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Six hundred sixteen ewes of six strains were inoculated twice with ovalbumin in Fruends' incomplete adjuvant in order to investigate genetic variation in immune response. Blood samples were collected from the ewes 6 days post-second injection. Serum samples were also collected from their 709 lambs, born in June 1984, between four and 30 hours of age, to examine genetic differences in ability of lambs to passively obtain anti-ovalbumin antibodies. Titers of anti-ovalbumin antibodies were determined using kinetic Elisa techniques. Strain was not a significant source of variation in ewe active immune response, but sire within strain was highly significant. Age of ewe did not significantly affect anti-ovalbumin antibody titer in all ewes, but it did have a significant effect on the titer of only the pregnant

ewes ($P<.05$). Heritabilities of anti-ovalbumin titer from a paternal half-sib analysis were .27 +/- .17 for all ewes and .57 +/- .25 for only pregnant ewes. The effect of strain of lamb on lamb's passive immunity against ovalbumin was significant only at $P<.10$, but sire within strain was a highly significant source of variation. The quadratic regression of lamb anti-ovalbumin antibody concentration on lamb's age at bleeding was significant ($P<.05$), with maximum titer for lambs bled at 18 hours of age. The size of the litter in which the lamb was born also had a highly significant effect on the lamb's passive immunity, with titer decreasing as litter isze increased. The heritability estimate for lambs' anti-ovalbumin antibody concentration determined by a paternal half-sib analysis was .38 +/- .11, and from the sire variance component of a full-sib analysis it was .28 +/- .15. When lamb's passive titer was considered a maternal trait (dams nested within maternal grandsire within strain), the sire variance component was negative. The antibody concentration of lambs that died between bleeding and 120 days of age was significantly less than that of the entire population ($P<.005$).

# INHERITANCE OF ACTIVE AND PASSIVE IMMUNE RESPONSES IN SHEEP

by

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Once upon a time, in a land far far away, there was a shepherdess who was looking for some sheep (616 plus lambs, to be exact). As she went out searching for them she met the first of the three wise men (Bill Hohenboken) without whose help she would never have located the sheep and known what to do with the data once she got it. He sent her on her way looking for more help and lo and behold she came upon the second wise man (Steve Kaattari) who showed her the wonder of test tubes and pH meters and the like. But she still needed the sheep so she traveled further and came upon the third wise man (Jap Shrestha) who actually had the sheep. "But" the three wise men said (in unison) "you must practice first on lesser sheep, till we are sure you are ready for the big time!" That's where the three bears came in.

The three bears (Bob Klinger, Piper Klinger, and Howard Meyer) kindly helped the shepherdess practice on lesser sheep (30 Suffolks) till she was ready to work on the elite 616 Canadian sheep (plus lambs). But the shepherdess had money problems, so along came the fairy godfathers and their big bucks (C.Parker and D. Price and USDA, ARS cooperative agreement) and they helped financially support the shepherdess, the sheep, and the test tubes. There were still rough times ahead for the shepherdess, till the two wizards (A.A. Grunder and J. Gavora) in the far off foreign capital looked into their crystal ball and ABRACADABRA!!!, they crystallized the egg whites!

So the shepherdess traveled off to the foreign capital to meet the sheep. While there she met Snow White (Sue Leffler) who saved her from a convent and other unmentionable horrors and the nine dwarfs (Bruce, Roger, Ray, Mac, John, Steve, Frank, Dan and Barry) who taught her all about bleeding sheep and the Duke of Richmond.

The shepherdess traveled back to her kingdom with her 1339 blood samples, where the invaluable help of the three laboratory magicians (G. Zimmerman, T. Jones, R. Baker) and the computer hobbit (T. Brundage) and the sage counsellor (N. Muggli) made things finally start cooking.

Thanks also go to all the basement munchkins (Brenda, Deb, Carol, Kitty, Cathy, Rob, Victor, Fernando, Hajime, and Noelle) for putting up with the shepherdess' idiosyncrasies and bad temper (especially Rob). Of course no fairy tale is complete without a knight in woolly armour (Boo Boo) who said to the shepherdess, "Why don't you go get a job with test tubes and I'll stay home and watch the sheep!" And they lived happily ever after!

Tak Sa Mycket

THE END

# PREFACE

This study would not have been possible without the invaluable contributions of the co-authors. Dr. Stephen Kaattari's expertise in immunology was necessary for the planning of the study and the development of the Elisa assay for the laboratory analysis . Dr. Jap Shrestha coordinated the use of the ARC flock and supervised the actual execution of the injections, bleeding and sample handling. Dr. Bill Hohenboken's guidance and statistical assistance made this study possible.

TABLE OF CONTENTS

LIST OF TABLES

# INHERITANCE OF ACTIVE AND PASSIVE IMMUNE RESPONSES IN SHEEP

## Chapter 1

## INTRODUCTION

The health and survival of young lambs is dependent on their absorption of maternal immunoglobulins from colostrum (Campbell,1974; McGuire et al.,1983). Maternal immunoglobulins can be absorbed through the gut epithelium of young ruminants up to 24 hours postpartum (Stott et al., 1979) by a pinocytotic mechanism (Brandon,1976). Many factors may affect the concentration of immunoglobulins a lamb receives. These include breed, litter size and birth weight (Halliday, 1968;1974). Past research conducted at Oregon State University (Norman et al.,1981; Muggli et al.,1984) reported genetic variation among calves in ability to acquire and absorb colostral immunoglobulins.

