Granulomatous lymphadenitis associated with *Actinobacillus pleuropneumoniae* serotype 2 in slaughter barrows

Takemi Ohba, Tomoyuki Shibahara, Hideki Kobayashi, Arika Takashima, Masataka Nagoshi, Masanori Kubo

**Abstract** — This study evaluated the occurrence of granulomatous lymphadenitis and its association with *Actinobacillus* spp. in 151,653 slaughtered pigs. Markedly enlarged pulmonary hilar, mediastinal, mandibular or hepatic lymph nodes were detected in 6 castrated males. The cut surfaces showed multifocal yellow-white lesions. Histologically, gram-negative bacilli were visible in the centers of the lesions with asteroid bodies, epithelioid cells, and multinucleated giant cells. Dense fibrous connective tissue surrounded these granulomatous lesions. Immunohistochemically, the organisms reacted with polyclonal antibodies against *Actinobacillus pleuropneumoniae* serotype 2 in all 6 barrows. The organism was isolated from the lymph nodes of all 6 animals. The results indicate that the granulomatous lymphadenitis was associated with *A. pleuropneumoniae* serotype 2 and the disorder had a tendency to occur in slaughter barrows.


(Traduit par Isabelle Vallières)
emerged as an important opportunistic pathogen of high-health-status swine of all ages (12) and has been reported to cause a wide range of pathological conditions including septicemia, arthritis, pneumonia, enteritis, meningitis, abortion, endocarditis, and erysipelas-like lesions (11). Little is known about the virulence factors of Actinobacillus pleuropneumoniae, including RTX toxins, urease, and iron-regulated outer membrane proteins, might play a role in pathogenesis (13).

Actinobacillus porcitonsillarum has been considered to be a commensal non-pathogenic species usually found in the porcine tonsils (14), and is similar to Actinobacillus pleuropneumoniae, thereby making diagnosis of porcine pleuropneumonia more complex (15,16). Recently, A. porcitonsillarum was isolated from lesions of the lungs of 2 pigs in Spain (17) and induced multifocal granulomatous lymphadenitis accompanied by pneumonia in a growing-finishing pig in Japan (18). In the Japanese case, the pulmonary hilar and mediastinal lymph nodes were markedly enlarged (18). In such a situation, the pathogenesis and the epidemiology of the disorder associated with A. porcitonsillarum infection remain poorly understood (14).

This study determined the occurrence of granulomatous lymphadenitis and its association with Actinobacillus spp. in 151 653 slaughtered pigs. The sex predilection, location of infection, and histological findings were investigated.

**Materials and methods**

**Animals and macroscopical examination**

A total of 151 653 animals from 48 farms were examined macroscopically on the processing line in a slaughterhouse in Toyama, Japan from 25 August 2006 to 18 January 2008. Markedly enlarged lymph nodes were detected in 6 pigs (Tables 1 and 2) and were examined by histopathology, immunohistochemistry, and bacteriological culture.

**Histology and immunohistochemistry**

Tissue samples were collected from the lymph nodes (pulmonary hilar, mediastinal, mandibular, hepatic, superficial cervical, subiliac, inguinal, renal, popliteal, internal iliac, and splenic), lung, liver, kidney, and spleen within 30 min after death. They were fixed in 10% phosphate-buffered formalin and embedded in paraffin wax. Tissue sections (3 μm) were stained with hematoxylin and eosin (HE) and Gram stains.

Serial histological sections were prepared for immunohistochemical labelling with streptavidin-biotin-alkaline phosphatase (Histofine SAB-PO Kit; Nichirei, Tokyo, Japan). The primary antibodies were rabbit polyclonal antibodies to A. pleuropneumoniae serotype 2 at a dilution of 1 in 4096 (9).

The sections were lightly counterstained with hematoxylin and assessed by light microscopy. Simultaneously, sections of a piece of liver into which A. pleuropneumoniae serotype 2 had been injected were immunolabelled as positive controls. Negative controls were prepared by replacing the primary antibody with normal goat serum or phosphate-buffered saline. The diluted rabbit polyclonal antibodies to A. pleuropneumoniae serotype 2 immunohistochemically reacted with A. pleuropneumoniae serotype 2, but not with A. pleuropneumoniae serotypes 1, 5, and 11, A. porcitonsillarum, and A. suis.

**Bacteriological examination**

The tissue samples were streaked on both chocolate and horse blood (5%) agar plates supplemented with V factor [nicotinamide adenine dinucleotide (NAD)] and incubated in 5% CO₂ at 37°C for 48 h. Biochemical characteristics of the isolates grown on agar medium were identified with ID-test-HN-20 Rapid (Nissui Pharmaceutical, Tokyo, Japan), API 20A, API Staph, API Coryne, API 20 Strep, API 20E, and API NH (All bioMérieux Japan, Tokyo, Japan).

