

STUDY REPORTS

5. Title: Enterobacter cloacae infections in Gibbons.

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OBJECTIVE

The objective of the study is to determine the clinical and pathological characteristics of Enterobacter cloacae and to discover the method by which it becomes a lethal pathogen in gibbons.

DESCRIPTION & PROGRESS

This study is based on two cases of Enterobacter cloacae infections in gibbons that have occurred in the gibbon colony. The striking feature of the disease that this common intestinal inhabitant causes in gibbons is its virulence. In order to present the possible factors that may transform this organism into a virulent pathogen and to describe the characteristics of natural infections, each of cases that occurred is summarized.

1. Gibbon S-63. This mature female gibbon was purchased on June 30, 1966, splenectomized, and subsequently used in malarial transmission studies. Following work with malaria, this animal was used for dengue virus work which included infection by several types of dengue virus and the collection of a number of venous blood samples. On November 14, 1968, the animal suddenly died without showing any signs of prior clinical illness. At necropsy, the liver was enlarged and had rounded edges. On the lung surface diffuse light pink areas that were slightly raised over the rest of the lung were observed. The cerebral vessels of the brain appeared to be injected. Samples of liver, lung and blood were collected for bacterial and viral isolations. Histopathological examination showed that the lungs contained a widespread interstitial inflammatory infiltrate with large and small mononuclear cells and a few polymorphonuclear cells. Many short plump bacilli were found in blood vessels as well as intra- and extra-cellularly, throughout the body. Other organs such as heart, liver, kidney, lymph nodes, and the central nervous system were congested. Enterobacter cloacae was isolated from each of the organs and blood samples submitted from the necropsy for culture.

2. Gibbon S-60 was also a mature splenectomized female. It was purchased on April 14, 1966 and was also used in malarial studies. This animal had a history of recurrent upper respiratory infections over a two year period that were treated with a number of antibiotics. In late 1967 the animal was also placed on the dengue virus study, and was treated again for a respiratory infection that continued intermittently for a period of time until November 1968. In late November the animal became progressively weak and lethargic and was returned to Bangkok where it became anorexic and suffered from diarrhea. Shortly after the return to Bangkok the right inguinal lymph node became enlarged and a complete blood count showed a neutrophilia with a degenerative left shift and hypochromic anemia. Because the condition of this animal was rapidly deteriorating, it was sacrificed on the 17th of December, 1968. The condition of the lungs was the most striking finding. They were dark red in color, dense, and non resilient; normal appearing lung tissue could not be found. A large ulcer with a diameter of about 5 millimeters was present on the surface of the tongue. The liver was swollen and a number of petechial hemorrhages were distributed uniformly on its surface. The mass located in the inguinal area was approximately 2 1/2 cm. across and was composed

of a thick fibrous wall and a necrotic center with semi-fluid contents. Microscopically, the lungs were almost completely consolidated. The airways were filled with polymorphonuclear and mononuclear cells, fibrin, necrotic debris, and masses of bacteria. The sinusoids of the liver were distended by swollen Kupffer cell and phagocytic inflammatory cells containing bacteria and debris. The ulcer on the tongue was filled in with granulation tissue which contained many bacilli; no inclusion bodies or fungi were observed. Interstitial inflammation was found in the kidney. Enterobacter cloacae was isolated in pure culture from the lungs, blood, liver, inguinal abscess, and heart blood. Both the pathological and bacteriological findings supported a diagnosis of a long standing bacterial infection with complications of pneumonia and chronic interstitial nephritis.

Enterobacter cloacae has been recovered a number of times from apparently normal as well as sick animals in the colony but has been thought until this time to be a non pathogenic inhabitant of the gut. However, because it was incriminated in two terminal cases of illness in gibbons it appeared, that a study of the pathogenicity of this organism in the primate colony was in order.

