AD NUMBER
AD843462

NEW LIMITATION CHANGE

TO
Approved for public release, distribution unlimited

FROM
Distribution authorized to U.S. Gov’t. agencies and their contractors; Administrative/Operational Use; JAN 1968. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701.

AUTHORITY
Fort Detrick/SMUFD ltr dtd 15 Feb 1972

THIS PAGE IS UNCLASSIFIED
DDC AVAILABILITY NOTICE

Reproduction of this publication in whole or in part is prohibited. However, DDC is authorized to reproduce the publication for United States Government purposes.

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/T10, Frederick, Maryland 21701

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland
A CASE OF EQUINE CEREBRAL CRYPTOCOCCOSIS IN LEOPOLDVILLE


V.Herin and R.Dormal Veterinary Laboratory, Leopoldville.

(Manuscript received on 30 November 1962)

Cryptococcosis, also known as torulosis or European blastomycosis, caused by Cryptococcus neoformans, is a disease of cosmopolitan distribution in man and animals. On several occasions, it had been reported in humans in the Congo. As far as we know, a naturally occurring infection among animals of that area has not yet been reported. We present a brief report on the first case of animal cryptococcosis in a horse at Leopoldville.

Clinical Observations

Early in October of 1961, the 10 year old mare "Kashtan" was examined because of its poor condition in comparison with other horses. The hide was dull. There were cutaneous lesions on the head. Between neck and shoulders and on the back a contagious dermatosis developed. Microscopic examination of the saddle and of the dermic exudate indicated the presence of eggs of Strongylus and Dermatophilus congolensis. A vermifuge treatment with phenothiazine and five injections of regular doses of penicillin-streptomycin produced an overall improvement of the general condition of the mare.

In December of 1961, abscesses appeared on the interior surface of the thigh; then, around the anus and one abscess formed on the lower portion of the tail. The abscesses ripened, grew to the size of a pigeon egg and contained viscous, yellowish pus. A test for Rivolta's cryptococcus (Histoplasma farciminosum) was negative.

The treatment was penicillin, combined with streptomycin, was in accordance with the results given by an antibiogram, and was given
simultaneously with potassium iodide. The abscesses, of which one was very deep near the anus, were cured without any difficulties in the docile mare.

In the evening of January 21, 1962, about one month after the last abscess had healed and after the general condition of the horse had improved, there appeared suddenly very serious nervous disorders after a normal ride. Violent myoclonic seizures affected all muscles from the head to the tail in decreasing intervals. These irregular spasms produced dyspnea, ataxia, rearing movements, facial grimaces and grinding of the teeth. Rectal temperature rose to 38°C; pulse was feeble, but regular and of normal frequency. The symptoms looked like the start of meningoencephalitis and a new round of treatment with penicillin-streptomycin was begun, but with a conservative prognosis. On the second day, the temperature reached 39.5°C and the nervous systems became weaker. Quite unexpectedly, symptoms disappeared almost completely after one week. There remained only isolated and occasional tremors; a hesitant gait and, especially, an intermittent weakness in the right leg, quickly corrected by the mare. The body temperature was normal and the general condition and main functions became again satisfactory.

By mid-February, the mare seemed cured and was ridden again. However, she went only for one ride. From that moment on, a syndrome of paralysis gradually developed and the mare was slaughtered on March 22.

In the meantime, research continued to establish the etiology of the disease. The effort was in vain. An examination of the blood ruled out infectious anemia, Nuttall's and Schweinsberg diseases.

Takata's test was strongly positive after 30 minutes. Total bilirubinemia was 2.32, direct bilirubinemia 0.80 mg/100 ml, urea 34 mg/100 ml, total protein 5.43 g/100 ml, but the dosage of polypeptides could not be determined. These analyses were done by the Biochemistry Division of the Princesse Astrid Institute in Leopoldville.

Sedimentation of the oxalated blood, determined according to Noelze, did not show any difference between healthy horses and the sick mare. Hemoglobin content was 10 gram. There were present among the leucocytes: lymphocytes 34.1%, monocytes 1%, neutrophils 63.2% and eosinophils 1%. These figures are normal for a horse.

Autopsy Results

An autopsy was performed on the slaughtered mare. Liver, spleen, kidneys, respiratory tract, heart, genitals and various ganglia appeared to be normal. The only lesions were found in the brain.

The left cerebral ventricle was strongly dilated and filled with a gelatinous, partly hemorrhaged pulp which adhered to the side of the wall.
A similar swelling was also observed on the right side, next to Ammon's horn. In different portions of the brain there existed numerous tiny foci and one major focus in the cerebellum, seen as a nut-like swelling in the section that was inspected.

Meninges, along their entire length, were strongly congested.

