Studies on

PROTOZOAN PARASITES OF PIGS WITH SPECIAL REFERENCE TO EXPERIMENTAL TRYPANOSOMIASIS CAUSED BY *Trypanosoma evansi* (Steel, 1885)

●

A THESIS

Submitted to the Faculty of

Veterinary Science and Animal Husbandry,

Magadh University,

In Partial Fulfilment Of The Requirements For The Degree Of

MASTER OF SCIENCE (VETERINARY)

By

Raj Nandan Prasad Sinha,

POST-GRADUATE DEPARTMENT OF PARASITOLOGY

BIHAR VETERINARY COLLEGE, PATNA.

DECEMBER, 1967.

Studies on

PROTOZOAN PARASITES OF PIGS WITH SPECIAL REFERENCE TO EXPERIMENTAL TRYPANOSOMIASIS CAUSED BY *Trypanosoma evansi* (Steel, 1885)

●

A THESIS

Submitted to the Faculty of

Veterinary Science and Animal Husbandry,

Magadh University,

In Partial Fulfilment Of The Requirements For The Degree Of

MASTER OF SCIENCE (VETERINARY)

By

Raj Nandan Prasad Sinha,

POST-GRADUATE DEPARTMENT OF PARASITOLOGY

BIHAR VETERINARY COLLEGE, PATNA.

DECEMBER, 1967.

SHRI L. N. MANDAL, B. V. Sc. & A.H., M.Sc.(Vet.), P.G.,
Professor of Food Hygiene & Dairy Science

&

Incharge Post-graduate,
Department of Parasitology,
Bihar Veterinary College, Patna.

Patna,

Dated 24th Dec., 1967.

Certified that the work described in this Thesis entitled " STUDIES ON PROTOZOAN PARASITES OF PIGS WITH SPECIAL REFERENCE TO EXPERIMENTAL TRYPANOSOMIASIS CAUSED BY Trypanosoma evansi (Steel, 1885) " is the bonafide work of RAJ NANDAN PRASAD SINHA, carried out under my guidance and supervision.

L mandal
24/12/67

(L. N. MANDAL)

C O N T E N T S

A C K N O W L E D G E M E N T S

The study in this thesis was made under the guidance and supervision of Shri L.N.Mandal, M.Sc.(Vet.), P.G., Professor of Food Hygiene and Dairy Science and Incharge Post-graduate Department of Parasitology, Bihar Veterinary College, Patna. The author expresses his deep sense of gratitude and indebtness for the encouragement and constant guidance.

The author is highly grateful to Dr. A.K.Verma, Ph.D. (London), Ex-Director, Livestock Research Station, Bihar, Patna for the valuable advice made available from time to time.

The author expresses his gratefulness to Dr. H. D. Srivastava, D.Sc., Head of the Division of Parasitology, I.V. R.I., Izatnagar, for supplying laboratory strain of T. evansi to carry out this experimental work.

Thanks are due to Shri R.K.Choudhary, B.V.Sc. & A.H., D.T.V.M.(Edin.) and Shri B.N.Prasad, B.V.Sc. & A.H., for their kind help in day to day laboratory work.

The author is deeply indebted to the Government of Bihar for financial assistance during the tenure of this work.

The author is also grateful to Mr. P.B.Kuppuswamy, B.A., G.M.V.C., B.V.Sc., P.G.(N.Z.), M.S.(Missouri), Principal, B.V. College and Heads of Various Department of this institution for providing necessary facilities, in doing the work.

R. N. P. Sinha.

INTRODUCTION

The antiquity of pig is evidenced by mention of it in mythology and religion. There are evidences of Indus people consuming pork as food in early civilization of Mohenjodaro in India. Later, in the Vedic and Pauranic period, swine has also figured in mythology and religion. Nowadays, porcine species is regarded as one of the most useful and valuable of the domestic animals being prolific breeder, good scavenger, and with its small omnivorous stomach well adapted for concentrated feeding. It excels all other animals in its feed conversion efficiency and it is said to increase one pound in live weight for every three and a half pounds of food consumed.

Pig industry is regarded as most economical as there is no wastage at any stage. Meat is utilized for consuming as raw pork and bacon, ham, sausages etc. are made out of the surplus. Non-edible offals are rendered for production of fat, animal feeds and fertilizers. Long back bristles of the pig make useful brushes of all sorts, whilst shorter hairs are utilized for making cushions and upholstery. Medicinal preparations are made out of the endocrine and other glands.

The importance of such a useful and economical industry was not recognised in India until recent years. Now, Government of India with the help of State Government has established two Bacon factories with regional pig breeding farm attached to each, one at Andhra Pradesh and the other at Ranchi. In India, pigs are maintained mostly in villages and suburbs of towns in most unhygienic conditions with a very poor production. Most of

the byproducts are wasted and not utilized due to lack of proper facilities to render them.

Efforts are being made to popularise and improve the pig industry by opening of pig breeding farms, with free distribution of boars and sows in the villages with monthly premium in cash to give incentive to the people for maintaining them on scientific lines. But, such a valuable industry is put to loss due to the nutritional deficiencies, bacterial, viral and parasitic diseases, to which they suffer most. Among them, the pigs are most vulnerable to suffer from parasitic diseases due to their scavanging habits. Protozoan parasites of pig also cause great loss to this industry. The common diseases of swine caused by protozoan parasites are balantidiosis, coccidiosis, amoebiasis, trypanosomiasis, eperythrozoonosis, toxoplasmosis and sarcocystosis; but of these diseases only coccidiosis, blantidiosis, amoebiasis and sarcocystosis are said to occur in India.

In India, no significant work has been undertaken regarding the protozoan parasites of pig except that of Ahluwalia (1959) and Gill (1960), who reported for the first time, the species of Coccidia occurring in swine. Although, most of the protozoan parasites donot necessarily kill the animal, but make them debilitated thereby, increasing the cost of production on one hand and depreciating their market value on the other. The occurrence of Balantidium coli, Entamoeba histolytica and Entamoeba coli are of great Zoonotic importance and have got great epidemiological significance due to the close association between human and swine population.

Pigs acts as a reservoir host, which pollutes the human surroundings and disseminate the infective materials on the ground.

According to 1966 Census, Bihar State has pig population of 6,64,248. Practically, no work has been done on the protozoan parasites of pig in this State, except the work of Sinha (1963), who studied the incidence of Coccidia and B. coli in pigs.

Taking into consideration the losses of the livestock caused by Trypanosoma evansi in India, the possibility of pig as carrier host of this organism was studied in this observation. Previously, only few workers in India and abroad took up this problem. In India, only Baldrey (1910) artificially infected Indian pig with T. evansi to demonstrate the possibility of pig being a carrier host of surra.

The present study was done with a good number of deshi and white yorkshire pigs to show the incidence of protozoan parasites, and to explore the possibility of pig acting as reservoir host of T. evansi for other domesticated animals. During this observation five sets of piglets were also artificially infected with a strain of T. evansi to study the clinical symptoms, prepatent period, parasitaemia, latent period and haematological studies.

REVIEW OF LITERATURE

COCCIDIA

Swine Coccidiosis was first reported by Rivolta (1877), who attributed to Eimeria Zurnii a severe enteritis which were epizootic among pigs (Novicky, 1945). It was pointed out by Biester and Murray (1929) that the literature dealing with Coccidiosis in swine prior to 1929 was interesting, inspite of its inadequate description and the confusion prevailing with regard to classification. Douwes gave the first accurate description of the swine Coccidiosis in his Inaugural Dissertation (1921). It was named as Eimeria deBliecki, which was corrected later on to Eimeria debliecki. He pointed out that a large and small variety occurred, but he did not assign any name for the larger variety. From the descriptions of Biester and Murray (loc. cit.), E. suis described by Noller (1921) and E. brumpti described by Cauchemez (1921), are taken as synonyms on the basis of priority.

Henry (1931) described three new species of Coccidia occurring in pigs in California in addition to E. debliecki. He named them E. scabra, E.perminuta and E.spinosa. He differentiated these species from E. debliecki by studying their morphological characters with size differences and sporulation time. Occurrence of these species was also confirmed by other workers in other countries.

Gallio-valerio (1935) from Switzerland reported the fifth species and named it E. scrofae. Pellerdy (1949) from Hungary described the sixth species and named it E.polita. Species of genus Isospora i.e. Isospora suis was described

by Biester and Murray (1934).

In India, Ahluwalia (1959) reported the occurrence of E. deblieckl. Gill (1960) has reported, for the first time, the occurrence of the species; E. scabra, E. deblieckl, E. perminuta, E. spinosa, E. polita and Isospora suis. He found two varieties of E. scabra and named them as E. scabra var scabra and E. scabra var ellipsoidalis. Sinha (1963) has indentified three species; E. deblieckl, E. scabra and E. perminuta and described their morphology. Mishra (1967) in his investigation has found four species; E. deblieckl, E. polita, E. perminuta and I. suis.

Morphology :- Henry (1931) has described the morphology, sporulation time and size range of the oocysts of Eimeria. He measured the length and breadth of the oocysts and sporocysts. The ovoidal oocysts of E. deblieckl measured 12.8 to 28.8/u in length and 12.8 to 19.2/u in width. The wall of the oocyst was double contoured and colourless. The size of the oocysts of E. scabra ranged from 22.4 to 35.6/u in length and 16.0 to 25.6/u in width. The oocystic wall had a characteristic brown colour and measured 1.5 to 2/u in thickness. The sporocysts measured 16 to 19.2/u in length and 6.4/u in breadth. The presence of stieda's body, sporocystic residual material and micropyle was also demonstrated. The oocysts of Eimeria perminuta were spherical or ovoidal in shape and measured 11.2 to 16.0/u in length and 9.6 to 12.8/u in breadth. The colour of the wall was yellow. The size of oocysts of E. spinosa varied from 16.0 to 22.4/u in length and 12.8 to 16.0/u width.

The cysts were brown in colour and entire surface of the cyst wall was studded with spines. The size of sporocysts varied from 9.1 to 11.7/u in length and from 5.2 to 6.5/u width. Pellerdy (1949) described the morphology of E. polita. The ellipsoidal oocysts measured 17 to 36 x 13 to 24/u in dimensions. The size of the sporocysts were 15 to 19 x 6/u.

