THE ROLE OF SOME BRUCELLA CARRIERS (STRAY DOGS AND RATS) IN MAINTENANCE OF BRUCELLA INFECTION

By

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ABSTRACT

The role of stray dogs and wild rats as brucella carriers as well as their role in spreading and maintenance of brucella infection were investigated. A total of 1640 sheep from different flocks and areas were serologically examined for brucellosis using Rose Bengal and Rivanol tests. At the same time, a total of 182 dogs and 120 rats were collected from both brucella infected and non-infected areas and examined serologically. In addition, tissue sections from their spleens were examined for brucella antigen using the immunoperoxidase technique. Tissue sections from spleens and udders of seropositive ewes were histopathologically examined where different pathological changes could be detected. Brucella antibodies could be detected in sera of 42 ewes as well as 18 dogs and 6 rats that were associated with brucella infected sheep. Brucella antigen could be detected in the spleen of 12 seropositive dogs and 4 rats as well as spleen of 20 ewes. *Brucella melitensis* biovar 3 could be isolated from the udder and spleen of 12 slaugthered seropositive ewes and spleen of 10 seropositive dogs and 2 rats. The epidemiological significance and the role of stray dogs and rats associated with brucella infected sheep were discussed.

INTRODUCTION

Brucellosis is a serious highly contagious disease and once infection is established in an animal population, the disease persists, occurs and reoccurs among animals without end inspite of the continous application of exhaustive trials to control the disease.

Several factors contribute in maintenance of brucella infection of which, the contagious nature of the disease, the survival of brucella organisms outside the animal host, **Radostits, (2000)**, the wide host range, latency, the capacity to survive within phagocytes of the host, **Nicoletti, (1989)** as well as the carrier state that may develop in other non preference hosts e.g. stray dogs and rats which may be associated with brucella infected animals.

Brucella organisms were isolated from dogs in farms where several cattle were serologically positive, **Palmer and Cheville, (1997)** and **Salem *et al.*, (1975)**. Serological and bacteriological evidences of brucella infection in rats collected from brucella infected farms was reported by **Hosein, (1987)**.

The present study was carried out to investigate the role of stray dogs and rats as brucella carriers and their role in maintenance of brucella infection among animals employing serological, bacteriological, histopathological and immunopathological techniques.

MATERIAL AND METHODS

Animals:

A total of 1640 sheep belong to 4 different flocks were employed in this study and subjected for serological, bacteriological, histopathological and immunopathological studies.

A total of 182 stray dogs and 120 rats were obtained from both infected and non infected sheep farms and subjected for serological, bacteriological, histopathological and immunopathological studies.

Samples:

Blood samples were collected from the jugular vein of 1460 sheep as well as from 182 dogs and 120 rats after sacrification. Blood sera were separated and used for serological examination.

Tissue specimens including udders and spleens were collected from slaughtered seropositive sheep. Spleens were collected from sacrificed dogs and rats. The obtained tissues were used for brucella isolation, immunopathological and histopathological examinations.

Serological examination:

Rose Bengal test was carried out according to **Morgan *et al.*, (1969)**. Rivanol test was carried out according to the method described by the National Veterinary Services laboratories, Ames. Iowa, USA (1984).

Isolation and identification of brucella organisms were carried out according to **Alton *et al.*, (1975)** using tryptose soya agar media (Difco, USA).

Tissue specimens were fixed in 10 % formaline solution and prepared for paraffin embedding. Paraffin sections 5 - 7 μ in thickness were stained with hematoxylin and eosin (H & E) and examined microscopically.

Deparaffinized sections of spleen of sheep, dogs and rats in which the endogenous peroxidase has been blocked were stained with Avidin-Biotin peroxidase technique (**Meador *et al.*, 1986**) using polyclonal antibodies prepared in rabbits as primary antibody, biotinyalted antirabbit IgG (Kriggard and Perry lab.) as secondary antibody, Streptavidin horse raddish peroxidase

(Gibco) and 3-amino - 9 - ethylcarbazol (AEC), (polyscinces) as a chromogenic substrate. Tissue sections were counter stained with Shadon' s hematoxylin then mounted.

RESULTS

Table (1): Serological examination of sheep.

Sheep flocks	No. of examined	No. of seropositive sheep
Flock 1	520	42
Flock 2	402	0
Flock 3	278	0
Flock 4	260	0
Total	1460	42 (2.88 %)

Table (2): Serological examination of stray dogs and rats.