**Diagnosis**

Microscopic examination of the gelatinous pulp of the brain revealed instantly the presence of a yeast with a characteristic capsule. Morphology indicated the genus *Cryptococcus*. The pathogen was isolated on glucose agar, grew well at 37°C and produced smooth, viscous, confluent colonies which slowly turned yellow over a period of time.

Intracerebral injection into white mice produced a fatal encephalitis within 3-7 days. Intracerebral pressure produced a swelling of the cranium and the meninges showed a strong hemorrhage. Cryptococci multiplied rapidly on the meninges, but were also isolated from nerve substance. The brain of one mouse was completely destroyed and transformed into a pulp filled with yeasts.

Two guinea pigs were inoculated intramuscularly with 1 ml of an emulsion of the gelatinous pulp, diluted 1:20 in physiological saline, before performing diagnostic microscopy. One specimen was a female which was inoculated with an emulsion free from antibiotics. She died after seven weeks. She gave birth to two young guinea pigs which are healthy and still alive after two months. The other specimen, inoculated with an emulsion containing penicillin-streptomycin, was a male, which died after eight weeks from lesions that were much more spectacular than those in the female guinea pig.

Both guinea pigs presented a picture of inflammatory swellings, grossly hemorrhagic at the spot of the inoculation, which became purulent in the female. Pink peritoneal exudate and grayish lungs with granulations were observed. The ureters of the male were swollen by a clear, gelatinous mass, but were free from yeasts. The urine, obtained from the bladder, was free from yeasts, when examined directly or after centrifugation.

Morphology, cultural characteristics and virulence indicated *Cryptococcus neoformans*. This diagnosis was also confirmed by an anatomocopathological examination.

**Histology**

1) Brain. The outer membrane of cerebrum and cerebellum contained multiple foci of cryptococci in different phases of development. Spleen, kidneys, and skin were also examined. We should have inspected the integrity
of the lungs, but did not do it, because we were not interested in them during the autopsy and were completely ignorant about the diagnosis. The microscopic aspect of the lungs was normal by inspection and palpation.

In the liver, a few yeasts were seen at a right angle to a large venous vessel, filled with blood. The liver cells contained strands of bile pigments.

The spleen was normal. No yeast was seen in sections of the kidneys, but the cortex contained microscopically small, hemorrhagic foci.

The skin, likewise, was free from pathogens. At our first visit, the skin was studded with very soft, wart-like growth, 2-3 mm in diameter and localized over areas of thin skin. Similar lesions existed in the buccal mucosa. They did not resemble cryptococcal lesions.

2) Female guinea pig. The abscess at the point of inoculation turned into a necrotic focus, containing pus and neutrophils. The yeast was found in the periphery of the lesion.

The liver contained an occasional pathogen without a local reaction.

The kidneys were more strongly affected. There actually existed masses of yeasts in the cortex, close to the fibrous capsule, while other yeasts occupied partly the glomerular spaces.

Heart and pericardiac sac were free from yeasts. Occasionally, a yeast could be seen in the blood of the heart cavity. Masses of cryptococci occurred in the fatty bodies and lymphoids, located near the base of the heart and lungs. The lungs were completely invaded. Death was probably due to asphyxiation.

3) Male guinea pig. The lesions differed somewhat from those of the female specimen. Lesions at the site of the inoculation and in corresponding ganglia were hemorrhagic, stuffed with cryptococci, but without any trace of purulent inflammation.

The liver showed a perisushepatic degeneration. However, no cryptococci could be seen in sections.

In kidney sections, a few yeasts were seen in the wall of the large vessels and in their immediate vicinity. The tubuli and Bowman's capsule contained granular matter, like the one seen in the ureters.

In both guinea pigs, cryptococci occurred abundantly on the surface of the peritoneum and often singly or in small colonies on the membrane or in the serum of abdominal organs.
Discussion

The origin of Cryptococcus neoformans, observed in a case of equine cryptococcosis, was not known, because of our failure to make an exhaustive study of the lungs of the mare which may have contained some older lesions. Injections of antibiotics may have stimulated a latent infection of the lungs or, more readily, the abscesses on the front legs. These abscesses may have served as an entrance for a new pathogen. In fact, Van Uden et al. isolated a culture of Cryptococcus neoformans from the caecal contents of a healthy horse among 252 horses examined.

Cryptococcosis comes readily to mind when one remembers the nervous involvement which developed without a fever or with only a slight fever. Gelatinous lesions were characteristically present, indicative of the causative organism and easily diagnosed. However, ante-mortem diagnosis is difficult because cases are rare. A study of cephalo-rachidic fluid from the occipito-atloidal space would have definitely led to the proper diagnosis of the illness.

Summary

A case of equine cryptococcosis was observed in Leopoldville. This was the first time this disease had been found in an animal in the Congo. Macroscopic and post-mortem microscopic lesions observed in the horse and in inoculated laboratory animals were described in detail.

Bibliography