Balantidium

Balantidium coli is of great medical and veterinary importance. It parasitises man and animals and produces disease called balantidiosis. It is transmissible from man to pig and *vice versa*. The disease has been of great vital importance due to the close association between man and pig. This parasite was first discovered in man by Malmsten (1857) from two dysentery patients in Stockholm, who called it *Paramoecium* (?) *coli*. It was Stein (1863), who placed this parasite in genus *Balantidium*. As stated by Wenyon (1926) *Balantidium* was first observed in pigs by Louckart (1861 a & 1863). This observation of the parasite was confirmed by many other workers. Hegner (1924) reported *Balantidium spp.* in the intestine of Maryland sheep. Hegner (1934) described six new species of *Balantidium* from parrots, monkeys, ostrich and camel. Dikmans (1948) first reported the presence of this parasite probably *B. coli* from a dog in North Carolina. Kennedy and Stewart (1957) has reported a case of balantidial dysentery in man in Northen Ireland. He also conducted survey of healthy pigs, which were slaughtered in Belfast and found that the incidence of *B. coli* was 74%. Jayasuriya (1959) recorded the incidence of balantidiosis amongst animals for the first time in Ceylon. Appasov (1960) in Russia, found this parasite common in pigs between the age of 2 months and 7 years. He examined the faeces of human beings including the piggery workers and found that the incidence of this parasite was most common in piggery workers. Ewing and Bull (1966) found severe chronic canine

diarrhoea associated with Balantidium - Trichuris infection.

In India, Sinton (1923) recorded the first human case suffering from Balantidium infection. Knowles (1928) has made an comment that monkeys and pigs in India, are very commonly parasitised with Balantidium. Biswas and Kanungo (1959) and Patnaik (1960) from Orissa, has reported chronic dysentery in cattle associated with this parasite. Sinha (1963) has reported high incidence of this parasite in domestic pigs in Bihar, for the first time. Mishra (1965) has also reported incidence of this parasite in pigs at Mathura.

Morphology :- According to Wenyon (1926), Brumpt (1909) and Walker (1913) gave the experimental evidence regarding the identity of porcine and human Balantidia. McDonald (1922) showed that two species of this parasite occurred in swine, B. coli and B. suis. B. suis has more slender body and straight macronucleus. It is specific to pig and not transmissible to man, whereas B. coli of pig is transmissible to man and vice versa. Hegner (1934) discussed the differentiation of the species of Balantidia by measurement of length and breadth of the body and macronucleus of the vegetative forms. He described that B. coli has an average length of 70.9/u and a breadth of 58.9/u, the ratio of breadth and length being 1:1.2. B. suis has an average length of 74.1/u and a breadth of 52.2/u, the ratio of breadth to length being 1:1.42. He concluded that Balantidium from pigs may belong to two species, B. coli and B. suis, but conclusive evidences are still lacking. Levine (1940, 1940 a) has pointed out that Balantidium from swine

changes dimensions upon cultivation and a single strain could resemble B. coli, if it was full fed and B. suis, if it was starved. Lamy and Roux (1950) studied the clone culture and found both B. coli and B. suis from a culture started with a single organism. He considered suis forms to be conjugant and coli forms trophozoites (quoated by Levine, 1961). Auerbach (1953) concluded from his cytological and cultural studies that the two forms B. coli and B. suis were not different species. Levine (1961) has said that B. suis is a synonym of B. coli.

Sinha (1963) studied the morphology of the parasite by measuring 40 trophozoites from 4 pigs. The length of the trophozoites varied from 66 to 88/u and breadth from 48 to 66/u. The macronucleus was sausage shaped and measured 22 to 34 x 9 to 12/u in dimensions. The cysts were spherical in shape and measured 34 to 46/u in diameter. He concluded that the specimens were of B. coli only. Mishra (1965) also found only B. coli in his study.

Entamoeba

Smith (1910) in America discovered amoebae in sections of intestinal ulcers of large intestine of pigs. Prowazek (1912) gave the description of Entamoeba polecki from pigs. The nucleus was round, globular and possessed a clearly marked nuclear membrane. The endosome was generally eccentric in position and surrounded by a chromatin ring. Hartmann (1913) examined the material sent by Smith and proposed the name Entamoeba suis of the pig amoebae, and admitted its identity with Entamoeba polecki. Nieschulz (1924) accepting the description of Entamoeba suis, described a new species from pig and called it Entamoeba debliecki. Kessel (1928) accepted the name E. polecki, but he believed that E. suis and E. debliecki are both the same as E. polecki. Kessel (1928) successfully infected pigs with Entamoeba histolytica from human source. He also found Entamoeba coli in natural and experimental infection in pigs. Frye and Meleney (1934) found the occurrence of E. histolytica, E. polecki, E. suis, E. debliecki, Endolimax nana and Iodamoeba butschlii in pigs. Hoare (1940) agreed to recognise Entamoeba suis and E. debliecki as valid species. But Noble and Noble (1952) described E. suis from pigs and disagreed with the opinion of other workers, who have reported two species of Entamoeba from pigs on morphological grounds. Hoare (1959) has reviewed the literature on Entamoeba of pigs and considered large forms as E. suis and small forms of Entamoebae to be a separate species, Entamoeba debliecki. Levine (1961) has agreed with the opinion of Noble and Noble (loc. cit.) and described E. suis. Mishra (1967) found single

nucleated amoebic cysts from piglets and described them as E. suis.

Morphology :-

Entamoeba suis - Hoare (1959) has given the size of the cysts of E. suis as 12 - 15 microns. According to Levine (1961) the size of cysts varies from 4-17 microns in diameter. The nucleus varies in appearance. The endosome is central and large. There is a homogenous ring of peripheral chromatin within the nuclear membrane. Chromatoid bodies are present in the shape of rods and irregular granules.

Entamoeba coli - According to Levine (1961) trophozoites measure 20 - 30 microns in diameter. The nucleus has an eccentric endosome larger than that of Entamoeba histolytica and a row of chromatin granules around its periphery. The cyst measures 10 - 33/u in diameter and has 8 nuclei when matured. The cysts contain slender splinter like chromatoid bodies.

Trypanosoma

Historical account :- Valentin of Bruce discovered first trypanosome in the blood of Trout (Salmofario) in 1841. It was Gruby (1843) who introduced the name Trypanosoma for the parasites of frog. Gros (1845) found trypanosomes in the blood of mammalian host in Russia for the first time. Chaussat (1877) found trypanosomes in the black rat but mistook them as nematode larvae. Lewis (1878) described the common trypanosome of rat in Calcutta naming it Trypanosoma lewisi. Evans (1880) reported the first pathogenic trypanosome causing "Surra" in the Indian livestock. He found the parasite in the blood of horses, camels and mules suffering from "Surra" in Dera Ismail Khan (Punjab). He mistook this parasite for Spirillum, but later on he recognised it to be a flagellate. He succeeded in producing the disease in dog and camel by subcutaneous inoculation of organisms. Steel (1885) found similar organisms in the blood of mules in Burma and named them Spirochaeta evansi. He also transmitted the disease in monkey and dog. Crookshank (1886) in London examined the blood films sent by Evans and described in detail the chief characters of the parasite and changed the name T. evansi (Steel, 1885). Trypanosoma theileri was also recorded in the blood of cattle by Lingard (1904) and Rao and Mudaliar (1934). Stirling (1921) found trypanosomes in the blood of bullock in India and regarded them as Trypanosoma congolense; and Mudaliar (1945) described a small trypanosome from the blood of female buffaloe and called it T. evansi var ravi; but their findings have not been

confirmed as yet.

Surra is an important disease of Livestock in India, mostly found in Punjab, United Province, Central Province, Rajasthan, Andhra Pradesh, Madras, Bombay, Bengal, Assam and Bihar as reported by Lingard (1899), Chetti (1922), Cross and Patel (1922), Ajawani et al (1933), Swaminathan (1933 - 1934), Rao and Mudaliar (1934), Mahajan (1934), Rajagopalan (1937), Mudaliar and Ray (1947), Raju and Swaminathan (1947) and Manjrekar (1950).

Susceptible hosts :- This disease is known to occur in horses camels, mules, elephants, cattle, buffaloes and rarely in dogs, fox, tiger, goats and pigs. In cattle and buffaloe surra takes hyperacute as well as chronic form. In hyperacute form it is very virulent and generally occurs in the form of outbreaks causing heavy mortality up to 90%. In milder type the animal, though apparently healthy, shows parasites in peripheral circulation at varying intervals and act as reservoir host. In horses, surra is extremely fatal. In camel, the disease runs a chronic course and the duration may be as long as three years; hence it has been called "Tribarsa". Wenyon (1926) reported that cats and pigs are also susceptible to T. evansi infection. Boehringer and Boehringer (1960) found pigs susceptible to T.equinum infection.

Experimental infection, Prepatent period and clinical Symptoms :- Experimentally, surra has also been proved

pathogenic for number of other mammals like mice, bat, guineapig, rabbit, cat, sheep and pig. (Laveran and Mesnil, 1907; Knowles, 1927; Krijgsman, 1933; Kraneveld and Mansjoer, 1947; Ray and Harbans, 1948; Yutuc and Sher, 1949; and Castillo and Joaquin, 1955).

Baldrey (1910) inoculated a pig with 2 c.c. of blood from a surra pony. Trypanosomes appeared in pig's blood five days afterwards; remained present for five days and then disappeared. They reappeared again 18 days after inoculation and remained for three days. They remained absent for two months after this last reappearence. The pig did not show any clinical symptoms and any appreciable rise of temperature throughout the observation. During the latent period a guineapig was inoculated with 1 c.c. of blood from the pig which was killed, gave a positive result after an incubatory period of 18 days. Kuppuswamy (1941) inoculated two pigs with surra blood which failed to take up the infection. Dejesus et al (1949) inoculated pigs with T. evansi and found that surra ran a chronic course and the trypanosomes appeared in the blood intermittently. They did not find any clinical signs, other than an elevation of temperature in pigs. They also observed that pigs act as reservoir host of surra. Cabrera and Lui (1956) artificially infected five pigs with T. evansi and did not find any visible signs of disease in the infected pigs. Soltys (1963) showed that pigs are highly resistant to infection with T. evansi.

