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Note

Equine Melioidosis in Iran

By

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Melioidosis is a glanders-like disease which is caused by a Gram-negative, short, and motile bacterium *Pseudomonas pseudomallei* or *Malleomyces pseudomallei*. It resembles closely *Pseudomonas mallei* or Pseudomonas aeroginosa. It was originally described by Whitmore et al. (1) in Rangoon. The organism is pathogenic for rodents (2), cats and dogs (3), pigs (4), cattle (5), goats (6), sheep (7), horses (8), and man (1).

First, in 1925, Stanton et al. (8) described equine infection, and the authors isolated from the pus of an abscess of a horse a micro-organism which resembled *P. pseudomallei*. Davie and his co-workers (9) in 1952 described three fatal cases of Melioidosis in race horses in Malaya. Generally, however, literature on Melioidosis in the equine is rare.

This report is to describe an outbreak of Melioidosis which occurred in horses and mule at the Razi Institute in May and June, 1969.

HISTORY

Two horses and one mule had clinical symptoms for one month. These consisted of anorexia, purulent discharge from nasal cavities and mouth, distention of the abdomen and pneumonia. The horses lost considerable weight, and treatment with antibiotics was not effective. All died in an interval of two weeks.

BACTERIOLOGICAL FINDINGS

The disease was thought to be either glanders or melioidosis, based on gross and microscopic pathological changes. Culture from abscesses of liver, spleen, nasal cavities and lungs on differential artificial media were made. These included glycerine agar, glycerine broth, blood agar (5% sheep red blood cells), and selective media. A bacterium with the following characteristics was detected.

On glycerine agar within 2 days at 37° C., there appeared some smooth, and glistening surface, colonies. On blood agar there was a hemolytic zone. The broth culture after 24 hours at 37° C. contained a growth of moderate turbidity and a slight powdery deposit. The culture was covered by a veil. On agar slope, after 24 hours at 37° C. the culture was abundant, confluent, raised, greyish-yellow, mucoid, spreading with glistening, beaten copper surface and undulate edge.

Microscopic examination of the Gram-stained organisms showed small, slender, Gram-negative rods, with granular type of staining and some bipolar forms, like those observed in fresh slide preparations from the lung and nasal tissues of the horses and mule. The organism was motile and biochemically active. It produced acid in glucose, manitol, lactose, sucrose and dulcitol. Indole and SH2 production were negative. Grouth was in the presence of Cristal Violet, vancomycin-colistin-nystatin, MacConkeys, and 2% NaCl agars, and there was gelatin liquifaction. Nitrates were reduced. There was no growth on cetyl-trimethylammonium bromide, 4% NaCl, and sodium azide agars. Urease was not produced, while cytochrome oxidase was positive.

The isolated organisms with above characters were identified as *Pseudomonas pseudomallei*. Our finding was confirmed by Dr. A.D. Alexander (Personnal communication).

Inoculation of the organism into laboratory animals produced characteristic symptoms and death. After subcutaneous inoculation of a small amount of the broth culture into guinea-pigs, death occurred in 7-9 days. Postmortem findings consisted of focal necrosis and small abscesses in liver and spleen and a marked thickneing of the tunica vaginalis. There was a swelling of testicles which appeared in 2 to 3 days post-inoculation and within a week they were markedly enlarged. There was a milky discharge from the eyes and nose of the inoculated guinea-pigs. A contraction of the omentum to the greater curvature of the stomach was also present in guinea-pigs inoculated interaperitoneally. In rabbits, a subcutaneous inoculation caused a local ulcerative lesion and swelling of the local lymph glands. They died within 2 to 3 weeks. A few greyish nodules were present in the lungs and there were ulcers on the nasal mucosa. After intravenous injection, numerous nodules developed in the spleen and liver but death was delayed for some weeks. The organism could be recovered from liver, spleen, kidney, peritoneal cavity, and heart blood of inoculated animals.

Two fillies were inoculated subcutaneously with 10ml of a broth culture but no clinical symptoms were observed. The serum agglutinated a culture of *P. pseudomallei* one week after inoculation.

GROSS PATHOLOGY

In the horses there were nodules on the legs which were 1-2 cm. in diameter. There were ulceration in the skin of the legs along the lymphatic ducts. In the mule, numerous nodules were found under the skin of much of the body along the lymphatic ducts and some were located over the legs. Severe oedema was present in the prepuce and there was orchitis.

In the nasal cavities there were several erosions and ulcers with uneven surfaces. Some of these ulcers were covered by a yellowish-green creamy exudate. There were granulomatous lesions which were scattered throughout the mucous membranes.

