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Melioidosis in Imported Non-Human Primates

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Abstract

In 1969, five cases of melioidosis in three separate outbreaks were diagnosed in nonhuman primates in the United States. In the first outbreak, two stump-tailed macaque monkeys *(Macaca arctoides)* developed signs of the disease approximately 6 months after purchase. A third animal, a chimpanzee *(Pan troglodytes)*, probably acquired its infection from one of these monkeys. Two other unrelated cases involving a pig-tailed monkey *(Macaca nemestrina)* and a rhesus monkey *(Macaca mulatta)* were diagnosed. These monkeys had been imported 3 years and 6 months, respectively, prior to the recognized onset of their disease. These cases represent the first known occurrences of spontaneous melioidosis in nonhuman primates in the United States.

Introduction

Melioidosis, a serious and frequently fatal zoonosis, is endemic to Southeast Asia. The causative agent, *Pseudomonas pseudomallei*, appears to be a normal inhabitant of the soil and water. It can readily be isolated from recently cultivated areas such as rice and oil palm fields.[8]

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Despite the apparently widespread distribution of the organism, clinical human disease associated with it is not common; however, subclinical infection is. As many as 15 percent of certain Southeast Asian population groups have antibody to *Ps. pseudomallei*.[9]

The disease in man has been referred to as the "Vietnamese time bomb", because it may not become clinically apparent for months or years after the patient has left an endemic area. It is this property that concerns us here.

While melioidosis has been noted previously in a variety of nonhuman primate species, those cases were all diagnosed in endemic areas.[5,7,10,11] In 1969, three separate occurrences of the disease were noted in the United States in monkeys that had been imported at least 6 months earlier.

First Outbreak

Case 1: Nineteen stump-tailed macaque monkeys *(Macaca arctoides)* from Thailand were obtained on July 11, 1968, by an aeromedical research laboratory in New Mexico from a dealer in California. On July 25, one of them developed an abscess on the left arm and axilla and pyoderma with lymphadenitis of the left arm. He was treated with kanamycin but his response was not recorded. On October 17, he was one of four monkeys sent to a hospital in New Mexico for renal clearance studies. There, when he was first taken from his cage on November 19, he was noted to be slightly anemic. A serum sample in December was an abnormal light green color. In January an indurated lesion was noted in the left axilla with a 1 cm hard subcutaneous mass. The surface was ulcerated and exuding a serous fluid. Cultures at that time were negative. Whether this was the same lesion as noted in the previous July is not known. The lesion had improved by February 14 without therapy, but on February 24 the animal was anorectic and uninterested in his surroundings. On March 3, a 5 cm mass was seen over the left clavicle, but this was apparently unrelated to the axillary lesion. At this point the animal was killed. At necropsy, the mass was found to be a subcutaneous abscess. Another abscess was found in the retroperitoneum extending from the femoral triangle to the thorax. Both abscesses contained a milky white material from which *Ps. pseudomallei* was isolated. This isolate, as well as those in the following cases, was confirmed at the National Communicable Disease Center, and at the Walter Reed Army Institute of Research.

The abscess wall was composed of necrotic tissue, masses of fibrin with diffusely infiltrating neutrophils, and areas of hemorrhage. In the lungs, there was marked distention of the alveoli by a protein - rich, cell - free edema fluid. There was also marked perivascular and peribronchiolar edema. The liver was remarkable for the apparent dissociation of the hepatocytes in a fashion similar to that classically described for leptospirosis. There was in addition a focally prominent, chronic inflammatory infiltrate in the portal triads. None of the other organs contained significant histologic alterations.

Case 2: Because of the potential severity of this disease in man, an epidemiologic investigation was initiated. A serologic survey was conducted of the 4 remaining stump-tail monkeys at the hospital, and all the stump-tail monkeys at the aeromedical research laboratory, as well as personnel who had contact with these monkeys. Five animals — two at the hospital and three at the research laboratory — had titers that in man would be indicative of infection (titer of 1:80 by hemagglutination technique or 1:8 by complement fixation technique). Only one of the suspect animals had been ill. The ill monkey — a male — was from the same shipment as the index case. In November 1968 he was used in a study in which a permanent subcutaneous catheter was implanted in addition to an electrode device in its skull. In February 1969 a series of abscesses had developed along the subcutaneous route of the catheter. These were opened, drained, and cultured. *Staphylococcus*

aureus and organisms identified as belonging to the klebsiella-aerobacter group were recovered. The abscesses were refractory to treatment. In May, *Ps. pseudomallei* was isolated from these. The other four animals (two from an earlier shipment from the same company that sold the index case, and two from other shipments of undetermined origin) never showed signs of overt illness.

