

## **CANINE CUTANEOUS LEISHMANIASIS: FIRST REPORT IN A GERMAN SHEPHERD POLICE DOG IN DIYALA GOVERNORATE-IRAQ**

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### **ABSTRACT**

A 5- year old male German shepherd police dog was presented to the clinic with good general condition and few circumscribed itchy skin lesions. Blood parameters were within normal range and the case was diagnosed as ringworm and antifungal treatment was prescribed. After few months the dog was brought again to the clinic, he has been subjected to complete body hair shaving and generalized small skin pustules was found scattered over the whole body. The case was diagnosed as atopic dermatitis; local and general corticosteroids and antihistamine were given to the animal. The condition get worse, the dog showed loss of condition and skin lesion continued with signs of dehydration, loss of appetite, emaciation, paleness of mucous membranes, There was no response to treatment with systemic and local antibiotics, multivitamin supplement, external and internal parasite treatment. At that time, blood examination revealed a decrease in hematocrit (Hct) and hemoglobin (Hb) values with a slight increase in total leukocyte count. Giemsa-stained impression smears prepared from various skin lesions revealed the presence of amastigotes of *Leishmania spp.*; free or inside macrophages; and the case was finally diagnosed as cutaneous canine leishmaniasis. As treatment for Leishmaniasis was not attempted, the animal condition gradually deteriorated and ended with its death.

**Key words:** German shepherd dog, canine cutaneous leishmaniasis, Iraq.

### **INTRODUCTION**

Canine *leishmaniasis* (CanL) due to *Leishmania infantum* is a major global zoonosis; it is potentially fatal to humans and dogs. Dogs comprise the main reservoir of infection to humans (Gramiccia and Gradoni, 2005). Canine *Leishmaniasis* is endemic in more than 70 countries in the world. It is present in regions of southern Europe, Africa, Asia, South and Central America (Baneth *et al.*, 2008). Reports of the WHO (2010); mentioned that *Leishmaniasis* is widely distributed around the world; and about 350 million are considered at risk in addition of some 2 million new cases occurring yearly in 88 countries. Dogs and

rodents are the major reservoirs of cutaneous *leishmaniasis* (CL), while wild carnivores such as jackals, foxes and wolves are reservoirs of human visceral *leishmaniasis* (VL) (Mohebbi *et al.*, 2005). Infection in dogs may be subclinical and manifested as a self-limiting disease, or it may be severe, and sometimes fatal even if treated (Solano-Gallego *et al.*, 2011). Subclinical infection is not necessarily permanent and factors such as immunosuppressant or concomitant diseases could break the equilibrium and lead to the progression of the clinical disease in dogs (Baneth *et al.*, 2008 ; Solano-Gallego *et al.*, 2009). Previously, CanL was considered rarely fatal (Barnes *et al.*, 1993), but now and in the Mediterranean countries, where infection rates are up to 63% (Petanides *et al.*, 2008); CanL is considered one of the leading causes of death in dogs (Dujardin *et al.*, 2008). It can be diagnosed by direct observation of the parasites in skin scrapings, impression smears or skin biopsies stained with Giemsa, Leishman or Wright stain. Amastigotes are the easiest to find in recent or active lesions (Barnes *et al.*, 1993). Diagnosis of canine *leishmaniasis* can also be attempted by serology (IFA test, ELISA) which is preferred because antibody titers are mostly directly related to severity of clinical signs (Cabral *et al.*, 1998). Cutaneous *Leishmaniasis* is transmitted via the bite of female sand flies belonging to the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Koutinas *et al.*, 1993). Many factors such as environmental condition, density of dogs in a cage with the presence of a reservoir host or the intermediate host (Sand fly) is important in acquiring visceral and cutaneous *leishmaniasis* (Jafarishourijeh *et al.*, 2006); although direct dog-to-dog transmission through bites or wound is suggested but not proven modes of transmission. It may explain the presence of autochthonous CanL clinical cases in non-endemic areas in the absence of apparent vectors (Shaw *et al.*, 2008).

