STUDIES ON *HABRONEMA MEGASTOMA* (RUDOLPHI, 1819) INFECTION IN HORSES

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CERTIFICATE

Shri K. Narendra Kumar Jain has satisfactorily prosecuted the course of research and that the thesis entitled "STUDIES ON HABRONEMA MEGASTOMA (RUDOLPHI, 1819) INFECTION IN HORSES" submitted, is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by him for a degree of any University.

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CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON HABRONEMA MEGASTOMA (RUDOLPHI, 1819) INFECTION IN HORSES" submitted in partial fulfilment of the requirements for the degree of MASTER OF VETERINARY SCIENCE (PARASITOLOGY) of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by Shri K. NARENDRA KUMAR JAIN under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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ABSTRACT

The investigation on the incidence, gross and histopathological changes in the tumours caused by *H. megastoma* and certain immunological studies of this infection in horses was explored. A total of 24 stomachs of horses were examined on autopsy, out of which 13 horses revealed the presence of *H. megastoma* tumours showing 54.16 per cent incidence.

The size of the nodules varied from 2 x 2.5 x 2.2 cm to 12 x 10.5 x 9.6 cm and the number of nodules ranged from 1-7 per horse. Mixed infection of *H. muscae*, *P. equorum* and Gastrophilus bots with *H. megastoma* was noticed in 7 horses. Symptoms of colicy pains and other digestive disturbances were noticed in a few horses, which on autopsy revealed *H. megastoma* tumours in the stomach. The tumours were mostly

located in the fundic and cardiac glandular region with one case in the pyloric region. Five of the positive cases showed suppurative infection.

The histopathological changes in the glandular mucosa showed congestion, oedema, infiltration of inflammatory cells with hyperplasia of the glands. The tunica propria was thickened with fibrous connective tissue and inflammatory cells. The submucosa revealed maximum changes with infiltration of eosinophils, mononuclears, proliferating capillaries and connective tissue proliferation. The cut section of the worm was surrounded by necrotic debris and inflammatory cells. In addition few cells showed irregularity in shape with mitotic figures. The tunica muscularis showed infiltration of inflammatory cells with degenerative changes.

The immunological studies made by agar gel diffusion and counter current immunoelectrophoresis employing whole worm saline extract of *Ha. megastoma* antigen against hyperimmune serum raised in rabbit, revealed three antigenic fractions. But none of the suspected sera of horses showed positive reaction. Immunoelectrophoretic study revealed three precipitin arcs. Indirect haemagglutination test showed a titre of 1 : 128 with hyperimmune serum raised in rabbit when compared to the range of low titre of 1 : 2 to 1 : 8 with suspected horse sera.

ABBREVIATIONS

CC	:	Cubic Centimeter
CCIEP	:	Counter current immunoelectrophoresis
Cm	:	Centimeter
IHA	:	Indirect haemagglutination
Kg	:	Kilogram
mA	:	Milli amperes
ml	:	Millilitre
mcg	:	Microgram
mm	:	Millimeter
NRS	:	Normal Rabbit Serum
NSS	:	Normal Saline Solution
PBS	:	Phosphate buffer saline
RBC	:	Red blood corpuscle
rpm	:	Revolutions per minute

CHAPTER I

INTRODUCTION

In India, over two million horses/Ponies are being used as work animals to pull carts/tongas and carry pack loads in remote areas. Also horses are being used in racing which is becoming a leading spectator sport and an important recreational activity throughout the world and in the game of polo, a sport which is increasing in popularity. At the same time horses are being widely used by the army men. Of late, the quality of the horses in India have improved enormously and as a result we have better blood lines available known as Indian thoroughbred horses.

Internal parasites have adopted themselves quite well to a comfortable existence within the body of the horse. They occur in many varieties and in vast numbers within an individual animal. Parasitism is insidious in its damage. It robs nutrition and damages tissues throughout the body. Many fine horses with tremendous genetic background fail to reach their potential every season because they have been stunted, crippled or outright killed by the parasites.

