

# Experimental *Salmonella*-associated Conjunctivitis in Cats

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## ABSTRACT

Cats were infected experimentally with *Salmonella typhimurium* via the conjunctiva. Clinical signs consisted of lacrimation, conjunctivitis, blepharospasm, prominent nictitating membrane and scleral injection. These signs were accompanied by an absolute neutrophilia and conjunctival smears indicative of moderate to severe suppurative inflammation. Ocular signs disappeared by day 6 postinfection. *Salmonella typhimurium* was cultured intermittently from the inoculated conjunctivae and rectal swabs through day 7. At necropsy, mesenteric and cervical lymph nodes were enlarged. Histopathological findings included chronic conjunctivitis and lymphoid hyperplasia in cervical and mesenteric lymph nodes. This study confirms that *S. typhimurium* can cause a primary conjunctivitis and that the ocular route of infection can lead to fecal excretion of *Salmonella*, in the absence of other clinical manifestations.

**Key words:** *Salmonella*, cat, conjunctivitis.

## RÉSUMÉ

Cette expérience consistait à injecter *Salmonella typhimurium* dans la conjonctive de chats qui manifestèrent ultérieurement les signes cliniques suivants : lacrymation, conjonctivite, blépharospasmes, proéminence de la membrane nictitante et injection sclérale. Ces signes s'accompagnaient d'une neutrophilie absolue, tandis que des impressions conjonctivales rece-laient des indices d'une inflammation

purulente modérée, ou même grave. Les signes oculaires disparurent en l'espace de six jours. L'examen bactériologique des conjonctives inoculées et d'écouvillons rectaux permit d'isoler *S. typhimurium*, par intermittence, pendant les sept jours ultérieurs à l'injection. Lors de la nécropsie, les ganglions lymphatiques cervicaux et mésentériques présentaient de l'hyper-trophie. L'histopathologie révéla de la conjonctivite chronique et une hyper-plasie lymphoïde des ganglions précités. Cette expérience confirme le fait que *S. typhimurium* peut causer une conjonctivite primaire et que la voie d'infection oculaire peut entraîner l'élimination de salmonelles dans les fèces, en l'absence d'autres manifestations cliniques.

**Mots clés :** *Salmonella*, chat, conjonctivite.

## INTRODUCTION

*Salmonella*, an ubiquitous enteric bacterium has a wide geographic and host distribution. Various serotypes of *Salmonella* are important agents in zoonoses and have been acquired by humans from pets with or without diarrhea, including cats and dogs (1-4). Until recently, there have been only scattered reports of salmonellosis in cats. However, it is now recognized that infections caused by *Salmonella*, including septicemia and nosocomial outbreaks of disease, can occur in cats stressed by hospitalization, concurrent disease, or surgery (5,6). The typical clinical situation is acute gastroenteritis, manifested by vomiting and diarrhea, and accompanied by dehydra-

tion, moderate fever, anorexia, pale mucous membranes and malaise. However, conjunctivitis and abortion can also be primary clinical signs associated with salmonellosis (7,8). Although the mode of transmission is usually by ingestion of the organism, infection via the conjunctiva is a distinct possibility. The coat of the cat may be contaminated with oral or fecal *Salmonella*, thus grooming habits of the cat may predispose the cat to primary conjunctival infection, with the conjunctiva serving as a route of entry. Indeed, *Salmonella typhimurium* has been isolated from inflamed conjunctiva of a naturally infected cat (7).

The purpose of the following study was to ascertain if *Salmonella typhimurium* could induce a primary clinical conjunctivitis and whether the conjunctiva could serve as a portal of entry for a more generalized infection or fecal dissemination of *Salmonella* in the cat.

