Rhodococcus equi気管内感染モルモットの病理学的観察

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Pathological Findings of Guinea-pigs Infected Intratracheally with *Rhodococcus (Corynebacterium) equi*

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ABSTRACT. The lungs of 32 guinea-pigs inoculated intratracheally with *Rhodococcus equi* were examined histopathologically in order to study the pathogenesis of *R. equi*-pneumonia. The most characteristic lesion was suppurative bronchopneumonia, which developed as follows: Congestion, serous exudation and mild infiltration of macrophages carrying Gram-positive bacteria and multinucleate giant cells 1 day postinoculation (pi), infiltration of numerous neutrophils and macrophages in the alveoli 2–5 days pi, focal accumulation of necrotic cells and infiltration of mononuclear cells phagocytizing degenerative neutrophils with a few multinucleate giant cells 6–10 days pi, small accumulation of mononuclear cells surrounded by normal alveolar tissues 12–16 days pi. The lung lesions in *R. equi*-infected guinea-pigs healed more easily as compared with those in the foals.—KEY WORDS: *Corynebacterium equi*, foal pneumonia, lung abscess, *Rhodococcus equi*, suppurative pneumonia.

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Rhodococcus (Corynebacterium) equi is known as a pathogen of foal pneumonia which is often accompanied by suppurative involvements of the alimentary canal, associated lymph nodes and joints (1–3, 6, 8–15, 20, 22, 24). The lung lesions caused by *R. equi* take the form of suppurative bronchopneumonia (1–3, 5, 6, 8–17, 20, 22, 24).

It is difficult to observe the early lung lesions because the lesions often have progressed before the affected animal shows clinical symptoms. No detailed descriptions have been done on the progression of the microscopic lesions in laboratory animals inoculated with *R. equi*, though many experiments were made to study the pathogenicity of the bacteria (1, 2, 4–8, 12–15, 17–21, 23, 24).

We were successful in reproducing constant lung lesions characterized by suppurative bronchopneumonia in guinea-pigs by the intratracheal inoculation of *R. equi*. In the present work, the lung lesions in them were studied histopathologically in order to elucidate the pathogenesis of *R. equi*-pneumonia.

MATERIALS AND METHODS

Animals: Thirty-two male guinea-pigs of the Nisseiken No.2 strain, weighing 400–600g, were used. They were reard in the separate wire cages and fed commercial assorted pellets.

Inoculum: *R. equi* CE-220 strain isolated from spontaneous suppurative bronchopneumonia of a foal (9,18) was used for the inoculum. This strain was incubated for 24 hours at 37°C on the nutrient agar and suspended in physiological saline.

Inoculation: An aseptic incision of the skin of the neck was performed in all animals under chloroform anesthesia. The sternothyro-hyoideus muscle was separated and the trachea was exposed. Then the bacterial suspension containing 2×10^8 *R. equi* in 0.2m*l* physiological saline was inoculated into the trachea of each animal. The

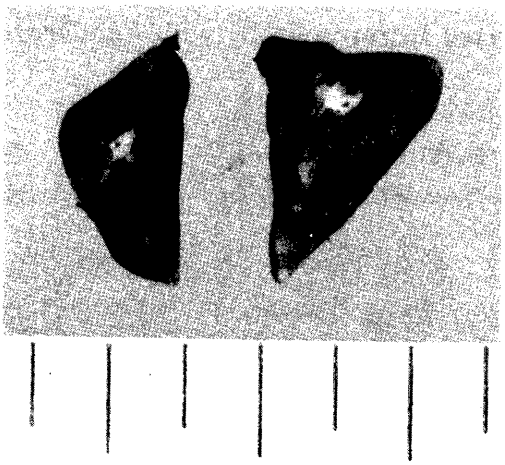

Fig. 1. Cut surface of lungs 1 day pi. Congestion (dark red area).

