OES—Form 8
(Nov. 29, 1954)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1957

(Three copies to be given to the OES examiner)

1. PROJECT: (Fund, number, and title): HATCH 34, A STUDY OF SOME OF THE PHYSIOLOGICAL, SEROLOGICAL AND IMMUNOLOGICAL PROPERTIES OF VIBRO FETUS. S-30

2. DEPARTMENTS AND COOPERATING AGENCIES: Other stations participating in the Southern Regional Project S-30.

3. PERSONNEL: J. G. Lecce, E. F. Busch, June Daoud

4. PROGRESS OF RESEARCH HIGHLIGHTING PRINCIPAL ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Investigations on the metabolic properties of V. fetus showed that a combination of a good cell yield and a high metabolic activity was obtained by growing, under increased CO_2 and decreased O_2 tension at 37°C, cells in deep heart infusion broth at pH 7.0. Cells harvested from this media, washed with phosphate buffer, were free of endogenous activity and active in the presence of exogenous substrate. In order to have a metabolic system that would serve as a standard for comparing the activity of one strain of vibrio versus various substrates and in addition to use this information to compare the activity among the various strains or isolates of vibrio, it was necessary to arbitrarily pick one metabolic system (lactic dehydrogenase) and rigidly standardize various optimal conditions for this system. Then, under these standard conditions the activity of other oxidizable substrates was compared. This gave a pattern of dehydrogenase activity for one strain. Under this same set of conditions the activity of other strains was determined for the same oxidizable substrates. To date 4 catalase-positive strains have been subjected to this regime, and all 4 are similar in their metabolic activities.

5. USEFULNESS OF FINDINGS (when results may justifiably be expressed in terms of public benefits):

An aid in further defining the species V. fetus

6. WORK PLANNED FOR NEXT YEAR:

To continue to determine the physiologic and metabolic properties of V. fetus.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

None

8. Prepared by J G Lecce Approved .... (Director).

Date .... Date ....

November 7, 1957

MEMORANDUM TO: Dr. R. L. Lovvorn
Director of Research
Experiment Station, Campus

We understand from Dr. E. G. Batte, who recently attended the Annual Meeting of the Technical Committee for Regional Project RR-S-30, that this project has been broadened from the consideration of Vibrio Fetus alone to the consideration of diseases affecting the reproduction of cattle and sheep. We understand the new title of this Regional Project will be "CERTAIN DISEASES AFFECTING REPRODUCTION OF CATTLE AND SHEEP."

The project recently developed by Dr. L. C. Ulberg entitled "REPRODUCTIVE INEFFICIENCY IN THE BOVINE" which has been assigned the number H-147, could appropriately serve as a contributing project from this station to the Regional Project mentioned above. This would replace our previous contributing project entitled "A STUDY OF SOME OF THE PHYSIOLOGICAL, SEROLOGICAL AND IMMUNOLOGICAL PROPERTIES OF VIBRIO FETUS" H-34 & RR-S-30, which is being phased out at the end of the current fiscal year. We would like to recommend that Project H-147 be submitted as our contributing project to this Regional Project and that we continue cooperating with and participating in this Regional effort.

We shall appreciate your advising us if this change in contributing projects meets with the approval of your office.

J. W. Pou, Head
Department of Animal Industry

JWP:nr

cc: Dr. E. G. Batte
Dr. J. E. Legates
Dr. L. C. Ulberg

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION PROJECT
CONTRIBUTING TO
THE SOUTHERN REGIONAL PROJECT, S-30

1. PROJECT: (H-34) RRS-30 A Study of Some of the Physiological, Serological, and Immunological Properties of Vibrio Fetus.

2. COOPERATING AGENCIES AND PRINCIPAL LEADERS: Other stations participating in the Southern Regional Project S-30. J. G. Lecce, Leader.

3. NATURE OF WORK AND PRINCIPAL RESULTS OF THE YEAR: In attempting to determine some of the physiologic and metabolic properties of V. fetus the following information has been learned.

The reduction of triphenyl tetrazolium chloride (TTC) in a Thunberg tube by resting cells of a typical strain of Vibrio fetus in the presence of Na lactate was chosen as a model system that was standardized with respect to the following optima: harvest time, pH of reduction reaction, TTC concentrations, cellular concentration and substrate concentration. Under these standard conditions other substrates were tested for their electron donating capacity. This yielded a pattern of oxidative activity for 1 strain. The pattern for this strain was compared to the pattern of 26 other strains examined in a like manner. Fourteen of these strains were considered V. fetus since they were catalase positive and were isolated from either aborted fetuses, or from the genital tract of cows or bulls that were associated with infertility problems. Six similar catalase positive strains of ovine fetal origin were also designated V. fetus. The remainder of the strains studied for oxidative action were 3 catalase positive strains isolated from pigs and 4 catalase negative strains isolated from bovine genital tracts. It was determined that the oxidative capacity of vibrios isolated from farm animals was quite similar regardless of whether they were isolated from different hosts, different anatomical locations, or different H_2S-catalase types from the same host and same anatomical location. Only lactate, formate, pyruvate, α ketoglutarate and succinate out of 31 substrates tested served as electron donors with each strain. There was nearly always 3 times more TTC reduced in the tube containing formate than lactate. In order to reach a degree of reductions comparable to lactate the tube with pyruvate required a reaction time 3 times that of lactate while α ketoglutarate was incubated 6 times longer. The degree of reduction with succinate was markedly lower than the other 4 substrates.

