# General Session III

Infectious Disease Update

Bruce Wilkie, *Presiding*



# Bovine Salmonellosis

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## Introduction

Generally speaking, bovine salmonellosis in terms of etiology, epidemiology, pathogenesis, clinical findings, therapy and prevention, is well described in standard texts (3, 14). However, recent research in the bovine relative to pathogenesis, epidemiology and prophylaxis in particular has challenged the validity of some of the conclusions about bovine salmonellosis derived from studies in other species.

In this presentation I will try to give a brief overview of Bovine Salmonellosis with particular emphasis on those topics or concepts which have been or need to be further clarified. At the outset I must acknowledge the research of Dr. R. C. Clarke, a graduate student at the University of Guelph, who studied *S. typhimurium* infection in the calf and with whom I had the pleasure to work. Our discussions about bovine salmonellosis, his research and that of others actively involved in the field have influenced my re-evaluation of certain aspects of this very important and complex disease.

**Losses to the livestock industry which have been estimated at $53 million dollars annually, not including poultry losses of $77 million dollars per year, indicate the economic impact of this disease on animal agriculture. The description of salmonellosis as the most prevalent disease of animals transmitted to man responsible for a $625 million dollar loss annually in human productivity emphasizes the importance of salmonellosis as a zoonotic disease (9).**

## Etiology

Although more than 2000 antigenically different serotypes of Salmonella organisms have been identified around the world, bovine salmonellosis commonly is caused by a limited number of pathogens. Some, like *S. typhimurium* and *S. newport,* are considered non-host adapted species, a factor of great importance relative to species transmission of the disease, whereas *S. dublin* is considered specifically host adapted to the bovine. *S. typhimurium* can truly be regarded as an ubiquitous organism and this together with its wide host range (man, domestic and wild animals, rodents and birds) make it a formidable bacterial pathogen. Obviously, the trend towards more intensive animal husbandry techniques facilitates the spread of these organisms and particularly *S. typhimurium* which has been described as the most common Salmonella pathogen in the U.S.A. and Canada (16).

## Epidemiology

This aspect of bovine salmonellosis has received increased attention over the past few years. The net result of this work has been a reaffirmation of the importance of animal reservoirs, environmental and feed contamination, specific epidemiological characteristics of certain serotypes of Salmonella and the importance of animal management all of which, often acting in concert, produce severe clinical disease.

Data from around the world would suggest an infection rate in dairy cows of between 5 and 15% (3). *S. typhimurium* is the principal pathogen in cattle in North America causing which is much more common in Britain and Europe, causes serious continuous disease problems particularly in calves. This difference between the two strains has been attributed to the prolonged carrier state induced by *S. dublin.* Such carrier animals may shed the organism intermittently, or continuously with or without clinical signs of disease or become latent carriers with residual infection localized to the mesenteric lymph nodes, the liver or the tonsils. Subsequently, stress factors such as transportation, irregular and or inadequate feeding and watering, changes in dietary composition or formulation, overcrowding, pregnancy, parturition, severe unaccustomed exertion and intercurrent disease can reactivate shedding of the organism and or cause disease (14). Obviously, farming practices that require the assemblage and transport of young calves with subsequent

close confinement particularly in loose-type housing systems will be conducive to high levels of environmental contamination and rapid spread of the organism and disease. Also, the potential for disastrous outbreaks of salmonellosis associated with contaminated feedstuffs particularly bone meal, fish meal, meat meal, etc., is well recognized (27).

The importance of high stocking densities on pastures combined with environmental influences that optimize survival of the organism for up to 7 months (5) and or the spreading of fresh surry on pastures significantly increases the danger of disseminating Salmonellosis (13).

Given the ability of the salmonella organism to persist in the animal and its environment, the potential for contamination of feed stuffs during processing either directly from the addition of infected organic material such as bone meal or indirectly by contamination with infected rodent droppings, and the trend in animal agriculture to confinement and maximum possible stocking densities, it should not be surprising that the incidence of bovine salmonellosis seems to have increased significantly over the last 30 years (3).

