

## نتائج موجبة لأختبار الروزبنكال في الخيول

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### ملاحظات قصيرة

لوحظت حالات متفرقة في مجموعة من الخيول شملت ناسور الحارك، التهاب المفاصل وتورم كيس الصفن. سحبت عينات دم من بعض هذه الحالات ومن خيول تبدو سليمة لقياس خضاب الدم وأجراء اختبار الروزبنكال ( Rose Bengal test ). كذلك تم سحب نموذج من السائل في كيس الصفن. أظهرت الفحوصات أن مستوى خضاب الدم كان ضمن الحدود الطبيعية (11.6-16.2)غم/100سم<sup>3</sup>. وان خمسة خيول من عشرة (50%) أظهرت نتيجة موجبة لأختبار الروزبنكال وكانت من الجنسين وبأعمار مختلفة ، ولم تقتصر على الخيول التي أظهرت ناسور أو تورم . ولم تعزل جراثيم من السائل المصلي الرائق والحاوي على كريات دم حمراء وخلايا قاحية والمسحوب من كيس الصفن لأحد الخيول .

A STUDY ON THE BACTERIAL  
DISSEMINATION AND EXPERIMENTAL  
PATHOLOGY OF SALMONELLA PARATYPHI  
– B INFECTION IN WHITE MICE

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SUMMARY

An attempt to simulate paratyphoid fever-B, in the experimental animals has been carried out in white mice. Paratyphoid bacilli type-B was isolated from the febrile patients in the Ibn-Al-Khatib hospital. One LD50 was enough to produce disease in the white mice, however 10 doses were used for mice. The microorganism was inoculated intraperitoneally in to the experimental animals; the course of the dissemination within the animals was clarified by sacrificing two mice at specific intervals after inoculation and for 30 days. The organs of the

sacrificed and dead mice were examined for the presence of microorganisms. The microorganisms were found in the spleen, liver and mediastinal lymph node for 29, 25 and 17 days postinfection respectively, whereas, the microorganisms were persistent in the lungs and heart blood for 11 days and in the brain and kidneys for 7 days postinoculation. The pathological changes were initiated as a neutrophils aggregates and microabscesses in the spleen, liver and mediastinal lymph node. During the second week post infection, these microabscesses and neutrophils aggregates will be replaced by mononuclear cells (lymphocytes and macrophages) to form granulomatous type lesion in these organs and to become more chronic and fibrosed, during third and fourth week post-infection.

Also mild miscellaneous pathological lesions were demonstrated during the first week in brain kidney and in the lungs and gradually disappeared.

## INTRODUCTION

Paratyphoid fever remains an important public health problem in many developing countries, it is associated with high numbers of deaths occur

annually in the world (1). Human beings are the only reservoir and host for this disease which is caused by Salmonella paratyphi type A, B or C (2). In our country as in other parts of the developing countries, febrile illness other than typhoid and paratyphoid are common e.g.: Brucellosis, tuberculosis, meningitis, hepatitis and certain other acute fevers with an overlapping clinical pictures leading to diagnostic confusions (3). Although the clinical and the epidemiological picture as well as diagnostic procedures of paratyphoid have been well studied, some aspects related to the role of bacteria in the disease process and the pathological changes associated with the disease still require some illumination. Thus, the objective of this study was to fulfill a requirement hypothesis that typhoid and paratyphoid can be simulated in a suitable laboratory animal model that provides a picture for human typhoid and paratyphoid, the present study aims at the followings:

- 1- Study the disease process, including the bacterial dissemination through the organs of the white mice experimentally infected with Salmonella paratyphi - B.

2- Study the pathological changes associated with this experimental disease process in the white mice.

