Studies on Tyzzer's disease in rats
A. Schaich Fries and O. Svendsen

Lab Anim 1978 12: 1
DOI: 10.1258/002367787780953297

The online version of this article can be found at:
http://lan.sagepub.com/content/12/1/1

Published by:
SAGE
http://www.sagepublications.com

On behalf of:
Laboratory Animals Ltd

Additional services and information for Laboratory Animals can be found at:

Email Alerts: http://lan.sagepub.com/cgi/alerts
Subscriptions: http://lan.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav

>> Version of Record - Jan 1, 1978

What is This?
Studies on Tyzzer's disease in rats

A. SCHAICH FRIES* & O. SVENDSEN†

*Department of Veterinary Virology and Immunology and Department of Pathology, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen V, and †Department of Pharmacology and Toxicology, H. Lundbeck & Co. A/S, Ottiliavej 7-9, DK-2500 Copenhagen-Valby, Denmark

Summary

An outbreak of an epidemic disease occurred in a specified-pathogen-free (SPF) breeding colony of rats. The clinical signs and the post-mortem findings were characteristic for Tyzzer's disease. The causative agent, Bacillus piliformis, was demonstrated microscopically in ileum, liver and myocardium, and transmitted to mice where its pathogenicity appeared to be similar to that of another strain isolated from mice.

B. piliformis from spontaneously-infected rats was demonstrated by indirect immunofluorescence technique. By means of the same technique it was found that the fluorescence antibody titre obtained of the individual sera from spontaneously-infected mice, rats and rabbits was the same, whether the antigen employed was organisms isolated from rats or mice.

By testing sera from healthy rats in 3 different colonies by use of immunofluorescence technique, antibodies were found in several sera.

Only few cases of spontaneous Bacillus piliformis infections in rats have been reported. Epidemics of a disease in Sprague-Dawley rats characterized by marked dilatation of the ileum have been reported by Geil, Davis & Thompson (1961) and Hottendorf, Hirth & Peer (1969). Granulomatous inflammation of the ileal tunica muscularis, acute focal myocarditis, acute hepatitis and focal hepatic necrosis were consistent microscopic findings. Pibteus mirabilis was isolated from the intestinal content and abdominal organs, but attempts to reproduce the disease with Proteus mirabilis were unsuccessful (Hottendorf, et al., 1969).

A case of Tyzzer's disease in a young Holzmann rat was diagnosed by demonstration of B. piliformis in the liver and the intestinal wall (Stedham & Bucci, 1969, 1970). At the same time an outbreak of Tyzzer's disease was reported in Sprague-Dawley rats receiving adrenocorticotropic hormone (Yamada et al., 1969). Jonas, Percy & Craft (1970) demonstrated by transmission experiments B. piliformis as the causative agent in a disease syndrome in rats, characterized by megaloileitis (Fig. 1), focal hepatic necrosis and myocardial inflammation.

In a colony of specified-pathogen-free (SPF) Sprague-Dawley rats, an epidemic disease appeared in the barrier-maintained breeding unit. The disease caused high morbidity (75-100%) and low mortality (5-10%) among young animals. The clinical symptoms and the gross pathological findings were characteristic of Tyzzer's disease caused by B. piliformis, but visible liver necroses were not present. The demonstration of this bacterium by microscopic examination of organs from diseased animals and by immunofluorescence techniques is reported in the present study. The isolated strain was maintained by serial passages in mice and was compared with regard to antigenicity and pathogenicity to a strain isolated from mice. The diagnostic use of the isolated strains in serological testing is also reported.

Materials and methods

Animals

The rats examined were supplied from an SPF colony of Sprague-Dawley rats (Mol: SPRA (SPF 68 Han)). NMRI/Bom/SPF mice, 4-5 weeks old, were used for transmission and serial passages. Mice from this breeding colony are routinely checked for absence of B. piliformis infections. The animals were housed and fed as previously described (Fries, 1977a).

Rat sera for testing of antibodies were supplied from the above (diseased) colony (A), from a large SPF breeding colony (B), and a small non-breeding colony (C). The sera originated from 12 different strains or stocks of rats of different ages, and practically all from clinically healthy animals.

