Haemonehus Baspanosus (Molin, 1860).

Railliet and Henry, 1909, with Special Reference to the Effect of Hydrocortisone Administration on Natural And Acquired Resistance in Kids

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Thesis

Submitted to the Faculty of Veterinary Science
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR
in partial fulfilment of the requirements
for the degree of
MASTER OF SCIENCE (VETERINARY)
1971

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March 17th, 1972.

Certified that the thesis, entitled "STUDIES ON HAEMONCHUS BISPINOSUS (MOLIN, 1860) RAILLIET AND HENRY, 1909, WITH SPECIAL REFERENCE TO THE EFFECT OF HYDROCORTISONE ADMINISTRATION ON NATURAL AND ACQUIRED RESISTANCE IN KIDS" embodies the results of work actually carried out by Dr. Ajit Kumar Sinha, B.V.Sc.& A.H., under my supervision and guidance for the award of the degree of Master of Science (Veterinary-Parasitology) of the Rajendra Agricultural University, Bihar.

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(B.N.Sahai)

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shelter the author could carry out his study smoothly.

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INTRODUCTION

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INTRODUCTION

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INTRODUCTION

Haemonchus contortus is often responsible for undermining the health of these animals. Martin and Ross (1934) has estimated that 2000 females of this species suck a minimum of 29 cmm. of blood per day from the host. The minimum withdrawal of blood by H. contortus is itself a sufficient proof to explain the severe anaemia, progressive debility and other pathogenic effects caused by them.

For a long period haemonchosis in sheep and goats was thought to mean a predominent infection with <u>H. contortus</u>. The infection was considered to be of immense importance in general Animal Husbandry practice, in view of its blood sucking habits. Although economic loss by these worms alone is not specified, it has been estimated that normal burden of parasites were responsible to reduce growth rate upto 30 percent (Soulsby, 1963).

Sahai and Deo (1964) for the first time described yet another species, Haemonchus bispinosus Molin, 1860 from Indian sheep and goats. The parasite was originally reported from Mazama nana in Brazil. Although, extensive studies have been done on various aspects of Haemonchus contortus, no work has been done on H. bispinosus except on morphology of adult and larval stages (Sahai & Deo, 1964; Dutt & Sahai, 1966; Sahai, 1966; Padmavathi et al, 1971).

It is fairly known that administration of cortisone

reduces immunogenesis in animals in various helminthic infections (Dhar & Singh, 1970). Since cortisone has been adopted as a common drug in clinical practice, it is of interest to know the influence of hydrocortisone on immunity due to H. bispinosus infection in goats.

With the objective in view, studies have been designed to determine the effect of hydrocortisone on natural and acquired resistance of <u>Haemonchus bispinosus</u> infection in kids, to establish pure infection of <u>H. bispinosus</u> and <u>H. contortus</u> for their specific identity and also a new indigenous compound "Wopell" was tested for its anthelmintic value against haemonchosis in goats. These researches have been carried out in the Department of Parasitology, Bihar Veterinary College, Rajendra Agricultural University, Bihar, Patna and results have been presented in this thesis in partial fulfilment for the requirements of M.Sc.(Vet.) degree of the Rajendra Agricultural University, Bihar.

MATERIALS AND METHODS

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MATERIALS AND METHODS

Source and collection of parasites:

For the purpose of the survey of helminth parasites of goats, the materials were collected from goats sacrificed in the Department of Biological products, Livestock Research Station, Bihar, Patna; from the goats slaughtered in villages near by the Bihar Veterinary College, Patna and from slaughter houses of Bankipur, Patna, Phulwarisharif, Dinapur and near by places. All their visceral organs and other tissues were carefully aexamined for the presence of helminths and their larvae. The large flukes, roundworms and tapeworms were isolated and preserved immediately after collection, but other small worms were collected from the intestinal contents preserved and stored for subsequent examination.

The methods adopted for collection were the same as are commonly employed for collection of helminths (Sahai 1960, 1967). In cases, where mucus were found to be attached to worms, the same was removed by adding 1% caustic soda to the normal saline as suggested by Sahai (1967).

Fixation and preservation:

Some specimens of the tapeworms and trematodes were flattened and fixed for whole mounts.

Fixatives used were hot solution of formalin or

steaming 70% alcohol. After 2 to 3 hours the parasites were transferred to a fresh fixative solution.

Staining and mounting :

The stains used for whole mount preparations of trematodes and cestodes were acetic alum carmine and borax carmine prepared and used in the usual manner. The worms were either mounted in D.P.X. or Canada balsam.

Clearing for Microscopical Examination :

For microcopical examination of the preserved nematodes, it was essential to clear them. The following media were used for clearing the parasites.

Beechwood creosote.

As its refractive index is quite high and the small specimens became too much transparent, only large and medium sized nematodes were cleared in this chemical. The parasites were transferred to this medium directly from the preservatives. It was observed that there was much shrinkage of the parasites just after putting them in the clearing agent, but after two to three hours they bulged out to their natural size owing to the penetration of the clearing agent and became transparent. The results were quite satisfactory for large round worms.

Lactophenol.

This clearing agent was prepared on lines suggested

by Taylor (1935) as follows.

Carbolic acid. 20 ml.

Lactic acid. 20 ml.

Glycerine. 40 ml.

Distilled water. 20 ml.

Lactophenol has got the property of softening the cuticle and counteracting the shrinkage. It was used for small and medium sized worms. The parasites were transferred to the lactophenol directly from preservative. It was found that there was voilent shrinkage in the beginning but after twelve hours the specimens plumped out to their normal size. It gave quite satisfactory results for morphological study.

Glycerine-Alcohol.

This clearing agent also gave satisfactory results for smaller nematodes. The mixture was prepared by mixing glycerine and 70% alcohol in the ratio of 1:19. The specimens preserved in 70% alcohol were transferred to this clearing agent and the alcohol was allowed to evaporate. After 5 to 6 days, the specimens became transparent. This reagent was best used for clearing the larval stages of <u>Haemonchus</u> species.

Preparation of cultures.

Water culture.

The gravid worms were macerated on a slide in a drop

of water, and the eggs, thus, recovered were sieved in a petridish, containing tap water to which a few drops of 2% formalin was added. Regular studies on the development of egg were made. The water was changed daily by pipette and fresh water with a few drops of 2% formalin was substituted. The water was areated daily atleast once. The culture was maintained at room temperature (Sahai, 1960).

Faecal culture.

Sahai (1960, 1965) has devised a simple method for faecal culture on the principle of Vaidyanathan's (1943) technique and Whitlock's (1956) inner tube method. In the present study the method described by Sahai (1965) was adopted which is as follows :- The pellets of the faeces were sterlized in hot air oven, for 48 hours. The dried pellets were transferred to a small petridish and freshly teased eggs implanted on their surface. This petridish was then kept in another bigger petridish containing water. The water in between the two dishes served as a moat. The outer dish was covered with another dish of equal size, to avoid dirt particles. The infective larvae climbed up the wall of smaller petridish and migrated into the water out side. The apparatus devised, served as a moist chamber, and moisture needed for the development of larvae was automatically controlled. The larvae took about 6 days to complete development and migration. These larvae were free from debris and could be collected easily.

Charcoal faeces culture.

This culture was prepared in the laboratory by mixing about 3 parts of sterlized powdered faeces with 2 parts of bone charcoal powder. Enough mixture was put to fill one third of jar. This was moistened by enough water to maintain sufficient humidity for the development of larvae. The eggs of <u>Haemonchus</u> spp. were implanted on the surface of the mixed charcoal faeces powder and the jar was covered with a lid. The culture was kept in the laboratory temperature.

It was found that in such cultures the larvae developed to the infective stage in about one weeks time. The infected larvae could be located or spotted in the condensed dew drops of moisture on the inner surface of the walls of the jar, where they had migrated. As mentioned in faecal culture method this is also based on the migratory habits of the infective larvae of the parasites and has been found to give satisfactory results in the present investigation. This method was also adopted by previous workers (Looss, 1911; Darling, 1911; Fulleborn, 1921; Africa, 1931; Sahai, 1960).

Collection of infective larvae.

For studying the larval stages and dosing the experimental animals, the infective larvae were collected in water from the faeces charcoal culture. Precautions were taken to recover the larvae without contamination from the culture. For this, the sides of the jar were repeatedly washed with water by

help of a brush and the contents were poured in a beaker.

Standardisation of dose.

To standardise the dose of infective larvae to be given to the experimental animals. The following method was adopted. The infective larvae were collected in water in a 250 ml. beaker. After a thorough stirring, one ml. of this water was taken on a slide and examined for the presence of larvae. The number of larvae present in 1 ml. of water was then counted under a bionocular microscope. Three such samples from the homogenous solution of water were taken and the larvae were counted. The average of three such counts multiplied by the total volume of the water gave the total number of infective larvae contained in the dose to be administered (Sahai, 1960).

Mode of infection.

Requisite doses of the infective larvae were administered to the experimental animals by the standard method of drenching. Sufficient care was taken to avoid spilling of the infective dose by the animals.

Experimental animals and their maintenance.

The experimental animals used for infection were two months old kids supplied by contractor. They were maintained under parasite free conditions throughout experimental studies.

Each of the kid was given 4 ounces and 2 ounces of the concentrates mixture daily in the morning and evening respectively.

In addition, 3 pounds of green leaves were provided to each of them. They were also given ½ lb. of milk in morning and evening. All the kids were muzzled throughout the experimental period except when they were given feed and water.

Examination of faeces.

Starting from the 10th day post infection, the faecal samples of the infected kids were examined every day as per method described by Sheather (1923) later adopted by Bhalerao (1935) to detect the presence of eggs in the faeces. For quantitative examination Stoll's (1930) egg count technique as modified by Faust (1949) was employed.

Measurements.

The measurements of the eggs and the adult worms were taken with the help of an eye piece micrometer. The measurements given refer to the range shown by 25 specimens and averages are given in the parentheses.

HAEMATOLOGICAL TECHNIQUE

Blood Cell Count.

(a) The following fluids were used for the blood cell count.

Diluting fluids.

I. For Erythrocytes

Gower's solution :-

Sodium Sulphate - 12.5 grams.

Glacial acetic acid - 33.3 ml.

Distilled water - Upto 200 ml.

II. For Leutocytes

Acetic acid solution:-

Glacial acetic acid - 4 ml.

Distilled water - 100 ml.

Preparing the samples for cell count.

The haemocytometer pipette was filled upto the desired mark with the oxalated blood. The tip of the pipette was cleared and it was then inserted into the diluting fluid. Care was taken to avoid air bubbles, while pipetting the diluting fluid in the pipette. The diluted blood was thoroughly shaken for about 2 minutes by holding it between the thumb and the forefinger of either hand.

Red Cell Count.

The R.B.C. count was usually made from blood diluted 200 times. The pipette was filled with blood upto 0.5 mark and the diluting fluid drawn upto the 101 mark. The counting chamber

was covered with clean cover slip. The pipette was shaken thoroughly and the clear diluting fluid from the capillary part of the tube was blown out. A drop of blood was made to trickle in between the coverslip and grooved area of the slide. Overflow of the diluted blood was checked. Diluted blood was shaken properly to make the fluid homogenous. Before the examination of the preparation it was allowed to stand atleast two minutes. The slide was examined under low power microscope to find out whether the cells were eventy distributed. Again the counting was done under high power (40x) microscope.

The R.B.C. located in four corners and in one central square were counted and the calculation of the number of R.B.C. per cubic millimeter of blood was made with following formula:-

Number of R.B.C./cmm =

Number of cell counted x dilution x 4000 Number of small squares counted

To avoid counting of the same cells twice the corpuscles on the top and left lines of the squares were counted.

White Cell Count &

The procedure for this is the same as for the R.B.C. count. The pipette with White bead was used and the blood was drawn upto the 0.5 mark. It was then filled with the diluting fluid upto 11 mark. The pipette was shaken thoroughly and the counting chamber was filled as described previously.

The cells present in four corner squares in the

haemocytometer were counted under a low power (10x) microscope. The white cells were clearly evident by their refractile appearance, and by the slight colour given to them by the diluting fluid.

The counting of cells were the same as before. To find out the number of W.B.C./cubic millimeter of blood, the total number of counted cells were multiplied by 50. The number of W.B.C./cmm was then calculated by the following formulla.

Number of W.B.C./cmm =

W.B.C. counted x dilution x 10

Number of squares counted.

Differential count of leucocytes.

Preparation of stained blood film.

A thin blood film was made on the slide. The slide was flooded with methyl alcohol for five minutes. The methyl alcohol was poured out and was again flooded with dilute Giemsa stain. The stain was diluted with distilled water in the ratio of 1:10 and allowed to stand on the film for 20-30 minutes. The slide was then washed in running tap water. After washing, the slide was kept in between the folds of a filter paper for drying.

Examination of stained blood film.

