# EXPERIMENTAL EVIDENCE OF RESISTANCE TO *HAEMONCHUS CONTORTUS* INFECTION IN SHEEP

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# TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF TABLES (cont'd)

# INTRODUCTION

Internal parasite infections are a major source of economic loss to the sheep industry of the world. For example, the U. S. Department of Agriculture (1965) estimated annual losses from internal parasite infections in sheep in the United States at approximately 25 million dollars.

According to Soulsby (1965), there has been very little critical study of the economic losses caused by internal parasite infections, but there is ample circumstantial evidence of the role that *Haemonchus contortus* plays in causing this loss. It is a ubiquitous parasite, found wherever sheep are raised in the world, and it is the only nematode parasite of sheep that causes a recognizable acute disease, haemonchosis. The economic losses are the result of the whole blood constituent losses due to hemorrhage caused by the voracious feeding habits of *H. contortus*. For example, Martin and Ross (1934) estimated blood loss at 30 ml. per day in a sheep with an infection of 2000 *H. contortus* females, and Clark *et al.* (1962) calculated that a single adult *H. contortus* consumed 0.05 ml. per day. Therefore, anemia is one of the clinical symptoms regularly associated with *H. contortus* infections. The degree of anemia is readily estimated by the determination of the hemoglobin content of the blood. It has been shown that hemoglobin levels are negatively correlated with the level of *H. contortus* infection in sheep (Loggins *et al.*, 1965).

The treatment of *H. contortus*-infected sheep with various anthelmintic drugs is one method currently used in an effort to reduce worm populations and thus prevent economic losses. However, the cost of such drugs and the labor necessary for administration increases the operating costs of the sheep enterprise. Also, the efficiency of many drugs in reducing the *H. contortus* levels in sheep may vary and periodic treatments are required. Finally, toxicity of the drugs to the host and the danger of tissue residues is of serious concern in their routine use.

Management practices, such as rotational grazing and dry-lot feeding, have been proposed as methods of controlling *H. contortus* infections. However, since Cook and Conway (1966) reported that the period from infection with third stage larvae to the onset of ova production was only 14 to 17 days and Ransom (1907) reported that ensheathed larvae are very resistant to freezing and drying and thus remain viable for several months, rotational grazing may not be effective. The grazing period would need to be short and the interim period long, resulting in low efficiency of pastures. On the other hand, the mechanical harvesting and transporting of forages to sheep in dry-lot is effective in controlling H. contortus infections, but involves a large expense and therefore is not commonly used.

Control of H. contortus infections through genetic selection is a method which does not increase operating costs, since genetically resistant sheep would require no special treatment or handling. The existence of a genetic resistance to H. contortus has been suggested by the low H. contortus levels in Florida Native sheep as compared with Rambouillet sheep in a report by Loggins, Swanson and Koger (1965). The Florida Native sheep is a crossbreed which has developed through natural selection, under Florida conditions, over the past 300 years.

The objective of this study was to attempt to elucidate this apparent genetic resistance to H. contortus infection in the Florida Native sheep, by the use of certain laboratory techniques. In addition to hematological, immunological and parasitological measurements, production data on the experimental sheep flock was collected. Finally, all data was subjected to statistical analysis to determine the significance of the results.

# LITERATURE REVIEW

## Life Cycle and Pathogenesis of Haemonchus contortus

The nematode H. contortus is a parasite of sheep which normally inhabits the abomasum. The life cycle of H. contortus was first worked out by Ransom in 1906 who reported that the ova which were passed in the feces, became embryonated and hatched within 14 to 19 hours, under suitable conditions of heat and moisture, releasing first stage larvae. The larvae are quite active and feed for 10 to 12 hours at which time they moult into the second stage, which is also an active, free-living, feeding stage. About three days after hatching, the larvae moult into the third, or infective, stage which migrates up blades of grass and are thus available for ingestion by grazing sheep.

Upon ingestion, a dialyzable factor, or factors, present in the sheep rumen stimulates the release of "exsheathing fluid" from a region near the excretory cell of the larvae which enables the larvae to break out of the sheath (Sommerville, 1957). The larvae pass to the abomasum and undergo two additional moults reaching the sexually differentiated young adult stage (Silverman and Patterson, 1960). Cook and Conway (1966) found that the period from infection by the third stage infective larvae until onset of reproduction as an adult H. contortus varied from fourteen to seventeen days.

Boughton and Hardy (1935) observed that the parasites attached themselves to the stomach wall by a peculiar striking motion of the head and neck, and that they remained attached for about twelve minutes. The parasites detached themselves from the stomach wall leaving minute hemorrhages which continued for a maximum of seven minutes. Andrews (1942) reported that blood appeared in the feces six to ten days after sheep were given H. contortus infective larvae. Fourie (1931) concluded that the anemia observed in sheep experimentally infected with H. contortus was purely hemorrhagic in character, since he was able to reproduce the same blood picture in healthy lambs by periodic bleeding from the jugular vein.

## Resistance of Sheep to Haemonchus contortus

### Immunology

Reports on resistance to parasitic nematode infections support the view that an immune response, probably humoral in nature, is stimulated by the excretions and/or secretions of the invading parasites (Taliaferro, 1940; Campbell, 1955; and Chipman, 1957).

Stoll (1929) first reported on a "self-cure" phenomenon in sheep with expulsion of H. contortus and protection of the animals thereafter against any significant amount of further infection. In his experiment, one of the two worm-free lambs was given 45 infective H. contortus larvae by mouth. Both lambs were then maintained on the same small pasture, with the infective lamb serving as the source of natural infection for the other and for repeated infection of itself. An initial build up of an infection as measured by ova counts in the feces and hemoglobin levels was followed by expulsion of adult H. contortus and protection of the lambs against natural and challenge reinfections with H. contortus infective larvae.

Gordon observed that "self-cure" was usually seen when there was a fresh growth of pasture, along with an increased H. contortus larval intake, but was of short duration and often insufficient to promote a rapid increase in body weight. Sheep which had expelled an infection of H. contortus were not always resistant to later reinfection. A sheep which underwent "self-cure" on one occasion may have succumbed to haemonchosis when the next outbreak occurred a few weeks later.

Stewart (1950b) found that the intake of large doses of infective larvae of H. contortus was the exciting cause of the "self-cure" phenomenon. Stewart (1953) concluded that "self-cure" did not result in a release of heterologous antibodies into the blood stream of the sheep. There was a reaction of the host associated with allergic sensitization and an edematous condition of the mucous membrane of the abomasum of the host after the administration of larvae. The abomasum of lambs which had not been exposed to H. contortus larvae previously remained flaccid and normal when massive doses of exsheathed larvae were injected into the abomasum (Stewart, 1955). In lambs hypersensitized by previous infections with *H. contortus*, the abomasum showed increased peristalsis and segmentation within ten minutes of the injection of the massive doses of the exsheathed larvae. Within one hour, the abomasum was pale, edematous and had contracted in diameter. The reaction of sheep resistant to *H. contortus* was similar to that of the previously infected sheep.

Soulsby *et al.* (1959) found that at the time of the "self-cure" phenomenon, the majority of the existing adult *H. contortus* population was in the small intestine and in a state of disintegration, although third and fourth stage larvae of varied sizes were present in the abomasum. This finding supports the evidence presented by Soulsby and Stewart (1960) that the main antigenic stimulation of the "self-cure" phenomenon was derived from substances released by larvae during the third moult.

The rate of development of the parasitic phase of *H. contortus* is dependent upon the age and immunological state of the host. Gordon (1948) observed that adult animals were generally more resistant but, in the field, it usually was not possible to separate the effects of age from those of a previous infection. The differentiation and development of larvae was more rapid in susceptible lambs than in older susceptible sheep (Silverman and Patterson, 1960). In their report the differentiation and development of larvae in resistant sheep became inhibited at the fourth and fifth stages and were expelled from the sheep.