The growth in heart infusion broth of 11 out of 12 bovine catalase positive strains was inhibited by the addition of 0.8% glycine while none of 5 ovine catalase positive strains were inhibited.

Up until 1955 motile, microaerophilic vibrio isolated from either aborted bovine and ovine fetuses, or bovine and ovine genital tracts were regarded as members of the genus and species, V. fetus. Since then, these vibrios have been divided into 2 catagories:

(1) catalase positive-H_2S negative vibrios were considered pathogenic, true, V. fetus, (2) catalase negative-H_2S positive vibrios were considered saprophytes belonging to a yet unnamed vibrio species. Three pieces of evidence indicate that this is not a meaningful separation: (1) Akkermans et al. (1956) isolated what they considered pathogenic vibrio in aborted bovine fetuses. These vibrio were catalase positive and H_2S positive, (2) Bond's (1957) discovery that catalase activity correlated with colonial type indicates catalase positive-H_2S negative vibrio and catalase negative-H_2S positive vibrio are progeny derived from the same parent organism, (3) the data in this report indicates that ovine and bovine catalase positive-H_2S negative genital vibrio, bovine catalase negative-H_2S positive vibrio and porcine dysentery vibrio are all quite similar when judged by their oxidative capacity. This similarity in oxidative capacity seems an important characteristic when one considers the number of different oxidative patterns that might conceivably occur with 31 different substrates, and the fact that the strains studied have ranged in isolation dates from 1939 to 1956. This data also lends support to the conjecture that vibrios isolated from the intestinal tract of farm animals might be related to V. fetus.

It is proposed in the light of recent data cited above that catalase and H_2S activity in genital vibrio is too tenuous a characteristic to differentiate a species. Differentiation based on 2 activities also would seem inconsistent with the accepted practice of regarding both ovine and bovine genital vibrio as V. fetus since they appear to differ in regards to antigens, glycine tolerance, pathogenicity, and host origin.

Differences surely exist between groups of vibrios and no doubt within groups. This point is well established and accepted. However, the author would like to question the wisdom of emphasizing these differences at the expense of similarities. Perhaps a framework of knowledge that included the possibility that the various vibrios are closely related as well as possessed of individual characteristics would more accurately reflect the facts of nature. It is true that the data in this report and elsewhere indicate that there are variations among these vibrios with respect to antigens, glycine tolerance, salt tolerance, catalase activity, colonial types etc. However, these same vibrios do have common characteristics as is evidenced by their oxidative. activity. It is conjectured that the vibrios that have been found associated with the intestinal tract and genital tract of farm animals are closely related except for slight variations that may be a reflection of their environment.

4. APPLICATION OF FINDINGS: An aid in further defining the species V. fetus.

5. WORK PLANNED FOR NEXT YEAR: Termination of project.

6. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR: A study of some of the metabolic properties of Vibrio fetus and other related vibrios isolated from animals. In preparation.

7. APPROVED:

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION PROJECT
CONTRIBUTING TO
THE SOUTHERN REGIONAL PROJECT, S-30

1. PROJECT: (H-34) RRS-30 A Study of Some of the Physiological, Serological, and Immunological Properties of Vibrio Fetus.

2. COOPERATING AGENCIES AND PRINCIPAL LEADERS: Other stations participating in the Southern Regional Project S-30. J. G. Lecce, Leader.

3. NATURE OF WORK AND PRINCIPAL RESULTS OF THE YEAR: In attempting to determine some of the physiologic and metabolic properties of V. fetus the following information has been learned.

   1. Preparation of washed resting cells of suitable metabolic activity.
      Good cell crop yields were obtained from blood plates; however, these cells did not have the desired metabolic activity. A combination of a good cell yield and a high metabolic activity was obtained by growing cells in deep heart infusion broth cultures at pH 7.0. Best results were obtained if the broth was freshly steamed to remove dissolved gasses, equilibrated to 37^{0} C., inoculated, and incubated under increased CO_2 and decreased O_2 tension at 37^{0}C. Cells harvested from this media, washed with phosphate buffer, were free of endogenous activity and active in the presence of exogenous substrate.

   2. Standardization of a metabolic system.
      In order to have a metabolic system that would serve as a standard for comparing the activity of one strain of vibrio versus various substrates and in addition to use this information to compare the activity among the various strains or isolates of vibrio, it was necessary to arbitrarily pick one metabolic system (lactic dehydrogenase) and rigidly standardize various optimal conditions for this system. Then, under these standard conditions the activity of other oxidizable substrates were compared. This gave a pattern of dehydrogenase activity for one strain. Under this same set of conditions the activity of other strains were determined for the same oxidizable substrates. To date 4 catalase-positive strains have been subjected to this regime, and all 4 are similar in their metabolic activities.

