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PATHOLOGICAL FINDINGS ASSOCIATED WITH EXPERIMENTAL *MYCOBACTERIUM BOVIS* INFECTION IN RABBITS.

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During the 2nd week postinoculation of thirteen rabbits with Mycobacterium bovis. Tuberculous lesions appeared in the lungs, liver, spleen, kidney, mediastinal and hepatic lymph nodes and in the omentum with an equal distribution in these organs. During the 4th week postinoculation, these tuberculous lesions increased in size to become well developed granulomas with caseated centeres. These granulomas persisted to the 6th, 8th and 10th weeks postinoculation and became more encapsulated later on. Three rabbits died during the 7th week postinoculation due to generalized tuberculosis.

الآفات المرضية الناجمة عن الإصابة التجريبية بعصيات السل البقري في الأرانب

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الخـــلاصـــة

بينت هذه الدراسة، خلال الأسبوع الثاني من الحقن التجريبي لثلاثة عشر أرنب بعصيات السل البقري، آفات تدرنية بدائية في الرئات و الكبد و الطحال و الكلى و العقد اللمفاوية المنصفية و الكبدية و في الثرب و بتوزيع متساوي للآفات في هذه الأعضاء. لقد ازداد حجم هذه الآفات و لتصبح أورام حبيبية تدرنية متطورة و ذات مراكز تنخر تجنبي في الأسبوع الرابع من الحقن. لقد استمر وجود هذه الآفات الورمية الحبيبية التدرنية في الأسابيع السادس، الثامن و العاشر من الحقن و لتصبح محاطة بمحفظة ليفية و هلكت ثلاثة أرانب في الأسبوع السابع من الحقن نتيجة انتشار مرض التدرن في كافة أعراضها.

INTRODUCTION

Tuberculosis (TB) is a chronic zoonotic disease and it still remains an important public health problem. It is responsible for 3 millions deaths annually in the world (1). Bovine tuberculosis is widely spread in tropical areas that cause a potential contribution to the prevalence of human tuberculosis.

Also, there is increase in the incidence of human tuberculosis that is caused by Mycobacterium bovis (2). One report of WHO was estimated that in 1990 about 7.5 millions people (one third of world population) infected with tuberculosis of human and bovine types (3). Many workers were reported that the rabbits were mostly susceptible to bovine type of the tubercle bacilli (4) which is responsible for infection of cattle as well as human beings. For this reason, the aims of this study were the following:

1. Study the pathogenesis of the disease experimentally induced in rabbits.
2. Study the gross and microscopical lesions associated with this disease in rabbits.

MATERIALS AND METHODS

Thirteen rabbits, 4 months old, 450-500gm of weight, provided by AL-Kindi Company For Veterinary Drugs and Vaccine Production were used. The animals were reared together to check them for complete health. All of these animals were tuberculin negative. These animals were experimentally injected with *Mycobacterium bovis* suspension by intraperitoneal route and the suspension of this microorganism was prepared according to Converse et al. method (5).

Briefly, a local pure strain of *Mycobacterium bovis* was obtained in Stonebrink's medium. Then, the microorganism were grown in ungar broth for 16 days. The growth was precipitated by centrifugation and washed 3 times by phosphate buffer saline. A suspension of this microorganism (1mg of *Mycobacterium bovis* per 1ml of phosphate buffer saline) was made and intraperitoneally injected in laboratory animals.

Two rabbits were killed at the end of 2nd, 4th, 6th, 8th and 10th weeks postinoculation, and all the morphological and gross lesions were recorded and representative pieces from the lesions were fixed in 10%. neutral buffered formalin, processed routinely and embedded in paraffin; cut at 5μm thickness and stained with hematoxylin and eosin and with Ziehl-neelsens's stain and examined under the light microscope.

