ECTOPIC SITES FOR TRICHOMONAS VAGINALIS IN LABORATORY MICE

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ABSTRACT

Host-Trichomonas vaginalis relationship was investigated by establishing an infected laboratory mouse model by a local strain. BALB/c mice were inoculated S/C (5 mice) and I/P (5 mice) with a medium containing Trichomonas vaginalis. At the same time, another mice were inoculated S/C (5 mice) and I/P (5 mice) with a medium only to serve as a control groups. There were ascites and multiple abscesses on the visceral organs during post-mortem of mice injected intraperitoneally (I/P). There was a localized abscess at the site of subcutaneous (S/C) injection of T. vaginalis. The histopathological changes of the internal organs and skin were studied and reported for the infected and control mice .Conclusion: There were pathological changes in mice infected with T.vaginalis local strain. Such animal model is useful for pathological, biochemical and therapeutic investigations.

INTRODUCTION

Trichomonas vaginalis is a flagellate protozoan that effects the urogenital tract of men and women.[1] It is transmitted by sexual intercourse[2,3] or by non-venereal means like toilet facilities or harrowed contaminated towels or underclothes.[2,4] In women it causes vaginitis and cystitis. In men it causes urethritis and prostatitis. [2] In this study mouse model for experimental *T. vaginalis* infection was established for the first time in Iraq, which would be suitable for immunological, pathological and therapeutic investigations on this organism.

MATERIALS AND METHODS

Female and male BALB/c mice of 4-6 weeks old were used after a full medical examination:

A medium containing *Trichomonas vaginalis* at a concentration ($5x10^6$-$10x10^6$ organism/ml) was inoculated subcutaneously (S/C) into the shoulder of 5 mice. At the same time another 5 mice were inoculated (S/C) with (1ml) medium only and served as a control group.[5] A medium containing *T. vaginalis* at a concentration (10^7 organism/ml) was inoculated intraperitoneally (I/P) into 5 mice. At the same time another 5 mice were inoculated (I/P) with (1ml) medium only and served as a control group. [6] All mice were anticipated for 16 days to observe any abnormal changes which might appear on them.

Post-mortem of experimental mice: -

On day 16 post-infection, post-mortem was carried out for the infected and control mice. They were killed with ether and dislocation of spinal cord, pinned out and a longitudinal median incision made to expose the underlying viscera. Pieces of infected organs were put on selective media of *T. vaginalis* (Difco No.0911.02).

Other pieces of infected organs and organs from control mice (liver, spleen, kidney, large and small intestine, heart, lung and skin), were removed and fixed with (10%) formalin for histopathological examination.

Before post-mortem was done:

The peritoneal fluid was collected from mice, which were injected I/P and a smear was prepared for *T. vaginalis* identification.

The peritoneal fluid was cultured in selective media of *T. vaginalis* to look for this organism.

Tissue processing:[7]

After the organs were fixed in 10% formaline for 24hr, the organs were dehydrated through graded ethyl alcohol; in 70% alcohol for 3-5hr. and then in 100% alcohol for 24hr. The organs then cleared in Xylene for 3-5hr. and then after the organs transferred from clearing agent, they were put on a bath of molten paraffin wax in oven at 50°C for 24hr. and finally embedded in paraffin wax.

Sections, 5 microns in thickness were cut and stained by Hematoxyline and Eosin stain, and finally mounted in DPX.

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RESULTS

The prominent pathological changes associated with *T. vaginalis* infection in mice will be described in the following order: -

1. Intraperitoneal injection of mice by T. vaginalis

Five mice were injected (I/P) by *T. vaginalis*; two of them were died on 5th day of post-infection and others were survived until day 16th which was the date of the post-mortem.
There was ascites in the abdominal cavity, when the peritoneal fluid was collected and examined cytologically; it revealed large number of pus cells but no evidence of *T. vaginalis*.

On post-mortem examination, there was multiple abscess formations with the variable size were found on different organs. The largest one was found on the liver and spleen (Fig. l).
When the pieces of these organs with abscess were put in selective media there was a growth of *T.vaginalis* in the media.
Histopathological changes due to *T. vaginalis* infection will be described in the following organs:-

a. Liver:
Microscopical examination showed necrotic areas with abscess formation (Fig.2), both within the liver parenchyma and on the surface of the liver. The inflammatory infiltrates mainly concentrated in and around the portal tract (Fig.2) (portal inflammation), with focal inflammatory areas scattered within the hepatic lobules (lobular inflammation), with feathery degeneration of hepatocytes.

b. Spleen:
Multiple abscesses were demonstrated throughout the splenic tissue (Fig.3).

c. Kidney, small and large intestines:
Microscopical examination of these organs revealed acute and chronic inflammatory cells infiltration.

