NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
PROJECT OUTLINE

Project No. S-198
Date Submitted 2-14-58
Approved 3-26-58
Revised

1. Title **Calf Scours I. Studies on Chemotherapy**

2. Objective(s)
   (a) To determine the incidence of coliform bacteria in diarrhea of calves.
   (b) To determine the in vitro sensitivity to selected chemotherapeutic agents of coliform isolates obtained from scouring calves.
   (c) To determine the efficacy in vivo of commercial antidiarrheals selected on the basis of in vitro activity of their active ingredients.

3. Reasons for undertaking Investigations*

Scours is frequently a highly infectious septicemic disease which affects calves during the first few weeks of life. Calf scours is a major problem on many dairy farms, often causing death losses ranging up to 25%. Calves may survive, only to terminate in unthriftiness with or without pneumonia. In addition, localized infections of the middle ear, joints, etc. may be seen in the chronic septicemic form of the disease.

Coliform microorganisms have been incriminated as the major bacterial agents involved in calf scours by many previous investigators. Further information concerning the type of coliform organisms involved, the efficacy of various chemotherapeutic agents as determined by in vitro tests, and the in vivo confirmation of the efficacy of the better compounds to control calf scours is necessary.

*Including economic justification

4. Previous work and present status of investigations in the field of this project:

Udall[2] studied the calf scour syndrome and stated that the disease may result from bacterial infection or from unknown causes. Other workers[3],[4],[5] also found bacterial species involved. Among the infectious agents, Smith and Little[6] isolated a highly virulent coliform organism from calves dying from acute septecemia during the first 3 days of life. Coliform organisms were considered the cause of scours in eighteen reports covering the years from 1893 to 1955 as cited by Henning[11]. One of these (Jensen 1893) reported a bacteremia occurred in calf scours and the organism was B. Coli Commune. Henning[11] cited the importance of various other serological types of Bact. Coli as being particularly important in the calf scours problem.

Viruses have also been implicated in calf scours[7],[8],[9].

Contaminated maternity stalls, calf barns and yards act as well-recognized sources of the infecting agents. Episodes of the most fatal forms of calf septicemia and scours often cease when cattle are allowed to calve at pasture, or in buildings previously unoccupied by the herd in which calves are dying.

Dehydration is a common sequel in calves extremely sick with scours. McSherry and Grinyer[10] advocated a balanced electrolyte solution in the treatment program to overcome the deficiencies that they discovered were present from a blood chemistry study.

5. Outline of Procedure:

A minimum of sixty day-old calves of the various breeds will be assembled and penned individually in housing with concrete floors presently available on the veterinary research farm. If possible, the three main dairy breeds of this area will be represented as follows: 30 Holsteins, 15 Guernseys, and 15 Jerseys.

The calves will be fed a standard limited milk ration and calf starter free choice on a schedule similar to practices of local dairy farmers, except that nipple feeding pails will be washed in cold water only. The concrete floors will be hosed down twice daily when the calf is scouring and in other instances once daily.

The nature of the stool will be recorded twice daily and rated as normal (OK), loose (1), watery (2), watery with excess of mucous, foamy, or bloody (3). Rectal temperatures will be recorded twice daily on all calves. On arrival and when a calf shows a stool classified for the first time as a (2) or (3), a bacteriological culture on brilliant green agar and eosin - methylene blue will be made on the blood and of the rectal contents obtained by means of a 5 inch glass speculum. A third culture of the stool and blood will be taken ten days after the onset of scours in each calf. Colonial morphology and hemolytic activity on blood agar of the isolated bacterial species will be recorded. Activity on triple sugar iron slants will also be determined and noted. Coliform isolates will be purified and identified by accepted microbiological methods.

Calves that die will be necropsied and the spleen tissue cultured on media as indicated for fecal cultures above.