The slide was examined under a low power (10x) microscope to see whether the film was homogenously stained. It was

then examined under an oil immersion (100x) objective in a drop of microscopic oil. During the examination of slides first 50 cells were studied from each edge and the percentage of different cells counted was recorded. The cells typed in the blood studies were lymphocytes, monocytes, neutrophils, and eosinophils.

Cell volume determination.

The haematocrit tube was filled upto the 10th mark with the oxalated blood with the help of capillary pipette.

The tube was then centrifuged at 3000 r.p.m. for about 40 minutes. The percentage of cell volume was determined by multiplying the compact volume of the red cell by 10.

Determination of Haemoglobin percentage.

The blood was collected in oxalated conical flask for stock. Then it was pipetted out in Zeiss Ikon Haemometer pipette upto the lower mark and was diluted with the decinormal solution of hydrochloric acid by sucking it in the pipette upto the mark located above the bulb. The mixture was well shaken and the diluted blood was then transferred to the Haemometer tube. It was thoroughly mixed in the tube sucking the pipette several times. The diluted blood was kept in the Haemometer tube for 5 minutes to develop colour. The percentage of haemoglobin in the blood was then determined by matching the colour, with the colours on the band of Haemometer. The reading was directly obtained from the scale after matching the colour. The

haemoglobin content of the blood has been obtained in grams/

Anthelmintic and dose.

"Wopell" an anthelmintic of Indian herbs Research and Supply Co., Saharanpur (U.P.), was used in the experimental chemotherapeutic trial. The powder was given with sweat vehicle orally in two doses each of 2 gm/kg. body weight with the interval of one week.

Criteria of therapeutic activity.

Therapeutic activity was based on the presence or absence and also their number of eggs in the faeces before and after treatment as well as the number of worm recovered at Post-mortem examination of treated and control kids.

INCIDENCE AND NATURE OF HEIMINTHIC INFECTIONS IN GOATS.

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INCIDENCE AND NATURE OF HELMINTHIC INFECTIONS IN GOATS.

Review of Literature.

Considering the importance and utility of Indian goats, the literature on the helminth parasites of this animal in Indian is rather scanty. For the first time in India, Bhalerao (1932) reported the occurrence of 6 nematodes, viz., Varestrongylus pneumonicus n. sp. from bronchi and Ostertagia orientalis n. sp., O. accidentalis Ransom, 1907, O. circumcineta Stadelmann, 1894, Haemonchus contortus Rud, 1803 and Oesophagostomum venulosum Rud, 1807, from gastro-intestinal tracts. While describing common worms of sheep and goats in India, Bhalerao (1934) have listed the following helminths: Trematoda - Fasciola gigantica Cobbold, 1855, F. hepatica Linnaeus, 1758, Dicrocoelium dendriticum Rud., 1819, Paramphistomum cervi Schrank, 1990, Schistosoma indicum Montgomery, 1906, S. spindalis Montgomery, 1906, S. bovis Sonsino, 1876; Cestoda - Moniezia expansa Rud., 1810, M. benedeni Monřez, 1879, Avitellina centripunctata Rivolta, 1874, A. sudanea Woodland, 1927, A. lahorea Woodland, 1927, Stilesia globipunctata Rivolta, 1874, S. vitata Railliet, 1896, Cysticereus tenuicollis Pallas, 1766, C. ovis Cobbold, 1869, Coenurus cerebralis Gervais, 1847, Hydatid cyst and Nematoda - Ascaris lumbricoides Linnaeus, 1758, Bunostomum trigonocephalum Rud., 1808, B. phlebotomum Railliet, 1900, Oesophagostomum columbianum Curtice, 1890, Dictyocaulus filaria Rud., 1809, Trichocephalus ovis Abilgaard, 1795, Varestrongylus pneumonicus

Bhalerao, 1932, Ostertagia orientalis Bhalerao, 1932, O. accidentalis, O. circumcincta, H. contortus, and O. venulosum.

Rao (1939) recovered two species of nematodes viz.,

Trichostrongylus columbriformis Giles, 1892, and Trichostrongylus axei (Cobbold, 1879) Railliet and Henary, 1909 from the
abomasum and duodenum of goats in Madras. This was the first
record of T. axei in India. Srivastava (1939) reported the
occurrence of F. gigantica from lung of the goat from the Indian
subcontinent. This appears to be the first of report of this
liverfluke from lung of the goat. Malkani and Prasad (1941)
have described nasal schistosomiasis due to S. nasalis from the
goats of Bihar.

Bhalerao (1942) described a monostome, Cymbriforma indica n. sp., occuring very commonly in the alimentary canal, of sheep and goats at Muktesar, Uttar Pradesh. He had also collected Capillaria bilobata Bhalerao, 1933 from the small intestine of a goat. A survey of the helminth parasites of goats in Uttar Pradesh was carried out by Pande (1942) who reported the occurrence of 21 helminths: Trematodes - P. cervi, C. cotylophorum Fischoeder, 1901, Gastrothylax crumenifer Creplin, 1847, F. gigantica, S. indicum; Cestodes - C. tenuicollis, Coenurus cerebralis, Leske, 1780, M. expansa, Stilesia hepatica Wolffhugel, 1903, S. globinunctata, S. vittata, Avitellina centripunctata, A. lahorea, A. sudanea, and Nematodes - H. contortus, T. colubriformis, B. trigonocephalum, O. columbianum, Trichuris ovis Abildgaard, 1795, Protostrongylus rufescens Leuckart, 1865, V. pneumonicus. He examined a total number of 120 goats and

observed that most commonly occurring worm was <u>T. ovis.</u> Next in order of frequency were <u>H. contertus</u> and <u>O. columbianum</u>, <u>S. globipunctata</u>, <u>C. tenuicollis</u>, <u>T. colubriformis</u>, <u>B. trigonoce-phalum</u>, Paramphistomes, <u>S. vittata</u> and <u>A. centripunctata</u>, <u>A. lahorea</u>, <u>F. gigantica</u>, <u>A. sudanea</u>, <u>S. indicum</u> and <u>M. expansa</u>.

<u>P. rufescens</u>, <u>V. pneumonicus</u>, <u>S. hepatica</u> and <u>C. cerebralis</u> were also encountered, but only on one occasion in case of each parasite.

During an exhaustive survey of the helminth parasites of domesticated ruminants in Central Province, Berar and Central India, Moghe (1945) recorded 10 trematodes, 10 cestodes and 10 nematodes from goats. They were Cotytophorn ovalum Harshey, 1934, C. cotylophorum, G. crumenifer, P. cervi, F. hepatica, F. gigantica, S. spindalis, S. haematobium, S. indicum, S. bovis from class Trematoda; S. vittata, S. globipunctata, A. lahorea, A. woodlandi, A. centripunctata, M. mexpansa, M. benedeni, C. cellulosae, C. tenguicollis from the Class Cestoda and H. contortus, B. phlebotomum, B. trigonocephalum, O. columbianum, Gaigeria pachyselis Railliet and Henry, 1910, Gongylonema pulchrum Molin, 1857, G. verrucosum Giles, 1892, Cooperia curticei Railliet, 1893, Ostertagia ostertagi Stiles, 1892, O. circummcincta from the class Nematoda.

During an examination of ruminant from Lahore, Peshawar Karanchi, and Muktesar (Uttar Pradesh), Sarwar (1945) recorded two nematodes from the abomasii of goats. He obtained Marshallagia marshalli (Ransom, 1907) Orloff, 1933 at Mukteswar and Haemonchus longistipes Railliet and Henry, 1909 from Peshawar

Karanchi and Lahore.

Srivastava (1945), during the survey of the incidence of helminth parasites of ruminants in India, reported the widespread occurrence of helminth parasites in sheep, goat, cattle and buffaloes in the Punjab, North West Frontier Province and Sind. He recorded five trematodes and five nematodes from goats. They were <u>Fasciola</u> species (16%), <u>D. dendriticum</u> (6%), <u>C. cotylophorum</u> (20%), <u>P. cervi</u> (32%), <u>G. crumenifer</u> (30%), and nematodes <u>P. rufescens</u> (17%), <u>Dictyocaulus filaria</u> Rud., 1809 (14%), <u>H. contortus</u> (27%), <u>Bunostomum</u> spp. (23%) and <u>Oesophagostomum</u> spp. (18%).

Sarwar (1947) described two species of lung worms,

Varestrongylus capricola Sarwar, 1944 and Protostrong/ylus

indicus Sarwar, 1944 from Indian goats. Varma (1953) reported
a new species of liver fluke, F. indica from goats, cattle and
buffaloes in Bihar. Mudaliar and Alwar (1947) reported Skrjabinema ovis, Skrjabin, 1915, O. venulosum, Gaigeria pachyscellis,

T. colubriformis and Trichuris alcocki Linstow, 1906 from goat
in Madras.

A systematic survey of helminth parasites of domesticated animals in Uttar Pradesh, Bihar, Bengal, Assam and Orissa was carried out by Thapar (1956), who reported the occurrence of 13 trematodes, 14 cestodes and 13 nematodes from goats. The trematodes were C. cotylophorum, D. dendriticum, Eurytrema pancreaticum (Janson, 1889) Looss, 1907 F. gigantica, Fischoederius cobboldi Poirier, 1883 F. elongatus Poirier, 1883,

G. crumenifer, Homalogaster paloniae Poirer, 1883 P. explanatum Creplin, 1847 P. orthocoelium, S. indicum. The cestodes were Avitellina centripunctata, A. lahorea, A. sudanea, A. woodlandi, C. cerebralis, C. tenuicollis, Echinococcus cysts, Echinococcus granulosus (larva), M. expansa, M. denticulata, M. benedeni, T. gaigeri, T. hydatigena and the nematodes were B. trigonocepha lum, G. pachyscelis, G. pulchrum, H. contortus, Nematodirus filicollis, Nematodirus spp. (female only), O. asperum, O. columbianum, O. circucincta, Setaria labiatopapillosa Aless, 1838, Strongyloides papillosus Wedl, 1856 T. colubriformis, T. ovis and V. pneumonicus. Out of these parasites only five trematodes viz., C. cotylophorum, F. cobboldi, F. gigantica, G. crumenifer, S. indicum; five cestodes viz., A. lahorea, C. tenguicollis, Echinococcus cyst, M. expansa, T. hydatigena; and four nematodes viz., B. trigonocephalum, H. contortus, O. columbianum and T. ovis were recorded from Bihar.

Varma (1957) had made an extensive survey on the nature, incidence and geographical distribution of amphistomes in Bihar (India). During this study, he examined 154 goats and observed G. crumenifer (48.7%), as the most common and widespread parasite, C. cotylophorum (47.4%) and Calicophorum calicophorum Fischoeder, 1901 (14.8%). He believed that C. calicophorum was reported for the first time from sheep and goats in India.

Sinha (1958) studied the incidence of helminthic infection of goats in eastern region of Uttar Pradesh. He examined 450 goats and reported the presence of 19 helminths comparising 4 species of trematodes: C.cotylophorum, F.gigantica,

G. crumenifer, S. indicum; 6 cestodes: Moniezia spp., M. denticulata, S. globimpunctata, Avitellina spp., C. tenuicollis, C. gaigeri and 9 nematodes; H. contortus, O. columbianum, O. venulosum, B. trigonocephalum, G. pachyselis, T. colubriformis, Trichuris spp., Skrjabinema ovis and Setaria cervi.

During the survey of helminthic infection of goats in Uttar Pradesh, Bengal and Orissa, Sood (1960) recorded 6 species of trematodes, 4 species of cestodes and 13 species of nematodes. The trematodes were P. cervi, C. cotylophorum, G. crumenifer, S. indicum, D. dendriticum, Ogmocotyle indica (Bhalerao, 1942) Ruiz, 1946; Cestodes were M. expansa, M. benedeni, Stillesia spp. and Avitellina spp. and nematodes were O. columbianum, O. venulosum, T. colubriformis, G. pachyscelis, B. trigonocephalum, O. circumcincta, C. punctata, H. contortus, Strongyloides papillosus, T. ovis, T. globulosa, Gongylonema pulchrum and T. ovina.

Pande, Rai and Bhatia (1961) reported a large number of Spirocerca lupi from the aorta of goats and pony in India for the first time. Alwar and Lalitha (1961) observed the occurrence of O. asperum and Setaria cervi from goats in Madras. Sinha (1962) examined 106 goats at Patna (Bihar) and reported two trematodes, five cestodes and five nematodes. They were G. crumenifer (37.8%), C. cotylophorum (40.55%), M. expansa (15.1%), M. benedeni (1.88%), A. centripunctata (16.1%), Cysticercus tenuicollis (21.7%), H. contortus (39.62%), B. trigonocephalum (2.83%), O. columbianum (34.9%), O. venulosum (14.1%) and Trichuris spp. (31.13%).

