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A Virus Diarrhea of Newborn Calves

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A Virus Diarrhea of Newborn Calves

Arlan W. McClurkin, D.V.M., Ph.D.

The diarrhea of newborn calves in Wisconsin is characterized by a watery, yellow diarrhea which begins 1 to 2 days after birth. The age at which the calf is exposed, the feeding of colostrum, and the method of exposure are critical factors determining the character of the clinical manifestations of the experimental disease. The fact that the aerosol method of exposure was the means by which the acute type of the disease has been produced suggests that this is largely an air-borne disease. The etiological agent of this disease has been characterized as a virus. Serial transmission of a bacterial free inoculum in calves together with serial passage in the gravid uterus of mice and guinea pigs have been accomplished.

Further support for the viral nature of the agent is provided by its ability to pass through Schleicher and Schuell membrane ultra filters of 50 to 80 millimicron effective pore size. Furthermore, electron microscopy of purified material revealed a virus-like particle, present in infectious material, strengthening the support for the viral nature of the agent.

The inability to grow except in the presence of living cells, and the resistance to certain antibiotics also attest to the viral character of the agent.

The clinical terminology which has been used in describing this acute diarrhea disease is defined briefly as follows:

1. Peracute fatal — those animals which died within 24 hours of exposure.
2. Acute fatal — those animals which died two or more days following exposure.
3. Sub-acute non-fatal — those animals which exhibited signs of the disease for several days, then began to recover.
4. Abortive — those animals which showed signs of disease for only a day or less. A few of these animals appeared to be in considerable distress only for 12 to 18 hours, following which they were active and had no more diarrhea.
5. Inapparent or subclinical — those animals which showed no evidence of diarrhea and which remained in apparent good health. Because facilities did not permit the management of unlimited numbers of animals, many calves were sold when they attained a marketable condition. Some were destroyed because of the time required for recovery.

The aerosol method of transmission has been utilized in the characterization of the virus. The experimental observations and the conclusions drawn therefrom are largely based on experimental work dur-
ing the period from September, 1952, to December, 1955; a total of 288 calves was employed.

Studies of the comparative gross and histopathological changes among apparently healthy non-exposed calves and experimentally exposed calves were undertaken in an attempt to ascertain what changes in the calf tissues could be attributed specifically to the virus. The non-exposed calves, which served as controls, were from herds which appeared to be free of the clinical disease, but in which the infection could have been present in subclinical form.

The purpose of the investigation reported here has been to identify and characterize a virus capable of initiating an acute disease of newborn calves similar to the naturally occurring condition commonly seen on the farms.

**PLAN OF EXPERIMENT**

**Procurement and Maintenance of Susceptible Calves**

All calves were separated from the dam and the rest of the herd as soon after birth as possible. The newborn calves were placed in a separate building on the farm, or weather permitting, tied outside. They were brought to the laboratory from the farm at the earliest convenience, usually one to 10 hours after birth.

Colostrum was withheld and the animals were less than 24 hours of age when they were exposed. Upon arrival at the laboratory a blood sample was taken. The serum, separated by centrifugation of the clotted blood, was tested for the presence of globulins by the serum turbidity test, using dilute zinc sulfate, as developed and described by Aschaffenburg (1-5).

In the earlier part of the investigation the calves were held outside separated from each other by tying to stakes approximately 50 feet apart. Later in the fall the experimental animals were maintained in separated units of an improved Rokefeller Foundation-type isolation building. Employing nipple pails the calves were fed on warm pasteurized skim milk at the rate of three to four pounds twice daily.

**Method of Exposure**

Exposure to the virus was effected with a No. 166 vaponefrin reflux nebulizer that produced an infective mist which the calf breathed while confined in a metal chamber of 14 cubic feet capacity. The nebulizer was operated with air pressure at 10 pounds per square inch. The droplet size was not measured. The chamber was cleaned after use by flushing with water at 180°F.

