Descriptive Epidemiology of *Salmonella* Infection in Minnesota Herds

Robert A. Robinson, *DVM*
Jeffrey B. Bender, *DVM*
Clinical and Population Sciences
College of Veterinary Medicine
University of Minnesota, St Paul, MN

Introduction

Salmonella infection and its clinical manifestations, (Salmonellosis), are of very real concern to both the dairy and beef industries in Minnesota, both from the economic impact of the disease itself and also the human, or public health aspects. Both meat-associated[1] and milk and cheese[2,3] associated outbreaks have been documented as occurring in the Midwest in the last decade. Obviously these incidents do not help promote the sale of animal products even though the actual fault may lie with the food processor or the consumer.

How significant and widespread is *Salmonella* infections in cattle herds in Minnesota? In the following brief paper, I will attempt to put in perspective what we know and probably more important what we don't know about this perplexing and often elusive problem.

Sources of Data on Clinical and Asymptomatic Infections in Minnesota

For any infectious disease, accurate data on its prevalence and incidence is very dependent on the accuracy (sensitivity and specificity) of the tests available, both at the herd and individual animal level. Unfortunately with *Salmonella* infections we are still searching for the ultimate test. Given that there have been significant improvements in the availability of improved culture media for Salmonellas, together with some promising biotechnological techniques, we are still limited in our ability to isolate and identify the bacteria especially when they are present at low levels. Serological tests (primarily ELISA's) show promise particularly for certain serotypes such as *S. enteritidis* in chickens and *S. dublin* in cattle. The type of specimen available for screening herds depends on the husbandry system. For example milk sox (filters) and drain swabs (were there is a milking parlor) have been used as a relatively crude technique to screen herds for the presence of *Salmonella* excreting cows. These techniques coupled with intensive sampling of young calves probably represent the best compromise we currently have to assess the overall herds status. Unfortunately the available tests lack sensitivity to measure the infection status of the individual animal particularly for movement purposes. A single negative test (fecal for example) has very limited value. Nevertheless taking these factors into consideration, our available data sources can be divided into random and non-random sources.

(a) Random Studies of Asymptomatic Infection

Only two bovine studies have been done in Minnesota that the author is aware of and both involved calves. The first was carried out in 1980 by Opuda[4] who, using fecal swabs, found only 13 out of 1,400 calves (1% approximately) (1-12 months of age, primarily males) positive for *Salmonellas* (*S. London, S. typhimurium, S. litchfield, S. heidelberg* and *S. newport*). A separate sample of 200 of these calves was followed through to slaughter and two isolates (1%) of *S. typhimurium* were made from the mesenteric lymph nodes.

In 1991-92, the National Animal Health Monitoring System (NAHMS) undertook a 3-month study of 74 randomly selected herds in Minnesota. In addition a parallel study of 29 herds randomly selected from calf submissions to the Veterinary Diagnostic Laboratory were also monitored for three months. One component of this study involved sampling a proportion of calves under 8 weeks of age. During the study period *Salmonella* isolation isolates were recovered from 9/311 (0.29%) calves in the NAHMS herds and 3/155 (0.19%) isolates were recovered from the University study respectively.

All the positive samples in the NAHMS study came from one herd, i.e. 1/74 (1.35%) herd infected while 3/27 (11%) University herd were positive. Interestingly 7 of the original University study herds were *Salmonella* positive based on laboratory isolations from live calf or necropsy materials.

As mentioned earlier, the value of a single fecal

Paper presented at the Minnesota Dairy Health Conference, May 17-19, 1993. Sponsored by the College of Veterinary Medicine, University of Minnesota.

swab or sample to assess the *Salmonella* status of an individual animal is of limited value but nevertheless these results suggest that the prevalence of *Salmonella* sp. either at the herd level, or after commingling at market are not particularly high. This is in contradistinction to *Cryptosporidium* sp. for example, where the results of the NAHMS study (to be published) would suggest that, based on a number of samples taken, almost all herds are infected.