Studies on the host-parasite relationship of H. contortus suggested that important antigens were released during growth and development of the parasite as it penetrates, moults and matures in the host (Silverman, 1965). No evidence of host tissue responses to the larvae in either susceptible or resistant sheep was observed up to the ninth day after infection (Silverman and Patterson, 1960). Damage to host tissue first occurred after the tenth day when young adults began to burrow into the mucosa. Fourth and fifth stage larvae showed the greatest antigenic activity and were the most susceptible to the adverse effects of serum from resistant animals, in vitro, while neither third stage larvae nor adult worms showed any apparent reaction to such serum (Silverman, 1965).

Several methods to elicit resistance in sheep to H. contortus are reported in the literature. Severe anemia and death following challange doses of infective larvae were prevented by previous infection with immature stages of H. contortus (Stoll, 1942; Christie et al., 1964 a; Christie et al., 1964b and Dineen et al., 1965). Stewart (1950 a) reported that ground, mature H. contortus, ground infective larvae, and heat-killed larvae did not stimulate detectable antibody responses. Jarrett et al. (1959, 1961) showed that vaccination with irradiated larvae produced a resistance sufficient to withstand large challenge doses of infective larvae.

Experimental bleeding (Bemrick *et al*., 1958) and mineral supplementation (Weir *et al*., 1948; Richard *et al*., 1954 a; and Richard *et al*., 1954 b ) appeared to increase resistance of lambs to *H*. *contortus* infections. Emerick *et al*. (1957) postulated that supplementation of the ration with cobalt increased the synthesis of vitamin $B_{12}$ in the rumen in response to the severe drain of blood by *H*. *contortus*.

## Hematology

Anemia is one of the clinical symptoms associated with haemonchosis in sheep. Georgi and Whitlock (1967) reported a positive correlation between exposure to *H*. *contortus* infection and onset of erythrocyte loss in sheep, supporting the assumption that blood loss leading to anemia was caused by *H*. *contortus.*

Bemrick *et al*. (1958) concluded that one of the most important factors in the development of a resistance to challenge infections with *H*. *contortus* in lambs was the hemorrhage produced by the blood sucking habits of the worms. Shutt and McDonald (1965) reported that experimental anemia, maintained by daily bleeding, provoked a marked increase in the rate of hemoglobin synthesis, about $3\frac{1}{2}$ times the normal rate.

Loggins *et al*. (1960) found that hemoglobin levels in sheep varied significantly between breeds. Holman (1944) and Becker and Smith (1950) observed no significant differences between breeds of sheep with regard to blood constituents studied. However, Loggins et al. (1965) reported breed differences in H. contortus ova counts and adult H. contortus counts at necropsy. These latter results suggest that the breed differences in hemoglobin levels may be confounded with the degree of resistance to H. contortus infections. For example, Florida Native sheep had higher hemoglobin levels and lower H. contortus ova counts and worm counts at necropsy than Rambouillet sheep.

Parasitology

Christie et al. (1964 c) presented ova count data which suggested that host resistance involved a resistance to the establishment of H. contortus. Suppression of ova production per H. contortus female had not occurred, and retardation of development of larvae was the major effect of control on the parasitic burden (Dineen et al., 1965). However, Kingsbury (1965) reported that counts of helminth ova in the feces of an infected animal was not a reliable measure of the level of infection. Counts ranged from 500 to 2000 ova per gram of feces regardless of the actual population of sexually mature worms in sheep with some large populations yielding counts of less than 1000 ova per gram of feces.

Christie and Brambell (1966) observed significantly lower worm populations in lambs protected by previous H. contortus infections than in uninfected controls following a challange dose of H. contortus infective larvae. Loggins et al. (1965) reported lower worm counts in Florida

Native sheep than in Rambouillet sheep. No significant relationship was found between the degree of infection at the time "self-cure" occurred and the degree of reduction in worm burden resulting from "self-cure" (Gordon, 1948). "Self-cure" operated to some extent in all the sheep, irrespective of their worm burden.

## Hemoglobin Types of Sheep

Hemoglobin, a complex molecule, consisting of iron, a porphyrin ring and globin, has been studied for more than 30 years (Kitchen, 1965). Hemoglobin differences reside in the protein moiety (globin) which comprises 95 percent of the hemoglobin. The globin of hemoglobin consists of two pairs of polypeptide chains which form a tetramer. In adult sheep hemoglobins, the polypeptide chains pairs are designated the alpha and beta chains. The two beta chains are replaced by two gamma chains in fetal hemoglobin. The abbreviation "Hb" will be used in this report to designate specific hemoglobin types.

The heterogeneity of types of sheep hemoglobins has been well established. Harris and Warren (1955) found three electrophoretically distinguishable types in a group of ewes: a) a single relatively fast-moving hemoglobin, b) a single relatively slow-moving hemoglobin, and c) both the relatively fast-and relatively slow-moving hemoglobins. Evans *et al*. (1956) designated the three hemoglobin types as Hb A, Hb B and Hb AB, respectively. Helm *et al*. (1957) described two hemoglobins in Dutch sheep;

the faster-moving hemoglobin which they designated as Hb II and the slower-moving hemoglobin which they designated as Hb I. Although no direct comparison was made, the two hemoglobins appeared similar to Hb A and Hb B, respectively.

Preliminary evidence has indicated that these types are genetically determined in a simple Mendelian manner (Evans et al., 1956). The genes for the two hemoglobins (Hb A and Hb B) in sheep are allelic and they are co-dominant. This type of inheritance was also shown by Huisman et al. (1958). In sheep heterozygous for Hb A and Hb B both hemoglobin types were equally distributed among all red blood cells (Moore et al., 1966).

Breed differences in gene frequencies of Hb A have been reported by several authors and are summarized in Table 1. The frequency of Hb A ranged from 0.99 in the Norwegian Spael to 0.01 in the English Leicester. Evans et al. (1957) reported that gene frequencies in different flocks of the same breed were in good agreement. In general, the lowland breeds of British sheep were predominantly of Hb B, while Hb A was more conspicuous in the mountain and hill breeds, possibly indicating that the hemoglobin type may be of some adaptive significance. This theory was supported by differences in gene frequency in the Romney Marsh breed under the different environmental conditions of Great Britain and Australia (Evans and Blunt, 1961). Similar differences were observed when gene frequencies in the Down or Shortwool breeds from Great

TABLE 1

Gene Frequency of Hb A in
Some Breeds of Sheep

| Breed | Gene Frequency of Hb A | Location of Flock | Reference |
|---|---|---|---|
| Scottish Blackface | .77 | Great Britain | Evans _et_ _al_., 1957 |
| North Country Cheviot | .43 | Great Britain | Evans _et_ _al_., 1957 |
| South Devon | .26 | Great Britain | Evans _et_ _al_., 1957 |
| English Leicester | .01 | Great Britain | Evans _et_ _al_., 1957 |
| Merino | .38 | Australia | Evans _et_ _al_., 1958 |
| Rambouillet | .79 | France | Evans _et_ _al_., 1958 |
| Romney Marsh | .11 | Great Britain | Evans and Blunt 1961 |
| Romney Marsh | .44 | Australia | Evans and Blunt 1961 |
| Spael | .99 | Norway | Efremov and Braend 1965 |
| Cheviot | .51 | Norway | Efremov and Braend 1965 |

Britain were compared with the gene frequency in the Southdown breed in New South Wales. No obvious differences in any other characteristics associated with different hemoglobin types within the same breed have been detected.

Two other hemoglobin types have also been detected in sheep, which are under special conditions.