4. APPLICATION OF FINDINGS: An aid in further defining the species V. fetus.

5. WORK PLANNED FOR NEXT YEAR: To continue to determine the physiologic and metabolic properties of V. fetus.

6. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR: None.

7. APPROVED:

PROCEDURES FOR THE ISOLATION AND IDENTIFICATION OF
BACTERIA THAT MAY BE IMPORTANT IN BOVINE MASTITIS

James G. Lecce

The ease of evaluating the significance or importance of bacteria isolated from milk samples is directly related to the quality of the sampling technique and the time lapse from sampling to plating. It, therefore, follows that the interests of those that are concerned with this problem are best served when a minimum of time lapses from the careful sampling of the udder to the inoculation of bacteriological media.

Weekly Routine Composite Milk Samples (4 quarters per vial)

I. Within one hour of sampling

A. With 5 mm. loop, streak blood agar quadrant with milk from vial containing composite sample of 4 quarters of cow.

B. With a 0.01 ml. syringe, spread 0.01 ml. of the above samples over an area of 1 sq. cm. for leucocyte counts. Stain. Leuococytes may be counted at the convenience of technician.

II. 18-24 hours after plating

A. Examine and process plates. It may be necessary to continue incubating for 48-72 hours negative plates and plates containing slow growing streps. This continued incubation of negative plates will afford us an opportunity to detect growth and characteristic hemolysis of Corynbacterium pyogenes.

B. Gram stain all significant colonies. What determines a significant colony naturally depends on relative numbers of colonies, type of colonial morphology and most of all the technician's experience. For instance, one fast-spreading-proteus-type colony among many Beta-type streps would not be significant, however, conversely, this colonial type in great and dominant numbers may be significant. When in doubt, stain the colony.

1. Gram positive coccus

a. Micrococcus pyogenes (Staph), record hemolysis and report the hemolytic strains.

b. Streptococcus, record hemolysis, emulsify colony in 1 ml. broth and inoculate a drop of this suspension into Hippurate, Mannitol and Sorbitol. (If growth is crowded, etc. so that an emulsion can not be made, carefully pick a colony with a needle into T. soy broth. Incubate overnight, then place a drop into Hippurate, Mannitol and Sorbitol.

TABLE I

	Group	Type Hemolysis	Hippurate	Mannitol	Sorbitol
S. agalactiae	B	β±, α±, γ±	+	-	-
S. dysagalactiae	C	α	-	-	±
S. uberis	?	γ±, α±, β±	+	+	+
S. pyogenes (animalis)	C	β	-	-	+
S. pyogenes*	A	β	-	-	-

* S. pyogenes (human) should be kept and provisionally placed into Human group A by using the bacitracin test.

24-48 hours after the above have been inoculated with the strep, read for acid production in Mannitol and Sorbitol, and for benzoic acid production in Hippurate.

Test for the hydrolysis of sodium hippurate

Reagent: Ferric chloride ($FeCl_3$-6 H_2O) ---------- 12 gms.
Hydrochloric acid ------------------ 2 ml.
Distilled water ------------------100 ml.

Medium: a) If sodium hippurate broth is prepared in cotton plugged tubes, the level of the medium in each tube should be marked with a wax pencil. During storage and incubation of the medium, some evaporation occurs, and the concentration of sodium hippurate in the medium is increased. Since the concentration of sodium hippurate must be exactly 1%, make up the loss due to dehydration by adding distilled water up to the wax pencil mark after incubation and before the ferric chloride test is performed. If this precaution is not taken, a false positive is obtained.

b) If sodium hippurate broth is prepared in screw-capped tubes, it is not necessary to mark the level of the broth, since there is no dehydration during storage or incubation and no water loss to be restored.

Procedure: Transfer 0.8 ml. of a 48 hour sodium hippurate broth culture to a Wasserman tube. Add 0.2 ml. of the ferric chloride reagent. Mix immediately and observe for several minutes. A permanent precipitate indicates the presence of benzoic acid and hydrolysis of the sodium hippurate.

Since sodium hippurate is first precipitated and later redissolved by the amount of reagent specified, and since benzoic acid is also redissolved by a greater excess of the reagent, it is necessary to have the reagent and the medium balanced, and to measure the amounts used in the test quite accurately. A control test with sterile medium should always be made.

c. Diptheroids-Corynbacterium pyogenes, pin point colonies usually requiring 48 hours to yield a narrow zone of Beta hemolysis. (Caution-Often times streps grown on solid media on primary isolation may stain similarly to diptheroids.) Note: Listeria are morphologically similar to C. pyogenes except the Listeria are motile. Most of the non-hemolytic diptheroids are probably contaminates.

d. Gram negative rods - With a needle pick the colony of the gram (-) bacillus and stab the butt of a T.S.I. agar slant. Examine T.S.I. slants in 24-48 hours. If a medium has alkaline slant (red) and acid and no gas, butt (yellow), inoculate urea media with the growth from the T.S.I. slant. The urea media will help identify the organism as Proteus, Salmonella, Shigella, Paracolon. T.S.I. (Triple sugar-iron agar) contains 1% lactose, 1% sucrose, 0.1% dextrose and 0.2% ferrous ammonium sulfate in a nutrient agar base and phenol-red as an acid-base indicator.