In the aforementioned studies, total immunoglobulin concentrations were measured, whereas in the current study, we measured antibodies specific to a specified antigen. In order to use an antigen to which the ewes would not have had any previous exposure, ovalbumin was chosen. Studies in mice (Biozzi et al.,1975) have shown a relationship between ability to respond to ovalbumin and ability to respond to actual pathogens. Six hundred sixteen ewes were twice inoculated with ovalbumin. Their active immune reponse and the passive immune response of their 709 lambs were quantified.

The objectives of this experiment were to examine variation in active responses of ewes and passive responses of lambs. Environmental factors possibly affecting ewe's response (age, inbreeding coefficient, breeding weight, pregnancy status and stage of gestation) and those possibly affecting lambs' passive antibody titer (age of dam, age at bleeding, litter size, birth weight and dam's titer) were examined. Strain differences and heritabilities of active immune response of ewes and of passive immune response of lambs (when considered both as an individual and a maternal trait) were potential genetic effects. In addition, the relationship between lamb titer and survival to 120 days of age was examined.

Chapter 2

# INHERITANCE OF ACTIVE AND PASSIVE IMMUNE RESPONSES IN SHEEP[1,2]

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## Summary

Six hundred sixteen ewes of six strains were inoculated twice with ovalbumin in Fruends' incomplete adjuvant in order to investigate genetic variation in immune response. Blood samples were collected from the ewes 6 days post-second injection. Serum samples were also collected from their 709 lambs, born in June 1984, between four and 30 hours of age, to examine genetic differences in ability of lambs to passively obtain anti-ovalbumin antibodies. Titers of anti-ovalbumin antibodies were determined using kinetic Elisa techniques. Strain was not a significant source of variation in ewe active immune response, but sire within strain was highly significant. Age of ewe did not significantly affect anti-ovalbumin antibody titer in all ewes, but it did have a significant effect on the titer of only the pregnant ewes. Heritabilities of anti-ovalbumin titer from a paternal half-sib analysis were .27 +/- .17 for all ewes and .57 +/- .25 for only pregnant ewes. The effect of strain of lamb on lamb passive immunity against ovalbumin was significant only at $P<.10$, but sire within strain was a highly significant source of variation. The quadratic regression of lamb anti-ovalbumin antibody concentration on lamb's age at bleeding was significant ($P<.05$), with maximum titer for lambs bled at 18 hours of age. The size of the litter in which a lamb was born also had a highly significant effect on the lamb's passive immunity, with titer decreasing as litter size increased. The heritability estimate for lambs' anti-ovalbumin antibody concentration

determined by a paternal half-sib analysis was .38 +/- .11, and from the sire variance component of a full-sib analysis it was .28 +/- .15. When lamb's passive titer was considered a maternal trait (dams nested within maternal grandsire within strain) the sire variance component was negative. The average antibody concentration of lambs that died between bleeding and 120 days of age was significantly less than that of the entire population (P<.005). (Key Words: Sheep, Immune Response, Heritability, Immunoglobulin).

## Introduction

The health and survival of young lambs is dependent on their absorption of maternal immunoglobulins from colostrum (Campbell,1974; McGuire et al.,1983). Maternal immunoglobulins can be absorbed through the gut epithelium of young ruminants up to 24 hours postpartum (Stott et al., 1979) by a pinocytotic mechanism (Brandon,1976). Many factors may affect the concentration of immunoglobulins a lamb receives. These include breed, litter size and birth weight (Halliday, 1968;1974). Past research conducted at Oregon State University (Norman et al.,1981; Muggli et al.,1984) reported genetic variation among calves in ability to acquire and absorb colostral immunoglobulins.

In the aforementioned studies, total immunoglobulin concentrations were measured, whereas in the current study, we

measured antibodies specific to a specified antigen. In order to use an antigen to which the ewes would not have had any previous exposure, ovalbumin was chosen. Studies in mice (Biozzi et al.,1975) have shown a relationship between ability to respond to ovalbumin and ability to respond to actual pathogens. Six hundred sixteen ewes were twice inoculated with ovalbumin. Their active immune reponse and the passive immune response of their 709 lambs were quantified.

The objectives of this experiment were to examine genetic and environmental variation in active responses of the ewes and in passive responses of lambs. Environmental factors possibly affecting ewe's response (age, inbreeding coefficient, breeding weight, pregnancy status and stage of gestation) and those possibly affecting lambs' passive antibody titer (age of dam, age at bleeding, litter size, birth weight and dam's titer) were examined. Strain differences and heritabilities of active immune response of ewes and of passive immune response of lambs (when considered both as an individual and a maternal trait) were potential genetic effects. In addition, the relationship between lamb titer and survival to 120 days of age was examined.

## Materials and Methods

Population. Six hundred-sixteen ewes from six strains at the Agriculture Canada Animal Research Centre (ARC), Ottawa, Ontario were used in the study. These strains were formed in 1974 and at the time

of formation, their breed composition was primarily as follows (in decreasing order of importance):

Strain 1: a synthetic paternal strain including Ottawa Synthetic (Suffolk x Leicester x North Country Cheviot x Romnelet), Ile de France, Suffolk and Leicester,
Strain 2: a synthetic maternal strain including Finnish Landrace, Shropshire and Suffolk,
Strain 3: a synthetic maternal strain with a breed composition similar to strain 2 but also including Dorset, East Freisian and several other breeds in small percentages,
Strains 4 and 5: random-bred purebred Suffolks and Finnish Landrace, respectively, and
Strain 6: a control strain with breed composition similar to Strain 3.