The tracheal mucosa was covered with a yellowish-gray creamy exudate. The lungs were moderately oedematous and severely congested. There were fibrotic areas 5 x 6 cm. which covered the fore portion of the diaphragmatic lobes. Palpation revealed numerous small nodules scattered throughout the parenchyma. In the horses the cut surface showed numerous whitish-gray areas 2 – 3 mm. in diameter. In the mule there

were red nodules with brownish caseation .There was severe oedema in the chest and inguinal regions.

The liver was slightly enlarged and friable. It was yellow and a lobulipattern was visible. The spleen was enlarged and had a pulpy parenchyma.

Subcapsular hemorrhaegs were present in the kidneys. The heart muscle was striated and some petechial hemorrhages were observed on the epicardium.

HISTOLOGICAL CHANGES

The histological changes in different organs were as follows:

Turbinates: Blood vessels were engorged with red blood cells and most were obstructed with thrombi. The mucous membranes showed vacuolizations and some degeneration close to the region of the ulcers. There were extensive inflammatory reaction of predominantly neutrophiles, monocytes and lymphocytes with extensive necrosis. Focal liquefaction necrosis was also present.

Nasal Cavity: The emboli partially obstructed the veins and small capillaries. There were submucosal nodules with slightly calcified centers surrounded by a wide zone of neutrophiles and peripheral histiocytes and some giant cells. The nodules were encapsulated and surrounded by thin layers of connective tissue. Smaller nodules were in the submucosa which consisted of a focus of intense neutrophilic infiltration and cellular necrosis with nuclear debris in the center. These were surrounded by histiocytes and mononuclear cells.

Lungs and Bronchioles: The lungs had severe hyperemia and oedema. The oedema was intra-lobular and intra-alveolar. There were nodules with extensive necrotic cells and nuclei debris. There was a wide neutrophilic zone around the periphery. These nodlues were surrounded by engorged vessels and various degrees of hemorrhage and oedema. There are some alveoli filled with fibrinous exudation which was surrounded by a zone of hyperemia and oedema.

The bronchioles were surrounded with extensive submucosal oedema and desquamation of epithelial cells.

Kidneys: These were congested and the capillary vessels were engorged with red blood cells, especially in corticomedulary junction. Several small focal hemorrhages were observed. The urinary tubular epithelial cells were partially degenerated into the lumen.

Liver: The liver was severely oedematous and the sinusoids were distended with exudate containing numerous neutrophiles and monocytes. The cells were in a narrow columnar shape due to pressure on the sinusoids by the oedema. They were in various stages of necrosis. Some focal necrosis with mononuclear cell infiltrations was present. Proliferation by fibrous connective tissue in the portal area and in small bile-ducts between the lobules was present. Some vacuoles had empty spaces while others were filled with homogenous eosinophilic substances.

DISCUSSION

Two cases of Melioidosis have previously been diagnosed in goats and sheep in Iran at the Razi Institute, Dept. of Animal Pathology. The goats were brought from the State Farm near the Institute and the sheep were from southern part of the country.

The epizootiology of this disease and the origin of contamination is unknown.

More than 80% of horses in the Institute have been received from the army during many years and the rest of them were purchased from the zone which never had a report of Melioidosis. There is no evidence of Melioidosis having been recognized in equines in Iran and neighbouring countries.

Since the outbreak, about 100 mice and rats have been trapped from the horse stables. All these mice and rats were killed and cultures made from internal organs, urine and faeces. All were negative for *P. pseudomallei*.

According to Blanc and Baltazards (10), the rat flea (*Xenopsylla cheopis*) and mosquito (*Aëdes aegypti*) and other arthropods might be important in the transmission of Melioidosis. It is possible that these arthropods were the source of the infection, although the role of mice and rats can not be overlooked.

Glanders in horses is the only disease which can be confused with Melioidosis. Since horses and mules are tested with mallein before entering into the Institute and subsequent annual tests are performed with negative results, it seems unlikely that glanders is present. *P. pseudomallei* was the only pathogenic organism isolated from clinical cases.

During this outbreak 600 horses and mules existing in the Institute were tested by mallein and whitmorine. Approximately 5% of the horses and mules have shown positive reactions with mallein and whitmorine. They were negative approximately one year previous to a mallein test. The sera of all positive animals agglutinated *P. pseudomallei* and *P. mallei* antigens in high titers.

SUMMARY

Melioidosis in horses and mules used for serum production at the Razi Institute is described. Two horses and one mule were bacteriologically examined. Postmortem examinations showed abscesses and nodules in the skin, lungs, spleen, nasal cavities and *Pseudomonas pseudomallei* was isolated from infected tissues of each animal.

The pathogenicity of the isolated organisms was tested in guinea-pigs, rabbits and mice.

Equine Melioidosis is the first reported in Iran. The possible role of rats and mice and also arthropod vectors in the epizootiology of this disease is mentioned.

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