Fermentation of the carbohydrates is indicated by a change from red to yellow (acid).

Gas is indicated by the formation of bubbles in the butt.

If lactose and/or sucrose is fermented, the slant becomes yellow.

If dextrose is fermented while lactose and sucrose are negative, the butt is yellow and slant remains red. The slant stays red because at this low concentration (0.1%) of dextrose aerobic oxidative metabolism utilizes the acid as quickly as it is produced, hence, the area in contact with air stays red while the acid from the fermented dextrose in the essentially anaerobic butt changes the indicator to yellow.

Hydrogen sulfide production is indicated by blackening of the medium due to iron sulfide precipitation.

Red color in the urea media indicates the organism has split urea into ammonia which in turn makes the medium alkaline.

See Table II for interpretation of the reactions on T.S.I. and Urease Media.

Acute Mastitic Milk Samples from Infected Quarters (one infected quarter per vial)

These samples are to be handled as the weekly routine composite milk samples except in addition the sensitivity of the etiological agent to antibiotics will be determined.

Personnel:

Supervisor E. F. Busch
Technician D. D. Blanchard

Glassware-Media Preparation June Daoud

TABLE II

INTERPRETATION OF REACTIONS ON T.S.I. AGAR AND UREASE TEST BROTH

T.S.I. AGAR				Urease Test
T.S.I Group	Reaction	Indicates	Possible Organisms	Broth
1	Yellow slant, yellow + gas in butt	Dextrose = acid + gas, Lactose and/or sucrose = acid + gas	E. Coli	-
			A. aerogenes	-
			Paracolon bacilli (sucrose = positive)	-
1a	Same as above plus blackening	Same as above plus hydrogen sulfide	E. freundii	-
			Proteus vulgaris	+
2	Red slant, yellow plus gas in butt	Dextrose = acid + gas, Lactose and sucrose = negative	Salmonella (H_2S-)	-
			Paracolon bacilli (sucrose - negative	-
			Proteus morgani	+
2a	Same as above plus blackening	Same as above plus Hydrogen sulfide	Salmonella (H_2S+)	-
			Bethesda-Arizona groups	-
			Proteus mirabilis	+
3	Red slant, yellow butt no gas	Dextrose = acid, no gas, Lactose and sucrose = negative	S. typhosa*	-
			Shigella	-
			Paracolon bacilli	-
			Providence group (29911)	-
			Proteus rettgeri	+
3a	Same as above plus blackening	Same as above plus hydrogen sulfide	S. typhosa*	
4	Red slant red butt	Dextrose = negative or not fermented anaerobically Lactose and sucrose = negative	Pseudomonas	-
			Achromobacter	-
			Flavobacterium	-
			H. parapertussis	-
5	Yellow slant, no change in butt in 24 hours; yellow butt in 48 hours	Lactose = negative Dextrose and sucrose = slight acid	Pasteurella multocida	-

* Salmonella typhosa usually shows only slight hydrogen sulfide production on T.S.I. agar, and frequently fails to produce any blackening of this medium. Organisms suspected of being S. typhosa which fail to blacken T.S.I. agar should be checked for hydrogen sulfide production on SIM medium.

Also, strains of Salmonella typhosa have been isolated which occasionally produce a small amount of gas in the butt of T.S.I. agar, and so will erroneously fall into T.S.I. group 2 or 2a.

H-34 & RR-S-30 VETERINARY

A STUDY OF SOME OF THEPHYSIOLOGICAL, SEROLOGICAL, ETC. OF VIBRIO FETUS

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
PROJECT OUTLINE

Project No. RM-70
Date
Submitted January 17, 1956
Approved March 9, 1956
Revised

1. Title A study of some of the physiological, serological and immunological properties of Vibrio fetus.

2. Objective(s)

(1) To determine the growth characteristics of V. fetus as a means for obtaining better isolation procedures, metabolically active resting cells, and stable, standard diagnostic antigens.
(2) To correlate serum antibody titer with mucous antibody titer and the effect of these titers on the immune state of the host (cows).

3. Reasons for undertaking Investigations*

One of the abortion problems of cows and sheep is due to a bacterium, Vibrio fetus (1). It has been stated that breeding difficulties due to this specific microorganism accounts for a loss of 172 million dollars a year to U. S. cattlemen (2). It is possible that this estimate is too high since the present diagnostic procedures do not afford us a means for eliminating vibrio as a cause of a particular breeding problem while it is possible to eliminate other etiological agents. Because of the lack of a definitive test, the tendency in such a situation would be to implicate vibrio. This could create a convenient pigeon hole for breeding problems of ill-defined origin which in turn would lessen the pressure to more clearly define these problems.

