Prevalence of Pneumocystis carinii in Slaughtered Pigs

Masahiro FUJITA, Takahisa FURUTA, Takashi NAKAJIMA, Fukuichi KURITA, Choji KANEUCHI, Katsumoto UEDA, and Manabu OGATA

Gunma Meat Inspection Center, Higoshi, Tamamura-machi, Sawa-gun, Gunma 379-21, 1st Department of Parasitology, Institute of Medical Science, The University of Tokyo, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108, 2nd Department of Veterinary Public Health, Azabu University, Sagamihara, Kanagawa 229, and 3rd Department of Veterinary Public Health, Institute of Public Health, Shiroganedai, Minato-ku, Tokyo 108, Japan

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Pneumocystis carinii is known to infect the lungs of a wide range of animals including man and it causes fatal pneumonia in immunocompromised hosts [1]. Recently, great attention has been paid to a zoonotic nature of Pneumocystis infection. However, there are only a limited number of reports throughout the world on the occurrence of P. carinii in domestic animals [2].

The purpose of this investigation was to examine the prevalence of P. carinii in slaughtered pigs. During the period of November 1986 to May 1987, a total of 223 crossbred-Landrace pigs about 6 months of age were subjected to the investigation at a slaughterhouse in Gunma Prefecture. They were 120 healthy pigs of approximately 100 kg of body weight and 103 growth-retarded pigs of 50 to 70 kg of body weight.

A five-gram lung sample was dissected from macroscopically normal dorsal area of the left caudal lobe. Lung impression smears from the cut surface were made first on a glass slide and then a small block of lung tissue was homogenized with 20 ml phosphate buffer saline (PBS) in a glass homogenizer and the homogenate was centrifuged at 800 g for 15 min. The sediment was suspended in 5 ml PBS and a portion (50 μl) of the suspension was smeared on a glass slide over a 1 cm² area (homogenate smear). The remaining suspension was mixed with 10 ml of PBS containing 0.1% collagenase (type 1, Sigma) and digested by incubation at 37°C for 4 hrs in a water bath. After digestion, the homogenate was passed through a 100 mesh-steel sieve and washed 3 times with PBS by centrifugation. The sediment was suspended in 10 ml of detergent solution containing 1% Triton X-100 (Sigma), 5% sodium deoxycholate (Sigma), and 1% sodium dodecyl sulfate (Sigma) in PBS and kept at 4°C overnight. After 3 washings with PBS, the sediment was resuspended in 1 ml PBS, and 10 μl of the suspension was smeared (collagenase-digested homogenate smear). All 3 different smears were stained with Giemsa and/or toluidine blue-O (TBO) [2].

On some samples which were found to have relatively high numbers of cysts by the TBO stain, the indirect fluorescent antibody (IFA) method [3, 4] was applied to the collagenase-digested homogenate smears. Rabbit anti-rat P. carinii serum was used as primary serum. It was prepared by hyper-immunizing a rabbit with sonicated P. carinii cyst suspension obtained from the cortisone-treated rat lung [3]. Serum titer for staining was 1:2560 against the rat-P. carinii cyst sample [4]. The air-dried smear was treated with the serum (1:256) and then stained with fluorescein isothiocyanate-labeled goat anti-rabbit IgG serum (H and L specific, Capple Labs., diluted at 1:20 as indicated) as secondary serum.

P. carinii cysts were detected by TBO stain (Fig. 1) and by Giemsa stain in which 8 intracytic bodies were discernible (Fig. 2). The TBO stain detected the cysts more effectively than Giemsa stain because of color differentiation. Specific fluorescence was confirmed in the preparations stained by IFA method (Fig. 3). When normal rabbit serum was used in place of the primary serum, no such fluorescence was seen. The collagenase treatment seemed to remove tissue residue and thus made it easier to detect P. carinii cysts by TBO or Giemsa stainings and to distinguish specific fluorescence from nonspecific background by the IFA method. It is known that P. carinii is sometimes difficult to distinguish from some kinds of fungi by the morphology and staining characteristics. Positive results obtained in the IFA staining may make the present diagnosis satisfactory.