Of the common stomach worms of equines, Viz., Habronema megastoma (Draschia megastoma), Habronema muscae and Habronema microstoma reported from all over the world

(Gaiger, 1910; Datta, 1933; Rai, 1960; Pandey, et al., 1981 and Lyons et al., 1983). Habronema megastoma is known to cause tumorous growth in the stomach.

Gastric habronemiasis is relatively common and although it may cause sporadic deaths most affected horses shows no signs of illness. H. megastoma has been known to cause parasitic nodules in the stomach of horses with resultant gastric mal-functions leading to ill health despite standard nutritional supplement and management. Further these nodules invariably cause colic by way of obstruction at the pyloric region leading to complications and death (Canard, 1937). Horses of all ages a e susceptable but the disease is most common in adults.

In most of the horses the lesions cause only a mild chronic gastritis. In rare cases ulceration is followed by perforation with the development of local peritonitis and death (Soulsby, 1965).

The usual conventional methods of diagnosis are not adequate to detect the infection of habronemiasis. Hence an attempt has been made to explore the possibility of serodiagnosis. Further, perusal of literature indicated paucity of information on various aspects of H. megastoma infection in horses. Therefore, the following investigation is proposed to be undertaken.

CHAPTER II

REVIEW OF LITERATURE

2.1 INCIDENCE

Descazeaux and Morel (1933) reported habronemiasis in 12 horses using biological method. *H. megastoma* worms were noticed in 7 horses and *H. muscae* in all the 12 horses.

In Denmark, stomachs of 172 horses was examined by Nielsen (1933) and noted all the three Habronema species. He considered *H. megastoma* was most dangerous, since it produces nodules in the stomach of horses.

Canard (1937) conducted autopsies on horses at Diego-Suarez and found that 90 per cent of the horses had tumours caused by *H. megastoma*. He stated that in 6 cases, death was resulted from the complications initiated by the tumours.

By employing modified xenodiagnostic technique, Desales and Jansen (1945) reported habbonemiasis in 85 (97.70%) out of 87 horses.

Gorshkov (1958) noticed various species of Habronema occuring in horses in different provinces of USSR. He observed that Central European part was predominated by *H. megastoma* (12 to 57%) than *H. muscae*. South Eastern area predominated by *H. muscae* (22 to 100%) than *H. megastoma* (13 to 75.8%). He also mentioned that the rate and intensity

of infection depends on climatic conditions, management of the animals and treatment of manure.

Rai (1960) reported that 70 per cent of the horses examined at postmortem revealed presence of nodular growths in the stomach caused by H. megastoma.

Occurence of cutaneous and gastric habronemiasis in Phillippines and Thailand horses was noticed by DeJesus (1963). He observed that the horses introduced into Thailand were more susceptible to Habronema infection than the indigenous breeds.

Waddell (1969) examined 280 stomachs of horses and found 115 (41%) horses positive for H. megastoma.

Presence of D. megastoma and H. muscae in a horse in Czechoslovakia was reported by Knezik and Belak (1972).

Reddy et al. (1976) reported two cases of D. megastoma and H. muscae infection in horses causing lesions in stomach, liver, spleen, kidneys and heart.

In Uzbek, 20 donkeys were examined by Zhdanova (1976) and found H. megastoma in 5 donkeys.

Scialdo (1977) examined stomach of 200 horses slaughtered in Texas, USA. He noticed H. megastoma infection in 48 horses, H. muscae in 22 horses. The number of D. megastoma worms were found upto 19 per horse.

Gaur and Reddy (1978) surveyed habronemiasis in 15 horses and ponies and reported 20 per cent incidence.

Alcaino et al. (1980) screened stomachs of 419 horses slaughtered in Central Southern area of Chile. The infection rate of H. megastoma was 22.9 per cent, H. muscae was 17.7 per cent and G. nasalis was 86.6 per cent.

Macruz et al. (1981) noticed Habronema infection in 76 horses, of which 22 horses had mixed infection with parascaris, Strongylus, Anaplocephala or Gastrophilus.

In Morocco, 94 horses were examined for stomach worms by Pandey et al. (1981). They found all the horses were negative for H. megastoma lesions. H. muscae occured in 95.8 per cent and H. microstoma in 75.6 per cent of horses.