To determine the pathogenicity of the Enterobacter cloacae, two gibbons, B-60 and B-72, were inoculated with 20% tissue suspensions made from the lungs of gibbon S-60 and suspensions of an Enterobacter cloacae isolate cultured from the same animal. Gibbon B-60, an adult male, was given 1 ml. of the lung suspension intravenously, four drops intranasally, and four drops on the pharyngeal mucosa. B-72, an adult male, was given the suspension of Enterobacter cloacae by identical routes. The clinical course of the experimental infection was followed closely. Gibbon B-72 died less than 48 hours after the administration of the organism. The death was preceded at least 24 hours by lethargy and obvious malaise. A moderate increase in temperature of 103.2°F. was recorded at the time clinical signs of illness were first observed and remained elevated until death. The gross findings at the necropsy of this animal were unremarkable. However, Enterobacter cloacae was recovered from the lungs, liver, and heart blood. Gibbon B-60 developed a hemiplegia about 36 hours after inoculation with the organism and was unable to move his left arm and leg. As gibbon B-72 had, this animal had showed signs of lethargy and depression and its temperature reached 104°F. 48 hours after inoculation. Because the condition of this animal was obviously terminal it was sacrificed at 55 hours following inoculation after withdrawing a blood sample for bacteriological examination. A pure culture of Enterobacter cloacae was cultured from the blood. Post mortem examination of this animal showed large focal areas of liver necrosis, hyperemic vasculature on the cerebral surface of the brain and a necrotic area deep in the thalamus that was 1 centimeter in diameter. Focal necrosis was also observed in the rectum and stomach and the vermiform appendix was inflamed. Histopathologic and bacteriologic examinations of the material taken from both animals indicated that deaths were caused by Enterobacter cloacae.

The pathogenicity of this organism in mice was established by innoculating random bred albino mice with suspension of Enterobacter cloacae organisms isolated from the lungs of gibbon S-60. Both tissue and bacterial suspensions were diluted serially to dilutions of 10^{-10} , and mice were inoculated both by the intraperitoneal and intranasal routes. Mice inoculated intraperitoneally all died within three days at dilutions as great as 10^{-8} . The virulence of this organism was not as great when given intranasally although deaths occurred at dilutions as great as 10^{-6} . Enterobacter cloacae was consistently isolated from all mice randomly selected for bacteriological examination. Because the possibility existed that virulence in mice might be due to endotoxin formed in the bacterial cultures, a second titration of the organism was run to exclude this possibility. A culture of Enterobacter cloacae was grown in brain heart infusion broth for 24 hours and the resulting culture was washed, centrifuged, and resuspended three times in phosphate buffered saline. The culture was then immediately inoculated intraperitoneally into groups of mice in dilutions ranging from 10^{-1} to 10^{-10} . Plate counts of each dilution of the organism were performed and appropriate calculation showed that the LD₅₀ of Enterobacter cloacae treated in this manner was a dilution of $10^{-8.3}$.

Further work to be conducted will be directed towards determining the approximate dose of Enterobacter cloacae that is pathogenic for gibbons and by what route natural infections may occur. Initially, two gibbons will be inoculated with a mouse LD₅₀ weight equivalent dose of Enterobacter cloacae. The intranasal route will be tried first and the intravenous route will be tried later if the intranasal inoculation proves to be unsuccessful in producing disease.

It must be concluded from current clinical findings and laboratory results that Enterobacter cloacae is sometimes a virulent pathogen for gibbons. This conclusion is justified by the occurrence of two cases of infections with Enterobacter cloacae in gibbons that terminated in fatal illness. Organisms that were isolated from both these cases were identified as Enterobacter cloacae in two different laboratories. Koch's postulates were fulfilled in incriminating Enterobacter cloacae as the etiological agent when both gibbons and mice died following reinoculation in in vitro cultures of the organism. Previous bacterial surveys have indicated that Enterobacter cloacae is a normal inhabitant of the intestinal tract of most of the primates in the colony and is detected in approximately 75% of the gibbon stools randomly sampled. Both of the naturally infected animals had a history of repeated blood sampling. It therefore seems very likely that infections could easily be introduced at the time blood is withdrawn from the animals for experimental purposes. In support of this theory is the large abscess from which Enterobacter cloacae was cultured in S-60 adjacent to the femoral vein; the site where blood samples are most frequently drawn. The course of the disease in gibbon S-60 was not acute as it was in S-63 and this is probably because of the suppressive effect of the antibiotics that were given for treatment of the respiratory infection. Although the possibility of airborne infection exists, the infrequent occurrence of this disease suggests that Enterobacter cloacae is accidentally introduced when blood samples are drawn.

SUMMARY

The natural occurrence of two cases of terminal illness caused by Enterobacter cloacae in gibbons is described. Laboratory work following the necropsy of these animals has established the pathogenicity of this organism for both gibbons and mice. Further work is being conducted to determine by what route natural infections may occur, what doses of the organism are necessary to cause natural infections, and what the predisposing factors to this type of infection are.