An attempt was made to elicit disease in the four clinically normal animals by stressing with steroids, and then all five animals were killed. Only the clinically ill animal had any lesions.

At necropsy, a yellow-green exudate was oozing from around the electrode device implanted in the skull of the ill monkey. The exudate originated from a layer of hemorrhagic soft tissue between the implant and the calvarium. The brain was uninvolved. In the right flank just above the wing of the ileum, there were four contiguous sinus tracts discharging a yellow exudate. These were 2 to 5 mm in diameter at their orifice. The tracts arose from areas of poorly encapsulated abscessation in the subcutis. These were histologically similar to those of Case 1. The only other significant lesions were in the liver. The liver was massively enlarged with rounded edges and had many small yellow absecesses varying from less than 1 mm to 2 cm in diameter distributed randomly in all lobes. The right lobe adhered to the abdominal wall. The larger liver abscesses were typically lined with yellow friable material and contained a thick yellow exudate. The smaller ones appeared solid. The gall bladder was distended with 20 ml of thick, yellow-green pus. Its wall was replaced by a thick layer of yellow tissue with a rough surface. A band of pale pink tissue extended across the lumen of the gall bladder (Figure 1).

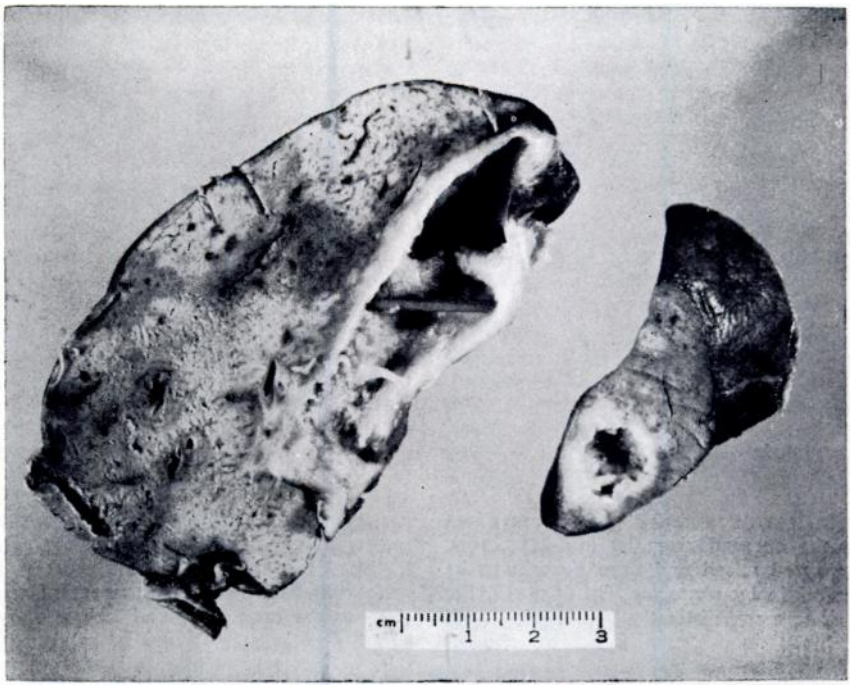

FIGURE 1. *Focal areas of hepatic necrosis and massive necrosis of the gall bladder wall. Note the band of proliferative connective tissue extending across the lumen of the gall bladder.*

The wall of the gall bladder was thickened and fibrotic. The adjacent liver parenchyma had undergone degeneration and fibrous replacement. The mucosal surface of the gall bladder was no longer apparent having been replaced by a layer of necrotic tissue infiltrated with neutrophils and macrophages.

The earliest liver lesions appear as neutrophilic aggregations usually in the area of the triads. The later lesions have a central core of necrosis bounded by a loose network of connective tissue containing many neutrophils and macrophages. The central necrotic zone is filled with a loose network of fibrillar material with intermeshed macrophages and neutrophils. These larger lesions exert pressure on the surrounding parenchyma producing a concentric pattern of compression. The liver appeared to be reacting to a general toxic influence. As in case 1, there was a tendency to dissociation of hepatocytes. There were early thrombi in some of the small veins.