There was no effect seen in these organs.

2. Intraperitoneal injection by medium only

Five mice were-injected (I/P) by medium only, no one was died and there was no ascites. On post-mortem examination, abscess was not found in any organs.
Histopathological examination showed no evidence of acute and chronic inflammations in the ***liver, spleen, Heart, lung, kidney, small and large intestines***

3. Subcutaneous injection by T. vaginalis

Five mice were injected (S/C) by *T.vaginalis*, one mouse was died on 10th day of post-infection, and other mice developed localized abscess at the site of injection, where later on ulcerated and opened on the 14th day of post-infection (Fig-4).

After post-mortem, the microscopical examination showed ulceration of the epidermis (Fig-5) and abscess formation in the dermis with acute and chronic inflammation.

4. Subcutaneous injection by medium only

After such injection, all five mice were survived without abscess formation at site of injection. On histopathological examination ulceration, abscess formation and inflammation all were not seen.

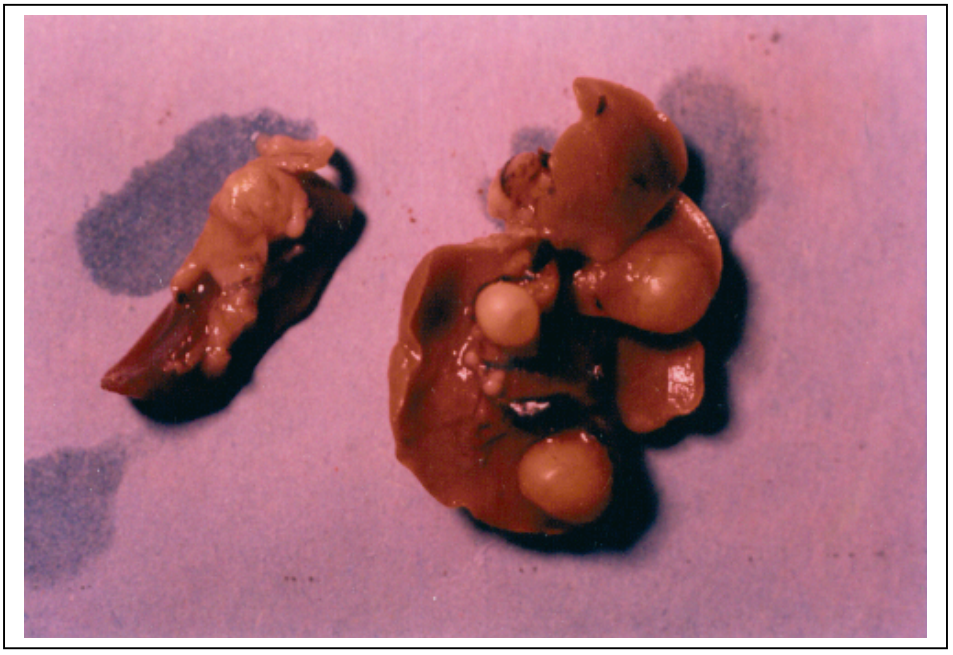

Fig 1. Many abscesses in the liver and spleen. The largest one found on the liver and spleen.