## MATERIALS AND METHODS

White mice, weighing 15-20 Gms, were obtained from Al-Kindi Company for Veterinary drugs and vaccines production. The mice were healthy and reared on concentrated food for two weeks before being used. A local strain of Salmonella paratyphi - B, isolated from the febrile patients, in the Ibn-Khatib hospital and their LD50 doses corresponded to  $5 \times 10^7$  bacterial cell, was used for this reason. A logarithmic phase of Salmonella paratyphi - B in trypticase soy broth at  $37^\circ\text{C}$  was taken, washed once in phosphate buffer saline, a suspension of a viable count of  $10^9$  bacterial cell/ml was obtained. Fourty four mice were intraperitoneally injected with 0.5 ml of Salmonella paratyphi - B suspension containing  $5 \times 10^8$  bacterial cell (10 LD50). Two inoculated mice were sacrificed every two days including the dead mice for the period of one month. All the sacrificed and dead mice were studies for the purpose of:

- 1- Isolation of Salmonella paratyphi - B from the different organs of the experimentally infected mice, looking for bacterial dissemination in the organs.
- 2- For pathological study, small representative pieces from all the organs of infected mice were fixed in 10% neutral buffered formalin, processed routinely, cut at 5- $\mu$  thickness and stained with hematoxylin and eosin (H&E).

## RESULTS

1- Dissemination of Salmonella paratyphi - B in the organs of experimentally infected white mice:  
During 30 days of experimental infection of mice with Salmonella paratyphi - B, an extensive dissemination of this microbe was found in the different organs at different intervals postinfection (PI). It is evident (Table - 1) that the spleen, liver and mediastinal lymph node found to be the main target organs of invasion, whereas, kidneys, lungs and brain were slightly invaded by this microbe. The spleen had the longest period of infectivity which lasted for 29 days postinfection, whereas, infectivity lasted for 25 days and 17 days in the liver and mediastinal lymph node

respectively. The lungs and heart blood harbored the organism for 11 days. The organisms were isolated from the kidneys and brain during first 7 days post infection.

Table -1: Distribution of *Salmonella paratyphi* - B through the organs of white mice after intraperitoneal inoculation

Intervals (PI) *	Spleen	Liver	Mediastinal lymph node	Lungs	Heart blood	Kidneys	Brain
1 <sup>st</sup> day	+	+	+	+	+	+	+
3 <sup>rd</sup> day	+	+	+	+	+	+	+
5 <sup>th</sup> day	+	+	+	+	+	+	+
7 <sup>th</sup> day	+	+	+	+	+	+	+
9 <sup>th</sup> day	+	+	+	+	+	-	-
11 <sup>th</sup> day	+	+	+	+	+	-	-
13 <sup>th</sup> day	+	+	+	-	-	-	-
15 <sup>th</sup> day	+	+	+	-	-	-	-
17 <sup>th</sup> day	+	+	+	-	-	-	-
19 <sup>th</sup> day	+	+	+	-	-	-	-
21 <sup>st</sup> day	+	+	-	-	-	-	-
23 <sup>rd</sup> day	+	+	-	-	-	-	-
25 <sup>th</sup> day	+	+	-	-	-	-	-
27 <sup>th</sup> day	+	-	-	-	-	-	-
29 <sup>th</sup> day	+	-	-	-	-	-	-

\* PI: Postinfection.

\*\* Two mice were sacrificed for each interval.



## 2- The Pathological Findings:

Different pathological Findings appeared in this experimental disease process were as follows:

### The spleen:

During the first week postinfection, there was extensive infiltration of neutrophils and edema in white and red pulps of the spleen. Also there is extensive congestion and in certain cases these neutrophils infiltration form a microabscesses (Fig.-1).

During the second week, these neutrophils infiltrations and microabscesses will be replaced by lymphocytes and macrophages (mononuclear cells) to form granulomatous type lesion which becomes more chronic and fibrosed during third and fourth week postinfection (Fig.-2). Also in certain cases there was extensive hyperplasia of white pulp and in the reticuloendothelial cells lining the red pulp.

