Contribution to the Study of Bovine Paralytic Rabies in Venezuela

Aportacion Al Estudio de la Rabia Paralitica Bovina en Venezuela

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PARALYTIC rabies of cattle is an infectious disease known in South America since 1911, when Carini first described it in Brazil. In subsequent years it has been diagnosed successively in other South American countries, and nowadays it has spread over almost the whole South American continent. A peculiarity of this disease is that it is transmitted by blood-sucking bats which feed on animal as well as human blood. This fact has been proven beyond doubt by a number of authors during the years 1931 to 1936. The agent of the condition is a neurotropic virus, in many respects identical with the virus of canine rabies. It possesses, however, some biological and immunological characteristics which the canine virus lacks. This is due, in the opinion of workers in this field, to the continuous passage of the virus in bats.

In Venezuela paralytic rabies was diagnosed for the first time in 1938. As it was the cause of heavy losses of cattle the Government decided to undertake a big campaign against it, consisting principally in the vaccination of cattle in the diseased areas. The author, while in the service of the Ministry of Agriculture and Animal Husbandry, had the opportunity to study the disease in the field as well as in the laboratory and, in 1939, started the production of the vaccine.

The work in regard to the artificial transmission of the disease has been carried out on cattle, goats, pigs, and dogs. The conclusions from these experiments may be summed up as follows:

1. Cattle and goats are very susceptible to paralytic rabies virus.
2. Pigs and dogs are very resistent to the virus.
3. Paralytic rabies virus may be transmitted to large domestic animals with security only by the intracerebral route. The transmission was successful in only about 70 per cent of animals when the virus was given intradermally, rubbed into the scarified skin or nasal mucous membrane or injected into the peripheral nerves. All other routes were insecure.
4. Dogs and pigs were susceptible to the virus only by the intracerebral route.
5. The incubation period of artificially infected calves oscillated between 8 and 39 days, depending on the route of inoculation, the concentration of virus, and the kind of animal.
6. The presence of paralytic rabies virus in the blood of one sick cow could be demonstrated.
7. Virus was present in the saliva of sick calves. However, using

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calves as experimental animals, the virus could not be found in the salivary glands, pancreas, suprarenal glands, the liver or extracted from cows dying of paralytic rabies.

9. Experiments to detect the presence of the virus in ticks collected from sick animals were negative, though white mice were used as experimental animals.

During our studies on the clinical course of the disease in artificially infected, as well as in spontaneously ill animals, we never observed a furious form of rabies. Some calves which were wild and attacked people before being inoculated, became more aggressive with the first signs of the disease. However, this increased aggressiveness soon abated when the first symptoms of paralysis became evident. When the virus was injected into the peripheral nerves, the animals showed pain in the injected leg as the first symptom. Similarly, the intradermal injection of the virus was followed by intense itching and tenderness of the inoculated site. The animals tried to rub, lick and bite the place of inoculation; later on, however, they were very anxious not even to touch it. Symptoms of paralysis often became evident as early as one hour after the first signs of the disease. Paralysis of the jaws was never observed in large-sized animals or in artificially infected dogs. The disease lasted 3 to 6 days, but the majority of animals died at the end of the fourth or at the beginning of the fifth day.

Post mortem examination gives the same results as canine rabies, the only difference being that we never found any foreign bodies in the stomach, not even of artificially infected dogs. Negri bodies were present in the brains of about 70 per cent of the animals, and they were always rather small. White mice were very useful for the biological diagnosis of the disease. They were very susceptible, and the incubation period of 8-12 days, as compared with that of 14--22 days in rabbits and guinea pigs inoculated with the same material, made a quick diagnosis possible.

Grown-up calves, 6-9 months old, were used for production of the vaccine. The intracerebral inoculation was performed by the frontal route. The frontal drilling of the skull of calves is easy. They withstand the operation well and there are no complications, provided the brain tissue has not been injured by the trepan.