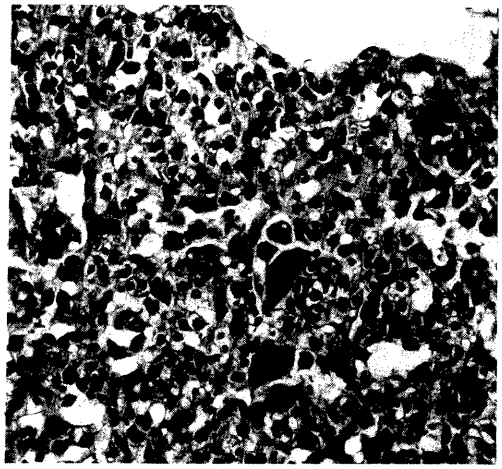

Fig. 2. Lung 1 day pi. Exudated macrophages with multinucleate giant cells in alveoli. HE. ×200.

lips of the wound of the skin were sutured by the silk thread.

Experimental design: Two animals were killed 1, 2, 8, 10, 12 and 14 days postinoculation (pi). One animal was also killed 3, 4, 5, 6, 7, 9 and 16 days pi. Thirteen of the 32 animals died during the experiment; an animal each died 3, 4, 5, 8, and 9 days pi, and 4 animals each 6 and 7 days pi. Both killed and dead animals were examined in the following way.

Bacteriological examination: At necropsy samples from the lungs, spleen, liver, kidneys and heart of all animals were submitted to routine bacteriological examination.

Pathological examination: At necropsy the tissue samples from the lungs, spleen, liver, kidneys, heart, bronchial lymph nodes and several portions of the small and large intestine were fixed in 10% buffered formalin solution and embedded in paraffin. Thin sections were stained with hematoxylin and eosin (HE), Gram's method, azan and periodic acid-Schiff (PAS).

Electron microscopic examination: The lung samples fixed in the formalin were cut into 2 mm cubes, postfixed in 1% osmic acid (OsO_4) and embedded in Epon 812. Ultrathin sections stained with uranyl acetate and lead citrate were observed under an electron microscope (JEOL-100CX).

RESULTS

The animals were observed clinically, and the body weight and body temperature were recorded daily for 16 days pi. The body weight of all animals decreased between 1 and 10 days pi. All animals showed emaciation with dyspnea and the body temperature rose up to 39.5°C in all animals 2–10 days pi with the exception of a few animals which showed critical condition

R. equi was recovered from the lungs of animals killed and died between 1 and 16 days pi. A large number of the bacteria were isolated particularly from the animals 3–6 days pi. *R. equi* was also recovered from liver, spleen and kidneys of many animals 1–8 days pi.

The predominant lesions were restricted to the lungs in all cases during the experimental period. The lesions in both the killed and dead cases at the same day showed similar figures.

1 day pi: The lungs of 2 killed cases had extensive dark red areas (Fig. 1).

Microscopically, the affected lungs were

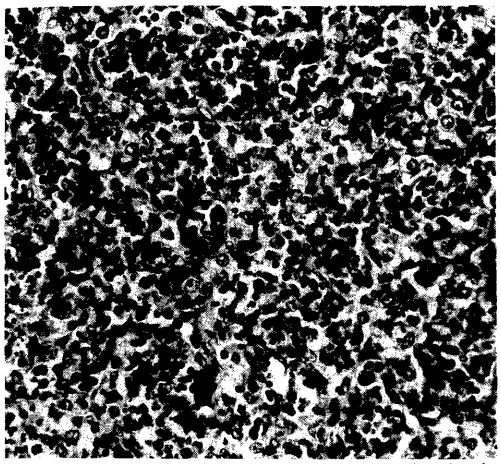

Fig. 3. Lung 2 day pi. Massive cell exudation having numerous neutrophils and macrophages in alveoli. HE. ×200.

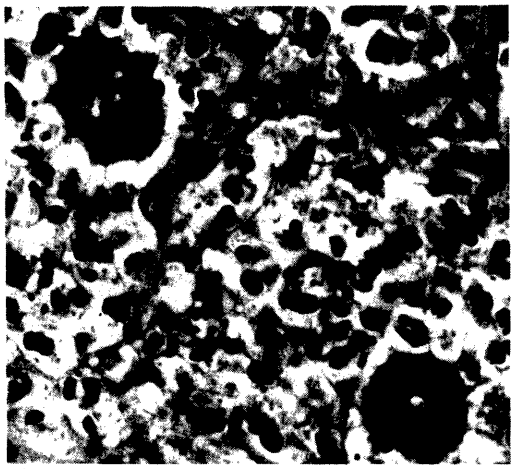

Fig. 4. Lung 2 days pi. Macrophages containing numerous Grampositive bacteria in alveoli. Gram stain. ×1,000.