Katiyar and Varshney (1963) reported very high incidence of amphistomiasis in sheep and goats of Uttar Pradesh. The amphistomes associated with the outbreak were <u>G. crumenifer</u>, <u>C. cotylophorum</u>, <u>P. cervi</u>, <u>P. explanatum</u> and <u>F. elongatus</u>. While investigating the pathogenesis of <u>Ogmocotyle</u> indica, Sharma Deorani (1965) reported 28.9% infection in sheep and goat of Uttar Pradesh.

During a survey of stomach worms of sheep and goats at Bareilly (Uttar Pradesh) Sahai and Deo (1964) reported two species of Haemonchus viz., H. contortus and H. bispinosus

Molin, 1860 a parasite originally reported from Mazama nana in Brazil, was reported by these workers for the first time from sheep and goats. Sahai (1966) observed that percentage of H. bispinosus was much more than H. contortus in sheep and goats.

Dutt and Sahai (1966) also confirmed the occurrence of H. bispinosus in Indian goats and sheep.

Mishra and Ruprah (1968) examined 120 goats at Hissar (Haryana) and reported the percentage of helminthic infection in them. Twenty species of helminths were encountered during their survey, but the most common parasites were <u>T. ovis</u>, Haemonchus spp., <u>O. columbianum</u>, <u>M. denticulata</u>, <u>S. globipunctata</u> and <u>Thysaniezia giardi</u> Moniez, 1879. In routine diagnostic examination in Tripura, Majumdar (1969) examined 293 faecal samples from goats and sheep and reported 4 trematodes, 7 cestodes and 108 nematodes in them.

Tripathi (1970) studied the seasonal variation of egg

that infective larvae of <u>Haemonchus</u> spp. were maximum and which was followed by <u>Strongyloides</u>, <u>Trichostrongylus</u>, <u>Oesophagostomum</u>, <u>Bunostomum</u> and <u>Cooperia</u> spp. respectively. Total number of larvae were maximum in South West Monsoon and Post Monsoon seasons. Hot Weather was found to be unfavourable for the development and survival of larvae in general.

Rajmohan and Paily (1971), during routine postmortem mexamination of goats in Kerala reported <u>Strongylus</u> (68.7%), <u>Moniezia</u> spp. (6.4%), <u>Trichuris</u> (2.8%), <u>Amphistomes</u> (1.9%) and <u>Fasciola</u> (0.09%).

In addition check lists of helminth parasites of goats have been published by Bhalerao (1935), Gaiger (1910, 1915), Alwar and Lalitha (1961), Ramanujachari and Alwar (1954), Mudaliar and Alwar (1947). Besides these surveys and check list occurrence of helminth parasites of goats have also been reported from time to time.

Results and Discussion.

In an examination of 162, 160 goats were found to be infected with one or other helminth parasites, only two goats were found to be free from parasitic infection. The number of hosts examined was not very large though it was sufficient to indicate the high rate of parasitism in goats.

The collection represents a total number of 16 species of helminths. Out of which there were 5 species of trematodoes, 5 of cestodes and 6 of nematodes as listed below :-

TREMATODA

- 1. Fasciola gigantica Cobbold, 1855.
- 2. Cotylophoron cotylophorum (Fischoeder, 1901)
 Nasmark, 1937.
- **3. <u>Carmyerius spatiosus</u> (Brandes, 1898)

 Nasmark, 1937.
 - 4. Gastrothylax crumenifer Creplin, 1847.
- *5. Ceylonocotyle spp.

CESTODA

- 1. Moniezia expansa Rud., 1810.
- *2. Stilesia globipunctata Rivolta, 1874.

- **3. Echinecoccus granulosus Batsch, 1786.
 - 4. Hydatid cyst.
 - 5. Cysticercus tenuicollis Pallas, 1766.

NEMATODA

- 1. Oesophagostomum venulosum Rud., 1809.
- 2. Oesophagostomum columbianum Curtice, 1890.
- 3. Trichuris ovis Abildgaard, 1795.
- 4. Trichuris globulosa Linstow, 1901.
- 5. Haemonchus contortus Rud., 1803.
- 6. <u>Haemonchus bispinosus</u> (Molin, 1860)
 Railliet and Henry, 1909.

The results of the incidence has been presented in Table 1, 2, 3 and Plate 1 indicates the incidence of trematodes, cestodes and nematodes. Table 2 is showing the percentage of helminthic infection. Table 3, Plate 1 and Figure 1 indicate the percentage of incidence in three groups of helminths viz., trematodes, cestodes and nematodes. Plate 1 and Figure 2 are showing the percentage of Haemonchus contortus and Haemonchus bispinosus infection in goats.

^{*} First record in goats in Bihar.

^{**} First record in this host.

Table 1.

Showing the incidence of Helminth infections in goat in Bihar.

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	14	1	+	ı	+	+	+	1	+	+	ı	+	+	ı	1	1	1	1	ļ	1	+
	13	+	+	1	+	+	1	+	1	+	ı	+	+	+	+	1	+	+	+	+	1
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	11									-	32	-									
	17	1	+	+	1	+	+	+	+	+	+	+	+	ı	+	+	+	4	+	ı	+
	16	+	+	+	+	+	+	+	+	1	+	+	+	+	+	+	+	+	+	+	+
	15	+	1	1	-1	1	1 1	1	+	+	+	1	+	1	1	1	1	1	1	+	+
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	13	+	+	+	+	1	1	+	1	1	+	+	+	+	+	+	+	1	+	+	ı
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17	+	+	. +	+	+	+	+	
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2	1	B	1	1	1	1	1	
-	156	157	158	159	160	161	162	

Con'd. Table

Showing the percentage of helminthic infection (no. of goats examined were 162).

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Name of parasites	Location	Percentage.
Showing one percenting		
Fasciola gigantica.	Liver and bileduct.	11.8
Cotylophoron cotylophorum.	Rumen and Reticulum.	47.5
Carmyerius spatiosus.	Rumen.	1.2
Gastrothylax crumenifer.	Rumen, Reticulum.	32.09
Ceylonocotyle spp.	Rumen.	0.6
Moniezia expansa.	Small intestine.	23.4
Stilesia globinpunctata.	Small intestine.	12.9
Echinococcus granulosus.	Small intestine.	0.6
Hydatid cyst.	Liver and lungs.	1.85
Cysticercus tenguicollis.	Peritoneal and abdominal cavity.	24.5
Oesophagostomum venulosum.	Colon.	14.4
Oesophagostomum columbianum.	Colon.	50.0
Trichuris ovis.	Caecum.	48.1
Trichuris globulosa.	Caecum.	27.1
Haemonchus contortus.	Abomasum.	74.6
Haemonchus bispinosus.	Abomasum.	67.2

Table 3

Showing the percentage of Trematoda, Cestoda and Nematoda.

No. of	Trema	toda	Cesto	oda	Nen	atoda
animals examined	No. of	Per-	No. of	Per-	No. of	Perce-
examined	infear	toge	(animais)	to cen-	(animais)	ntage.
	ted	age.	ted	l vage	ted (
					Ì	
162	121	74.6	67	31.4	138	85.1

reported and described from different localities in lette by

The project examination revealed the intertion in 77

The present study revealed the infection only in 2 goats (1.2%) which appears to be first report of this parasite in goats.

4. Gastrothylax crumenifer.

It is also a common trematode of Indian sheep and goats. In India, Pande (1942) recorded its occurrence for the first time from the rumen and reticulum of goats. Thereafter several workers (Moghe, 1945; Thapar, 1956; Varma, 1957; Sood, 1960; and Sinha, 1962) had recorded its occurrence in the different regions of Indian subcontinent.

The present investigation revealed the infection in 52 out of 162 goats (32.09%) examined. Thapar (1956) reported (30.4%) G. crumenifer infection in goats of Bihar. Varma (1957) and Sinha (1962) reported that the percentage of the infection in goats in Bihar was 48.2% and 37.84%, respectively.

5. Ceylonocotyle spp.

During the survey one goat was found to harbour a few specimens of <u>Ceylonocotyle</u> (i.e. 0.6%). Although it appears to be first report of <u>Ceylonocotyle</u> in goats, specific identification could not be done. Several species of <u>Ceylonocotyle</u> have been reported from Indian cattle and sheep (Gupta, 1958, Mukherjee, 1963).

CESTODA

1. Moniezia expansa.

It is one of the most common cestodes of goats.

TREMATODA

1. Fasciola gigantica.

It is a very common liver fluke of domestic ruminants in Indian subcontinent. The history of liver fluke is traceable as early as 1397, but Gaiger (1910) was the first scientist who reported the incidence of <u>F. gigantica</u> in ruminants in Uttar Pradesh, Punjab and Himalayan regions. Thereafter, it has been reported and described from various localities in India by several workers (Bhalerao, 1934; Pande, 1942; Moghe, 1945; Srivastava, 1945; Thapar, 1956).

In the present investigation only 19 goats (11.8%) were found to harbour the parasite.

2. Cotylophoron cotylophorum.

It is also a most common trematode parasite of goats. It was first recorded by Pande (1942) and thereupon it has been reported and described from different localities in India by several workers (Moghe, 1945; Srivastava, 1945; Thapar, 1956; Varma, 1957; Sood, 1960; Sinha, 1962).

The present examination revealed the infection in 77 out of 162 goats (47.5%) is examined. Sinha (1962) had reported that the percentage of infection in Bihar was 40.56%.

3. Carmyerius spatiosus.

It is a parasite of cattle, buffaloes and antelopes.

Bhalerao (1934) was first person to record its occurrence from goats of Indian subcontinent. Thereafter, many scientists (Pande, 1942; Moghe, 1945; Thapar, 1956; Sood, 1960; Sinha, 1962) have noticed its occurrence from goats in India.

The present study revealed the infection in 38 out of 162 goats (23.4%) examined. Sinha (1962) reported that the percentage of the infection in Bihar was 15.1.

2. Stilesia globipunctata.

In India, Bhalerao (1934) reported for the first time its occurrence from goats. Later on many workers (Pande, 1942; Moghe, 1945; Sinha, 1958; Mishra & Ruprah, 1968) have noticed its occurrence from goats in India.

During the present survey this parasites was observed in 21 out of 162 goats (12.9%) examined, which appears to be first report in Bihar.

3. Hydatid cyst.

It is larval stage of <u>Echinococcus granulosus</u>, which has been extensively reported from different visceral organs of Indian ruminants including goat.

In the present study, also it has been recovered from 3 out of 160 goats (1.85%) examined.

4. Echinococcus granulosus.

It is one of the most common tape-worms of carnivora.

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4. Echinococcus granulosus.

It is one of the most common tape-worms of carnivors,

In the present investigation only one goat (0.6%) was found to harbour two specimens of this parasites in its intestine.

5. Cysticercus tenuicollis.

In India, it is also one of the most common bladder worm of goats and its occurrence has been reported by many parasitologists and zoologists.

Thirty eight goats out of 162 (24.5%) examined in the present study haboured these bladder worms in the peritoneal and abdominal cavities of goats in Bihar. Sinha (1962) reported it from 21.7% of the goats examined in Bihar.

NEMATODA

1. Oesophagostomum venulosum.

This nodular worm was reported from Indian ruminants for the first time in Punjab by Gaiger (1910). Thereafter, Bhalerao (1932) had recovered these worms from sheep and goats at Muktesar, Uttar Pradesh. Sinha (1962) reported its occurrence for the first time in goats of Bihar.

During the present survey, 14.4% of goats examined were found to be infected by this parasites, whereas Sinha (1962) had its occurrence from 14.1% of goats in Bihar.

2. <u>Oesophagostomum columbianum</u>.

This species of nematode is a common parasite of goats

in India, and has been reported by several authors (Gaiger, 1910; Bhalerao, 1934; Pande, 1942; Moghe, 1945; Srivastava, 1945; Thapar, 1956; Sinha, 1958; Sinha, 1962; Mishra & Ruprah, 1968; Tripathi, 1970).

Out of 162 goats examined, 81 (50%) were found to harbour the parasite in the colon. Srivastava (1945) reported its occurrence from 18%, Sinha (1958) from 49.3% and Sinha (1962) from 34.9% of the goats examined by them.

3. Trichuris ovis.

It has been observed to be a common whip-worm of sheep and goats by previous workers in India.

In the present investigation, 77 goats (48.1%) were found to harbour the parasite in caecum. Sinha (1958) had reported <u>T. ovis</u> in 93.3% of goats in Eastern region of Uttar Pradesh and Rajmohan & Paily (1971) had found it in 2.8% of goats examined by them.

4. Trichuris globulosa.

In India, <u>T. globulosa</u> was first reported by Gaiger (1910) from dromedary in Punjab. Thereafter, it was observed to be a common nematode of ruminants (Sood, 1960; Sinha, 1962).