For the identification of A. pleuropneumoniae, a polymerase chain reaction (PCR) (19) was employed to detect the genes encoding the species-specific protective outer membrane lipo-protein (omlA) of the species. In addition, serotyping was carried out with slide agglutination tests using rabbit hyperimmune sera against reference strains of the first 12 NAD-dependent

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**Table 1. Granulomatous lesions in the lymph nodes in slaughtered pigs and occurrence of pleuropneumonia on 5 farms of origin**

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number of pigs slaughtered</th>
<th>Number and percent (%) of pigs with lymph node lesions</th>
<th>Number and percent (%) of pigs with pleuropneumonia</th>
<th>Vaccination against A. pleuropneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3817</td>
<td>1 (0.026)</td>
<td>267 (7.0)</td>
<td>2×; serotypes 1, 2, 5</td>
</tr>
<tr>
<td>B</td>
<td>7151</td>
<td>2 (0.028)</td>
<td>849 (11.9)</td>
<td>Unvaccinated</td>
</tr>
<tr>
<td>C</td>
<td>2040</td>
<td>1 (0.049)</td>
<td>221 (10.8)</td>
<td>Unvaccinated</td>
</tr>
<tr>
<td>D</td>
<td>14 701</td>
<td>1 (0.007)</td>
<td>2511 (17.1)</td>
<td>Unvaccinated</td>
</tr>
<tr>
<td>E</td>
<td>5633</td>
<td>1 (0.018)</td>
<td>309 (5.5)</td>
<td>1×; serotypes 2, 5</td>
</tr>
<tr>
<td>Total</td>
<td>33 342</td>
<td>6× (0.018)</td>
<td>4 157 (12.5)</td>
<td>NA</td>
</tr>
</tbody>
</table>

* All 6 pigs were castrated males.

**Table 2. Macroscopic lesions in 6 pigs**

<table>
<thead>
<tr>
<th>Pig number</th>
<th>Farm</th>
<th>Date (d/mo/y)</th>
<th>Lymph nodes that were enlarged</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>25/08/2006</td>
<td>Mandibular</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>10/10/2006</td>
<td>Pulmonary hilar, mediastinal</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>31/10/2006</td>
<td>Mandibular</td>
</tr>
<tr>
<td>4×</td>
<td>D</td>
<td>02/07/2007</td>
<td>Pulmonary hilar, mediastinal</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>26/07/2007</td>
<td>Pulmonary hilar, mediastinal</td>
</tr>
<tr>
<td>6</td>
<td>E</td>
<td>18/01/2008</td>
<td>Pulmonary hilar, mediastinal</td>
</tr>
</tbody>
</table>

* This pig also had cysts in both kidneys.

b This pig had pneumonia, and white foci in the liver and kidney.
serotypes of *A. pleuropneumoniae* (9). The specificity of the 12 antisera against individual serovar antigen was confirmed (9), and there was no cross-reaction with *A. suis* antigens.

**Results**

**Lymph node lesions associated with *A. pleuropneumoniae* serotype 2**

Markedly enlarged lymph nodes were detected in 6 of 151 (0.004%) pigs from 5 of 48 (10.4%) farms. On the basis of macroscopic (Table 2), histological, immunohistochemical, and bacteriological evidence, the enlargement of the lymph nodes was determined to be caused by *A. pleuropneumoniae* serotype 2 infection. Although the numbers of slaughtered male (49.3% to 52.5%) and female pigs were almost equal for the 5 farms, all 6 affected pigs were castrated males (Tables 1 and 2). No differences among farms were detected for the incidence of granulomatous lymphadenitis. The rejection rates due to porcine pleuropneumonia for the unvaccinated pigs (farms B, C and D) were higher than for the vaccinated pigs (farms A and E) (Table 1). On the 5 farms, animals showing signs of respiratory disease were immediately treated with antibiotics.

**Gross lesions associated with *A. pleuropneumoniae* infection**

Macroscopically, enlargement of pulmonary hilar and mediastinal (4/6), mandibular (3/6), and hepatic (1/6) lymph nodes was detected in the 6 barrows (Table 2). They were 20 to 80 mm in diameter, and firm. On their cut surfaces, multifocal yellow-white foci were seen in all 6 barrows (Figure 1). In pig 4, white foci associated with *A. pleuropneumoniae* serotype 2 were detected in the lung. Similar small foci were also seen in the liver and kidney. Although bilateral polycystic kidney (pig 2) was seen, *A. pleuropneumoniae* was not detected in the lesions by immunohistochemistry. Neither ecto- nor endo-parasites were observed in any pig macroscopically and histopathologically.