There were 139, 187, 183, 26, 43 and 38 ewes in strains 1 through 6, respectively. In strains 1, 2 and 3, one ram is utilized per 12 ewes. In strains 4,5 and 6, one ram per two ewes is utilized.

The average inbreeding coefficient of the ewes was 2.0% and ranged from 0.0% to 12.0%. The ewes were of three age groups, either 1.00, 1.75 or 2.50 years of age at lambing. From the June, 1984 lambing, 723 lambs born to 378 of these and were also used in this study. The remaining ewes had been exposed to rams but did not conceive or produce live lambs.

Management. The ewes, which are maintained as a specific pathogen free flock, are managed under total confinement. An accelerated lambing schedule allows each ewe the opportunity to lamb every 8 months, in January, September and June. Estrus was synchronized using progestagen impregnated sponges and pregnant mare serum gonadotropin, and breeding spanned approximately 23 days. Lambs are kept with their dams for 8-24 hours and then removed and raised artificially on milk replacer. Lambs are weaned from milk replacer at 21 days of age. The population and its management are described in greater detail in ARC Technical Bulletin No. 2 (1980).

Experimental Procedures. The objectives of the experiment were to evaluate genetic and environmental effects on the ability of a ewe to mount an active immune response to a specific antigen, and of her lamb(s) to passively acquire those specific colostral immunoglobulins. The antigen used to stimulate this active response in the ewes was ovalbumin. The ovalbumin was purified by A.A. Grunder from whites of eggs from specific pathogen free chickens, located at ARC, using the method of Kabat and Mayer(1967).

On April 5 and 6, 1984, approximately 2 months prior to lambing, 616 ewes were injected with 5 mg ovalbumin in 2 cc of a 1:1 emulsion of physiological saline and Fruend's incomplete adjuvant. A second injection of 2 mg ovalbumin in adjuvant-saline emulsion was administered 4 weeks later, on May 3, 1984. Six days after the second injection, 10 cc of blood was collected from each ewe via puncture of the external jugular. After the blood clotted, samples were

centrifuged at 1,100 x g for 8 minutes, and serum was collected. Sodium azide was added as a preservative to yield a final solution of 0.02% sodium azide, and samples were stored at -20 degrees C.

Five cc of blood was collected from each of the 723 lambs born during the June, 1984 lambing . Blood was collected from each lamb via puncture of the external jugular at the time that the lamb was removed from its dam. Lamb blood samples were handled in a manner similar to the ewe blood samples. The protocol for antigen concentrations and timing of inoculations was determined in pilot work.

Laboratory Analysis. The serum samples were assayed for specific antibodies to ovalbumin using kinetic Elisa techniques (Tsang et al,1980). In kinetic Elisa, the estimation of antibody titer is based on the rate of the enzyme-substrate reaction (or the regression coefficient of absorbance on time) rather than an endpoint measurement of the reaction. Costar half-area Elisa plates (#3690) were purchased from Hyclone Laboratories, Logan, UT. Anti-sheep Ig G (heavy and light chains) conjugated to horseradish peroxidase was purchased from U.S. Biochemical Corporation, Cleveland, OH. ABTS (2-2' Azino-di-(3-ethylbenz-thiazaline-6-sulfonic acid) and 30% hydrogen peroxide, which were used in the substrate solution, were purchased from Sigma Chemicals Co., St. Louis, MO.

The optimum amount of ovalbumin (point of saturation) to give maximum binding of antibodies, for assaying both the ewe and lamb samples, was determined by a titration of ovalbumin in coating buffer

while holding the dilution of serum in serum diluent constant. This optimum amount was determined to be 10 ug/ml. The peroxidase conjugate was similarly titrated out in conjugate diluent to determine the optimum conjugate dilution, which was found to be 1:100.

A preliminary Elisa was run on approximately 100 ewe and 150 lamb serum samples to identify high (h), medium high (mh), medium low (ml) and low (l) samples which could be combined to form four pools to generate a standard curve. Two groups of pools were made, one for lambs and one for ewes. Using the kinetic Elisa technique, the pooled samples (h,mh,ml,l) were titrated out in serum diluent to determine the optimum dilution. A dilution which is on the linear portion of the titration curves (slope vs. dilution) from all four pools is optimum. The dilutions of sera used were 1:550 for ewe samples and 1:275 for lamb samples.

The titration curves of the four pools were plotted, slope vs. increasing dilution. The dilution, when expressed as a decimal, gives the ml of sera necessary to achieve that dilution. The 50% point, or the dilution corresponding to half the maximum Y value (slope), was set equal to one Elisa unit. This was then converted into Elisa units per ml. The titers of the four pools were expressed as Elisa units in this manner. The standard curve for each plate was plotted as each pool's slope vs. the natural log of each pool's predetermined Elisa unit. (Logs were used to help achieve linearity). The Elisa units corresponding to each individual sample were obtained from the standard curve by taking the antilog of the log Elisa unit which

corresponded to the slope of that individual sample.