As an approach to the question of the role of intestinal coliforms to scours, a study of the serological relationship of isolated coliforms to their host will be made. Isolates from feces, blood and/or spleen will be used.

Isolated colonies of coliforms will be grown in broth cultures and administered orally to 3-day old non-scouring calves to attempt to induce the

disease. Multiple doses of 10cc of broth culture will be used. An equal number of untreated calves and calves given heat-killed cultures will be maintained as controls.

Calves moribund or that die will be necropsied and gross lesions noted. Pathological tissues for histopathological study will also be collected. A summary of the gross lesions and the histopathology will be compiled.

For the purpose of determining chemotherapeutic agents that offer promise in controlling calf scours, in vitro sensitivity test of selected materials will be run on coliform isolates recovered in the study. Promising chemotherapeutic agents from in vitro tests will be tested in vivo for confirmation of their effectiveness.

The following experimental design will be used to study antidiarrheals for the control of calf scours.

Experimental Design

	Test Compound A	Test Compound B	Placebo C
Calves 1st replication (natural scours)	1 calf	1 calf	1 calf

A minimum of twenty replications will be run in the course of the study. Calves showing a stool classified as a (2) or a (3) will be assigned at random to one of the treatments shown in the experimental design. Each calf will be treated morning and night for two days only in the initial studies. Additional studies will be run to evaluate different methods of administration of the chemotherapeutic agents. Rectal temperatures and severity of scours will be recorded twice daily.

Each surviving calf will be on experiment until 21 days of age and will be weighed on day 1 and day 21 of the experiment. Criteria of evaluation will be days with scours, severity of scours, and weight gains during the 21 days, and the death losses attributable to scours during the experimental period.

6. Probable Duration of Project: 2 years

7. Date of Initiation: As soon as approved

8. Personnel:

Name	Department	Relation to Project
J. G. Osborne	A.I.	Leader
R. D. Mocherie	A.I.	Cooperator
E. G. Batte	A.I.	Advisor
J. J. McNeill	A.I.	Advisor

9. Coöperation:

a. Interdepartmental

none

b. Other Agencies

Eaton Laboratories, Norwich, N. Y.

10. Financial Support:

a. Proposed Budget ..1957.... to ..1958..

Items	ALLOCATION OF FUNDS				
	Hatch	Regional Research	State	Other Eaton Labs.	Total
1. Salaries					
2. Labor				2735.00	
3. Travel				250.00	
4. Equipment & Supplies				2665.00	
5. All Other				50.00	
Total				5700.00	

b. Proposed Future Budgets:

Year	Salaries	Total Expenditures	Estimated Income
1958-59		5000.00	

11. General Remarks:

Project No. .....

SIGNATURES OF APPROVAL

1. Approval of Project Leaders

Date 18 Mar 58 ..... L. Clark Osborne .....

Title Leader

Date .....

Title .....

Date March 18, 1958 ..... Edward Ball .....

Title Owner

2. Approval of Heads of Departments or Coöperating Agencies

Date 22 Mar 58 ..... J. W. Stone .....

Head, Animal Industry

Date .....

Head, ...

Date .....

Head, ...

3. Approval of Director

Date 3/26/58 ..... R. L. Harrison .....

Director, North Carolina Agricultural Experiment Station

4. Approval of U. S. D. A.

Date .....

Chief, Office of Experiment Stations

attach to File Copy.

MEMORANDUM OF UNDERSTANDING

Between the

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION OF N. C. STATE COLLEGE

And the

SMITH, KLINE & FRENCH LABORATORIES

PHILADELPHIA, PA.

1. PROJECT TITLE: Control of Gastro-Intestinal Parasites of Ruminants
2. PROJECT LEADERS: E. G. Batte, W. E. Frazier, Veterinary Section, Animal Industry Department, N. C. State College, Raleigh.
3. OBJECTIVE: To screen promising compounds as anthelmintics against nematode parasites of ruminants.
4. PROCEDURE: Naturally infected sheep will be purchased and parasite load determined. The compound will be given to sheep and post-mortem examination made on third day to determine efficacy.

