Tonsillar Abscess Caused by "*Tonsillophilus suis*" Infection in Fattening Pigs

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"*Tonsillophilus suis*", which Azuma and Bak [2] isolated from sulfur granules in tonsils and a submandibular lymph node of pigs, has been classified into the genus *Incertar sedis* [5]. Some experimental studies by using this organism have been done [3, 4, 7, 8].

Recently Shiozawa *et al.* [9] reported the incidence of tonsillitis due to "*T. suis*" infection in pigs at a slaughter house in Japan. However, a pathological study on the natural infection in pigs is rare [4].

This study was carried out to clarify the nature and significance of tonsillar lesions caused by "*T. suis*". During a period of one year, June 1989 to May 1990, 37 tonsil samples (tonsilla veli palatini) were obtained from 6 month-old pigs at a slaughter house. All the pigs were from the same piggery. All the samples were examined macroscopically, fixed in 20% buffered formalin, and cut serially from the anterior to the posterior margin (Fig. 6). Paraffin embedded sections were stained with hematoxylin and eosin (H-E). For sections having tiny abscesses

Gram [6], Grocott's [10] and Kossa's stains were applied. Rabbit antisera against "*T. suis*" 648 isolated from our materials [1] and "*Actinomyces suis*" 101 were used as primary antibodies. Immunoreactions were visualized by the avidin-biotin-peroxidase complex (ABC) method using Vector stain ABC kit, PK 4001 (Vector Laboratories, Burlingame, CA, U.S.A.). Controls for the ABC method were carried out by omitting the primary antiserum.

"*T. suis*" lesions were defined by the following criteria; 1) the existence of microbial elements with or without clubs in the tiny abscesses, 2) by ABC method, positive for anti "*T. suis*" but not for "*A. suis*".

The tonsils did not show any macroscopical abnormalities, except the presence of plant-fibers in the crypts, which might originate from feedstuff. "*T. suis*" lesions were recognized as yellowish white spots in slices of 3 cases after fixation.

Histopathologically, lesions extended from the crypt-surface to the deep portion of the tonsils (Fig. 1A). Crypts with coalesced tiny abscesses were considerably distended, with desquamation, swelling and partial flattening of cryptal epithelia. The lesions consisted of microbial

Fig. 1. A: Microbial elements with plant-fibers in a tonsillar crypt abscess. H-E stain. $\times 100$. B: Positive immunoperoxidase stain for "*T. suis*" in a serial section of A. $\times 100$.

Fig. 2. Densely flourishing stick like thalli and globose bodies (arrows). Grocott's stain. Bar indicates 10 μ m and inset $\times 1,000$.

Fig. 3. Numerous radially developing filaments originating from thallus. Grocott's stain. Bar=10 μ m.

Fig. 4. Agglomeration of multilocular tubers. Grocott's stain. Bar=10 μ m.

Fig. 5. Zoospores in tubers and in the surrounding areas. Grocott's stain. Bar=10 μ m.

elements, around which there was marked accumulation of neutrophils. Macrophages, eosinophils, lymphocytes and plasma cells were scattered in the adjacent tissue. Radially projecting clubs were frequently found around the "*T. suis*" colony. By Kossa's stain, black-stained calcium deposits were observed in the crypt abscess. The fundamental structure of the microbial elements was similar in all the cases though there was some diversity from animal to animal and from lesion to lesion. The microbial elements were characterized by 4 main types; densely flourishing stick like thalli (2–5 μ m) with globose body on its tip (Fig. 2), numerous radiating filaments (0.3–0.5 μ m) originating from the thallus (Fig. 3), agglomeration of multilocular tubers (10–40 μ m) in the periphery of the lesions (Fig. 4) and zoospores either within the tubers, or dispersed in their surrounding areas (Fig. 5). The thalli and tubers were stained with eosin whereas the zoospores and filaments were stained with hematoxylin. Most of the zoospores and filaments were Gram-positive. Kossa's stain revealed black-stained cal-

cium deposits in the thalli and tubers. The four elements described above were also confirmed by Grocott's stain.

Immunohistochemically, "*T. suis*" was stained positively with anti "*T. suis*" serum diluted 1:4,096 with phosphate-buffered saline (PBS) (Fig. 1B), but not with anti "*A. suis*" serum diluted 1:2,048 with PBS.