While there does not appear to have been any systematic study of *Salmonellas* either in bulk raw milk or milk filters carried out in Minnesota, the FDA Center for Microbiological Investigations in Minneapolis reported in 1987 that *Salmonellas* were isolated from 32/678 (4.7%) milk samples from bulk milk tanks in Wisconsin, Michigan and Illinois.[5] Canadian studies in Ontario have shown a cumulative incidence of 9.0% per year on repeated monthly milk sampling and that the presence of *Salmonella* in bulk milk supplies is dynamic.[6] While there are a number of published reports of persistent udder infection with various *Salmonella* sp. it is presumed that the majority of isolates in milk are the result of fecal contamination of the udder.

(b) Non-Random Studies: Clinical Disease

The primary source of information on clinical Salmonellosis is the State Veterinary Diagnostic Laboratory. Where a *Salmonella* sp. is isolated from live animal specimens (primarily fecal samples or swabs) or necropsy material, this is submitted to the national Veterinary Services Laboratory at Ames, IA for confirmation, serotyping and phage-typing in certain cases. Together with the actual isolate, information on the owner's name and address, source animal species, number of animals in the herd or flock, morbidity, mortality, age groups affected, and whether *Salmonella* were considered to be primary or secondary infections is included. No distinction is made between beef or dairy. Obviously there are many possible biases in this database depending in part on whether or not veterinarians decide to submit samples, or if they are consulted on individual cases. Nevertheless we believe that herd outbreaks usually result in professional intervention so that while the NVSL Database cannot be considered as a randomly selected sample of cattle or herds in the U.S.A., it does represent the most comprehensive and consistent collection of data on bovine *Salmonellas* on a nationwide basis.

A review of the descriptive epidemiology of Bovine 17,488 *Salmonella* serotypes from (1982-1991) has recently been published.[7] Of the 10 most common serotypes, *S. typhimurium* (38.7%) and *S. dublin* (16.8%) are, as expected, the most common serotypes, isolated from cattle. See Table 1.

Table 1. Ten Most Common *Salmonella* Serotypes Isolated from Cattle (1982-1991)

SEROTYPE	NUMBER OF ISOLATES (%)
Typhimurium	4812 (27.5)
Dublin	2933 (16.8)
Typhimurium (variety Copenhagen)	1958 (11.2)
Newport	771 (4.4)
Anatum	669 (3.8)
Montevideo	513 (2.9)
Cerro	462 (2.6)
SAL 9, 12:Nonmotile*	426 (2.4)
Muenster	361 (2.1)
Agona	275 (1.6)
All other isolates	4308 (24.7)
TOTAL	17488 (100)

*A serogroup D isolated which cannot be definitively identified as a recognized serotype.

Data from the national study were also examined with respect to typical morbidity, mortality, and case fatality rates, age and serotype of *Salmonella*. While the overall morbidity was similar in both young and old animals, the mortality as expected was higher in the calves. Somewhat surprisingly although *S. dublin* is reputed to be more pathogenic for cattle than other serotypes, this is not borne out in the summary data as shown in Table 2.

Table 2. Morbidity, Mortality and Approximate Case-Fatality Rate by Age Group and Type of *Salmonella* (Source Ref. #7)

Type of Salmonella	AGE					
	Immature & Prenatal			Mature & Mixed		
	Morbid (%)	Mortal (%)	Approx Case Fatality (%)	Morbid (%)	Mortal (%)	Approx Case Fatality (%)
S. typhimurium	13.3	7.3	55.8	12.3	3.5	28.5
S. dublin & other Group D variants	10.5	4.6	43.8	12.3	3.2	2.6
Other Salmonella	11.5	4.9	42.6	11.4	3.7	32.5
All Salmonella	11.8	5.6	47.5	11.9	3.5	29.4%

Data from 1983-1992 cattle isolates in Minnesota have been analyzed separately. The results are shown in Table 3.

Table 3. Bovine Salmonella Isolations: Minn. Vet. Diag. Laboratory (1983-1992 Inclusive*)

	S. dublin	Non-motile Group D	S. enteritidis	All Group D Salmonellas	S. typhimurium	Other Salmonellas
1983	2	--	--	2	32	4
1984	2	--	--	2	23	1
1985	1	--	--	1	8	8
1986	1	--	--	1	5	5
1987	--	--	--		19	9
1988	3	6	1	10	37	16
1989	8	1	5	14	52	14
1990	29	10	16	55	44	18
1991	30	35	8	73	23	8
1992	40	43	5	88	11	23

Note that these have been grouped into *S. typhimurium*, Group D *Salmonellas* (which includes *S. dublin*, *S. enteritidis*) and the so-called 9,12 non-motile variants (which are almost certainly variants of *S. dublin*) and "other" *Salmonellas*. As can be seen, there has been dramatic increases in the Group D *Salmonellas* during the last decade, and currently these *Salmonellas* are the major causes of bovine salmonellosis in this state. During this period, the number of bovine accessions to the laboratory has remained fairly constant at 7-8,000/ year so the increase is probably very real.