RESULTS

During the 2nd week postinoculation, there were minute white foci distributed on the lungs, liver, spleen, kidney, mediastinal and hepatic lymph nodes and on the omentum. These necrotic foci, histologically consisted of an aggregation of macrophages, neutrophils and edema (Fig-1). During the 4th week postinoculation, these lesions gradually progressed and increased in size and histologically consisted of a central area of caseation which was mostly predominant in the lung tissue (Fig-2), surrounded by a wide zone of epithelioid cells (Fig-3), few Langhans's giant cells and lymphocytes. During the 6th week postinoculation, the areas of caseation were became predominant all over the organs (Fig-4) and surrounded by a wide zone of epithelioid cells (Fig-5), few langhan's giant cells (Fig-6) and extensive lymphocytes infiltration with few fibroblasts proliferation and encapsulation of these lesions. During the 8th

and 10th weeks postinoculation, there was an increase in the thickness of fibrous tissue layer encapsulating the lesions in the different organs. All these tuberculous lesions did not involve calcification in the different stages of this disease process and all histological sections, stained with Ziehl-Neelsen's stain were showed positive acid fast bacilli, indicating presence of these bacteria in the different stages of the disease in the all infected organs. Three rabbits had died at 7th week postinoculation due to generalized tuberculosis.

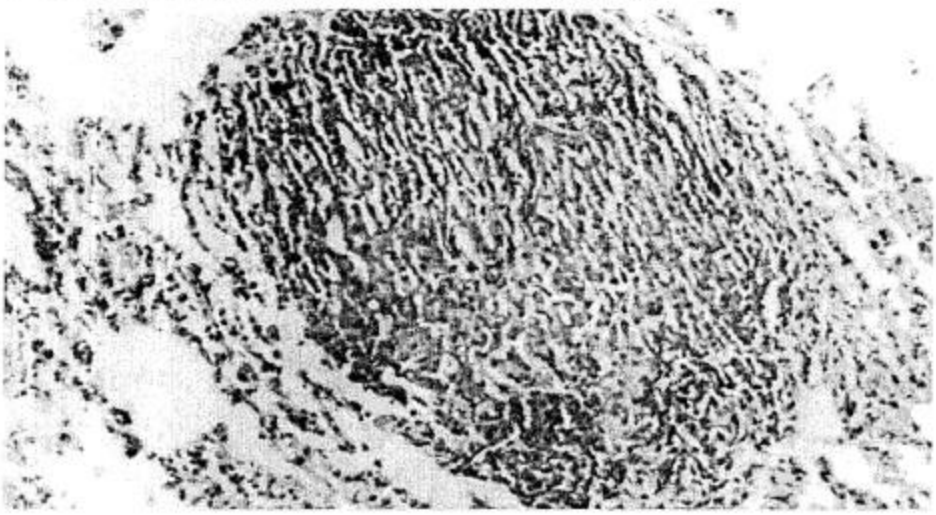

Fig-1: Lung tissue with a primary tuberculous lesion. Note aggregates of macrophages, edema, some lymphocytes and neutrophils, during the 2nd week postinoculaton. (H & E) x 125.

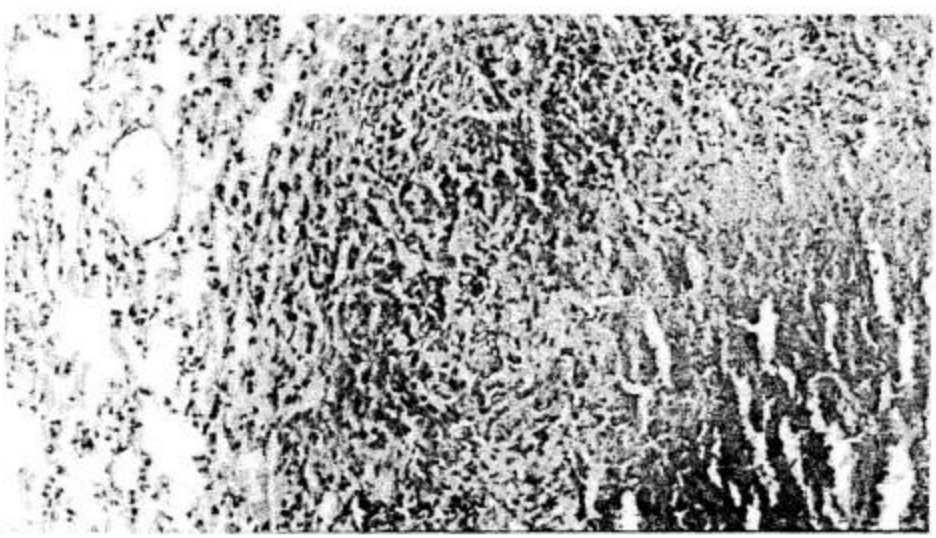

Fig-2: Lung tissue with a well developed tuberculous granulomatous lesion with a central caseous necrosis, during the 4th week postinoculation (H & E) x 125.