Oral intake is the accepted common route of infection. Experimentally in calves it has been documented that oral doses of greater than $10^8$ organisms are required to produce a consistently fatal disease (7, 30, 31, 21, 4) and significant invasion and intestinal mucosal damage in calf gut-loop preparations (4). These facts and the recognition that this disease is endemic on some farms further emphasize the importance of environmental contamination in the overall syndrome and the need for the veterinary practitioner to be familiar with and prepared to apply the basic principals of epidemiology to the diagnosis, treatment and prevention of bovine salmonellosis.

## Pathogenesis

Following ingestion, the organisms penetrate directly through the intestinal mucosa. Concomitant invasion of the lymphoid tissue of the posterior pharynx and spread systemically via lymph and blood has been clearly demonstrated in esophagectomized calves (7). Residual infection in fixed macrophages and other cells at this site of entry may explain the ease of culturing the organism from the posterior pharynx in some carrier animals. Although either route could initiate infection and disease and account for the development of the carrier state, the massive proliferation of Salmonella that results in gross contamination of the environment and disease transmission occurs in the distal small intestine and large bowel.

Our understanding of the details of this aspect of the disease is still wanting. Based on studies in cattle and other species it has been suggested that a critical dose of organisms of a strain capable of colonizing the intestine and invading enterocytes and causing intestinal secretion is required before infection with Salmonella produces clinical disease (14). Recent work (4) in calves has confirmed the first two of these requirements and demonstrated that *S. typhimurium* produces a severe enteritis with partial atrophy of villi, erosion of the mucosa and extensive inflammatory infiltration of the ileum by 6 hours post-infection compared to the 12 hours previously reported in the guinea pig by Takeuchi (26). Interestingly, Clarke identified more severe lesions in the ileum compared to the jejunum and large bowel in spite of the fact that comparable numbers of organisms were present in tissue and intestinal content at all sites studied.

The exact explanation for the extensive intestinal inflammatory reaction, depletion of lymphoid elements in lymphoid follicles, and vascular damage with the development of fibrin thrombi has not been provided to date. No doubt, enterotoxic and cytotoxic factors (2) and Salmonella endotoxin will be implicated with or without additional as yet unidentified factors perhaps related to the immune response of the animal. Also, in spite of the severe intestinal damage to ileal gut-loops in calves following inoculation with $10^9$ *S. typhimurium* organisms, Clarke observed very little fluid accumulation in the loop compared to the volume produced by enterotoxigenic *E. coli* inoculated under comparable experimental conditions (25). Why? One would postulate several explanations but no one really knows!

Undoubtedly, intermittent seeding of the intestine from the gall bladder, mesenteric lymph nodes and macrophages in the lamina propria and gut associated lymphoid tissue accounts for the intermittent or continuous shedding of organisms by clinically normal carriers.

## Clinical Findings

The common clinical syndromes produced by Salmonella infection in the bovine include acute and chronic enteritis, abortion, septicemia, polyarthritis, pneumonia, endarteritis and dry gangrene.

Before discussing each of these syndromes it is important to recognize that many cattle must develop subclinical disease. The most important consequence of this is the establishment of the carrier state and the potential to shed organisms and under certain circumstances develop clinical disease.

