy and epistaxis are less common. Among the 53 dogs infected with the second batch, 36 showed no symptoms, representing a rate of 67.92% of healthy carriers. This result can be compared to that of a study carried out in central Morocco in which it was noted that 73.33% of HIV-positive dogs were asymptomatic [17]. Ait Oudhia, *et al.* in 2011, in the Algiers region, observed that 58.8% of the dogs having presented a positive serology were asymptomatic and only 15.4% had presented more than three clinical signs [3].

In France, at the end of the 1980s, one in two dogs with HIV showed clinical signs, today only one in four or only five dogs shows signs [30].

Another study on canine Leishmaniosis carried out by Harrat, *et al.* in the Algerian (Algeria) found a very different result, since 70% of dogs with HIV showed symptoms [21].

In order to compare two diagnostic techniques for canine Leishmaniosis, we randomly selected a sample of 90 dogs from which we carried out, in addition to the IFI serology, a search for parasitic DNA by conventional PCR. Sixty-seven samples were carrying the *Leishmania* genetic material, which gives us a positivity of 74.44%. The sera from the same sample, tested by the IFI serological technique, were positive in 15 cases (titer > 1/80), i.e. a rate of 16.66%. These rates correspond well to those described by other authors, such as Lachaud, *et al.* in a study of canine Leishmaniosis by PCR, on 263 dogs the prevalence was 79% (by PCR) and 29.6% by IFI [27]. Mary in 1994 observed in Marseille, an endemic area for canine Leishmaniosis, an 80% rate of dogs carrying the parasitic DNA [31].

Coulibaly cited by Bourdoiseau, *et al.* (2004), gives the results of a survey of veterinarians in the south of France: 10 to 20% of dogs are positive by the usual serology, the prevalence reaches 80% by gene amplification (PCR) and half of the animals “positive” to PCR are asymptomatic, either because they are in incubation, or because they are “resistant” or finally because they evolve towards healing [13].

If we compare the results obtained by PCR and those obtained by serology we can note that there are 28 concordances against 48 discrepancies; Mc Nemar’s statistical test applied to the two diagnostic approaches shows a significant discrepancy between the results obtained by the PCR and those obtained by IFI (p-value < 0.0001).

The importance of the rate of PCR positive dogs must be put into perspective in the endemic area. The main problem lies in demonstrating the cause and effect relationship between the presence of the parasite’s DNA and Leishmaniosis/disease. According to Gradoni (2002), a possible positivity to PCR in the absence of other signs, in an endemic area does not necessarily mean that he develops the infection [18].

However, we can assume that the real rate of seropositive dogs is clearly higher than 10.13% given by serology alone by IFI at the 1/80 dilution, a part of the dogs with a doubtful serology is probably positive especially on 14 doubtful dogs at IFI, 12 had a positive PCR.

Identification of parasitic strains

The introduction, in the 1980s, of Iso-enzymatic typing, currently considered as the taxonomic tool of choice in the area of Leishmaniosis, made it possible to distinguish within the *L. infantum* species from numerous zymodemes [36].

Out of 30 samples seeded on NNN medium and rabbit serum (20 from blood cultures and 10 from ganglionic juice), only one strain was able to evolve normally and could be identified as belonging to the MON-1 zymodem. The other samples were soiled during the collection or during subcultures.

The identified strain has the reference: MCAN/DZ/2007/LIPA 5207 and comes from a 2-year-old male Rottweiler dog from the Boghni region. The animal had a positive IFI at 1/80, the PCR was positive and clinically it showed signs of loss of hair loss from skin lesions and onychogryphosis.

In dogs, the MON-1 zymodem has always been largely predominant [4]. Ait Oudhia in 2011, in a bibliographic review reports the polymorphism of the iso enzymes of 1023 strains of *Leishmania infantum* isolated from dogs of the Mediterranean basin, a total of 12 zymodemes was identified: MON-1, MON-24, MON-34, MON-72, MON-77, MON-80, MON-98, MON-105, MON-108, MON-199, MON-199 var NP1130 and MON-281, among which, 6 were present in Algeria. The MON-1 zymodem being predominant (86.5% of the strains) [3].

Conclusion

Canine Leishmaniasis is doubly worrying: it is first of all a serious disease, often fatal for the dog itself, moreover, it is even more worrying from a public health point of view since numerous epidemiological studies demonstrate a close relationship between the incidence of cases of human visceral Leishmaniasis and the prevalence (clinical and serological) observed in dogs.

Through this study, we have shown that this zoonosis is still endemic in the focus of Kabylia, which remains one of the most active Leishmaniasis (visceral and canine) in the western Mediterranean. Across Kabyle territory, the prevalence of canine Leishmaniasis is very heterogeneous: it is very high in the corridor that forms the depression of Dra El Mizan and quite low on the coast, along the Sebaou river and in the villages perched on the Kabyle Massif, it is average. This distribution of the prevalence of canine Leishmaniasis completely follows the distribution of vector sandfly species, described by entomological studies done in this region.

The age and breed of animals appear to affect the seroprevalence of this disease, although opinions are divided. It seems that it is rather the dog's lifestyle that determines the infection (exposure to sandflies in the outside environment). Sex on the other hand does not seem to play a role.

The clinical signs are numerous and not always specific, we note above all that nearly $\frac{3}{4}$ of the seropositive animals were asymptomatic, therefore susceptible to infection and infection and thus to participate in the creation of new foci or in the extension of the current foci in being direct sources of parasites for sandflies and indirectly for humans.

The research of the parasitic DNA by the PCR gave us a rate of 74.44% of positive dogs, percentage which it is necessary to take with precaution in particular in endemic zone, because the problem is to demonstrate that there is a relation cause and effect between the presence of parasitic DNA and Leishmaniasis disease. PCR can be used to confirm a positive serology in dogs with obvious clinical signs or serious analytical alterations.

The identification of Leishmaniasis by the isoenzyme method allowed us to characterize a single strain which belongs to the MON-1 zymodem.

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