### The liver:

During the first week postinfection, the lesions were composed of multifocal aggregates of neutrophils and edema in sinusoids and in their adjacent hepatic tissue (Fig.-3). Also there is extensive congestion of hepatic tissue leading to acute sinusoidal dilation. During the second week postinfection these neutrophils infiltration with their microabscesses will be replaced by mononuclear cells (Lymphocytes and Macrophages) forming a granulomatous type lesions (Fig.-4), which becomes more chronic and fibrosed during the third and fourth week postinfection.

### Mediastinal Lymph Node:

During the first week postinfection, both the cortical and medullary regions of the lymphoid tissue showed features of acute lymphadenitis characterized by infiltration of the neutrophils, edema and few macrophages in addition to extensive congestion of blood vessels in cortical and medullary regions. During the second and third week postinfection, the neutrophils infiltration will be replaced by mononuclear

cells (Lymphocytes and Macrophages) leading to the hyperplasia of the lymphoid follicles and in the reticuloendothelial cells lining the medullary sinuses.

Other Organs:

The lungs:

It shows extensive congestion and mild acute interstitial pneumonic lesions.

The Brain:

It shows extensive congestion and perivascular leukocytic cuffing (Fig.-5).

The Kidneys:

It shows extensive congestion with the microthrombi in the corticomedullary regions , these microthrombi contain bacilli (Fig.-6).

## DISCUSSION

1- Dissemination of Salmonella paratyphi - B in the organs of experimentally infected mice:

Salmonella paratyphi - B is enteropathogenic bacteria with marked host specificity, strictly pathogenic for human or higher primates but normally not

pathogenic for mice and other laboratory animals. However, to produce infection in such unnatural host, a large amount of microorganism should be inoculated in to the animal's (4). These findings were confirmed by this work as an extensive disease produced in mice when Salmonella paratyphi - B inoculum contains  $5 \times 10^8$  bacterial cell. Similar results were reported by others who showed that typhoid like disease was produced in mice, guinea pigs and in rabbits using other related strain of Salmonella typhi (5); (6) and (7).

This study revealed that following the intraperitoneal injection of Salmonella paratyphi - B with the massive dose of  $5 \times 10^8$  bacterial cell, a heavy growth of Salmonella paratyphi - B occurred in peritoneal cavity and in the draining mediastinal lymph node. The microorganism reached in to thoracic duct and to the blood circulation, they were isolated from the blood circulation, spleen, liver, mediastinal lymph node, lungs, kidneys and brain, one day postinfection.

These results go along with those reported by (8), (9), who isolated these microorganisms from these organs within 2-3 minutes to 4 days postinfection, and with the results obtained by (10) who isolated another related organisms Salmonella typhi from the

blood after one minute postinfection and after 3 hours from spleen and liver. This variation in dissemination might be attributed to the difference in the dose volume and route of administration, in this study  $5 \times 10^8$  bacterial cell, intraperitoneally injected, whereas, the dose was  $5 \times 10^9$ , orally used by (8) and  $2 \times 10^6$  intravenously used by (9). In the present study following intraperitoneal injection of Salmonella paratyphi – B in to white mice a persisting systemic growth occurred in the most of infected mice, some infected mice died within 72 hours postinfection probably due to endotoxemia, others harbored the infection in spleen for 29 days, in the liver for 25 days and in the mediastinal lymph node for 17 days postinfection. These findings are in accord with those reported by (8), (9) , for the same organism and with other related type organism ( Salmonella typhi ) (10) and (7) .

### PATHOLOGICAL FINDINGS:

This study revealed that the main lesions were initiated in the spleen, liver and mediastinal lymph node of the infected mice and the lesions were edema characterized by infiltration of neutrophils and

in addition to congestion of blood vessels. The neutrophils infiltration with the microabscess formation in these organs will be reported by (11, 12) in murine Salmonellosis caused by Salmonella typhimurium and by (7) in mice experimentally infected with Salmonella typhi (another related bacteria). Also similar pathological findings were demonstrated in infants (13) and in adults (14) infected with typhoid bacilli (another related organism).