The present investigation revealed the presence of this parasite in 44 goats (27.1%) out of 162 examined. Sinha (1962) observed its occurrence in 31.13% of goats examined in Bihar.

5. Haemonchus contortus.

This species of nematode is a common parasite of Indian ruminants and has been reported by many workers from goats (Gaiger, 1910; Bhalerao, 1932; 1935; Baylis, 1936; Pande, 1942. Moghe, 1945; Srivastava, 1945; Thapar, 1956; Sinha, 1958; Sinha, 1962; Sahai & Deo, 1964; Sahai, 1966; Mishra & Ruprah, 1968 and Tripathi, 1970; Padmavatti et al, 1971).

Out of 162 goats examined, 120 (74.6%) were found to harbour this parasite in the abomasum. Srivastava (1945) reported it from 27%, Sinha (1958) from 78.61%, Sinha (1962) from 39.62% and Sahai (1966) from 71% the goats examined by them.

6. Haemonchus bispinosus.

Molin (1860) described this parasite from Mazama nana, a members of the family Cervidae, from Brazil. The validity of this parasite was questioned till Khera (1954) recovered and described H. bispinosus from the abomasum of Cervux axis in India. Sahai and Deo (1964) reported its occurrence from sheep and goat for the first time. Thereafter, Dutt and Sahai (1966), Sahai (1966) and Padmavatti et al (1971) have reported its occurrence from Indian sheep and goats.

In the present investigation, 67.2% goats were found to harbours the parasite. Sahai (1966) studied the incidence of haemonchosis in sheep and goats in Uttar Pradesh and reported the infection in 77% of sheep and goats examined by him.

EFFECT OF HYDROCORTISONE ON NATURAL AND ACQUIRED RESISTANCE IN KIDS TO THE NEMATODE, HAEMON-CHUS BISPINOSUS (MOLIN, 1860) RAILLIET AND HENRY, 1909.

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Review of Literature.

Work on parasitic immunology has received special attention in recent years, though the papers on immunity concerning helminth parasites are available as early as Stoll (1928). Several workers have reported that acquired and innate resistance in sheep, goats and cattle develops against Haemonchus species (Stoll, 1929; Taylor, 1934; Chandler, 1939, 1953; Taliaferro, 1940; Mayhew, 1940, 1941; Stewart, 1950a, 1950b, 1950c, 1950d; Soulsby, 1956, 1957, 1958, 1960, 1968; Lisenco, 1956; Roberts, 1957; Allen and Samson, 1961). Some of them have also described self cure and protection phenomena in haemonchosis in domestic animals (Stoll, 1928, 1958; Stewart, 1955; Soulsby, 1960; Soulsby and Stewart, 1960). Also sufficient work has been done to study the effect of different chemical and physical agents in relation to parasitic immunity (Ross and Gordon, 1933; Roberts and Keith, 1959).

Kass and Finland (1953) described that administration of Adrenocorticotropic Hormone, Hydrocortisone, Cortisone, Predisolone or related steroids generally lowers the resistance

of animals to latent and induced bacterial, viral, rickettesial, protozoal, fungal and helminthic infection. They opined that cortisone and hydrocortisone suppress inflammatory response and bring about the inhibition of the antibody production and alteration of the Reticulo-endothelial function. They also described the mechanisms by which ACTH, Cortisone, and Hydrocortisone may reduce resistance to infection which are -

- 1. Inhibition of inflammation
- A. Decreased capillary permeability
- B. Decreased cellular exudation and infiltration
- C. Decreased exudation of fluid and protein
 - D. Decreased phagocytosis by polymorphonuclear leucocytes.
- E. Decreased fibrogenesis and repair.
 - 2. Inhibition of antibody production.
 - 3. Negative nitrogen balance.
- 4. Alteration of reticulo-endothelial function.

The work on the effect of Cortisone and Hydrocortisone in relation to the parasitic immunity in various animals is reviewed.

Weinstein (1953) studied the effect of cortisone on the immunogenic character against <u>Nippostrongylus muris</u> against in the white rats and reported that it suppresses the formation of antibody in the serum. During the course of experimental trichinelliasis in laboratory mice, Stoner and Godwin (1953) observed a slight increasing susceptibility to <u>Trichinella</u> infection after ACTH therapy and with cortisone therapy, the mortality showed a 45% increase.

Galliard and Berdonneau (1953) showed the effect of cortisone and ACTH on strongyloidosis in dogs. They also confirmed the observation of previous workers that cortisone increase susceptibility of dog also for Strongyloides infection. Again, Stoner and Godwin (1954) studied the effect of ACTH and cortisone on acquired immunity against Trichinella infection in mice. They observed that in ACTH therapy slight increasing in mortality rate than untreated control animals infected with T. spiralis. Cortisone and Cortisone combined with antibiotic produced a significant breakdown of the acquired immunity to reinfection.

The influence of cortisone on the immune response against <u>Nippostrongylus muris</u> in white rats was carried out by Weinstein (1955). He proved that white rats given infective larvae of <u>N. muris</u>, majority developed into adults of larger size in case of cortisone treated rats than that of untreated rats.

Coker (1955a) investigated the effect of cortisone treatment on normal defence of mice against <u>Trichinella spiralis</u> infection. He reported that <u>T. spiralis</u> infection in cortisone treated but non-immunised mice persisted for a longer period in the intestine as well establishment of more larval worms in the muscles than that of control mice. His experiments have also

reported the view that non-immunized mature mice developed a delayed acquired immunity against initial <u>T. spiralis</u> infection. Again, he (1955b) described that the cortisone therapy eliminated the cellular reaction in the intestinal wall which customarily occurs just prior to the operation of acquired immunity. He gave this hypothesis during his study about the cellular factor in acquired immunity against <u>T. spiralis</u> in mice.

Later, in (1956) he gave a hypothesis that immunized mice became non-immuned due to the effect of cortisone. These could not be immunized, though it was possible in control mice to develop immunity in 11 to 14 days post-infection.

Nicol and Bilbey (1956) observed that the depression of the Reticulo-endothelial system occured during the early stage of cortisone treatment in guinea pigs accompanied by a fall of the total leucocytic counts in the blood.

Roman (1956) studied the effect of cortisone against innate resistance of <u>Strongyloides ratti</u> in guinea pigs and observed that cortisone breakdown the natural immunity.

Markell (1957) also studied the effect of cortisone treatment on the immunity to subsequent reinfection with <u>Trichinella</u> in the rat, and confirmed the hypothesis given by other workers that it breaks down the immunity. During studies on influence of cortisone in white rats against <u>Litomosoides</u> carinii, Olson (1958) was also of opinion that cortisone reduces immunity. Again in 1959 he studied the cellular response against

L. carinhi infection in white rats influenced by cortisone. Cortisone injection effectively suppressed the cellular response and the capsulation of the larvae in the planal cavities of white rats, but did not increase the percentage of larvae completing migration.

Ritterson (1959) stated that golden hamsters were not naturally resistant against <u>Trichinella spiralis</u> infection, though Chinese hamsters were resistant. He reduced natural immunity of Chinese hamsters by cortisone therapy.

Weinmanmand Hunter (1959) observed the effect of cortisone on the <u>Schistosoma mansoni</u> infection in mice. After their experimental studies, they concluded that the cortisone treated animal showed small but statistically significant reduction in worm burden. Times of cortisone administration had no effect on worm burden.

Cross (1960) studied on natural resistance against in Nematospiroides dubius in white rats and the effect of cortisone on this resistance. N. dubius normally develops in the white mouse, and white rat was naturally resistant to this infection. But due to the effect of cortisone therapy, this infection could be produced in white rat and white mouse in similar intensity. Berubik (1960) demonstrated that hydrocortisone sodium succinate given to hamsters and guinea pigs did not render them susceptible to infection with larvae of the sheep and rabbit strains of Strongyloides papillosus. The hamsters and guinea pigs had strong natural immunity against these strains.

Weinmann and Rothman (1961) had described the effect of cortisone on acquired and innate resistance against Hymenolepis nama in mice. They reported that cortisone acetate treatment allowed massive reinfection of H. nama in mice whereas after cessation of cortisone treatment, mice recovered the ability to resist reinfection. Parker (1961) had carried experimental studies to find out the effect of cortisone on immunogenic response against the infection of Nippostrongylus brasiliensis in the guinea pigs. They concluded that the treatment of guinea pigs with various doses of cortisone rendered them less resistant against N. brasiliensis infection. Untreated male guinea pigs harboured more number of larvae than that of untreated females and likewise cortisone treated males harboured more than that of cortisone treated female.

Dunsmore (1961) observed the effect of whole body irradiation and cortisone on the development of Ostertagia species in sheep, and concluded that the percentage of larvae showing arrested development was 8.5% in the cortisone treated sheep, 18.7% in the irradiated sheep and 43.2% in the control group. He suggested that both treatments interfered with an immune response of the sheep and that arrested development was the result of this immune response.

Olivier (1962) studied the effect of cortisone on natural resistance against <u>Taenia taenia formis</u> and reported that cortisone had more profound effect on larval survival and can make the hosts highly susceptible. The cortisone must be given before the 12th day of infection for its effectiveness. It was

effective when it was used for 8 or even 4 days. He postulated that the cortisone was effective at least in part, because of its inhibition of antibody production.

Zaiman et al (1962) investigated the effect of cortisone acetate on the defensive mechanisms of mice in <u>Trichinella</u> larvae infection. He inoculated per os with 6400 larvae of <u>T</u>. <u>spiralis</u> in young male mice and then gave single intraperitoneal injection of water, 90% sucrose and cortisone acetate at 25 and 2.5 mg./kg. body weight. The average survival times were 42.5 hours following the larger dose and 50.4 hours after the smaller dose. Animals, given water or sugar survived for an average of 71.8 hours.

Villarejos (1962) contradicted the previous hypothesis and concluded that cortisone did not increase the susceptibility of the rats nor decrease the resistance of the older animals to infection with <u>E. histolytica</u>. It appeared that non of the mechanisms through which cortisone decreases host resistance against other type of infection defined by others as a lowering effect upon the serum gamma globulin levels, upon the phagocytic activity of Reticulo-Endothelial systems and upon the total and differential leucocytic counts.

Mathies (1962) observed the effect of cortisone on host parasite relationship in <u>Aspiculuris tetraptera</u>. He reported that cortisone at a dose of 75 mg./kg. body weight significantly increased the susceptibility of mice against <u>A. tetraptera</u> infection and the effect was not destroyed by the concomitant administration of estradiols at a dose of 0.16 mg./kg. body weight.

and Collette
Campbell_(1962) studied the effect of cortisone against
Trichuris muris infection in albino mice and reported that
mice which had some degree of innate resistance against T.
muris became more susceptible when cortisone was given.

Briggs (1963) observed the effect of cortisone on natural and acquired resistance against Litomosoides carinii infection. He compared the growth and development of L. carinii in cotton rats and white rats. When about 400 larvae were given in divided doses, less than 10% were recovered from white rats, while 46 to 58% were recovered from the cotton rats 7 week after infection. Both hosts had seral antibodies which produced precipitates around the mouth of larvae in suspension. It was found by serial killing after single doses of largae that migration to the serosal cavities took place in the first week in the cotton rat but that in the white rat the larvae were killed before reaching these cavities. In neither host, there was any antibody demonstrable before the 3rd week. administration of cortisone either before or at the time of infection resulted in many more worms reaching the serosal cavities of white rats and developing there.

Johnson and Hansen (1964) reported the influence of hydrocortisone on susceptibility of chicken against <u>Ascaridia</u> galli. He reported that cortisone treated leghorn cockerels and Delaware hampshire birds had developed an average of 37.2 and 22.5 worms where as control had only 13.1 and 1.5 worms respectively. The hydrocartisone appeared to interfere with the hosts response to the "tissue phase" period of this parasite.

Ogilwie (1965) observed the daily treatment of rats with steroid compound, prednisolone during initial infection of Nippostrongylus brasiliensis and reported that prednisolone prevented acquired resistance either completely or at an early stage. All manifestation of acquired resistance were suppressed by daily administration of prednisolone or betamethasone.

Singh and Rao (1968) investigated the effect of cortisone on laboratory animals against Cysticercus fascioloris infection. During their investigation they have utilized 4 rats, 4 guinea pigs, 2 hamsters and 2 rabbits. Cortisone was given intramuscular for 12 days and on the 5th day these animals were given 300-350 eggs of T. taeniaformis. One rat after 20 days post-infection showed greater development of cysts when compared with control, but other rats, 30 days after infection harboured much less developed cysts, when compared with control. The rats autopsied after 35 and 45 days of infection harboured bigger and better developed cysts than control. The rabbits were found to be refractory even under cortisone stress. One out of 4 guinea pigs subjected to cortisone, developed only one cyst in the liver when autopsied 45 days after infection.