**Preparation of Inoculum**

When adaptation of the virus to the gravid uterus of mice and guinea pigs was under investigation, proof of the presence of the virus in the rodent tissues was accomplished by the successful exposure of susceptible calves to an aerosol prepared from gravid mouse or guinea pig uterus with fetal fluids, membranes and fetal viscera. The tissue was minced in a Waring blender without addition of fluid and placed in 50 ml. screw cap vials and held overnight at -40°C. The tubes containing the tissue were placed directly in the centrifuge on the following morning. Centrifugation at 3,000 rpm was started while the suspension was in the frozen condition and continued for 30 minutes, as the material thawed. A variable quantity of supernatant fluid was pipetted off and stored at -40°C as the undiluted virus bearing tissue. When an inoculum was prepared, portions of the virus bearing tissue were thawed and passed through Seitz ST pads to remove larger particles. Absence of bacteria in the filtrate was routinely determined by means of inoculation into glycollate fluid medium which was incubated at 37°C for 48 hours. Occasionally fluid and solid Difco PPLO media, and blood agar were used to demonstrate the sterility of the filtrate.

When experimentally infected calf lung or bovine lung from apparently healthy animals was tested for virus content, the lung tissue was minced in a Waring blender with the addition of a minimal amount of saline and the fluid removed by initiating centrifugation while the tissue was in the frozen condition.

**Preparation of Virus for Study of Physical Characteristics**

In studying the physical characteristics of the virus, suspensions of several virus bearing tissues were filtered, and subjected to different environmental conditions, or treated with various chemicals. The treated virus suspension was evaluated for pathogenicity and infectivity by exposing the naturally susceptible host, the newborn colostrum-free calf, to an aerosol of the manipulated material. The concentration and quantity of the treated test material was the same as that, which prior to manipulation had proved lethal. If disease failed to develop from the exposure, or only an abortive attack occurred the virus was considered to have been removed or inactivated.

To facilitate study of the physical characteristics of the virus, purification by differential centrifugation was employed. Lung tissue from calves manifesting signs of the acute disease was suspended in a minimal quantity of saline by means of a Waring blender. The suspension was placed in 50 ml. plastic vials and held at 56°C for one hour, then frozen. Centrifugation at 3,000 rpm in an International centrifuge was initiated while the tissue was in the frozen condition. This facilitated separation of the fluid. The supernatant fluid was decanted and extracted overnight at 4°C with twice its volume of diethyl ether. The ether fraction was separated by means of a separatory funnel and discarded. Extraction of the ether from the remaining fluid was accomplished by holding at room temperature under a reduced atmosphere of 0.5 mm of mercury until all ether was evacuated.

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Sedimentation of the larger particles from the aqueous fraction was accomplished by centrifugation in a Sorvall type M angle centrifuge running at maximum speed for 45 minutes. The supernate was then placed in rotor number 30 of the Spinco ultra centrifuge model L and the particles sedimented at 25,000 rpm for four hours with an average centrifugal force of 54,450 times gravity. The supernate was discarded and the pellet resuspended in saline. Centrifugation of the suspension at 2,500 rpm in an International centrifuge size 1 type SB for five minutes removed the larger particles which were set aside for estimation of virus content. The supernatant fluid was sedimented in the number 40 rotor of the Spinco for three hours at 50,000 rpm, an average of 39,310 times gravity. The supernate was discarded and the pellet resuspended in distilled water, the suspension was centrifuged at 2,500 rpm for five minutes to remove the larger particles, which were set aside. The supernate was again sedimented, for three hours at 25,000 rpm in rotor number 40 at an average of 41,190 times gravity. The supernate was discarded and the pellet resuspended in distilled water. The larger particles which did not go into suspension readily were removed by centrifugation at 2,500 rpm for five minutes. The supernate was then alternately frozen and thawed four times using low speed centrifugation for 30 minutes in the Sorvall angle head centrifuge after each thawing. This process produced a precipitate which was separated and set aside. A small portion of the supernate was diluted 1:100 and placed in a number 166 vaponefrin nebulizer with an equal number of particles of possible virus nature, for the purpose of retaining the larger droplets, and slightly under focus negatives were selected. Air pressure at the rate of eight pounds per square inch was applied to the nebulizer with a centrifugal pressure pump. After the nebulizer and the tube had been flushed with mist for 30 seconds, collodion treated 200 mesh electron microscope specimen screens were exposed to the mist for one minute at a distance of one inch below the end of the glass tubing. The specimen screens, with the virus preparation, were shadowed at an angle of 11 degrees with uranium and examined with an R.C.A. E.M.U. 2 electron microscope for the presence of particles of possible virus nature. Through-focus series were taken in most cases, and slightly under focus negatives were selected for reproduction.