Harris and Warren (1955) identified the hemoglobin of normal fetuses before birth from ewes of all three phenotypic hemoglobin types, but found only one type present which they designated Hb F. Drury and Tucker (1962) reported that 23 of 24 lambs at birth had both adult hemoglobin and Hb F, the latter being the major component. As the lambs grew older, the adult hemoglobins increased in amount until at about 30 days of age when no fetal (Hb F) hemoglobin could be detected.

The other new hemoglobin was observed in cells from the top erythrocyte layer of centrifuged blood of sheep with Hb A (Blunt and Evans, 1963). Vliet and Huisman (1964) reported a similar type of hemoglobin in sheep following experimental bleeding and called it Hb C. Braend _et_ _al_. (1964) observed an electrophoretic hemoglobin band, from a seven-month old anemic lamb, with a rate of migration on starch gel which was slower than that of Hb B. This hemoglobin type was named Hb N. Although no direct comparison has been made, based on rates of migration, Hb C was probably the same as Hb N.

Braend and Efremov (1965) found small amounts of Hb N in 99 of 105 Norwegian (Spael) sheep, all of Hb A type. This suggested that Hb N may be a normal rather than an abnormal component. Efremov and Braend (1966) found that Hb N occurred in relatively higher amounts in lambs than in adults, with one-month-old lambs having an Hb N content as high as 30 percent of the hemoglobin.

In animals subjected to extreme experimental blood loss, the Hb A was replaced entirely by Hb C, whereas the production of Hb B apparently was not affected . Under conditions of moderate blood loss the replacement of Hb A by Hb C was only partial (Vliet and Huisman, 1964). Following bleeding, little or no Hb A was observed in the young cells of the AB population, but Hb C appeared instead. Later, Hb A reappeared but Hb C persisted in the blood for at least 2 months (Drury and Tucker, 1965). No Hb B variant has been observed under anemic condition due to parasitic infections (Efremov and Braend, 1966) or experimental bleeding (Vliet and Huisman, 1964). Blunt (1965) reported that a variant of Hb A produced during experimental anemia (probably Hb C) was located almost entirely in the reticulocytes. It was postulated that this variant is a relatively unfinished hemoglobin associated with the immature erythrocytes produced after a severe anemic stress. Schapira _et al_. (1962) concluded that the fraction of hemoglobin associated with young erythrocytes could have been either a special type of hemoglobin from red cells with a short lifespan, or a young, unfinished hemoglobin, which would eventually acquire the properties of the adult type.

The genetic control of the Hb A variants has not been completely determined. Braend and Efremov (1965) proposed that one of the structural genes controlling Hb N is closely linked to one of the structural genes controlling Hb A. Hb N commonly occurred with Hb A, but in very small quantities. Beale et al. (1966) concluded that the process by which Hb C forms the major proportion of the hemoglobin in anemic sheep of Hb A type is due to an increase in means or rate of synthesis of hemoglobin types rather than to the activity of a new gene. Wilson et al. (1966) indicated that the synthesis of the beta chain of Hb C is controlled by a structurally different and "silent" gene, which is activated during severe anemia. The beta chain of Hb C was probably the product of a distinctive gene related to the beta chain of Hb A through gene duplication and remained linked in coupling (Boyer, 1967).

The hemoglobin proteins have been observed to differ in many physical and chemical properties. In addition to the varieties demonstrated by electrophoresis, differences were found to exist in oxygen affinity (Kernohan, 1961), in specific gravity and percent of dry matter (Mounib and Evans, 1959), in resistance to alkali (Blunt, 1965) and in amino acid composition (Helm et al., 1957).

Helm et al. (1957) found that Hb II in the sheep contained higher amounts of glutamic acid, threonine and serine, and lower amounts of aspartic acid, glycine and alanine. No differences in the peptide patterns of the alpha chains of the three adult hemoglobins were observed, but several amino acid differences in the beta chains of Hb A and B were observed (Muller, 1961 and Naughton et al., 1963). Vliet and Huisman (1964) reported that Hb A, B, C, and F shared the same alpha polypeptide chain, but the non-alpha chains of each of the four hemoglobin types were distinctly different. Huisman _et_ _al_. (1965) presented data which supported this hypothesis. Wilson _et_ _al_. (1966) and Boyer _et_ _al_. (1967) have determined amino acid sequences of the non-alpha chains of the four hemoglobin types, and have shown minimum differences in residues present.

While a considerable amount of research has been reported on the physical and biochemical properties of the various hemoglobin types, little has been reported on the association of hemoglobin types with resistance to parasitic diseases. Evans _et_ _al_. (1963) presented evidence that sheep with Hb A harbored fewer adult worms than sheep with Hb AB following infection with _H_. _contortus_. The trend in ova counts and worm counts at the height of _H_. _contortus_-induced anemia suggested an interaction between hemoglobin type and susceptibility to _H_. _contortus_ might exist, the animals with Hb A being the less susceptible.

Evans and Evans (1964) showed a relationship between hemoglobin types and hematocrit values. The mean hematocrit values for Hb A were greater than those for Hb B, with the mean hematocrit values for Hb AB intermediate.

King _et_ _al_. (1958) found no significant differences in reproductive performance or growth rate of ewes of different hemoglobin types. The number of lambs produced by ewes of different hemoglobin types did not differ significantly, although there was some suggestion that hemoglobin heterozygotes produced a slightly larger number of lambs. Evans and Turner (1965) reported that ewes with Hb A had fewer lambs born or weaned than those with Hb AB or B, while the difference between Hb AB and B was slight. The main source of difference in lambs born in the various flocks was in the proportion of multiple births. The superiority of the Hb B ewes appeared to be associated with the production or survival of lambs from multiple births.

## MATERIALS AND METHODS

### Basic Design and Management Practices

The basic design was a 2 X 2 factorial, with two foundations (120 Florida Native ewes and 60 Rambouillet ewes) and two levels of resistance to _H_. _contortus_ (high and low). Assignment to the high or low resistance level groups was based on the mean value of periodic hemoglobin determinations over a two-year period. Animals from each breed were ranked according to this mean hemoglobin value and divided at the median into two equal groups. Those ewes with hemoglobin means above the median were assigned to the "high resistance" groups; those below into the "low resistance" groups.

TABLE 2

Experimental Design
Number of Ewes per Resistance Group

| Foundation | Resistance<br>High | <br>Low |
|---|---|---|
| Florida Native | 60 | 60 |
| Rambouillet | 30 | 30 |

The entire flock was maintained on 25 acres of permanent Coastal Bermudagrass pastures at all times as one flock except for a 45-day breeding season which began on July 1 of each year. This continuous grazing of permanent pastures assured a high exposure rate to H. contortus. Supplemental feeding of Coastal Bermudagrass hay (free choice) and one to two pounds of a corn-soybean meal concentrate per head was provided the ewe flock when pasture conditions were inadequate.

Anthelmintic treatments were initially eliminated from all groups to allow genetic resistance potential to be manifested. As a result, death losses increased in the Rambouillet ewes to the extent that the low resistance group was in danger of being eliminated from the experiment. Consequently, very anemic Rambouillet ewes were treated with phenothiazine in an effort to reduce the adult H. contortus load and prevent death. All drenched ewes were allotted to the low resistance group. Therefore, survival without anthelmintic treatment became the criterion for selection in the Rambouillet ewes.

Ewes were culled on age and failure to fit into the resistance group for which they were selected. Natural selection, manifested in death losses, was so strong in the Rambouillet ewes that no culling was permissible.

All physically sound ewe lambs were kept as replacement ewes. Replacement lambs were placed with the ewe flock after the breeding season to challenge them with natural infections of H. contortus. Mean hemoglobin level from weaning to 18-months of age was used to determine the resistance group into which a replacement ewe was placed.