Animals	No. of examined animals	No. of seropositive animals
Dogs associated with infected sheep	64	18
Dogs not associated with infected sheep	118	0
Total	182	18 (9.89 %)
Rats associated with infected sheep	42	6
Rats not associated with infected sheep	78	0
Total	120	6 (5 %)

Table (3): Correlation between the results of Br. isolation, immunoperoxidase technique and serological examination.

Animals	No. of examined animals	Seropositive	Immunoperoxidase + ve	Br. isolation*
Sheep	1460	42	20	12
Dogs	182	18	12	10
Rats	120	6	4	2

* *Brucells Melitensis* biovar 3.

DISCUSSION

Serological examination of 1460 sheep belong to four flocks classified them into one brucella infected flock and three brucella free flocks. Brucella antibodies could be detected in 42 (2.88 %) sheep of the brucella infected flock using Rose Bengal and Rivanol tests.

In this study, a total of 182 dogs and 120 rats were collected and examined for brucellosis, out of them 64 dogs and 42 rats were obtained from the brucella infected farm or from neighboring areas, while the remaining dogs and rats were obtained from brucellosis free area. Brucella antibodies could be detected in 18 (9.89 %) dogs and 6 (5 %) rats of those obtained from the infected area. On the other hand, all dogs and rats that were collected from brucella free flocks or areas were serologically negative for brucellosis. The role of dogs and rats as brucella carriers was previously reported by **Salem *et al.*, (1975) and Hosein, (1987)** who reported the presence of serologically reactor dogs and rats in brucella infected cattle farms.

An avidin-biotin peroxidase technique in this study could specifically stain and demonstrate the brucella antigen in the spleen of 20 sheep, 12 dogs and 4 rats (Figs. 1, 2 & 3). Spleen is considered by several authors as an important target for brucella organisms (**Bosseray, 1992 and Pough, 1989**). The obtained results indicate the high degree of colonization of brucella organisms in this organ (Figs. 1,2 & 3). The applied technique proved high sensitivity by using high dilution of the primary antibody, it also permits visulization of highly specific reaction as well as the localization of the antigen in infected tissues. The technique also eliminates the risk of infection from handling cultures of brucella organisms. .

Brucella melitensis biovar 3 could be detected from the udder and supramammary lymph node of 12 sheep, spleen of 10 dogs and 2 rats. Isolation of the same brucella biovar from sheep, dogs and rats from the same area may refer to the nature of the epizootic process of the disease and the possibility of transmission of infection among these animals. **Palmer and Cheville, (1997)** reported that naturally acquired brucella infection can occur in dogs associated with infected cattle. They isolated brucella organisms from dogs in a farm where several cattle were serologically positive.

Brucella infection in dogs and rats as proved by serological examination, brucella isolation and immune detection of brucella antigen in the tissues of spleen using immunoperoxidase technique may be attributed to the close contact of these animals with infected sheep and feeding on the aborted material or exposure of such animals to infection during the first few days following abortion when large numbers of brucella organisms are present which is considered the primary hazard in brucella infected flocks or herds.

Histopathological changes observed in udder tissues consisted of edema of the interstitial connective tissues and degeneration on the epithelial cells lining the lactating acini. In addition, aggregation of mononuclear cells mainly lymphocytes and plasma cells occurred in the interlobular connective tissues (Fig. 4). Similar findings were reported by **Deeb *et al.*, (1994)**.

In the spleen diffuse infiltration with polymorphnuclear leucocytes an red and white pulp were seen. Scattered cellular necrosis in subcapsular region in red pulp were observed. Accumulation of neutrophils and epithelioid cells adjacent to the lymphoid follicles were also observed (Fig. 5). Similar results were reported by **Deeb *et al.*, (1994), Montaser, (1995) and Enany *et al.*, (1997)**.