Michel and Sinclair (1969) observed the effect of cortisone on the worm burden of calves infected daily with Ostertagia ostertagi. They concluded that daily treatment with P-methazone, a cortisone derivative greatly reduced the ability of calves to produce antibody and a number of side effects appeared.

Sinclair (1970) studied the pathogenicity of F. hepatica

in corticosteriod treated lamb. He described that the enhanced pathogenicity and accelerated growth of flukes in dexamethasone treated lamb and there was evidence that the effect of drug continued into the period of reinfection, though the acquisition of resistance was not adversely affected.

Dhar and Singh (1970) had carried out experimental studies on the effect of cortisone on the natural and acquired resistance in lamb to the <u>Oesophagostomum columbianum</u> and reported that administration of cortisone, at the dose rate of 50 and 25 mg./kg. body weight daily to lambs for a period of 10 days resulted in the breakdown of the innate and the acquired resistance of lambs to <u>O. columbianum</u> infection. The degree of the breakdown of the innate and the acquired resistance appeared to depend upon the amount of cortisone administered. Possibly the breakdown in the innate and acquired resistance was due to the interference in the cellular defence mechanism of the host.

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Experimental Study.

In the present investigation 55 to 60 days old kids were used. They were maintained under parasitic free condition throughout the experimental period. All the six kids were muzzled throughout the experimental period except when they were given feed and water. These kids were divided into two groups, each having three kids.

Group-I (Immunized group).

Each of three kids in this group received a total of 10,000 infective larvae of H. bispinosus in 16 per os feedings. Doses were given on every alternate day. The first 7 immunizing dose comparised 400 and the last 9 of 800 infective larvae given in each dose (Table 4). Two of the kids (Nos. 1 and 2) were treated with hydrocortisone 7 days after they had received the last immunizing dose. The dose of hydrocortisone administered per os was 30 and 15 mg./kg. body weight daily for a period of 10 days to kid Nos. 1 and 2 respectively. These two kids (Nos. 1 and 2) and kid No. 3 (control) which was immunized but not treated with hydrocortisone was challenged with 10,000 infective larvae of H. bispinosus 12 days after the last immunizing dose. The immunized kids under hydrocortisone treatment had already received 5 daily treatment of the drug at the time they were challenged. Hydrocortisone was administered further 5 days to these kids after the challenge (Table 5).

Group-II (Non-immunized group).

In this group kid Nos. 4, 5 and 6 were used. Among them kid Nos. 4 and 5 were treated with hydrocortisone per os at the dose rate of 30 and 15 mg./kg. body weight for a period of 10 days, respectively. Kid No. 6 which was not treated with hydrocortisone served as control. All the three kids either treated or untreated were challenged with 10,000 infective larvae of H. bispinosus. Kid Nos. 4 and 5 were infected after they received 5 dose of hydrocortisone.

Weekly weight of the animals were taken throughout the experimental period. Clinical signs were observed daily. Haematological counts were made weekly throughout the experimental period.

TABLE 4.

of. and number of infective larvae given for immunization. the date, dose bispinosus Showing

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Tho the contraction of the contr	800 10,000	800 10,000	800 10,000	
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71.0	800	800	800	
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2.00	400	400	400	
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Results.

A. Parasitological findings.

The results of the effect of hydrocortisone at the rate of 30 and 15 mg./kg. body weight per day for 10 days during the course of infection in the immunized and non-immunized kids have been shown in Table 5 and Plate 2.

In the immunized and non-immunized kids treated with hydrocortisone and subsequently challenged with 10,000 infective larvae, a larger percentage of parasites established themselves in the abomasum, when compared to their respective controls, which were not treated with hydrocortisone. The kids both immunized and non-immunized, which were treated with 30 mg./kg. body weight of hydrocortisone daily for 10 days, showed comparatively many more parasites in the abomasum than the kids which received 15 mg./kg. body weight of hydrocortisone each day for 10 days. In two kids of the immunized group, received 30 and 15 mg./kg. body weight of hydrocortisone daily for 10 days, the total number of infective larvae succeeded in establishing in them, were 5.62% and 3.49% respectively, as against 0.26% in the control. Similarly, in the non-immunized group 18.64% and 13.15% of the total number of infective larvae fed reached the adult stage in the abomasum of kids received 30 and 15 mg./kg. body weight of hydrocortisone daily for 10 days as against 9.21% in the control kids.

In the immunized group, hydrocortisone treated kid

Nos. 1 and 2 received 30 and 15 mg./kg. body weight daily for 10 days showed gradual increase in its egg counts when compared to the control. The egg counts were more in kid No. 1 which received higher dose of hydrocortisone than kid No. 2 which received lesser dose.

The egg counts for the animals in the non-immunized group in the present investigation could not be made except the control, since these animals succumbed to the challenge exposure before the parasites could reach the sexual maturity.

B. Clinical Findings.

Kid No. 3 (control) which was immunized but not treated with hydrocortisone showed no clinical signs and not only surjived and challenge infection but also showed gained in body weight as apparent from Table 5, Plate 3 and Fig. A. In the hydrocortisone treated immunized kids, however the signs varied with the amount of hydrocortisone administered. Kid No. 1, which received 30 mg./kg. of hydrocortisone showed loss of appetite, anaemia, intermittent diarrhoea and loss of body weight on the 11th day and died 26th day after the challenge exposure. In kid No. 2 which had received 15 mg./kg. of hydrocortisone showed slight diarrhoea, loss of appetite and loss of body weight 16th day of challenge infection. As Plate 3 Fig. A indicated that fall of body weight of kid No. 1 was more pronounced than in kid No. 2.

The animals of the non-immunized group did not survive

the challenge infection and died except the control (kid No.6) which showed the symptoms of diarrhoea, loss of appetite, and anaemia after 10 days of challenge exposure. Kid No. 4, received 30 mg./kg. of hydrocortisone showed diarrhoea, loss of appetite after 5 days and marked anaemia after 8 days of challenge dose, loss of body weight in kid No. 4 was more pronounced than in kid No. 5. The animal died on 19th day of challenge exposure. Again kid No. 5 received 15 mg./kg. of hydrocortisone showed similar symptoms as was shown by kid No. 4 but died on 21st day of the challenge.

C. Haematological Findings.

A transitory lymphocytosis, neutropenia, monocytosis and eosinophilia was observed along with a mild increase in the number of leucocytes due to immunization as evident vide Table 6, 7; Plate 4; Fig. A, B, C, D and Plate 5; Fig. A.

Immunized animals when treated with hydrocortisone revealed significant reduction in haemoglobin percentage. Erythrocytosis and leucopenia were well marked. Besides, there were distinct neutrophilia, lymphocytopenia and complete absence of eosinophils, there was reduction in P.C.V.% also as shown in Table 6, 9; Plate 4; Fig. A, B, D and Plate 5; Fig. A, B, C, D.

When these hydrocortisone treated immunized kids were challenged by 10,000 infective larvae of <u>H. bispinosus</u>, they revealed further reduction in haemoglobin percentage along with leucocytes and lymphocytes with marked neutrophilia. Progressive

reduction in percentage of erythrocytes and packed cell volume were also observed as evident from Table 6, 10; Plate 4; Fig. A, B and Plate 5; Fig. A, B, C, D.

Immunized non treated kid when challenged with 10,000 infective larvae showed almost similar haematological change as in treated kids except that the haematological alterations were more mild and eosinophil was not completely absent but decrease in number as shown in Table 6, 11; Plate 4; Fig. A,B,C,D and Plate 5; Fig. A,B,C,D.

In the non-immunized kids there was significant reduction in the haemoglobin percentage and erythrocyte level due to parasitic infection. There was also leucocytopenia, lymphocytopenia, monocytosis, neutrophilia and mild eosinophilia. Some degree of reduction in packed cell volume was also noted as evident from Table 7, 11; Plate 6; Fig. A,B,C,D and Plate 7; Fig. A,B,C,D.

The effect of hydrocortisone on blood of 30 mg. and 15 mg./kg. treated animals was almost similar. There was increase in the number of circulating erythrocyte as well as reduction in haemoglobin percentage. Leucocytopenia was significantly marked. The number of circulating neutrophils showed definite increase. Administration of hydrocortisone caused total disappearance of eosinophils from circulation as shown in Table 7, 9; Plate 6; Fig. A,B,C,D and Plate 7; Fig. A,B,C,D.

When these animals were challenged with 10,000 juveniles of H. bispinosus after hydrocortisone treatment,

they showed almost similar haemotological changes as in untreated but infected kid except that the haematological alterations were more severe. It fact, these animals did not survive the infection after 3 week. The only difference observed in hydrocortisone treated kids were that it did not show reduction of circulating erythrocytes rather there was transitory increase in the number of erythrocytes and there was no eosinophilia upto 3rd week post infection as evident in Table 7, 10; Plate 6; Fig. A,B,C,D and Plate 7; Fig. A,B,C,D.

Parasitological findings of the immunized and non immunized kids following administration of Hydrocortisone and subsequent challenge with 10,000 infective juveniles of H. bispinosus*.

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a. B. G were taken 50 day post-infection but mean is presented in the table * = + =

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Haematological findings of the non-immunized kids following administration of Hydrocortisone and subsequent challenge with 10,000 infective juveniles of H. bispinosus*.

***************************************	Kid	No.6 (control)	contr	(10		~(X)>	Kid	No.4 (No.4 (treated	d with		Kid	No. 5 (treated	(treat	ed with	
	Before Trea infection.	Trea-After I tmentiwith 1 Ctive Ctive Inst 121 Weekiw	Mith With Ctiv Of H Neek	r cha. 10,00 e juv bisp Znd week	After challenge with 10,000 infe- ctive juveniles of H.bispinosus 1st 2nd 13rd 14th week week week	4 + th	Before infec- tion.	theart theart	Trea-After challer thentwith 10,000 in the juvenil of H. Dispinos itst i 2nd k- iveek week ive	r challenge 10,000 infe e juveniles bispinosus 2nd k-3rd	infe- les les sus srd	Before Tre tion tion	Trea-(After then the thing is t		After challenge entiwith 10,000 inf tive juveniles H. bispinosus. Ist & 2nd & 3 meek	inge infec- es of s. Trd week.
Haemoglo- bin/gm.%	10.6	1	8.2	8.0	7.2	7.0	10.4	8.2	6.2	5.6	4.4	11.0	10.2	8 8	7.4	6.2
Erythro- cytes mill,	12.8	J	12.2	11.4	11.0	9.5	12.9	14.5	16.5	16.4	13.45	13.75	15.45	15.90	14.45	- 62
Leucocytes thousand/ cmm.	14.70	1	14.0	13.6	14.0 13.6 12.0 10.2	10.2	13. 15.	8.4	7.9	7.2	6.3	13.7	10.0	4.6	6.5	8
Lympho- cytes%	58	1	41	39	36	34	09	36	32	28	24	61	45	33	29	27
Neutro- phils%	35	1	45	44	46	46	34	96	59	62	64	32	46	57 5	59	61
Monocy- tes%	7	ı	0	10	10	10	9	80	6	10	12	9	6	10 1	2	12
Eosino- phils%	ı	1	4	7	ω	10	ī	1	1				•	1	ı	1
P.C.V.%	41.2	1	39.8	38.2	39.8 38.2 37.4 36.2	36.2	42.0 41.2		38.4	36.2	30.1	42.2	41.8	40.0 36.8	8.9	32.0

62

dose rate of 50 mg./kg.body weight daily to kid No. 4 and 5 for a period of 10 days. Five of the Hydrocortisone were infection per os to both kid. The controls (kid No.6) did not Hydrocortisone was administered at the dose 15 mg./kg.body weight daily to kid no. given before and 5 after the challenge receive any Hydrocortisone.

Haematological findings of the kids following immunization with infective juveniles of H. bispinosus.*

	X	Kid No.	-4				Kid No.					Kid No.	3		
	Before!	During		immunization 2nd 3rd	4th	Before	During		1 1-00	4 th	Before	During		immunization	on 4th
	tion.	week				tion.	week	week	week	eek	ction. week	week			week
	1														
Haemoglobin/ gm.%	10.6	10.4	10.6	10.4	10.6	10.2	10.0	10.0	10.2	10.0	10.4	10.2	10.2	10.4	10.4
Erythrocytes mill/cmm.	12.6	12.8	13.2	13.4	13.8	12.4	12.4	12.9	13.6	13.8	13.4	13.4	13.2	13.2	13.5
Leucocytes thousand/	13.5	14.0	14.8	14.8	14.9	12.9	13.6	13.8	14.6	14.5	11.4	14.6	15.6	15.2	14.9 -
Lymphocytes%	58	66	64	62	59	60	64	63	61	60	56	65	64	57	56 -
Neutrophils%	35	20	19	16	27	33	100	16	14	13	36	20	15	14	13
Monocy tes%	7	7	00	9	9	7	9		11	1	00	00	10	10	10
Eosinophils%	ļ	6	9	13	15	1	9	10	14	16	ı	7	11	19	20
P.C. V.%	40.2	40.8	41.2	41.6	41.6	41.0	41.8	42.2	42.2	41.2	41.0	41.2	41.4	41.4	41.6

^{*} Each kid in this group was immunized by repeated administration of H. bispinosus infective juveniles per os on every alternate day. In all each kid received a total of 10,000 infective juveniles in 16 immunizing doses, the first 7 comprised 400 and the last 9 of 800 infective juveniles fed in each dose.