Haematology :- Laveran and Mesnil (1907) observed in dogs suffering from "Nagana" (caused by T. brucei), diminution

in the number of red blood corpuscles with the first appearence of the trypanosomes. Usually the red blood corpuscles appeared normal but there were at times changes in shape (polychromatophilia). The red blood corpuscles were deficient in haemoglobin . There was increase in the number of leucocytes except in the eosinophils. In the beginning the number of polymorphs increased while the mononuclear cells diminished relatively in dogs. But, it was also observed in the same dog the increase of mononuclear cells from 15-48%. They also reported increase of buffy coat during parasitaemia. Krijgsman (1933) reported decrease in the erythrocyte count in mice and rats infected with T. evansi and this persisted throughout the course of disease. French (1937) observed sedimentation rate to be much more rapid in donkeys than in cattle and sheep. Poindexter (1939) recorded in rats suffering from T. equiperdum infection, increase in large lymphocytes. Nicolle and Simons (1939) observed the sedimentation rate to be markedly increased in guineapig, inoculated with T. equiperdum, T. brucei and T. evansi, in each case. Kaltenbach (1954) studied the leucocytic response in natural surra cases of camels and horses and experimental surra of rabbits and mice. In natural chronic infection the degree of regenerative leucocytosis and number of large lymphocytes ran parallel to the number of circulating trypanosomes, without any change in the number of monocytes. In rats the infection was acute showing increased number of neutrophils followed by regenerative leucocytosis and finally lymphocytosis. Edward et al (1956) stated rapid fall of

erythrocyte count in sheep, goat and horses, infected with T. vivax, T. congolense and T. brucei. In other experiments the erythrocyte sedimentation rate was found normal in goats, but increased in sheep after inoculation with T. brucei. Cabrera and Lui (1956) artificially infected five pigs with T. evansi and studied the nuclear shift index and other haematological indexes. Resistance to the infection was clearly reflected in the differential white blood response. Monocytosis and eosinophilia were observed during the patent period, while the lymphocytes were but moderately reduced compared to subpatent level. The decrease in the lymphocytes even in the 5+ grade of parasitaemia was but moderate, while the eosinophils and monocytes were increased over and above the normal and subpatent levels. The lymphocyte - eosinophil - monocyte relationship was observed inspite of neutrophilia and a continuing left shift. The increase in eosinophilic and monocytic elements indicated that the defensive forces in pig were strong and acting intensely. Samadar et al (1962) found in goat experimentally infected with surra organisms, decrease in erythrocyte count, packed cell volume, haemoglobin percentage and increase in neutrophils throughout the infection. There was decrease in mean corpuscular volume, leucopenia and microcytic hyperchromic anemia. Srivastava (1965) examined the blood of dog artificially infected with surra and found reduction in red blood counts, decrease in packed cell volume showing macrocytic type of anemia. He indicated increase in sedimentation rate and neutrophilia throughout the infection. Mandal (1965) experimentally infected dogs with T. evansi and

studied daily in detail the progressive course of the infection, clinical symptoms, haematology and sugar analysis. During this observation, he reported in the fall of red blood corpuscles, haemoglobin percentage, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and percentage of sugar in the blood. Increase of buffy coat layer was observed during parasitaemia. The total count of white blood cells showed irregular rise and fall. In the initial stage there was neutrophilia with a decrease of lymphocytes. The condition reversed at the peak of infection and neutrophila predominated during the period of death.

MATERIALS AND METHODS

Intestinal Protozoan Parasites

(1) Collection of materials :- Faecal samples intended to detect the protozoan parasites were collected from caecum and rectum of deshi pigs examined postmortem, from a local salughter house at Dujra, where pigs were slaughtered for sale of pork. Faecal samples were also collected from deshi pigs from Bihar Veterinary College area and other adjoining villages for examination. In addition, fresh faecal samples were collected from large white yorkshire pigs at Government Cattle Farm, Dumraon for examination in the Farm campus. In each case faeces were collected per rectum and kept in glass tubes and were brought in the laboratory for examination.

(2) Examination of faecal samples -

(a) Direct smear method :- A small quantity of faeces was taken on a clean glass slide and two thin films were made, one in physiological saline solution and other in iodine solution (filtered saturated solution of iodine in 1% Potassium iodide).

(b) Centrifugal sedimentation method :- A small quantity of faeces was taken in mortar and pestle and thoroughly mixed with tap water. It was poured in centrifuge tubes and centrifuged for 5 - 8 minutes at 1000 r.p.m. This method was useful for the concentration of Coccidian oocysts.

(c) Centrifugal floatation method :- After sedimentation the clear supernatant fluid was thrown off and the sediment was resuspended in saturated solution of sugar. It

was centrifuged for 5 - 7 minutes, and kept in rest for 5 minutes. The upper scum was removed and transferred on a clean microscopic slide with the help of a beaded glass rod. This method was found fairly useful for concentration of coccidian oocysts.

(3) Staining of Protozoa :- Smear from fresh faecal sample was made on a clear microscopic glass slide and fixed in Schauddin's fluid. After that the staining was done by means of Heidnhain's Iron Haematoxylin method.

(4) Preservation of faecal samples :- Faecal samples positive for intestinal protozoan parasites were preserved in 5% formol saline solution for further examination.

(5) Sporulation of Coccidian oocysts :- Positive samples of coccidia were immediately placed in 2.5% Potassium dichromate solution in large petridishes for sporulation at room temperature. Constant watch was kept over the process of maturation of oocysts by examining the contents of the petridishes under the microscope at the end of every 24 hours.

(6) Measurements:- Measurements of protozoa were taken with the help of ocular and stage micrometers.

Trypanosome

1. Strain :- Laboratory strains of T. evansi was brought from Indian Veterinary Research Institute, Izatnagar, and maintained by serial needle passage in guineapigs, rabbits, mice and white rats. This strain was used in this experiment.

2. Experimental animals, their care and management :- Five deshi piglets aged about two and half months were purchased locally and used in this experiment. These piglets were fed on gram, wheat bran and groundnut cakes with plenty of water. Before starting the experiment, their stool and blood were examined and they were dewormed with suitable anthelmintics. Their temperature was recorded daily before and after infection.

Besides these, guineapigs, mice, white rats and rabbits were used for maintaining the strain of T. evansi in the laboratory. They were fed on gram and wheat bran.

3. Site of blood collection :-

(a) Piglet :- Blood samples for examination, were collected from the anterior vena cava by holding the pig on its back. About 2.5 c.c. of blood was collected and immediately kept in dried oxalated solution in small penicillin vial. The oxalated blood was used for all haematological studies. In case of passage of the parasites citrated blood was used.

(b) White rat :- Rat showing teeming trypanosomes in the peripheral circulation was used for drawing blood direct from the heart.

(c) Mice :- Blood from mice showing teeming trypanosom-es was collected from the heart directly.

Tubes for collecting blood were prepared by pipetting 0.5 ml. of a solution of Potassium oxalate and Ammonium oxalate (Pot. oxalate - 0.8 gm. Ammonium oxalate - 1.2 gms and water 100 c.c.) into a small empty penicillin vial and then evaporated to dryness in a hot air oven.

4. Dose and site of inoculum :- Turk's fluid (Glacial acetic acid - 3 c.c., 1% solution of aqueous gentian violet - 1 c.c. and distilled water 100 c.c.) was used for dilution of blood and the trypanosomes were counted with the help of standard haemocytometer. Infective inocula containing 2,00,000 to 2,50,000 trypanosomes were injected to each piglet intra-peritoneally. One piglet was inoculated with the strain maintained in white rat and the four piglets were inoculated with the strain maintained in the mice.

5. Study of Parasitaemia :-

(a) Wet blood smears - were examined daily for the presence of parasites under dry high power lens.

(b) Stained smears :- Blood smears made from the inoculated animals were stained with Penoptic staining method (combined Leishman's and Giemsa's), for the presence of parasites and also for differential leucocytes counts.

6. Haematological studies -

(i) Counting of total erythrocytes :- Blood sample was diluted exactly 1:200 with a special diluting pipette using Hayem's fluid (Mercuric chloride 0.5 gm., Sodium chloride 1.0 gm., Sodium sulphate 5.0 gm. and distilled water 200 c.c.). The diluted blood was placed in improved Neubauer counting chamber and red blood corpuscles were counted in a

measured volume and calculated per cubic centimeter.

(ii) Counting of total leucocyte :- Blood sample was diluted 1:20 in Turk's fluid in white blood diluting pipette. Counts were made with improved Neubauer ruling and the total count per cubic centimeter was finally calculated.

(iii) Differential leucocytic count :- Blood smears were stained by Penoptic method and were examined for different types of white blood corpuscles. Two hundred cells were counted for each determination.

(iv) Estimation of haemoglobin :- For this Sahli's standard technique was adopted. N/10 HCL was placed into a graduated tube upto the level of 2 gms. or 10% mark. The special Sahli's pipette was filled up to 20 mark with oxalated blood and immediately it was discharged into the tube containing acid. It was then mixed with a glass rod and after a lapse of 5 minutes distilled water was added drop by drop to the mixture and the colour was compared with the standard tube. The reading was recorded when the colour of the tube had the same colour tinge and intensity as the standard tube. Haemoglobin was estimated in gram per 100 c.c. Haemoglobin estimation was done within an hour after collection of the blood.

(v) Determination of erythrocyte sedimentation rate:- The erythrocyte sedimentation rate was determined with oxalated blood using Wintrobe haemocrit tubes. Immediately after collection of blood, haemocrit tube was filled with the blood upto zero mark with the help of pipette. The tube was placed vertically at room temperature. The amount of

sedimentation was noted at the end of one hour.

(vi) Determination of Packed cell volume :- The packed cell volume was determined by means of Wintrobe haemocrit tubes. Fresh oxalated blood was filled in the tube upto 10 mark and centrifuged for one hour at 3,000 r.p.m. The packed cell volume was noted.

(vii) Determination of mean corpuscular volume :- For determination of mean corpuscular volume, the volume of packed cell per 100 c.c. of blood was divided by the number of million of erythrocytes per cubic m.m. and multiplied by 10.

(viii) Determination of mean corpuscular haemoglobin :- For determination of M.C.H. in micro-micrograms the haemoglobin in gram per 100 c.c. was divided by erythrocytes in million per cubic m.m. and multiplied by 10.

(ix) Determination of mean corpuscular haemoglobin concentration :- To determine the mean corpuscular haemoglobin concentration, haemoglobin in gram per 100 ml. of blood was multiplied by 100 and divided by percentage packed cell volume. It was expressed in percentage.

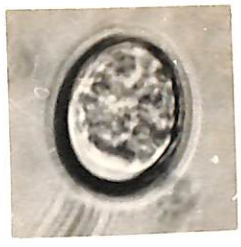


PLATE NO.I.

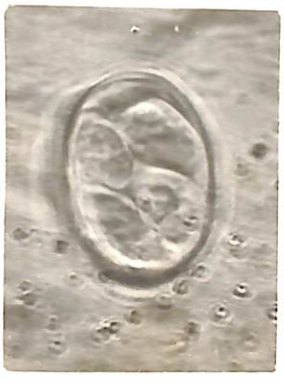


PLATE NO.II.