## MATERIALS AND METHODS

### ANIMALS

Four, four month old cats from the same litter were used. These animals were reared in accordance with federal guidelines (Guide for the Care and Use of Laboratory Animals, Revised Edition; U.S. Department of Health, Education, and Welfare, National Institutes of Health; NIH Publication No. 80-23, 1978). The cats were fed a commercial chow *ad libitum* and provided with fresh drinking water. Animals were housed individually in metal cages in a room maintained at 22°C, 50% relative humidity, and 12 hour:12 hour light:dark cycle. All

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animals had been asymptomatic and were clinically normal on physical examination prior to experimentation. In addition, blood was drawn for hematology and serology; the sera were stored at  $-70^{\circ}\text{C}$ . Rectal swabs were cultured for *Salmonella* using hektoen enteric agar (HE), tergitol-7 agar with triphenyltetragolium salts (T-7-T) and selenite enrichment broth (Scott Laboratories, Fiskville, Rhode Island 02823, USA). Feces were examined for evidence of endoparasites via ova flotation. The lower palpebral conjunctiva of both eyes of each cat was cultured for bacteria using the following media: mycoplasma agar and broth, blood/MacConkey agar (BAP/Mac) biplate, chocolate agar (Choc), thioglycollate (thio) broth, HE, T-7-T and selenite broth. Conjunctival scrapings were obtained from both eyes of each cat, stained with Giemsa and Wright's stain and submitted for cytological examination.

#### INOCULATION

The *Salmonella typhimurium* isolate used for inoculation was originally obtained from the conjunctiva of a cat with ocular salmonellosis (7). The fornix of the lower conjunctiva of the left eye of each cat was instilled with 0.05 mL of a six hour culture of *S. typhimurium* in heart infusion broth. The eyelid was then held closed for one to two minutes. The same procedure was used on the right eye with 0.05 mL of 0.9% sterile saline.

The four animals were designated as follows: cats B and C were each given  $9 \times 10^3$  colony forming units of *S. typhimurium* (low dose); cats A and D each received  $9 \times 10^5$  colony forming units (high dose). Quantification of inocula was performed by serial dilution and plating onto blood agar.

#### ANIMAL MONITORING

Cats A and B were monitored for seven days; cats C and D were observed for 26 days. Daily recordings were made of rectal temperature, appearance of the eyes and conjunctivae and general condition of each cat. Conjunctival scrapings for cytology were taken on days 1,2,4,6 postinoculation from each eye. Both eyes were cultured daily for one week and on days 13 and 26 postinoculation from cats C and D. Rectal swabs were taken

from each live cat on days 4, 7, 13 and 26. All swabs were cultured on HE, T-7-T and selenite broth. Blood was drawn for hematology and serology at the same time that swabs were taken; sera were stored at  $-70^{\circ}\text{C}$ .

Cats A and B were euthanatized with intravenous pentobarbital one week postinoculation. Cats C and D were similarly killed 26 days postinoculation. Samples of tissues or swabs for *Salmonella* culture were taken from conjunctiva of both eyes, mouth, spleen, gall bladder, heart blood, liver, lungs, mesenteric lymph nodes and colon of each animal. Each sample or swab was cultured on HE, T-7-T and selenite broth. A necropsy was performed on each cat. Representative samples of all body organs and tissues were preserved in neutral buffered formalin and processed by standard methods. Sections were cut at  $5 \mu\text{m}$  and stained with hemotoxylin and eosin.

#### SEROLOGY

Preinoculation and postinoculation serum samples from each cat were tested for "H" and "O" antibodies to *S. typhimurium* using tube agglutination procedures (9).

## RESULTS

All four cats appeared clinically normal until day 2 postinoculation. At this time the two cats receiving the high dose of *Salmonella* (cats A and D) exhibited slight reddening of the inoculated conjunctiva which progressed to moderate to severe conjunctivitis over the next five days. Other signs evident in the inoculated eye of both cats were lacrimation, blepharospasm and a prominent nictitating membrane (Figure 1a and b). On days 5 and 6, cat A had marked scleral injection of the inoculated eye and cat D had a mucopurulent discharge from the inoculated eye. One of the two cats (C) receiving the low dose of *Salmonella* exhibited a slight ocular discharge and mild reddening of the inoculated conjunctiva; these signs were noted intermittently during days 5-12. Cat B, which also received the low dose of *Salmonella*, had no ocular signs. Only one cat (A) displayed clinical signs in the noninoculated eye, which consisted of a slight discharge and reddening of the conjunctiva on day 5.

All cats were afebrile, active and alert and had normal feces during the



Fig. 1a. Cat D: Blepharospasm and mucopurulent discharge five days after conjunctival inoculation of *S. typhimurium*.



