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Identification of Actinobacillus spp Cell-Surface Antigens Using Monoclonal Antibodies

Kall S. Symons
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Identification of Actinobacillus spp Cell-Surface Antigens Using Monoclonal Antibodies

by

Kall S. Symons

A senior project submitted in partial fulfillment
of the requirements for graduation
from the Honors Program
with the degree of

BACHELOR OF SCIENCE

in

Bioveterinary Science

Utah State University
College of Agriculture
Department of Animal, Dairy, and Veterinary Sciences
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U.S.U. Honors Senior Project
College of Agriculture, ADVS Dept.
Bachelor of Science in Bioveterinary Science

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Why this project sponsored
by Dr. Mark C. Healey
should meet the requirements
of a U.S.U. Honors Senior Project

Personnel: Kall S. Symons

Duration of Project: January, 1986 - January, 1987

Summary:

Though a senior project is required for graduation from U.S.U. with the Honors Program, there are no written guidelines describing the requirements of a U.S.U. Honors Senior Project. This proposal seeks to show how the present research project from the College of Agriculture should meet the intended project requirements of the Honors Director.

Budget:

Materials and laboratory space will be provided without charge to personnel or the Honors Program through funding from the Utah Agriculture Experiment Station (No. 3016) and the Utah State Department of Agriculture (No. 84-0246). Personnel will not receive a salary for time spent on the project, but the time will apply to university credits.

Identification of Bacterial Antigens: Why this project sponsored by Dr. Mark C. Healey should meet the requirements of a U.S.U. Honors Senior Project

The honors senior project has two purposes. First, it introduces an undergraduate student to aspects of research that are a part of graduate work, preparing the student for a career in research, if desired. Second, it qualifies a student for acceptance into a graduate program by providing evidence, other than grades and test scores, of the students talent. A project sponsored by Dr. Mark C. Healey, designed to identify Actinobacillus spp outer membrane antigens, fulfills these two purposes of the Utah State University Honors Senior Project.

INTRODUCTION

This identification project is important because a vaccine against epididymitis in purebred virgin ram lambs (lamb epididymitis) needs to be developed. This vaccine is needed because two forms of ram epididymitis exist. Different bacterial pathogens cause both forms of this disease. Brucella ovis causes the first form in breeding rams in range herds. The second form, found in virgin ram lambs in purebred flocks, is caused primarily by Actinobacillus spp. Colorado Serum Company produces a vaccine purported to control B. ovis, but a vaccine against Actinobacillus spp is unavailable.

The proposed project will assist in research aimed at making an Actinobacillus spp vaccine available by identifying antigens on the outer membranes of approximately 20 Actinobacillus spp isolates cultured from the epididymides of infected ram lambs. This identification will be done by testing the 20 Actinobacillus spp isolates with a panel of monoclonal antibodies prepared against one field isolate of Actinobacillus sp (As8C strain). Identification will be done by both the indirect fluorescent antibody (IFA) test and the enzyme-linked immunosorbent assay (ELISA), so a comparison of the results of these two tests can be made. This proposal discusses the literature review, objectives, procedures, implications, and qualifications for doing these tests to complete the senior research project requirement of Utah State University's Honors Program.

LITERATURE REVIEW

The two genera of bacteria responsible for epididymitis in rams are Brucella ovis and Actinobacillus spp[1,2,3]. Brucella ovis causes epididymitis in adult breeding rams[1] while Actinobacillus spp causes epididymitis in purebred virgin ram lambs[2,3]. The non-Brucella form of the disease has been reported in literature since 1950[4]. Serological assays for B. ovis were compared by Cox et al[5] in 1977. Watts[6] reported that epididymitis had been a problem in southern Australia since 1949. The disease is identified by palpating ram testes and locating areas of scar tissue formation located on the epididymides. When the infection

is unilateral additional rams with associated maintenance costs, must be maintained by the sheep producer. If the ram is infertile or sterile, the producer must cull a potentially valuable animal. These losses are greatest in purebred herds where the production of wool or meat does not help offset the loss of the reproductive potential.