Antisera tested against sheep albumin and bovine serum albumin and control sera (sera from non-immunized sheep) tested against ovalbumin gave similarly low readings (slopes), which were used as the zero endpoint. Any serum sample with a similar or lower slope was considered devoid of anti-ovalbumin antibodies.

The intra-assay coefficient of variation (CV) was obtained as the average of the CV's of all samples ( each sample was run in quadruplicate). The inter-assay coefficient of variation was determined from a pooled sample run on each plate. The average intra-assay CV was 6.3% and the inter-assay CV was 17.6%. The average coefficient of determination for all standard curves was .96. Twenty samples also were run on different days and the correlation between the two readings was .98.

Statistical Analysis. Statistical analyses were performed by least-squares analysis of variance (Harvey, 1975). Data from all ewes, all pregnant ewes and all lambs were analyzed separately. The mathematical model for ewe's active titer included strain of sire, sire of ewe within strain, age of ewe, pregnancy status and appropriate regression effects. For lamb's passive titer, the mathematical model included strain of sire, sire or maternal grandsire of lamb within strain, age of dam, litter size and regression effects. For this paternal half-sib analysis, the actual average genetic relationship among each sire's progeny (including both full and half

sibs), which was calculated to be 29%, was used rather than the traditional 25%. In the analysis of ewe's active titer, the actual average genetic relationship of "paternal half-sib" ewes was 27%.

The average genetic relationship was calculated by computing the numbers of full-sib and half-sib pairs in each paternal half-sib family, and then summing over all families. The total proportion of pairs of full-sibs weighted by the genetic relationship of full-sibs (.5) plus the total proportion of pairs of half-sibs weighted by the genetic relationship of half-sibs (.25) yielded the actual average genetic relationship.

Lambs' passive titers also were analyzed by a full-sib analysis, with progeny nested within dams and dams nested within sires, in order to investigate the importance of maternal and(or) dominance genetic effects.

All effects were assumed to be fixed except sire or maternal grandsire within strain, ewes within sires or maternal grandsires and the residual. Heritability estimates of anti-ovalbumin antibody titer were computed from these analyses. Titers were not normally distributed, therefore all analyses were conducted on $\log_{10}$ titer in order to normalize the data. Least-squares means were obtained by computing the anti-log of the $\log_{10}$ least-squares mean titer.

In preliminary analyses of active antibody titers of ewes, other potential sources of variation were examined. The linear effects of

ewe inbreeding coefficient, breeding weight and time between antigen injection and lambing (as a measure of stage of gestation) on anti-ovalbumin antibody concentration were analyzed. For lamb passive antibody titer, other sources of variation examined were the linear effects of time between injection and lambing , birthweight and linear and quadratic effects of age in hours at bleeding. In a separate lamb analysis, ewe's active titer was analyzed as a non-interacting covariate on lamb's passive titer.

Seventy-seven of the lambs in this study received supplemental bovine colostrum at birth. This colostrum was given when a ewe did not have enough colostrum for her lambs. This supplement would affect a lamb's total immunoglobulin concentration and possibly its survival but not its anti-ovalbumin titer, since those antibodies could only be received from the lamb's dam. Therefore these supplemented lambs were retained in all the major analyses. They were removed from an additional analysis conducted to examine the relationship between lamb's passive titer and survival to 120 days of age, as supplementation would affect their survival potential.

## Results and Discussion

Environmental Influences on Ewes's Titer. The least-squares means and regression coefficients for factors affecting ewe anti-ovalbumin titer are presented in table 1. The effect of

pregnancy status, measured as the number of fetuses in utero (0 to 5), was non-significant. In a supplementary analysis, pregnancy status was also analyzed simply as pregnant vs. non-pregnant, which also had no significant impact on a ewe's antibody titer. The least-squares means for pregnancy status do indicate a general tendency for ewe titer to decrease as the number of lambs in utero increases. Presented on Table 1 are the regressions of ewe's antibody titer on inbreeding coefficient and breeding weight which also were not significant.

The effect of ewe age on titer was non-significant in the analysis of all ewes but was significant ($P<.05$) in the analysis of only the pregnant ewes. Lie (1979) found an age effect on the antibody titers of young bulls (between 113 and 420 days of age) to human serum albumin. The effect was positive and curvilinear with titers leveling off after 180 days of age. In the analysis of pregnant ewes, there was no definite trend for the effect of age on titer, but there were only three age groups, with the youngest animal being at least 365 days of age. The ewes in the middle age group (1.75 years) had lambed as ewe lambs the previous October (8 months earlier), which could possibly have been an added stress on them explaining their lower titer. Accelerated lambing has been shown to affect lambs' passively acquired immunoglobulin concentration (Halliday,1976). Therefore it is possible the 1.75 year old group's own titer could have been affected by lambing only 8 months previously.

The effect of stage of pregnancy at time of injection, as reflected by time between injection and lambing, on ewe titer also was examined in the analysis of pregnant ewes. The effect was not significant. Breeding in this flock was synchronized; therefore lambing occurred over a short period of time (28 days) which led to little variation in stage of gestation at the time of injection.

