

SEROPREVALENCE OF KALA-AZAR AMONG HUMANS AND DOGS IN YEMEN

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Kala-azar (visceral leishmaniasis) is a major cause of fever in the world, and is estimated to affect 100 million people worldwide.¹ Kala-azar was first reported from the northern part of Yemen over 90 years ago.² Sporadic cases of the disease, however, are now widely reported from all over the country, including the governorates of Sana'a, Taiz, Ibb, Al-Hodeidah, Hajjah, Damar, Sa'adah, Al-Mahweet, Mareb, and Al-Jawf. Nearly 4047 cases, mostly in young children, have been reported during the last decade.³ The scarce information of the disease epidemiology in Yemen showed that the causative organisms were *Leishmania donovani* complex and *L. infantum* complex, and vectors were *Phlebotomus orientalis* and *P. arcticus* for *L. infantum* and *L. donovani*.^{4,6} In addition, a few other infantile cases were reported from the northern part of Yemen.^{7,8} Michie⁹ described four adult cases from the southern part of Yemen.

Canine leishmaniasis was discovered by Nicole and Comte in 1908,¹⁰ and since then reports of infected dogs have come from almost all human visceral leishmaniasis foci in the Mediterranean area.¹¹⁻¹³ Dogs are an efficient reservoir host because parasitized fixed macrophages are so abundant in the dermal layer that parasites are readily taken up by feeding sandflies.^{14,15} Canine leishmaniasis was reported by Rioux et al. from human visceral leishmaniasis focus in the Taiz area of Yemen.

The objectives of this study were to determine the seroprevalence of kala-azar among children in selected localities in Yemen, and to identify the natural reservoir of human kala-azar in these areas, focusing particularly on dogs, with a view to determining infection rates among them and their role in the transmission of the disease to humans.

Materials and Methods

The study was conducted in December 1993 in three

selected villages where cases of kala-azar had been reported during the year, and also in the city of Sana'a. The study population consisted of volunteer schoolchildren in Beni-Mansur (Al-Haymah, Sana'a province), Huth (Sana'a province) and in Sana'a city. Based on an expected kala-azar prevalence of 10% and the worst acceptable value of 4%, not less than 270 randomly selected subjects were needed out of a total estimated 100,000 children, at a confidence level of 99.9%.

A systematic random sampling of every fifth child was done. A sample of 285 subjects were selected, comprising 68 subjects from Beni-Mansur, 85 from Huth, 75 from Sharis and 57 from Sana'a city.

All study participants completed an epidemiological questionnaire and gave informed consent to participate in the study. Basic demographic data, including age, sex, address, and altitude of the house, were obtained, as well as potential risks factors for kala-azar.

Serological Assay

A 5 mL aliquot of venous blood was allowed to clot and the serum was separated and stored at -20°C until serological analyses were performed. All the sera were tested for kala-azar using the ELISA technique, according to guidance on leishmania antibody ELISA from the Institute of Zoology, London, U.K.

Collection and Testing of Canine Specimens

Dogs were captured in two areas where cases of human visceral leishmaniasis had been identified. Feral dogs are abundant in the study areas, living close to man and wandering around farm buildings and houses, where they are considered to be a nuisance by the local population. (Official permission was obtained to capture 16 feral dogs, eight from the Sharis area and another eight from the Beni-Mansur area.)

A tented field laboratory was set up at each location for the purpose of providing a base where the animals could be examined. The dogs were examined externally for signs of leishmania infection, then dissected for the following investigations:

1. Smears were prepared from any skin lesion detected, and from the livers and spleen of each dog. These were fixed with methanol, stained with Giemsa stain and examined microscopically for the presence of amastigotes.

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Accepted for publication 15 September 1997. Received 13 July 1996.

2. Biopsy specimens were collected aseptically from the livers and spleen, then inoculated into biphasic culture medium (locally prepared from nutrient agar containing 10% whole rabbit blood overlaid with Lock's buffers containing 100 U_g/mL gentamicin). The inoculated cultures were incubated at 21°C for up to six weeks and examined weekly for the presence of promastigotes.

3. Serology: Blood was collected from each dog and sera were separated and stored at -20°C until assayed. The sera were tested for the detection of antibodies against kala-azar by ELISA test. The test was calculated with respect to positive sera from dogs infected with leishmania and negative sera from dogs free of leishmaniasis. Forty percent absorbance values of positive sera were considered positive.

Results

Out of the 285 sera examined from the four localities, 99 (34.7%) sera showed a reaction equal to or greater than 1:200.