A total of 363 thoroughbred horses of 1 to 26 years age were autopsied by Lyons et al. (1983) and observed infection rates of D. megastoma adult 62 per cent, D. megastoma immature 13 per cent and H. muscae adult 38 per cent. They noticed gross lesions caused by D. megastoma in 58 per cent of the cases examined. The highest number of D. megastoma (adult) found was 4,100 per horse and the number of lesions observed grossly varied from 1 to 12 per horse. Further, Lyons et al. (1984) examined 396 stomachs of horses for D. megastoma worms. 63 per cent of the horses

showed lesions in the glandular region of the stomach and 95 per cent of the lesions were ± 50 mm in diameter. Prevalence of lesions was more in 1 to 7 year aged horses (81%), with overall 41 per cent of lesions in 4 years age group.

Shamsul Islam (1985) reported that out of 35 horses examined in Jambia, 10 (28.57%) were positive for H. megastoma. The maximum number of Habronema worms recovered from each horse was 1,423.

Bauer (1986) noted the non-occurence of D. megastoma out of 89 horses examined in Northern Germany.

2.2 CLINICAL SIGNS

Scott (1932) mentioned a brief account of clinical manifestation in a horse with colic which revealed H. megastoma tumours on autopsy.

Thomas (1944) reported a case of colic in a horse whose autopsy revealed, tumour at the pyloric region of the stomach.

Clinical signs like gastritis, anaemia, reduction in haemoglobin per cent with digestive disturbance and periodic increase in the temperature of the horses affected with H. megastoma was reported by Gorshkov (1946).

Arnold (1959) reported that recurrent colic and chronic debility was noticed in the horses, which had parasitic nodules in the fundic or cardiac portion of the stomach, when compared to the horses which had nodules in the non-glandular region. He considered Habronema abscesses as a primary focus of septic infection.

Soulsby (1965) stated that colicy pain was more noticeable in horses affected with H. megastoma when the tumour occured near the pyloric region. Further, he opined that the tumours might cause acute haemorrhage or perforation of the stomach wall leading to acute peritonitis and death. On the contrary James (1984) held the opinion that Habronema spp. rarely cause colic but this species can be incriminated as a predisposing cause to gastric perforation of rupture.

Misra (1984) observed colicy pain in a horse which on postmortem revealed a granuloma at the pyloric end caused by D. megastoma with rupture of the stomach wall.

Jubb and Kennedy (1985) stated Habronema nodules generally produce no clinical disturbance. He considered that rarely these nodules lead to abscessation or perforation when secondarily infected with pyogenic bacteria.

Draschia megastoma caused the development of nodules upto 4 cm diameter, with central abscessation in the cardiac region, was reported by Hanna (1986). Heavy burdens may

lead to non-specific digestive disturbance, weight loss and weakness with mild colic during feeding in Drachia infection.

2.3 GROSS PATHOLOGY

Scott (1932) reported that the tumours in the wall of stomach were about the size of a man's fist with several openings from which the worms were expelled out on pressure.

Thomas (1951) stated that H. megastoma cause tumours of the size of a hens egg which appeared like honey comb with small communicating cavities with an opening into the stomach which contained greyish caseous mass. He concluded that the nodules were due to reaction of the submucous tissue to the irritation of the worms.

Morgan and Hawkins (1953) noticed that the parasite penetrate deeply into the mucous membranes and produced tumour like abscess. The nodules measured from 1 to 4 inches in diameter and centrally possessed many worms with pus or cheese like mass. They stated that nodules may occur anywhere in the stomach but usually located in the non-glandular area.

The tumours caused by H. megastoma contain cavities in which the worms live and have one or more openings on the surface of the tumours (Lapage, 1956).

Arnold (1959) reported occurence of Habronema tumours along with suppurative changes in the glandular portion of the stomach.

The tumours were as large as hens egg size and usually seen at the junction of the cardiac and the glandular portion of the stomach. The tumours contain necrotic and caseous material with worms in the fistulous tracts which lead from the lesion to the lumen of the stomach (Soulsby, 1965).