Warwick _et_ _al_. (1949) reported that it was necessary to have intense selection for resistance to _H_. _contortus_ on both sides of the pedigree to make progress through selection. Rams were selected on mean hemoglobin level. Hemoglobin type of the ram was included in selection of sires for the 1967 breeding season. An attempt was made to increase the frequency of Hb A in the high resistance groups and the frequency of Hb B in the low resistance groups by the selection of the sires on hemoglobin type in 1967 (Table 3). Two rams were placed with each breed by resistance sub-group except for the high resistance Rambouillet group which had only one ram in 1967. Replacement rams were selected from ewes which best fit their respective resistance group (from ewes with highest hemoglobin levels in the high resistance groups and from ewes with lowest hemoglobin levels in the low resistance group.

A "sample flock" which consisted of one-sixth of each breed-resistance group was selected at random from within the breed-resistance groups and maintained with the remainder of the flock at all times. The sample ewes were used to study the relationships between hemoglobin levels, as an indicator of resistance to _H_. _contortus_, and total gamma globulin levels, ova counts and hemoglobin types. The ewes were sampled at one-month intervals for one com-

TABLE 3

Hemoglobin Type of Sires for 1967 Breeding Season

| Breed | Number | Hemoglobin Type | Resistance Group to Which Mated |
|---|---|---|---|
| Florida Native | N20 | AB | Low |
|  | N21 | B | Low |
|  | N80 | A | High |
|  | N81 | AB | High |
|  | N82 | AB | Spare |
| Rambouillet | R01 | B | Low |
|  | R02 | AB | High |
|  | R80 | B | Low |

plete year (December, 1966, to November, 1967). When a ewe from the sample flock died, it was replaced by a comparable ewe from the same breed-resistance group.

## Resistance of Sheep to *Haemonchus contortus*

### Immunology and Hematology

Blood samples were collected from the entire sheep flock at two-month intervals from an ear vein. Blood samples were collected into heparinized capillary tubes and hemoglobin pipettes.

A microtechnique using heparinized capillary tubes was employed for packed cell volume determination. Each tube was filled with blood, sealed at one end with plastic clay and centrifuged at 11,500 rpm for five minutes in a Model MB centrifuge.[1] At the completion of the cycle, the tubes were placed in a microcapillary tube reader[2] and the packed erythrocyte column measured as packed cell volume percent for each sample.

The acid-hematin technique of Cohen and Smith (1919) was employed for hemoglobin level determination. In a hemoglobin pipette, 0.025 ml. of whole blood was added to 5.0 ml. of one percent hydrochloric acid solution and allowed to stand at room temperature for one hour. The percent transmission of the sample was measured in a spectrophotometer[3] at a wave-length of 525 millimicrons and converted into grams of hemoglobin per 100 ml. of blood.

At one-month intervals, two blood samples from each ewe in the "sample flock" were obtained by venipuncture of the jugular vein with a 20-gauge disposable needle. A whole blood sample was collected into a 4 ml. Vacutainer$^R$ tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and a second sample was collected into a 10 ml. Vacutainer$^R$ tube containing no anticoagulant, to

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[1] International Equipment Company, Needham Hts., Mass.

[2] *Ibid*.

[3] Bausch and Lomb Spectronic 20.

obtain serum. In the laboratory, packed cell volumes and hemoglobin levels were determined by the methods described above.

The method of Jager and Nickerson (1948) was employed for the determination of total serum gamma globulins. One ml. serum and 0.5 ml. saturated ammonium sulfate were placed in a 15 ml. centrifuge tube, shaken and refrigerated at four degrees C. over night. The suspension was then centrifuged, the liquid removed, and 3.0 ml. of 33.3 percent saturated ammonium sulfate added to the precipitate. After stirring and centrifuging the suspension, the liquid was removed and the precipitate was dissolved in ten ml. of 0.85 percent sodium chloride. Five ml. of the sodium chloride solution was added to five ml. biuret reagent and the percent transmission of the solution was measured in a spectrophotometer[1] at a wavelength of 540 millimicrons and converted into grams gamma globulin per 100 ml. of serum.

Parasitology

Fecal samples were collected from each ewe in the flock in July and September, 1966, to measure the effectiveness of selection. Fecal samples were also collected from the sample ewes at monthly intervals from December, 1966, to November, 1967. Ova counts were determined by the McMaster slide flotation technique (Whitlock, 1948).

Since mixed, natural parasitic infections were used,

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[1] Bausch and Lomb Spectronic 20.

larval identification was necessary to determine the types of nematode infections present. About five grams of feces were cultured at room temperature for seven days to permit development of the larvae. The larvae were collected with a Baermann funnel (Morgan and Hawkins, 1949) and identified as to species (Skerman and Hillard, 1966).

Abomasal worm counts were obtained from most of the sheep which died and from 20 Florida Native ewes culled and slaughtered in January and February, 1968, to determine _H_. _contortus_ incidence in the flock. All adult _H_. _contortus_ present in two 20-ml. aliquots of the abomasal contents and washings were counted and multiplied by the appropriate factor to obtain total worm counts.

## Hemoglobin Types of Sheep

After hemoglobin levels and packed cell volumes were determined on the whole blood samples from the ewes of the "sample flock" in February, 1967, the red blood cells from the remainder of each sample were saved for hemoglobin type determination. Blood samples were obtained from the remainder of the flock, using the same technique. The red blood cells were washed with 0.85 percent physiological saline and hemolyzed with distilled water to release the hemoglobin for electrophoresis.

A modification with vertical gel of the starch gel method described by Smithies (1955) was used for hemoglobin type determinations in this study. A 0.06 M Tris-

EDTA-borate buffer with a pH of 9.0 (12.1 grams Tris, 0.9 grams boric acid, 1.6 grams $Na_2$ EDTA and distilled water to bring buffer volume to 2000 ml.) was used to suspend the hydrolyzed Connaught starch. A 0.12 M barbital buffer with a pH of 8.6 (24.7 grams sodium barbital, 3.4 grams diethylbarbituric acid and distilled water to bring buffer volume to 1200 ml.) was used as the electrode buffer. The power supply was set to deliver 250 to 300 volts with the milliamps not exceeding 50 and allowed to run 1½ to 2 hours. The gel was then cut to the desired size, removed from the tray and sliced with a cutter set at 4.0 mm. depth. The two halves were separated and stained with aniline stain.

Lambs were weighed at 70-days of age to determine production of the ewes with each hemoglobin type. Ewes were weighed at two-month intervals to determine if weight differences are present between hemoglobin types.

# RESULTS

## Resistance of Sheep to *Haemonchus Contortus*

The deviations in the number of ewes per breed-resistance group are shown in Table 4. Death losses

TABLE 4

Deviations in the Number of Ewes and Death Losses by Resistance Groups

| | Florida Native | | Rambouillet | |
|---|---|---|---|---|
| Dates | High | Low | High | Low |
| | Number of ewes | | | |
| August, 1966 | 60 | 60 | 31 | 31 |
| October, 1966* | 60 | 60 | 26 | 30 |
| February, 1967 | 58 | 59 | 22 | 29 |
| April, 1967 | 56 | 57 | 19 | 28 |
| Replacement ewes added | 4 | 4 | 3 | 5 |
| June, 1967 | 59 | 58 | 21 | 29 |
| August, 1967* | 59 | 58 | 15 | 34 |
| October, 1967 | 59 | 58 | 15 | 27 |
| February, 1968 | 59 | 58 | 14 | 24 |
| | Death losses | | | |
| October, 1966 | 0 | 0 | 3 | 3 |
| February, 1967 | 2 | 1 | 4 | 1 |
| April, 1967 | 2 | 2 | 3 | 1 |
| June, 1967 | 1 | 3 | 1 | 4 |
| August, 1967 | 0 | 0 | 0 | 1 |
| October, 1967 | 0 | 0 | 0 | 7 |
| February, 1968 | 0 | 0 | 1 | 3 |
| Total death loss | 5 | 6 | 12 | 20 |

*Reallotment of Rambouillet ewes

reduced the number of ewes in each group below the number described in the basic design of the study. Forty-six percent of the Rambouillet ewes died during the two years, while only nine percent of the Florida Native ewes died during the same period of time. Fourteen Rambouillet ewes had survived without anthelmintic treatment.