Lung tissue from a healthy newborn calf was also prepared and examined by electron microscopy.

Pathological Changes

In order to make a comparative study of the gross and histopathological changes of apparently healthy non-exposed calves and experimentally exposed calves eight healthy calves were sacrificed for tissue studies. These were taken from three different herds in which there had been no clinical evidence of disease among the calves during that particular year. Six of the calves were under 24 hours of age, one was two and one-half days old and the eighth was 10 days old at the time of sacrifice. The calves were stunned, exsanguinated and, at autopsy, appropriate sections of various organs were removed and placed in 10% neutralized formalin in preparation for paraaffin imbedding and histological examination.

Random lung samples were taken from 70 cows and 66 calves at the time of slaughter in a local packing house. All specimens were from carcases that had passed United States Department of Agriculture Research Service inspection. Twenty-eight experimental calves showing signs of acute and subacute disease were sacrificed or autopsied immediately after death. Appropriate sections of tissue were prepared for histological examination.

Some Physical Characteristics of the Virus

Investigations to determine the stability of the virus to heat, ether, formalin, low and high pH, storage at -20°C, drying at 25°C for one year and to chloromycetin, terramycin, penicillin and streptomycin are recorded in Table 1. The results suggest that the virus is stable to various environmental factors and chemicals.

Preliminary work indicated that the virus would pass through Seitz ST and EK filter pads through sintered glass UF filters and HA millipore filters of 500 mu effective pore size. A further attempt to evaluate the size of the virus particle was accomplished by passing 10 ml. of a 10⁻² dilution of a purified lung tissue suspension through Schleicher and Schuell membrane filters of 10 to 50 mu as well as 50 to 80 mu effective pore size. The calf exposed to the virus material which was passed through the filter of 50 to 80 mu effective pore size developed the acute disease indicating the virus was less than 50 to 80 mu in diameter. The calf exposed to the virus material which was passed through the 10 to 50 mu effective pore size remained healthy indicating that the virus had been retained.

An unfiltered portion of the purified lung tissue suspension, which had been demonstrated to contain a lethal quantity of the virus in the 10⁻¹ dilution, was found on examination by electron microscopy for possible virus particles to contain a uniformly spherical particle of 17 mu diameter (Figure 1). This particle was not found when normal lung tissue was prepared and examined in the same manner, thus indicating that the particle...
Table 1. Characteristics of Survival of the CPE Virus, Using Susceptible Calves as an Indicator.