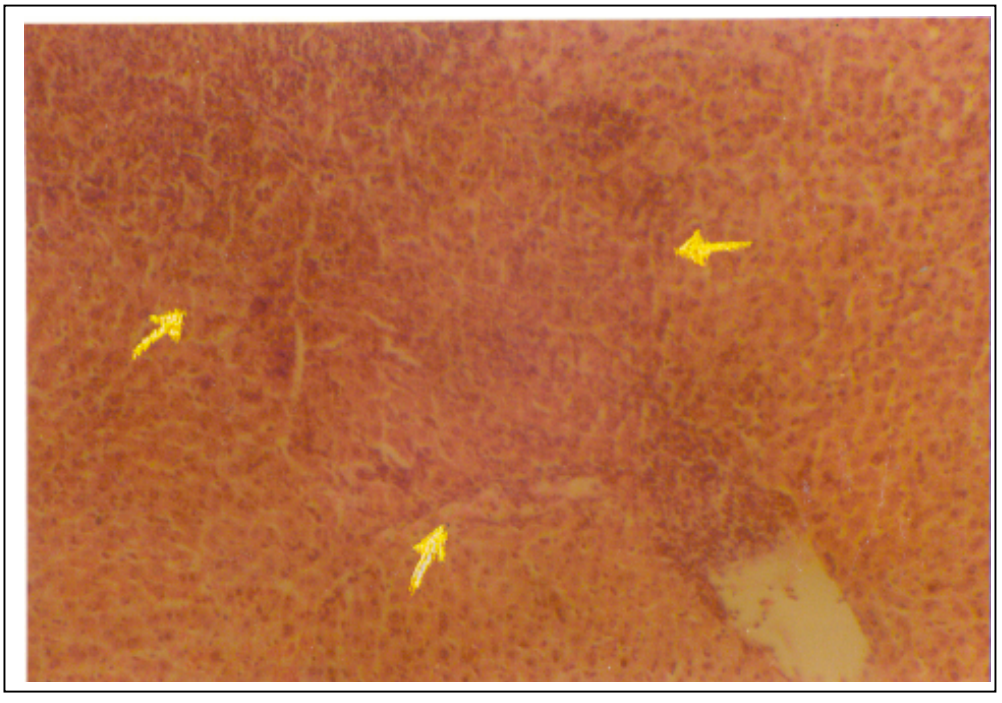

Fig 2. Necrotic area (↗) with abscess formation in liver parenchyma (X10)

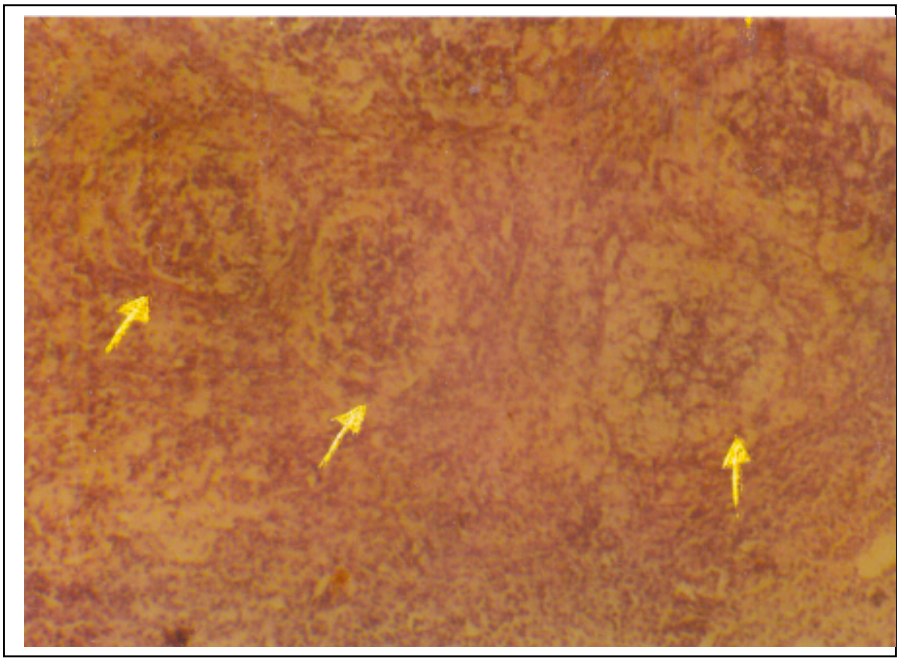

Fig 3. Multiple abscesses (↗) throughout the splenic tissue (X10)

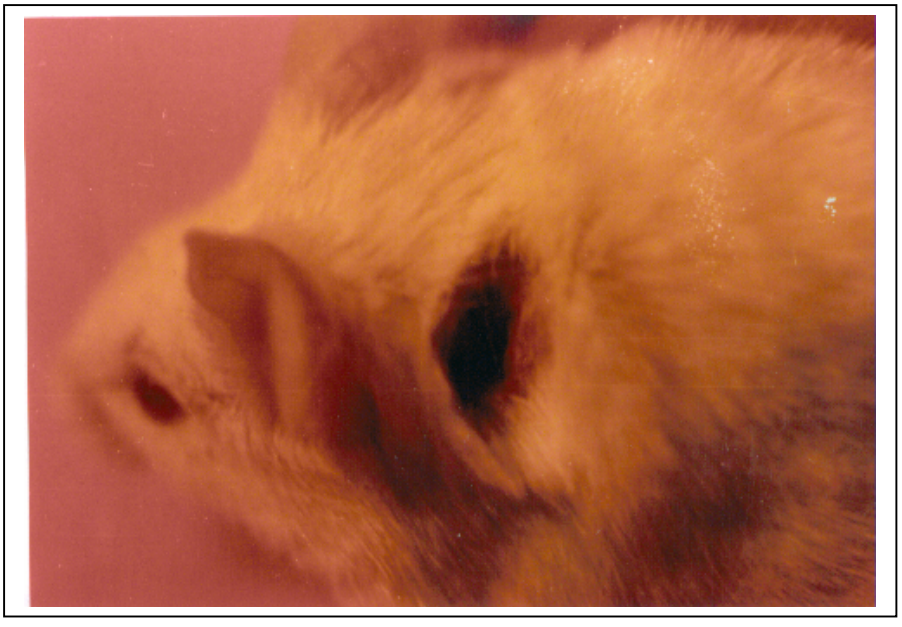

Fig 4. Ulcerated and opened skin abscess on the 14th day post-infection.