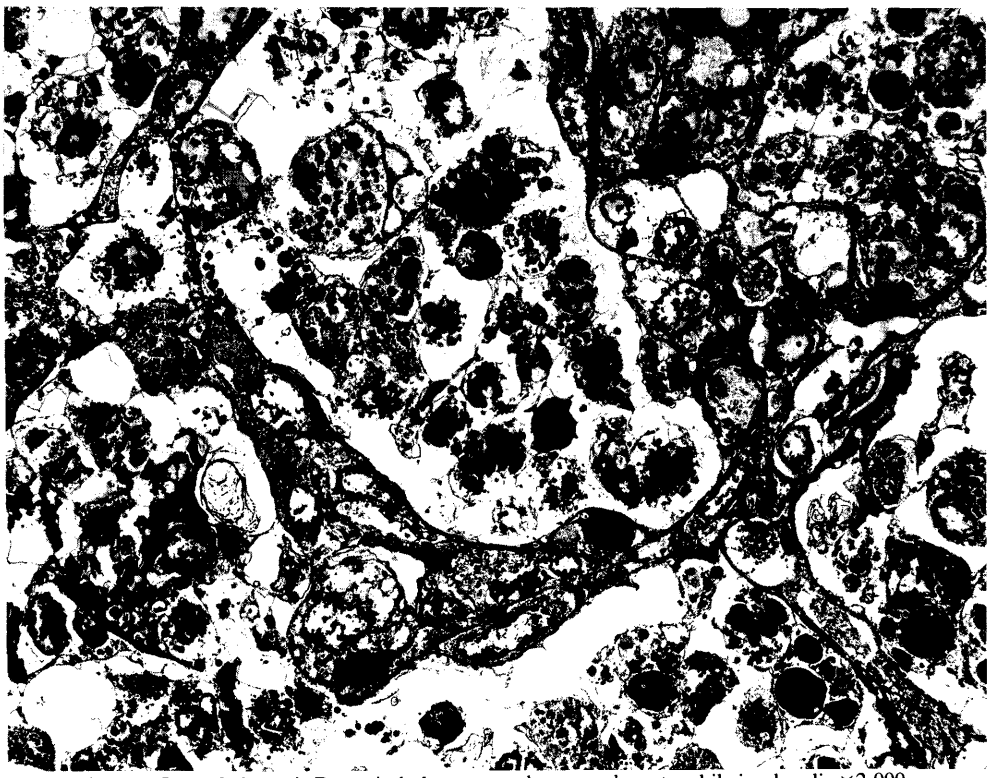

Fig. 5. Lung 2 days pi. Bacteria-laden macrophages and neutrophils in alveoli. ×2,000.

atelectatic with congestion, serous exudation and infiltration of macrophages containing Gram-positive bacteria which were enclosed by PAS-positive substance. Multinucleate giant cells appeared occasionally (Fig. 2) but the bacteria were seldom demonstrated in them.

Epithelial cells of the trachea were hyper-

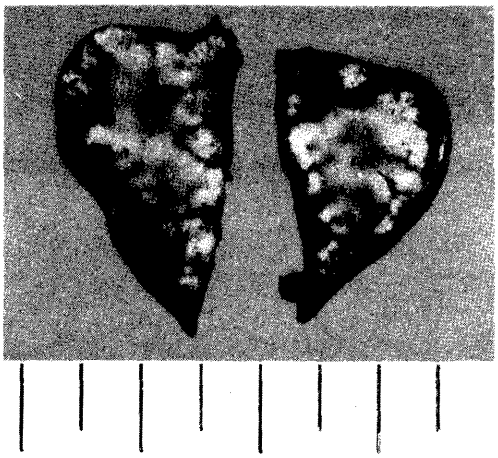

Fig.6. Cut surface of lungs 6 days pi. Extensive consolidated lesions demarcated by narrow red areas.