OBSERVATIONS.

Coccidia

A survey was made to know the incidence of coccidia in deshi and yorkshire pigs. In the present work the percentage of infection in deshi domestic pigs was found to be 57.26%. The incidence of coccidia in yorkshire pigs examined at Dumraon Farm was found to be 44%. In this study, 138 postive samples of coccidia from deshi pigs and 55 samples from yorkshire pigs were sporulated for specific identification. One hundred oocysts were measured in each case except E. spinosa. (Table No.1)

Five species of Eimeria viz: Eimeria debliecki, Eimeria scabra, Eimeria perminuta, E. polita and E. spinosa were identified in the positive samples. E. spinosa was not found from yorkshire pigs. Most of the pigs showed multiple infections with two or three species. E. debliecki, E. scabra, and E. perminuta were found to be common in deshi as well as yorkshire pigs. The percentage of infection with different species is shown in Table No.2.

The description of the species studied are given as follows :-

(1) Eimeria debliecki Douwes, 1921.

The oocysts were ovoidal in shape and measured 16 to 30 microns in length and 12 to 22 microns in breadth. The average dimensions were 22.8 microns x 17.5 microns. The oocystic wall was colourless and composed of two layers. It measured 0.9 to 1.1 microns in thickness. Micropyle and polar

PLATE NO.III.

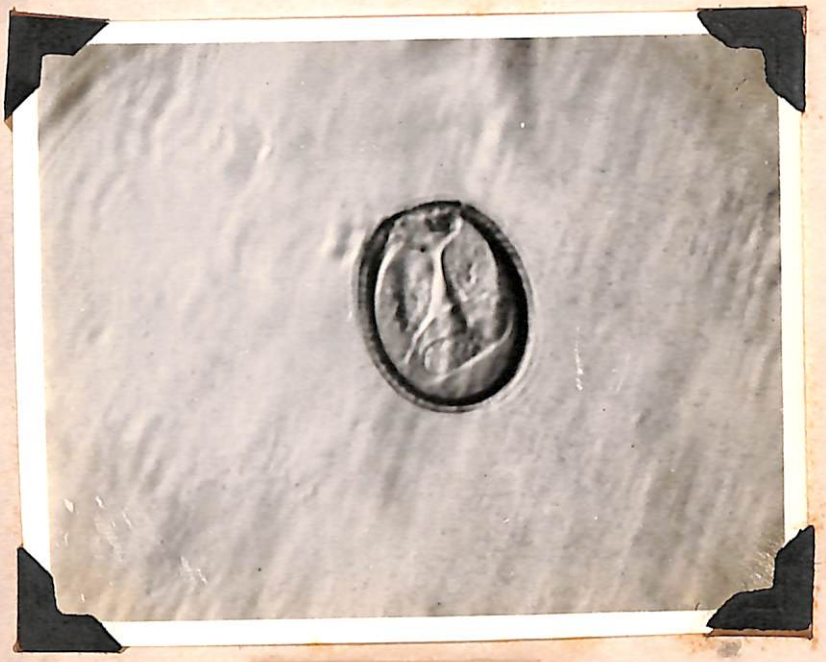


PLATE NO.IV.

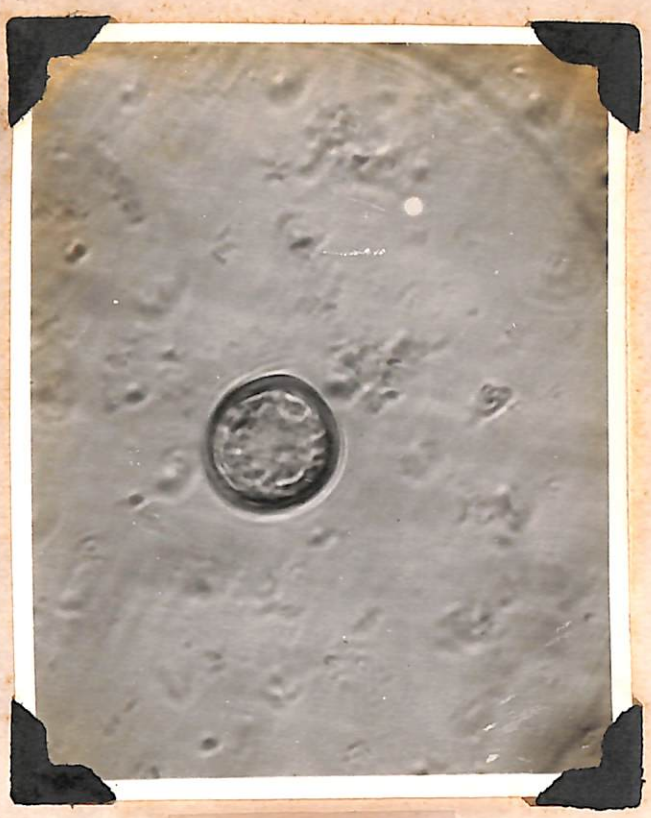


PLATE NO.V.

PLATE NO.VI.

granule were absent. The oocystic residual body was absent. The stieda's body and sporocystic residual body were present. The sporocysts were ovoidal or ellipsoidal in shaope and measured 11 to 18.4 microns in length and 5.7 to 8.9 microns in breadth. The average size of the sporocyst was 12 microns x 7 microns. The sporulation time varied from 6 to 7 days at room temperature. (Plate I & II, Fig. I & II).

(2) Eimeria scabra Henry, 1931.

The oocysts were ovoidal or ellipsoidal in shape. The size was variable measuring 19 to 34 microns by 14 to 23 microns in dimensions. The average size was 25 by 18 microns. The oocystic wall was thick, rough and brown in colour. The wall measured 1.4 to 2.2. microns in thickness. A micropyle and oocystic residual body were absent. An oocystic polar granule was present. The stieda's body and sporocystic residual body were distinctly present. The sporocysts were ellipsoidal or ovoidal in shape measuring 12 to 19 microns x 6.5 to 9 microns in dimensions. The average size was 14 microns x 7.5 microns. One hundred sporocysts were measured. The sporulation time was 9 to 11 days at room temperature.(Plate III & IV, Fig. III & IV).

(3) Eimeria perminuta Henry, 1931.

The oocysts were spherical or oval in shape and varied in size from 11 to 16.8 microns x 10 to 15 microns. The average dimension was 14.2 x 12.4 microns. The oocystic wall was rough and yellowish brown in colour. A micropyle and oocystic residual body were absent. A polar granule was distinctly observed. The sporocysts were ovoidal in shape

PLATE NO.VII.

PLATE NO.VIII.

and measured 6.5 to 8.5 microns in length and 5 to 7.5 microns in breadth. The average size was 7.3 microns x 5.8 microns. One hundred sporocysts were measured. The sporulation time was 9 to 10 days at room temperature. (Plate V & VI, Figure V & VI).

(4) Eimeria polita Pellerdy, 1949.

The oocysts were ellipsoidal or cylindrical in shape and measured from 17 to 36.5 microns in length and 13.5 to 25 microns in breadth, with an average dimensions of 27 microns x 17 microns. The oocystic wall was smooth, but occassionally roughened, pinkish brown in colour. The wall measured 1.1 to 2.3 microns in thickness. A micropyle and stieda body were absent. Oocystic residual body was absent, but sporocystic residual body was present. A polar granule was present. The sporocysts were ellipsoidal in shape and measured 14 to 19 microns in length and 6 to 9 microns in breadth, with an average dimensions of 15.9 microns x 6.9 microns. One hundred sporocysts were measured for this study. The sporulation time was found to vary between 10 to 12 days at room temperature. (Plate VII & VIII, Fig. VII & VIII).

(5) Eimeria spinosa Henry, 1931.

The oocysts were spherical or ovoidal in shape and varied from 18 to 22 microns in length and from 16 to 18 microns in breadth. The oocysts were brown in colour and entire surface of the wall was covered with spines. The spines were wider at the base and tapered to a point. The cytoplasm of the unsporulated oocysts was extremely heavy in

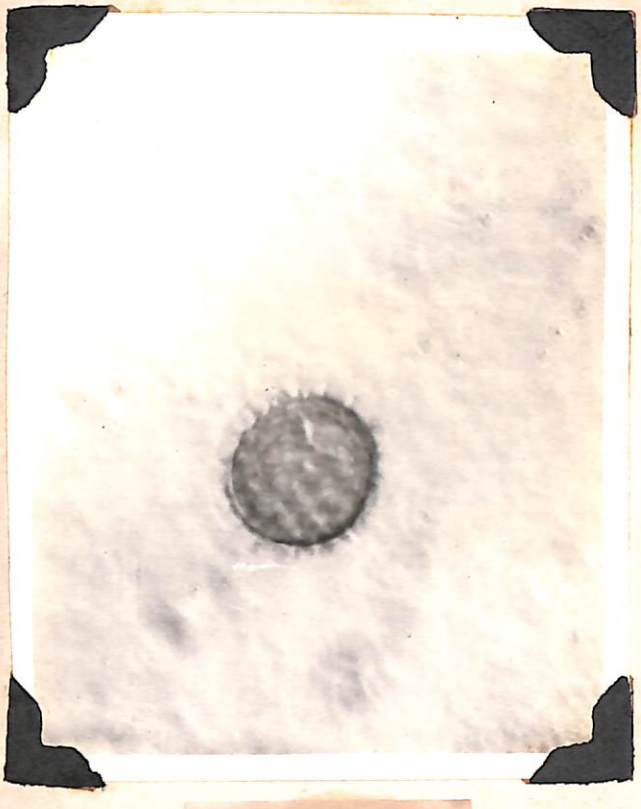


PLATE NO.IX.

appearence and was made up of relatively larger granules. Fifty oocysts were measured in this study. The oocysts could not sporulate even in 15 days time at room temperature. (Plate IX, Figure IX).