Nieberle and Cohrs (1967) reported that H. megastoma penetrate the lumen of the stomach boring through the mucosa into the submucosa where they remain in balls formed of several worms rolled up together in regular chambers embedded in greyish yellow porridge like purulent mass of debris. The worms cause chronic inflammatory connective tissue proliferation and nodules increase in size and reach hazelnut to that of hens egg projecting into the lumen of stomach. The cavities communicates with the lumen of the stomach and open on the summit of the projecting tumours. These nodules were generally observed in the fundic region close to the margoplicatus.

Large gastric tumours containing adult worms were observed by Ashizawa et al. (1973). They noticed 12 granulomatous nodules of about 1 cm in diameter in the glandular part of the stomach with larval forms of the worms.

Damodaran and Ramachandran (1973) observed a small sessile nodular growth about 1.5 cm in diameter in the fundic portion of the stomach, adjacent to the margoplicatus.

Macruz et al. (1973) examined 82 Habronema nodules in the glandular stomach of 76 horses and isolated beta hemolytic streptococci bacteria from 74 nodules. Also few cases showed coliforms and other bacteria.

Reddy et al. (1976) observed several irregular polyploid growths of variable sizes and one of the conspicuous growth measured 5.0 x 3.5 x 2.5 cm. They noticed large number of fine hair like worms in the growths.

Tumours ranging from 1 to 8 in number with size varying from pea to cricket ball were noticed by Radhakrishna Reddy et al. (1978-79). The cauliflower like tumours were perforated by sinuses, with openings into the gastric lumen.

Draschia megastoma usually form distinct indurated submucosal nodules over the glandular mucosa, close to the margoplicatus. The nodules varied in size from 1 cm in diameter and contained fistulous tracts which communicated with the gastric lumen through a common pus filled opening (Mansmann et al., 1982).

Misra (1984) noticed a firm mass of tissue of about 8.5 x 6.0 cm size at the pyloric end of the stomach. The mass was fibrous, hard to cut and had caseated mass. Cultural examination revealed E. coli organisms.

2.4 HISTOPATHOLOGY

Soulsby (1965) stated that the sections of the tumours showed marked proliferation of the glandular tissue with chronic inflammatory changes in the muscularis mucosa and submucosa. In the later stages fibrosis and atrophy of the glandular tissue occurred with marked eosinophilic infiltration.

Nieberle and Cohrs (1967) reported that the wall of the nodules consisted of newly formed connective tissue with three zones. The innerside zone was hyalinized, swollen and contained few cells, the middle zone was heavily infiltrated with the lymphocytes and plasma cells. The outer zone contained fibrous connective tissue capsule infiltrated with eosinophils. The mucous membrane and muscularis mucosa exhibited no changes in the region of nodules. Cicatrisation was observed when the worms left the nodule. They further described that when the worms die, the mass of debris gets inspissated and calcified.

Histopathology of the Habronema tumours was studied by Ashizawa et al. (1973). They stated that the larvae of H. megastoma penetrates into the stomach wall and cause the formation of individual, craters like nodules within which they develop. The nodules had a wall of thin granulation tissue surrounded by hyperplastic submucous connective tissue with areas of leucocytic infiltration. Further, they mentioned that as the development proceeds, the nodules become united to form the large tumours in association with the adult worms.

The microscopic structure of the nodular growth caused by H. megastoma near the margoplicatus of the stomach was described by Damodaran and Ramachandran (1973). The columns of stratified squamous epithelium from the margoplicatus had infiltrated into the glandular portion of the stomach and united to form an anastomosing pattern, enclosing islands of fibrous connective tissue. The mucosa and submucosa showed cellular infiltration, predominantly eosinophils and lymphocytes and the muscular coat had moderate degree of cells. The gastic glands were atrophied, scattered and the mucosal surface was necrosed and ulcerated.