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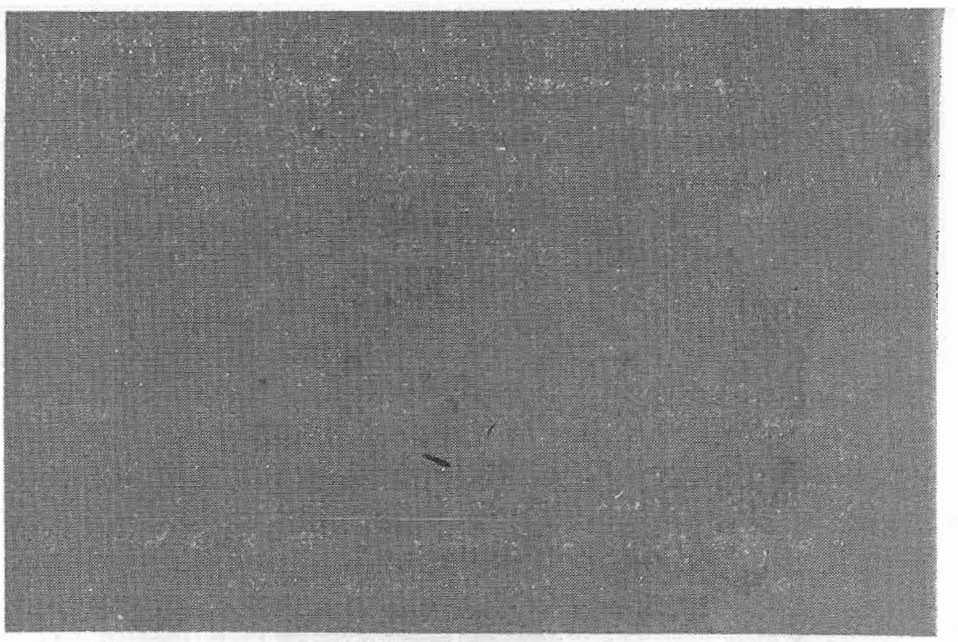

Fig. (1): Spleen of mice showing small scattered reddish aggregates (positive immunoperoxidase reaction) 1 x 160

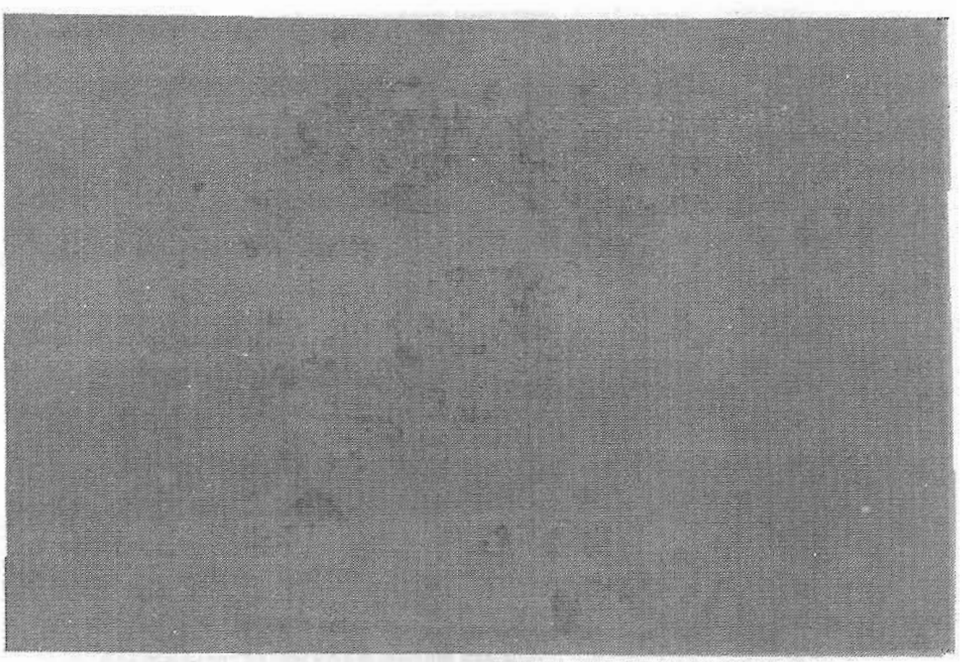

Fig. (2): Spleen of dog showing red stained patches (positive reaction of immunoperoxidase technique) 1 x 400