RESPONSIBILITIES OF COOPERATING AGENCIES:

A. The North Carolina Agricultural Experiment Station agrees to provide such office and laboratory space and supplies as may be needed and may be available. They further agree to furnish the personnel necessary to properly plan and conduct the research work and to make periodic progress reports to Smith, Kline and French.

B. Smith, Kline and French agrees to place at the disposal of North Carolina Agricultural Experiment Station funds in the amount of $1500 to be disbursed in accordance with the fiscal regulations of North Carolina Agricultural Experiment Station in support of these investigations.

C. It is mutually agreed that rights to publication or formal release of the data obtained will be retained by the North Carolina Agricultural Experiment Station, and prior to publication or formal release by the North Carolina Agricultural Experiment Station, no publication or formal release of the data shall be made without its knowledge or consent. Smith, Kline and French may use results of investigations conducted under the provisions of this Memorandum as it may elect, except that the name of North Carolina Agricultural Experiment Station at North Carolina Station College shall not be used in commercial advertising.

This memorandum shall become effective August 1 and shall be effective for a period of one year.

Date	Edwin G. Batte Project Leader
Date	W. Rutherford, Jr. Head, Department of Animal Industry
July Date	J. F. Brown Director, North Carolina Agricultural Experiment Station
7-17-58 Date	S. Stoney Simons Smith, Kline & French Laboratories Research and Development Division

File

December 4, 1957

MEMORANDUM TO: Dr. J. W. Pou

Subject: Comments by Matrone, McNeill, and Thomas concerning project submitted by Dr. Osborne on Calf Scours.

Page 1 -

Title - suggest title be made less all-encompassing; doubt seriously whether the etiology and chemotherapy of scours can be even begun in one 2-year project.

Objective -

(a) This seems like an impossible task. It is next to impossible to say with any degree of certainty that one or a few strains of the many types of coliforms are the causative agents. This is particularly true since coliforms are sure to be found in the feces anyway. The implication of one or several strains as the causative agent would, by itself, be a large project.

(b) How is it to be determined just what organisms isolated are pathogens? Also, is a screening program a proper objective?

(c) Is it possible to evaluate anti-diarrheals on only 2 days of treatment?

Reasons - this paragraph appears to be a part of the review of the history of the disease which should better be placed in section 4. This paragraph does not seem to have a convincing statement as to why such a project should be undertaken at N. C. State College at this time.

Page 2 -

Para. 2 - was virulent org. isolated from blood?

Para. 3 - do not see pertinence of discussion on viral agents. No work is proposed on this problem. Completely irrelevant.

Page 4

Outline of procedure -

In the previous section the author cites evidence indicating that virulent strains of E. coli are organisms most frequently implicated in calf scours. From an infected calf it is proposed to isolate organisms by streaking fecal samples on E.M.B. and B.G.B. These media permit the growth of coliforms only. Selected colonies will be cultured and their sensitivity to antibiotics and nitrofurans will be observed. The reviewer sees no merit in this procedure. The only organisms isolated will be coliforms, and since these organisms are notoriously resistant to antibiotics it is difficult to see what useful information can be obtained. Likewise, there seems to be no point in studying activity on TSI agar. This medium is used to differentiate between typhoid, paratyphoid, and dysentery organisms, and all of these will have been eliminated in the original plating on E.M.B. and B.G.B. Also, it is believed that these organisms are not implicated in calf scours.

The author does not indicate what he intends to do with this in vitro information after he gets it.

Page 5 -

Treatment for 2 days seems very short time. How old will these treated calves be?

What is purpose for keeping surviving calves on experiment for 21 days?

JJMcN:msb

J. J. McNeill

MEMORANDUM OF UNDERSTANDING

Between the

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION OF N. C. STATE COLLEGE

And the

EATON LABORATORY

NORWICH, N. Y.