Although the organism was readily cultured from all the lesions, it was difficult to identify them with certainty in Brown and Brenn stained sections.

Case 3: In May 1969, a young female chimpanzee *(Pan troglodytes)*, which also had an electrode device implanted in its skull, showed signs of fever, anorexia, dehydration and difficulty in walking. A liver biopsy indicated hepatitis, which correlated with a high serum transaminase. It was treated with antibiotics and supportive therapy. On June 7, several abscesses were found on its wrists and hands. A serum sample drawn at that time was light green and had a hemagglutination titer of 1:2560 for *Ps. pseudomallei*. The animal subsequently died, and *Ps. pseudomallei* was isolated.

This animal was imported in April 1966 from Africa, a non-endemic area. While it had never had direct contact with monkeys, the chimp was housed in the same area as case 2 for a time. There is a strong probability that the infection was spread from the monkey to the chimp during clinical examination of their respective electrode implants.

After this case occurred, all the animals in the aeromedical laboratory colony were examined bacteriologically and seriologically. To date, no new cases have been found.

Second Outbreak

On October 7, 1968, a male rhesus monkey *(Macaca mulatta)*, received on October 1, at the National Institutes of Health in a shipment of 50 from India, had soft stools, for which he was given tetracycline, nitrofurazone, and a commercially made oral feeding mixture. On December 2, he was issued to a psychology laboratory, where on December 18, he underwent a craniotomy with excision of parts of the cortical sensory areas. Sensory testing was started on January 15, 1969, but the monkey proved difficult to test and train.

In late April round scabs were noted at the surgical scar; by mid-May, they appeared raised and were thought to be underlain by abscesses. On May 19, the lesions were distinctly suppurative, and at that time similar processes were noticed on the skin of the chest and leg. *Pseudomonas pseudomallei* was isolated from the lesions.

The animal was killed on May 28. At necropsy, a 2 cm raised, fluctuant, subcutaneous abscess was observed on the left dorsal aspect of the head, directly over the site of the previous frontoparietal craniotomy. The abscess contained thick pale yellow pus. A similar subcutaneous abscess was located on the left side of the chest, approximately 1 cm lateral to the nipple, and was connected by a fistulous tract to a larger 3 by 4 cm abscess in the left axilla, apparently involving the axillary lymph nodes. Internally, multiple 0.5 to 1 cm abscesses were observed in the liver, spleen, pancreaticosplenic lymph nodes, and in the superior gastric nodes. One of the liver abscesses was contiguous with the wall of the gall bladder. Two 1 cm subpleural abscesses were found on the dorsal aspect of the right apical lung lobe, and lesions were found also in several mediastinal lymph nodes. The pus in the internal lesions was thin and dull white or dull green.

Histologically, the lesions had the appearance of poorly encapsulated abscesses.

Third Outbreak

Melioidosis was diagnosed in October 1969 in a 9-year-old female pigtail monkey *(Macaca nemestrina)* being used in behavioral research in Seattle. The animal had been semi-isolated for 3 years, but in May was caged near the rest of the colony while the floor was being repaired in their observation compound. The monkey had been well except for unexplained lameness in the right leg and mild anemia in August 1969, for which she received iron parenterally. On September 18, she had extensor paralysis of the right leg compatible with a radial nerve injury, mild swelling of the right eyelid, and generalized lethargy, and she was placed in isolation.

Within several days, a large fluctuant swelling developed over the frontal bone, extending over the orbital crest to involve the right palpebrum. The swelling was excised and 10 ml of a thick, grey-white fluid was drained. The abscess was flushed and packed with a furacin seton, and sodium ampicillin therapy was started. Lincomycin was then used after a preliminary culture of the abscess demonstrated a coagulase positive staphylococcus and a hemolytic streptococcus sensitive to lincomycin.

On October 3, three fistulous tracts, discharging a purulent material, were noted on the medial aspect of the upper right forearm. About this time, *Ps. pseudomallei* was identified in the original culture.

The animal died on October 13. A serum sample taken several hours before death showed a hemagglutination (HA) antibody titer for *Ps. pseudomallei* of 1:2560. A necropsy revealed 1) complete resolution of the abscess over the right frontal bone and eyelid, 2) a marked accumulation of purulent material over fascial planes on the proximal medial aspect of the right arm, 3) a large unilocular abscess beneath the right mammary gland which extended in a proximal-lateral direction to the axilla, with no gross anatomic connection between the infections on the right arm and the mammary gland, and 4) the right lower lobe of the lung contained two 2 x 2 cm firm nodules which were granulomatous in consistency.

