Etiologic Studies on Bovine Winter Dysentery

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Introduction

Winter Dysentery (WD) is a highly contagious, acute enteric disease of cattle characterized by a brief attack of severe diarrhea and sometimes dysentery (1). It occurs primarily in stabled dairy cattle in Northeastern and North Central United States and Canada, although similar diseases have been reported in Australia (4), Sweden (3), France (2), Israel (8), and England (10). As the name implies, it occurs in late fall, winter, or early spring. The most severely affected cattle are the two and three year old pregnant and milking heifers, with older cows usually showing less severe involvement. Severe intestinal hemorrhage occurs in 5-10% of affected cattle. Mortality is uncommon and most cattle survive without treatment unless dehydration or hemorrhage occurs. A more detailed description of the clinical signs and epidemiology is presented in the companion paper (7).

Etiology

The etiology of WD is unknown. Scientists in the early 1930's indicated that *Vibrio jejuni* was the etiological agent (5), but more recent studies have failed to substantiate this (2,3,9,). Viruses have been isolated and incriminated in WD-like disease in Canada (9), France (2), and Israel (8).

The apparent transmissibility of the disease by fomites, contact, or fecal suspensions, the extreme contagiousness, the mode of spread through a herd, and their febrile response seen early in the disease in some cattle (7,11) would indicate that it is infectious. Certainly bovine practitioners, cattle dealers, and inseminators who have been incriminated for spreading WD from farm to farm are easy to convince of its contagiousness.

Over the years a number of microbiologists have attempted to determine the etiology of WD. To say the least, it has been a "tough nut to crack."

The senior author has been involved in studies to determine the etiology of WD since 1965. In the fall of 1972, the investigation was expanded to provide a multidisciplined approach with six primary investigators, including a clinician, an epidemiologist, a gastroenterologist-clinician, a physiologist, an immunologist, and a microbiologist. Eight additional investigators contributed to the program in their specialties. While funds are limited, it is the start of a program that hopefully in time will solve the mystery of WD.

This report summarizes attempts to determine the etiologic agent of WD. Approximately twenty herd outbreaks have been investigated, some with only a cursory examination and some with a detailed herd investigation including taking temperatures and an examination of all cattle three times a day for one week. Feces, serum, blood,and sometimes nasal swabs were taken from three to six animals in each herd for bacteriologic, virologic, and transmission studies.

Vibrio

Since the reports in the early 1930's (5) *Vibrio jejuni* was considered to be the etiologic agent of WD for several years. In recent years, attempts at isolation of *V. jejuni* from clinical cases have been consistently negative. Several investigators (2,3,9) concluded from their studies that *V. jejuni* was not the cause of WD.

In our studies, when rigorous microbiologic techniques were applied, vibrios could not be isolated from fecal, gut content, or blood specimens collected from several WD outbreaks. It is our opinion that *Vibrio jejuni* was not involved in these outbreaks. This conclusion is analogous to recent findings that *Vibrio coli* once considered the cause of swine dysentery has a less significant causal role than the spirochete *Treponema hyodysenteriae.*

Other Bacteria

Routine culturing of feces and blood have failed to isolate any significant bacteria. Feces have contained a variety of organisms as would be excepted, but have been consistently negative for *Salmonella spp.* Blood cultures usually were negative but a *Bacillus* organism was isolated from blood samples of one outbreak. The exact role of this organism is unknown, but inoculation of one heifer with this isolate (Table 3) failed to produce immunity to subsequent challenges. Studies are continuing to evaluate this organism and anaerobic bacteria in WD.

Viruses

The clinical disease and epidemiologic pattern of WD are consistent with an acute viral disease. Viruses were isolated from WD-like diseases in Canada (9), Israel (8), and France (2). The French isolate was shown to be an enterovirus, but the other two viruses were not characterized.

Numerous attempts were made to isolate viruses from feces, nasal swabs, blood, and tissues from naturally occurring WD outbreaks and experimentally produced cases using embryonic bovine cell cultures of kidney, spleen, thyroid and lung.

Cultures of blood and nasal swabs were negative for cytopathogenic viruses. Most fecal samples were negative, but isolation of cytopathogenic viruses resembling bovine enteroviruses (BEV) were made from several fecal samples from three WD outbreaks.

