Aborted Lesions of a Pig Associated with *Arcanobacterium abortisuis* and the Immunohistochemical Features

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**ABSTRACT.** A microorganism isolated from an aborted sow’s placenta has been proposed as a novel species, *Arcanobacterium abortisuis*. The lesions in the placenta were suppurative and necrotic placentitis, whereas lesions of three fetuses were suppurative bronchopneumonia. Gram and Grocott-positive organisms in these lesions were immunolabeled with anti- *Arcanobacterium abortisuis* antibody. This is the first case report, which showed the association of *Arcanobacterium abortisuis* with the aborted lesions of a pig.

**KEY WORDS:** *Arcanobacterium abortisuis*, immunohistochemistry, porcine abortion.

Recently, we isolated a diphtheroid-shaped organism from an aborted sow’s placenta, and have proposed as a novel species, *Arcanobacterium abortisuis* sp. nov. (accession no. DDJB AB305159; ATCC BAA-1522, DSM 19515 and JCM 14813) [1]. To date, *Actinomycetaceae* isolated from pigs has been known to have three genera and ten species. In the genus *Actinomyces*, *Actinomyces bovis* [5], *Actinomyces hyovaginalis* [3], *Actinomyces israelii* [18], *Actinomyces naeslundii* [18], *Actinomyces suimastitidis* [8], *Actinomyces viscosus* [4] and unpublished *Actinomyces* sp. strain Chiba 101 (*Actinomyces* sp. Chiba 101: This strain has very similar morphological and microbiological properties to those of *Actinomyces suis* reported by Franke (1973) and Grässer (1962) [15], and forms actinomycotic abscesses in swine tonsillar crypts and mammary glands [13]) have been reported. The other species are *Arcanobacterium pyogenes* [17], *Arcanobacterium* sp. strain (TO16177) (*Arcanobacterium* sp. TO16177) [14] and *Nobaculum suis* (former *Actinomyces suis*, *Eubacterium suis*) [9, 12]. Among them *Actinomyces hyovaginalis* [6], *Actinomyces naeslundii* [16] and *Actinomyces suis* [21] have been reported as infective agents, which cause porcine abortion. In the present study we report the first case of porcine abortion associated with *Arcanobacterium abortisuis*, and it’s pathological and immunohistochemical features.

Porcine abortion had occurred off and on during six months in one pig farm, raising approximately 600 sows, in Chiba Prefecture Japan. These sows were commonly injected with live vaccine against Japanese encephalitis virus, Aujesky’s virus and porcine reproductive and respiratory syndrome virus. A placenta and three fetuses of a sow, which aborted at day 87 of her fifth gestation, were examined for diagnosis.

At autopsy, placenta, brain, heart, lung, liver, spleen, kidneys and superficial lymph nodes were removed and fixed in 20% neutral buffered formalin. Fixed tissues were then embedded in paraffin, sectioned 3 to 4 µm thick, and stained with haematoxylin and eosin (HE). The sections were stained by Gram’s and Grocott’s methods to show the microorganism.

In order to examine immunohistochemical cross-reactivities of rabbit hyperimmune serum against *Arcanobacterium abortisuis* between *Arcanobacterium abortisuis* and ten *Actinomycetaceae* isolated from pigs mentioned above, antigens were prepared from ten bacterial species listed below: Seven species of *Actinomyces* were *Actinomyces bovis* (ATCC 13683), *Actinomyces hyovaginalis* (BM 1192/5), *Actinomyces israelii* (ATCC 43320), *Actinomyces naeslundii* (ATCC 12104), *Actinomyces suimastitidis* (CCUG 39276), *Actinomyces viscosus* (ATCC 15987) and *Actinomyces suis* sp. Chiba 101; two species of *Arcanobacterium* were *Arcanobacterium pyogenes* (ATCC 19411) and *Arcanobacterium* sp. TO16177; and *Actinobaculum suis* (DMS 20369). *Arcanobacterium abortisuis* (strain Murakami) [1] was used as a positive control.

For preparation of the tissue samples, nine *Actinomycetaceae* mentioned above except for *Arcanobacterium* sp. TO16177 were directly injected into the lung of sacrificed mice and tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. *Arcanobacterium* sp. TO16177 was directly injected into swine liver followed by the same histopathological process described above [14]. As the primary serum, rabbit hyperimmune serum against *Arcanobacterium abortisuis* was used and diluted stepwise in 0.01 M phosphate-buffered saline with 0.05% Tween 20. Immunohistochemical technique was performed with the streptavidin-biotin conjugation method using a Histofine SAB-PO(R) Kit (Nichirei, Inc., Tokyo, Japan) following the manufacturer’s instructions. The serial sections were stained without the primary antibody and used as negative controls. In addition, the sections from the fetuses and the placenta which contained the purulent lesions were also stained without the primary antibody and used as negative controls.
immunostained using the same method.