Acute enteritis is characterized by a high fever, fluid diarrhea, with or without mucus, blood or casts. The degree of dehydration, malaise and survival in this form of the disease is probably as dependent on factors such as disease resistance of the host, concomitant stresses including other infectious and non-infectious disease processes as it is on the strain of the organism. Obviously the number of organisms ingested will be very important relative to the onset and severity of clinical signs. Calves that survive this syndrome may subsequently develop polyarthritis, pneumonia or endarteritis with the development of dry gangrene involving the distal extremities such as the tips of the ears or tail or the distal fetlock area. This latter syndrome may not become apparent to the casual observer for 2-4 weeks after the initial diarrheic episode (17). Pregnant cows that survive this

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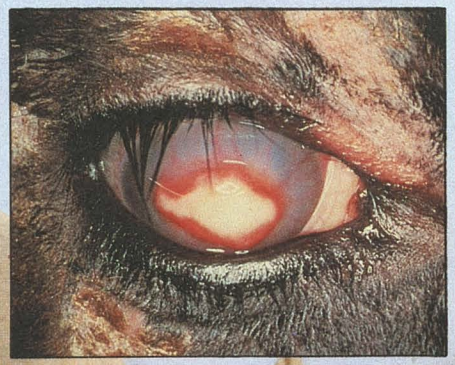

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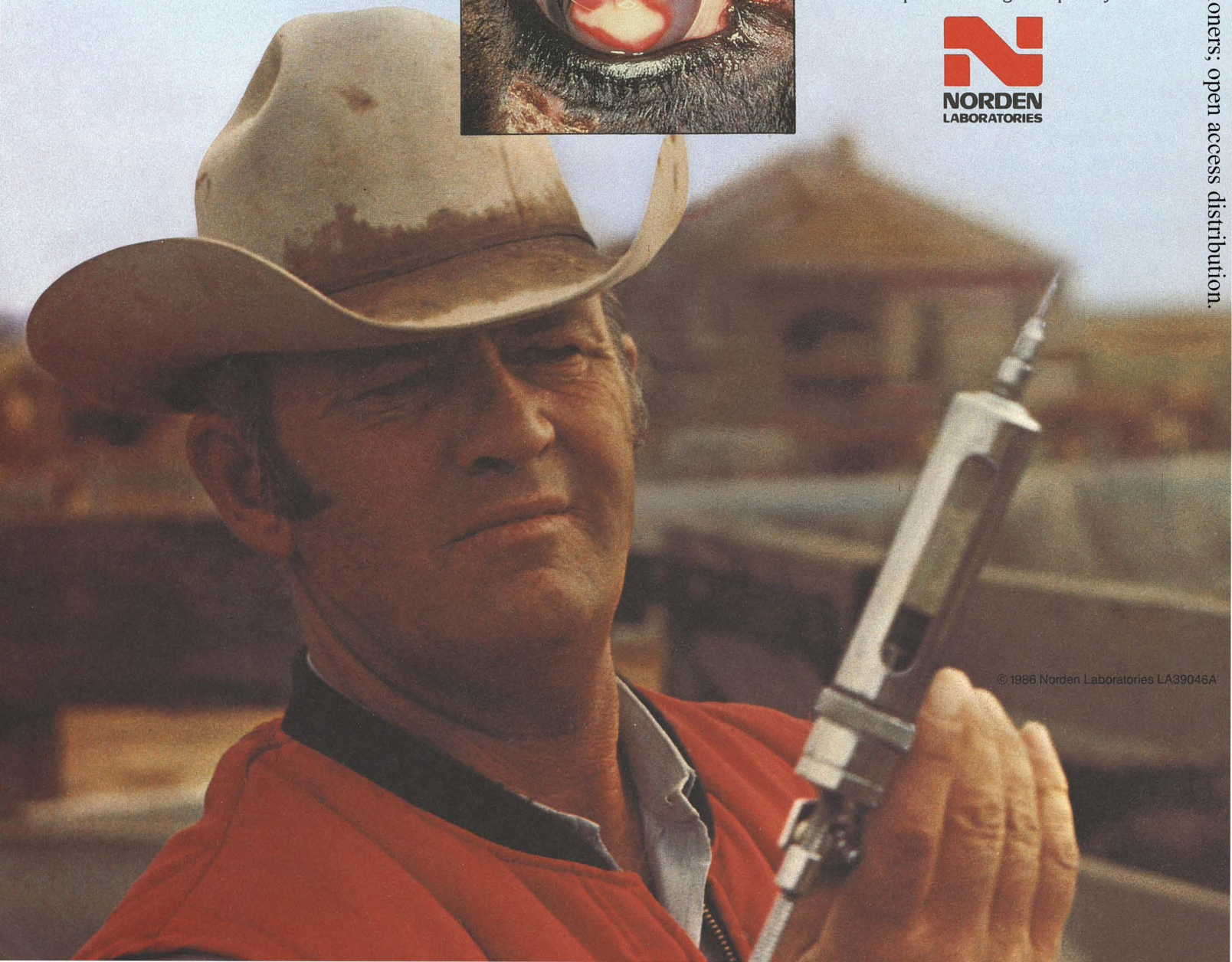