The author decided to use a local paralytic virus for the production of the vaccine. He supposed that this virus, having certain biological characteristics in which the canine virus is lacking, may possess some different immunological properties as well. Before using the local virus for the elaboration of the vaccine, it was fixed in calves by a number of continuous intracerebral passages. Later on a second paralytic rabies fixed virus was added for use in the production of vaccine.

The first fixed virus originates from the southern parts of Venezuela, from the State of Bolivar. The incubation period of the first intracerebral passage in calves was up to 18 days. Then it became quickly shorter and attained 7 days in the 4th passage. It decreased to 6 days in the 12th passage and remained so up to the 52nd passage, when it fell to 5 days, where it has constantly stayed up to the 275th passage. The clinical course of the disease in the first two passages was identical with that of the disease acquired in the field, the duration being from 4 to 6 days. With

the number of passages increasing, the short prodromal symptoms of irritation disappeared, and paralysis, extending quickly over the whole body, became the first symptom of the disease. The salivation, a notable feature of the disease in the first passages, also disappeared quickly. The duration of the disease became shorter and shorter, so that by the 12th passage, some animals died 24 hours after first showing symptoms of the disease. Negri bodies, found in the first two passages in the brains of almost all the animals, became rarer from the 3rd passage on and were never found after the 7th passage. The virulence of the virus for calves increased with the number of passages. The lethal dose of virus for calves after the 2nd passage was the dilution infective, namely 1:4000 after the 2nd passage, and 1:5000 after the 63rd up; it may be said that the Bolivar virus began to show the characteristics of a fixed virus after the 12th passage.

The second strain of paralytic virus, fixed in calves, was isolated in an area where paralytic rabies of cattle was diagnosed in 1938. It is called *Maturin* virus. The incubation period in the first two passages was 10 and 11 days, respectively. It fell to 7 days in the 7th passage and to 6 days in the 14th passage. It remained at 6 days up to the 252nd passage. As happened with the Bolivar virus, the increasing number of passages of the *Maturin* virus also led to more marked symptoms of paralysis and to a shorter duration of the disease. This, after the 12th passage, became consistently short (2 days) and decreased after the 63rd passage to even one day in some animals. Negri bodies disappeared after the 5th passage. The dilution of the *Maturin* virus, still infective, was 1:4000 after the 2nd passage, and 1:5000 after the 63rd passage.

As soon as the Bolivar virus showed signs of being fixed, the production of our anti-rabies vaccine was started. The brains and spinal cords of calves killed when in an advanced stage of the disease were roughly ground in a meat chopper and diluted with an equal amount of physiologic saline containing 2 per cent of phenol. This suspension was further ground to a fine emulsion in a colloid mill and then attenuated for 6-24 hours at 37°C. and for two days at room temperature in a dark room. The vaccine was then further diluted with saline containing 1 per cent of phenol, so that the final product consisted of a 15 per cent emulsion of the nerve tissue. Sterility was assured with the 1.5 per cent phenol, so that the final product was innocuous and protection tests were carried out and, when satisfactory, the vaccine was released.

The antigenic properties of the vaccine were examined according to Habel's mouse test, the Bolivar fixed virus being used as challenge virus. The protection conferred by the different batchs of our vaccine was always higher than that required for a standard vaccine in the United States of America. Gallia and Kubes showed that the vaccine containing Bolivar paralytic rabies fixed virus conferred on mice better protection against the Bolivar virus than a vaccine containing Pasteur fixed virus. Their work seems to be confirmed by Colonel Raymond Randall, V.C., Director of the U.S. Army Veterinary School.

The vaccine containing Bolivar virus keeps its antigenic properties unchanged for 6 months, when stored in a dark cool room. The vaccination gives very good results in the fight against paralytic rabies in Venezuela. The disease is now well controlled and has ceased to be a problem because of the preventive vaccination of all susceptible animals.