## Immunology and Hematology

Mean packed cell volumes and hemoglobin levels of the ewe flock obtained at two-month intervals are shown in Table 5. The Florida Native ewes maintained higher levels in both values than the Rambouillet ewes. Within breed, the high resistance group levels were higher than the low resistance group levels. The Rambouillet ewes in the high resistance group were anemic for the last two sample periods.

Mean packed cell volumes, hemoglobin levels and total gamma globulins of the sample ewes are shown in Table 6. Breed and resistance group differences in packed cell volumes and hemoglobin levels similar to these for the whole flock were observed. Florida Native ewes had higher total gamma globulin levels than the Rambouillet ewes, indicating a positive correlation between resistance and total gamma globulins. Within breeds, the low resistance groups had higher total gamma globulin levels than the high resistance groups, indicating a negative correlation between resistance and total gamma globulins. A non-significant, positive relationship (0.02) was observed between total gamma

## TABLE 5

### Mean Packed Cell Volumes and Hemoglobin Levels of the Ewe Flock

| Dates | Florida Native | | Rambouillet | |
|---|---|---|---|---|
| | High | Low | High | Low |
| | Packed cell volume (%) | | | |
| October, 1966 | 34.8 | 31.4 | 31.3 | 25.4 |
| December, 1966 | 31.7 | 29.2 | 26.4 | 25.3 |
| February, 1967 | 23.9 | 27.0 | 25.4 | 24.7 |
| April, 1967 | 27.8 | 26.2 | 25.9 | 25.9 |
| June, 1967 | 28.5 | 26.0 | 24.4 | 21.9 |
| August, 1967 | 31.1 | 29.0 | 28.4 | 24.6 |
| October, 1967 | 32.7 | 29.7 | 27.8 | 22.6 |
| December, 1967 | 29.9 | 27.7 | 22.4 | 23.4 |
| February, 1968 | 29.4 | 26.0 | 20.4 | 25.8 |
| | Hemoglobin level (g./100 ml.) | | | |
| August, 1966 | 8.45 | 7.52 | 7.85 | 7.28 |
| October, 1966 | 9.56 | 8.00 | 8.33 | 5.77 |
| December, 1966 | 9.03 | 8.15 | 7.13 | 6.91 |
| February, 1967 | 7.17 | 6.93 | 6.13 | 5.95 |
| April, 1967 | 8.23 | 7.85 | 7.40 | 7.39 |
| June, 1967 | 8.29 | 7.40 | 6.85 | 5.98 |
| August, 1967 | 9.01 | 8.29 | 8.15 | 7.00 |
| October, 1967 | 7.64 | 6.94 | 5.88 | 5.10 |
| December, 1967 | 6.78 | 6.37 | 4.04 | 4.18 |
| February, 1968 | 6.72 | 6.04 | 4.54 | 5.99 |

### TABLE 6

### Mean Packed Cell Volumes, Hemoglobin Levels and Total Gamma Globulins of the "Sample Flock"

| Month | Florida Native | | Rambouillet | |
|---|---|---|---|---|
| | High | Low | High | Low |
| | Packed cell volume (%) | | | |
| December | 28.4 | 25.8 | 22.8 | 20.4 |
| January | 26.6 | 22.3 | 21.8 | 15.5 |
| February | 28.4 | 24.9 | 22.5 | 15.3 |
| March | 27.3 | 24.7 | 20.8 | 20.1 |
| April | 26.7 | 22.8 | 22.3 | 21.6 |
| May | 27.2 | 24.4 | 24.7 | 21.9 |
| June | 27.4 | 24.2 | 23.8 | 22.1 |
| July | 27.9 | 25.2 | 30.7 | 24.6 |
| August | 28.8 | 27.6 | 29.8 | 23.8 |
| September | 28.4 | 26.3 | 28.5 | 24.2 |
| October | 31.5 | 27.5 | 28.7 | 24.6 |
| November | 31.8 | 26.6 | 28.8 | 22.7 |
| | Hemoglobin level (g./100 ml.) | | | |
| December | 9.6 | 8.6 | 6.8 | 5.7 |
| January | 9.2 | 7.4 | 7.0 | 5.3 |
| February | 10.0 | 9.5 | 8.3 | 5.1 |
| March | 10.2 | 9.1 | 6.7 | 6.0 |
| April | 10.1 | 8.7 | 8.1 | 7.8 |
| May | 9.5 | 8.6 | 8.2 | 7.8 |
| June | 9.2 | 8.1 | 7.3 | 6.9 |
| July | 8.8 | 8.2 | 10.4 | 8.1 |
| August | 10.7 | 7.5 | 9.7 | 7.4 |
| September | 9.5 | 8.6 | 9.3 | 7.6 |
| October | 10.4 | 8.5 | 8.4 | 7.8 |
| November | 10.4 | 8.6 | 9.9 | 7.5 |
| | Total gamma globulins (g./100 ml.) | | | |
| December | 0.81 | 1.02 | 1.12 | 1.28 |
| January | 1.29 | 1.69 | 1.04 | 1.17 |
| February | 1.95 | 2.28 | 1.45 | 1.60 |
| March | 2.16 | 2.28 | 1.65 | 2.01 |
| April | 2.04 | 2.27 | 1.98 | 1.98 |
| May | 2.20 | 2.25 | 1.97 | 1.99 |
| June | 1.91 | 2.23 | 1.81 | 1.54 |
| July | 2.23 | 2.07 | 1.57 | 1.96 |
| August | 2.36 | 2.23 | 2.02 | 2.07 |
| September | 1.62 | 1.95 | 1.42 | 1.60 |
| October | 1.80 | 2.08 | 1.45 | 1.54 |
| November | 1.64 | 1.78 | 1.18 | 1.39 |

globulins and hemoglobin levels.

Parasitology

Mean H. contortus ova counts, taken in July and September, 1966, to determine the effectiveness of selection, are shown in Table 7. While large breed differences in the means exist, within breed resistance group differences were generally small. No helminth ova were observed in the samples from a large percent of the ewes in all groups. Over ninety percent of the Florida Native ewes had counts of less than 200 ova per gram of feces. The Rambouillet high and low resistance groups had 53.8 and 36.7 percent of the ewes, respectively, with counts of less than 200 ova per gram of feces. Only

TABLE 7

Helminth Ova Counts from Ewe Flock*

| | Florida Native | | Rambouillet | |
|---|---|---|---|---|
| | High | Low | High | Low |
| Mean ova per gram of feces | | | | |
| July, 1966 | 93 | 42 | 534 | 577 |
| September, 1966 | 80 | 163 | 1288 | 3310 |
| Percent samples with less than 200 ova per gram of feces | 92.5 | 91.7 | 53.8 | 36.7 |
| Percent samples with more than 1,000 ova per gram of feces | 2.5 | 1.7 | 21.2 | 41.7 |

* >94% H. contortus

about two percent of the Florida Native ewes had counts of more than 1000 ova per gram of feces. The Rambouillet high and low resistance groups had 21.2 and 41.7 percent of the ewes, respectively, with counts of more than 1000 ova per gram of feces.

Breed differences in the mean ova counts of the "sample flock" can be divided into two periods (Table 8). During the first period, December through June, the ova counts were lower in the Florida Native ewes than in the Rambouillet ewes. During the second period, July through November, the differences were small. In the Florida Native flock, the high resistance group had lower ova counts than the low resistance group. A significant, negative relationship (-0.32, $P < 0.01$) was observed between ova counts and hemoglobin levels. Over 90 percent of the ova in the feces were identified by larval cultures as H. contortus.