syndrome may abort or deliver a calf that rapidly succumbs to Salmonella septicemia.

Chronic enteritis in the bovine may occur either subsequent to acute enteritis or as the primary problem. Characteristically affected animals in addition to showing continuous to intermittent often mild diarrhea have a poor appetite and tend to become wasted, rather poor-doing animals. Such calves in veal units are commonly euthanitized. Morbidity and mortality in herd outbreaks will vary considerably with the production facility (veal unit with individual crates versus veal calves reared in group pens, dairy cows confined in a stanchion barn versus loose housed cattle, etc.) the level or exposure to the causitive organism, the strain of organism and the effect of ongoing stress factors on disease resistance and feed and water intake etc. (5).

The occurrence of acute enteric Salmonellosis in veal calves is a nightmare both to the owner and the veterinarian. Most of the important stressors that predispose to Salmonellosis are operative in this type of production unit: young age, dietary and management changes and invariably some calves with little or no passive immunity and or because of concurrent disease problems a compromised active immune response. It shouldn't be surprising that periodically salmonellosis is disseminated amongst such calves either assemblage of transport prior to arrival at the unit or in group pens in the unit through fecal contamination or faulty management, feeding or medication practices. Generally outbreaks that occur within two weeks of arrival in the unit are the most severe with virtually 100% morbidity, very, very poor response to treatment, and mortality ranging from 40-90%. In this type of outbreak I suspect the organism was widely disseminated amongst the calves during assemblage and or transport prior to arrival at the veal unit. Outbreaks that occur 2-6 weeks after arrival in the unit may not be associated with such high morbidity and mortality rates but the impact of the problem is severe none-the-less. Again I suspect that contamination prior to arrival in the unit has occurred, but often the management (group penning, common feeding utensils and equipment, etc.) is such that it facilitates the development of clinical disease. Outbreaks that occur after 6 weeks in the unit tend to be much less severe and can usually be traced to a management problem within the unit. Regardless, the diagnosis should be confirmed by fecal culture and the isolate should be phage typed for future reference particularly if one is concerned about residual or carry-over infection in the facility.

## Diagnosis

The only definitive diagnostic test for bovine salmonellosis is bacteriological culture from the feces or intestinal tissues and associated lymph nodes at necropsy. However, culture of *S. dublin* has been reported to be particularly difficult in the early stages of the syndrome and in severely affected calves (32). Four consecutive daily fecal samples that can be cultured immediately or delivered to the laboratory quickly in the appropriate transport medium should optimize the chances for a diagnosis of Salmonellosis if the samples are collected prior to antibiotic therapy. Otherwise, one cannot and should not in my opinion be too dogmatic about negative culture results in cattle. Other diagnostic tests such as serology and hematology are not specific enough in my opinion for use in individual animals, although the former test may be of value in assessing the status of a herd subsequent to an epizootic to identify animals from whom feces should be cultured. In such circumstances it is recommended that feces be collected every two weeks for a total of three samples from each suspect animal for culture specifically for Salmonella (19). The difficulties associated with the diagnosis of clinically and subclinically affected animals are recognized and need much more research. In the event of a herd outbreak it is particularly useful from an epidemiological standpoint to have the organism phage typed by the Center for Disease Control in Atlanta, Georgia. This often is the only way to conclusively implicate a particular source of infection or contamination.