In Iraq CanL has been diagnosed in stray dogs as early as the beginning of the last Century (Chadwick and Mchattie, 1927), in 1980, another study was conducted on seroepidemiology of visceral *leishmaniasis* in children resident in villages South Baghdad using indirect fluorescent antibody test (IFAT) and ELISA, ELISA was found more sensitive than IFAT. Parasitological and serologically study was also conducted in the same study on 151 jackals and 65 dogs, largely strays. Neither from these nor from a limited sample of rodents could the parasite be isolated, though several jackals were seropositive (Daoud, 1980). In humans, the incidence of cutaneous *leishmaniasis* (CL) declined during the anti-malaria control, but when this was stopped in the mid-1960s, the incidence greatly increased. During the Gulf War (1991), case numbers of

Visceral *Leishmaniasis* (VL) and CL peaked with an incidence of 45.5/100,000 population.

Extensive people movement and migration of none immune population into endemic foci during the war together with poor sanitary conditions and an increase in the sand fly and vector population are important underlying causes for the rise in cases of *Leishmaniasis*. The incidence of both forms went down after 2004, but recently, *leishmaniasis* has again become a rapidly increasing health problem. In a study conducted on cutaneous *leishmaniasis* in humans in Al- Haweja / Tikrit Governorate, the incidence rate in human was 45 cases per 10,000 (Al- Samarai and Al- Obaidi, 2009). In Diyala Governorate, the number of VL cases doubled, while CL caused several outbreaks in 2008 and 2009 (WHO, 2003). Cases are in a continuous increase, from a total of 307 cases of CL in 2009 to 4243 in 2017 has been reported in different districts particularly Khanaqin (Diyala Health Department/ unit of zoonotic diseases). As dogs are considered an important reservoir for this disease, more clinical and serological studies should be conducted to estimate the prevalence of this zoonotic protozoal parasite in dogs in this Governorate. This is the first report on canine cutaneous *leishmaniasis* in Diyala Governorate /Iraq.

## MATERIALS AND METHODS

**Patient:** A 5- year old male German shepherd police dog was presented to the veterinary clinic in the Faculty of Veterinary Medicine in Diyala University, at different periods of 2013 and 2014. Clinical and laboratory examination of different blood and skin lesion samples were conducted regularly and in each visit. The main complaint was persistent, chronic, untreatable cutaneous skin lesions.

**Laboratory examination:** Blood samples collected in EDTA tubes from cephalic vein were subjected to manual complete blood count including; hematocrit (Hct), hemoglobin (Hb), erythrocyte count, total leukocyte count, differential leukocyte count and evaluation of platelet number. Erythrocyte sedimentation rate, total plasma protein and fibrinogen values were sometimes estimated. Scrapings from skin lesions were subjected to direct KOH preparation examination for detection of ring worm and mange. Impression smears from skin ulcers were stained with Giemsa stain for protozoa and with Zeihl Neelson's acid fast stain for cutaneous tuberculosis. Blood sample and impression smears prepared from various skin lesions were taken from the animal and sent for laboratory examination. All laboratory tests were conducted according to Coles (1986).

**Ultrasound examination:** In the last visit, the animal was subjected to general ultrasound examination.

## RESULTS

### Case history and clinical signs

When the animal was first admitted to the Faculty clinic; he appeared active and in a very good general condition, small localized itchy skin lesions were found in the elbow region (Fig. 1 and 2); blood, feces and skin scrapings from affected area were collected and sent to the clinical pathology laboratory in the Faculty. The case was first diagnosed as ring worm and antifungal treatment was prescribed for the dog; the animal was under regular treatment for external and internal parasites affections.

After about two months the dog was brought again to the faculty, he had been subjected to complete, generalized hair shaving; skin lesions as pustules and papules appeared scattered over the whole body and there was no response to antifungal treatment. Blood and skin scrapings collected from skin lesion were subjected to direct examination for mange and ringworm; both were negative and the case was diagnosed as atopic dermatitis.