<table>
<thead>
<tr>
<th>Calf Number</th>
<th>Environment</th>
<th>Clinical Signs</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>H171</td>
<td>56 C for 30 minutes</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H175</td>
<td>56 C for 60 minutes</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H181</td>
<td>56 C for 180 minutes</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H184</td>
<td>56 C for 90 minutes</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H100</td>
<td>56 C for 360 minutes</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H122</td>
<td>-20 C for 6 months</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H163</td>
<td>-20 C for 11 months</td>
<td>abortive</td>
<td>recovered</td>
</tr>
<tr>
<td>H275</td>
<td>-20 C for 29 months</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H274</td>
<td>dried at 25 C for 12 months</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H191</td>
<td>phosphate buffer, pH 5.4</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H795</td>
<td>phosphate buffer, pH 5.4</td>
<td>subacute</td>
<td></td>
</tr>
<tr>
<td>H266</td>
<td>hydrochloric acid, pH 1.3 for 1 hour at 25 C</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H267</td>
<td>hydrochloric acid, pH 1.3 for 1 hour at 25 C</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H271</td>
<td>NaOH, pH 13 for 1 hour at 25 C</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H273</td>
<td>formalin, 2% for 1 hour at 25 C</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H177</td>
<td>diethyl ether at 4 C for 24 hours</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H188</td>
<td>diethyl ether at 4 C for 24 hours</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H189</td>
<td>diethyl ether at 4 C for 24 hours</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H250</td>
<td>Chloromycetin 250 mgm, 25 C for 2 hrs.</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H270</td>
<td>Chloromycetin 250 mgm, 25 C for 2 hrs.</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H124</td>
<td>Terramycin 250 mgm, 37 C for 1 hour</td>
<td>subacute</td>
<td>recovered</td>
</tr>
<tr>
<td>H98</td>
<td>Penicillin 100,000 units</td>
<td>acute</td>
<td>died</td>
</tr>
</tbody>
</table>

seen in the infected lung material was the virus. In subsequent work at the University of Wisconsin it has been found that a similar particle is also present in purified guinea pig fetal and uterine tissues which had been inoculated with the calf diarrhea virus.

Protection of the Calf with Colostral Whey

In a search for a possible explanation of the apparent discrepancy between the ability of colostrum to prevent the disease in the laboratory and its variable ability to prevent the disease in the field, as reported by farmers in the area, an investigation of the time relation between feeding colostral whey and exposure of the calf to the virus was undertaken. The results presented in Table 2 suggest that the protective principle in colostrum must be consumed by the calf prior to the intake of lethal quantities of the virus if the colostrum is to protect the calf.

Gross Pathological Findings

The studies of the comparative gross and histopathological changes of apparently healthy non-exposed calves and experimentally exposed calves revealed some interesting alterations in the non-exposed animals. The clinically healthy calves which were sacrificed at 2 and one-half days of age or earlier had one
Table 2. Protection Afforded to Calves by Feeding 500 ml. of Colostral Whey Before and After Exposure to Lethal Virus.

<table>
<thead>
<tr>
<th>Calf Number</th>
<th>Administration in Relation to Exposure to the Virus</th>
<th>Clinical Signs</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>H190</td>
<td>5 hours pre-exposure</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H219</td>
<td>12 hours pre-exposure</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H220</td>
<td>8 hours pre-exposure</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H285</td>
<td>6 hours pre-exposure</td>
<td>none</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H214</td>
<td>immediate pre-exposure</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H192</td>
<td>1 1/2 hours post-exposure</td>
<td>abortive</td>
<td>recovered</td>
</tr>
<tr>
<td>H199</td>
<td>1 1/4 hours post-exposure</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H202</td>
<td>1 1/4 hours post-exposure</td>
<td>acute</td>
<td>remained healthy</td>
</tr>
<tr>
<td>H203</td>
<td>1 1/2 hours post-exposure</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H206</td>
<td>1 1/4 hours post-exposure</td>
<td>subacute</td>
<td>recovered</td>
</tr>
<tr>
<td>H216</td>
<td>1 1/4 hours post-exposure</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H197</td>
<td>4 hours post-exposure</td>
<td>acute</td>
<td>died</td>
</tr>
<tr>
<td>H198</td>
<td>4 hours post-exposure</td>
<td>acute</td>
<td>died</td>
</tr>
</tbody>
</table>

outstanding lesion. There were numerous small but well defined hemorrhages on the surface of the lungs and the mucosa of the abomasum. The hemorrhages were present in all except one of the calves under two and one-half days of age but not in the 10-day old calf or in the three calves 5 days of age which were exposed to an aerosol of chicken embryo fluids.