## Necropsy Findings

The severe necrotic enteritis involving the terminal jejunum, the ileum and the large bowel with either a watery, blood-tinged intestinal content or a dipheritic pseudomembrane is quite characteristic. Likewise swollen and edematous mesenteric lymph nodes are described as a common finding both in spontaneous disease (14) as well as experimental disease (4).

## Differential Diagnosis

Salmonella induced acute or chronic enteritis in the calf over 3 weeks of age can be suspected if the diarrhea contains evidence of frank blood, excess mucus and casts although in calves over 17 days of age with blood and mucus and no casts coccidiosis or helminthiasis would have to be ruled out. The common causes of neonatal calf diarrhea (enterotoxigenic *E. coli,* rota and corona virus) tend to occur in younger calves and the response to rehydration therapy in such calves is remarkable compared to that seen in calves with salmonellosis. The severely ill attitude in such calves in spite of rehydration therapy and the tendency towards rapid body wasting is characteristic of salmonellosis and bovine virus diarrhea. The diagnosis of BVD in the absence of typical mouth lesions would necessitate a thorough post mortem examination.

With an individual cow or at the start of a herd outbreak it is necessary to differentiate salmonellosis from other causes of acute diarrhea without mouth lesions such as rumen overload, winter dysentery, helminthiasis, copper deficiency/molybdenum excess, Johnes disease and heavy metal poisoning, and from diarrheal diseases with mouth lesions such as bovine virus diarrhea and malignant head catarrh.

## Treatment

For the treatment of bovine salmonellosis, I prefer a combination of intensive fluid therapy with an alkalinizing potassium containing polyionic electrolyte solution with or without additional hypertonic (5%) sodium bicarbonate and broad spectrum antibiotic therapy. The choice of antibiotic ideally should be based on the sensitivity of the organism and be administered in full therapeutic doses. For this reason, one should always attempt a fecal culture prior to antibiotic therapy and specifically handle the sample to facilitate the culture of the organism. This should optimize the possibility of an isolation and provide an antibiotic sensitivity pattern if the response to the initial therapy is poor. Based on sensitivity, Trimethoprim Sulfa, Ampicillin or Gentamycin can be used systemically and nitrofurazone can be used orally although acute toxicity in calves under 2 weeks of age and chronic toxicity in all ages of calves has been recognized with Nitrofurazone (8).

Replacement and maintenance fluid therapy must be based on the critical assessment of the degree of dehydration. Severely ill animals in my experience generally have little or no rumen motility particularly in the early stages of the disease and do **not** respond to large volumes of electrolyte solution administered orally. With such animals it is usually necessary to catheterize one or both jugular veins for continuous infusion. Once rehydrated and stabilized (24 to 72 hours) or in less severely affected cases it may be possible to maintain adequate hydration by the oral route. Depending on the severity of the diarrhea, the degree and rate of dehydration and the size of the animal it may require 20-80 liters of fluid per day to attain and maintain a normal state of hydration. Ideally acid base and electrolyte parameters particularly Na+, K+ and Cl- and PCV and total serum proteins should be monitored regularly. Severely affected animals are prone to develop metabolic acidosis, electrolyte deficiencies and hypoproteinemia which must be corrected if the chance of survival is to be optimized. From these comments about therapy it should be apparent why many of these cases are referred to a veterinary medical teaching hospital.

The medical management decisions required when a severe outbreak of Salmonellosis occurs in a veal unit are unique and difficult. One must immediately institute management strategies to try and control further spread of infection; stop all introductions of new animals into the unit; segregate sick from healthy animals if calves have been in group rather than individual pens; establish a feeding and medication protocol that ensures that healthy animals are always cared for first with disinfected feeding utensils and other equipment; and ensure that carcasses, waste and waste disposal procedures do not cause contamination of the healthy calves, the feed supply, or the workers.