It seems obvious from the published literature (3,4,5,) and from personal conversations with diagnosticians that serology leaves much to be desired as a means for establishing diagnosis, control or understanding for the disease, vibriosis. Perhaps the enigma associated with the diagnostic serology is due to a lack of fundamental information concerning the physiological, serological and immunological properties of this group of organisms. A certain precision of reproducibility is needed before stable, standardized antigens and infecting agents can be produced. Stable, standardized cells must be produced before diagnostic serology can be meaningful. Certainly an approach directed at learning some of the basic serologic and metabolic characteristics of vibriosis would aid in any attempt to give serology meaning.

*Including economic justification

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

Past investigators have directed much time and energy towards characterizing the mode of transmission and the site of infection for this vibrionic disease (1,3,6). Most probably the disease is venereal in nature. That is, the vibrio organism gains entrance into the host via the genital tract and demonstrates a predilection for mucosal epithelium by setting up a local, mild, chronic inflammation there. At this point, the disease has little importance to the economy of the host. However, with the advent of pregnancy, an organism of low pathogenicity assumes importance in the role of a potential aborting agent. Another aspect of venereal diseases is the transmission of the infection to the male genitalia and from there to other females <u>ad infinitum</u>.

In general with venereal diseases of this type (those that have little or no bacteremia, e.g., gonorrhea in humans), diagnosis based on serology is difficult if not impossible since localized inflammations of body surfaces rarely engender the production of high and consistent antibody titers. In these instances, diagnosis of vibriosis are being used less and less as more and more investigators realize that serological results are inconsistent with field and clinical observations. These techniques are based on the detection of agglutinins in the cow's serum and also in the vaginal-cervical mucus. Reliability in these tests is questioned since there is a lack of correlation among mucus titers blood titers and clinical evidence (4). The pathogeneses of this disease undoubtedly contributes to the difficulty of evaluating serological data. Other contributory factors could reside in our ignorance concerning the basic nature of this group of organisms (vibrios). For example, there might be variation in antigens due to the lengthy and inefficient procedure used for the growth of the antigens (1). It is known for instance, that some

continued page 2A

5. Outline of Procedure:

<u>PART I</u> It seems obvious from the above that there is a lack of basic information concerning the organism <u>per se</u> and also the host's response to the organism. It is for these reasons that we feel an important key to the problem of vibriosis is to be found in a study of the growth characteristics of these organisms. For example, such things as the optimal pH of various media, advantages and disadvantages of commercially available dehydrated media, the effect of adding natural supplements and intermediate metabolites to growth media, the proper balance of atmospheric N_2 to H_2 to O_2 to CO_2 for optimal growth will be determined. From this, we would expect to uncover more efficient ways of isolating and growing these organisms. Such information would assist us in authoritatively correlating serological response with the presence of infection, as well as in the production of standardized antigens. This information would also lend itself to a study of the metabolic activity of resting cells. A study of the metabolic activity of resting cells would aid in determining homogeneity within the group and also might supply clues as to possible sources of energy for growth. Before any such study on growth is contemplated, <u>FIRST</u> an accurate means of estimating the number of organisms and the stage of growth of these organisms will be sought.

<u>PART II</u> The second phase of the problem will concern itself with correlating humoral antibodies with mucous antibodies and their effect on infection and immunity. Before this can be approached, we must produce an antigen that is not only stable in presumed normal fluids, but can also excite production of a high level of circulating antibodies. This strain should also have the capacity to infect a cow or to cross antigenically with a strain that can infect that host. Oil-based adjuvants similar to those used in influenza and polio immunization will be used in attempting to obtain high circulating antibody titers. Methods for increasing

strains vary from batch to batch in their stability in presumed normal sera (1,4). Only recently it has been learned that antigenic difference exist within the group, and there is a lack of fundamental information concerning their metabolic homogeneity (7). Little wonder that the serological data is confusing when one contemplates these contributory factors coupled with the characteristics of the infection.

The same reasons that make serological diagnosis difficult also lend themselves to poor control of the disease by way of vaccines. Work, however, with human influenza indicates that a correlation exists between circulating antibody and antibody found on the respiratory mucous membrane (8). Here mucous antibodies are found when the host has a high titer of circulating antibody. These mucous antibodies may engender immunity to the disease by aborting the infection.

vaginal mucous titers will be investigated. Perhaps mechanical irritation of the vaginal-membranes, or the presence of a low grade infection other than vibrio, or the use of a drug can cause humoral antibodies to spill over onto the mucous membrane. When and if the investigators are satisfied that mucous antibody levels have been produced, these experimental animals, along with controls, will be challenged with an infecting dose of vibrio *via* the genital route. The criterium of infection will be the subsequent isolation of the organisms. The controls will serve a dual function: (1) to determine efficacy of the challenge, (2) to study the pattern of development of serum and mucous antibodies when stimulated by means that approximate natural infection. What this pattern can mean in terms of the immune state of the host will be investigated.