**Fig. 1b.** Close up of Figure 1a. Prominent, reddened nictitating membrane, scleral injection chemosis and mucopurulent discharge.

course of the study. One cat (A) exhibited slight anorexia and dehydration corresponding to the period of most severe ocular signs on day 5.

Leukocytosis with an absolute neutrophilia were noted in both dosage groups, the higher dose of *Salmonella* associated with slightly higher cell counts. These hematological values peaked at days 4 and 7 postinfection, and were: 24,100-40,300 leukocytes/mm<sup>3</sup>, 19,700-33,850 neutrophils/mm<sup>3</sup>.

*Salmonella typhimurium* was isolated from the conjunctiva of the inoculated eye or the rectum during the first week, regardless of dose of bacteria (Table I). All *S. typhimurium* isolates had antibiotic resistance patterns identical to that of the *S. typhimurium* inoculum (7). Other *Salmonella* species or other bacterial pathogens were not isolated. One cat (C) yielded *S. typhimurium* from the eye receiving sterile saline. None of the cats possessed antibodies to *Salmonella* H and O antigens either preinoculation or through day 26.

Cytological examination or conjunctival scrapings from the cats before inoculation revealed small numbers of epithelial cells with few or no leukocytes. After day 2 postinfection, conjunctival scrapings had an

increased number of cells with a preponderance of neutrophils, a few epithelial cells, lymphocytes, and macrophages and many extracellular bacteria. These findings persisted through day 6 and were observed from scrapings taken from each inoculated conjunctiva. Conjunctival scrapings exhibiting the greatest numbers of neutrophils, i.e. most severe inflammatory response, were from the cats receiving the high dose inoculum.

Cats A and D (high dose) had slight enlargement of cervical and mesenteric lymph nodes at necropsy. Histologically, these cats had foci of lymphoid hyperplasia and mixed leukocytic infiltration of the lymphoid tissue associated with the nictitating membrane of the inoculated eye of each cat. Cat D had diffuse mononuclear cell infiltration of the lacrimal gland associated with the nictitating membrane of the noninoculated eye. There were also occasional, small foci of mononuclear cell infiltration of the lungs and trachea. A slight to moderate degree of lymphoid hyperplasia was present in cervical and mesenteric lymph nodes from both cat A and D. Other findings in cat A included mild, focal, polymorphonuclear cell infiltration of the Peyer's patches and lamina propria of the ileum and the mesenteric lymph nodes.

Only one cat (B) receiving the low dose inoculum had gross or histological abnormalities. The cervical lymph nodes were grossly enlarged and histologically exhibited moderate lymphoid hyperplasia. Numerous, small, perivascular aggregates of lymphocytes and focal accumulations of alveolar macrophages were found in the lungs.

Swabs taken at necropsy from the inoculated conjunctival sac of cat A yielded *Salmonella typhimurium* and *Staphylococcus epidermiditis*. *Streptococcus fecalis* was cultured from the liver of cat D. All other specimens cul-

**TABLE I.** Isolation of *Salmonella typhimurium* from Experimentally Infected Cats

Days PI <sup>a</sup>	High Inoculum Dose Cat		Low Inoculum Dose Cat	
	A OS/R	D OS/R	C OS/R	B OS/R
0 <sup>b</sup>				
1	+ <sup>c</sup> /ND <sup>d</sup>	+/ND	-/ND	-/ND
2	+/ND	+/ND	+/ND	-/ND
3	+/ND	+/ND	+/ND	-/ND
4	+/+	+/+	+/+	-/+
5	+/ND	+/ND	+/ND	-/ND
6	+/ND	+/ND	+/ND	-/ND
7	+/-	+/+	+/+ <sup>e</sup>	-/-
13		-/-	-/-	
26		-/-	-/-	

(The sites sampled for *S. typhimurium* were the left eye (OS), the right eye (OD) and the rectum (R))

<sup>a</sup>PI = Postinoculation

<sup>b</sup>Cultures taken preinoculation all negative for *S. typhimurium*

<sup>c</sup>+ = *S. typhimurium* isolated; - = no growth

<sup>d</sup>ND = Not done

<sup>e</sup>*S. typhimurium* also isolated from right eye. All other cultures from OD from all cats were negative

tured from all cats were negative for *S. typhimurium*.