**Histological and immunohistochemical findings associated with *A. pleuropneumoniae* infection**

In the 6 barrows, lymphatic lesions were characterized by multifocal granulomatous inflammation surrounded by dense fibrous connective tissue (Figure 2). The granulomas consisted of large numbers of neutrophils, eosinophils, epithelioid cells, foreign body-type giant cells, and abundant collagen fibers, aggregating around asteroid bodies with or without gram-negative rod-shaped organisms. The microbial clumps were consistently located in the centers of the asteroid bodies (Figure 3a). Immunohistochemically, the distribution of *A. pleuropneumoniae* antigen (Figure 3b) corresponded closely to that of the granulomatous lesions with asteroid bodies.

In pig 4, similar granulomatous and suppurative lesions were also detected in the lung. Multifocal suppurrative vasculitis was detected together with *A. pleuropneumoniae* antigen in the liver. Suppurative nephritis was seen, but no discrete granuloma or asteroid bodies were seen in the hepatic and renal lesions. In the other 5 pigs no lesions containing *A. pleuropneumoniae* antigen were seen except in the lymph nodes.

**Isolation of *A. pleuropneumoniae***

*A. pleuropneumoniae* serotype 2 was isolated from the enlarged lymph nodes in all 6 pigs and from the lung in pig 4. All the...
isolates reacted strongly and specifically with the antiserum against *A. pleuropneumoniae* serotype 2. *Staphylococcus* species (pigs 1 and 6) and *Moraxella* species (pigs 1 and 6) were also isolated from the lymph nodes. No other pathogenic bacteria were isolated.

**Discussion**

In this study, *A. pleuropneumoniae* serotype 2 was the only infectious agent common to the 6 pigs with granulomatous lymphadenitis. Until recently, no association between *A. pleuropneumoniae* and granulomatous lymphadenitis has been found in the literature (9) and this is the first report of the isolation of *A. pleuropneumoniae* from granulomatous lesions of the lymph nodes. Moreover, this is the first survey of granulomatous lymphadenitis due to *Actinobacillus* spp. in slaughter pigs.

Surprisingly, all positive animals were barrows. Only 1 similar granulomatous lymphadenitis (9) and another similar lymphadenitis caused by *A. porcitonsillarum* (18) have been previously reported. These 2 cases were also in 6-month-old slaughter barrows. The present study, along with the previous findings, indicate that granulomatous lymphadenitis due to *Actinobacillus* spp. in slaughter pigs.

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*Actinobacillus pleuropneumoniae* is the etiologic agent of porcine pleuropneumonia, and the lesions are usually confined to the thoracic cavity (4,7,21,22). The distribution of the present lesions was different from that of experimental acute cases in young pigs (10). Hepatic *A. pleuropneumoniae* infections were accompanied by pneumonia (7 of 11 pigs) (9). The present granulomatous lymphadenitis, however, was not accompanied by pneumonia in 5 of the 6 barrows. In pig 4, *A. pleuropneumoniae* was not isolated from the liver and kidney, but the hepatic and renal suppurative lesions were closely associated with vasculitis and *A. pleuropneumoniae* antigen. The results suggest that hematogenous spread of *A. pleuropneumoniae* had occurred in pig 4. Given that the organisms were only detected in the systemic lymph nodes in the other 5 pigs, lymphatic spread appeared to have occurred as well. The histological and bacteriological findings in this study indicate that there is long-term survival of *A. pleuropneumoniae* in the lymph nodes and in the tonsils (16), lungs (23), and liver (9). The most frequently affected lymph nodes were the pulmonary hilar, mediastinal and mandibular ones.

Histopathologically, it is difficult to distinguish between lymphadenitis due to *A. pleuropneumoniae* and that due to *A. porcitonsillarum* (18) because both are characterized by gram-negative bacilli, asteroid bodies, granuloma, and dense fibrous connective tissues. The differentiation of *A. pleuropneumoniae* from *A. porcitonsillarum* is still complicated (16). An accurate clinical and histopathological examination followed by bacteriological examinations was necessary to establish the final diagnosis. The number of pigs with these lesions is small and the incidence rate (0.004%, 6/151,653) was lower than that of granulomatous hepatitis due to *A. pleuropneumoniae* (0.0164%, 11/66,894) (9). *Actinobacillus porcitonsillarum* was not isolated.
in this study; therefore, granulomatous lymphadenitis due to *A. porcitonsillarum* is the rarer of these 2 disorders. One important differential diagnostic criterion for granulomatous lymphadenitis in pigs is *Mycobacterium* infection [mostly *Mycobacterium avium* (24), *M. intracellulare* (24), or atypical mycobacteria (25)]. The present gross and histological lesions clearly differed from the tuberculous lesions of swine *Mycobacterium* infection. The tuberculous lesions are mainly observed in the mesenteric lymph nodes and those in the head (24,25), and they lack dense fibrous connective tissues and asteroid bodies.

The crux of this report is that the granulomatous lymphadenitis was associated with *A. pleuropneumoniae* serotype 2 and the disorder had a tendency to occur in slaughter barrows.

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**References**