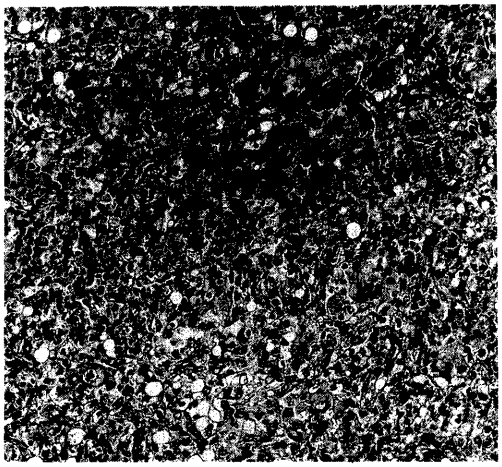

Fig. 7. Lung 6 days pi. Necrotic changes of exudated cells in alveoli. HE. ×200.

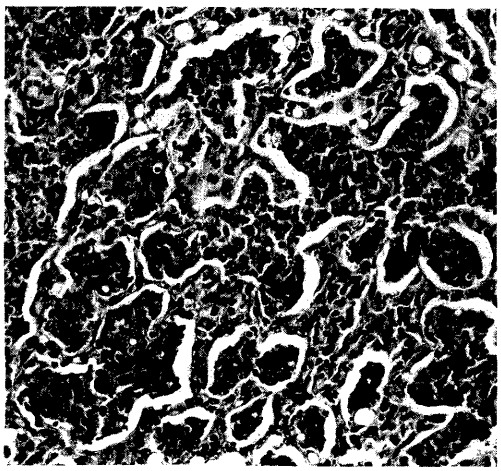

Fig. 8. Lung 8 days pi. Exudation of mononuclear cells in alveoli. HE. ×200.

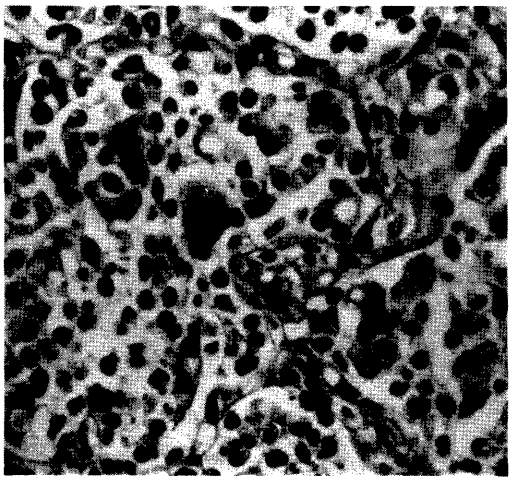

Fig. 9. Lung 7 days pi. Mononuclear cells with a multinucleate giant cell in alveoli. HE. ×400.

plastic or necrotic with numerous bacteria. The bronchial epithelial cells enlarged or desquamated in the lumen. There was perivascular infiltration of lymphocytes in the interstitial tissues.

2–5 days pi: The lungs of 2 cases killed 2 days pi had grayishwhite lesions, about soy-bean size, surrounded by the dark red areas in the cranial lobes. The extensive consolidated lesions were present in 6 cases. The cut surface of the lesions showed white firm or caseous appearance demarcated from dark red area.

Microscopically, the lesions were characterized by suppurative bronchopneumonia. Massive neutrophil and some macrophage exudation was present in the alveolar spaces (Fig. 3). The lesions with multinucleate giant cells as observed 1 day pi remained in 1 case 2 days pi. The macrophages contained numerous Gram-positive bacteria in cases 2–4 days pi (Fig. 4). The bacteria-laden macrophages decreased in number and the neutrophils showed degenerative and necrotic figures in cases 5 days pi. Under electron microscope,