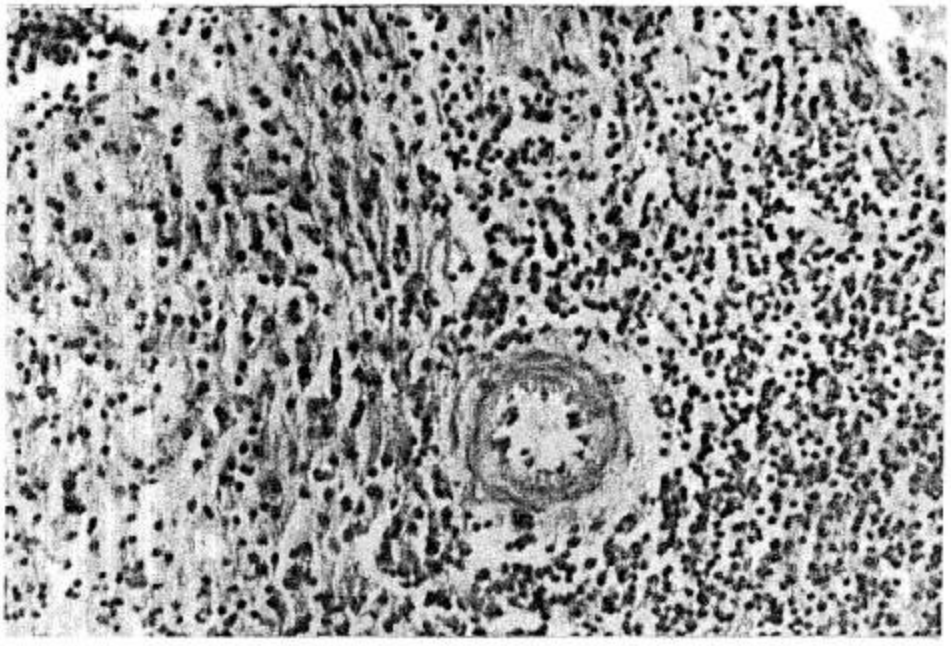

Fig-3: Spleen tissue with early tuberculous granulomatous reaction consisted of aggregation of epithelioid cells in the white pulp, during the 4th week postinoculation (H & E) x 250.

