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REPORT ON SOME PULMONARY LESIONS PRODUCED BY THE BACILLUS OF HEMORRHAGIC SEPTICÆMIA OF CARABAOS.

By PAUL G. WOOLLEY, M. D., *Pathologist Biological Laboratory.*

The lesions caused by the bacillus of hemorrhagic septicæmia in cattle are legion. Subcutaneus and lymphatic suppurations, gastro-intestinal ulcerations and hemorrhages, widespread subcutaneous and subserous edemas, pathologic joint conditions, and varying types of pulmonary changes are frequently seen, sometimes alone, but usually accompanied by ecchymoses. During the epidemic of hemorrhagic septicæmia through which the Government carabaos have lately passed we had opportunities to study many of these.

Among the animals dead of the prevailing infection was one in whose lungs were lesions so like those of contagious peri-pneumonia that we were at some loss to make a positive diagnosis until careful pathologic and bacteriologic examinations had been made. Unfortunately the autopsy had to be done under such unfavorable circumstances and so hurriedly that there are necessarily some gaps in the protocol which can not be filled, yet the known clinical facts, together with the bacteriologic and pathologic findings, leave no room for doubt as to the nature of the disorder.

Case I. Pleuro-pneumonia.—The animal was a fairly well-nourished carabao which had arrived in Manila (from Shanghai) three days prior to coming under our observation. It had seemed well and had acted in a perfectly normal manner since landing. On the evening before death the overseer had noticed nothing peculiar about it. There was no cough and it ate and drank. The next morning the animal was found dead in its stall.

Since its arrival in Manila it had been with a herd which had come from China at the same time, and, while a few of the others had died, it was proven that the cause of death had been hemorrhagic septicæmia and they had shown no lesions similar to those found in the animal under discussion. Since its death other members of the

herd have died also, but in these there have been no lesions resembling pleuro-pneumonia.

The autopsy was done early in the afternoon of the day of death. On opening the body there was none of the subcutaneous gelatinous edema which has been so characteristic of the cases of hemorrhagic septicæmia studied by us; neither were there any extravasations of blood in the subcutaneous tissues. The abdominal cavity showed nothing remarkable, though the liver presented a number of abscesses in which flukes were found.

The remarkable lesions were in the thoracic cavity. When this was opened a quantity of pale clear amber fluid gushed out. In the residual liquid in the pleural cavities were some fibrinous shreds. The pleural surfaces were, for the most part, covered with a well-marked fibrinous exudate which could readily be peeled off, leaving a reddened, congested, roughened surface. The pleura itself was thickened and edematous. The subpleural tissues were in places filled with a sero-gelatinous exudate, and this condition was most marked under the mediastinal surfaces. The mediastinal connective tissues were completely filled with the same gelatinous material. The pericardium in its whole extent was lined with a fibrinous exudate and its surfaces were separated by a serous fluid containing flakes of fibrin.

The lungs were not collapsed, but contained air only in the anterior and apical portions. Cut surfaces of the organs were firm and red, in some places very dark, and divided by fine and coarse bands of what appeared like edematous connective tissue, so that the whole section had a marbled appearance. These bands varied from one-eighth to one-half of an inch in thickness and were in places quite saturated with serum and even honeycombed with small cystic areas filled with a bluish-looking, gelatinous material. There were no hemorrhages in the thoracic organs except in the heart. The right auricle of this organ was nearly black with large and small confluent hemorrhages. The mediastinal and prescapular glands were enlarged, pale, and showed areas of necrosis.

Bacteriologic.—Smears made from the heart's blood, from the liver, lungs, and prescapular lymph glands, showed a considerable number of small, oval, polar-staining bacilli. Cultures were made on agar, from the heart's blood and from the bands in the lungs. Within twenty-four hours small, translucent, shining, moist colonies appeared on the surfaces, resembling dewdrops. The organisms

comprising these colonies were short rods, which when stained with 1–10 carbol fuchsin or carbol thionin, showed well-marked polar staining. All the cultures made at autopsy gave the same kind of growth, and the organism was present in pure culture. The other features of this bacillus were that it did not stain by Gram's method, did not form spores, did not liquify gelatin, and did not coagulate milk. In peptone solution, after twenty-four to forty-eight hours' growth at 37° C., it gave a well-marked "cholera-red" reaction.