Next, based on the clinical parameters of the severity of diarrhea and dehydration, physical strength and appetite of the calf, and the presence of other concurrent disease(s) such as pneumonia, septic arthritis, and omphalitis one should euthanize those animals with little or no hope of survival. This will help to limit further contamination and allow the owner to concentrate the therapy and the nursing care on animals that are most likely to respond.

Finally, one must institute the use of oral fluid therapy with an alkalinizing, polyionic, glucose containing electrolyte solution at the rate of 1 to 4 liters, 2 to 3 times a day, depending on the amount of fluid required to rehydrate the calf and maintain it in a reasonable state of water and electrolyte balance. I prefer that the calf drink this electrolyte solution spontaneously 15-30 minutes prior to the feeding of the regular ration. In my opinion, this seems to optimize the absorption of the solution compared to mixing the electrolyte solution with the ration. Calves can be force-fed the electrolyte solution via esophageal feeder. Anoretic force-fed calves or calves that drink spontaneously and develop abdominal distension fail to show any improvements in their state of hydration within 24 to 36 hours or deteriorate in terms of their physical strength and become recumbent should be euthanized. Given the limited sensitivity pattern of the pathogen and the need for mass medication, the decision to use antibiotics should be based on known antibiotic sensitivity patterns. The tendency for owners to resort to the use of Procaine Pencillin, Tetracyclines and Sulfas for "something to try" should be discouraged. Also, the mentality that reasons that the use of newer synthetic Pencillins, or Gentamycin or combined Sulfa preparations (often at suboptimal doses because of the high cost of the drug) rather than electrolyte solutions because it is "easier and probably just as effective" must be challenged and changed.

## Prevention

There is a greater and growing awareness amongst veterinarians and farmers of the potential of introducing salmonellosis into a herd either through the purchase of, or exposure to, carrier animals or a contaminated environment. Often, however, little thought is given to the isolation or at least segregation from the herd of newly purchased animals, animals being returned to the herd from a high risk environment, or animals that suddenly develop diarrhea. Likewise, serious efforts at routine disinfection often occur after a problem develops, rather than as a routine preventive medicine strategy.

Much research has gone into attempts to develop an effective vaccine. The efficacy of killed vaccines in controlled studies in cattle (10, 11, 22, 23) like killed human typhoid vaccines is poor. When properly tested the protection in terms of survivial and or the development of clinical signs is incomplete. Also, vaccine administered either orally or parenterally did not decrease the fecal shedding of the challenge organism by the vaccinate (20). Attempts to date to produce vaccines from ribosomal extracts of *S. typhimurium* and *S. dublin* have not been successful (12) but

further research is needed.

A live, rough mutant vaccine of *S. dublin* (strain 51), (24, 18) has been shown to reduce mortality but not morbidity following vaccination and vaccinates did shed the organism for up to 14 days after oral vaccination. This vaccine is used in Britain and Europe where *S. dublin* infection is a serious endemic problem, but it is not and will not be marketed in North America.

Much research has been directed toward the development of live *S. typhimurium* vaccines for calves based on selected mutant strains such as gal E. mutants (28, 29, 1), aro A mutants (20, 23) and dap mutants (4). Although some of these vaccines have prevented death losses, none have prevented the development of fever or diarrhea in vaccinates subsequent to an adequate oral experimental challenge with pathogenic organisms.

Continued efforts to find an appropriate mutant strain for the manufacture of a live vaccine are needed. The ideal live vaccine must be stable, capable of preventing mortality and morbidity when the vaccinate is exposed to an adequate challenge with virulent Salmonella, and enable the vaccinate to eliminate the challenge organism quickly from its body so as to minimize or preferably prevent the carrier state.

**I for one await the development of this vaccine with great anticipation.**

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