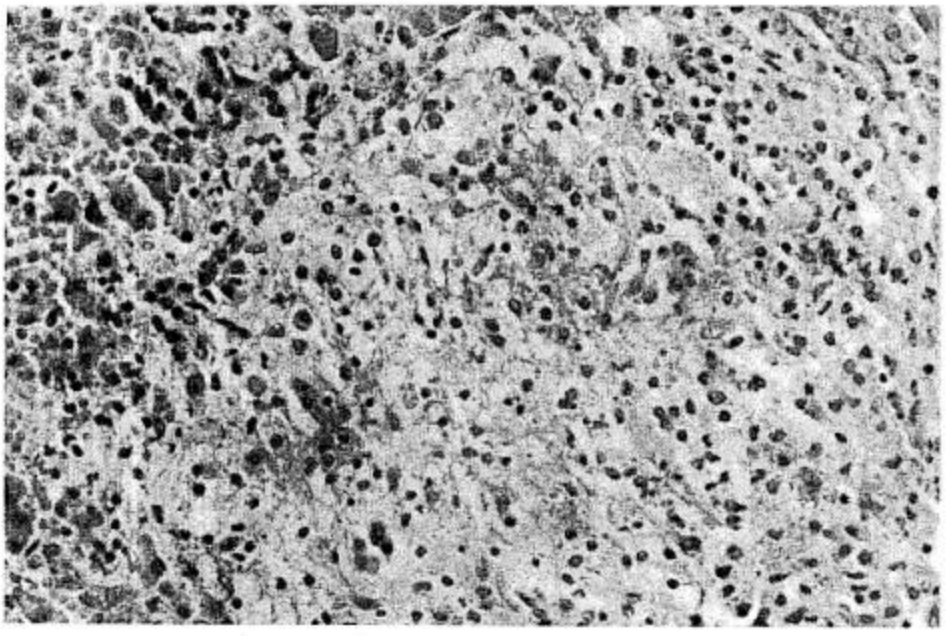

Fig-4: Liver tissue showing early tuberculous granulomatous reaction in the form of aggregation of epithelioid cells with a caseation, during the 6th week postinoculation (H & E) x 125.

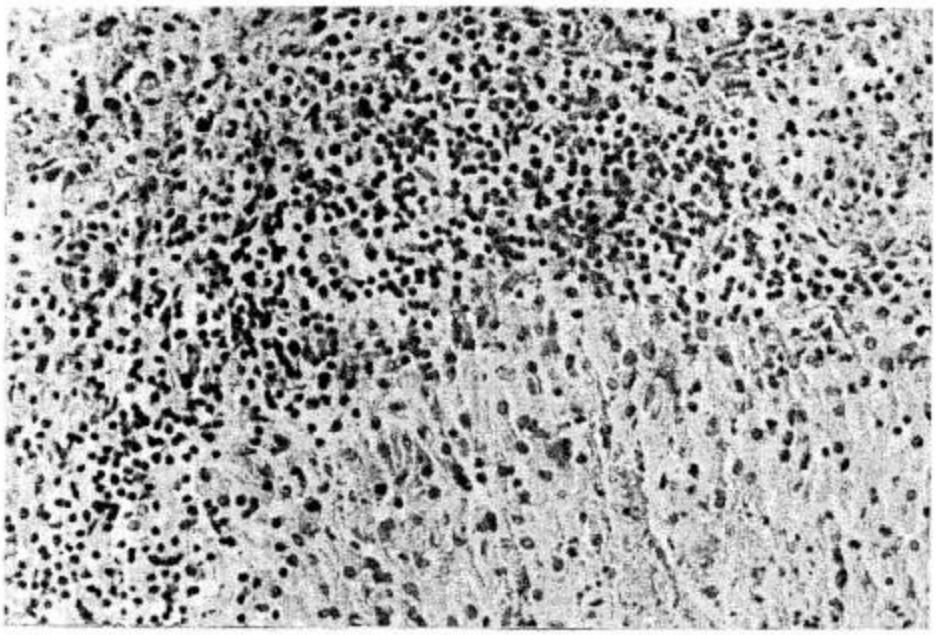

Fig-5: Spleen tissue showing a well developed tuberculous granuloma, which consists of aggregation of epithelioid cells and lymphocytes with central caseous necrosis, during 6th week postinoculation. (H & E) x 250.