TABLE 9

Haematological findings of immunized and non-immunized kids following Hydrocortisone treatment*.

	roup	reatment.	10.2	15.5	10.0	45	46	6	1.46	41.8
	Non-immunized group	tk Before kinfection	11.0	13.7	13.7	61	32	9	1	42.2
		Treatmen	8,2	14.5	8.4	36	56	ω	1	41.2
	Kin	Before infection.	10.4	12.9	13.5	09	34	9	86 T	42.0
		Trea-	8.4	15.6	11.4	44	44	12	1	38.4
	No. 2	After immun (zatio	10.0	13.8	14.5	09	13	11	91	41.2
	group Kid	Treat Before thent infection.	10.2	12.4	12.9	09	33	7	1	41.0
		Treat tment	8.6	16.8	10.2	40	50	10	1	36.8
STATE OF STREET	Immunized No. 1	After immuni- zation	10.6	13.8	14.9	59	17	6	15	41.6
0	Kid	Before After	10.6	12.6	13.5	. 58	35	7	1	40.2
			Haemoglobin/ gm.%	Erythrocytes mill/cmm.	Leucocytes thousand/cmm.	Lymphocytes%	Neutrophils%	Monocytes%	Eosinophils%	P.C.V.%

* 5th day post treatment.

Haematological findings of immunized and non immunized Hydrocortisone treated kids following challenge with 10,000 infective juveniles of H. bispinosus.

.C. V.%	osinophils%	Monocytes%	Neutrophils%	Lymphocytes%	Leucocytes thousand/cmm.	Erythrocytes mill/cmm.	Haemoglobin/gm.%	
36.8	ı	10	50	40	10.2	16.8	8.6	tment-
34.2	ı	11	54	35	8.7.	16.8	6.8	8 * 0 0 B A
34.2	1	11	58	32	. 8.5	16.2	6.0	Immun ter chal th 10,00 ive juve H.bispi stl 2nd
32.8	1		60	29	5 7.0	13.8	5.4	Immunized g No. 1 No. 1 10,000 infe e juveniles bispinosus 2nd 3rd week week
32.0	1	12	61	27	6.9	8 12.8	5.0	lenge lenge O infe- niles nosus 3rdi 4thi
38.4	1	12	44	44	11.4	15.6	8.4	Treamen
37.4	ı	13	48	39	9.7	15.7	7.6	to No
36.9	I	14	50	36	9.2	15.1	6.4	er challe h 10,000 H. bispir H. bispir k week we
W	1	13	53	34	8.5	13.9	6.2	challen 10,000 i juvenil bispino 2ndi 5r weekjwee
5.4 34.8	1	14	52	4	7.4	13.4	5.4	challenge 10,000 infectiventles juveniles bispinosus 2nd 3rd 4th weekjweekjweekj
41.2	1	00	56	36	8.4	14.5	8.2	tment
	ı	9.	59	32	7.9	16.5	6.2	I I Decoded accorded and I
38.4 36.2	ı	10	62	28	7.2	14.5 16.5 16.4 13.4	5.6	n-immunize o 4 er chall- e with 000 infec- e juvenile H.bispino- tl 2nd 3r klweeklwee
30.1	ı	12	64	24	6.3	13.4	4.4	Non-immunized No. 4 After chall- ange with 10,000 infec- tive juveniles of H. bispino- sus. *1st[2nd[3rd] week[week]
41.8	1	9	46	45	10.9	15.4	10.2	group Trea- tment
40.0	1	10	57	3 5	9.4	15.4 15.9 14.4 13.8	8.8	XXXXXXXXXX
36.8	1	12	59	29	9.2	14.4		No. 5 After chall- ange with 10,000 infe- ctive juve- niles of H. bispinosus. *1sti2nd (3r) *eek[week]week[week]
40.0 36.8 32.0	i	12	61	27	8.7	13.8	7.4 6.2 9	chall- ith infe- juve- of H. losus. nd (3rd

^{*} The animals received Hydrocortisone 5 doses after infection.

TABLE 11

Haematological findings of immunized and non immunized kids following challenge with 10,000 infective juveniles of H. bispinosus*.

	Administration of the party states	AND PROPERTY OF STREET, STREET	ending of the second se	Octobra and Person Spinster, or other Persons								
	~~	173	Immı	Immunized.	93				Non-	Non-immirad	2	
	0		Kid	No. 3		-		The same	K i	Kid No. 6	n n	
	Before !!	After immu-	After chal infective bispinosus	After challenge with infective juveniles bispinosus.	nge with	with 10,000	0	Before After challenge with infective juveniles	After	challenge ive juveni		10,000 of H.
		etion.	1st week	2nd week	3rd Week	4th week	week		Week	week.	3rd Week	4th Week
Haemoglobin/gm.%	10.4	10.4	10.2	9.6	9.1	0	0					
Erythrocytes/mill/cmm.	13.4	13.5	12.5	12.1	12.2	12.2	12.1	12.8	12.2	11.4	11.0	9.2
Leucocytes thousand/cmm.	11.4	14.9	14:0	13.2	13.88	13.0	13.0	14.7	14.0	13.6	12.0	10.2
Lymphocy tes%.	56	56	51	50	44	43	38	58	41	39	36	34
Neutrophils%	36	13	25	27	30/	32	36	35	45	44	46	46
Monocytes%	00	10	10	10	1	11	12	7	10	10	10	10
Eosinophils%	Ф	20	14	13	15	14	14	1	4	7	00	10
P.C.V.%	41.0	41.6	40.6	40.2	40.2	39.2	39.2	41.2	39.8	38.2	37.4	36.2

^{*} Untreated control.

Discussion.

Kass and Finland (1953) in reviewing the literature found that administration of either cortisone, hydrocortisone or ACTH increased the susceptibility of the host to various infective agents.

The present work showed that the administration of hydrocortisone increased the susceptibility of non-immunized and immunized kids against <u>Haemonchus bispinosus</u> infection. This was evident by establishing more number of adult parasites in the abomasum of the treated kids than that found in the controls in immunized and non-immunized kids.

The results are in complete agreement with those obtained by Weinstein (1953, 1955) in Nippostrongylus muris infection in white rats; Stoner and Godwin (1953, 1954), Coker (1955, 1956), Markell (1957) and Ritterson (1959) in Trichinella spiralis infection in mice, rats and golden hamsters, respectively; Olson (1958) and Briggs (1963) in Litomosoides carinii infection in white rats; Cross (1960) in N. dubius infection in white rats; Weinmann and Rothman (1961) in Hymenolepis nana infection in mice; Dunsmore (1961) in oestertagiasis in sheep; Parker (1961) and Ogilvie (1965) in Nippostrongylus brasiliensis infection in guinea pig and rats respectively; Olivier (1962) in Taenia taeniaeformis in mice; Campbell and Collette (1962) in Trichuris muris infection in albino mice; Mathies (1962) in Aspiculuris tetraptera infection in mice; Johnson and Hansen (1964) in Ascaridia galli infection in chicken; Michel and

Sinclair (1969) in <u>Ostertagia ostertagi</u> infection in calves and Dhar and Singh (1970) in <u>Oesophagostomum columbianum</u> in lambs.

The results revealed that the breakdown of innate and acquired resistance in kids against <u>Haemonchus bispinosus</u> are directly in relation with the amount of hydrocortisone administered to hosts.

The present study though based on the study of a small number of animals have suggested that the degree of breakdown of the innate and acquired resistance to H. bispinosus infection is possibly directly related with the amount of hydrocortisone administered to the animals. Thus, animals received hydrocortisone 30 mg./kg. body weight daily for 10 days revealed more number of established parasites in the abomasum when compared with the animal received hydrocortisone only 15 mg./kg. body weight daily for 10 days. Further, in the immunized animals the breakdown of the well established acquired immunity of kid No. 1 which received hydrocortisone 30 mg./kg. body weight daily for 10 days, as against kid No. 2 which received only half of the above dose, revealed that the degree of breakdown of acquired resistance was dependent upon the amount of hydrocortisone administered in immunized animal.

While studying the mortality and survival time of immunized mice treated with cortisone acetate, Zaiman et al (1962) reported that 25 mg./kg. caused death in mice with in 42.5 hours whereas 2.5 mg./kg. caused death in 50.4 hours. Similarly, Dhar and Singh (1970) found that cortisone acetate in dose of 50 mg./kg. body weight caused much degree of the breaddown of the innate

and acquired resistance against Oesophagostomum columbianum in lambs in comparison with that of 25 mg./kg. body weight.

Haematological investigation.

Haemoglobin and Erythrocytes.

In the present study the experimental animals fed with 10,000 larvae of H. bispinosus showed reduction of haemoglobin percentage and erythrocytopenia. The observation that H. bispinosus causes anaemia was in agreement with the observation of other workers in haemonchosis / Kholoshchanov, 1953; Andersen et al, 1960; Sahai, 1966; Silverman et al, 19707. Martin and Ross (1934) studied that 2,000 H. contortus suck a minimum of 29 ml. blood per day from the host, is itself a sufficient proof to explain the severe anaemia. The reduction of haemoglobin percentage was more marked than erythrocytopenia which suggested that probably determination of haemoglobin will be a better parameter to know the severity of Haemonchus infection. As regards the mechanism of anaemia in infected animal it appeared that Haemonchus species inhibited the synthesis of haemoglobin (Silverman et al, 1970) in individual erythrocytes and depressed erythropoiesis in bone marrow. Whitlock (1950) focused attention on the anaemia associated with haemonchosis which could be complicated by a deficiency of iron or some other nutrient essential for the function of haemopoietic system. LaPage (1956) described Haemonchus induced anaemia to the blood sucking activities of the parasites. Schalm (1950) reported

decreased erythropoiesis and Baker and Douglas (1957) observed reduced survival time of erythrocytes in the vascular system.

erythrocytosis and lowered haemoglobin percentage. Reduction in haemoglobin and increase erythrocytes in circulation was more marked after administration of hydrocortisone in dose of 30 mg./kg. body weight as compared to the animal given hydrocortisone in dose of 15 mg./kg. body weight. Similar findings have been reported by Yoffey (1954), Nicol and Bilbey (1956).

Yoffey (1954) (as cited by Nicol and Bilbey, 1956) pointed out that cortisone stimulated the production of erythrocytes in male guinea pigs. This might be true in present investigation with kids. Though the erythrocytes were produced and thrown in the circulation in greater number, the haemoglobin content was reduced which indicated the failure of haemoglobin synthesizing mechanism and not inadequate erythropoiesis.

Immunization of kids with <u>H. bispinosus</u> 3rd stage larvae did not cause any significant change either in haemoglobin percentage or in erythrocytes. However, when the immunized untreated control were challenged with 10,000 larvae of <u>H. bispinosus</u> no apperent untoward effect could be observed except a slight decrease in haemoglobin percentage and erythrocytes level and that immunity was more or less complete. The alteration in haemoglobin percentage and erythrocytes on hydrocortisone administration to immunized animals were similar to non-immunized animals, although the changes were less severe. It indicated

that administration of hydrocortisone may cause blood reactions in both immunized and non-immunized animals.

Leucocytes.

Infection of H. bispinosus larvae to non-immunized animals caused eosinophilia and neutrophilia in the present experiment. Kholoshchanov (1953) observed slight leucopenia, neutropenia, lymphocytosis and a marked eosinophilia in lambs due to H. contortus infection. However, Delaune and Mayhew (1940) and Sahai (1966) noted leucopenia, neutrophilia, monocytosis, slight eosinophilia and lymphocytopenia in calves, kids and lambs, respectively. The leucocytic reaction to H.contortus in the present experiment was very much similar to that reported In the present experiment granulocytes like by Sahai (1966). neutrophils and eosinophils increased in circulation. Both these cells are derived from a common progenitor myloblast in the bone marrow. It is quite possible that metabolites of larvae or parasites of H. bispinosus may have stimulated the proliferation of myoblast or the differentiation of myloblastic cells descendents into neutrophils and eosinophils. It is also possible that the granulocytes already formed in the bone marrow may have been pumped to the circulation in greater number due to the presence of H. bispinosus infection.

In the present study lymphocytopenia was an important feature of <u>H. bispinosus</u> infection. Lymphocytes are normally formed in germinal centres of lymphoid organs and thrown in the circulation via lymphatic channels. Since lymphoid organs like

lymph nodes and spleen were not examined in the present experiment it was difficult to speculate whether the parasites reduces the formation of lymphocytes in lymphoid organ or it causes harm to the circulating lymphocytes.