Mean necropsy H. contortus counts obtained on ewes which died are shown in Table 9. The stomachs of two of the high resistance Florida Native ewes contained large amounts of sand. Several of the low resistance Rambouillet ewes were drenched with phenothiazine shortly before death. These factors reduced the mean counts of the respective groups.

Necropsy H. contortus counts for Florida Native ewes culled during each of the two months were very low (Table 9). The entire stomach contents were screened in January since the samples failed to show any worms. The mean counts from

## TABLE 8

### Helminth Ova Counts* And Larval Cultures from "Sample Flock"

| Month | Florida Native | | Rambouillet | |
|---|---|---|---|---|
| | High | Low | High | Low |
| | Ova per gram feces | | | |
| December | 1680 | 3070 | 6920 | 6220 |
| January | 2070 | 2710 | 6920 | 8660 |
| February | 167 | 344 | 9525 | 6400 |
| March | 50 | 511 | 3800 | 400 |
| April | 70 | 430 | 400 | 1467 |
| May | 40 | 390 | 1467 | 3175 |
| June | 388 | 588 | 900 | 1660 |
| July | 70 | 356 | 167 | 350 |
| August | 60 | 310 | 50 | 317 |
| September | 67 | 457 | 33 | 283 |
| October | 70 | 256 | 300 | 471 |
| November | 475 | 370 | 1000 | 960 |
| | Larval cultures (% *Haemonchus contortus*) | | | |
| December | 100 | 99 | 99 | 98 |
| January | 96 | 95 | 98 | 99 |
| February | 97 | 100 | 100 | 100 |
| March | 95 | 98 | 100 | 95 |
| April | 98 | 94 | 99 | 100 |
| May | 96 | 93 | 85 | 93 |
| June | 92 | 96 | 92 | 97 |
| July | 92 | 86 | 95 | 95 |
| August | 86 | 94 | 96 | 98 |
| September | 83 | 79 | 97 | 95 |
| October | 94 | 88 | 100 | 75 |
| November | 92 | 91 | 97 | 99 |

*>94% *H. contortus*

TABLE 9

Necropsy *Haemonchus contortus* Counts On Ewes Which Died and Culled Ewes

| | Florida Native | | Rambouillet | |
|---|---|---|---|---|
| | High | Low | High | Low |
| Ewes which died | | | | |
| Number sampled | 5 | 5 | 10 | 9 |
| Mean *H. contortus* counts | 10880 | 12500 | 12230 | 11311 |
| Culled ewes | | | | |
| January, 1968 | | | | |
| Number sampled | 4 | 4 | | |
| Mean *H. contortus* counts | 5 | 4 | | |
| February, 1968* | | | | |
| Number sampled | 6 | 6 | | |
| Mean *H. contortus* counts | 283 | 550 | | |

*Many immature worms present

the ewes culled in February were higher than those culled in January. Many of the worms found in February were immature worms. Both groups of cull ewes, however, had much lower mean *H. contortus* counts than the ewes which had died. Two ewes slaughtered in February were very anemic (hemoglobin levels of 3.8 and 4.4 grams percent) but had only 1400 and 2100 *H. contortus*, respectively, at necropsy. While these were the two highest counts, they were considerably lower than the counts from ewes which had died.

## Hemoglobin Types of Sheep

Hemoglobin types were determined on blood samples from the 30 sample ewes (Table 10). A greater incidence of the Hb A gene was observed in the Florida Native ewes than in the Rambouillet ewes. No Hb A ewes were found in the Rambouillet sample group. There was a trend toward greater incidence of the Hb A gene in the high resistance groups than in the low resistance groups. Hb C was found in one very anemic Rambouillet ewe, along with Hb AB.

TABLE 10

Hemoglobin Types of
"Sample Flock" by Resistance Groups

| Breed | Resistance Group | Hemoglobin Type A | AB | B | ABC |
|---|---|---|---|---|---|
| Florida Native | High | 2 | 7 | 1 | - |
| | Low | 3 | 3 | 4 | - |
| Rambouillet | High | - | 1 | 3 | 1 |
| | Low | - | 1 | 4 | - |

Figure 1 is a photograph which shows the various hemoglobin types observed in the sheep flock. The samples migrated for two hours on a power source delivering 290 volts and 48 milliamps, and were stained with aniline stain. The origin of the migrations is at the top of the starch gel. The Hb A fraction migrated the greatest distance from the origin, the Hb C fraction migrated

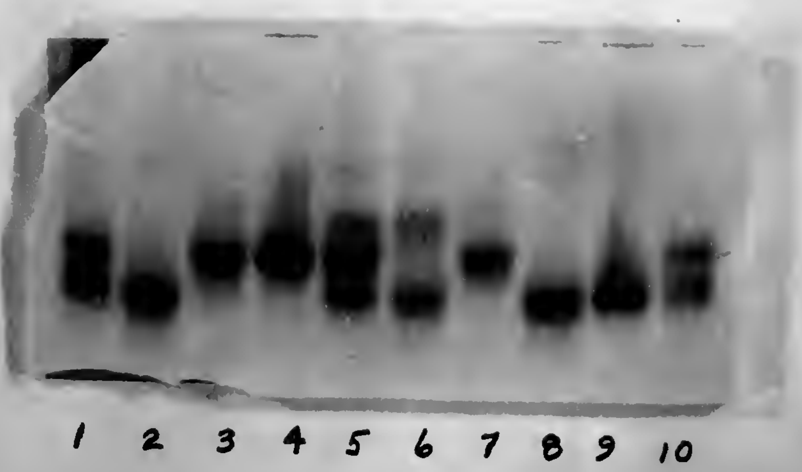

Figure 1. Starch gel showing hemoglobin types found in Florida sheep

the shortest distance from the origin and the Hb B fraction migrated the intermediate distance from the origin.

Samples 1 and 10, from six-month old Florida Native lambs, were Hb AB. The dam of the first lamb had Hb A (sample 2). Another dam-daughter pair (samples 3 and 4, respectively) had Hb B. Hb C was found in only two sheep; the Rambouillet ewe mentioned above with Hb ABC (sample 5), and a 10-year old Florida Native ewe with Hb AC (sample 6). A Rambouillet ewe had Hb B (sample 7) and a Florida Native dam-daughter pair (samples 8 and 9, respectively) had Hb A. Two of the lambs (samples 4 and 9) are from one-month old lambs. No Hb F was present in either sample. The stained masses above the hemoglobin fractions are impurities that were not removed during preparation.

The number of ewes per hemoglobin type in each resistance group are shown in Table 11. Breed differences for hemoglobin type were large and highly significant. No Hb A ewes were present in the Rambouillet flock, while 39 percent were present in the Florida Native flock. The gene frequencies for Hb A were 0.190 for the Rambouillet ewes and 0.558 for the Florida Native ewes.

It was observed that the aged Florida Native ewes (9-, 10- and 11-years of age) had a high incidence of Hb A but 15 of the 18 ewes were in the low resistance group. The assumption was made that ewes which live to this age must have some resistance and, therefore, the low

TABLE 11

Hemoglobin Type of
Ewe Flock by Resistance Groups

| Breed | Resistance Group | Hemoglobin Type | | | | |
|---|---|---|---|---|---|---|
| | | A | AB | B | AC | ABC |
| Florida Native (less than 9-years of age) | High | 21 | 24 | 11 | - | - |
| | Low | 14 | 14 | 18 | - | - |
| Florida Native (9-, 10- and 11-years of age) | High | 2 | 1 | - | - | - |
| | Low | 8 | 3 | 3 | 1 | - |
| | gene frequency for Hb A | | 0.558 | | | |
| Rambouillet | High | - | 6 | 9 | - | 1 |
| | Low | - | 12 | 22 | - | - |
| | gene frequency for Hb A | | 0.190 | | | |

hemoglobin percents may be due to some aspect of aging. An examination of the teeth of several of these ewes revealed very poor, or almost complete absence of teeth. Because of these facts, the Florida Native ewes were classified into two age groups.