1. PROJECT TITLE: Calf Scours I. Bacteriology and Chemotherapy

2. PROJECT LEADERS: J. C. Osborne, R. D. Mocherie, E. G. Batte and Katherine Prestwood, Veterinary Section, Dept. of A. I., N. C. State College, Raleigh.

3. OBJECTIVE:

(a) To isolate and catalog the pathogenic bacterial genera present in feces of calves suffering from scours.

(b) To study the efficacy of 5 nitro-2 formaldehyde semioxamazone in combination with bismuth subsalicylate (Entefur)* for the control of infectious scours of young dairy calves.

(c) To compare the value of Entefur with a commonly used antidiarrheal as a therapeutic agent for the control of calf scours.

4. EXPERIMENTAL ANIMALS AND PROCEDURES

Sixty day-old bull calves will be purchased and housed in existing barns on the veterinary research farm at N. C. State College. If possible the three main dairy breeds of this area will be represented as follows: 30 Holsteins, 15 Guernseys, and 15 Jerseys.

The calves will be fed a standard ration and on a schedule similar to practices of local dairy farmers. The design of experimental treatments is as follows:

* Trademark Eaton Laboratories, Norwich, N. Y.

Experimental Design

	Treatment and Pens		
	A Entefur	B Commonly used antidiarrheal	C Placebo
Calves 1st replication (natural scours)	5	5	5
Calves 2nd replication (induced scours)	5	5	5
Calves 3rd replication (induced scours)	5	5	5
Calves 4th replication (induced scours)	5	5	5
Totals	20	20	20

A - 1 Entefur bolus a.m. and p.m. to effect
B - Daily treatment to effect
C - 1 placebo bolus a.m. and p.m.

The 1st replication will include cases of white scours that occur paturally during the 1st five days after birth. From each case of natural occurring scours attempts will be made to induce scours in 3, 6 or 9 calves (depending upon the number of cases occurring and calves on hand) by feeding in milk an aliquot of a 24 hr. old tryptose broth culture made from feces from the natural occurring cases. In the event natural cases are numerous, fewer attempts to induce scours will be made. Treatments A, B, and C will be initiated as soon as scouring is observed. Just prior to the initiation of treatment a bacteriological culture of the fecal material in the rectum will be made of each calf. Colonial morphology and hemolytic activity of isolated bacterial species will be noted.

RESPONSIBILITIES OF COOPERATING AGENCIES:

A. The North Carolina Agricultural Experiment Station agrees to provide such office and laboratory space and facilities and supplies for this investigation as may be needed, and as may be available to the North Carolina Agricultural Experiment Station. They further agree to furnish the personnel necessary to properly plan and conduct the research work contemplated and to make periodic progress reports to Eaton Laboratory.

B. Eaton Laboratory agrees to place at the disposal of the North Carolina Agricultural Experiment Station funds in the amount of $3500.00 to be disbursed in accordance with the fiscal regulations of the North Carolina Agricultural Experiment Station in support of these investifations.

C. It is mutually agreed that rights to publication or formal release of the data obtained will be retained by the North Carolina Agricultural Experiment Station, and prior to publication or formal release by the Station, no publication or formal release of the data shall be made without its knowledge or consent. The Eaton Laboratory may use results of investigations conducted under the provisions of this Memorandum for such purposes as it may elect, except that the name of the North Carolina Agricultural Experiment Station or North Carolina State College shall not be used in commercial advertizing.

This memorandum shall become effective September 15, 1957 and shall be effective for a period of two years.

9/16/57
Date

J. Clark Bloom
Project Leader

17 Sept 57
Date

J. W. Fox
Head, Department of Animal Industry

Oct. 29 1957
Date

R. Bowdoin
Director, North Carolina Agricultural Experiment Station

Date

Eaton Laboratory
Research & Development Dept.