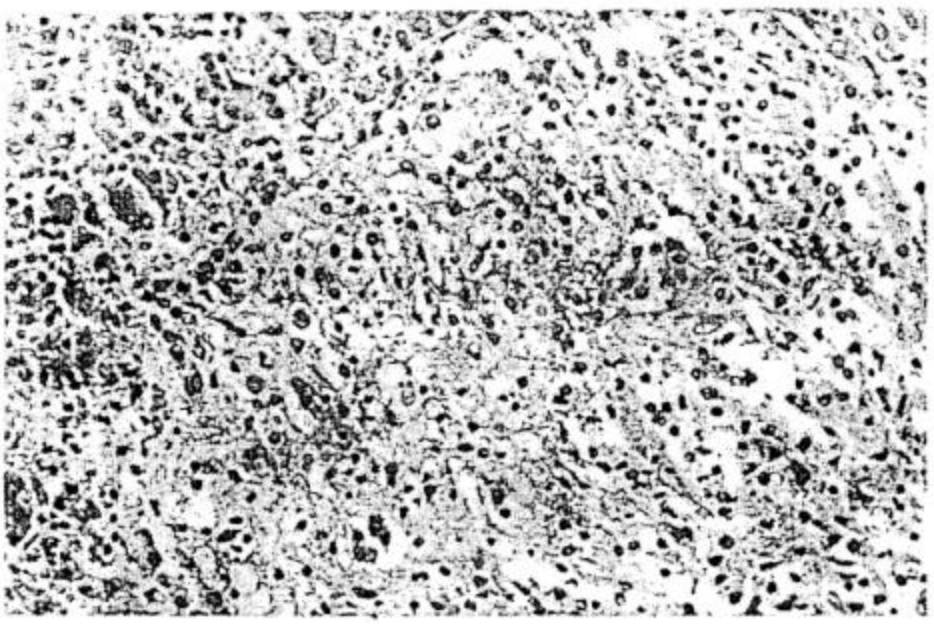

Fig-6: Hepatic lymph node tissue with a well developed tuberculous granuloma, consisting of a central caseous necrosis surrounded by aggregation of epithelioid cells, lymphocytes and few gaint cells, during the 6th week postinoculation. (H & E) x 125.

DISCUSSION

A limited work only had been done on tuberculosis in rabbits, and thus results obtained in this study showed an equal distribution of the tuberculous lesions on the lungs, liver, kidneys, spleen, mediastinal and hepatic lymph nodes and on the omentum. This equal distribution of tuberculous lesions in these organs indicated that the Mycobacterium bovis was disseminated into these organs with similar numbers, following the intraperitoneal injection method of these microorganism, whereas, the microorganisms were induced more tuberculous lesions in the lungs than in liver and mesenteric lymph nodes in cows contracting the disease through inhalation, a findings that has reported previously in cattle infected with pulmonary tuberculosis (6,7).

In this study, following the intraperitoneal inoculation of the Mycobacterium bovis, the microorganism was introduced and replicated inside the peritoneal macrophages and neutrophils causing destruction of these phagocytic cells and reintroduce and replicate in other peritoneal macrophages which carry them into mediastinal ymph nodes (which receive the afferent lymphatic fluid from peritoneal cavity), then the microorganisms reach through the lymphatic fluid into thoracic duct and finally by hematogenic dissemination in the different organs which was mostly evident in this study. The multiplication of the microorganisms in the macrophages at the immunocompetent animals may have been resulted in a degree of systemic macrophages and lymphocytes activation; the activated macrophages were present in the form of epithelioid cells and few langhan's giant cells in the present study; a similar findings has been reported previously in guinea pigs (8).

In this study, the primary tuberculous lesions were observed during the 2nd week postinoculation in the lungs, liver, spleen, kidneys, mediastinal and hepatic lymph nodes and in the omentum. These primary tuberculous lesions consisted of an aggregation of macrophages, some neutrophils, edema and lymphocytes. During the 4th week postinoculation, the number of the lymphocytes and activated macrophages were increased (the neutrophils were decreased and disappeared). These lymphocytes (T cells) produced different lymphokines which in turn activate macrophages derived from blood monocytes to become epithilioid type cells and to form a compact cluster or granuloma (9) which was predominant in different organs in this study. The centers of these granulomas contained a caseation which gradually increased in size; the caseation was consisted of a mixture of necrotic tissue and dead macrophages; occurred due to replication of the Mycobacterium bovis in these granulomatous cellular reactions, causing destruction's of these cells (macrophages). Also, the caseation may be resulted from releasing of cytotoxic lymphokines and hydrolytic enzymes by T lymphocytes and activated macrophages (10).

The granulomatous reaction which was main tuberculous lesion in the present study was an indication of emergence of the cell mediated immunity in which recognition of mycobacterial antigens by macrophages and stimulation of these antigen specific T lymphocytes (11,12) for the migration of the macrophages and T lymphocytes to the sites of mycobacterial invasion and liberation of different cytokines responsible for these granulomatous reactions. Later on, all these granulomatous lesions were encapsulated by fibrous tissue, which indicated the final defense mechanism of the body against this microbial infection (13). Three

rabbits had died during the 7th week postinoculation due to generalized tuberculosis, which was resulted from complete dissemination of *Mycobacterium bovis* through the blood stream in the different body organs causing generalized tuberculosis. A similar finding has been observed in rabbits experimentally infected with the *Mycobacterium bovis* (5,6).

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