Administration of hydrocortisone to non-immunized and immunized animals induced almost similar changes in the number and proportion of leucocytes, although the changes were more severe in non-immunized animals. The main alteration were fall in leucocytic counts, lymphocytopenia, neutrophilia and total disappearance of eosinophils from circulation. This finding was in accordance with other observations of the workers (Nicol and Bilbey, 1956; Jones, 1966; French and Macfarlane, 1970).

When hydrocortisone treated immunized and non-immunized animals were challenged with 10,000 larvae of H. bispinosts, the blood alterations were very much exaggerated. It was no surprise to see changes in the number and proportion of leucocytes to be much severe in hydrocortisone treated animals because both larvae and hydrocortisone induced similar effect on circulating leucocytes. The only significant difference was that the parasites caused eosinophilia while hydrocortisone caused total disappearance of eosinophils from circulation. This effect of hydrocortisone was not altered even when larvae of H. bispinosus were fed to animals.

The immunized untreated kid (control) showed similar changes as the non-immunized kid (control) when challenge with 10,000 infective larvae but the changes in leucocyte were mild

of nature. The haematological findings were in consistance with the detection of few eggs in the faecal samples. It was interesting to note that even immunized animals showed temporary changes in the number and proportion of leucocytes which indicated that probably immunization with <u>H. bispinosus</u> wasincomplete.

The cellular response has revealed only a slight reduction in lymphocytes and neutrophils as compared with the immunized group. It is difficult to conclude whether the resistance following immunization is cell mediator or antibody mechanism, though the later seems to be unlikely as reported by Stewart, 1953, Roberts, 1957, Soulsby, 1960. A more detailed investigation could throw some light on such mechanism. The increase in the eosinophilic counts in the immunized un-treated animals suggest some participation of hypersensitivity, which was annuld by hydrocortisone therapy. Stewart (1953) and Soulsby (1960) have also reported increased hypersensitivity reaction in infection rate with H. contortus, and increase in the amount of free histamine. Therefore, the eosinophilic reactions observed in the present study agrees with the previous workers.

The exact manner in which cortisone or hydrocortisone acts is not known, although it has been shown that cortisone brings about a suppression of the inflammatory response, inhibition of antibody production and alteration of the reticulo-endothelial function (Kass and Finland, 1953). As reported by Soulsby (1968), Antibody has got no role regarding the immunity in case of <u>H. contortus</u> infection, it seems reasonable to presume

that the hydrocortisone interfered with the cellular mechanism of the host in bringing about a lowering of the innate or acquired resistance to <u>H. bispinosus</u> infection.

STUDIES ON THE IDENTITY OF HAEMONCHUS CONTORTUS

AND HAEMONCHUS BISPINOSUS BASED ON

GENETICAL DEVELOPMENT.

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STUDIES ON THE IDENTITY OF HAEMONCHUS CONTORTUS AND HAEMONCHUS BISPINOSUS BASED ON GENETICAL DEVELOPMENT.

Review of Literature.

Haemonchus bispinosus (Molin, 1860), Railliet and Henry, 1909, a stomach worm originally described from Mazama nana in Brazil and was differentiated from a well established stomach worm, H. contortus (Rudolphi, 1803), Cobbold, 1898 on the basis of variation in vulvular flap and size of spicules. Le-Roux (1929) has also described three new species of Haemonchus on the characters of spicules in males and vulvular flaps in Bhalerao (1932) refused to accept the character of variation in vulvular flaps sufficient to create species. Almeida (1933, 1935) considered H. bispinosus as "species inquirendum". Place (1893) described a new species Strongylus placei, recovered from abomasum of calf suffering from diarrhoea. He has given no description except length of the parasite 1 to This was made synonym of S. contortus by Ransom (1893) (cited by Sahai and Deo, 1964) who transfered it to the genus Haemonchus in 1911. Thereafter, Roberts et al (1954), however, upheld its validity and separated it from H. contortus on the above mentioned character viz., knob-like vulvular flap. Since, then this parasite has been retained as H. placei by Roberts and Bremner (1955), Roberts (1957) and Rao and Hiregoudar (1959).

Khera (1954) reported H. bispinosus from Cervux axis (deer) for the first time in India, on the character of vulvular

flap. Again, a similar parasite from sheep and goats were recovered at Bombay and described as H. placei by Rao and Hiregoudar (1959).

Das and Whitlock (1960) discussed the taxonomy of H. contortus based on the morphology of adult worms collected from three different geographical zone. They created one new variety and two new subspecies on the basis of vulvular flap formula and geographical distribution. They were H. contortus contortus from sheep of Queensland, Australia, H. contortus cayugensis from sheep of New York, U.S.A. and H. contortus var utkalensis from sheep and goats Orissa, India.

During a survey of <u>Haemonchus</u> species at Barailly,

Uttar Pradesh, Sahai and Deo (1964) for the first time described

H. <u>bispinosus</u> from Indian sheep and goats on the characters of
spicules in males and vulvular flaps and opening of the vulva
in females. Since, <u>H. placei</u> described by Roberts <u>et al</u> (1954)
had similar morphological characters was put a synonymous to

H. <u>bispinosus</u>.

In pure infections of <u>H. contortus</u> and <u>H. bispinosus</u> given to a lamb and a kid, revealed in them a mixture of <u>H. contortus</u> and <u>H. bispinosus</u> in the same proportion as was found under natural conditions. Still, no definite conclusion was drawn and was suggested to carry out further work to determine if they are two species or variation of <u>H. contortus</u> (Scientific Report, Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, 1963-64).

Fotedar and Bambroo (1965) obtained large number of Haemonchus from the intestine and stomach of sheep in Kashmir. They reported H. contortus var kashmirensis, a new species on the basis of morphological difference. They opined that vulvular lips were inconspicuous but a linguiform process was invariably present.

Dutt and Sahai (1966) confirmed the earlier views of Sahai and Deo (1964) that H. placei is synonymous to H. bispinosus. They also stated that there was no justification for assigning worms morphologically similar to H. bispinosus but occurring in domestic animals to a different species (H.placei) as was done by Roberts et al (1954), since in the genus Haemonchus there is little host specificity. They surprised that how several workers have ignored H. bispinosus which not only has priority over H. placei but the description of which conforms atleast in very important character, to that of their specimens. On this basis the parasite reported by Rao and Hiregoudar (1959) as H. placei and Bhatia (1960) as H. longistipes from sheep, they dropped them as synonymous to H. bispinosus. Again, Sahai (1966) studied the embryonation and morphology of different stage of H. contortus and H. bispinosus.

Rao and Rahman (1967) while studying the morphology of H. contortus, collected from sheep and goats in Banglore observed some difference in vulvular flap formula, than that described by Das and Whitlock (1960). So, they created a new variety H. contortus var bangalorensis.

Padmavathi et al (1971) reported that all the females of H. contortus exhibited linguiform vulvul appendage and all the female of the H. bispinosus exhibited knob type vulvul appendage and they bred true to their character of vulvular flaps, the linguiform and knob type respectively.

Experimental study and Results.

Three kids (Nos. 14, 15 and 16) aged about 4 months were maintained under parasitic free conditions. Two kinds (Nos. 14 and 15) were administered with 1500 infective larvae of <u>H. contortus</u> and <u>H. bispinosus</u>, respectively and third (No.16) kid was maintained as non-infected control. All the animals were sacrificed 30 days post-infection to collect the adult worms for morphological study.

The results of the experimental infection are presented in Tables 12 and 13.

One kid (No. 15), received 1500 infective larvae of H. bispinosus was autopsied and thorough search for worms reveated 62 adult parasites in the abomasum (4.13%) out of which 34 were females and 28 were males. Second kid (No.14), received the same number of infective larvae of H. contortus revealed 78 (4.53%) parasites in the abomasum on autopsy. Out of 68, 42 were females and 26 were males.

All the female worms of <u>H. bispinosus</u> infection exhibited knobbed type vulvular flaps with the opening of vulva at the anterior end of the vulvular process whereas in <u>H. contortus</u> the vulvular flaps were invariably linguiform type. Control kid (No.16) did not develop any infection.

The measurements of the adult worms and egg collected from these worms are given in Table 13.

TABLE 12.

Showing the percentage of parasites observed in kids following challenge with 1500 infective juveniles of H. contortus and H. bispinosus.

kids.	Number of infective larvae dosed.	tion.		tological Females	Males	Perc- lenta- lge.
14	1 500	H. bispinosus (knob type).	62	34	28	4.13%
15	1 500	H. contortus (linguiform type).	68	42	26	4.53
16*	ra t	Late of Form, e of prevent	-	inch, m and sto	ee 5 17/	

PROFES TO

^{*} Non infected control.

TABLE 13

Showing the measurement (in mm.) of adult females, male, and eggs of <u>H. contortus</u> and <u>H. bispinosus</u>.

Observation (Haemonchus of Measurement Range		Haemonchus Measurement Range	National Committee of the Committee of t			
Females	ni na steunt						
Total length.	20.200 to 29.400	24.120	21.350 to 27.400	25.200			
Width.	0.244 to 0.364	0.294	0.294 to 0.425	0.365			
Length of oesophagus.	1.080 to 1.680	1.520	1.090 to 1.620	1.420			
Length from cervical papillae to anterior extermities.	0.410 to 0.540	0.440	0.290 to 0.420	0.380			
Type of vulval appandage.	Linguiform conspicuou		Knob, short and stumpy.	t No t (pal)			
Males		with voter t					
Total length.	13.980 to 17.840	16.210	14.200 to 18.600	14.900			
Width.	0.180 to 0.250	0.214	0.180 to 0.310	0.250			
Length of oesphagus.	1.250 to 1.460	1.340	1.260 to 1.610	1.320			
Length from cervical papillae to anterior extermities.	0.310 to 0.410	0.380	0.320 to 0.380	0.350			
Length of gubernaculum	0.192 to 0.198	0.194	0.125 to 0.194	0.164			
Length of the stem of Y shaped dorsal ray.	0.085 to	0.087	0.082 to 0.094	0.088			
Length of spicules. Eggs.	0.410 to 0.430	0.425	0.432 to 0.471	0.452			
Length.	0.062 to	0.072	0.065 to	0.078			
Width.	0.081 0.032 to 0.051	0.037	0.082 0.038 to 0.057	0.042			

Discussion.

Identity of Haemonchus contortus and Haemonchus bispinosus is still not clearly established. LeRoux (1929) created three new species of Haemonchus on the characters of spicules in males and on situation of vulvular opening and variations in vulvular processes in females. But, Bhalerao (1932) did not attach any importance to the variation of vulvular process for differentiation of species. Roberts (1941) observed tongue-like and knob-like vulvular processes in the specimens, belonging to the genus Haemonchus, obtained from sheep and cattle, respectively and also in mixed infection. He attributed the reduction of tongue-like process to a knob either as an evolution of H. contortus in two species or as some physiqogical difference between cattle and sheep. On the situation of vulva anterior to the short knob-like vulvular process, Molin (1860) created Strongylus bispinosus which was later transferred to the genus Haemonchus by Railliet and Henry (1909). Place (1893) recovered a similar parasite from calf suffering from diarrhoea and named it as Strongylus placei without giving any detail description. Ransom (1893) made this parasite synonymous to S. contortus and later in 1911, he transferred it to the genus Haemonchus. Roberts et al (1954), however, maintained its validity and separated it from H. contortus on the basis of knoblike vulvular process. Sahai and Deo (1964) and Dutt and Sahai (1966) have emphasised that H. placei should be regarded as a synonym of H. bispinosus. Dutt and Sahai (1966) opined that

Roberts et al (1954) erred in naming the species as H. placei.

Das and Whitlock (1960) created two new subspecies and one new variety of H. contortus on the basis of vulvular flap formula and geographical distribution. Specimens, recovered from sheep of New York, U.S.A. having under 30% linguiform, under 15% knobbed and over 60% smooth vulvular flaps, have been described as H. contortus cayugensis, where as recovered from sheep of Queensland, Australia, having over 80% linguiform, under 10% knobbed and under 10% smooth vulvular flaps, have been described as H. contortus contortus. Specimens collected from sheep and goats of Orissa, India, having 20-55% linguiform, 40-60% knobbed and under 20% smooth vulvular flaps, were named as H. contortus var utkalensis. It is surprising that Das and Whitlock (1960) who had cited Almeida (1935), should have ignored H. bispinosus which is metioned above has been created on the basis of situation of vulva and vulvular flap.