Reddy et al. (1976) noticed that mucous membrane appeared much thickened due to marked fibrosis and accumulation of large number of leucocytes, particularly eosinophils,

separated by fibrous bands. The mucosal glands showed degeneration and necrosis. Oedema was observed in tunica propria and muscular layers and the later appeared partly detached from submucosa. In addition marked congestion of blood vessels was noticed in this region. The serous and muscular layers showed leucocytic infiltration particularly eosinophils. They concluded that the constituents of tumourous growths were predominantly mononuclear cells, eosinophils and fibrous connective tissue cells. The tissue reaction indicated a mixed granulomatous inflammation.

Radhakrishna Reddy et al. (1978-79) noticed granulomatous reaction around the section of the worm with central core of necrotic and cellular detritus. They also noted eosinophilic infiltration.

Misra (1984) reported that the histopathological examination of the firm mass of tissue in the stomach revealed granulomatous tissue reaction akin to parasitic infection. The reaction consisted of intense infiltration of mononuclear cells with proliferation of fibroblasts and the gastric glands showed foci of infiltration of lymphocytes.

Jubb and Kennedy (1985) reported that the D. megastoma burrows into the submucosa to produce large tumour like nodules. In the submucosa the worms provoke a granulomatous reaction with cellular infiltration of eosinophils.

2.5 IMMUNOLOGICAL ASPECT

2.5.1 Agargel diffusion test and immunoelectrophoresis

Perusal of the available literature indicated meagre information on the varied immunological tests adopted in the immunology of *Ha. megastoma*. However, some workers have utilized these techniques for the immunodiagnosis of different helminthic infections.

Kagan (1956) revealed the nature and specificity of various ascarids antigen using double diffusion agar technique with homogenous antisera raised in rabbits. He observed 9, 7, 6 and 5 precipitation bands in the ascarids of pig, man, cat and dog, respectively.

Using Ouchterlony technique, Coloman and Fotorny (1961) observed 4 groups of precipitation bands to the whole worm extract of *Hymenolepis nana* with its homologous antiserum.

Biguet *et al.* (1962) analysed the saline antigenic extract of *Onchocerca volvulus* with homologous hyperimmune serum by immunoelectrophoresis and revealed 14 antigenic fractions.

Geyer (1967) analysed the freezed dried extract of whole adult *Fasciola hepatica* with its homologous antiserum raised in rabbit employing immunoelectrophoresis and found 23 precipitation lines.

Gaur and Deo (1972) analysed the whole worm antigen of *A. suum* by precipitin ring test and agar gel diffusion test and reported more than one antigenic component was present in the antigen.

Zyngier (1974) conducted Ouchterlony plate test with the extracts of *Toxocara canis* and antiserum raised in rabbits and revealed 7 antigenic bands.

Sincai and Pora (1976) noticed seven precipitation lines in gel diffusion test with *Ascaridia galli* whole extract against homologous immune serum.

Choi and Lee (1979) observed 9 precipitation bands in the Ouchterlony test and 11 bands in the immunoelectrophoresis for the crude *F. hepatica* antigen.

2.5.2 Counter current immunoelectrophoresis

Hillyer (1975) reported that counter electrophoresis test was more advantageous over the immuno diffusion test as the precipitation can be observed after 30 minutes.

Enayat and Pezeshki (1987) compared the counter electrophoresis test with IHA test for detection of antibodies in experimentally infected guinea pigs with *Toxocara canis* and concluded that the counter electrophoresis test could be performed rapidly and with ease, but the titres were lower than that of IHA test.

Kaliraj et al. (1977) detected 1 to 3 precipitation bands in the 16 sera of men carrying microfilaria of W. bancrofti by employing CCIEP test with antisera raised in rabbits.

Pathak et al. (1984) conducted CCIEP test with 40 sera samples of pig infected with Cysticercous cellulosae using water soluble extracts of scolex and cyst wall of T. solium as antigen. They observed a sharp and thick concave precipitin band at the point of interaction after 90 minutes in 39 sera (97.5%). The precipitin reaction was best with Barbitone buffer at pH 8.6.

Xu and Zhai (1985) conducted CCIEP test using suspected horse sera for cerebrospinal filariasis with the purified immature Setaria digitata antigen and noted better results.