Autopsy and histopathologic examination

Prior to the examination, the animals were divided arbitrary into 4 groups according to the severity of the clinical manifestation—initial stage, stage of slight disease, middle stage and pronounced stage—and 4 animals from each group were killed by chloroform anaesthesia and autopsied. Specimens of tissue for histological examination were taken from jejunum, ileum, colon, liver and heart. The tissue specimens were fixed in neutral phosphate-buffered 10% formalin, embedded in paraffin wax, sectioned and stained with Harris' haematoxylin and eosin, Giemsa and Warthin-Starry silver stains (Armed Forces Institute of Pathology, 1960).

Maintenance of B. piliformis in mice

Maintenance of the isolated B. piliformis strain was performed as previously reported (Fries, 1977b).
Immunofluorescence microscopy
Demonstration of B. piliformis by indirect immunofluorescence technique. Smear preparations of rat livers were incubated with rabbit and mouse antisera. These sera were obtained from spontaneously-infected colonies of mice and rabbits. An FITC-labelled anti-mouse globulin produced in rabbits (Fries, 1977a) was used in tests employing mouse antisera. For tests employing rabbit antisera an FITC-labelled antirabbit globulin (DAKO immunoglobulins; Dakopatts A/S, DK-2000 Copenhagen F, Denmark) produced in swine was used in dilution 1:30 (Fries, 1977a).

Demonstration of antibodies. For demonstration of antibodies in rat sera an FITC-labelled IgG fraction to rat immunoglobulin produced in rabbits (RARa/FITC; Nordic Immunological Laboratories, Tilburg, The Netherlands) was used in dilution 1:40. A B. piliformis strain (M strain) obtained from a spontaneous case of Tyzzer's disease in mice was used as antigen. The microorganism was maintained by mouse passages (Fries, 1977b). Infected liver homogenates were incubated with positive and negative rat sera followed by staining with normal rabbit serum or FITC-conjugated normal rabbit serum as control for specificity of the test.

Experiments and results
Rats from the spontaneously diseased colony

Macroscopic findings. In the most pronounced stages of disease, changes similar to those described by Jonas et al. (1970) were found (Fig. 1), but without necroses in the livers. No other changes appeared in these animals. In organs from animals in less diseased stages no lesions were seen.

Microscopic findings. In the most diseased stage changes similar to those described by Jonas et al. (1970) were found: the myenteric plexi were usually vacuolated or missing. A slight lymphocytic infiltration around the hepatic portal tracts was the only finding in the organs from the less severely diseased animals. In Giemsa or Warthin-Starry silver stained sections, bacterial rods were demonstrated in ileum, liver (Fig. 2) and heart.

Table 1. Microscopical (mic) and bacteriological (bact) examinations of diseased rats grouped according to stages of clinical manifestations

<table>
<thead>
<tr>
<th>Stage of disease</th>
<th>initial</th>
<th>slight</th>
<th>middle</th>
<th>pronounced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heart</td>
<td>(+)</td>
<td>(+)</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

The overall morphologic appearance of this microorganism was consistent with the appearance of B. piliformis (Fries 1977b). The results obtained from the examination of the different groups are shown in Table 1.

Provocation with prednisolone
10 Sprague-Dawley rats, 5 weeks old, with lightly distended abdomen, were received from the infected colony. In order to activate a possible B. piliformis infection as previously done in mice (Fries, 1977b), each rat received 5 mg prednisolone subcutaneously on each of 2 consecutive days. The rats developed severe signs of disease. The most pronounced symptoms were found among animals being in initial stages of the disease when the prednisolone was given. These animals died 3-6 days after the treatment. The remaining rats were killed.

Macroscopic findings. Changes, corresponding to those observed in the untreated, spontaneously-diseased animals were found—but more pronounced. In addition, pale disseminated foci were also present scattered throughout the liver.

Fig. 1. Marked dilated terminal jejunum and ileum (A) in a spontaneously diseased rat.
Studies on Tyzzer’s disease in rats

Microscopic findings. The overall microscopic findings were similar to those described for untreated animals (Fig. 2).