S. dublin was first detected in California and has subsequently spread throughout much of the cattle raising areas of the country. It was first detected in Minnesota in 1980, when four outbreaks were recorded. Investigations at that time indicated that at least one of these initial outbreaks involved calves purchased from New York State. Since then it appears to have spread throughout many counties in Minnesota, presumably via movement of calves or adult animals. *S. dublin* is primarily a cattle associated serotype but sporadic isolates have been recorded in Minnesota from a cat, swine and lizards.

Among the other group D *Salmonellas*, *S. enteritidis*, although primarily of concern to the poultry industry because of egg contamination, it does occasionally occur in cattle as shown in Table 2. The situation with the non-motile Group D *Salmonellas* is somewhat confusing. Inability to demonstrate the flagellar antigens makes precise identification impossible but in some instances following prolonged and repeated subculture, some isolates are confirmed as *S. dublin* or *S. enteritidis*. In some herds dual isolations of *S. dublin* and *S. enteritidis* or non-motile Group D's have been made, indicating either dual infections or some bacterial genetic changes.

It is of interest that none of the random calf sampling programs detected the presence of *S. dublin* even though it could be argued that its occurrence now constitutes an "epidemic" in Minnesota. Presumably herd introductions occur when either recovered "carrier" or excreting animal is introduced or an animal incubating the infection.

The major clinical syndromes associated with *S. dublin* and other Group D *Salmonellas* include:[8]

(a) Enteric Form: - This is similar to disease caused by other *Salmonellas* with high fever, watery feces containing fibrin and, on necropsy, reddening of the intestinal mucosa, multifocal areas of mucosal erosion and adherent fibrin tags.

(b) Respiratory Form: - This form is often seen and may confuse even experienced practitioners who would not normally include Salmonellosis in their list of differentials for respiratory disease. Diarrhea may be absent or occur as a terminal event. On necropsy the lungs are heavy and wet, do not collapse and sero-sanguinous fluid exudes from the cut surfaces. Petechial and ecchymotic hemorrhages are seen on serosal surfaces. An interstitial pneumonia is present on histological examination. A few calves may also show icterus and yellowing of the liver.

(c) Neurologic Form: - This is seen as a more chronic form of the disease than the enteric and respiratory forms. There may have been prior antibiotic treatment and *Salmonella* may only be isolated from CNS. Recumbency, opisthotonos, and paddling are common signs.

Survivors of calfhood *S. dublin* infections are often unthrifty and may also exhibit signs and lesions of arthritis. Approximately 93% of *S. dublin* and other Group D *Salmonellas* in Minnesota have been made from calves and the remainder from adult animals. With regard to the latter it is important to note that abortion can occur. *S. dublin* has now become one of the leading causes of abortion in the United Kingdom following the eradication of brucellosis.

There appears to have been a diminution in the occurrence of *S. typhimurium* infections in the last several years, while there has been an apparent increase in the occurrence of other serotypes, such as *S. agona, S. montevideo, S. brandenburg, S. newbrunswick, S. newington*, etc. Some of these serotypes are found as contaminants in meat and bone, which is increasingly being utilized as a source of by-pass protein in lactating dairy cattle. Specific links of these products have been difficult to establish although field investigations of one recent outbreak revealed *S. mbandaka* as cause of illness in adult cows and the same organism being recovered from rendered animal fat component of their diet. It is worth noting that *S. dublin* other Group D Salmonellas and *S. typhimurium* are virtually never recovered from feed sources.