One testicle or both testes from a ram with epididymitis becomes swollen near the site of the lesion. The swelling is caused by a bacterial infection that may become so severe as to occlude the sperm duct. This prevents the normal passage of semen, resulting in ram infertility or sterility. In 1958, Lawrence[7] showed that the incidence of infection in flocks ranged between 10% and 74%. Watts[6] reported that the lambing percentage in a flock was noticeably lower when the ratio of infected to normal rams was greater than 1 to 10. Therefore, the results of Lawrence and Watts show that epididymitis is a significant problem for sheep producers.

A vaccine to control Brucella ovis-induced epididymitis in adult rams was produced by Cutter Laboratories in Berkeley, CA, in 1967[1]. In an experiment by Brinton et al[1] in Wyoming, this vaccine was purchased under the trade mark Ramedol. According to Dr. Healey[8], a similar vaccine is now produced by Colorado Serum Company. This commercial vaccine has been ineffective in controlling the disease in purebred herds[1]. The reason for this failure is that the type of bacteria responsible for epididymitis in range herds (B. ovis) is different from the bacteria in purebred herds

(primarily Actinobacillus spp)[1,2,3]. Thus, within the past few years, the need to develop a vaccine for lamb epididymitis has arisen.

Dr. Healey's method for developing a new, bacterial-extract vaccine is different from the method used to produce traditional bacterial vaccines used in veterinary medicine. The B. ovis vaccine produced to control epididymitis in range rams is a whole-cell vaccine. In making this vaccine, a single strain of B. ovis is grown in culture. The bacteria are subsequently killed, an adjuvant added, and the crude, whole-cell preparation is used as the final product for the vaccination of rams. However, this method was judged to be too crude for present technology. The new vaccine to be used to control Actinobacillus spp-associated epididymitis was developed by extracting antigens from the bacterial outer membrane by use of a non-ionic detergent[9].

OBJECTIVES

The student expects to learn some very practical methods and also more about the theories of vaccine production. The IFA test and ELISA are often used in contemporary diagnosis and research in microbiology, and experience with these tests is valuable.

The proposed project will increase our knowledge of Actinobacillus spp by comparing results obtained from the IFA test and ELISA. This comparison may be used to decide on future screening methods in extension work. The project will also help Dr. Healey[9,10] further his work in developing an Actinobacillus spp vaccine. Identification of the

surface antigens involved will help Dr. Healey choose cultures to be used in making a multi-spectrum vaccine. The skills learned and results obtained by doing these tests have application in many areas of biotechnology.

The atmosphere of an immunology lab, having the opportunity to interact with a few of the university's best scientists and technicians, is very motivating. A student working in this lab may expect to develop interest in veterinary medical research while learning more about the concepts of vaccine production.

PROCEDURES

Monoclonal antibodies were produced by Dr. Mark C. Healey[9] against an isolate of Actinobacillus spp (As8C), that was identified as the pathogen of lamb epididymitis. This bacterium was isolated from the epididymis of an infected ram lamb. Monoclonal antibodies were produced by first inoculating mice with this specific bacteria isolate. After the mice developed antibodies to the bacteria, the cells in the spleen that were producing the antibodies were removed. These cells were fused with myeloma cells that lack the ability to grow in a restrictive medium. Fused cells that had both spleen and myeloma genes (hybrid cells or hybridomas) continued to grow in the restrictive medium while all other cells died. The resulting hybridoma cells were isolated and three useful cell lines were discovered to produce monoclonal antibodies targeted to antigens on the outer membrane of As8C.

These three monoclonal antibodies have been used to analyze the outer membrane antigens extracted from Actinobacillus spp bacteria. The ideal bacterial extract vaccine should contain antigens found on a broad spectrum of Actinobacillus spp that naturally cause lamb epididymitis. Thus, it should be possible to develop a vaccine representative of most Actinobacillus spp bacteria without containing antigens that are not useful in protecting the ram from epididymitis.

In the proposed IFA test, each of 20 isolated Actinobacillus spp strains will be applied to the surface of several glass slides as described by Cox et al[5]. The bacteria will be covered by several dilutions of ascitic fluid from each of the three monoclonal antibodies developed previously, and incubated for one hour at 37° C and 100% relative humidity. The monoclonal antibodies will bind to target antigens found on the outer membrane. The slides will then be rinsed in distilled water and washed for 30 minutes in phosphate buffered saline solution with gentle stirring.