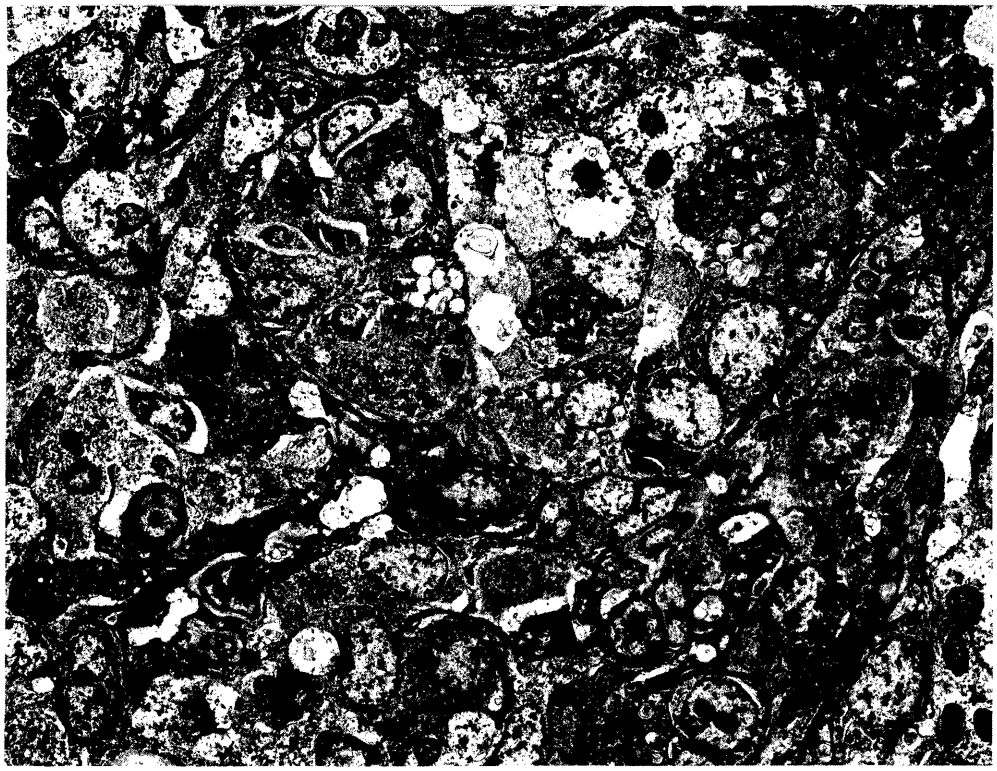

Fig. 10. Lung 8 days pi. Macrophages containing degenerative neutrophils in alveoli. ×2,000.

the bacteria in the macrophages were oval or pleomorphic in shape (Fig. 5). In some cases, serous exudation in the alveolar spaces and obstruction by neutrophils, macrophages, desquamated epithelial cells and necrotic materials in the bronchi and bronchioles were observed. The interstitial connective tissue was slightly edematous.

6–10 days pi: The affected lungs in 13 of 17 cases were extensively consolidated with multiple yellowish granules on their surface. The remaining 4 cases had white consolidated lesions in the cranial or caudal lobes. Their cut surface showed white caseous appearance surrounded by the dark red areas (Fig. 6). The yellowish granular lesions contained suppurative or caseous materials.

Microscopically, focal accumulation of degenerative and necrotic cells was present without encapsulation by fibrous tissue (Fig. 7). In the alveolar spaces neutrophils and macrophages having bacteria were replaced by mononuclear cells characterized by abundant cytoplasm (Fig. 8) with a few multinucleate giant cells (Fig. 9). Although these monoclear cells did not contain bacteria, they had degenerative neutrophils in their cytoplasm under electron microscope (Fig. 10).

12–16 days pi: The soy-bean sized white soft lesions were observed in 4 cases killed 12–14 days pi and a hemp-seed sized lesion was observed in a case killed 16 days pi. The cut surface of the lesions showed a sponge-like appearance with obscure delineation (Fig. 11).

Microscopically, there were small lesions consisting of mononulear cells and a few multinuleate giant cells surrounded by compressed alveolar septa (Fig. 12).

The bronchial lymph nodes were enlarged

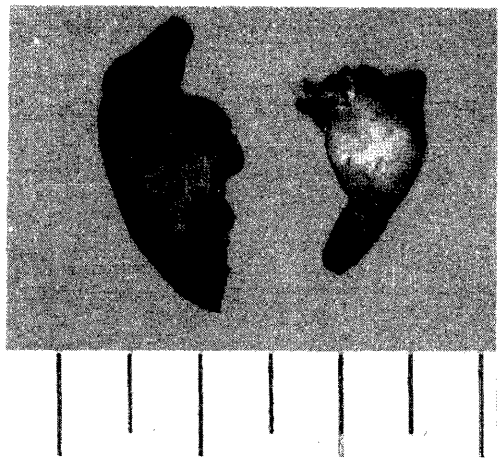

Fig. 11. Cut surface of lung 12 days pi. Lesions showing a sponge-like appearance and obscure outlines.