Genetic Influences on Ewe's Titer. Strains reached significance only at $P<.10$ and only in the analysis of all ewes (table 1). Strain 1, the synthetic paternal strain was highest and Strains 5 and 6, the Finnish Landrace and control strains were lowest. In the pregnant ewe analysis, strains was not a significant source of variation, but in both analyses sires were highly significant. The heritabilities and standard errors for ewe antibody titer against ovalbumin are presented in table 2. The $h^2$ for titer estimated from all ewes, using an average genetic relationship of 27%, was .27 +/- .17. This is the $h^2$ for antibody titer of the class IgG 6 d post-second injection. Van der Zijpp et al. (1983) reported that heritabilites for active titers in poultry differed depending upon the day post-second injection that blood samples were collected. In their study the paternal half-sib heritability of 2-mercaptoethanol resistant antibody titer (all classes of immunoglobulins except IgM) against sheep red blood cells on the seventh day post-second injection was .28 +/- .18. Lie (1979) reported a paternal half-sib heritability of titer to human serum albumin (all Ig classes) 8 days post-second injection in

young bulls of .15+/-.19. In mice the $h^2$ of antibody response against sheep red blood cells was estimated by three methods to range from .32 to .39 (Biozzi et al.,1979).

From the analysis of only the pregnant ewes, $h^2$ was estimated to equal .57 +/- .25. If this is a real difference the increase in $h^2$ between the analysis of all ewes and the analysis only of pregnant ewes might be caused by the added stress of pregnancy accentuating genetic variation for antibody titer.

Environmental Factors Affecting Lamb's Titer. The least-squares means for effects on lamb's titer are presented in table 3. The effect of lambing date on lamb's passive titer was examined to investigate the possibility that stage of gestation at time of injection affected the concentration of antibodies a lamb received. The effect of lambing date on lamb's titer was only significant at $P<.10$. Halliday (1974) found an effect of lambing date on total immunoglobulin concentration that a lamb received, with lambs born earlier in the season having higher Ig concentrations. Halliday's study measured total Igs which would include antibodies to all antigens to which a ewe had been recently exposed; in contrast our study examined only anti-ovalbumin antibodies, to which ewes should not have had exposure prior to inoculation. In our study, lambing season only spanned approximately 28 days; consequently seasonal variation was not expected.

Birthweight had a positive linear impact on lamb's titer, which was not significant. The regression coefficient was .0244 $\log_{10}$

titer/kg. Muggli et al. (1984) reported no association between birthweight and passively acquired IgG concentration in young calves. Halliday (1968) in lambs and Cabello and Levieux (1981) in calves reported a negative correlation between birthweight and total Ig concentration after suckling.

The effect of age of dam on lamb's titer was not significant. Even so, the youngest ewes had lambs with the highest titers, as shown in table 3. This result is in contrast to the results of Norman et al. (1981) and Muggli et al. (1984), who both reported older cows having calves with higher concentrations of total immunoglobulins. In this study there were only three age groups, all of which were fairly young. It is possible the younger animals had lower volumes of colostrum produced and consequently higher concentrations of antibodies. The lambs in our study usually were removed from their dams by 24 hours of age. Therefore it may not have been important for a ewe to produce large volumes of colostrum, as long as she produced enough for her lambs in those first crucial hours.

Litter size had a highly significant effect on lamb titer ($P<.001$, table 3). The range of means is from 3.428 Elisa Units (EU) for single born lambs to 1.235 EU for individuals from litters of five. The correlation between titer and litter size was -.95 with $r^2 = .90$. These results are similar to those of other researchers who have reported a negative relationship between litter size and total Ig concentration in young lambs (Halliday, 1968, 1974; Hunter et al., 1977).

Age of the lamb at bleeding had a significant non-linear effect on lamb titer ($P<.05$). The average bleeding age was 15.7 hours, with a range form 4 to 30 hours. The relationship between titer and bleeding age was curvilinear, increasing from 4 to 18 hours and then declining after 18 hours of age. Muggli et al.(1984) found a negative regression of total IgG concentration on bleeding age between ages 24 and 48 hours in calves. Halliday (1971) reported a rapid increase in total IgG concentration of lambs from 0 to 12 hours and then a gradual increase from 12 to 18 hours, with a decline after 18 hours of age. The decline of titers after 18 hours of age is probably due to catabolism of immunoglobulins and the equilibration of immunoglobulins in extravascular spaces (Bush et al.,1971; Logan et al., 1972).

The regression coefficient of lamb's titer on ewe's titer was .3193 $\log_{10}$ titer/$\log_{10}$ titer ($P<.001$). Related back to actual titer, a change of one Elisa unit in ewe's titer from 2.0 to 3.0 EU corresponds to a .301 EU increase in lamb's titer and when ewe's titer increases from 4.0 to 5.0 EU there is a .214 EU increase in lamb's titer.

The effect of sex of lamb on lamb's antibody titer was non-significant. The least squares means for passive antibody titer for ram and ewe lambs were 2.411 EU with standard error of .267 and 2.526 EU with standard error of .298, respectively.

Genetic Influences on Lamb's Titer. Strains approached significance for differences in lambs passive titer only at $P=.10$. Strain 5 (purebred Finnish Landrace) was highest for lamb titer with

Strain 4 (purebred Suffolk) lowest . Halliday (1968) reported breed differences for IgG concentration of lambs, with Finnish Landrace lambs being higher than lambs of either Merino x Cheviot or Scottish Blackface breeds ($P<.01$).