Chi squared analysis (Snedecor, 1962) of the differences between high and low groups within the Florida Native ewes (less than 9-years old) approached significance at the 5 percent level. Hb A was more prevalent in the high resistance group and Hb B was more prevalent in the low resistance group.

The effects of hemoglobin type on hemoglobin level, packed cell volume and weight of the ewes are shown in

Table 12. Hemoglobin type had a highly significant effect on hemoglobin level and packed cell volume. Ewes with Hb A had higher mean hemoglobin levels and packed cell volumes than ewes with Hb B (Table 13). Differences between ewes with the Hb A and ewes with Hb Ab were small. The effect of hemoglobin type on the weight of the ewes (Table 12) was not significant.

TABLE 12

Least Squares Analyses for Hemoglobin Level, Packed Cell Volume and Body Weight of Ewes

| Source | d.f. | Hemoglobin Level | Packed Cell Volume | Weight of Ewes |
|---|---|---|---|---|
| Total | 701 | | | |
| Hb type (T) | 2 | 21.14** | 396.41** | 338.53 |
| Season (S) | 5 | 65.29** | 287.86** | 9185.64** |
| T x S | 10 | 1.72* | 6.17 | 36.55 |
| Animal (A) | 116 | | | |
| A:T | 114 | 3.46** | 48.01** | 412.06** |
| AS:T | 570 | .75 | 6.35 | 32.09 |

$^*P < 0.05$

$^{**}P < 0.01$

Season had a highly significant effect on all three traits (Table 12). The hemoglobin type by season interaction was significant only for hemoglobin level. Theoretically no good error term was present to test the sig-

## TABLE 13

Mean Hemoglobin Level, Packed Cell Volumes and Weights of the Ewes by Hemoglobin Type of the Ewes

| Sample month (season) | Hemoglobin Type A | Hemoglobin Type AB | Hemoglobin Type B |
|---|---|---|---|
| Hemoglobin level (g./100 ml.) | | | |
| February | 7.50 | 7.08 | 7.02 |
| April | 8.60 | 8.04 | 7.59 |
| June | 7.92 | 8.05 | 7.49 |
| August | 8.84 | 8.90 | 8.07 |
| October | 7.29 | 7.56 | 6.98 |
| December | 6.60 | 6.79 | 6.15 |
| Average | 7.79 | 7.74 | 7.22 |
| Packed cell volume (%) | | | |
| February | 29.7 | 28.2 | 27.4 |
| April | 28.6 | 27.4 | 25.4 |
| June | 28.1 | 27.5 | 25.7 |
| August | 31.0 | 30.8 | 28.3 |
| October | 31.9 | 32.0 | 29.1 |
| December | 29.5 | 29.1 | 27.2 |
| Average | 29.8 | 29.2 | 27.2 |
| Weight of the ewes (pounds) | | | |
| February | 89.5 | 92.1 | 92.3 |
| April | 79.3 | 80.6 | 80.7 |
| June | 81.7 | 83.0 | 82.9 |
| August | 85.9 | 88.8 | 87.5 |
| October | 90.9 | 94.0 | 90.8 |
| December | 104.6 | 107.0 | 103.1 |
| Average | 88.7 | 90.9 | 89.6 |

nificance of the mean squares for animals within hemoglobin type (A:T). The best estimate (Henderson, 1960) was the mean square for animal X season within hemoglobin type (AS:T). The ratios of these two variances were large, clearly demonstrating a significant difference between animals of the same group.

The relationship between hemoglobin type and reproductive performance of Florida Native ewes is shown in Table 14. Ewes with Hb B had a higher percent of twin births than ewes with Hb A, although Chi squared analysis did not show the difference to be significant. Ewes with Hb A had the highest percent of single births. Ewes with Hb B had the lowest percent barren ewes in 1967, while ewes with Hb A had the lowest percent barren ewes in 1968.

TABLE 14

The Relationship Between Hemoglobin Type and Reproductive Performance of Florida Native Ewes

| Reproductive Performance of ewes | Lamb Crop | Hemoglobin Type A | AB | B |
|---|---|---|---|---|
| barren ewes (%) | 1967 | 4.8 | 7.3 | 3.3 |
| | 1968 | 20.9 | 26.2 | 25.8 |
| | Total | 12.9 | 16.9 | 14.7 |
| single lamb (%) | 1967 | 83.3 | 75.6 | 70.0 |
| | 1968 | 69.8 | 64.3 | 61.3 |
| | Total | 76.5 | 69.9 | 65.6 |
| twin lambs (%) | 1967 | 11.9 | 17.1 | 26.7 |
| | 1968 | 9.3 | 9.5 | 12.9 |
| | Total | 10.6 | 13.2 | 19.7 |

29 | $\frac{2}{14.5}$

Least squares constants of 70-day weights of Florida Native lambs are shown in Table 15. Type of birth had a highly significant effect on the 70-day weight of the lambs. Lambs from single births were 6.03 pounds heavier than lambs from twin births. Neither hemoglobin type of the ewe or of the lamb had significant effects on the 70-day weights of the lambs.

TABLE 15

Least Squares Constants for 70-day Weight (Pounds) by Type of Birth, Hemoglobin Type of Dam and Lamb

| Variable | 70-day Weight | Variable | 70-day Weight |
|---|---|---|---|
| | | Type of Birth** | |
| General Mean | 32.43 | single lambs | 3.015 |
| | | twin lambs | -3.015 |
| Hemoglobin type of the lamb | | Hemoglobin type of the dams | |
| Hb A | -0.235 | Hb A | -0.03 |
| Hb AB | -0.745 | Hb AB | 1.34 |
| Hb B | 1.905 | Hb B | -1.31 |
| N.D.* | -0.925 | | |

*Lambs disposed of before blood samples could be taken for hemoglobin type determination

**$p < 0.01$

# DISCUSSION

## Resistance of Sheep to *Haemonchus contortus*

Death loss in the Rambouillet ewes was higher than in the Florida Native ewes even though many of the Rambouillet ewes were drenched with phenothiazine and none of the Florida Native ewes were drenched. Fourteen Rambouillet high resistance ewes had survived without anthelmintic treatment. The fact that some Rambouillet ewes had survived without anthelmintic treatment would indicate that variability in resistance level is present in the Rambouillet ewes.

## Immunology and Hematology

The packed cell volume and hemoglobin level within animals showed a large amount of fluctuation. This fluctuation may be caused by the "self-cure" phenomenon as reported by Stoll (1929).

Large breed differences were present in the packed cell volume and hemoglobin level data. These results agree with the results of Loggins *et al*. (1965) in which Florida Native ewes had higher hemoglobin levels than Rambouillet ewes. Holman (1944) and Becker and Smith (1950) observed no significant breed differences for packed cell volumes or hemoglobin levels. The breeds used in the present study and by Loggins *et al*. (1965) were different in their adaptability to Florida conditions. The Florida Native breed was developed by natural selection under Florida production conditions (Jilek, 1966). The Rambouillet breed was imported from Texas and Alabama and was not well adapted to Florida production conditions. The breed differences would then appear to be the result of differences in parasitic burdens.

The gamma globulin fractions of blood serum are generally associated with an immune response. The results, obtained in this study, which show that total gamma globulin levels were not associated with hemoglobin levels, would suggest either that there was no immune response present or that relative increases and decreases of specific fractions of gamma globulins were associated with immunity.

Parasitology

Within animal observations on ova counts showed a large amount of fluctuation during the course of this study. The decrease in ova counts was more rapid than the increase in packed cell volume or hemoglobin level and these results also suggest that a "self-cure" phenomenon was operating.

