Pulmonary Lesions in Guinea Pigs Experimentally Infected with *Actinobacillus pleuropneumoniae* (A.p.) serovar 1

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(Received 10 June 1998/Accepted 2 October 1998)

ABSTRACT. Pathological studies were carried out on the lungs of guinea pigs intratracheally inoculated with 4.6×10^6 colony forming units (CFU)/head of *Actinobacillus pleuropneumoniae* serovar 1. All animals in the highest dose group died within 24 hr post inoculation (hpi) and showed pulmonary lesions being hemorrhagic in nature while all animals in the lowest dose group were killed as scheduled at 11 days post inoculation (dpi) and showed only hyperplasia of peribronchial lymphoid tissues. In the middle dose group, two died within 24 hpi, two died at 9 dpi, and the remaining one was killed at 11 dpi. Two guinea pigs which died at 9 dpi showed fibrinonecrotic pleuropneumonia which is the most characteristic acute pulmonary lesion in swine, and has not yet been reproduced in laboratory animals up to the present time. This suggests that guinea pigs may be a useful laboratory animal for studying the pathogenesis of *Actinobacillus pleuropneumoniae* infection in swine.—**KEY WORDS:** *Actinobacillus pleuropneumoniae* serovar 1, fibrinonecrotic pleuropneumonia, guinea pig.

J. Vet. Med. Sci. 61(2): 163–165, 1999

Swine is the only natural host fully susceptible to *Actinobacillus pleuropneumoniae* (A.p.) and an acute infection of A.p. in swine is characterized by extensive fibrinonecrotic pleuropneumonia [1]. Although there are many studies on experimental A.p. infection in laboratory animals, especially in mice [2, 4, 5, 7, 8], fibrinonecrotic pleuropneumonia has not been reproduced in laboratory animals up to the present time. Recently, we found the lesion in a small number of guinea pigs experimentally infected with A.p. serovar 1. This paper describes the pulmonary lesions observed in that experiment.

Twenty 10-week-old female guinea pigs of Hartley strain which had been serologically confirmed to be free from any pathogens including A.p. were used. The animals were housed in plastic cages in an animal room controlled at $23 \pm 2^\circ\text{C}$ with $55 \pm 5\%$ relative humidity, and given pelleted diet and water *ad libitum*. They were acclimatized for a week before A.p. inoculation.

A field strain of A.p. serovar 1 which was isolated from a pig with pleuropneumonia and grown in Nicolet medium (agar PPLO; Difco, supplemented with nicotinamide adenine dinucleotide or fresh yeast extract plus fresh horse serum) for 16 hr at 37°C was suspended in phosphate buffered saline (PBS) and adjusted to the following alternative dosage: 9.2×10^8 , 9.2×10^7 , and 9.2×10^6 colony forming units (CFU)/ml. Five animals were inoculated intratracheally with 0.5 ml of each solution, respectively. Lung samples were collected from animals which died or were killed under moribund state as well as from animals which were killed as scheduled at 11 days post inoculation (dpi). The remaining 5 animals which were inoculated with medium solution in the same way were killed at 11 dpi and served as controls.

The lungs obtained from each animal were fixed in 10% neutral buffered formalin, and 4 μm -paraffin sections were stained with hematoxylin and eosin (HE). In addition, routine bacteriological examination was performed on lung

samples obtained from each animal.

All animals in the highest dose group died within 24 hpi. In the middle dose group, two animals died within 24 hpi, two died at 9 dpi, and the remaining one was killed at 11 dpi, respectively. On the contrary, all animals in the lowest dose group survived until 11 dpi showing no clinical signs.

Pulmonary lesions of animals which died or were killed within 24 hpi were hemorrhagic in nature, and the lungs showed consolidation without pleural changes. Two animals in the middle dose group which died at 9 dpi showed a typical gross picture of lobar fibrinous pleuropneumonia with pericarditis (Fig. 1). At the cut surface, well demarcated necrotic areas were observed beneath the pleura as well as around the airways (Fig. 2). All animals which were killed as scheduled at 11 dpi had no gross pulmonary changes.