6. Probable Duration of Project: Two (2) years

7. Date of Initiation: Immediately

8. Personnel:

Name	Department	Relation to Project
James C. Lecce	Animal Industry	Leader
Flora G. Bourdeau	Animal Industry	Cooperator
J. C. Osborne	Animal Industry	Advisor

9. Coöperation:

a. Interdepartmental None

b. Other Agencies None

REFERENCES

1. Plastridge, W. N., L. F. Williams, H. L. Easterbrooks, E. C. Walker and R. N. Beccia. 1951 Vibriosis in Cattle. Storrs Agr. Expt. Sta. Bull. 281.

2. Ed. 1955, Infertility: Look for Vibriosis. Agri. Res. June, p. 14.

3. McEntee, K., D. E. Hughes and H. L. Gilman. 1954, Experimentally Produced Vibriosis in Dairy Heifers. The Cornell Vet. 44:376-394.

4. Hughes, D. E., 1953. A Study of the Diagnosis of Bovine Vibriosis with Special Reference to the Detection of Agglutinins in the Vaginal Secretions. Cornell Vet. 43:431-444.

5. Harsh, Hadleigh and E. A. Tunnicliff. 1955, The Diagnostic Significance of the Agglutination Reaction for Vibriosis in Sheep. J. Am. Vet. Med. Assoc., 126:100-103.

6. Easterbrooks, H. L. and W. N. Plastridge. 1952, Experimental Transmission of *Vibrio fetus* in Diluted Semen and by Contact. J. Am. Vet. Med. Assoc., 120:199-201.

7. Price, K. E., L. J. Poelma and John E. Faber, Jr. 1955, Serological and Physiological Relationship Between Strains of *Vibrio fetus*. Am. J. Vet. Res. 16:164-169.

8. Francis, T., H. E. Pearson, E. R. Sullivan and P. N. Brown. 1943, The Effect of Subcutaneous Vaccination with Influenza Virus Upon the Virus Inactivating Capacity of Nasal Secretion. Am. J. Hyg. 37:294-300.

Project No. ....

10. Financial Support:

a. Proposed Budget 2/1/56 .... To 6/30/56

Items	ALLOCATION OF FUNDS						
	Bankhead-Jones	R & M	Purnell	Adams	State	Other	Total
1. Salaries							
JAMES G. LECCE						2625.	
FLORA BOURDEAU						1717.	
2. Labor						200.	
3. Travel							
4. Equipment & Supplies		1650. 350.				1650 1800. 3450	
5. All Others						75.	
Total		2000.				6417.	8417.

b. Proposed Future Budgets:

Year	Salaries	Total Expenditures	Estimated Income
1956-57	$12,000.00	$18,000.00	
1957-58	$12,000.00	$18,000.00	

11. General Remarks:

 Project No. RM-70

SIGNATURES OF APPROVAL

1. Approval of Project Leaders

Date 2/8/56 James G. Leece

Title Leader

Date 2/8/56 Flora J. Baurdeau

Title Cooperator

Date 2/8/56 J. Clark Osborne

Title advisor

2. Approval of Heads of Departments or Coöperating Agencies

Date 2/11/56 J. W. Pou

Head, Animal Industry

Date

Head,

Date

Head,

3. Approval of Director

Date Feb 28, 1956 H. A. Stewart

acting Director, North Carolina Agricultural Experiment Station

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

Date MAR 9 1956 J. W. Wellington

~~Chief, Office of Experiment Stations~~

ACTING Director
State Experiment Stations Division
AGRICULTURAL RESEARCH SERVICE

NORTH CAROLINA STATE COLLEGE
School of Agriculture
RALEIGH

Department of Animal Industry

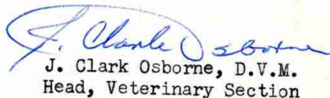
February 8, 1956

Dr. J. W. Pou
Polk Hall
Campus

Dear Dr. Pou:

I am attaching herewith the project entitled, "A Study of some of the Physiological, Serological and Immunological Properties of Vibrio fetus", that has been previously approved by the Director's office. I submit the project as a new number project and we request that every effort be exploited to get Federal support for this project for the remainder of this fiscal year based on the following break-down: Salary, for time spent on the project--James G. Lecce, $\frac{1}{4}$, $650.00, Flora Bourdeau, $\frac{1}{4}$, $420.00, Lucy Ponder, 1/5, $300.00. Supplies and Equipment, needed for the remaining five months of the fiscal year are itemized as follows: High Speed Centrifuge, $535.00; Spectronic 20 Colorimeter, $300.00; Water Bath, $138.00; Incubator Stand, $35.00; Small Refrigerator, $200.00; Platform Scales, $60.00; Office File, $65.00; Test Tubes, Glassware and Media, $500.00, for a total expenditure for equipment and supplies of $1833.00 . Contractual item to install additional electrical outlets, $75.00.

The above request is based on a break-down by the project leader and I urge that these funds be made available if at all possible in order to get this research properly underway.



J. Clark Osborne, D.V.M.
Head, Veterinary Section

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October 27, 1955

MEMORANDUM TO: Dr. J. C. Osborne

Enclosed is a copy of the proposed project entitled "STUDIES ON BOVINE VIBRIOSIS. III Growth Requirements and Immunity," together with suggestions from Dr. Stewart concerning additional information and possible revision in the statement of Objectives that the Experiment Station Office would like to have before final approval of the project.