From the paternal half-sib analysis, sire was a significant source of variation in lamb's titer ($P<.001$) as was true in the full-sib analysis. The heritability for lamb titer from the paternal half-sib analysis (table 5) was .38 +/- .11. This heritablity estimate would be biased by any dominance genetic and (or) maternal effects contributing to similarity among full-sibs. This bias is evident when this $h^2$ of lamb titer is compared with the $h^2$ estimated from the paternal half-sib resemblance from the full-sib analysis (.28 +/-.15).

Muggli et al.(1984) reported a paternal half-sib $h^2$ for IgG concentration in calves of .03 +/- .09 for a selection line Hereford population and .13 +/- .19 for an Angus, Hereford and Red Poll population of cattle. Norman et al.(1981) reported $h^2$ for IgG concentration in calves at 24 hours of age to be .52 +/- .28. The $h^2$ estimated from the current study falls between these estimates. Important differences between these studies should be noted; the current study involves sheep rather than cattle, and also involves the antibody to a specific foreign protein and not just total IgG concentration.

The $h^2$ estimated from the dam component of the full-sib analysis of variance greatly exceeded 1.00. The sire variance

component was .0005 compared to the ewe variance component of .0070, the dam variance component being approximately fourteen times greater than the sire variance component. These results indicate that the lamb's passive titer is a trait which is greatly affected by maternal effects and(or) dominance genetic effects, most likely a preponderance of the former.

Halliday (1973), using embryo transfer techniques, reported Finnish Landrace lambs were superior for total IgG concentration to lambs of other breeds, regardless of the breed of surrogate dam. The current study indicates there is a genetic component of the lamb's titer but that the titer is also greatly influenced by maternal effects.

These maternal effects do not appear to be genetically controlled, as can be seen when $h^2$ is estimated by considering titer a ewe as opposed to a lamb trait, using, in other words the maternal grandsire variance component to estimate $h^2$. As reported in table 4, the maternal grandsire component of variance was negative. The maternal effects which influence lamb titer therefore appear to be environmental rather than genetic in origin. Lamb's passive titer is a complex trait, involving the ewe's own titer, the ewe's ability to concentrate antibodies in colostrum, total colostrum production and maternal behavior along with traits of the lamb (such as vigor). Therefore it is difficult to break this trait down into its component parts which are subject to both genetic and environmental influences. Muggli et al. (1984) reported $h^2$ of calf IgG concentration

estimated from the maternal grandsire component of variance to be .23 +/- .17 for a Hereford population, while the maternal grandsire variance component was negative in the other cattle population investigated.

The intra-litter correlation among lambs' titer was .56 +/- .04 ($P<.01$). The intra-litter correlation was estimated in a supplementary analysis with ewes nested within strains and lambs nested within ewes. This is similar to the intra-litter correlation for total IgG concentration in lambs reported by Halliday (1974) of .52 ($P<.001$).

Heritabilities of Other Traits. Heritabilities of litter size (-.03) and ewe body weight (.63 +/- .25) estimated from this population as reference heritabilities were comparable to those generally reported (Lasley, 1972).

Mortality. Lambs were coded as 0 if they died before 120 days (for any reason) or 1 if they survived to 120 days. A supplementary analysis was run to test whether death by 120 days was associated with higher or lower titer early in life. The least-squares mean titer for lambs which survived until 120 days was 3.544 EU, compared to 2.713 EU for those lambs which did not survive ($P<.005$). The overall least-squares mean was 3.100 EU. All lambs that received supplemental colostrum were excluded from this analysis, as the supplement would have improved their survival potential but not their titer. Muggli et al. (1984) reported significant differences between the Ig G concentrations of calves who died during the calving season and those

of the entire population. Many other studies have shown that low levels of immunoglobulins are related to increased death rates of young animals due to pathogens (eg., Sawyer et al., 1977; Mc Guire et al.,1983).

The total mortality of lambs between bleeding and 120 days of age was 10.8%. As shown in table 5, those lambs with titers less 1.824 EU (1 SD below the mean) had a mortality rate of 22.1%, or more than twice that of mortality in the population at large. Lambs with titers greater than 6.938 EU (3 SD above the mean) did not have the lowest but rather approximately average mortality. The lambs with the lowest mortality percentage were those whose titers fell between 4.376 EU and 6.938 EU (within plus 1 SD to plus 3 SD of the mean). This may reflect stabilizing natural selection (where both extremes are selected against) for the ability of lambs to passively acquire antibodies of the IgG class from their dams.

Conclusions. The results of this experiment are to some extent a function of the population studied. The flock was a specific pathogen free flock in which estrus in ewes was synchronized, ewes were on an accelerated lambing schedule and lambs were artificially raised. Further research is needed in other populations whose management is more characteristic of that found in commercial practice.

Most environmental effects that were examined were not important sources of variation in ewe's titer. There was a moderate heritability estimated for active immune response to ovalbumin in

ewes. Selection for immune response, therefore is likely possible, but further work must be done examining immune responses to known pathogens and associations of humoral with cellular responses and with production traits.