Corticosteroids, antihistamine and multivitamins were prescribed for the dog. In January 2014, the dog was presented again; he appeared in a very bad condition, emaciated dehydrated with pale mucous membranes, loss of appetite, failure of hair regrowth, hyperkeratosis, dermatitis, ulcerative lesion on different parts of body mostly in the lateral aspect of the legs, most parts of the tail with extensive involvement of the back of the animal (Fig. 3, 4"A, B" and 5).



**Fig. 1. Failure of hair regrowth, localized skin lesions on lateral aspect of legs and emaciation**





**Fig. 2. Emaciation, alopecia with multiple skin pustules and ulcers on right side of the animal**



**Fig. 3. Extensive skin lesions along back of the animal with alopecia, crusting, hyperkeratosis and ulcers**



A



B

**Fig. 4. (A and B) Alopecia and multiple pustules on ventral and dorsal side of tail**



**Fig. 5. Severe emaciation and dehydration after about nine months of misdiagnosis and improper treatment**

### **Laboratory examination**

Blood parameters obtained from the animal at three different visits are listed in table 1. When the animal was first admitted in March-2013, most blood parameters were within normal limits, with the exception of MCV, MCH and MCHC that showed a slight decrease. Similar results were obtained in the second admission; blood parameters detected in the last visit in January 2014, revealed a decrease in hemoglobin, hematocrit, MCV and MCH; with an increase in leukocyte count, slight neutrophilia with mild left shift, marked monocytosis and an increase in platelet count. Basophilic polychromasia indicative for reticulocytosis or nucleated RBCs were not observed in stained blood films. Skin scrapings obtained from the animal in the first visit was positive for arthrospores of ring worm, other samples collected later were negative for both ring worm and mange. Fecal examination was negative for parasite eggs.

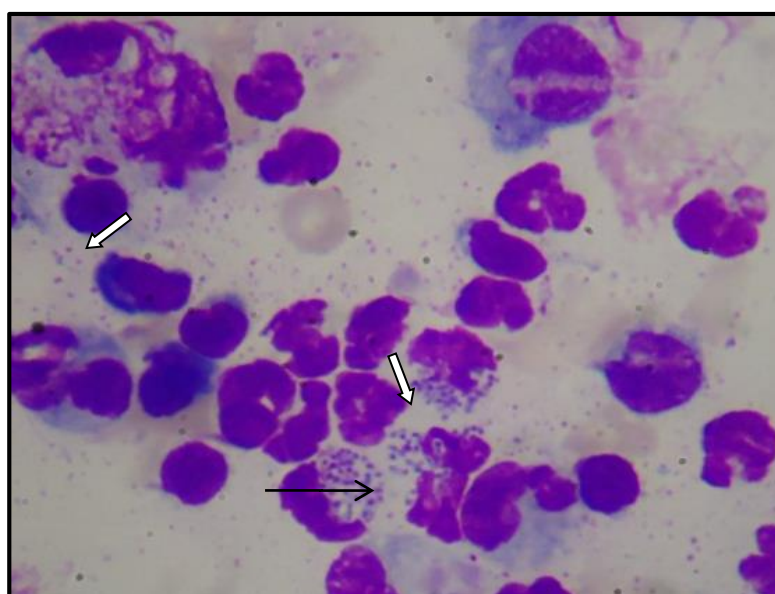
Impression smears prepared from various skin lesions and exudate were negative for the presence of acid fast bacilli. Interpretation of cytology requires time and expertise for the detection of *Leishmania* amastigotes when parasites are in low numbers and freed from the cells.

Microscopical examination of Giemsa stained impression smears revealed the presence of *Leishmania* amastigotes; they were detected in low number of microscopical fields; plenty of amastigotes, free and inside cytoplasm of macrophages were detected (Fig. 6 and 7).