After air drying, these antigen-bound antibodies will be exposed to a second antibody specific to them (antiserum to mouse IgM or IgG), which in turn is conjugated to a fluorescing chemical called fluorescein isothiocyanate. After incubation, the slides will be rinsed, washed, and air dried. A coverslip will be applied over the bacterial smear using Moial as a mounting medium. When observed under an ultraviolet microscope, the bacteria will fluoresce and can

be used to analyze the specificity of the antibodies against the particular strain. This process is explained in the book entitled: Fluorescent Protein Tracing by R. C. Nairin[11] and Dr. Mark C. Healey's article in the American Journal of Veterinary Research[10].

The ELISA will be done on the same 20 Actinobacillus spp isolates used for the IFA test, according to the method of Healey et al[10]. A 96-welled plate will be coated for the ELISA and an automated reader will be used to get quantitative results. To coat the plates, a suspension of one of the 20 isolates in 0.06M $NaCO_3$ buffer will be left in the plate over night at room temperature. The plate will be washed with distilled water. The sources of ascitic fluid containing the three monoclonal antibodies used for the IFA will also be used to make several serial dilutions to cover the bacteria on the plate and incubated for two hours at 37° C. After incubation, the plate will again be washed. A dilution of horseradish peroxidase-conjugated goat antibody to mouse immunoglobulins will be placed in each well. The plate will again be washed and then color will be allowed to develop by adding o-phenylenediamine substrate to individual wells. Known positive and negative sera obtained from immunized and non-immunized mice, respectively, will be used simultaneously as controls with all ascitic fluids.

IMPLICATIONS

If the project results are positive, then they may be used by Dr. Healey in one of his papers with my name as a co-author[12]. This will increase my qualifications for

graduate work. If possible, the results will also be reported by me at the Scholars Day paper sessions on May 9, 1987. I hope that by doing this project, professors in the ADVS Department will have interest in sponsoring other undergraduate research projects. As this proposal will appear as an introduction to the project report, I hope that other Honors Students can use the project schedule information in the appendix to know how they may get involved in undergraduate research opportunities.

QUALIFICATIONS

I am qualified to perform this antigen identification project because I have worked for Dr. Healey and know the location and use of the materials and instruments in his laboratory. I have had general instruction in the physical, chemical, biological, and agricultural aspects involved in Dr. Healey's work. The specific theories and procedures used during this project should be reasonably possible for me to understand in a short period of time.

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CURRICULUM VITAE

EDUCATION

Graduated in top 2% from Sky View High School in 1982.
Currently in the Utah State University Honors Program.

PROFESSIONAL SPECIALTIES

BIOTECHNOLOGY: Major program of study at Utah State University is Bioveterinary Science.

CHINESE LANGUAGE: Chinese language studied for 4 years, including 16 months in Taiwan.

PROFESSIONAL EXPERIENCE

LABORATORY TECHNICIAN: Three years experience in an immunology laboratory.

VETERINARIAN'S ASSISTANT: Over 300 hours experience with animals in clinical and field work.

CHINESE LINGUIST: Over two years experience as an interrogator, interpreter, and translator for the Utah Army National Guard.

PUBLICATIONS

"What is a 'liberal education'?" in the Utah State University Honors' Journal **In Print**, 10 pages, 1983.

"Identification of Actinobacillus spp Cell-Surface Antigens Using Monoclonal Antibodies" as a bound Utah State University Honors Senior Project, 35 pp., 1987.

"Characterization of Actinobacillus species outer membrane antigens using monoclonal antibodies" as a co-author with Mark C. Healey, DVM, PhD, in the American Journal of Veterinary Research, 1987.

Date of Birth: February 3, 1964 Single, no children

PROJECT REPORT

Please note:

I. This report is in the style of the American Journal of Veterinary Research.

   A. The information on the first page is there to be printed across the top of the article.

   B. The remaining information is to be printed in a two-column format.

   C. The information on the second page would be printed as footnotes.
      1. The report was typed using WordPerfect which has footnoting capability.
      2. To follow the style of an original research paper to be submitted to a journal, standard footnoting was not used.