In the serologic examination of the remaining 20 animals in the behavioral group, seven had HA titers to *Ps. pseudomallei* ranging from 1:80 to 1:2560. These animals were all killed and examined no lesions compatible with melioidosis were present nor was the causative agent isolated. Serology and fecal cultures of all other animals in the colony likewise failed to uncover any further cases.

Serologic Survey

When initiating the epidemiologic study of the first outbreak, we did not know what importance to attach to the low titers in many of the contact animals. To set a baseline, serum samples were obtained from a variety of macaques imported from endemic areas as well as some born in this country. All the serum

TABLE 1. *Melioidosis in non-human primates — 1969.*

Case	Species	Natural Habitat	Date Imported	Onset Illness*
1	*Macaca arctoides*	S. E. Asia**	July 1968	Jan. 1969
2	*Macaca arctoides*	S. E. Asia**	July 1968	Feb. 1969
3***	*Pan troglodytes*	Africa	April 1966	May 1969
4	*Macaca mulatta*	India**	Oct. 1968	April 1969
5	*Macaca nemestrina*	S. E. Asia**	1966	Aug. 1969

*When signs of illness first recognized
**Endemic area of melioidosis
***Infection probably acquired from case 2 .

samples were checked by a hemagglutination (HA) technique.[9]

Serum samples came from 284 monkeys — 250 imported animals and 34 born in the United States. The imported group included 135 *Macaca nemestrina*, 93 *Macaca arctoides* and 22 *Macaca irus*. The animals born in this country were all *M. nemestrina*.

Hemagglutination titers for *Ps. pseudomallei* were present in 239 (95.6 percent) of the imported animals. Eight had HA titers of 1:80, and three had 1:160 titers (Table 2). All of the 34 monkeys born in the United States had titers of less than 1:80 (Table 4). The distribution of titers had no apparent correlation with year of importation (Table 3) or age in the case of animals born in the United States (Table 4).

TABLE 2. *Hemagglutination titers for* Pseudomonas pseudomallei *in clinically normal imported macaque monkeys.*

	Hemagglutination Titer						
Species	<1:20	1:20	1:40	1:80	1:160	>1:160	Total Animals
M. nemestrina	60* (44.4)	41 (30.3)	29 (21.4)	4 (2.9)	1 (0.7)	—	135
M. arctoides	42 (45.2)	33 (35.5)	15 (16.1)	3 (3.2)	—	—	93
M. irus	12 (54.5)	7 (31.8)	—	1 (4.5)	2 (9.1)	—	22
Total	114 (45.6)	81 (32.4)	44 (17.6)	8 (3.2)	3 (1.2)	—	250

*Number of animals (precent of species group)

TABLE 3. *Hemagglutination titers for* Pseudomonas pseudomallei *in clinically normal imported macaques with known importation dates.*

	Hemagglutination Titer						
Year Imported	<1:20	1:20	1:40	1:80	1:160	>:160	Total Animals
1969	36* (43.4)	28 (33.7)	13 (15.7)	4 (4.8)	2 (2.4)	—	83
1968	28 (57.1)	10 (20.4)	10 (20.4)	1 (2.1)	—	—	49
1967	11 (34.4)	12 (37.5)	9 (28.1)	—	—	—	32
1966	28 (46.6)	21 (35.0)	9 (15.0)	1 (1.7)	1 (1.7)	—	60
1965	5 (41.7)	5 (41.7)	2 (16.7)	—	—	—	12
1964	—	1 (100)	—	—	—	—	1
Total	108 (45.6)	77 (32.5)	43 (18.1)	6 (2.5)	3 (1.3)	—	237

*Number of animals (percent of group imported that year)

The HA titers were determined for four of the culture-positive monkeys. These ranged from 1:320 to 1:10,240. In two of the outbreaks, 24 contact animals were examined at necropsy for evidence of melioidosis. Four of this group had been stressed with steroids prior to necropsy in an attempt to induce acute disease. Nine of the 24 contact animals had HA titers ranging from 1:80 to 1:2560 — five had 1:80, one 1:160, one 1:320, one 1:1280 and one 1:2560. The remainder had titers of less than 1:80. In addition, two animals with HA titers of 1:20 or less had complement fixation titers of 1:16. In none of the contacts were lesions compatible with melioidosis seen; nor was the organism recovered (Table 5).