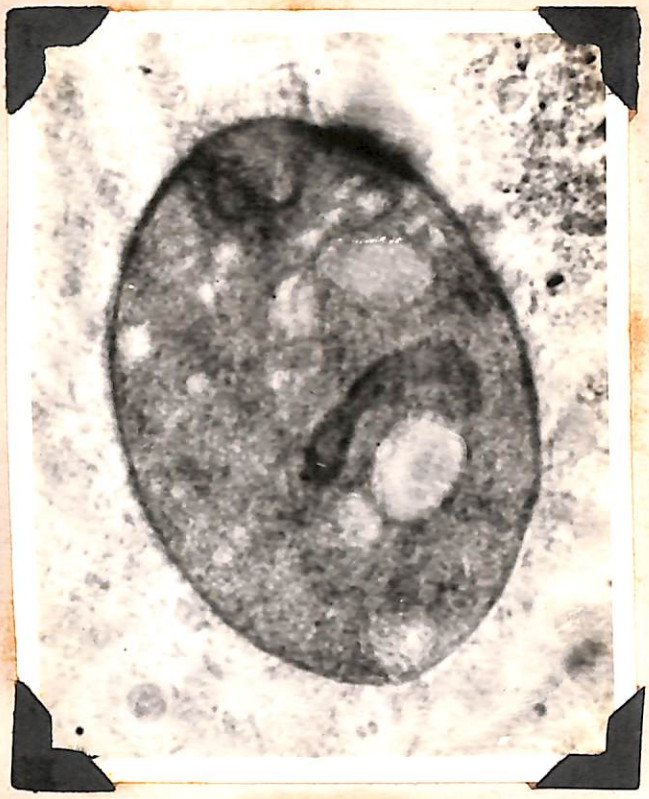


PLATE NO.X.

Balantidium

A survey was made to study the incidence of *Balantidum coli* in deshi and yorkshire pigs. In the present work, the incidence of this parasite was found to be 57.67% in deshi and 40% in yorkshire pigs. The presence of parasite was not associated with any clinical symptoms. The author did not find any gross pathological changes in the caecum and colon of the pigs examined postmortem.(Table I)

The morphology of unstained and stained specimens was studied. In the present study,100 trophozoites from ten pigs were measured. The trophozoites were ovoidal in shape with many longitudinal rows of cilia. A small peristome was present at the anterior end of the body which was lined with coarser cilia. Cytostome and cytopharynx were located at the end of the peristome. A distinct cytopyge was present at the posterior end of the body. A contractile vacuole was present near the centre and one at the terminal end of the body. There were many food vacuoles present within the cytoplasm. The trophozoites measured from 66 to 89 microns in length and 48 to 68 microns in breadth. Macronucleus was distinct and sausage shaped and measured from 27 to 34 microns in length and 5.7 to 10.3 microns in breadth. The micronucleus was small and vesicular. The cysts were spherical to ovoid in shape. They measured from 33 to 44 microns in diameter. (Plate X, Fig. X & XI).

Entamoeba

The present study was made to find out the incidence of amoebic infection in deshi and yorkshire pigs. Altogether 241 faecal samples from deshi pigs and 125 samples from yorkshire pigs were examined and the incidence of amoebic infection was found to be 29% and 21.6% respectively. Out of 70 postive samples of Entamoeba from deshi pigs, 62 samples were found to be positive for Entamoeba suis and 8 samples were positive for Entamoeba coli. The percentage of infection of E. suis and E. coli was found to be 25.72% and 3.31% respectively. All the 27 samples from yorkshire pigs were found to be positive for only E. suis. The percentage of infection of E. suis in yorkshire pigs was found to be 21.6%. E. coli was not found to be present in any of the sample examined from yorkshire pigs.

The species of Entamoeba were identified by studying the morphological characters of the cysts, which were encountered during the course of investigation. No trophozoites were found in any of the samples examined (Table No. I(a) and I(b).

The description of the species of Entamoeba are as follows :-

(1) Entamoeba suis Hartmann, 1913.

The cysts were spherical in shape and measured from 4.5 microns to 15.3 microns in diameter. The average diameter of the cyst was 10.2 microns. The nucleus was distinct with prominent central endosome. The chromatin granules were present in the inner surface of the nuclear

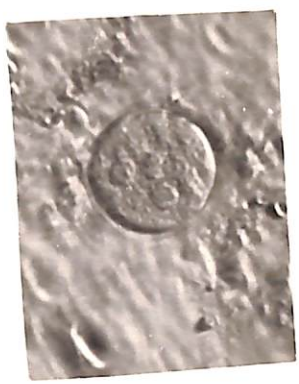


PLATE NO.XI.

membrane. The feature became clear after treatment with gram's iodine solution. Chromatoid bodies of different shapes were present. In some cysts glycogen vacuoles were present. Altogether fifty cysts were measured in this observation. (Fig. XII to XVII).

(2) Entamoeba coli (Grassi, 1879)

Casagrandi and Barbagallo, 1895.

The cysts were oval to spherical in shape and measured from 14 - 26 microns in diameter. Eight nuclei were present in each cyst. The nucleus possessed eccentric endosome and prominent nuclear membrane. The nuclear membrane was lined with coarse chromatin granules around its periphery. Chromatoid bodies were absent. But, in few cyst it was found in the form of splinters or fragments. Altogether fifty cysts were measured in this study. (Plate XI, Fig. XVII to XXI).

Table No.I(a)

Table showing the percentage of infection with different Protozoa in domestic deshi pigs examined at Patna.

Sl. No.	Name of the Protozoa.	No.of pigs examined.	No.found infect-ed.		Total infect-ed.	Percent-age of infection.
			Light.	Heavy.		
1.	Coccidia	241	30	108	138	57.26%
2.	Balantidium coli	241	22	117	139	57.67%
3.	Entamoeba (E. suis E. coli)	241	20	50	70	29%

Table No.I(b)

Table showing the percentage of infection with different protozoa in yorkshire pigs examined at Dumraon Farm.

Sl. No.	Name of the Protozoa.	No.of pigs examined.	No.found infect-ed.		Total infect-ed.	Percent-age of infection.
			Light.	Heavy.		
1	Coccidia	125	11	44	55	44%
2	Balantidium coli	125	9	41	50	40%
3	Entamoeba (E. suis)	125	6	21	27	21.6%

Table II (a)

Table showing infection of deshi domestic pigs with different species of Eimeria examined at Patna.

Sl. No.	Name of Eimerian spp.	No. of samples examined.	No. found infected.	Percentage of infection.
1	E. debliecki	138	61	44.22%
2	E. scabra	138	27	12.31%
3	E. perminuta	138	32	23.19%
4	E. polita	138	10	7.24%
5	E. spinosa	138	8	5.79%

Table II(b)

Table showing infection of yorkshire pigs with different species of Eimeria examined at Dumraon Farm.

Sl. No.	Name of Eimerian spp.	No. of samples examined.	No. found infected.	Percentage of infection.
1	E. debliecki	55	14	25.45%
2	E. scabra	55	19	34.54%
3	E. perminuta	55	16	29.0%
4	E. polita	55	6	10.9%
5	E. spinosa	55	Nil	Nil

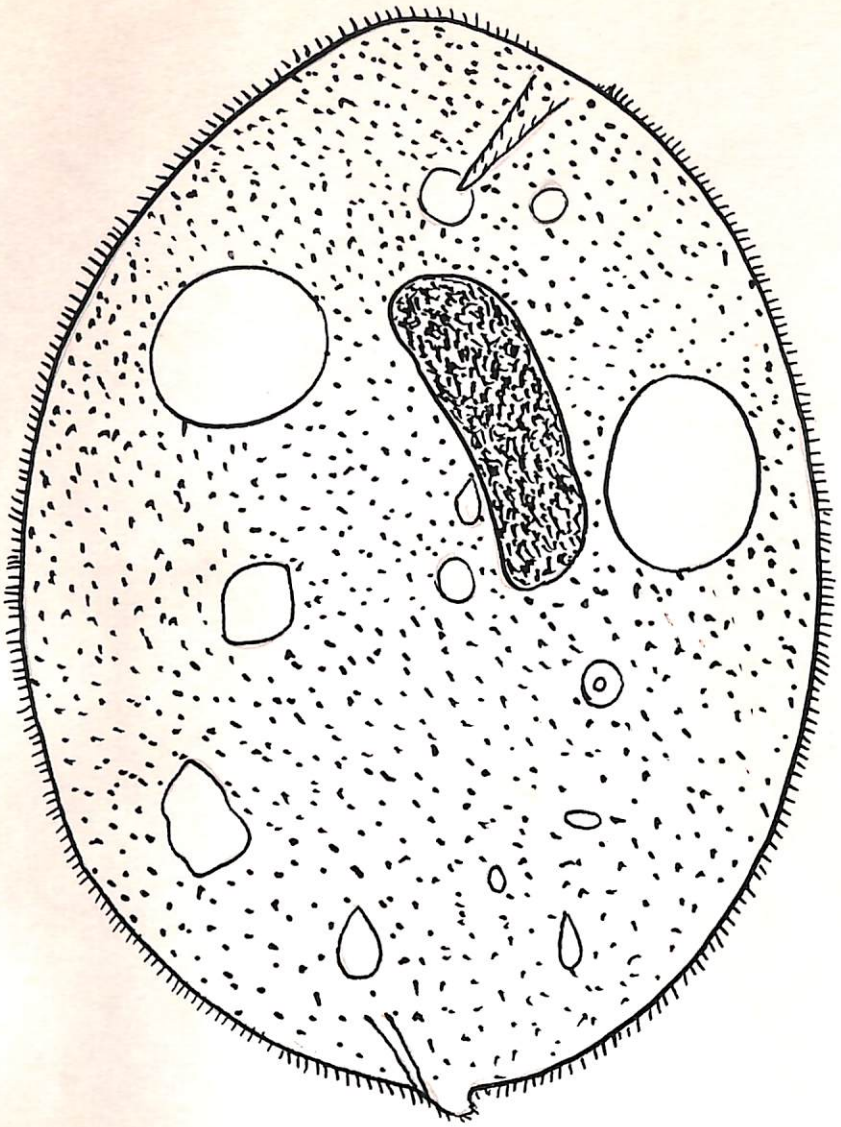


Fig. X.

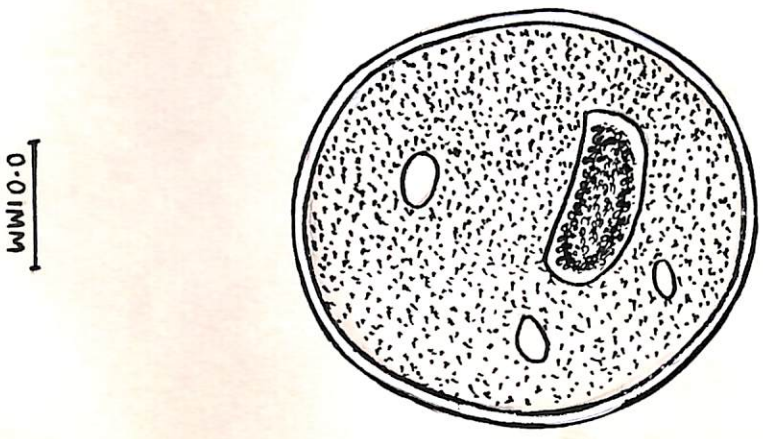


Fig. XI.