2.3.3 Indirect haemagglutination test

Sood et al. (1972) noted a titre of 1 : 4 in 6 cases and 1 : 12 in 2 cases by IHA test in 50 sera samples of dogs affected with hook worms, but they did not noticed any precipitation reaction by agar gel diffusion test or circumoval precipitin test.

Dinanath Kulkarni et al. (1975) studied the comparative efficiency of agar gel diffusion and passive haemagglutination test in the immunodiagnosis of Ascaridia galli. They

noted the titre of 1 : 320 and 1 : 640 and even higher titres in naturally infected birds. Agar gel diffusion test did not reveal any precipitin lines. They concluded that the IHA test was more reliable and sensitive than agar gel diffusion test.

Polidori et al. (1982) detected antibodies against T. saginata in 28 cattle with titres of 1 : 4 to 1 : 32 out of 1,000 cattle tested employing IHA test.

The IHA test conducted by Novakova et al. (1983) observed a titre of 1 : 20480 to 1 : 40960 to the purified soluble antigen from sexually mature A. suum with the rabbit immune sera.

Ratnam and Khanna (1983) conducted IHA test for the diagnosis of cysticercosis in cattle and buffaloes. Out of the total 200 cattle examined 13.5 per cent were positive by serological test whereas 5.5 per cent were positive by autopsy. Out of 100 buffaloes, 9 per cent were positive by the serological test but on autopsy no cysticerci could be noticed.

Wang et al. (1985) conducted IHA test for the diagnosis of fascioliasis in 150 cattle and compared with faecal examinations. They observed 111 sera samples were positive for fascioliasis, while 113 animals revealed the presence of fascioliasis by faecal examination.

CHAPTER III

MATERIALS AND METHODS

3.1 SOURCE OF MATERIAL

Twenty four carcass of horses referred to the Department of Pathology, College of Veterinary Science, Rajendranagar, Hyderabad for postmortem examination during the past one year i.e. from June, 1986 to May, 1987, formed the source of material for this study. The horses belonged to the Hyderabad Race Club, National Police Academy, A.P. Riding Club, Biological E. Limited and 1 (A) R & V Regiment (NCC), Hyderabad.

A detailed autopsy was conducted on all the carcasses. The stomach was opened on its greater curvature and the contents were washed thoroughly with normal saline solution. The stomach was examined for the presence of nodular growths and the gross lesions were recorded. The size, number and location of the nodules was noted. The nodules were incised and the parasites were collected in Normal saline solution. The total number of worms obtained from the nodules were noted. The nodules containing suppurative material was examined for micro-organisms.

3.2 Identification of the parasite

The parasites collected from the nodules were washed thrice with NSS. Some of the worms were dehydrated in 70

per cent alcohol and cleared in 1 per cent lactophenol for identification. For the preparation of antigen the parasites were further washed, four to five times and preserved in sterile Normal saline at -20°C.

The washed stomach content was centrifuged and the sediment was examined for the presence of ova/larva of the parasite.

3.2.1 Preparation of antigen

The H. megastoma worms were subjected to freezing and thawing. The worms were triturated with sterile NSS in a pestle and mortar which was kept in an ice-jacket. The resultant homogenous suspension was centrifuged at 3,500 rpm for half an hour. The clear supernatant fluid was removed in sterile screw cap tubes after adding a few drops of Merthiolate (1 : 10,000) as a preservative and stored at -20°C till use.

3.3 HISTOPATHOLOGY

Small pieces of tissues from the nodules were collected and preserved in 10 per cent formalin. Tissues were deformalinized, dehydrated, cleared in xylol and embedded in paraffin. Sections were cut at 4-5 micron thickness and stained with haematoxylin and eosin stain and mounted in D.P.X.

3.4 PROTEIN ESTIMATION

The protein content of the whole worm saline extract antigen was estimated by micro-Kjeldahl method.