Considering the foregoing data and the serious nature of the disease in man, a conservative interpretation of the HA titers was adopted. It was felt that titers of 1:40 or less were negative, 1:80 to 1:160 were suspect, and 1:320 or greater highly suspicious of active infection.

Because of the limited volume of monkey sera available and some technical problems, a comparable study of complement fixation serology was not possible.

TABLE 4. *Hemagglutination titers for* Pseudomonas pseudomallei *in clinically normal* Macaca nemestrina *born in the United States.*

Age Years	Hemagglutination Titer				
	<1:20	1:20	1:40	1:80 or more	Total Animals
3 or less	13* (92.9)	1 (7.1)	—	—	14
4 or more	13 (72.2)	4 (22.2)	1 (5.6)		18
Unspecified	—	—	2 (100.0)	—	2
Total	26 (76.5)	5 (14.7)	3 (8.8)	—	34

*Number of animals (percent of group)

TABLE 5. *Hemagglutination titers for* Pseudomonas pseudomallei *in non-human primates with culture confirmed cases or in contact animals subsequently necropsied.*

Category	No. Animals	HA Titer	Comment	Culture
Case	1	1:10,240	Case 1, outbreak 1	+
Case	1	1:320	Case 2, outbreak 1	+
Case	1	1:2560	Case 3, outbreak 1	+
Case	1	1:2560	Outbreak 3	+
Contact	2**	1:80-1:160	Outbreak 1*	—
Contact	7	1:80-1:2560	Outbreak 3	—
Contact	13	<1:80	Outbreak 3	—

*Stressed with steroids in an attempt to induce acute disease.

**Two additional contact animals in outbreak 1 had complement fixation titers of 1:16 for *Pseudomonas pseudomallei* but HA titers of 1:20 or less.

Disease in Human Contacts

In the three outbreaks, all personnel having contact with nonhuman primates were serologically screened for evidence of infection. Of 115 people serologically examined, ten had HA titers ranging from 1:80 to 1:320; the remainder had titers of 1:40 or less. No evidence of active human infection was found.

Discussion

The lesions observed in the current cases are consistent with those described previously in nonhuman primates as well as in man.[1,7,10,11] The lesions are similar in all involved organs. There is usually a white to yellow exudate which may have the "earthy" odor associated with the organism. About a central core of this purulent exudate, there is frequently a zone of fibrin and early proliferation of fibrous connective tissue containing many neutrophils, macrophages, and areas of hemorrhage. The tissue of the involved organ peripheral to this outer zone is often necrotic, infiltrated with neutrophils, and contains areas of hemorrhage. The lesions are not granulomatous though they were described as such in the earlier literature.[6] While *Ps. pseudomallei* is easily cultivated from the lesions, the organism is often difficult to demonstrate in histologic preparations.[6] We have found it easy to demonstrate the organism in sections cut from routinely processed, paraffin-imbedded tissue blocks using fluorescent antibody techniques.

An unusual clinical feature observed in cases 1 and 3 during the first outbreak was the light green serum which probably reflects acute liver damage. This is supported by the high serum transaminase levels in the chimpanzee.

The striking feature of this disease is the long incubation period which may be observed. In four of the cases, the animals had been out of an endemic area for 6 months to 3 years. In one case, in a chimpanzee, the animal had never been in an endemic area and probably acquired its infection in the laboratory. The pathogenesis of the spontaneous disease is not known. The apparent long incubation periods probably reflect an early acute or subclinical infection with consequent persistence of a nidus of infection, possibly as a focal osteomyelitis. Then in times of stress the persistent nidus breaks down producing an acute manifestation. Definite proof of such a sequence, however, has not been obtained.

In three of the five cases, first signs of the disease appeared at the site of a surgical procedure. In two, this was at the site of implanted foreign objects, and in one at the site of an old surgical wound. For this reason, one should be alert to the possibility of melioidosis when dealing with surgical wound infections in monkeys from endemic areas.

If an isolate from an animal is suspected of being *Ps. pseudomallei,* it should be handled with caution. Laboratory infections have occurred and can be quite serious.[4] All suspect isolates should be submitted to a laboratory experienced in handling the organism for identification or confirmation.

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