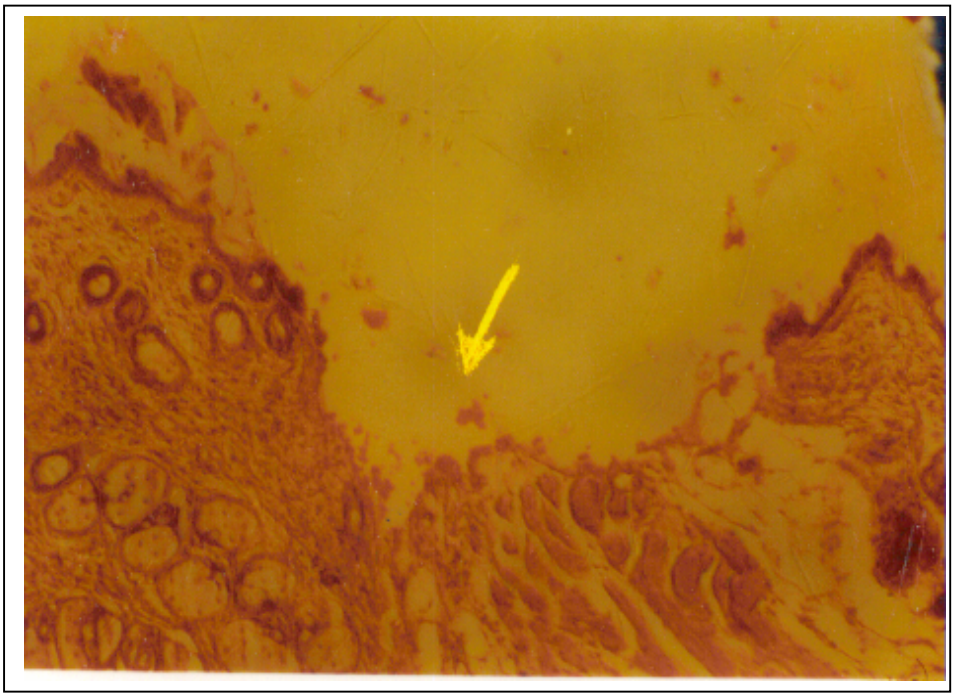

Fig 5. Ulcer formation (↙) on the epidermis (x10).

DISCUSSION

Interestingly, mouse model for experimental *T. vaginalis* infection was established for the first time in Iraq. Mice that injected I/P with a local strain of *T. vaginalis*, multiple abscesses were produced on their organs. Even there was no previous similar study in Iraq to compare with, these finding are interesting and provide a better understanding about host-*T. vaginalis* relationship. There was no definite explanation about the formation of multiple abscesses in mice and the pathogencity in natural host (human body), even in India, [8] but the variation in the pathogenicity level was observed among the isolates according to the severity of isolates. On the other hand, a definite correlation between clinical picture in natural host and pathogenicity in mice was not observed. [9-11] However, there was a varying volume of ascitic fluid (0.5ml to 2.5ml) and infection on various organs in contact with peritoneal fluid. The purulent discharge from the infected organs revealed plenty of trichomonads along with leucocytes. [9,10] The size of cutaneous abscess which was observed at the site of S/C injection with *T. vaginalis* may be related to the severity of vaginitis produced in the women from whom the *T. vaginalis* was isolated, [12] or related to a dose of trichomonas administrated S/C.[13] A better understanding of the cutaneous histopathological mechanism might facilitate future pathological, biochemical and physiological investigations in addition to trials on chemotherapy.

In conclusion, Mouse model was established to study the host-parasite relationship. There were pathological and histopathological changes during intraperitoneal and subcutaneous injection of mice with *Trichomonas vaginalis*.
Future experimental research is needed about the ectopic site infection in laboratory animals to understand the pathogenicity of *T. vaginalis* infection.
Experimental infection is useful for future pathological, biochemical and therapeutic investigations.

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