II. The summary would be printed at the beginning of the first column of the article. It is independently written as an abstract to also appear without the report.

III. This report will not be submitted for publication in a refereed journal of the veterinary profession. The student and his project advisor, Dr. Healey, decided to use the style of the American Journal of Veterinary Research, to give the student experience in writing this type of technical paper. However, a portion of these data will be included in a manuscript to be submitted to this journal in July, 1987. Kall Symons will appear as a co-author on that manuscript.

Identification of *Actinobacillus* spp Cell-Surface Antigens Using Monoclonal Antibodies

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Accepted as partial completion of Utah State University Honors Program graduation requirements November, 1987.

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The authors thank Dr. Hank H. Hwang, Dr. Wilford N. Hansen and Ms. Marian B. Smith for technical assistance concerning the IFA test. The administrative assistance of Dr. Douglas D. Alder, Dr. Joseph G. Morse and Ms. Lois J. Kelley is also appreciated.

[a]Calbiochem, La Jolla, CA.

[b]Dynatech Laboratories, Alexandria, VA.

[c]Hyclone Laboratories, Logan, UT.

[d]Bio-tek Instruments, Winooski, VT.

[e]Dynatech Laboratories, Alexandria, VA.

SUMMARY

The indirect fluorescent antibody (IFA) test and 96-well enzyme-linked immunosorbent assay (ELISA) were the serological techniques used in this project to identify Actinobacillus spp cell-surface antigens. Test results were also compared to earlier results obtained when using the Gilford ELISA.

Three monoclonal antibodies were used in the IFA test and the 96-well ELISA to identify cell-surface antigens on each of 19 Actinobacillus spp isolates cultured from the epididymides of infected ram lambs. In the IFA test, 1 monoclonal antibody (LG33) identified antigens on 13 of the isolates, while the other 2 monoclonal antibodies (LG17 and LG30) recognized antigens on only the As8C isolate. The 96-well ELISA failed to demonstrate the specificity of LG33 to antigens on the 13 Actinobacillus spp isolates identified by the IFA test. The 96-well ELISA suggested that LG30 also identified antigens on all 19 isolates, while LG17 recognized antigens on none of the isolates except As8C.

With the procedures used, the Gilford ELISA was preferred over the 96-well ELISA and the IFA test. There was no correlation between the IFA test and the 96-well ELISA, but a 70% correlation by the comparison of log transformations was found between the IFA test and the Gilford ELISA. Compared to both the 96-well and Gilford ELISA, the shorter preparation time of a bacterial sample for the IFA test and the slower rate of decomposition of IFA test results made it more convenient. However, the results

of the Gilford ELISA were the most precise. Because of its greater capacity and sensitivity, the 96-well ELISA, when read using an automatic reader, should have been the most precise serological assay for the diagnosis of Actinobacillus spp-induced epididymitis. However, problems in adjusting assay conditions to yield low background color for the 96-well ELISA made the data unreliable.

Actinobacillus is the major genus of bacteria responsible for causing lamb epididymitis[1-3]. However, not enough research concerning this bacteria has been done. For example, data identifying cell-surface antigens on species of *Actinobacillus* has not been compiled. Also, a comparison of serological assays used for the diagnosis of this economically important disease of purebred ram lambs in Utah has not been done.

The purpose of this project was to compile *Actinobacillus* spp cell-surface antigen data using the IFA test and 96-well ELISA, and to compare these 2 serological assays. By performing these serological assays on several *Actinobacillus* spp isolated from the epididymides of infected ram lambs, it was thought that these assays could reveal a cell-surface antigen common to all *Actinobacillus* spp. This common antigen could then be used to quickly diagnose the specific etiology of an epididymitis infection and aid in prescribing an effective vaccine.

It is difficult to prescribe an effective vaccine for epididymitis because 3 different genera of bacteria are responsible for this disease in rams. These genera are *Brucella*, *Actinobacillus*, and *Hemophilus*. *Brucella ovis* causes epididymitis in adult breeding rams[1] while *Actinobacillus* spp and *Hemophilus* cause epididymitis in purebred virgin ram lambs[2,3]. To control these epididymitis-causing bacteria, a vaccine recognizing the specific genus of bacteria causing the disease must be prescribed.