## DISCUSSION

The clinical signs and conjunctival smears in three of four cats experimentally inoculated via the conjunctiva with *S. typhimurium* are consistent with naturally occurring *Salmonella*-associated conjunctivitis in the cat (7). Features common to both the experimental studies and natural case include: 1) moderate to severe conjunctivitis of several days duration, with lacrimation, blepharospasm and prominent nictitating membrane, 2) scleral injection and mucopurulent ocular discharge from the infected eyes, 3) conjunctival scrapings of infected eyes yielded large numbers of neutrophils for six days postinfection. In addition, none of the inoculated cats with conjunctivitis nor the clinical index case reported previously had clinical signs of systemic illness. Furthermore, none of the cats developed a detectable antibody response to *Salmonella*, even though the pathogen was shed in the feces.

Previous attempts to experimentally infect healthy, six month old cats per os with  $10^8$  colony forming units of *S. typhimurium* failed to elicit clinical disease (6). However, *S. typhimurium* was isolated from rectal swabs for up to 35 days postinoculation. Only one of the six cats developed antibodies to *Salmonella* H and O antigens. In contrast, serum from four of 12 natural cases of feline salmonellosis described in the same report had significant antibody titers to *Salmonella* antigens 10-14 days after onset of clinical signs. It was inferred that cats with clinical enteric salmonellosis had systemic involvement and thereby mounted a detectable antibody response. Ocular signs and ocular lesions were not noted in any of the experimentally infected cats or natural cases (6).

Conjunctivitis caused by *Salmonella* has also been reported in the mouse (10) and the guinea pig (11). The usual route of infection in mice is by ingestion. However, the conjunctival route was more efficient experimentally, requiring fewer *Salmonella* to establish systemic infection

(12,13,14). As well as serving as a portal of entry for murine salmonellosis, *Salmonella*-associated conjunctivitis can also be a secondary manifestation of systemic disease (15).

In an epizootic of salmonellosis in a guinea pig colony, *Salmonella*-associated conjunctivitis was widespread (11). It was concluded that conjunctival swabs were more effective than rectal swabs in monitoring the transmission of *Salmonella* in guinea pigs. In subsequent experiments, instillation of a small inoculum of *Salmonella enteritidis* into the conjunctiva of healthy guinea pigs resulted in local inflammation followed by infection of the cervical lymph nodes and septicemia. Enlargement of the cervical lymph nodes was also noted in the two cats in the current study which received the higher dose of *Salmonella* and in one cat receiving the lower dose. Unfortunately, these nodes were not cultured. Clinical signs in experimentally infected guinea pigs were similar to our findings in the cat, and included purulent ocular discharge, marked lacrimation, blepharospasm and edema of the palpebra. All the guinea pigs developed splenic salmonellosis after conjunctival instillation of  $3.5 \times 10^2$  to  $3.5 \times 10^6$  microorganisms (11). This is in contrast to reported difficulty of infecting guinea pigs with *Salmonella* per os, which was attributed partly to intestinal motility and its inhibition of successful colonization (16).

The results of this study demonstrate the causative role of *Salmonella typhimurium* in primary bacterial conjunctivitis in the cat. Our study agrees with findings for the guinea pig and mouse in that the ocular mucous membranes of the cat can serve as the site of primary infection under natural and experimental circumstances. Furthermore, the conjunctiva of the cat can serve as a source of *Salmonella* excretion locally, as well as a potential portal for enteric infection or subsequent dissemination via feces. It would have been of interest to periodically culture blood from these cats postinoculation to detect a *Salmonella* septicemia prior to fecal shedding.

Only one rectal swab was taken from each cat before inoculation to determine a *Salmonella*-negative

state. Therefore, it is possible that these cats were not truly *Salmonella*-free since multiple sampling may be necessary to detect fecal shedding of *Salmonella*. However, this is most unlikely in this case because the cats were littermates and housed together since birth, no clinical signs were noted prior to experimentation, and the postinoculation *Salmonella* isolates all had antibiotic resistance patterns identical to that of the *S. typhimurium* inoculum. We have previously demonstrated that this multiple antibiotic resistance pattern is plasmid-mediated (7,17). It is not known how common the ocular route of infection/excretion is in the spread of *Salmonella* in the cat population or from cats to other susceptible hosts (including humans), but this possibility should not be overlooked.

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