Intrapleural injection of small amounts of broth culture ($\frac{1}{4}$ c. c. forty-eight-hour culture) killed a guinea pig in something less than twenty hours, and a post-mortem examination of the dead animal revealed a well-marked fibrinous pleuritis, a fibrinous pericarditis, and hemorrhages into the pericardium and pleura. The lungs were partially solidified. The pancreas was surrounded by a gelatinous tissue which produced the impression that the organ had been embedded in a perfectly clear gelatin. The organism was recovered in pure culture from the heart's blood and pleural exudate, and smears from the heart, liver, kidneys, spleen, and lungs showed apparently the same organism.

Pathologic.—In sections from the lungs the air spaces contained a granular material and an occasional leucocyte or desquamated endothelial cells. The blood vessels were all intensely congested and filled with red blood cells. The mucous membrane of the bronchi was desquamated in some places, and these tubes contained a fibrino-purulent material. The bands seen at autopsy were for the most part composed of fibrin and leucocytes, but the largest of them, those extending down from the pleura, also contained a considerable amount of fibrous tissue. The smaller bands ran in all directions across the lung tissue. Such bands were rather sharply outlined from the surrounding edematous lung tissue, but they contained the hyaline, degenerating remains of the air cells which they had involved and which were filled with leucocytes. It was in these fibrino-purulent bands that the bacteria might be seen in sections stained with methylene blue and eosin, and it was from one such band that the cultures described above were obtained. Occasionally about such bands a well-marked leucocytic infiltration was observed, so that the tissue appeared like that in the gray stage of hepatization in pneumonia. In such cases the bacilli were present in the air spaces. These bands were not homogeneous. Some

were composed of nearly solid masses of leucocytes and fibrin, but many of them were formed of an external layer of polymorphous cells, leaving an intermediate clear space, free of cells, but across which fine filaments of fibrin were interlaced. Occasionally, too, other larger cells of endothelial origin were enmeshed in this fibrinous lacework. Then, too, the congestion, which was general, was more intense about these bands. There were no signs of periarterial fibrosis, but on the contrary the blood vessels seemed normal save for the congestion.

The liver showed no more than well-marked congestion of the centers of the lobules.

It seems then that this may be considered as a pure case of the infectious pleuro-pneumonia, and not as one of the contagious type.

The facts in the clinical history seem to support Theobald Smith's theory of the etiology of the disease. The ocean trip, a rough passage, rough handling, all would tend to produce the primary broncho-pneumonia and emphysema upon which the later stages follow. In this case, the broncho-pneumonia was perhaps the first stage of the disease. The presence of a very infectious disease in the same herd would account for the presence of the causative organism in the lungs of the infected animal. But even without the bacilli of hemorrhagic septicæmia in other animals, the organisms might have invaded the weakened animal from the upper respiratory tract where they might have been present, and probably were, if the same conditions hold here as in the cattle which Moore examined. There is, too, a very good reason for the presence of these organisms in cattle here, if, as has been proved in other places, they are present in water. The health of the carabao, or water buffalo, depends to a great extent upon the daily bath, which is usually taken in a wallow, in the thick mud of which the animals immerse or embed themselves until only the ears, eyes, nose, and horns are visible. Frequently the whole head disappears from sight. Habits of this sort offer every inducement for such organisms as are present to enter the animal. However, we have not been able to demonstrate the bacillus of hemorrhagic septicæmia in the water or soil.

Case II.—The animal was very weak when first observed, but in fair physical condition otherwise. The conjunctivæ were somewhat congested, respiration was rapid, and the feces normal. Temperature, 40°.2 C. When taken off the truck at the Laboratory it

staggered a few steps and fell on its side. There were numerous bruises on the body, probably the result of a rough voyage across the China Sea. It ate food when placed near it and also drank, although it did not, apparently, suffer from thirst. It had no cough. During the next few days it became a little brighter and somewhat stronger. On June 6 it was again weak and could not stand up, the hind legs seeming to be especially feeble. It gradually became weaker and diarrhea developed, but with no traces of blood or mucus. Death occurred on the ninth day after landing.

The post-mortem examination showed a few patches of subcutaneous edema on the sides. There were a few small pericardial hemorrhages about the base of the heart. The lungs showed a number of subpleural nodules, which on section exposed granular areas similar to those seen in broncho-pneumonia in the stages of red and gray hepatization and suppuration. The suppurating areas were filled with a thick, granular, greenish-yellow, sticky material.