Important environmental effects on lamb's titer included litter size and age at bleeding which should be considered when similar experiments are conducted. Strains were a significant source of variation on lamb's titer with Strain 5 (purebred Finnish Landrace) being highest. Finnish Landrace sheep, which have large litter sizes, have been selected under systems in which many of the multiple birth lambs must be raised artificially. This may have resulted in selection for lambs with the ability to acquire sufficient concentrations of antibodies in a short time.

Selecting for increased ewe titer could possibly increase lamb's passive antibody concentration, but the relationship between ewe's titer and lamb's titer, though significant is not large. Many factors other than a ewe's own ability to respond to an antigen may affect her lambs' passive concentration of antibodies against that antigen. It is necessary for a ewe to have antibodies against a particular antigen in order for her lamb passively to acquire protection, but a ewe's active titer alone may not insure that her lamb will be protected. Maternal effects, particularly environmental ones, have an important impact on lamb's passive titer. Further research needs to be done to identify these factors.

Differences in mortality were found when lambs were grouped

according to titer. This relationship should be examined in other populations and experiments. There may possibly be an optimum lamb titer which is less than maximum. Lamb's passive antibody concentration, at least in this population, was heritable. Selecting for increased lamb titer would be possible, but we need to identify which levels of antibodies give the best protection before we advocate selection for increased active or passive titer.

TABLE 1. LEAST-SQUARES MEANS AND STANDARD ERRORS FOR ANTI-OVALBUMIN ANTIBODY TITER (ELISA UNITS) FOR ALL EWES AND FOR PREGNANT EWES

| | All ewes | | | | Pregnant ewes | | | |
|---|---|---|---|---|---|---|---|---|
| Effect | No. | Mean | SE | P | No. | Mean | SE | P |
| Overall | 616 | 3.238 | .048 | | 378 | 3.246 | .068 | |
| Strain | | | | .10 | | | | NS |
| 1 | 139 | 3.816 | .095 | | 71 | 3.604 | .134 | |
| 2 | 187 | 3.332 | .081 | | 132 | 3.240 | .097 | |
| 3 | 183 | 3.100 | .082 | | 109 | 3.086 | .107 | |
| 4 | 26 | 3.231 | .214 | | 7 | 3.485 | .417 | |
| 5 | 43 | 2.906 | .169 | | 35 | 3.115 | .189 | |
| 6 | 38 | 2.906 | .175 | | 24 | 2.991 | .221 | |
| Age | | | | NS | | | | <.05 |
| 1.0 yr | 162 | 3.093 | .088 | | 64 | 3.348 | .124 | |
| 1.75 yr | 204 | 3.153 | .139 | | 128 | 2.453 | .217 | |
| 2.5 yr | 250 | 3.481 | .104 | | 186 | 4.166 | .162 | |
| Pregnancy status | | | | NS | | | | |
| 0 | 238 | 3.393 | .336 | | | | | |
| 1 | 87 | 3.301 | .298 | | | | | |
| 2 | 158 | 3.202 | .278 | | | | | |
| 3 | 108 | 3.002 | .294 | | | | | |
| 4 | 20 | 2.623 | .449 | | | | | |
| 5 | 5 | 3.628 | .838 | | | | | |
| Pregnancy status | | | | NS | | | | |
| 0 | 238 | 3.378 | .074 | | | | | |
| 1 | 378 | 3.139 | .062 | | | | | |
| Regressions | | | | | | | | |
| Inbreeding | | .00009 | .009 | NS | | .0042 | .014 | NS |
| $\frac{\log_{10} \text{ titer}}{\% \text{ Inbreeding}}$ | | | | | | | | |
| Lambing date | | | | | | .0020 | .002 | NS |
| $\frac{\log_{10} \text{ titer}}{\text{day}}$ | | | | | | | | |
| Breeding weight | | -.0004 | .001 | | NS | -.001 | .002 | NS |
| $\frac{\log_{10} \text{ titer}}{\text{kg}}$ | | | | | | | | |

TABLE 2. HERITABILITY ESTIMATES OF EWE ANTI-OVALBUMIN TITER

| Population | $h^2$ | SE |
|---|---|---|
| All ewes, using ewe sire variance component | .27 | .17 |
| Pregnant ewes, using ewe sire variance component | .57 | .25 |

TABLE 3. LEAST-SQUARES MEANS AND STANDARD ERRORS FOR PASSIVE ANTI-OVALBUMIN ANTIBODY TITER (ELISA UNITS) FOR LAMBS

| Effect | No. | Mean | SE | P |
|---|---|---|---|---|
| Overall | 709 | 2.384 | .127 | |
| Strain | | | | <.10 |
| 1 | 98 | 2.687 | .128 | |
| 2 | 271 | 2.576 | .100 | |
| 3 | 209 | 2.561 | .102 | |
| 4 | 9 | 1.613 | .373 | |
| 5 | 68 | 3.150 | .150 | |
| 6 | 54 | 2.038 | .162 | |
| Age of dam | | | | NS |
| 1.0 yr | 103 | 2.597 | .131 | |
| 1.75 yr | 228 | 2.340 | .104 | |
| 2.5 yr | 378 | 2.230 | .100 | |
| Litter size | | | | <.001 |
| 1 | 76 | 3.428 | .148 | |
| 2 | 294 | 3.229 | .099 | |
| 3 | 265 | 3.042 | .108 | |
| 4 | 60 | 1.852 | .165 | |
| 5 | 14 | 1.235 | .309 | |
| Regressions | | | | |
| Lambing date | | $\frac{.0035 \log_{10} \text{ titer}}{\text{day}}$ | .002 | <.10 |
| Bleeding age | | | | |
| Linear | | .0053 | .002 | <.05 |
| Quadratic | | -.0008 | .0003 | <.05 |
| Birth weight | | $\frac{.0244 \log_{10} \text{ titer}}{\text{kg}}$ | .018 | NS |
| Ewes titer | | $\frac{.3103 \log_{10} \text{ titer}}{\log_{10} \text{ titer}}$ | .048 | <.001 |