Serologic studies plus experimental inoculation of cattle indicated that these isolates probably were not involved in the etiology of WD. Paired serum samples (collected at illness or prior to inoculation and three weeks later) from natural and experimental cases of WD did not have a rise in titer against the French enterovirus isolate "42". This would indicate that isolate "42" is not involved in the etiology of WD. The Canadian and Israeli viruses have not been compared with WD in New York State.

Direct cell cultures of kidney, spleen, lung, thyroid, and intestine were prepared from tissues of WD cases which were necropsied. These cultures were usually passaged three times, but in two cases such cultures were passaged weekly for 50 weeks. Buffy coat cultures were prepared from EDTA preserved blood samples by centrifugation and separation of leukocytes. Buffy coat cultures, co-seeded with embryonic bovine spleen cells, were passaged at least three times at weekly intervals.

A bovine syncytial virus (BSV) was isolated from direct cell cultures of lung and kidney of a downer cow that was euthanized one week after the onset of WD. This cow also had traumatic reticulitis. This virus was inoculated into two yearling heifers of unknown susceptibility to WD or BSV, intranasally in one case, and i.v. and orally in the other. No illness was observed for 14 days. Attempts were made to characterize the isolate (12), but detailed serologic tests were not possible due to technical difficulties arising from the virus.

Approximately 25% of normal cattle are carriers of BSV. BSV has not been shown to produce disease in cattle, and there is no evidence at present to link this isolate with WD. Therefore, the isolation of BSV from a WD infected cow will have to be regarded as coincidental unless further proof of its involvement is obtained.

Direct cell cultures often developed cytoplasmic vacuolization of cells, especially in the cultures that were passaged many times. This was not caused by BVD virus (which does produce vacuolization). When these cultures were examined, one felt that a virus should be present to cause these changes. However, examination of cultures by staining and electron microscopy failed to reveal evidence of a virus, and a heifer inoculated with one of these cultures failed to develop resistance to WD (Table 3). The cause of this vacuolization is unknown.

Previous studies (6) showed that BVD and IBR were not involved in WD. Screening studies with bovine parvovirus 1 (HADEN virus) indicate that this virus probably is not involved in WD because most acute serums had antibody and there was no evidence of a rise in titer following WD.

Mycoplasma

Mycoplasma were isolated from fecal samples from several outbreaks of WD, from nasal swabs of one heifer that also had respiratory disease, and from several blood and fecal samples after passage in cell cultures.

Inoculation of two heifers with mycoplasma isolated from a cell culture passaged blood sample failed to produce any clinical disease. However, the susceptibility of these heifers to WD could not be ascertained.

These data cannot rule out mycoplasma, nor can they incriminate them as being involved in WD.

Protozoa

Trypanosomes were observed frequently in buffy coat cultures prepared from blood samples of WD infected cattle. Some direct cell cultures of tissues also had trypanosomes. Pre-inoculation buffy coat cultures from experimental heifers had trypanosomes as frequently as did cultures from acutely infected cattle. Approximately 40% of

normal cattle in New York are carriers of trypanosomes (12). It would appear that trypanosomes are not involved in the etiology of WD.

Examination of feces failed to show coccidia or other significant intestinal parasites.

Transmission Studies

Thirty-two yearling or two-year-old heifers and one adult cow have been involved in transmission studies. Lacking an agent or serologic test, it is impossible to determine susceptibility to WD prior to challenge. To date it has not been possible to retain infectivity of fecal samples by freezing, thus a constant source of infectious challenge material has not been available. Negative transmission results using fresh material from WD cases cannot be interpreted as an absence of an agent in the inoculated material because the experimental animal may have been immune prior to challenge.

Transmission of typical clinical WD has been successful in five cases (Table 1). Four of these were inoculated orally or oral-intranasal with a fresh, unfrozen fecal suspension from WD cases. The fifth case was a heifer that was introduced into a herd on the first day of the outbreak. Infectivity was maintained through three serial passages.

Four other heifers (Table 2) (all purchased from the same herd as B-12 in Table 1) developed soft feces consistent with mild clinical WD. The incubation period was four days in one case and five days in the other three cases.

In these nine transmissions, the incubation period varied between three and five days, with a mean of 4.5 days.

An additional four heifers (Table 3) showed mild signs which may have been WD. Three of these heifers developed signs seven to eleven days after inoculation with feces that had been preserved by freezing at -60°C with 10% dimethylsulfoxide. Another (B-29) developed typical WD when challenged 12 days later indicating a failure of immunity to develop to the earlier exposure, and indicating that clinical signs seen after the initial inoculation probably were not due to WD.