**Table 1. Haematological parameters in successive periods of illness**

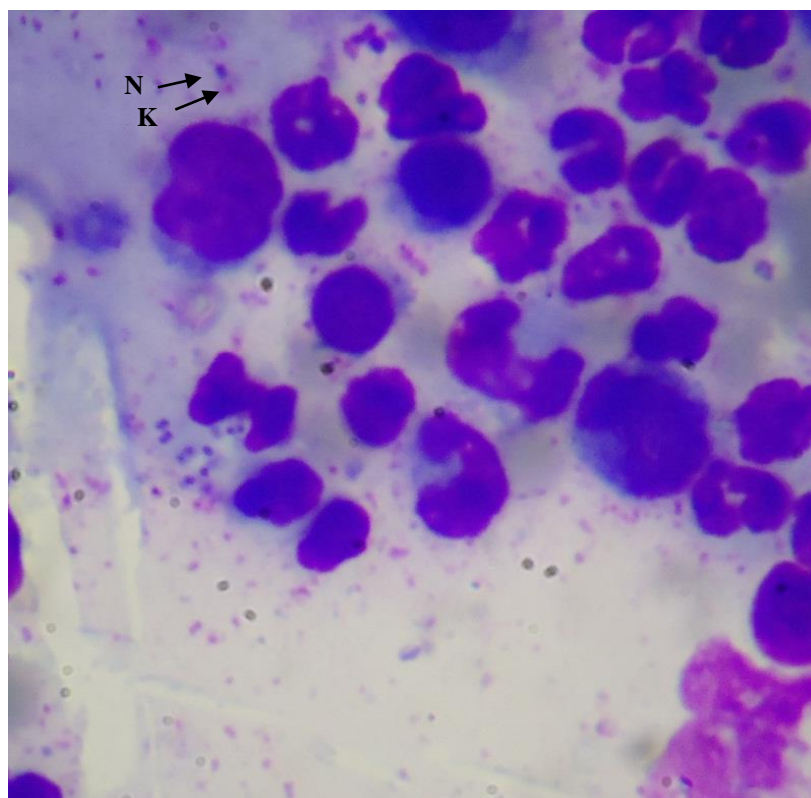
| Parameter  | 18/3/2013       | 10/5/2013      | 5/1/2014       | *Normal Range |
|--|-----------------|----------------|----------------|---------------|
| Hct %  | 40              | 40             | 30             | 37-55         |
| Hb g dl <sup>-1</sup>                              | 12.6            | 12.4           | 10             | 12-18         |
| RBCs x10 <sup>6</sup> μl <sup>-1</sup>             | 6.750           | 6.684          | 5.75           | 5.5-8.5       |
| MCV fl   | 59.7            | 59.8           | 52.2           | 60-77         |
| MCH pg   | 18.8            | 18.55          | 17.4           |               |
| MCHC g dl <sup>-1</sup>                            | 31.5            | 31             | 33.3           | 32-36         |
| Platelets x <sup>5</sup> 10 μl <sup>-1</sup>       | Adequate        | Adequate       | 9.7075         | 2-5           |
| Leukocyte count x <sup>3</sup> 10 μl <sup>-1</sup> | 5.75            | 5.55           | 17.65          | 6-17          |
| Band Neutrophil μl <sup>-1</sup>                   | 57.5<br>(1%)    | 166.5<br>(3%)  | 353<br>(2%)    | 0-300         |
| Segmented Neutrophil μl <sup>-1</sup>              | 4082.5<br>(71%) | 4107<br>(74%)  | 12708<br>(72%) | 3000-11500    |
| Eosinophil μl <sup>-1</sup>                        | 2.875<br>(5%)   | 0              | 0.706<br>(4%)  | 100-1250      |
| Basophil μl <sup>-1</sup>                          | 0               | 0              | 0              | Rare          |
| Monocyte μl <sup>-1</sup>                          | 862.5<br>(15%)  | 832.5<br>(15%) | 2118<br>(12%)  | 150-1300      |
| Lymphocyte μl <sup>-1</sup>                        | 460<br>(8%)     | 444 (8%)       | 1765(10<br>%)  | 100-4800      |
| ESR mm h <sup>-1</sup>                             | -               | -              | 26             | 0-15          |
| TPP g dl <sup>-1</sup>                             | 7.5             | 7.4            | 7.5            | 5.9 - 8.3     |
| Fibrinogen mg dl <sup>-1</sup>                     | -               | -              | 500            | 150-300       |

Referent data from (normal range) Meyer and Harvey (2004).