In line with the suggestions in Dr. Stewart's letter, it would be of assistance I believe to consult with Dr. W. J. Peterson and either Dr. Matrone or Dr. Wise concerning suggestions they might have for changes in the proposal. If I can be of any assistance in these suggested revisions please let me know.

In submitting this project for approval of the Experiment Station Office they were informed that the project had been reviewed by Dr. Bogg, Dr. Monroe and Dr. Speck and that this project is one we would like to consider as contributing to a Southern Regional Project on Vibriosis or Reproduction Problems in cattle should North Carolina participate in such a project.

J. W. Pou, Head
Department of Animal Industry

JWP:nsr
Enc.

NORTH CAROLINA STATE COLLEGE

SCHOOL OF AGRICULTURE • RALEIGH, N. C.

OFFICE OF THE DEAN AND DIRECTORS

October 24, 1955

File

MEMORANDUM TO: J. W. Pou

FROM: H. A. Stewart H. A. S.

We have received the proposed project, "Studies on Bovine Vibriosis. III Growth Requirements and Immunity". This project names Dr. J. C. Osborne as leader with Dr. J. G. Lecce and Dr. Flora Bourdeau as cooperators. We consider this project is not specific enough in the statement of the objectives nor in the outline of procedures to undertake what is proposed. The following questions come to mind:

1. Are there strains of the vibriosis organism? If so, and I believe it is true, which strain will be the one studied under the project and why? Are the antigenic properties of the various strains equal and unchanging or do they vary in virulence? It is difficult for me to understand how a study of the immunological properties of the organism can be evaluated until the production of the organism can be quantitized so that the dosage may be consistent from animal to animal.

I feel that the first objective indicated could be the basis for a very objective worthwhile study. If the term "growth requirements" is interpreted in a broad sense I am not certain that much would be gained from determining the specific nutrition requirements of one strain of this organism in culture media. In making such determinations this project could well be bogged down with a study of specific amounts and quantities of amino acids, co-enzymes, vitamins, minerals, and perhaps complex materials that are not chemically defined.

2. I cannot see howthe second objective can be approached objectively without information that does not seem available at present. I should like to suggest that the basic hypotheses upon which this project is based be outlined in the reasons for undertaking the study and that the present status of work with this organism be catalogued rather completely. On the basis of the hypothesis and our present knowledge a reasonable objective and procedures should follow. I have confidence that we have personnel now available in this section to do a creditable piece of work in this field. I should like to have those who will be held responsible for the conduct of the work have a position of responsibility and leadership in formulating the outline. I believe that any project which is proposed should be approved in its final form by bacteriologists and statisticians outside of the Animal Industry Department. If this project is to deal with growth requirements or the nutrition of the organism I feel that the project should meet with the approval of the Animal Nutrition Section and Dr. W. J. Peterson.

October 14, 1955

MEMORANDUM TO: Dr. R. L. Lovvorn
Director of Research
N. C. Experiment Station
Campus

Enclosed is the draft of a proposed project entitled, "Studies on Bovine Vibriosis. 111 Growth Requirements and Immunity," The work on Vibriosis has previously been conducted as a Subproject under Project S-78. Project S-78 was originally set up when research work in the Animal Disease area was initiated. Since several areas of work are now in progress, and since additional staff members are assigned to work in this area, we feel it desirable to divide the general project (S-78) into more specific projects. In addition to the persons who will be working with this project, the material has been reviewed by Dr. Borg, Dr. Monroe and Dr. Speck.

We would appreciate your consideration of this proposed project and approval or suggestions as to changes that should be incorporated into the present plan. Should North Carolina participate in a Southern Regional Project on Vibriosis or Reproductive Problems in Cattle, this would be the project that we would recommend submitting as the contributing project from this Station.

J. W. Pou, Head
Department of Animal Industry

JWP:nsr

Department of Animal Industry
NORTH CAROLINA STATE COLLEGE

In addition to personnel who will be participating

MEMORANDUM

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To Dr. J. W. Pou

ATTACHED PAPERS

- ____ Please note and return.
- ____ File.
- ____ For your records.
- ____ Speak to me concerning.
- ____ Please handle.
- ____ Please answer.
- ____ Note opinion and return.
- ____ Needs your signature.
- ____ Please approve.
- ____ Please give me all data.
- ____ Refer to me.
- ____ Please note and pass to next person.
- ____ Please reply, sending me a copy.

I am attaching the revised project on Bovine Vibriosis for your approval. This project has been ~~xxxxxxx~~ reviewed by Drs. Borge, Monroe, and Speck, and their suggestions given full consideration.

Date Sept. 26, 1955

Signed: J. Clark Osborne

TITLE: STUDIES ON BOVINE VIBRIOSIS. 111 GROWTH REQUIREMENTS AND IMMUNITY.

OBJECTIVES: (1) To investigate the growth requirements of Vibrio fetus microorganisms as a basis for more accurate diagnosis.