The hemorrhages did not appear to be related to the virus disease under study because in the acute and subacute experimental disease the calves were from 2 to 5 days of age at the time of death and the hemorrhages were usually not present. These observations suggest that the hemorrhages are somehow related to a natural process in newborn calves.

In describing the gross lesions of the acute type of the disease it can be said that there is no lesion which is pathognomonic. Dehydration was a prominent feature in the carcass of the animals that died after several days of diarrhea. In the peracute disease in which death occurred within 24 hours of exposure there was marked congestion and edema in all organs but in those animals that died from the second to the fifth day following exposure this feature was not prominent.

On examination of the lungs of the exposed animals and some of the non-exposed control animals there was a suggestion of a slight edema and atelectasis in the diaphragmatic lobes; however, the lungs were of uniform consistency and no nodules or areas of consolidation could be palpated. The bronchi were free of fluid or exudate. Occasionally petechiae were observed in the trachea and larger bronchi. The mucosa of the turbinate bone was congested in the acute but not in the subacute type of the disease.

The gut of the non-exposed calves contained no visible lesions except the petechial hemorrhages previously described. The same was true in the exposed calves which failed to develop clinical manifestations. However, the gastro-intestinal tract of the animals which developed clinical signs of disease, exhibited considerable variation in degree and extent of pathological change.

In the peracute type of the disease congestion and hemorrhagic enteritis usually extended throughout the abomasum and the small intestine, sometimes extending into the colon. In many acute
and subacute cases the pathological alteration of the gastro-intestinal tract was confined to localized areas of the small intestine while in other instances no obvious change could be observed.

The mesenteric lymph glands were normal in most instances although there was occasional edema. Except for congestion in the peracute disease no obvious lesions could be observed in the kidney, liver, spleen, brain, heart, muscle, adrenal gland, thyroid or pancreas.

Histopathological Findings

Definite histopathological lesions in the non-exposed control calves and the experimentally exposed calves was confined to the lungs and appeared to differ in degree rather than in process. Of 70 specimens collected from cow lungs at the time of slaughter 12 were considered essentially normal, while of 66 calf lungs collected at slaughter 10 were considered normal. The remaining specimens revealed varying degrees of interstitial pneumonitis, and the virus could be demonstrated in them. The outstanding histological picture in the lung was one of interstitial thickening, dilation of capil-

Fig. 1. Electron micrograph of a virus causing diarrhea in newborn calves. (x 160,000).

laries and congestion. The alveoli were clear and, with a few exceptions, the bronchi were clear. Occasionally there was sloughed bronchial mucosa and cells in the lumen of the bronchi but these were so rare that one would hesitate to include this alteration as part of the disease picture.

DISCUSSION

When studying an agent which can produce acute disease only in newborn colostrum-free calves, the observation of Smith and Little (7) and Aschaffenburg et al (1, 2, 3, 4, 5) must be considered. Smith and Little obtained all their calves from one farm near Princeton, New Jersey, during the months of October to May. No mention is made concerning the time within which the calves were separated from the herd and transported to the laboratory; however, 90% of the colostrum-free calves died without experimental exposure. The group of colostrum deprived calves reported by Aschaffenburg et al, were not isolated from other bovine animals and a mortality of 80% was reported. Neither Aschaffenburg et al nor Smith and Little appear to have considered the possible presence, in the atmosphere, of an agent capable of causing diarrhea and death in newborn calves.

Preliminary work revealing the presence of an infectious agent, which could produce the typical disease, in the lungs of apparently healthy cattle, and the effectiveness of the aerosol method of exposure as a means of transmission indicated that the virus was widespread and that apparently healthy animals in the herd may serve as a reservoir for the agent. The histopathological changes, which were considered as indicative of infection by the virus were present in a high percentage of the lung specimens of apparently healthy animals, thus giving further support to the probable high prevalence of carrier or reservoir animals.