After vaccines are developed, serological assays are used on many different bacterial isolates to determine how efficiently they recognize the target bacteria. For example, a vaccine to control B. ovis-induced epididymitis in adult rams was developed by Cutter laboratories at Berkeley, CA, in 1967[1]. Following the development of this vaccine, serological assays identifying B. ovis among the numerous bacteria cultured from the epididymides of infected ram lambs were used in 1977 by Cox et al[4]. These assays were preformed on several different isolates to determine how consistently the vaccine product covered the target bacteria found in nature. Since these tests depend on the antigens in the Cutter vaccine, they should be a measure of how efficiently the vaccine stimulated an immune response by sheep (antibodies) to B. ovis.

Although the Actinobacillus spp-induced epididymitis has been reported in the literature since 1950[5], a vaccine to control this bacteria in purebred virgin ram lambs was just developed by Healey et al.[6] in 1985. The following research will determine how well Healey's vaccine could be recognizing Actinobacillus spp. In this research, authors used both the IFA test and 96-well ELISA. An evaluation of the utility of these 2 tests was made. These 2 tests were also compared to the Gilford ELISA test which had previously been done on the isolates by Healey et al[6,7].

Materials and Methods

In 1985, Healey et al. developed 3 different hybridoma cell lines; LG17, LG30, and LG33. Each was shown to produce only 1 specific monoclonal antibody. One of these monoclonal antibodies hypothetically could be directed against a cell surface antigen common to all Actinobacillus spp. The procedures for the production of ascites fluids containing these 3 antibodies and the production of an antiserum containing polyclonal antibodies targeted against the cell-surface antigens on 1 Actinobacillus sp (As8C strain) have been previously described by Healey et al[6,7].

IFA test -- Indirect fluorescent antibody (IFA) titers to each of the 19 isolates were determined for each of the 3 ascites fluids using the method described by Healey et al[6]. The only significant difference in the procedure was the use of 20% Mowiol 4.88[a] in de-ionized, distilled water (DD H_2O) as a mounting medium[8,9].

ELISA -- Enzyme-linked immunosorbent assay (ELISA) titers to each of the 19 cultures were determined for each of the 3 ascites fluids using the method described by Healey et al[6] with the following differences in procedure. A polyvinyl chloride, U bottom, Microtiter, 96-well plate[b] was coated by filling individual wells with 0.2 ml of a solution containing 5 x 10^7 bacterial (Actinobacillus spp) cells/ml as determined by a constant optical density. A 0.1 ml amount of each of the other reagents was used. A horseradish peroxidase (HRPO)-conjugated goat antibody to mouse IgM or IgG (heavy and light chain specific)[c] was used

at a dilution of 1:1000. The plates were read with a 490 micrometer filter using an EL309 Microplate Autoreader[d].

Results

IFA test -- Using the IFA test the LG33 monoclonal antibody identified antigens on 13 of the 19 Actinobacillus spp isolates. Positive antibody titers ranged from 1,600 to 12,800 for LG33. There were no titers for LG17 or LG30 for any isolate except As8C which had a titer of 1,600 for both of these antibodies (Table 1). Problems with the IFA procedure were: (1) running together of reagents in different circles of the same slide, (2) extreme viscosity of mounting media, and (3) non-specific binding of the fluorescein isothiocyanate antibody marker.

ELISA -- Only Actinobacillus isolate S-15 failed to give an ELISA antibody titer. Positive ELISA antibody titers ranged from 1,600 to 12,800 for LG33 and from 200 to 12,800 for LG30 (Table 2). In contrast to the 96-well ELISA which consistently gave high titers for LG30 antibodies, the Gilford ELISA (Table 3) gave only low titers for LG30, except As8C and 29522. There were no titers for LG17 for any isolate except As8C which had a titer of 3,200 (Table 2). Problems with the 96-plate ELISA were: (1) non-specific HRPO-conjugate binding, (2) inconsistent optical density readings by the automatic reader, (3) ineffective HRPO-conjugate for IgM, and (4) difficulty in finding an optimum-utility 96-well plate.