Of the 37 pairs of tonsils examined, 17 (46%) had "*T. suis*" lesions. Out of them, 15 cases had 1 to 3 foci each, 1 animal had 6 foci and another one pig had 11 foci. To determine the distribution of the foci, both the left and right sides of each tonsil were divided into three equal parts (anterior, middle and posterior). The location of the 38 foci were plotted on a diagram (Fig. 6). Their distribution was as follows; 53% in the anterior, 39% in the middle and 8% in the posterior part. From this finding, the anterior and middle parts seemed to be infected more frequently by "*T. suis*", there being no significant difference in the number of foci between the left and right sides of the tonsil. Of the 38 foci, plant-fibers were detected in 12 (32%). Minute coccoid elements in

Table 1. Microbial elements in lesions of "*T. suis*"

Bak <i>et al.</i> [4] Porcine tonsil	Momotani <i>et al.</i> [8] Peritoneum and subcutis of mice	Present case Porcine tonsil
Mycelia showing transverse septation	Mycelia like element	Thallus
Chlamydospore like particle ND	Chlamydospore like structure ND	Globose body Filament
Mycelia showing transverse and longitudinal septation	Element with transverse or longitudinal septa	Multilocular tuber
Zoospore	Coccoid element	Zoospore

ND) Not detected.

Fig. 6. Distribution of the 38 foci in the tonsils. A: 20 foci (53%) in the anterior part. B: 15 foci (39%) in the middle part. C: 3 foci (8%) in the posterior part.

such plant-fibers were positive for anti "*T. suis*" serum in 3 (25%) of the 12 specimens.

Moreover, 25 (68%) of the 37 tonsils contained dual lesions of "*T. suis*" and actinomycotic infection. In Korea, Bak *et al.* [4] demonstrated actinomycotic abscesses (15%) containing "*T. suis*" in the 600 pairs of porcine tonsils. Shiozawa *et al.* [9] recognized that out of 1,322 tonsils examined from fattening pigs 178 (13.4%) had "*T. suis*" lesions. Infection rate was higher in the present study than that in the study by Bak *et al.* [4].

Histopathologically, the tiny abscesses without plant-fiber and the 4 different structures of the microbial elements were analogous to those of the experimentally induced cases [4, 7] (Table 1). It was possible to differentiate the "*T. suis*" lesions from actinomycotic lesions because the latter consisted of clubs associated with characteristic slender filaments. Biochemical properties of the isolated strain "*T. suis*" 648 differed from *Dermatophilus congolensis*, but coincided with "*T. suis*" W254 (ATCC 35846) [1, 5]. The immunohistochemical results suggest that the feedstuff has a significant role in

the mechanism of infection of this species. The characteristic microbial elements in "*T. suis*" infection and its immunostaining property give the evidence that "*T. suis*" might be a causative agent of tonsillar crypt abscesses in young pigs.

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REFERENCES

1. Azuma, R. 1988. *Proc. The 8th Seminar on Science and Technology: Respiratory and Reproductive System of Swine*. Tokyo, 151-161.
2. Azuma, R. and Bak, U. B. 1980. *Proc. 1980 Int. Pig Vet. Soc. Congress*. Copenhagen, 348.
3. Azuma, R., Takahashi, Y., Miyagawa, E., and Taniguchi, T. 1989. pp. 21-24. *In: Trends in Actinomycetology in Japan* (Koyama, Y. ed.), Soc. for Actinomycetes, Japan, Tokyo.
4. Bak, U. B. and Azuma, R. 1980. *Proc. 1980. Int. Pig Vet. Soc. Congress*. Copenhagen, 349.
5. Gordon, M. A. 1989. pp. 2409-2410. *In: Bergey's Manual of Systematic Bacteriology*, vol. 4 (Williams, S. T., Sharpe, M. E., and Holt, J. G. eds.), Williams & Wilkins, Baltimore.
6. Leaver, R. E., Evans, B. J., and Corrin, B. 1977. *Clin. Pathol.* 30: 290.
7. Momotani, E., Inui, S., Ishikawa, Y., and Azuma, R. 1985. *Mycopathologia* 91: 143-149.
8. Momotani, E., Yoshino, T., Ishikawa, Y., and Azuma, R. 1983. *Mycopathologia* 81: 99-105.
9. Shiozawa, M., Koizumi, H., Ito, M., Harada, Y., Imagawa, C., Murakami, S., Koeda, T., Azuma, R., and Fujiwara, H. 1991. *J. Jpn. Vet. Med. Assoc.* 44: (in press).
10. Tome, Y., Matsuoka, S., Yamamoto, M., and Sato, Y. 1988. *Stain Technol.* 63: 53-57.