Other transmission studies have been attempted in suckling mice, rabbits, and guinea pigs. Blood inoculated intraperitoneally on one to two-day-old suckling mice resulted in mortality in some cases, with a decreased mortality on subsequent serial passage. Mortality did not occur after the second

Table 1

Transmission Studies of Bovine Winter Dysentery. Positive Transmission*

Heifer	Inoculum				DPI*	
No.	Sample	Source	Treatment	Dose & Route	Signs	Signs
B-12	Feces	Pollock herd	Fresh susp.**	50 ml oral	5	Profuse watery diarrhea
B-31	--	Eddy herd	Contact	Herd contact	5	Diarrhea
B-30	Feces	Eddy herd	Fresh susp.**	45 ml oral/5 ml I.N.	3	Profuse watery diarrhea with blood
B-32	Colon	B-30	Fresh susp.**	45 ml oral/5 ml I.N.	5	Diarrhea
B-29b	Feces	B-32	Fresh susp.	100 ml oral/10 ml I.N.	4	Diarrhea with blood

I.N. = intranasal.
DPI = days postinoculation.
* = based on clinical signs typical of WD.
** = clarified by centrifugation, 2000 rpm for 20 minutes.

Table 2

Transmission Studies of Bovine Winter Dysentery. Probable Transmission.*

Heifer	Inoculum				DPI	
No.	Sample	Source	Treatment	Dose & Route	Signs	Signs
B-13	Blood pool	Pollock herd ized	Fresh heparin-	15 ml I.V.	5	Soft feces
B-14	Cecum & ileum	B-12 trifuged	4°C 3 days, cen-	50 ml oral	5	Soft feces
B-15	Cell culture**	B-12	None	60 ml I.V./100 ml oral	4	Soft feces
B-16	Cell culture***	B-12	None	20 ml I.V./20 ml oral	5	Soft feces

*Based on clinical signs consistent with mild WD.
**Direct cell culture of jejunun, ileum, and spiral colon, first and second transfer, with cells.
***Direct cell culture of ileum, third passage, with cells.
DPI = days postinoculation.
I.V. = intravenous.

Table 3

Transmission Studies of Bovine Winter Dysentery. Possible Transmission.

Heifer No.	Inoculum				DPI	
	Sample	Source	Treatment	Dose & Route	Signs	Signs
B-6	Feces	Smith herd	-60°C; 10,000*; 10% DMSO+PSM	25 ml I.V./25 ml oral	7	Diarrhea (clay colored), anorexia
B-8	Feces	B-6	-60°C; 10,000*; 10% DMSO+PSM	15 ml I.V./13 ml oral	10	Soft feces
B-19	Feces	Pollock herd	-60°C; 2,500*; 10% DMSO	4 ml oral/1 ml I.N.	11	Soft feces
B-29a	BEV-8	Mix herd	Fecal isolate BSp	5 ml I.V./5 ml oral I.N.		
	Cell culture	Kenney herd	BEL-4T**	25 ml I.V./25 ml oral-I.N.	3	Slight diarrhea, blood flecks
	Bacillus sp.	Mix herd	Blood isolate	10 ml I.V./15 ml oral-I.N.		

*Centrifugation rpm for 20 minutes at 4°C.
**Bovine embryonic lung, fourth passage, inoculated with feces.
DPI = Days postinoculation.
I.V. = Intravenous.
I.N. = Intranasal.
PSM = Penicillin, dihydrostreptomycin, and nystatin.
BEV = Bovine enterovirus.
DMSO = Dimethylsulfoxide.

passage. Additional research is needed to clarify the susceptibility of suckling mice to WD. No changes were produced in the limited number of rabbits or guinea pigs inoculated.

Transmission studies in embryonated eggs with blood from cattle with WD were attempted according to protocol of Komorov, et al. (8). Although embryonic deaths resulted in some cases, results were equivocal and no specific agent was identified. Again, further research is indicated.

Summary and Conclusions

The etiology of WD still remains in question. However, experimental transmission of cattle including three serial passages with fresh fecal suspensions supports an infectious etiology. *Vibrio jejuni* is *not* the cause of WD as previously believed. IBR, BVD, parvovirus, enteroviruses, and other cytopathogenic bovine viruses do not seem to be involved. While the epidemiology and clinical picture are consistent with an acute viral infection, the possibility of a non-viral etiology must be considered. Further research must be done using other approaches to determine the etiology of WD.

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