Under such conditions the problems of securing adequate control animals for the study of the virus has been difficult because the only true control is a colostrum-free newborn calf of the same origin obtained at the same time as were those
animals in which the virus was to be transmitted.

In the initial work it became apparent that if there was an infectious agent, a suitable method of obtaining controls would have to be found before a detailed study of the agent could proceed. Colostrum-free calves were used to facilitate the production of a disease in the laboratory which was similar to the natural disease as seen in the field. If the calves had received colostrum from the dam before being brought to the laboratory it was impossible to reproduce the acute type of disease which occurs under natural conditions. As the airborne nature of the disease became apparent and proper laboratory isolation for such an agent was accomplished it was observed that healthy colostrum-free controls could be obtained in the season of the year when barns were open and the herd was not stabled except at milking time. As a further precaution the calves were removed from the dam and the barn as soon as possible, in most instances within 10 to 30 minutes after birth.

With the newer understanding of the virus diarrhea a total of 82 colostrum-free calves represented controls for the project. Within the group some were not exposed to any material, others were exposed to inactivated material, and some to a sub-lethal dosage of the virus. Some of the animals exposed to various virus-bearing suspensions developed the abortive disease, those not exposed remained healthy, none died.

The fact that the seasons of the year in which the barns are kept open are coincidental with the warm seasons of the year might suggest that environmental temperature at the time the calf is born might be a critical factor. However, preliminary work indicated that keeping calves at temperatures from 20°F to 40°F in a current of air moving at 300 feet per minute did not produce the disease nor did it lower the resistance of the calf to the virus when various routes of inoculation and exposure were employed.

Although the prophylactic value of feeding colostrum prior to experimental infection was very evident the ability of colostrum to prevent the natural disease was not so apparent. The virus under study in the laboratory was isolated from natural field cases and, within the limits of possible strain variations, was presumed to be the same as the one which produced the disease in the field. Therefore, the only obvious difference between the two situations appeared to be that when colostrum was fed to the experimental calves it was fed two to eight hours prior to exposure whereas if the calves were born into an infective barn atmosphere exposure would begin from one to four hours prior to receiving colostrum. When the time relationship between experimental exposure and feeding colostrum was reversed, the results, as presented in Table 2 support this hypothesis. The benefit reportedly derived from the immediate feeding of colostrum at birth on the farms also suggests that exposure begins at once and the time differential between birth and colostrum feeding is a critical factor.

Preliminary work with antibiotics indicated that the use of penicillin, streptomycin, and terramycin had no effect on either the virus or the course of the disease in the calf. In fact the feeding of terramycin immediately following exposure to the virus increased the severity of the disease. Chloromycetin did not attenuate the virus in vitro; however, when fed to the calf at the rate of 500 mgm. per calf daily prevented symptoms from developing following exposure to the virus and, if symptoms were allowed to develop prior to feeding chloromycetin, caused a favorable termination of the disease.

Variations in rapidity of onset and severity of the disease have occurred both in the laboratory induced disease and in the disease that occurs in the field. To a limited extent variations in the severity of the disease can be produced by varying the intensity of exposure. In general, when the virus was diluted beyond the known lethal end-point a subacute and abortive disease resulted. However, it has not been possible to reproduce at will under experimental conditions, a peracute condition which terminates fatally within
24 hours after exposure. It is not known from our investigations whether this represents an individual variation in response to a single etiological agent or whether more than one agent or factor is involved in the condition. However, the type of disease in which the animals die within 48 to 72 hours after exposure and after manifesting a watery, yellow diarrhea is readily reproduced. The characteristic disease has been reproduced even though the virus had been passaged in rodent uterus, or purified by physical and chemical means. The virus is truly very stable under various adverse environmental conditions.