Discussion

The results suggest that none of the three monoclonal antibodies used recognize an antigen common to all 19 Actinobacillus spp isolates. Using the IFA test the LG33 monoclonal antibody identified antigens on 13 of the 19 Actinobacillus spp isolates. LG17 and LG30 antibodies both failed to recognize antigens on any isolate other that As8C. In repeated tests, 5 of the 6 isolates that failed to show LG33 antibody titers, also consistently failed to be recognized by hyperimmune mouse serum (HMS) antibodies. This suggests that none of the cell-surface antigens of As8C recognized by HMS are common to these 5 Actinobacillus spp isolates, demonstrating the importance of a vaccine containing a broad spectrum of Actinobacillus spp field isolates.

The following methods were used to solve the problems encountered when using the IFA test. Flooding was prevented by using fresh slides which were not scratched to assure the slide's surface was as hydrophobic[11] as possible. Viscosity was adjusted by not using glycerol (Table 4). Non-specific binding was recognized after several trial tests and evaluated during each subjective reading of the slides.

New methods are now being used to solve the problems encountered when using the ELISA. Non-specific HRPO-conjugate binding is now prevented by washing the bacteria coated plate 5 times with ELISA diluent. The diluent blocks the sites on the plate where the cells did not stick. The optical density readings are now more consistent because

only one plate is run at a time. This plate is left in the machine, preventing the site where the optical density is read from changing. The old HRPO-conjugate for IgM was thrown out, and a new bottle was purchased from Hyclone Laboratories. An Immulon 2^e, flat bottom, polystyrene plate is now used instead of the U bottomed plate that was used initially.

Compared to both the 96-well and Gilford ELISA, the shorter preparation time of a bacterial sample and the slower rate of decomposition of the IFA test results made it more convenient. The ELISA wells had to be filled with the bacteria solution and allowed to sit for 24 hours during the coating process. These wells also had to be incubated for 2 hours. On the other hand, the IFA plates were coated with bacterial smears in just a few minutes. Also, they had to be incubated for only 1 hour.

Because the conditions used in the 96-well ELISA procedure were incorrect, there was no correlation between the 96-well ELISA and the other two tests. For example, the 96-well ELISA results failed to demonstrate specificity of any of the 3 antibodies to antigens on the 13 Actinobacillus spp isolates identified by the IFA test. The unexpectedly high titers for LG30 using the 96-well ELISA can be explained by the presence of too much background color, or non-specific binding of the HRPO-conjugated antibodies.

Another example of the lack of correlation between these tests is the absence of LG17 titers when using the 96-well ELISA. Some consistently low titers for LG17 were

recorded using the Gilford ELISA. The lack of LG17 titers with the 96-well ELISA can be explained by the more recent discovery that the IgM HRPO-conjugated antibody used for this test was inactive. The inactivity of the IgM was not discovered during the assays because the design of the experiment failed to provide positive controls for Ig subisotypes.

The Gilford ELISA test was preferred over the 96-well ELISA and the IFA test. Though the greater throughput of the 96-well ELISA made it the most convenient assay when more than one isolate had to be tested at the same time, problems in adjusting experimental conditions to yield low background color for the 96-well ELISA made data generated by this assay unreliable. These problems also out weighed the greater objectivity of using an automatic reader to read the 96-well plate. The IFA was read subjectively. Thus, there was less variation of the results, as the presence of fluorescence could not be precisely determined. Very low amounts of fluorescence could not be seen. Therefore, low amounts of antigens on the cell-surface could not be identified. The presence of some antigens on the cell-surface of the 6 isolates not identified by the IFA test could be more common than the IFA data suggest.

The IFA results support Dr. Healey's work and will appear as part of a journal article by Healey et al[10]. He will use the IFA results because there was a correlation between the IFA test and the Gilford ELISA[12]. When the Gilford ELISA was done, 2 of the Actinobacillus spp

isolates, Y136 and A2, did not demonstrate an antibody titer using LG33. The IFA test also failed to recognize antigens on these 2 isolates. The positive LG33 antibody titers for the Gilford ELISA ranged from 200 to 51,200. Four isolates, 908, R75, Y136 and A2, did not demonstrate titers using LG30. The positive LG30 antibody titers for the Gilford ELISA ranged from 200 to 6,400. Only Y136 and A2 did not demonstrate antibody titers using LG17. The positive LG17 antibody titers ranged from 200 to 800 except for As8C which had a titer of 102,400.