The human health risks to farm workers and their families are obvious and should be emphasized whenever a practitioner is presented with a herd outbreak. A recent Canadian study investigated human fecal excretion of *Salmonellas* on dairy farms when *Salmonellas* had been isolated from the milk filters. Twenty two of 168 (13.1%) individuals were positive and all had consumed unpasteurized milk.[9] Raw milk consumption, particularly where the very young, the elderly or the immunocompromised are involved should be avoided at all costs. Handwashing, changing of footwear and outer clothing prior to entering the home should be emphasized.

Summary

Based on the limited random sampling of calves on farm, at market and at slaughter, the prevalence of excreting animals and infected herds appears to be relatively low. However, based on Diagnostic Laboratory results, there has been a very significant increase in the number of calf-hood epidemics of salmonellosis in Minnesota during the last decade. Most of this increase can be ascribed to *S. dublin* and closely related Group D variants. As these *Salmonellas* are bovine-adapted, it is presumed that herd introduction occur when calves, or adult cattle are purchased or otherwise commingled. Current testing procedures are still limited in their ability to determine both herd and individual animal status.

References

1. Holmberg, S.D., *et al.* 'Drug Resistant Salmonella from Animals Fed Antimicrobials' (1984). *New Eng. J. Med.* 311:617. 2. Ryan, *et al.* Massive Outbreak of Antimicrobial-resistant Salmonellosis Traced to Pasteurized Milk. (1987) *J. Am. Med. Assoc.* 258:3269. 3. Hedberg, C. W., *et al.* A Multistate Outbreak of *Salmonella javiana* and *S. oranienburg* Infections Due to Consumption of Contaminated Cheese. 1992. *J. Am. Med. Assoc.* 268:3203. 4. Opuda-Asba, J. (1981) Isolation of *Salmonella* from the Mesenteric Lymph Nodes and the Cecal Contents of Calves and Examination of Some Factors Influencing *Salmonella* Carrier Rate and Shedding Among Calves. MPH Thesis. Univ. of Minnesota. 5. McManus, C. and J. M. Lanier. *Salmonella, Campylobacter jejuni*, and *Yersinia enterocolitica* in Raw Milk. (1987) *J. Food. Prot.* 50:51. 6. McEwen, S. A. *et al.* A Prevalence Survey of Salmonella in Raw Milk in Ontario 1986-87. *J. Food Prot.* (1988) 51:963. 7. Robinson, R. A. *et al.* Descriptive Epidemiology of *Salmonella* Serotypes from Cattle in the USA (1982-91). (1992). Proceedings XXV. American Association of Bovine Practitioners and XVII World Buiatrics Congress, St. Paul, MN. 8. Woods, K. L., *Salmonella dublin* and Group D Non-motile Salmonellae in Calves (1993). Animal Health Newsletter. Wisconsin Dept. of Agriculture, Trade and Consumer Protection. Jan/Feb. issue. 9. West, A. M., *et al.* Factors Associated with the Presence of *Salmonella sp.* in Dairy Farm Families in Southwestern Ontario (1988) Canad. J. Pub. Health. 79:119.

Risk maps: results of a research carried out to evaluate the prevalence of cattle treated with hormones.

M. Severini, A. Vizzani, S. Dominici, and G. Bertorotta.
Obiettivi e Documenti Veterinari, (1990) XI (7/8), 41-45.

General criteria of elaborating territorial risk maps are proposed. The criteria were applied in an experimental research based on a monitoring program for illegal hormone treatment. About 50,000 cattle slaughtered in the region of Umbria during a period of 12 months were included in the program. The results show that the proposed method is easily adopted and is most suitable for mapping such risk.

Vaccination of pregnant cows with K99 antigen of enterotoxigenic *Escherichia coli* and protection by colostrum in newborn calves.

C. Valente, G. Fruganti, B. Tesei, A. Ciorba, P. Cardaras, A. Floris, and E. Bordoni.
Comp. Immun. Microbiol. Infect. Dis., (1988) 3/4, 189-198.

The immune response to the K99 was tested in 45 pregnant cows, subcutaneously vaccinated, for protecting the newborn calves. Serological tests were performed in the blood sera of all animals and in the milk and colostrum sera; hemogram, inhibition of the adhesion to the brush border and histological tests were performed. The calves from vaccinated cows survived the experimental infection after the ingestion of colostrum in spite of the fact that the calves from control dams died with diarrhea.