**Fig. 6. Giemsa stained smear from skin lesion exudates from a dog with clinical cutaneous leishmaniasis. (X100) A- High numbers of intracellular amastigotes (→) and extracellular *Leishmania* amastigotes (⇨)**





**Fig. 7. Giemsa stained smear from skin lesion exudates of the dog with clinical cutaneous leishmaniasis. (X100). Note the nucleus (N) and the kinetoplast (K) of extracellular amastigotes (arrows)**

**Table 2. Biochemical parameter of patient at last visit**

| Test                             | Unit                 | Normal range | Patient |
|----------------------------------|----------------------|--------------|---------|
| Glucose                          | mmol l <sup>-1</sup> | 4.3 - 6.7    | 5.8     |
| Urea                             | mmol l <sup>-1</sup> | 2.5 - 8.9    | 2.7     |
| Creatinine                       | μmol l <sup>-1</sup> | 44 - 124     | 63      |
| Alkaline phosphatase (ALP)       | IU l <sup>-1</sup>   | 10 -73       | 77*     |
| Alanine aminotransferase (ALT)   | IU l <sup>-1</sup>   | 15 -58       | 64*     |
| Aspartate aminotransferase (AST) | IU l <sup>-1</sup>   | 16 - 43      | 46*     |

Referent data from (normal range) Meyer and Harvey (2004).  
 \*= More than normal value

Normal plasma protein, elevated fibrinogen and ESR level was also detected (Table 1) in addition to normal level of glucose, serum creatinine and urea with elevated liver enzyme including ALT, AST and ALP (Table 2).

### DISCUSSION

Carlos, the dog of this case was a police dog, spending his life in conducting harsh tough duties between country borders and police checkpoints, this represents a continuous stress for these animals, in addition of exposing them to multi disease factors as insect bites. Cutaneous canine *leishmaniasis* is known by its variable clinical forms, starting from subclinical unapparent to



severe fatal form, Subclinical infection may not be static, it can turn to a severe type in cases of stress, immunosuppression and neglection (Baneth *et al.*, 2008); and this may explain the progression of *leishmaniasis* in this dog from mild unapparent to a severe fatal form.

Misdiagnosis of Carlos case was due to atypical clinical signs in the early stages of the disease, in addition to inability to detect amastigotes in primary impression smears prepared from cutaneous lesions until advanced stages of the disease commenced. This is highly expected in such types of infection with variable clinical outcomes (Ayele and Seyoum, 2016). The gradual onset and progression of clinical signs observed in this case agreed with that reported in almost all cases of cutaneous leishmaniasis studied in 150 dogs naturally infected by *Leishmania infantum* in a large endemic area of southern Italy (Ciaramella *et al.*, 1997). Anyhow, the variable and nonspecific clinical signs make the list of differential diagnoses for CanL wide and extensive (Solano-Gallego *et al.*, 2011).

It is worth noting that German Shepherd; in addition to many other pure breeds of dogs are more susceptible for developing cutaneous *leishmaniasis* than other breeds due to an identified genetic susceptibility (Franca-Silva *et al.*, 2003).

Late, advanced clinical signs observed in this case represented by progressive and generalized skin lesions together with devastating systemic clinical signs agreed with most frequent manifestation of this disease in dogs (Solano-Gallego *et al.*, 2009). The cutaneous form of the disease characterized by nodules, ulcers or scabs. Atypical skin lesions including pustular rashes, depigmentation, erythema multiform, hyperkeratosis has also been documented (Barnes *et al.*, 1993 ; Bailey and Lockwood, 2007 ; CFSPH, 2009).

Detecting characteristic *Leishmania* amastigotes inside macrophages and free in impression smears prepared from cutaneous exudates was the first and final indicator for the diagnosis of *Leishmaniasis* in this case.