TABLE 4. HERITABILITY ESTIMATES FOR LAMB PASSIVE ANTI-OVALBUMIN ANTIBODY TITER

| Method of Estimation | $h^2$ | SE |
|---|---|---|
| From 1/.25 times the sire of lamb variance component in a model in which progeny were nested within dams which were nested within sires | .28 | .15 |
| From 1/.29 times the sire of lamb variance component in a model in which progeny were nested within sires | .38 | .11 |
| From 1/.27 times the maternal grandsire of lamb variance component | -.15 | |

TABLE 5. MORTALITY PERCENTAGES OF LAMBS GROUPED BY PASSIVE ANTI-OVALBUMIN ANTIBODY TITERS

| Group | No. | % Mortality | SE | Confidence Interval |
|---|---|---|---|---|
| < 1.824[c] EU | 86 | 22.1[a] | 4.5 | 13.2 - 31.0 |
| 1.824 - 4.376[d] EU | 339 | 10.6[ab] | 1.7 | 7.3 - 13.9 |
| 4.376 - 5.652[e] EU | 70 | 1.4[b] | 1.4 | -1.4 - 4.2 |
| 5.652 - 6.938[f] EU | 42 | 4.8[ab] | 3.3 | -1.9 - 11.5 |
| >6.938[g] EU | 95 | 10.5[ab] | 3.2 | 4.2 - 16.8 |
| TOTAL | 632 | 10.8 | 1.2 | |

[a,b] Percentages with no superscripts in common differ ($P<.05$).

[c] Corresponds to minus 1 standard deviation.

[d] Corresponds to minus 1 standard deviation to plus 1 standard deviation.

[e] Corresponds to plus 1 standard deviation to plus 2 standard deviations.

[f] Corresponds to plus 2 standard deviations to plus 3 standard deviations.

[g] Corresponds to plus 3 standard deviations.

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# Chapter 3

## SUMMARY

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# APPENDIX

# APPENDIX

## Buffer Solutions For Assay

(1) Coating Buffer. $Na_2CO_3/NaHCHO_3$ buffer, pH 9.6, 1.59 g $Na_2CO_3$; 2.93 g $NaHCHO_3$ made up to 1 l with distilled water. Store no longer than 1 week. Store at $4^o$ C.

(2) Elisa Washing Solution. PBS, pH 7.2, 8.5 g NaCl, 0.22 g $NaH_2PO_4$, 1.19 g $Na_2HOP_4$ made up to 1 l with distilled water. Store at $4^o$ C.

(3) Serum and Conjugate Diluent. Tris buffer with 1.0% Bovine Serum Albumin (BSA). pH 7.4, 8.7 g NaCl, 0.372 g EDTA, 6.05 g Tris base, 0.5 ml Tween 20, 10 g BSA, made up to 1 l with distilled water. Add Tween 20 just prior to using. Store no longer than 1 week. Store at $4^o$ C.

(4) Substrate Diluent. Citrate buffer. pH 4.0, 10.51 g Citric Acid, made up to 1 l with distilled water. Make fresh every day.

(5) Stock ABTS solution. 548.7 mg / 25 ml of distilled water, 40 mM solution ABTS. Store in dark, at $4^oC$.

(6) Substrate. Dilute 75 ul of 40 mM ABTS in 10 ml citrate buffer. Dilute $H_2O^2$ to 3% (1:10) in citrate buffer. Mix 25 ul of 3% $H_2O_2$ citrate buffer in 10 ml ABTS and citrate buffer. Use soon after mixing. Store in dark, at $4^oC$.

Kinetic Elisa Protocol

(1) 100 ul of a solution of ovalbumin diluted in coating buffer (10ug/ml) was pipetted into each well of a plate.

(2) Coated plates were stored at least overnight and up to two weeks at $4^{o}C$ in a humid chamber.

(3) Plates were washed 4 times in PBS-Elisa wash.

(4) Each serum sample was diluted in serum diluent, and 100 ul was pipetted into each of four well. All four pooled samples were run in quadruplicates on each plate. Each plate contained a serum, conjugate and substrate control.

(5) Plates were incubated at room temperature for 1 hour in a humid chamber.

(6) Washing was repeated.

(7) Conjugate was diluted in serum diluent, and 50 ul was pipetted into each well.

(8) Plates were incubated for 30 minutes at room temperature in a humid chamber.

(9) Washing was repeated.

(10) 100 ul of substrate was added to each well.

(11) Plates were read on a Flowlabs Elisa reader at 414 nm at 0.5, 2.5 and 4.5 minutes after substrate was added to determine absorbance.

(12) The rate of reaction was determined by calculating the regression coefficient of absorbance on time for each serum sample.

(13) Elisa Units for each sample were determined from the standard curve which was run on each plate.