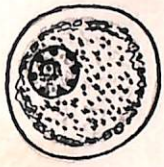
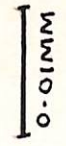


Fig.XII.

Fig.XIII.

Fig.XIV.



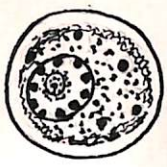


Fig.XV.

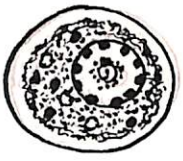


Fig.XVI.

Fig.XVII.

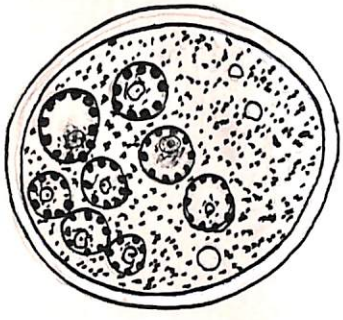


Fig.XVIII.

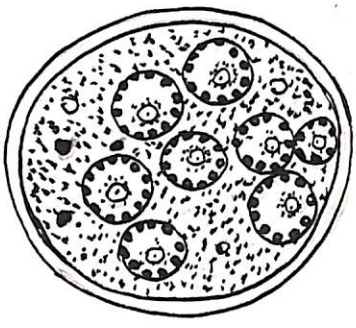
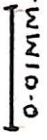


Fig. XIX.



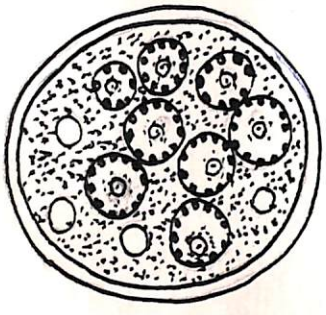


Fig. XX,

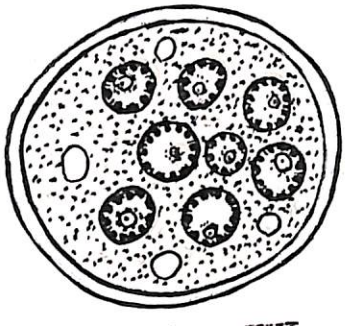


Fig. XXI.

Explanation of figures

(Camera lucida drawings)

Fig. I - Unsporulated oocyst of *Eimeria debliecki*.

Fig.II - Sporulated oocyst of *Eimeria debliecki* showing stieda's body and sporocystic residual body.

Fig.III - Unsporulated oocyst of *Eimeria scabra*.

Fig. IV - Sporulated oocyst of *Eimeria scabra* showing polar granule, stieda's body and sporocystic residual body.

Fig. V - Unsporulated oocyst of *Eimeria perminuta*.

Fig. VI - Sporulated oocyst of *Eimeria perminuta* showing polar granule.

Fig.VII - Unsporulated oocyst of *Eimeria polita*.

Fig.VIII - Sporulated oocyst of *Eimeria polita* showing presence of Polar granule and sporocystic residual body.

Fig. IX - Unsporulated oocyst of *Eimeria spinosa* showing spines on the oocystic wall and heavy cytoplasm made up of larger granules.

Fig. X - A trophozoite of *Balantidium coli* showing the cytopharynx, the sausage shaped macronucleus, the micronucleus, the contractile vacuoles, food vacuoles, the cytopyge and the ciliated covering.

Fig. XI - A cyst of *Balantidium coli* showing the macronucleus and food vacuoles.

Fig.XII to XV - *Entamoeba suis*, cysts showing the nucleus with a entral endosome, the peripheral ring of chromatin granules and chromatoid bodies of different shapes.

Fig. XVI & XVII - *Entamoeba suis*, cysts showing chromatoid bodies and glycogen vacuoles.

Fig.XVIII- to XXI - *Entamoeba coli*, cysts showing eight nuclei with a eccentric endosome and a row of coarse chromatin granules around the periphery of nuclear membrane, glycogen vacuoles. In Fig. No.XIX, chromatoid bodies are present in the form of fragments.

*****

Trypanosome

Piglet No.1.

(A) Parasitaemia, Temperature and Prepatent Period :- The piglet weighed 19 lbs. Temperature before inoculation was 104° F. On 26th June, 1967 diluted blood of white rat contain-ing trypanosomes was inoculated intraperitoneally into the piglet. On Ist July, 1967 trypanosomes appeared in the peripheral blood circulation with slight rise of temperature (105°F.). Blood smears were examined regularly on alternate days. The parasites were found on 3rd, 5th and 7th July in the stained blood smears. They were 4 to 5 in numbers in whole blood smear in each slide. The parasites were present for 7 days after their first appearence. Then, the parasites dis-appeared from the peripheral blood circulation on 9th July, and remained absent upto 26th July. On 28th July the parasites reappeared in the blood circulation. They were found till 4th August, 1967 on examination. On 6th August, the parasites again disappeared from the blood circulation and afterwards they did not reappear. The piglet was sacrified on 23rd September, 1967. (Table No.III).

(B) Biological tests :- During the latent period of infection, blood was examined regularly and blood smears were found negative for the parasites. During this period 4 mice were inoculated separately each with 1 c.c. of blood taken from the infected piglet, after 15 days of interval i.e. on 6th, 23rd August and 8th and 23rd September, 1967. In three mice, trypanosomes appeared in the blood circulation between

5 to 6 days and died after 10 - 11 days of infection. The 4th mice did not pick up the infection and remained healthy during the period of observation (Table No.IV).

(C) Clinical symptoms :- The piglet showed only slight rise of temperature (105° F.) after first appearence of the parasites in the blood circulation. No other clinical symptoms were found. The piglet remained quite healthy up to the time of slaughter.

Piglet No.2.

(A) Parasitaemia, Temperature and Prepatent Period :- The animal weighed 18 lbs. Temperature before inoculation was 104°F. The piglet was inoculated intraperitoneally with blood containing trypanosomes from mice on 28th June, 1967. Trypanosomes appeared in the peripheral circulation on 3rd July, 1967 with a slight rise of temperature (105°F). Blood smears were examined on alternate days to find out the presence of parasites in the blood circulation. The parasites were found on 5th, 7th and 9th July, 1967. The number of parasites were only 4 to 5 in the whole of the stained blood film. The parasites disappeared from the peripheral circulation from 10th July onward and reappeared in the blood circulation on 29th July. Blood smears examined on 29th, 31st July, 2nd, 3rd and 5th August, 1967 showed the presence of parasites. They were 1 to 2 in number in whole of the stained blood film examined. Then, the parasites again disappeared from peripheral circulation on 6th August, 1967 and remained absent till the date of slaughter of the piglet (23rd September, 1967).

(B) Biological tests :- During the latent period of infection blood smears were examined regularly, which were found to be negative for the parasites. During this period, four mice were separately inoculated I/P with 1 c.c. of blood collected from the infected piglet at intervals of 15 days i.e. on 7th, 23rd August, 8th and 23rd September, 1967. The trypanosomes were found to appear in the peripheral circulation between 5 to 6 days in three mice, which died between 10 to 11 days after infection. The fourth mice, which was inoculated at the last observation, did not pick up the infection and remained healthy upto the last date of observation (Table No. IV).

(C) Clinical symptoms :- The piglet showed only slight rise of temperature (105° F) after first appearence of the parasites in the blood circulation. No other clinical symptoms were found.

Piglet No.3.

(A) Parasitaemia, Temperature and Prepatent Period :- The weight of the piglet was 16 lbs. The piglet was inoculated intraperitoneally with diluted blood containing trypanosomes from a mice on 28th June, 1967. Temperature before inoculation was 104°F. Trypanosomes appeared in the peripheral blood circulation on 4th July, 1967. Blood smears were regularly examined every alternate days. The parasites were present in the blood circulation from 4th July, 1967 to 12th July, 1967. They varied in numbers from 4 to 5 in whole of the stained blood film examined. The temperature was 106°F during the above

period of parasitaemia. On 13th July, 1967 parasites disappear-ed in the blood circulation and remained absent upto 27th July, 1967. The temperature became normal during the absence of the parasites in the blood circulation. The parasites again appeared on 28th July and remained present in the blood circulation upto 3rd August, 1967. The parasites disappeared from the blood circulation on 4th August and remained absent till the date of slaughter of the animal (Ist October, 1967). (Table No.III).

(B) Biological tests :- During the latent period of infection blood smears were examined regularly and found negative for the trypanosomes. During this period, blood was collected from the piglet and four mice were injected intraperitoneally separately with 1 c.c. of blood at a intervals of 15 days i.e. on 14th August, Ist September, 16th September and Ist October, 1967. Trypanosomes appeared in the peripheral circulation between 5 - 6 days in three mice and they died between 11 to 12 days, after infection. The fourth mice, which was used at the last experiment did not pick up the infection and survived upto the time of last observation (Table No.IV).

(C) Clinical symptoms :- There was rise of body temperature (106° F), when the parasites were in the peripheral circulation. No other clinical signs of disease were found. The animal was in good health throughout the observation.

Piglet No.4.

(A) Parasitaemia, Temperature and Prepatent Period :- The weight of the piglet was 17 lbs. The body temperature was 102.8°F before inoculation. On 3rd July, 1967, blood from a mice containing trypanosomes was inoculated intraperitoneally into the piglet. The parasites appeared in the blood circulation on 7th July, 1967. There was no rise of temperature Blood smears from the infected piglet were examined on alternate days. The parasites remained present upto 12th July, 1967. The parasites disappeared from the peripheral blood circulation on 13th July and remained absent upto 31st July, 1967. On Ist August, 1967, the parasites reappeared in the peripheral blood circulation and the blood remained positive till 7th August. After that the parasites did not appear again during the observation. The piglet was slaughtered on 18th November, 1967. (Table No.III).

(B) Biological tests :- During this latent period blood was collected from the piglet and inoculated into 6 mice. One c.c. of blood was inoculated into each mice. In this observation 4 mice were inoculated intraperitoneally at intervals of 15 days and two mice were inoculated at intervals of 20 days, i.e. on 11th, 26th August, 12th, 28th September and 18th October and 9th November, 1967. In the first four mice trypanosomes appeared in the peripheral blood circulation between 5 - 6 days and they died between 11 - 12 days after infection.

In the 5th mice trypanosomes appeared in the blood circulation in 7 days and the mice died in 14 days after

infection. The sixth mice did not pick up the infection and remained in good condition till the period of observation. (Table No.IV).