Cultures were made from the lung abscesses on agar and blood serum. After twenty-four hours at 37° C. the agar tubes showed a growth of small, transparent, grayish, round colonies. The blood serum showed a very scanty growth of small colonies. Transfers were made from these tubes to various other media, and plates were also made. After a careful study of its morphologic and cultural characteristics, it appeared that the organism under consideration was a short bacillus with rounded ends, and nonmotile. Its measurements varied between 1 and 2 microns in length, and 0.3 and 0.5 micron in thickness. The largest forms were seen in glucose media, the smallest on potato. From the animal body it showed well-marked polar staining, although this was not so distinct in organisms grown on artificial media. It was stained easily with the usual watery aniline stains, but was not stained by Gram's or Weigert's methods. The rods, as a rule, occurred singly, often in pairs, occasionally in chains of five or six individuals. The appearance of the growths on the usual culture media was in no way characteristic. The colonies on agar were small, grayish, transparent, and well circumscribed, with little or no tendency to spread. On all the solid media approximately the same appearance was noticed. In gelatin no liquefaction was caused. In bouillon a granular deposit was formed on the sides and bottom of the tube.

During the first few hours of growth the whole medium was faintly clouded, but as the sediment was deposited the liquid became clear. After a few days the sediment became viscid, as could be shown by shaking the tube, when the precipitate rose, not in floccules, but in threads. In Dunham's peptone solution the same general characteristics were observed as in broth, but the growth was not so abundant. The cholera-red reaction was produced by the addition of sulphuric acid (free from nitrates) at the end of twenty-four to thirty-six hours. No phenol was detectable. No gas was produced in solid glucose or lactose media, and the reaction of the media was not changed. Milk was unaffected even after two weeks. No acid was produced, no coagulation occurred, and there was no reduction of litmus. Stab cultures in solid media showed nothing remarkable; the growth followed the line of inoculation closely, with no tendency to spread; it extended to the bottom of the punctures, finely granular and composed of small colonies. The surface growth was small, just surrounding the point of entrance of the needle.

This organism was pathogenic for monkeys, small birds, rabbits, and guinea pigs, when injected subcutaneously, intravenously, intrapleurally, or intraperitoneally. Death was the result of a septicæmia or acute sero-purulent inflammation with subsequent septicæmia.

Sections of the lungs in this case showed a general edematous condition with well-marked congestion. There were some areas of emphysema. The peribronchial tissue was infiltrated with red blood cells and leucocytes, which filled the air spaces and which were enmeshed in a network of fibrin. Occasional bands of fibroid tissue were met with, extending down from the pleura. Some of these showed infiltration with leucocytes. There was no perivascular fibrosis.

The pleura itself was thickened, but showed no evidence of chronic inflammation. The tissue beneath it was, however, infiltrated with small round cells, and showed well-marked, new vascular formation.

Case III.—A native horse. In this case no clinical history was obtained. The lung lesions corresponded with those of Case II. There was likewise a gelatinous edema about the base of the heart. Cultures were made from the small abscesses and nodules, and in smears and cultures an organism was present that agreed in every detail with the one from Cases I and II.

The sections from the lungs showed congestion. The air cells were either widely distended or filled with a fibrino-purulent material or a granular material which resembled coagulated albumen. As a whole, the fibrous tissue in the lungs was increased. There was a considerable subpleural accumulation of well-formed granulation tissue, and there was an increase of peribronchial fibrous tissue. The bronchi were filled with fibrino-purulent material, in some cases mixed with the desquamated lining cells of those tubes. In some places, too, the lining mucous membrane of the bronchi was thickened. Extending down from the pleura into the pulmonary tissue were some considerable bands of fibrous tissue, but in this case they showed but little round-celled infiltration and no leucocytes.

The smaller consolidated areas resembled the gray stage of hepatization. The larger ones were veritable abscesses, in the sides of which the hyaline remains of air spaces could be seen, but in the center no such remnants, but only the nuclear material and cells undergoing karyorrhexis appeared.

Therefore the cases cited above were examples of the invasion of the lungs by the bacillus of hemorrhagic septicæmia. How they gained access to the lungs we can not state with absolute certainty, but we suspect them to have come from the upper air passages and believe the pulmonary invasion was subsequent to a bronchitis in all three cases. It is tolerably certain, too, that in all of the cases death was the result of a terminal septicæmia incident to the entrance into the blood stream of the organisms which were present in the lesions of the lungs.