The highly significant, negative relationship between hemoglobin level and ova counts observed in this study is not in agreement with the observations of Kingsbury (1965), in which no relationship was observed. Ewes with very high ova counts had low hemoglobin levels. These extreme values increased the magnitude of the negative relationship. Ewes with low ova counts did not necessarily have high hemoglobin levels.

Mean necropsy worm counts from the Florida Native ewes which had died (11, 690 H. contortus) were much larger than the mean necropsy worm counts of the culled ewes which were sacrificed (250 H. contortus). This would indicate that necropsy count on ewes which die is not a good measure of the parasitic load of the flock. The worm burden of very anemic ewes would seem to increase greatly just before death. The precarious existence of the parasites may necessitate this increase prior to the death of a host.

There are two possible explanations for the lower necropsy worm counts in January than in February. First, the ewes culled in January were barren ewes or ewes which lost their lambs at an early age. These ewes were not stressed from parturition and lactation as were the lactating ewes. Two of the ewes which were sacrificed in January were anemic. However, the necropsy worm counts on these ewes were low. Second, "self-cure" may have reduced the number of worms in the ewes just prior to the sacrificing of the ewes in January. The number of immature worms present in the ewes sacrificed in February would indicate that "self-cure" had occurred and new infections were developing.

## Hemoglobin Types of Sheep

"Self-cure," a dynamic cyclic phenomenon, influences the hematological and parasitological values of a sheep. If resistance is measured by one of these parameters, the level of resistance will be influenced by the particular phase of the "self-cure" cycle of the animal. An animal sampled just prior to expulsion of the worms may therefore appear to have very little resistance to H. contortus since hematological measurements will be low and parasitological measurements will be high. On the other hand, the same animal sampled just prior to establishment of a new infection may appear to have a high resistance to H. contortus since hematological measurements will be high and parasitological measurements will be low.

One solution to this problem is to select a discrete variable which is correlated with resistance levels as the parameter for estimating resistance. The discrete variable examined in this investigation is hemoglobin type. The results of Helm et al. (1957) and Huisman et al. (1958) indicate that the hemoglobin types are genetically determined in a simple Mendelian manner. The only reported changes in an animal's adult hemoglobin type have involved the production of Hb C in very anemic sheep having Hb A (Blunt and Evans, 1963; Braend et al., 1964; Vliet and Huisman, 1964). If the hemoglobin types can be shown to be correlated with the resistance of sheep to H. contortus, the phase in the "self-cure" cycle of the animal when the sample is obtained will not influence the determination of the resistance levels.

The design of this experiment does not permit the determination of the inheritance of hemoglobin type. Multiple sires of different hemoglobin types were used in each breed group. Ewes with Hb A gave birth to lambs with either Hb A or AB and ewes with Hb B gave birth to lambs with either Hb B or AB. While the inheritance of hemoglobin type could not be studied in detail, no evidence was observed to refute the type of inheritance reported in the literature.

Hb C was observed in only two ewes, both of which also had Hb A. If the sheep had been sampled periodically through the year, it is possible that more sheep would have had some Hb C.

The facts that Florida Native ewes have a high frequency of Hb A and are adapted to Florida production conditions may indicate that Hb A sheep are adapted to the adverse conditions that are present in Florida. These results are in agreement with those of Evans and Blunt (1961) in which the frequency of Hb A or AB was higher under more adverse conditions than under less adverse conditions. Adaptation to Florida production conditions would include increased resistance to H. contortus infections.

The analyses of resistance group differences within the Florida Native breed and of the effect of hemoglobin type on hematological values indicate, likewise, that sheep with Hb A are more resistant to parasites than sheep with Hb B. Evans *et al.* (1963) reported that trends in ova counts and in worm counts *post mortem* at the height of the anemia induced by *H. contortus* suggest that an interaction between hemoglobin type and suceptibility to *H. contortus* may exist. The sheep with Hb A are the less susceptible sheep. Evans and Evans (1964) found that a close relationship exists between hemoglobin type and hematocrit values.

A large increase in the percent of barren ewes was observed in 1968 as compared to 1967. The percent ewes giving birth to twin lambs decreased in 1968 as compared to 1967. A loss of weight by the ewes prior to the 1968 lambing season may have caused this decrease in reproductive rates. The mean weight of the ewes was less than 90 pounds during the 1968 breeding season. Coop (1962) found that barrenness increased rapidly in ewes below 90 pound liveweight. Many of the ewes in the present study were below this critical weight.

The effect of hemoglobin type on production is mainly in the proportion of multiple births. The mean weight of the lambs from the Hb B ewes was lower than the mean weight of lambs from Hb A or AB ewes. Lambs born as twins weighed less at weaning than lambs born as singles (Shelton and Carpenter, 1957). While the individual lambs from Hb B ewes weighed less, there were more of them to increase the production per ewe bred over the Hb A and AB ewes. Evans and Turner (1965) observed that ewes of Hb A had fewer lambs born or weaned than those of Hb AB or B. Least squares analysis of the 70-day weights in 1967 showed that differences in the weights between the hemoglobin type groups was due mainly to the type of birth. The results of this study are in agreement with those of Evans and Turner (1965). The superiority of the Hb B ewes appears to be associated with the production or survival of lambs from multiple births.

# SUMMARY

This study was conducted over a two-year period using 60 Rambouillet and 120 Florida Native ewes. Each breed group was divided into high and low resistance groups using mean hemoglobin levels as indicators of resistance to _H_. _contortus_ infections.

Death loss was greater in the Rambouillet than in the Florida Native ewes. Only 14 of 70 (original allotment plus replacements) Rambouillet ewes survived without anthelmintic treatment.

Florida Native ewes were consistently higher in hemoglobin levels and packed cell volumes than Rambouillet ewes. Within breeds, the high resistance group was higher in both values than the low resistance group. A non-significant, positive relationship (0.02) was observed between total gamma globulins and hemoglobin levels.

Large breed differences were observed in mean _H_. _contortus_ ova counts. However, a large percent of the ewes in both breed groups had very low ova counts. A significant, negative relationship (-0.32, $P < 0.01$) was observed between ova counts and hemoglobin levels. This significant relationship can be explained by the very anemic condition of ewes with very high ova counts. Over 90 percent of the ova in the feces were identified by larval cultures as

*H. contortus*.

The mean necropsy worm count on ewes which died was 11,690 *H. contortus*. Breed differences for necropsy counts on ewes which died were small. The mean necropsy worm count on culled Florida Native ewes was only 250 *H. contortus*, indicating that the necropsy counts on ewes which died were not good measures of the parasitic burden of the flock.

A higher incidence of the Hb A gene was observed in the Florida Native breed and in the high resistance groups than in the Rambouillet breed and low resistance groups, respectively. Resistance group differences within the Florida Native breed approached significance at the five percent level.

Hemoglobin type had a highly significant effect on hemoglobin level and packed cell volume. Ewes with Hb A or AB had higher-mean hemoglobin levels and packed cell volumes than ewes with Hb B.

The effect of hemoglobin type of production was mainly in the proportion of multiple births. Twinning percent was higher in Florida Native ewes with Hb B than in Florida Native ewes with Hb A.

These results would indicate that ewes with Hb A may be more resistant to parasitic infections with *H. contortus* than with ewes with Hb B. Ewes with Hb B, however, may be more prolific and have greater production per ewe than ewes with Hb A.

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# BIOGRAPHICAL SKETCH

Anthony Francis Jilek was born July 11, 1942, in Barron County, Wisconsin. In May, 1960, he was graduated from Rice Lake High School, Rice Lake, Wisconsin. He attended Wisconsin State College, River Falls, from which he received the degree of Bachelor of Science in Agricultural Education in June, 1964.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1968

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