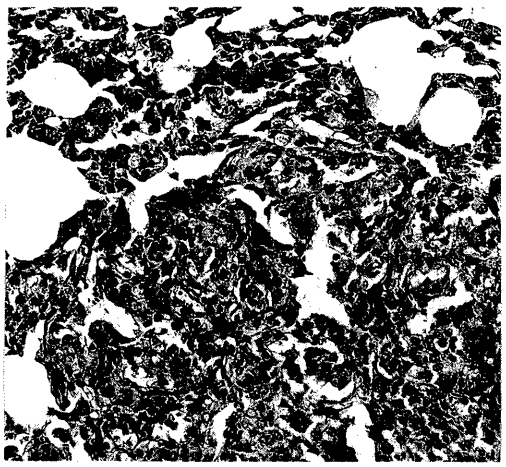

Fig. 12. Lung 14 days pi. A small lesion consisting of mononuclear cells and macrophages in alveoli. HE. ×100.

about three times as large as normal ones in cases 2–12 days pi.

Microscopically, the cortex and paracortex were edematous having hypocellular lymphoid follicles. There was an infiltration of macrophages in the sinus. In addition, focal accumulation of necrotic cells was present in the cortex of cases 7–8 days pi.

Mild degeneration of liver cells and infiltration of macrophages in the sinusoid were often present. In the spleen, lymphoid follicles of most cases were poor in lymphocytes with macrophage infiltration. Some cases had mild infiltration of lymphocytes and macrophages in the lamina propria of the intestine and the interstitium of the myocardium and kidneys. These lesions were observed throughout the experimental period.

DISCUSSION

In experimental suppurative bronchopneumonia in guinea-pigs macrophages in the lesions 1 day pi may be the important component preceding neutrophil infiltration and contribute to phagocytosis of the bacteria. The multinucleate giant cells, however, seem to be weak in the function of excluding the bacteria, because they seldom phagocytized the bacteria. Although the lesions observed 1 day pi have not been described in *R. equi*-infected foals, they may occur before the distinct symptoms.

Suppurative bronchopneumonia with many bacteria-laden macrophages 2–5 days pi resembled that of *R. equi*-infected foals in which the disease had run a more fulminating course (10). It has been described that bacteria-laden macrophages were always present in the chronic course of the foal (9) and *R. equi* appeared to survive in phagocytes by evading the damage (10). The ability of the macrophage for the clearance of *R. equi* may be higher in the guinea-pigs because the bacteria-laden macrophages were decreasing in number 5 days pi. The PAS-positive substance around the bacteria in the macrophages seems to be accordant with PAS-positive macrophages reported in *R. equi*-enteritis of the foal (3). It was not clear if this substance was responsible for protecting the bacteria from damage.

Lung abscesses were characteristic in foals infected with *R. equi* (1–3, 8–10, 12–15, 17, 20, 22, 24), In the present guinea-pigs abscess formation was not observed but focal accumulation of necrotic

cells was present without encapsulation. A decrease in the number of *R. equi* within a comparatively short time may be responsible for disturbing the development of chronic lesions including abscess formation.

Large mononuclear cells appeared 6–10 days pi may have a role in clearing off the necrotic neutrophils instead of phagocytosis of the bacteria judging from electron microscopy.

The lung lesions became smaller in size and consisted of small unorganized accumulations of the large mononuclear cells 12–16 days pi. In the previous work, the lungs of the foals which took the long clinical course had small organized lesions as the result of absorption of the contents in abscesses. The lesions of the guinea-pigs may be easy to heal owing to the lack of abscess formation.

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要　　約

Rhodococcus equi 気管内感染モルモットの病理学的観察：石野清之・中沢宗生・松田泉[1)]（農林水産省家畜衛生試験場，[1)] 農林水産省農業環境技術研究所）— *R. equi* による肺炎病変の推移を明らかにするために，*R. equi* を気管内接種された32頭のモルモットの肺を病理組織学的に検討した。接種による化膿性気管支肺炎は次のように推移した。接種後1日では，充血，奨液浸潤，多核巨細胞とグラム陽性菌を貪食したマクロファージの軽い浸潤，2—5日後では多数の好中球と食菌したマクロファージの浸潤，6—10日後では壊死細胞の集積，変性した好中球を貪食した単核細胞と少数の多核巨細胞の浸潤，12—16日後では正常肺胞にかこまれた単核細胞の小集積巣が見られた。*R. equi* を感染させたモルモットの肺病変は子馬の病変より修復傾向が強かった。