Three fetuses macroscopically revealed subcutaneous edema, congested lungs with/without hemorrhages, and significantly increased fluids in the body cavities. The placenta showed a cloudy surface accompanied by congestion.

The major histopathological lesions in the all three fetuses were suppurative bronchopneumonia. The alveoli and bronchial lumen were filled with a large number of degenerative immature neutrophils and bacterial clumps. Congestion and mild infiltration of macrophages were also observed around the lesions (Fig. 1a). In the placenta, although only a few neutrophils and macrophages could be seen in the embryonic connective tissue on the maternal side, on the allantoic side the tissue was thickened with a large number of degenerative neutrophils and macrophages showing necrotic and suppurative placentitis (Fig. 2a). Other lesions of these fetuses included mild infiltration of macrophages in the pericardium, congestion or hemorrhages in the cardiac parenchyma. The microorganisms in the lesions were positive for Gram’s and Grocott’s staining and revealed to be rod-shaped or frizzled hair-like filament (Fig. 1c).

In the Immunohistochemical analysis, the antigens of Arachnoderma abortisuis were clearly immunolabeled with 1:51,200 diluted antibody (Fig. 1b and 2b) on the filamentous microorganisms in the placentas and the placental lesions. The bacterial antigen of Arachnoderma sp. TO16177 was immunolabeled with 1:25,600 dilution. On the contrary, the other bacteria were not stained even with a dilution of 1:800 or less.

Although the lesions linked to viral infections were not found in the present case, it is not able to rule out the possible association with virus infections because of no virological examinations performed. However, it is reasonable to conclude that this may be the first report of porcine abortion primarily associated with Arachnoderma abortisuis, which is elucidated by pathological and immunohistochemical investigations.

The genus Arachnoderma has become independent from the genus Actinomyces, which had originally consisted of eight species: Arcanobacterium bernardiae [17], Arachnoderma bialowiezense [11], Arcanobacterium bonasi [11], Arachnoderma haemolyticum [2], Arcanobacterium hippocoleae [7], Arcanobacterium phacae [17], Arcanobacterium pluranimalem [10] and Arcanobacterium pyogenes [17]. These bacteria were isolated from the inflammatory lesions of human and/or animals, and Arachnoderma pyogenes has been known only as the causative agent of abscesses of pigs [19], but there has been no report about the abortion caused by Arachnoderma pyogenes. On the other hand, there have been three reports on porcine abortions caused by Actinomyces hyovaginalis [6], Actinomyces naeslundii [16] and Actinomyces suis [21]. The typical lesions of these aborted fetuses infected with Actinomyces naeslundii and Actinomyces suis were suppurative bronchopneumonia with intraleisonal bacterial clumps and pyogranulomatous bronchopneumonia, although no histopathology for Actinomyces hyovaginalis has been available so far. Those lesions in the previous reports are very similar to the case we presented here. However the previous reports were lack of the detailed description on the bacteriological investigations. In the present case, there were no cross-reactivities between the antigen against Arachnoderma abortisuis and all other antigens examined except for Arachnoderma sp. TO16177, which presented a strong positive reaction against Arachnoderma abortisuis antibody. Apart from the immunohistochemical investigation, Arachnoderma abortisuis (strain Murakami) showed 99.4% similarity to Arachnoderma sp. TO16177 [14] in 16S ribosomal DNA gene sequencing analysis based on a comparison of 677 bp (unpublished data). Therefore, it can be highly possible that Arachnoderma abortisuis is the same or closely-related to Arachnoderma sp. OT16177.

In the present study, Arachnoderma abortisuis was associated with the aborted lesions of a pig. On the other hand, it has been indicated that Arachnoderma sp. TO16177 was associated with multiple organ failures accompanied by acute hemorrhagic necrotizing splenitis in the pig [14]. Furthermore, in the very recent past, it has been confirmed that the microorganism isolated from the urogenital tract of nine pigs with varied clinical symptoms was Arachnoderma abortisuis, though it was unclear whether Arachnoderma abortisuis was the exact causative agent for the symptoms [20].
REFERENCES