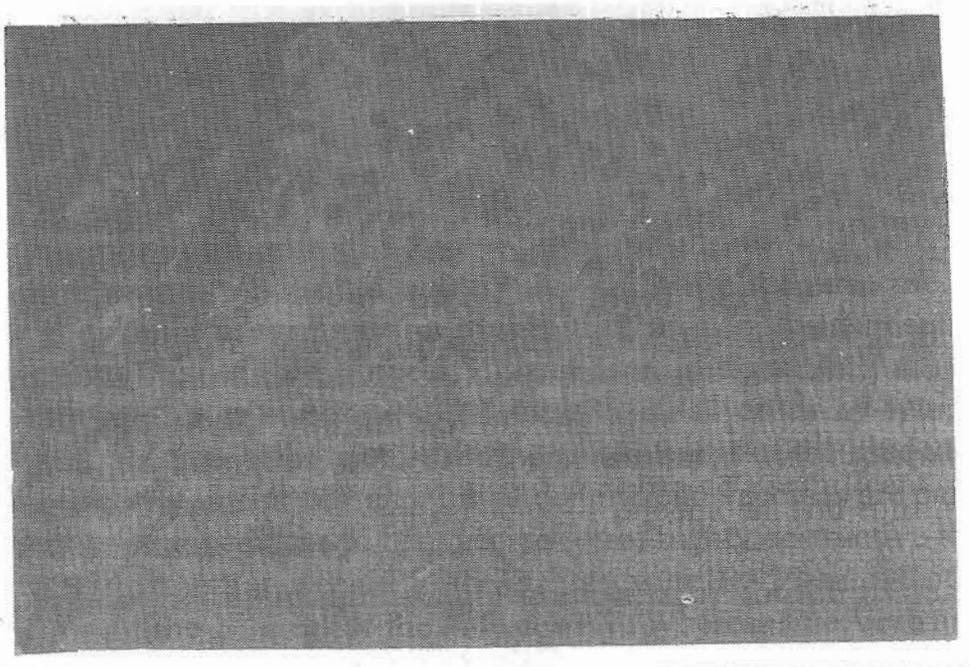

Fig. (3): Spleen of sheep showing red stained patches of different zones (positive reaction of immunoperoxidase technique) 1 x 400

Fig. (4): Udder of sheep showing infiltration of mononuclear leukocytes in the interstitial tissues between lactating acini of the udder (H & E) 1 x 400

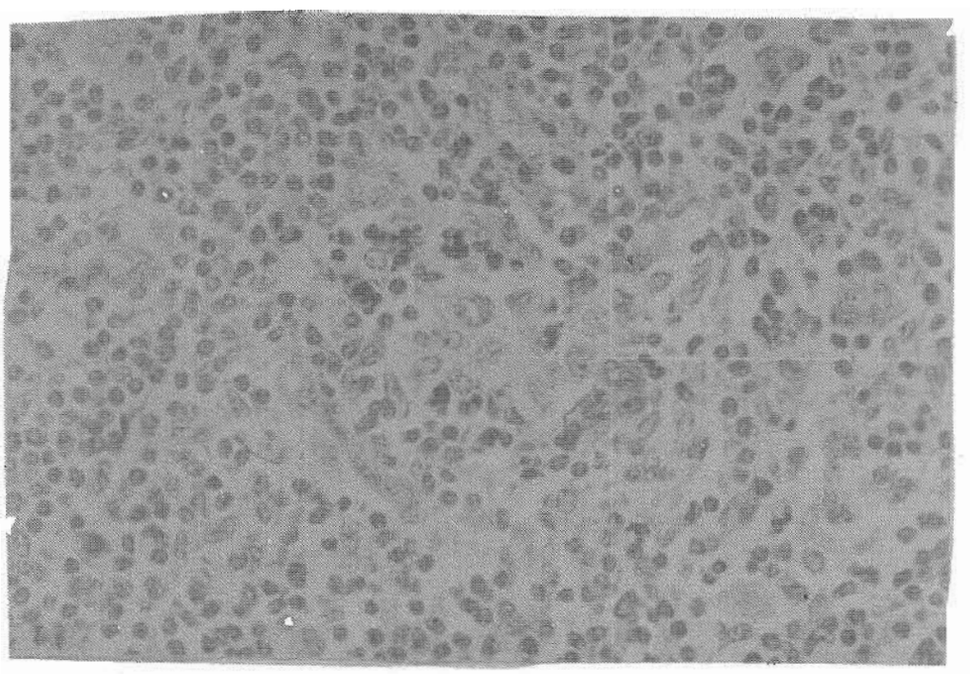

Fig. (5): Spleen of sheep showing leukocytic and epithelioid cells infiltration (H & E) 1 x 400