The detection rates of P. carinii cysts in the lungs of 120 healthy and 103 growth-retarded pigs in the impression, homogenate, and collage-
Fig. 1. *P. carinii* cysts in the smear of the homogenate of pig lung tissue. Toluidine blue-O stain. ×400.

Fig. 2. Intracystic bodies in the smear of the same material as that described in Fig. 1. Giemsa stain. ×1,000.

Fig. 3. *P. carinii* cysts in the smear of the collagenase-digested homogenate of pig lung tissue. Indirect fluorescent antibody method. ×400.

Collagenase-digested homogenate smears are shown in Table 1. *P. carinii* cysts were detected in the samples of the growth-retarded pigs but not in the healthy pigs. The highest detection rate for the growth-retarded pigs was 44.4%, which was obtained with the TBO-stained collagenase-digested homogenate smear. The mean detection rate in both the healthy and growth-retarded pigs was 20.6%. This rate is almost comparable to the 17.2% (5/29) reported by Shimizu et al. [5] for the swine in Tottori and Hyogo prefectures.

The mean numbers (log_{10}) of *P. carinii* cysts detected in the smear preparations stained with TBO are shown in Table 2. If the number, 2.27±0.12/g, in the homogenate smear is extrapolated to the entire areas of both lungs (about 350 g) of the growth-retarded pigs, approximately 4.81/lung is given. The extrapolated value is considered to be fairly high although it is lower than that (7.9±0.3/lung) in athymic nude rats [4].

The present results demonstrated that *P. carinii* is more prevalent in the growth-retarded

<table>
<thead>
<tr>
<th>Pig</th>
<th>Impression smear</th>
<th>Homogenate smear</th>
<th>Collagenase-digested homogenate smear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Giemsa</td>
<td>TBO</td>
<td>Giemsa</td>
</tr>
<tr>
<td>Growth-retarded</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1.0 (1/103)</td>
<td>13.6 (14/103)</td>
<td>2.9 (3/103)</td>
</tr>
<tr>
<td>Total</td>
<td>0.4 (1/223)</td>
<td>6.3 (14/223)</td>
<td>1.3 (3/223)</td>
</tr>
</tbody>
</table>

a) Toluidine blue-O.
b) % (no. of sample positive/no. of sample examined).
Table 2. Number of *P. carinii* cysts stained by toluidine blue-O in the lungs of growth-retarded pigs

<table>
<thead>
<tr>
<th>Pig</th>
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<th>Homogenate smear</th>
<th>Collagenase-digested homogenate smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth-retarded</td>
<td>1.20±1.10a</td>
<td>2.27±0.12b</td>
<td>1.14±0.29b</td>
</tr>
<tr>
<td>(n=14)</td>
<td>(n=39)</td>
<td>(n=10)</td>
<td></td>
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a) Numbers (log_{10}) of cysts per cm² of the smear (mean±S.D.).
b) Numbers (log_{10}) of cysts per gram of the lung tissue (mean±S.D.).

The growth-retarded pigs suggest that some defective immune functions were involved. The morphology of *P. carinii* cysts from the pigs was quite similar to that from mice, rats, and humans. Antigenic similarities among *P. carinii* from different origins have already been reported [6]. *P. carinii* from the pigs in this study also seems to have common antigens with those from other mammals.

**REFERENCES**


**要 約**

豚における *P. carinii* (Pc) の感染状況（短期）：藤田雅弘・吉田隆久①・中嶋 隆・栗田富久一・金内長司②・上田雄範③・尾形 学②（群馬県中央食肉衛生検査所, ①東京大学医科学研究科寄生虫研究部, ②麻布大学獣医学部病態生物学第二講座, ③国立公衆衛生院）—健康豚120頭および発育不良豚（ひね豚）103頭の腸における Pc の検出率はそれぞれ 0 %, 44.4 %であり, 陽性ひね豚における平均シスト数 (log_{10}) は2.27±0.12/gであった.