The study of the risk factors for the contraction of kala-azar will be reported separately. We found a high risk associated with residence in villages, ownership of dogs, open refuse dumps close to houses, stray dogs around the house and the presence of rodents in houses.

Leishmaniasis Infection in Feral Dogs

Sixteen feral dogs were examined, eight in Beni-Mansur and eight in Sharis, both of these being endemic foci for infantile kala-azar. Lesions of possible leishmania origin were observed on the ears of five dogs in the Beni-Mansur area, but no parasites were identified in the skin of these animals. Splenomegaly was observed in four and hepatomegaly in two dogs. Amastigotes were observed in Giemsa-stained smears of livers and spleens of four dogs. ELISA testing of canine blood indicated that 75% (six out of eight dogs) from Beni-Mansur were positive, compared with 25% (two out of eight dogs) from Sharis. This gives a prevalence of 50%. No growth occurred in cultured liver and spleen of dogs in NNN media, even for dogs found to be positive for amastigotes by liver and splenic aspiration.

Discussion

The serological survey of children living in endemic areas yielded a high-positive rate from 14% to 85%. These were higher than those reported by Al-Zahrani et al.¹⁶ in Saudi Arabia by the ELISA technique (3.2%) in areas endemic for kala-azar. The high prevalence of antibodies against kala-azar suggested that subclinical infections in man, particularly in the Sharis area, are common and important in the spread of kala-azar in Yemen. This is similar to the results reported by Pampiglione et al.,²⁴ where they found that 44% of the study population had antibodies in their blood and only 3% of them had clinical

infection. Cross-reacting antibodies are commonly seen in ELISA and numerous conflicting reports are found in the literature, quite often with discrepant results. The most frequently reported cross-reactions occur in areas where diseases such as Chagas' disease and African trypanosomiasis co-exist with kala-azar.^{17,18} As these two diseases do not occur in Yemen, this was not a problem in our setting, but cross-reactions from cutaneous leishmaniasis^{19,20} can affect specificity,

In spite of some reports of cross-reaction of anti-leishmania antibodies with malaria, TB, and Hansen's disease,¹⁸⁻²² Choudhry et al. did not observe any such cross-reactions.²³ The prevalence of antibodies to kala-azar noted in these asymptomatic populations may have been due either to past infection (apparently subclinical), current active or subclinical disease.

The data presented in our study suggest the involvement of *L. donovani* or *L. infantum* as an etiologic agent. It should be noted that there is an antigenic cross-reaction, but at a low titer, less than 1:200 with malaria, TB, Hansen's disease and cutaneous leishmaniasis.¹⁸⁻²² Thus, kala-azar remains the most likely cause of the responses noted.

In areas endemic for infantile visceral leishmaniasis, dogs mainly harbor *L. infantum*, although infections with *L. donovani* complex and other leishmania parasites have been reported from different areas.^{25,26} As in humans, the disease elicits strong humoral immune response in infected dogs and in recent years, canine serology has been frequently used not only to confirm patent infections but also to detect latent infections.

ELISA has been used to detect antibodies in dogs²⁸ and was considered more sensitive and specific than IFA,²⁹ but cross-reactions occurred with cases of cutaneous leishmaniasis and with Chagas' disease.³⁰

The prevalence of antibodies to kala-azar noted in the canine population may have been due either to infection with *L. infantum* complex and *L. donovani* complex,²⁵⁻²⁷ (past infection or current active disease), or to antigenic cross-reaction with cutaneous leishmaniasis. However, kala-azar remains the most likely cause of the responses noted.

The overall prevalence among dogs in our survey (50%) was higher than that reported in Saudi Arabia in endemic areas by ELISA test (19.3%),¹⁶ and far higher than that in Tunisia (6.03%).¹³

Conclusion

The human serological survey indicates the possible existence of subclinical infection, which may thus serve as a reservoir of infection. In addition, the human seroprevalence was higher than that reported from Saudi Arabia.

In spite of the small sample size of the canine survey, the existence of a reservoir of infection was confirmed and

suggests an alarming percentage higher than that of Saudi Arabia, and far higher than that of the Mediterranean region.

Kala-azar should be made a notifiable disease in Yemen and further field studies should be organized to define more clearly the foci of human kala-azar in Yemen, and to trace and confirm possible reservoirs among canines and other wild animals, as well as to study vectors.

The health authorities should take the following measures: 1) case finding, including a search for overt cases of kala-azar by spleen puncture and serology to detect recent infections which may or may not become overt; and 2) control of the human reservoir, which is especially important in epidemic situations. Case finding and treatment form the major component of any control scheme. Also, the canine reservoir may be controlled by reduction of the feral dog population.

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