Mild none regenerative anemia observed in late visit blood sample is due to chronic inflammatory nature of the disease and it has been reported in most cases of CanL all over the world (Baneth *et al.*, 2008). Mild leukocytosis, left shift and monocytosis run parallel with chronic granulomatous nature of this disease. Platelets were within adequate value through examining early samples, but thrombocytosis was detected in the last sample examined, these findings disagreed with that reported by others where thrombocytopenia and thrombopathy with bleeding disorders were frequently recorded. Some reported

that only 5- 15% of CanL dogs showed epistaxis, while others claimed that CanL accounted for 48% of cases in a recent retrospective study on 61 dogs with epistaxis in Northern Greece (Petanides *et al.*, 2008). Anyhow, bleeding abnormalities were not detected through the whole course of this case which runs parallel with absence of thrombocytopenia. Normal plasma protein in cases of dehydration indicated the possibility of hidden hypoproteinemia, while the increase in fibrinogen concentration runs parallel with the increase in ESR which is a non-specific indicator for chronic inflammatory diseases of animals. Normal serum Creatinine and urea indicated apparently normal renal function while general elevation in liver specific enzymes indicated liver impairment. Renal failure may be the only clinical sign recorded in dogs with visceral *Leishmaniasis* (Zatelli *et al.*, 2003); in the current case internal lesions were not detected using Ultrasonography.

Leishmaniasis is endemic in human in Iraq as many other countries in the Middle East (Al-Samarai, *et al.*, 2009) in the last decade, a flaring and reemerging of *Leishmaniasis* among humans has been detected. In Diyala Governorate; cases reported for cutaneous leishmaniasis is showing a gradual increase, from a total of 307 cases in 2009 to 4243 in 2017 has been reported in different districts particularly Khanaqin (Diyala Health Department/ unit of zoonotic diseases). As dogs are considered an important reservoir for this disease, more clinical and serological studies should be conducted to estimate the prevalence of this zoonotic protozoal parasite in dogs in this Governorate.

**Conclusion:** Canine cutaneous leishmaniasis was detected in a police dog which is well cared fore; further studies should be conducted on stray neglected dogs to be a reservoir for *Leishmania* sp.

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## داء اللشمانيا الجلدي الكلبى: أول تقرير في كلب نوع الراعي الألماني لكلاب شرطة محافظة ديالى، العراق

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### المستخلص

أحضر كلب بوليسي ذكر من نوع الراعي الألماني بعمر خمس سنوات الى العيادة التابعة لكلية الطب البيطري في جامعة ديالى وهو بحالة صحية جيدة مع وجود بعض الآفات الجلدية وحكاك. وقد شخصت الحالة مختبرياً كقوباء حلقيه (Ringworm) وأعطيت علاجاً مضاداً للفطريات. بعد بضعة شهور أحضر الكلب الى المستشفى مرة ثانية وقد أخضع الى حلاقة شاملة للشعر، أظهرت وجود آفات جلدية بشكل بثرات على كل مناطق الجسم. وقد شخصت الحالة سريرياً على أنها التهاب الجلد التأتبي (atopic dermatitis) واعطيت علاج الكورتيوزون مع مضادات الهستامين. ساءت الحالة تدريجياً بعد ذلك مع فقدان للشهية، وسوء الحالة العامة، وهزال وفقدان للوزن وشحوب للأغشية المخاطية. لم يستجب الحيوان للعلاج بالمضادات الحيوية، والفيتامينات المكملة ومضادات الطفيليات الخارجية والداخلية التي وصفت له. وقد أظهر فحص الصورة الدموية لتلك الفترة انخفاضاً في مستوى منفصل وخضاب الدم (Hct %, Hb g dl<sup>-1</sup>) مع ارتفاع بسيط في مستوى الخلايا الدموية البيضاء. وتم أيضاً مشاهدة أجسام طور الأماستيكوت الخاصة بطفيلي اللشمانيا (Amastigotes) في المسحات الطبيعية المحضرة من الآفات الجلدية والمصبوغة بصبغة الكيمز، بشكل حر او داخل هيولي خلايا البلعم الكبير. شخصت الحالة اخيراً على انها حالة ليشمانيا سز جلدية (Cutaneous leishmaniasis)، ولم يقدم العلاج المناسب للحيوان المصاب فساءت حالته الصحية وانتهت بوفاته.

الكلمات المفتاحية: كلب الراعي الألماني، اللشمانية الجلدية الكلبية، العراق.