(2) To further evaluate the immunological properties of Vibrio fetus microorganisms for the control of bovine vibriosis.

REASONS FOR UNDERTAKING THE STUDY: Vibriosis continues to be one of the major problems associated with lowered fertility in cattle. The International Congress on Animal Reproduction meeting in Copenhagen in December 1954 estimated the problem of sterility and lowered fertility to be a 435 million dollar world problem. They concluded that Vibriosis was responsible for approximately 40% of this loss. The disease has been found to be widespread in the United States, and in North Carolina as well. In dairy cattle the failure to reproduce a calf annually in V. fetus infected herds seems to result in a 25 to 40 per cent reduction in the herd milk production in the several outbreaks investigated. In the beef herd the disease is even more costly since failure to produce a calf from a cow each year represents zero annual production from each female unit. The importance of this disease has been recently emphasized nationally. Intensive research at many experiment stations has been initiated during the past decade upon the demand of the cattle industry.

PREVIOUS WORK AND PRESENT STATUS: Published literature emphasizes vibriosis as the leading cause of reproductive failures in the bovine. The disease is also a major problem in the ovine specie. The greatest single need for information at present, short of a

specific control measure, is for a truly accurate diagnostic method. Present bacteriological and serological methods are not sufficiently accurate to be satisfactory. Instability of antigen strains of *V. fetus* on laboratory media makes serological testing uncertain. Inability to get consistently a good growth or any growth of *V. fetus* from certain innocula clearly indicates the need for further studies on the exact growth requirements of this organism. Plastridge et.al. (1) in studying the growth requirements of *V. fetus* added glutathione to the complex thiol medium and reported this to be a satisfactory medium for growth. Later ~~Frank~~ Bryner et.al. (2) reported differentiation of pathogenic from non pathogenic *V. fetus* strains by the catalase test. Specific growth requirements of the pathogenic catalase positive strains have not been reported. Osborne and Bourdeau (3) reported enhancement of growth of *V. fetus* when certain hormones were added to semi-solid thiol medium. No reports of tests utilizing synthetic media have been made. The exact growth requirements of catalase positive *V. fetus* are needed to pave the way for more accurate diagnosis. Lack of this information is impeding studies on other phases of the disease such as mode of transmission. Published (4) and unpublished studies by the writer have shown *V. fetus* biologics to be of value in controlling vibriosis. Confirmation of the value of *V. fetus* vaccine in controlled experiments has been reported by Plastridge et;al. Further investigation to determine optimum methods for use and values of adjuvants are justified.

PROCEDURE: *To obtain objective* (1) A bacteriological medium will be made from C. P. synthetic ingredients that will approximate as

nearly as possible the complex medium now in use. If one can be developed that will support good growth of catalase positive V. fetus, then a systematic drop out of ingredients will be made in subsequent media to attempt to determine the exact nutritive requirements of this organism. Variation of the ratio of ingredients will be made where indicated.

To obtain Objective (2) Twenty-four heifers with the following background will be utilized as follows:

Group A. 9 weanling heifers out of vaccinated dams.

Group B. 9 weanling heifers out of non-vaccinated dams.

Group C. 3 weanling heifers out of vaccinated dams.

Group C will be raised in isolation from birth.

Treatments - Alum Ppted. catalase positive
V. fetus bacterin

Group A. 9
3 attenuated V. fetus Subcu.
3 attenuated V. fetus Intracervically.
3 attenuated V. fetus Subcu. and Intracervically

Group B. 9
Same as group A.

Group A and B will be maintained as separate groups throughout the trial.

Group C. 3 no treatment. This group will be maintained in isolation.

All groups will be bred artifically by the same technician.

Challenge of all groups will be made intravenously at the 4 to 5½ months stage of pregnancy, using 3.cc virulent catalase positive V. fetus microorganisms with a density of 76% light transmission on the colorimeter. The above dosage is predicted from the writers experience (unpublished) data and upon the experience of workers at the Storrs, Connecticut Station (unpublished)

data. The blood serum and cervical mucus titres will be demonstrated at two week intervals beginning six weeks prior to treatments and continuing three months post challenge.

PROBABLE DURATION OF PROGRAM: 2 years.

DATE OF INITIATION: Immediately.

PERSONNEL: J. C. Osborne, leader; James G. Lecce, cooperator; Flora Bourdeau, cooperator; R. H. Ferneyhough, cooperator.

COOPERATION:
INTERDEPARTMENTAL - Institute of Statistics.
OTHERS - None.

REFERENCES:

1. Flastridge et al - An improved method for preparation of vibrio fetus agglutination antigen. J. Bact. 57:657. 1949

2. Bryner, J. H. and A. H. Frank - A preliminary report on the identification of vibrio fetus. Am. Jour. Vet. Res. XVI: 76-78. 1955

3. Osborne, J. C. and Bourdeau, F. - The stimulated growth of vibrio fetus by use of hormones. J. of Bact. 70: 250. August 1955

4. Osborne, J. Clark, Avianized vibrio fetus vaccine and some preliminary observations on its use. Proc. AVMA, 1952 Pages 112-115.