In the light microscopic examination of the lungs of animals which died or were killed within 24 hpi, hemorrhage and edema with inflammatory cell infiltration in the alveoli were characteristics. In addition, perivascular lymphangiectasia and clusters of inflammatory cells outlining the interlobular connective tissues were observed in some animals.

In 2 animals in the middle dose group which died at 9 dpi, the pleura was covered with organized fibrinous exudates (Fig. 3). In the alveoli as well as around the peribronchial connective tissues, areas of ischemic necrosis which were well demarcated by a thick line composed of inflammatory cells were observed (Fig. 3). Within the necrotic foci, immature connective tissues and dystrophic calcification were observed. The pericardium was covered with fibrinous exudates.

In the lungs of animals which were killed at 11 dpi, only hyperplasia of peribronchial lymphoid tissues was detected.

A.p. serovar 1 was recovered from 80% of the lungs with gross lesions, and also from the pleura and pericardial exudates. A.p. was not isolated from animals which were

Fig. 1. Gross appearance of lungs of a guinea pig which was inoculated with 4.6×10^7 CFU/head of *A.p.* serovar 1 and died at 9 dpi. Lungs show a typical gross picture of lobar fibrinous pleuropneumonia.

Fig. 2. Cut surface of formalin-fixed lungs of a guinea pig which was inoculated with 4.6×10^7 CFU/head of *A.p.* serovar 1 and died at 9 dpi. A well demarcated zigzag line of inflammatory cells beneath the pleura (arrows) and an extensive area of necrotic tissue are seen. Deposition of organized fibrins is also detected on the pleural surface.

Fig. 3. Lungs of a guinea pig which was inoculated with 4.6×10^7 CFU/head of *A.p.* serovar 1 and died 9 dpi. Pleura is covered with organized fibrinous exudates. A thick line composed of inflammatory cells (arrow head) which corresponds to a zigzag line shown in Fig. 2 is also seen. HE, $\times 20$.

killed at 11 dpi.

As mentioned above, there are many experimental studies on *A.p.* infection in mice [2, 4, 5, 7, 8]. The striking difference between lesions in mice and those found in swine was lack of fibrin in murine model [7]. However the lesions observed within 24 hpi in mice were compatible with those

found in peracute infection in swine and related with endotoxin shock [2, 6], even though the acute lesion in mice was still hemorrhagic in nature whereas it was necrotizing hemorrhagic fibrinous pleuropneumonia in swine [1].

It was reported that guinea pigs, cotton rats and Afgan

pikas inoculated with *A.p.* died within 2 dpi and had pulmonary lesions which were hemorrhagic in nature and lacking in fibrinous exudate and pleuritis [5]. In the present study, all animals in the highest dose group died within 24 hpi, though the dose was lower than that used by Nakai *et al.* [5]. This may be explicable by the fact that *A.p.* serovar 2 used by Nakai *et al.* is said to be less pathogenic than serovar 1 used in our study [3].

Guinea pigs which died during the first 24 hpi had lung lesions resembling those found in mice [2, 7] and those in swine to some extent [1]. Namely, perivascular lymphangiectasia and clusters of inflammatory cells outlining the interlobular connective tissues in relation with the presence of *A.p.* are pathognomonic findings of *A.p.* infection in swine [1].

Two guinea pigs in the middle dose group which died at 9 dpi showed a typical picture of lobar fibrinonecrotic pleuropneumonia with pericarditis which is the most characteristic pulmonary lesion in swine with acute *A.p.* infection [1]. Such pulmonary lesion has not yet been

described in any laboratory animals up to the present time. This suggests that guinea pigs may be an appropriate laboratory animal for studying pathogenesis of *A.p.* infection in swine.

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