Component culture extracts for an optimum vaccine should be D-107, S-15, A-2, Y-136, S-6, D-56, and As8C. Though the Gilford ELISA gave low LG33 titers for D-107, S-15, S-6 and D-56, it is probable that a vaccine targeted against As8C cell-surface antibodies would fail to provide adequate immunity to protect ram lambs against infections by these four isolates. The other 12 isolates were antigenically similar to As8C. A broad-spectrum vaccine in which As8C cell-surface antigens were targeted should also provide immunity against the other 12 isolates.

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12. Sisson DV and Symons KS. Data for LG33 was entered on a VAX computer by Dr. Donald V. Sisson and Kall S. Symons. The data entered was that obtained previously when using the Gilford ELISA and IFA test. The correlation of the data obtained when using these two techniques was first determined using Minitab on the raw data. The coefficient of correlation for the raw data was .561. Upon consulting a table of coefficients of correlation, the coefficient of .561 was found to exceed the coefficient at the 5% level which was .456. Since the hypothesis for this test is that the correlation should be 0, by exceeding the coefficient the hypothesis is rejected and a correlation of the data is deduced. However, the coefficient of correlation for the raw data of .561 did not exceed the coefficient at the 1% level which was .575.

Because of the extreme variability of the data, the data were compared by using the transformation of the natural logarithm. The coefficient of correlation for the transformed data was .837. This coefficient does exceed the coefficient at the 1% level. From this coefficient it is evident that 70% of the variability is due to one set of data, or that one would expect the data to vary 30% of the time when comparing the Gilford ELISA and the IFA test.

APPENDIX

TABLE 1--IFA determination of ascites fluid antibody titers to *Actinobacillus* spp.

Actinobacillus spp	Monoclonal Antibody		
	LG17	LG30	LG33
As8C	1,600	1,600	12,800
29522	0	0	12,800
15768	0	0	1,600
4101	0	0	3,200
908	0	0	12,800
D56	0	0	0
R57	0	0	3,200
JJ5161	0	0	1,600
A22	0	0	3,200
S-6	0	0	0
Y136	0	0	0
A2	0	0	0
A11	0	0	3,200
A46	0	0	6,400
84-832(9)	0	0	3,200
84-832(5)	0	0	1,600
S-15	0	0	0
84-833	0	0	6,400
D107	0	0	0

TABLE 2--96-well ELISA determination of ascites fluid antibody titers to *Actinobacillus* spp.

Actinobacillus spp	Monoclonal Antibody		
	LG17	LG30	LG33
As8C	3,200*	6,400	51,200
29522	<200	1,600	12,800
15768	<200	1,600	3,200
4101	<200	1,600	1,600
908	<200	1,600	3,200
D56	<200	12,800	6,400
R57	<200	6,400	12,800
JJ5161	<200	3,200	12,800
A22	<200	1,200	6,400
S-6	<200	1,600	3,200
Y136	<200	6,400	3,200
A2	<200	1,600	3,200
A11	<200	400	1,600
A46	<200	1,600	6,400
84-832(9)	<200	3,200	6,400
84-832(5)	<200	3,200	1,600
S-15	<200	3,200	3,200
84-833	<200	200	12,800
D107	<200	3,200	1,600

*ELISA using LG17/B1 ascites fluid, the same batch as the IFA test. When using LG17/B8 ascites fluid, because the amount of LG17/B1 was small, the titers were all <200.

TABLE 3--Gilford ELISA determination of ascites fluid antibody titers to *Actinobacillus* spp.

Actinobacillus spp	Monoclonal Antibody		
	LG17	LG30	LG33
As8C	102,400	6,400	51,200
29522	200	600	51,200
15768	400	200	12,800
4101	200	200	12,800
908	200	<200	12,800
D56	200	200	<200
R57	800	200	1,600
JJ5161	200	200	1,600
A22	400	400	12,800
S-6	<200	200	800
Y136	<200	<200	<200
A2	<200	<200	<200
A11	200	200	3,200
A46	200	400	12,800
84-832(9)	400	200	51,200
84-832(5)	<200	800	12,800
S-15	200	300	200
84-833	200	200	102,400
D107	200	800	800

TABLE 4--Determination of Mowial 4.88 mounting medium.