It would appear that there is a slight discrepancy between the size of the virus particle as indicated by membrane filtration and by electron microscopy. The size of the particle seen by electron microscopy is 17 mu whereas the membrane filter of 10 to 50 mu effective pore size appeared to retain the virus, possibly suggesting that the particle was larger than 50 mu. According to Van Rooyen and Rhodes (8) however, membrane filters of 10 to 100 mu effective pore size will retain a particle from 0.3 to 0.5 of the effective pore diameter. By taking the factor of 0.3 to 0.5 into consideration calculation of the particle retained by the 10 to 50 mu membrane would be 3.3 to 16 mu or 5 to 25 mu. In as much as the calf exposed to an aerosol prepared from the 10 to 50 mu membrane filtrate remained healthy and the calf exposed to an aerosol prepared from the 50 to 80 mu membrane filtrate developed the acute disease it appears that there is remarkable correlation between the size as obtained from the electron microscopy and that obtained from filtration.

According to the present understanding of the nature of viruses the characteristics of the agent which can incite the calf diarrhea, as set forth in these investigations, thoroughly establishes it as a virus. However, current work by Moll (6) indicates that the incidence and severity of the clinical disease can be enhanced if the calf is exposed to both virus and E. coli.

**SUMMARY**

Infection with the calf diarrhea virus under study is manifested by a watery yellow diarrhea and interstitial pneumonitis in newborn calves. The age of the calf at exposure, whether or not colostrum has been fed and the method of infection are critical factors determining the nature of the experimental disease. The fact that exposure to aerosols has of the disease suggests that this disease has been required to produce the acute type is largely an airborne disease.

A study of a few of the characteristcics of survival indicate that the virus survives at 56°C for 90 minutes but not for three hours. It withstood drying at 25°C for 1 year, and storage at -20°C in saline for at least 6 months but not 29 months. It remained viable in hydrochloric acid at a pH of 1.3 for 1 hour at 25°C but not in sodium hydroxide at a pH of 13 for 1 hour at 25°C. The virus is also inactivated by two percent formalin for 1 hour at 25°C. The virus was resistant to deithyl ether for 24 hours at 4°C. The virus was not inactivated by penicillin, streptomycin, terramycin and chloromycetin when used in vitro; however, when chloromycetin was fed to the calf at the rate of 500 mgm. daily the calf responded very favorably.

Exposure of susceptible calves to filtrates of the virus suspension indicated that the virus could pass Schleicher and Schuell membrane filters of 50 to 80 mu effective pore size. Examination of purified virus suspension by electron microscopy revealed a 17 mu spherical particle in material prepared from diseased lung; material containing this particle was able to produce the acute disease.

An animal protecting substance, stable at 56°C for 30 minutes, present in colostral whey has been demonstrated by feeding the whey at least 2 hours prior to exposure of the calf to the virus. Feeding the colostral whey after exposure had very little value.

Epidemological studies indicated that the virus was present in the lungs of apparently healthy cattle, and it is believed that this is the probable reservoir of the virus. Spread from dam to calf and calf to calf may take place by means of infective virus particles in the contaminated atmosphere.

*Calf Diarrhea*

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Calf Diarrhea

(Continued from page 188)

REFERENCES


Recent experiments have shown that, administration of Newcastle Disease Virus through the drinking water gave a comparable response to that elicited among chickens vaccinated ocularly with a similar dose.

In the past quarter century, 4 to 5 years have been added to the average life span of a dog.

Calciphos organic calcium-phosphorus compound

From the Heart of Corn

Easily Assimilable Calcium

Protects against depletion of Mineral Reserves-Pregnancy-Lactation-Weaning-Anorexia

Helps Correct Mineral Deficiencies—Rickets—Skin Diseases—Nervous Disorders—Slow Union of Fractures

Daily Dose of Calciphos: For small animals 2 to 4 tablets, or 1 or 2 teaspoonfuls powder, mixed with food.

Calciphos: 10-grain tablets in bottles of 100 and 500. Powder in bottles of 5 oz.

WRITE FOR LITERATURE AND SAMPLE


BILHUBER-KNOLL CORP. ORANGE, NEW JERSEY

Iowa State College Veterinarian