Pure infection of <u>H. contortus</u> and <u>H. bispinosus</u> (based on vulvular flaps as described by Sahai and Deo, 1964), was given to a kid and a lamb maintained in the Animal shed of the Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, revealed that these species are not true to their characters, since both kid and lamb had mixed type of vulvular flaps. Still, no definite conclusion on validity of these species were given (Scientific Report, Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, 1963-64). Rao and Rahman (1967) have collected specimens of

Haemonchus from sheep and goats slaughtered at Banglore and described a variety of H. contortus var bangalorensis on the basis of vulvular flap formula, which was 67.9% linguiform, 32.1% knobbed and 0% smooth. Padmavathi, Reddy and Venkataratnam (1971) have described H. contortus and H. bispinosus two distinct species on the characters of their vulvular flap. They have also observed that H. contortus and H. bispinosus bred true to their characters when pure artificial infection was given to two different lambs separately.

In view of these confusions regarding validity of H. contortus and H. bispinosus in the present investigation infective larvae of these species were raised in faecal cultures, separately and were given to two different kids with one non-infected control. These kids were sacrificed 30 days post-infection and worms recovered separately, were examined. The non-infected control kid had no worm. The knobbed and linguiform vulvular flaps were found to breed true to their character, quite in agreement of with Padmavathi et al (1971). So, genetically also it has been established that H. contortus and H. bispinosus are two distinct species, though mixed infection in nature is quite common.

The present study is also suggestive of that the various new subspecies and new varieties of <u>H. contortus</u> described by Das and Whitlock (1960) and Rao and Rahman (1967) may be referable to <u>H. bispinosus</u>, as considered by Padmavathi <u>et al</u> (1971).

ANTHEIMINTIC EFFICACY OF WOPELL AGAINST HAEMONCHOSIS IN GOATS.

ANTHEIMINTIC EFFICACY OF WOPELL AGAINST HAEMONCHOSIS IN GOATS.

ANTHELMINTIC EFFICACY OF WOPELL AGAINST HAEMONCHOSIS IN GOATS.

General.

Although, extensive literature is available on anthelmintics against gastro-intestinal nematodes, only a few reports
are available on indigenous drug as an anthelmintic. Garg and
Mehata (1958) reported <u>Butea frondosa</u> (Palas) a superior anthelmintic than that of copper sulphate against <u>Haemonchus similis</u>
and <u>Bunostomum trigonocephalum</u> infections.

The Indian Herbs Research and Supply Co., Saharanpur, Uttar Pradesh, has produced in anthelmintic, "Wopell" from indigenous products. The ingredients are:

Kamala powder. 2 gm.

Pulv. Babarang. 2 gm.

Pulv. Palas Papra. 2 gm.

Pulv. Areca nut. 2 gm.

Pulv. Malefern. 2 gm.

The firm has recommended its use as an anthelmintic against common roundworms and tapeworms of all domestic animals.

Some reports are available for the individual ingredient of "Wopell" here and there. Birdwood (1936) tested

Embelia ribes (Babarang) and Mallotus philippinensis (Kamala)

against tapeworm infection and found them good anthelmintic

against tapeworm infection. In another trial, he observed Pulv. Arecanut as a good vermifuge. Mukerji and Bhaduri (1947) tried Butea frondosa (Palas), Embelia ribes (Babarang) and Mallotus philippinensis (Kamal) against hookworms, ascaris, Taenia and Hymenolepis infections in man and observed none of the drug effect against hookworms and tapeworm infections. However, they found Butea frondosa and Embelia ribes better than santonin and equally good to that of oil of chenopodium in the treatment of ascariasis. Garg and Mehta (1958) tested anthelmintic efficacy of the decoction of seeds of Butea frondosa and Embelia ribes against B. trigonocephalum and H. similis, in vitro. They observed Butea frondosa superior and Embelia ribes inferior to that of Copper sulphate solution against these infections. According to Gibson (1965) none of the substances (Areca nut, Kamala and Malefern) have been critically examined, though they are recommended for treatment of tapeworm infection in horses.

This product is new one and not much experimental trials are available in literature. However, Nanda (1971 - personal communication) has tried it in 15 calves, 2 buffaloes and one bullock suffering from ascariasis and found "Wopell" as a good vermicide.

The present study was aimed to investigate the anthelmintic action of "Wopell" against Haemonchosis in goats and result are reported hereunder.

Experimental Studies and Results.

Four non-descript type of kids aged about 10 weeks were maintained under parasitic free condition. They were provided an adequate and identical diet. All the kids were infected with 4000 infective larvae of H. bispinosus and H. contortus (Mixed infection). In order to find out the intensity of infection faecal egg count were made.

All the four kids (No. 9, 10, 11 and 12) infected with Haemonchus species were examined. Egg counts were made twice on 26th and 30th day of infection. On 31st day of infection "Wopell" in doses of 2 gm./kg.body weight was given orally to three kids (Nos. 9, 10 and 11) and one kid (No.12) was maintained as control. Three days after the administration of "Wopell", egg counts were found to be reducing in progressive manner whereas egg count of control kid was seen increasing. On the 7th day of post-treatment one kid (No.9) was sacrificed whereas other kids (Nos. 10 and 11) were given second dose of the "Wopell" (2 gm./kg. body weight). These kids (Nos. 10 and 11) also showed progressive reduction in egg count once again. On 15th and 16th days of initial treatment the rest two treated kids (Nos. 10 and 11) were sacrificed. Control kid (No.12) was also autopsied on 47th day of infection.

One kid (No.9) which received single dose of "Wopell" revealed 22.05% of dead parasites, whereas other kids (Nos. 10 and 11) which received the drug in two doses showed 41.05 and 32.5% dead parasites respectively. Control kid (No. 12)

Showing the efficacy of "Wopell" against haemonchosis in goats.

	~ 88 ~					
	Percentage of dead parasites	00	22.05	41.05	52.5	0.0
	No. of stra-		09	117	145	l lenotani
	Total Total Transoctal Transoctal	0	272	285	276	280
	Duration be tween infectio and auto psy (in days)		100 100	45	46	47
	Duration Duration of the between the treatment infection of the sand auto-land auto-sand auto-sa	×	C 0114	15	9	
	Duration be infect on and treatment (in days)	*	25	31	31	omy 35
	4		05 and	135	124	1424
	+ a dent + dent		1	224	196	1296
	treatmen in days +6 days	d	325	440	320	1248 1296
	Post-treatment faces in days) + 5 + + + + + + + + + + + + + + + + + +	en F	648	548	425	
	and ream		850	872	915	724 1040
×	7 0 7 0 9 0 9 0 1		648	728	784	428
	Fre-Route quen -cy.		Per	Peros	Peros	i
+ 1 0 m + 0 0 m ft	even en e		F % .	0	0	ı
	Do		2 gm/ 1 kg.body weight.	66	6 6	1
4.4	N O	(o	10	11	12*

* Control kid.

revealed all the worms alive as evident from Table

Discussion.

Haemonchosis in goat "Wopell" did not prove to be 100% efficacious but it provided only the clinical improvement in goats which is evident by the fact that both dead and living parasites were recovered from the abomasum of the treated kids.

Faecal egg counts, as evident from Table have not become nil in these kids but it has considerably reduced in treated kids in comparison with control. It is evident from the above mentioned Table 14 that the kid No. 9 which received single dose of 2 gm./kg. body weight of the drug showed only 22.05% dead worm while the other kids (Nos. 10 and 11) which received double the above dose showed 41.05 and 52.5% dead worm respectively.

So it is apparent that "Wopell" indigenous drug is an effective anthelmintic against heamonchosis in goats and can be recommended for field treatment. But its efficacy increases only when given in repeated doses, though 100% efficacy could not be achieved.

SUMMARY.

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SUMMARY.

INCIDENCE AND NATURE OF HELMINTHIC INFECTIONS IN GOATS.

A survey of the helminth parasites on 162 non-descript goats was conducted. 98.7% of the goats were found to be infected with one or more parasites. The percentage of infection of trematodes, cestodes and nematodes were 74.6, 31.4 and 81.5, respectively.

Five species of trematodes were recovered in the present study; Cotylophoron cotylophorum (47.5%), Gastrothylax crumenifer (32.09%), Fasciola gigantica (11.8%), Carmyerius spatiosus (1.2%), Ceylonocotyle spp. (0.6%).

Similarly the five species of cestodes were also encountered. Among them two were larval forms <u>Cysticercus tenui-collis</u> (24.5%) and hydatid cyst (1.85%). Others <u>Moniezia expansa</u> (23.4%), <u>Stilesia globipunctata</u> (12.9%) and <u>Echinococcus granulosus</u> (0.6%).

Six nematodes were recovered; <u>Haemonchus contortus</u> (74.6%), <u>Haemonchus bispinosus</u> (67.2%), <u>Oesophagostomum columbianum</u> (50.0%), <u>Trichuris ovis</u> (48.1%), <u>Trichuris globulosa</u> (27.1%) and <u>Oesophagostomum venulosum</u> (14.4%).

Carmyerius spatiosus, Ceylonocotyle spp., Stilesia globipunctata and Echinococcus granulosus are being reported for the first time from goats in Bihar. In the present study goat was found to be a new host for Echinococcus granulosus.

EFFECTS OF HYDROCORTISONE ON NATURAL AND ACQUIRED RESISTANCE IN KIDS TO THE NEMATODE HAEMONCHUS BISPINOSUS (MOLIN, 1860) RAILLIET AND HENRY, 1909.

The administration of hydrocortisone in doses of 30 mg. and 15 mg./kg. body weight for a period of 10 days was found to reduce both natural and acquired immunity against <u>Haemonchus</u> bispinosus in goats. However, the degree of breakdown of resistance was seen to be directly related with doses of hydrocortisone.

The breakdown effect was attributed to marked decreased in lymphocytes percentage and leucocytic counts with complete absence of eosinophils and also with increased number of erythrocytes. Thus, the effect of hydrocortisone in reducing the resistance might be due to an interference in cellular defence mechanism of the body.

STUDIES ON THE IDENTITY OF HAEMONCHUS CONTORTUS AND HAEMONCHUS BISPINOSUS BASED ON GENETICAL DEVELOPMENT.

Haemonchus contortus and Haemonchus bispinosus were identified on the basis of their vulvular flaps and faecal cultures from eggs collected by dissecting Haemonchus contortus and Haemonchus bispinosus separately, were prepared. Thus, different kids were infected with infective larvae of Haemonchus contortus and Haemonchus bispinosus, separately, which on autopsy revealed that these worms bred true to their characters. Thus, it was seen that genetically also these species are different, as was found morphology.

ANTHEIMINTIC EFFICACY OF "WOPELL" AGAINST HAEMONCHOSIS IN GOATS.

An indigenous drug "Wopell" was tried against haemon-chosis proved to be satisfactory but its efficacy was observed only when given in two doses @ 2 gm./kg. body weight each dose.



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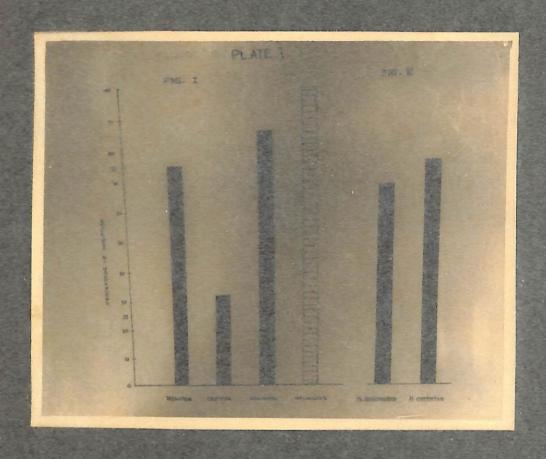
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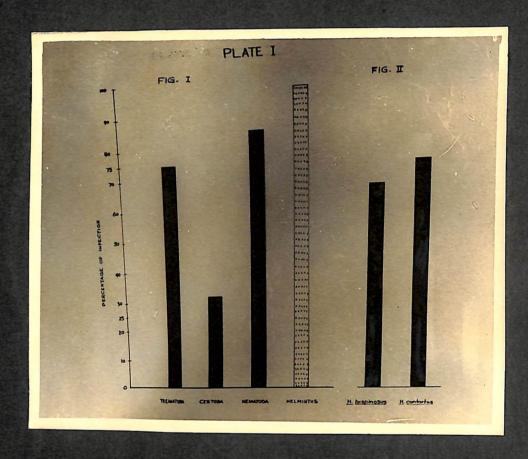
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PLATES

PLATES







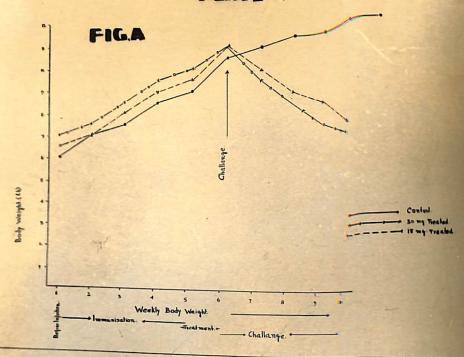


FIG.B