(C) Clinical symptoms :- No visible signs of disease were found during the observation. The pig was in good health upto the time of slaughter.

Piglet No.5.

(A) Parasitaemia, Temperature and Prepatent Period :- The piglet weighed 16 lbs. Temperature before inoculation was 104°F. The piglet was inoculated intraperitoneally with diluted blood of mice containing trypanosomes on 3rd July, 1967. Trypanosomes appeared in the blood circulation on 7th July, 1967 with a rise of temperature (106°F). Blood smears were examined. The parasites remained present upto 11th July. The temperature during parasitaemia was 106°F. The parasites disappeared from the blood circulation on 12th July, 1967 and remained absent upto 29th July. The temperature was normal during this period. The parasites reappeared on 30th July, 1967 and remained present upto 6th August, 1967. The parasites again disappeared from the peripheral blood circulation on 7th August, 1967 and did not appear in the circulation upto the time of slaughter (18th November, 1967) (Table No.III).

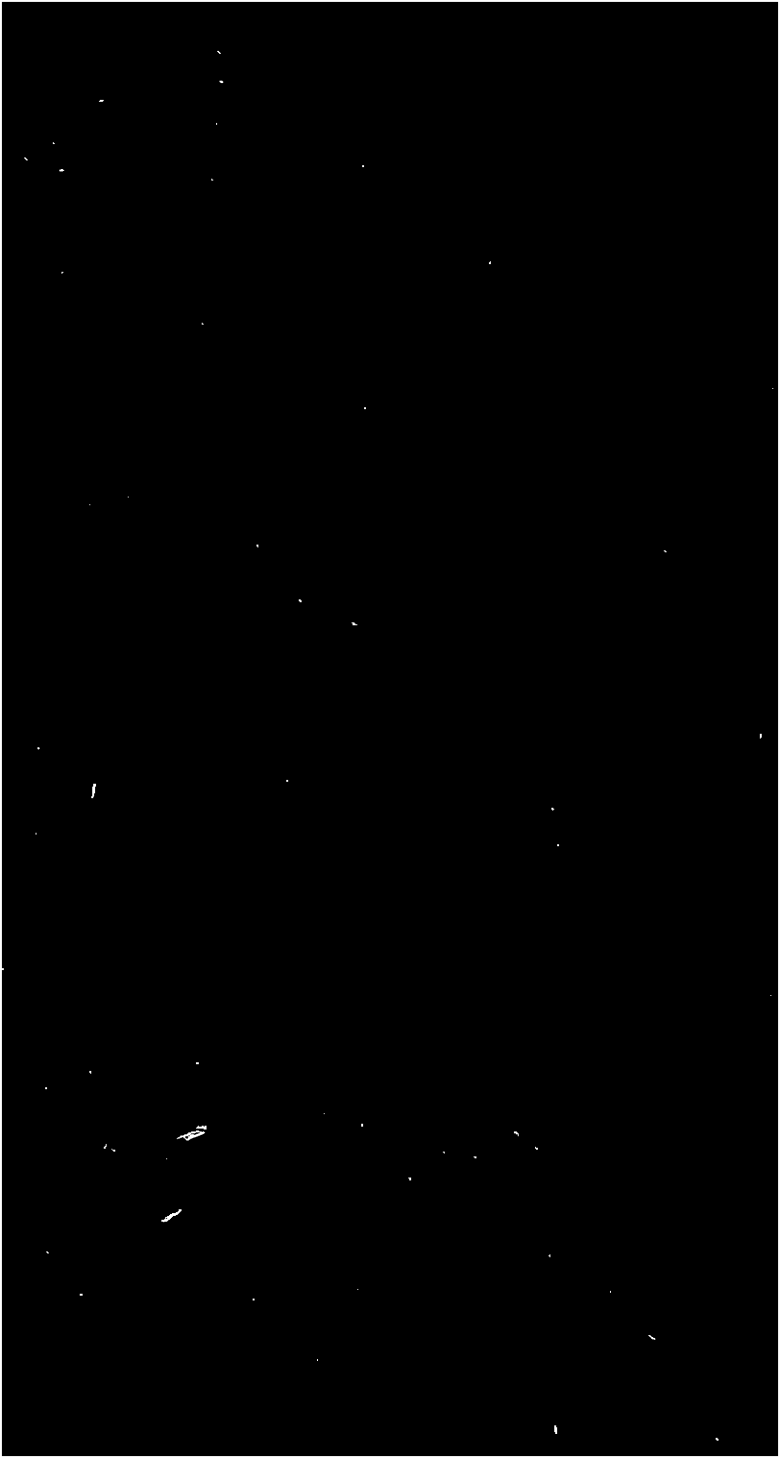
(B) Biological tests :- During the latent period of infection blood smears were examined and found negative for parasites. During this period, blood was collected from the piglet and inoculated into 6 mice. Out of 6 mice, 4 mice

were inoculated at intervals of 15 days and 2 mice were inoculated at an interval of 20 days i.e. on 11th, 26th August, 12th, 28th September, 18th October and 9th November, 1967. In the first four mice trypanosomes were found in blood circulation between 5 - 6 days and died between 11 - 12 days after infection. In the 5th mice trypanosomes appeared in the blood circulation in 7 days and died in 13 days after infection. The sixth mice did not pick up the infection and survived upto the time of last observation.(Table No.IV).

(C) Clinical symptoms :- There was slight rise of temperature (106°F) at the time of first appearence of the parasites in the blood circulation. The temperature remained normal for the rest of the period. No visible symptoms were found.

Macroscopical lesions

Visceras of all the five piglets were thoroughly examined after slaughter, but no gross pathological lesions were found in any organs. Impression smears from lungs, liver, spleen and lymph glands did not show the presence of parasites.



DISCUSSION AND CONCLUSION

Coccidia

Morphology :- The general morphological features in respect of size and shape of oocysts, presence or absence of micropyle, oocystic residual body, sporocystic residual body, stieda's body and polar granule for E. debliecki, E. scabra, E. perminuta and E. spinosa agree with the observations of Henry (1931). The descriptions of E. polita agree with that given by Pellerdy (1949). Pellerdy (loc. cit.) has mentioned the presence of micropyle in the oocyst of E. scabra. Later, Sinha (1963) has also found the presence of micropyle. The author,in the present study did not find the presence of micropyle in the oocyst of E. scabra, and thus, the specimens examined correspond with the findings of Henry (1931). The two varieties of E. scabra e.g. E. scabra var scabra and E. scabra var ellipsoidalis as reported by Gill (1960) were not found in the present studies. Hence, the author supports the views of Henry (loc. cit.) who did not find varieties of E. scabra.

Incidence :- In India, Gill (1960) has reported first time, six species of coccidia viz: E. scabra, E. perminuta, E. polita, E. spinosa, E. debliecki and I. suis from 20 pigs examined at Izatnagar. In Bihar, Sinha (1963) has reported only three species e.g. E. debliecki, E. scabra and E. perminuta and found that the incidence ofcoccidia was 57% in deshi pigs and 42.36% in yorkshire pigs. In the present study, the author found five species of coccidia in Bihar

viz: E. deblieckl, E. scabra, E. perminuta, E. polita and E. spinosa in the survey of 241 deshi pigs and 125 yorkshire pigs. The occurrence of E. polita and E. spinosa has been reported for the first time in this State. The percentage of infection has been found to be 57.26% and 44% in deshi and yorkshire pigs respectively. Multiple infections with E. deblieckl, E. scabra and E. perminuta have been observed. This is in conformity with the findings of Sinha (1963), who also observed the higher incidence and multiple infection of coccidia in deshi pigs in comparison to yorkshire pigs.

Balantidium

Morphology :- During the course of investigation vegetative and cystic forms of Balantidia from pigs were examined. Vegetative forms were oval in shape with longitudinal rows of cilia. The trophozoites measured from 66 to 89 microns in length and 49 to 68 microns in breadth. Macronucleus was distinct and sausage shaped. McDonald (1922) described B. coli and B. suis, two species from pigs. He pointed out that the vegetative forms of the latter are more slender in shape and measured from 35 to 120 microns in length and 20 to 60 microns in breadth. Hegner (1934) reported that the vegetative forms of B. coli are 70.9 x 58.9 microns in dimensions. Sinha (1963) studied the morphology of vegetative forms and reported the length and breadth between 64 to 88 microns and 48 to 66 microns. Thus, the author agrees that the species encountered in this State are Balantidium coli. During this observations, the other species, B. suis, which was also reported by other workers, has not been found.

Incidence :- In the present study, the incidence of B. coli in deshi and yorkshire pigs was found to be 57.67% and 40% respectively. Hence, the author agrees with the findings of Sinha (1963),who studied the incidence of this parasite in pigs in Bihar.

Entamoeba

Morphology :- Hoare (1959) has given the size of the cysts of Entamoeba suis (synonym; E. polecki) as 12 - 15 microns in diameter. Levine (1961) has mentioned the size of the cysts of E. suis as 4 - 17 microns in diameter. He has agreed with the opinion of Noble and Noble (1952) with regard to the morphology of the cysts. Mishra (1967) has found spherical uninucleate amoebic cysts measuring from 8.46 to 16.92 microns in diameter, which was attributed to be of E. suis. In the present study, uninucleate spherical cysts with prominent central endosome and chromatin granules arranged on the periphery of the nuclear membrane, were found. They measured from 4.5 to 15.3 microns with an average of 10.2 microns in diameter. The general morphological characters and measurements of the cysts correspond with those of Levine (1961) and Mishra (1967). Thus, it is concluded that the cysts found in this observation are of Entamoeba suis.

In the present investigation, 8 nucleated cysts of Entamoeba coli were also found. The measurements of the cysts were 14 - 26 microns in diameter. The nuclei possessed an eccentric endosome and a row of coarse chromatin granules in the periphery of nuclear membrane. Chromatoid bodies were absent, but in few cysts they were found in the form of fragments. These findings are in conformity with those of Smith (1910), Kessel (1928) and Levine (1961) in pigs.

Incidence :- The incidence of E. suis was found to be 25.7% in deshi pigs and 21.6% in yorkshire pigs. The

incidence was common in deshi and yorkshire pigs. The percentage of infection of E. suis was higher in deshi pigs than yorkshire pigs. E. coli was only found in deshi pigs. The author agrees with the findings of Mishra (1967), who recorded the incidence of E. suis from Mathura. In the present survey, the E. suis and E. coli in pigs was recorded for the first time in Bihar.