Mowial 4.88	Solvent	Glycerol	Viscosity
0%	PBS	Unknown%	This did not hold the cover slip in place.
30%	DD H_2O	0%	This was easy to pipette without bubbles.
30%	PBSS	0%	This made bubbles too easily when pipetting.
30%	DD H_2O	30%	This was slow to pipette and made bubbles.
30%	PBSS	30%	This pipetted very slowly and made bubbles.
20%	DD H_2O	0%	This was used for the experiment.

PROJECT SCHEDULE

There are seven aspects of my proposed project that are representative of graduate research and, therefore, fulfill the requirements of an honors senior project. They are: (1) choosing the topic, (2) finding an advisor, (3) deciding on a project, (4) interdepartmental communication, (5) literature search, (6) procedure problems, and (7) writing the results.

1. In November, 1985, I selected a senior project topic of interest to me that requires further research. Veterinary medicine became interesting to me during junior high school after I read books on the subject. During my first year of college, I worked for 300 hours over a period of 47 days with six different veterinarians who treated companion, farm, and exotic animals. My association with veterinarians involved developing new procedures to improve their practices. For example, when I was at the North Cache Veterinary Clinic, the veterinarians were developing an artificial insemination program for foxes, testing different amounts and schedules of uterine infusions for dairy cows, and starting computer services for clients. The process of developing these procedures interested me, and I am now investigating veterinary research.

2. In November, 1985, I also found a cooperating professor in my area of interest to serve as my advisor. With a reference from the Honors Program Director, I

approached Dr. Mark C. Healey who had been my Preveterinary Program Advisor. Dr. Healey was willing to help me select a research project.

3. In December, 1985, I decided on a specific research project. The field in veterinary medicine of most interest to me is veterinary immunology. I selected a project in this field of study, involving the IFA test and ELISA on 20 different bacterial isolates belonging to the genus Actinobacillus, because it could be completed in a 180 hour period to match the six credits I would receive. Dr. Healey assured me that it was simple enough for me to learn and understand at an undergraduate level. He had developed the procedures supporting the project enough to teach them to me without interfering too much with the work he was already doing.

4. In January, 1986, Dr. Alder decided that the project my advising professor and I had chosen would complete the senior project requirement of the Honors Program. During the processes of submitting my project idea, I communicated information between the honors director and my advising professor. This was difficult because of Utah State University's diversity, i.e. the research methods used in the various colleges on campus are different, especially between the Colleges of Humanities and Agriculture. Only a few vague requirements concerning creativity and length were given by Dr. Alder. I learned

that specific judgments concerning the needed amount of originality, purpose, design, and size of the project had to be decided by my advising professor and myself, because of the project's uniqueness when compared to most senior projects in the program. An initial, one page proposal met Dr. Alder's requirements for an honors project proposal. This four-part proposal is written to meet Dr. Healey's requirement for a semi-formal research proposal.

5. In January, 1986, I did a literature review of the research topic I selected. I spent 20 hours reading articles on the subject and writing a review of what I had learned. Dr. Healey allowed me to review the pertinent articles he has on file. I also did a computer search to determine the breadth of the field.

6. When I carry out the project, I will meet with procedural problems. I will have to learn how to find time in my schedule to get the work done to solve these problems.

7. The results obtained will have to be communicated to others. Also, statistical analyses will have to be done to determine the reliability of the results. Time spent in writing, editing and printing this proposal has taken over 40 hours of computer work thus far.

These seven aspects of my project are representative of graduate research and will fulfill one of the two purposes of an honors project. Relative to qualifying me for graduate work, the second purpose of an honors project, the

project will be bound as an honors thesis and kept in the honors lounge. If the results are good, there may also be an opportunity for the results to appear in a journal as part of a larger project. Along with symposia in which I may participate, these experiences will serve to contribute to the evidence of research ability shown by grades and test scores.