The neutrophils infiltration will gradually replaced by mononuclear cells (Lymphocytes and Macrophages) during the second week postinfection to form granulomatous type lesion which become more chronic and fibrosed during the third and fourth week postinfection.

The transformation in to the granulomatous type lesion were demonstrated by (11,12) in murine Salmonellosis caused by Salmonella typhimurium and also demonstrated by (7) in mice experimentally infected with Salmonella typhi (another related microorganism). Both of those workers explained that the granulomatous reaction as a providing efficient antibacterial mechanism in the form of macrophages armed with cytophilic antibodies.

Other lesions such as extensive congestion & perivascular leukocytes cuffing in brain and interstitial pneumonic lesions in the lungs and extensive congestion and microthrombi in the renal tissue were considered as a metastatic type of lesion evidently caused by Salmonella paratyphi – B through hematogenous dissemination.

Such metastatic lesions were also reported in typhoid patients by (14) and (13) and also reported by (7) in mice experimentally infected with Salmonella typhi (another related bacteria).

### Conclusions

The results of this study on mice experimentally infected with Salmonella paratyphi – B differ from the results of the previous study obtained by (7) on mice experimentally infected with Salmonella typhi by the followings-:

- 1-The multifocal microabscesses and granulomatous lesions produced by Salmonella typhi in the previous study, were seen in most of body organs including spleen, liver, mediastinal lymph node, lungs, kidneys and brain; whereas, in the present study there is focal microabscess and granuloma

- seen only in the liver and spleen and no involvement of other organs.
- 2- No involvement of bile ducts, gall bladder and intestinal tract by Salmonella paratyphi – B, through the experimental infection in mice, in the present study.
  - 3- Mild miscellaneous pathological lesions were seen in kidneys, lungs and brain comparable to sever lesions that seen in previous study cause by Salmonella typhi infection .
  - 4- The above findings indicate that the Salmonella paratyphi – B is of lower pathogenicity and virulence than for the Salmonella typhi.
  - 5- This report is considered to be the first report on the pathology of Salmonella paratyphi – B infection in mice.



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دراسة إنتشار الجراثيم المسببة لمرض البار اتايفونيد نوع ب-  
والافات الناجمة عنها في الخمج التجريبي في الفئران البيضاء

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### الخلاصة

في محاولة لفهم بعض جوانب مرض البار اتايفونيد في الانسان أجريت تجربة بأحداث المرض في الفئران البيضاء، حيث تم الحصول على عزلة من عصيات البار اتايفونيد نوع ب- من المرضى الراقدين في مستشفى ابن الخطيب في مدينة بغداد ، حيث حددت الجرعة المسببة بأمراضية % من الفئران ، حيث استخدمت جرعة كانت عشرة أضعافها للحصول على أشد حالات المرض وطأة . لقد أعطيت جرعة الجرثومة عن طريق الخلب لـ فأرا وتم قتل فأرين على مدى فترات محددة من التجربة و لمدة ثلاثون يوما حيث وجد أن الجرثومة تتجمع في الطحال ، الكبد والعقدة الليمفاوية المنصفية ولمدة ، و يوما على التوالي بعد الخمج التجريبي كما وتتجمع في دم القلب و في الرئتين لمدة يوما و في الدماغ و الكلى لمدة أيام. أما التغيرات المرضية فكانت بالبداية بشكل خراجات مجهرية وتجمع لخلايا العدلات في الطحال ، الكبد و العقدة اللمفاوية المنصفية و التي تتحول تدريجيا الى أورام حبيبية صغيرة من جراء إحلالها بخلايا وحيدة النوى (اللمفاوية و البلاعم الكبيرة) ، حيث تصبح أكثر تطورا في الأسبوع الثالث و الرابع ، كذلك سجلت آفات طفيفة متفرقة في الدماغ ، الكلى و الرئتين في الأسبوع الأول من الخمج التجريبي حيث اختفت تدريجيا .