Trypanosome

Prepatent period :- Five piglets were inoculated with T. Evansi and parasites appeared in the blood circulation in all the piglets after 4 to 5 days of infection. The present study thus confirms the observation of Baldrey (1910), who also reported the prepatent period to be 5 days.

Parasitaemia :- Parasitaemia i.e. the appearence of the trypanosomes in the infected piglets was studied by direct blood smear method and counting of trypanosomes in stained blood smear on every alternate days.

Parasites appeared in the blood circulation 4 - 5 days after infection in all the piglets. The parasites were 4 - 5 in whole stained blood smear. They remained in blood circulation for 7 - 8 days after first appearence and this was followed by disappearence of the parasites from the blood circulation. After a latent period of 14 - 18 days the parasites reappeared in the blood circulation for 8 days. The parasites again disappeared from the blood circulation and remained absent for the rest period of observations in all the piglets. But on biological test piglets were found positive for the parasites for 1 month in piglet nos.1, 2 and 3 and for 2 months 10 days in piglet nos.4 and 5, after the last disappearence of the parasites from the blood circulation. These observations were based on the examination of stained blood smears. The present study confirms the observations of Baldrey (1910), Dejesus et al (1949) and Cabrera and Lui(1956), but differs with Kuppuswamy (1941) and Soltys (1963), who

reported that pigs are highly resistant against the disease.

Temperature and clinical symptoms :- There was slight rise of temperature in all the piglets except piglet no.4, during parasitaemia. No other visible clinical symptoms were observed in any of the piglets, during study period of between 3 to 4½ months. All the piglets were apparently healthy and were in living condition. Thus, the author agrees with the findings of Baldrey (loc. cit.) , Dejesus et al (1949) and Cabrera and Lui (loc. cit.), who reported that clinical symptoms were not seen in the experimental pigs which were infected with T. evansi.

Biological tests :- During the latent period of infection, blood from all the piglets (piglet nos.1,2,3,4 and 5) was collected and 1 c.c. of blood was inoculated into each mice. In case of piglet nos.1, 2 and 3, four mice were inoculated separately with blood at intervals of 15 days upto a period of 1½ months from the last disappearence of the trypanosomes. All the mice died of surra showing trypanosomes between 10 - 11 days, except the 4th mice in each case, which did not pick up the infection and survived. In case of piglet nos. 4 and 5, six mice were inoculated separately with the piglet's blood at intervals of 15 days, except the last two mice, which were inoculated at intervals of 20 days, upto a period of 2 months and 7 days. All the mice, except the sixth, died of infection due to parasitaemia. The 6th mice in each case did not pick up the infection and survived.

With the above observations, it was concluded that blood

smears of infected piglets during latent period were negative for parasites. But, after biological tests in mice, it was found that organisms were present in the blood circulation and were able to produce disease in mice. Thus, the author agrees with the findings of Baldrey (1910) & De Jesus et al (1949) who reported that infected pigs may act as carrier host for this parasite.

Haematological studies :-

As regards erythrocyte count, haemoglobin estimation, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and erythrocyte sedimentation rate, no changes were observed in any of the infected experimental piglets. So far literature is concerned, such type of work has not been carried out in pigs. But in other animals infected with T. evansi, Krijgsman (1933), French (1937), Nicolle and Simons (1939), Edward et al (1956), Samadar et al (1962), Srivastava (1965) and Mandal (1965) reported decrease in erythrocyte count, haemoglobin percentage, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Increase of leucocytes counts were found in all the piglets in prepatent and patent periods. Then, there was a gradual decrease of leucocytes counts, which came to normal level in latent period. After that, no further change was observed.

Differential leucocytic count :- After inoculation of trypanosomes, the percentage of neutrophils increased with corresponding decrease of lymphocytes in all the piglets.

Then, the percentage of neutrophils decreased with corresponding increase of lymphocytes in the latent period of infection, except in piglet no.5, in which neither decrease of neutrophils nor increase of lymphocytes were found. There was slight increase of eosinophils in piglet nos.2 and 4. The increase of monocytes was also observed during this study. Thus, the author agrees with the present findings with those of Cabrera and Lui (1956).

SUMMARY

Faecal samples from 241 deshi pigs at Patna and 125 white yorkshire pigs at Dumraon Farm (Sahabad) were collected and examined for the protozoan parasites. The extent of incidence of different protozoan parasites in deshi and yorkshire pigs were studied. In deshi pigs, the incidence of Coccidia was found to be 57.26%, Balantidium coli 57.67% and Entamoeba (E. suis and E. coli) 29%. In yorkshire pigs the incidence of Coccidia was 44%, B. coli 40% and Entamoeba (E. suis) 21.6%.

The incidence of protozoan parasites were found to be higher in deshi pigs than yorkshire pigs. E. coli was found only in deshi pigs. The incidence of E. suis and E. coli was reported from pigs for the first time in Bihar.

138 faecal samples from deshi pigs and 55 samples from yorkshire pigs were sporulated at room temperature for specific identification. Five species; Eimeria debliecki, E. scabra, E. perminuta, E. polita and E. spinosa were identified. The occurrence of E. polita and E. spinosa was reported for the first time in Bihar. E. spinosa was not found in yorkshire pigs. E. debliecki and E. perminuta were common in deshi pigs, whereas E. scabra and E. perminuta were common in yorkshire pigs. Multiple infections were also found.

Experimental trypanosomiasis in piglets caused by Trypanosoma evansi (Steel, 1885) was studied. Five piglets were used in this experiment and observations were made on the course of infection, parasitaemia, clinical symptoms and

haematology. In addition, biological tests of latent surra of piglets in mice were also conducted.

The trypanosomes appeared in the blood circulation of the infected piglets after a prepatent period of 4 - 5 days. They remained in blood circulation for 7 - 8 days. Then, they disappeared from the circulation. After a latent period of 14 - 18 days, they reappeared in the blood circulation and remained for 8 days. Afterwards, they disappeared from the circulation and remained absent upto the last period of observation. There was slight rise of temperature at the time of first appearence of the parasites except piglet no.4. No other clinical signs of disease were noticed in any piglets. All the piglets were found healthy. They were slaughtered after a period of 3 months to 4½ months, after infection.

During the latent period of infection, blood from all the piglets were collected and inoculated into each mice at intervals of 15 days and 20 days. The mice died with the disease after a period of 10 - 12 days. The last mice in each case did not pick up the infection and survived. The death of the mice with the disease showed that trypanosomes were present in the blood circulation of the infected piglets in the latent period. Thus, the possibility of pigs as carrier host of surra is demonstrated.

The blood picture showed that there was no change in total red cell count, haemoglobin percentage, packed cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and erythrocyte sedimentation rate in all the piglets. The total counts of white blood cells

showed rise in the beginning with a gradual decrease and came to normal without any further changes. Neutrophilia was observed with corresponding moderate decrease of lymphocytes in the prepatent and patent period of all the piglets. The percentage of neutrophils gradually decreased with a corresponding slight increase of lymphocytes in the latent period, except piglet no.5, in which there was neither decrease of neutrophils nor increase of lymphocytes. The percentage of eosinophils was found to be slightly increased in piglet nos. 2 and 4 only. Slight monocytosis was noticed in all the piglets.

No gross pathological lesions were found in the internal organs of the experimental piglets, which were slaughtered after the end of the observations.

REFERENCES

1. Ahluwalia, S.S.,(1959c) - " A note on Coccidial infection in domestic pig". Science and Culture., 25, 314 -315.

2. Appasov, R.N., (1960) - "Intestinal parasites of man and pig in the Alma-Ata area". Vet.Bull., 31, 319 (1961).

3. Auerbach, E., (1953) - Jour. Morph., 93, 405 - 445, cited by Levine (1961).

4. Ajawani, G.A., Thillanyagam, P.M. & Pillai,K.T.,(1933) - " A case of Surra in a dog". Indian J. Vet. Sci., 10, 132.

5. Biester, H.E. and Murray, C.(1929) - " Studies in infectious enteritis of swine. IV. Intestinal Coccidiosis". Jour. Am. Vet. Med. Assoc., 75, No.6, 705.

6. Idem. (1934) - " Studies in infectious enteritis of swine. VIII. Isospora suis n. sp. in swine". Ibid.,85, 207.

7. Idem. (1934) - " Studies in infectious enteritis of swine. The occurrence of I. suis n. sp. in swine a preliminary note." Ibid., 84, 294.

8. Brumpt, E.,(1909) - " Demonstration du role pathogene du Balantidium coli, enkystement et conjugaison de cet infusoire." C.R.Soc. Biol. Vol. LXVII,P.103

9. Baldrey, F.S.H.,(1910) - " Transmission of Surra." The Journal of Trop.Medi., V, 595 - 596.

10. Boehringer, E.G. & Boehringer, I.K.,(1960) - " Susceptibility of pigs to T. equinum". Vet. Bull.,31, 15 (1961).

11. Cabrera, D.J. & Lui,T.J., (1956) - " The nuclear shift index and other haematological indexes in Surra." Am. Jour. Vet. Res., 17, 617 - 625.

12. Chaussat, (1877) - "Trypanosomes & Trypanosomiasis" cited by Laveran & Mesnil (1907).

13. Crookshank, E.M., (1886) - "Flagellated protozoa in the blood of diseased and apparently healthy animals". J.Roy. Mic. Soc., 6, 913.

14. Chetti, C.R.V., (1922) - "Trypanosomiasis in cattle." Madras Vet. J., 17, 2 - 5.

15. Cross, H.E. & Patel, F.G., (1922) - "Camel Surra". Dept. Agri. Pb. Vet. Bull. No.8.

16. Castillo, A.M. & Joaquin, Z.B., (1955) - " Studies on sugar. 1. The blood sugar level and the effect of glucose infection on rats infected with T. evansi." Philippine J. Anim. Indust., 16, 189.

17. Dikmans, G., (1948) - "The dog, canis familiaris, a new host of Balantidium sp." Porc. of Helmin. Soc. of Wash., 15, 40 - 41.

18. Dejesus, Z., Cabrera, D.J. and Gonzales, F.Z., (1949). - "Studies on Control of Surra. II. The pig as probable reservoir host of surra trypanosomes." U.P. Nat.Applied Sci. Bull. Vol. 9, pp. 341-354.

19. Ewing, S.A. & Bull, R.W., (1966) - " Severe chronic canine diarrhoea associated with Balantidium - Trichuris infection". Jour.Am.Vet.Med. Ass., 149, 519 - 520.

20. Evans, G.H., (1880) - "Report on Surra disease in the Dera Ismail Khan district". Punjab Govt. Milit. Dept., no.493, 4467.