Transmission experiments
A liver with macroscopic lesions was collected from one of the prednisolone-treated rats. A homogenate was prepared (Fries, 1977b) and injected intravenously into 5 mice, each mouse receiving 0.2 ml. Prednisolone (2 mg) was simultaneously injected by the subcutaneous route. Mice serving as controls were treated with prednisolone alone. The mice died 4-5 days after the inoculation. Necropsy revealed several necrotic foci in the livers, and numerous microorganisms were found in smear preparations. No mouse in the control group died. The isolated strain of B. piliformis named the R strain, has since been maintained for 13 serial passages in mice. During these passages no differences in pathogenicity have been observed, however, that after formalin treatment even livers from uninfected animals may in-duce antibody formation if inoculated into mice (Fries, 1977a).

Contrary to the findings of Fujiwara, Kurashima, Magaribuchi, Takenaka & Yokoiyama (1973), it was found that pathogenicity of the strain isolated from rats appeared identical, when tested in mice, to that of the strain originating from mice.

Comparison between M and R strains of Bacillus piliformis
The fluorescence antibody (FA) titre of positive sera from spontaneously infected mice, rats and rabbits (5 individual sera from each species) was determined in 2 series of tests. In the 1st, mouse liver homogenate from animals inoculated with the M strain was used as antigen. In the 2nd series mouse liver homogenate from animals inoculated with the strain isolated from rats was used. The same FA titre for the individual serum from the mice, the rats and the rabbits was found whether the M or the R strain of B. piliformis were used as an antigen (Table 2).

Table 2. Comparison between FA titres in mouse, rabbit and rat sera by using the M or R strain as antigen
The titres indicated are the means of the FA titres in 5 different sera. The range is indicated in brackets.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mouse</th>
<th>Rabbit</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>M strain (312 passages)</td>
<td>336 (240-480)</td>
<td>402 (160-640)</td>
<td>402 (160-640)</td>
</tr>
<tr>
<td>R strain (12 passages)</td>
<td>336 (240-480)</td>
<td>402 (160-640)</td>
<td>402 (160-640)</td>
</tr>
</tbody>
</table>

Serological examinations
By testing sera from rats in the infected colony (A), antibodies to B. piliformis were demonstrated by the FA technique in 66 of 68 tested sera, with titres of 80-2580. Antibodies were found in 50 of 147 tested sera from the large colony B, titres 10-1280. Of 18 sera from colony C, 6 reacted positively.

Discussion
The morphological findings of the present study are very similar to those described by Gell et al. (1961), Hottendorf et al. (1969), and Jones et al. (1970). Macroscopically visible liver necroses were not found as frequently as reported in spontaneously-infected mice (Fries, 1977b), and the microorganisms were more difficult to demonstrate than in the liver of mice.

B. piliformis was demonstrated in rat liver when antisera from mice or rabbits were used in the indirect immunofluorescence technique. Furthermore, the FA titres of mouse, rabbit and rat sera from different infected colonies were the same whether the M or the R strain of the microorganism was used as an antigen. These observations indicate that the antigenic properties of the M and R strains are similar.

Fujiwara et al. (1973) found that using different strains of B. piliformis isolated from rats and mice gave quite different results in the indirect fluorescence test. The antisera used in the study of Fujiwara et al. were obtained from mice immunized with formalin-treated infected liver homogenates. It has been observed, however, that after formalin treatment even livers from uninfected animals may induce antibody formation if inoculated into mice (Fries, 1977a). These antibodies react with antigens...
from infected livers. If the livers from uninfected animals were inoculated without formalin treatment, no such antibodies could be demonstrated.

The sera used in the present study were obtained from spontaneously-infected, untreated animals. Furthermore, in spite of the fact that these sera were obtained from 3 different colonies without any mutual contact, no antigenic differences were observed.

The few reports on Tyzzer's disease in rats suggest that this disease is uncommon in rats. However, the results of the present work indicate that *B. piliformis* infections are not uncommon in the rat, and many infections may occur without clinical manifestations in rat colonies. Small lymphocytic infiltrations similar to those observed in the spontaneously-diseased rats of the present study are often encountered in livers of rats used in routine toxicological studies, and may indicate exposure to *B. piliformis*. However, these changes may also be caused by other agents, and the problem deserves further investigations.

Acknowledgement
The study was supported by grant 513-5015, Danish Agricultural and Veterinary Research Council.

References