3.5 PREPARATION OF HYPERIMMUNE SERUM

Two healthy rabbits aged about 3-4 months weighing 2 kg, maintained under strict hygenic conditions and normal diet, were used for hyperimmunization. The rabbits were immunized with the whole worm saline extract antigen. The antigen and Freunds Complete Adjuvant (Difco, U.S.A.) each 0.5 CC was mixed thoroughly till a hard milky white suspension was formed. It was injected I/m to the rabbits at weekly intervals for four weeks. Five days after the last injection the rabbits were bled intracardially and the serum was collected in the sterile screw cap tubes to which a few drops of Merthiolate (1 : 10,000) was added and preserved at -20°C till use.

3.6 COLLECTION OF SERA FROM SUSPECTED HORSES

Random sera samples from 60 suspected horses were collected in the sterile serological tubes to which a few drops of Merthiolate (1 : 10,000) were added and preserved at -20°C till use.

3.7 IMMUNOLOGICAL ASPECT

3.7.1 Agar gel diffusion test

The procedure adopted was essentially same as described by Ouchterlony (1958). One per cent Agarose was prepared in NSS containing a few drops of Merthiolate (1 : 10,000). Four ml of the molten agarose was poured on clean microscopic slides and was allowed to set for one hour at 4°C. A standard protocol of 5 wells were cut each having a diameter of 4 mm, spacing 4 mm apart from the central well. The floor of the wells was sealed with the molten agarose.

In each test antigen was charged in the central well and hyperimmune serum or suspected horse sera in the peripheral wells. Normal Rabbit Serum (NRS) was used as control in each case. After charging the wells, the slides were kept in humid chamber at room temperature and the results were recorded after 24 and 48 hours. The negative results were declared after 96 hours of observation. The test was standardised with hyperimmune serum against the whole worm saline extract antigen.

3.7.2 Staining of gel slides

The slides which showed precipitation lines were kept in NSS for 24 hours. Gels were finally dried overnight at 37°C by keeping a wet filter paper over the surface of the

gel. After drying of the gels, filter paper was removed by wetting. The slides were later stained by keeping in 0.5 per cent Amidoblack stain for 15 minutes and destained with 5 per cent acetic acid.

3.7.3 Electrophoresis

One per cent agarose was prepared in Barbitol buffer (pH 8.6). Four ml quantity was poured on microscopic slides and allowed to set at 4°C for one hour. A single well was punched at one end of the agarose slide and the gel was sucked out. The well was charged with the *Hi. pneastoma* antigen. Each electrode compartment of electrophoretic tank was filled with 50 ml of Barbitol buffer (pH 8.6). Slides were kept in the electrophoresis tank and contacts were made to electrode compartments with Whatman No.1 filter paper wicks and the electrophoresis apparatus was fastened to power pack. Electrophoresis was allowed to proceed for one and half hour with a current flow of 5 mA per slide. Finally the slides were fixed in 10 per cent glacial acetic acid and stained with 0.5 per cent Amidoblack and destained with 5 per cent acetic acid. The antigenic fractions were recorded.

3.7.4 Immunoelectrophoresis

One per cent agarose was prepared in Barbitol buffer (pH 8.6). Four ml of quantity was poured on the microscopic

slides and was allowed to set. A trough at the centre of the gel slide and wells on either side of it was punched. The gel from the wells was sucked out. One well was charged with the antigen and the other with NRS as control. Contacts were made and the operation was carried out for one and half hour with a current flow of 5 mA/slide. A drop of Bromophenol blue was added to the antigenic well as a marker.

After electrophoresis the gel from the trough was sucked out and charged with hyperimmune serum. Double diffusion was allowed to proceed overnight in a closed humid chamber at room temperature. Observations were made for the presence of arcs after 24 hours and 48 hours and the slides were stained by 0.5 per cent Amidoblack.

3.7.5 Counter current immunoelectrophoresis

Four millilitre of 1 per cent agarose in Barbitol buffer (pH 8.6) was poured on the slide and allowed to set. Parallel wells 4 mm in diamter spaced 6 mm apart in the gel were cut. The antigen was charged in the wells towards the cathode side and hyper immune serum/suspected sera of horses was charged towards anode side. Electrophoresis was carried out for one hour with a current flow of 5 mA/slide. The number of precipitation lines were noted immediately after the test.

3.7.6 Indirect haemagglutination test

The indirect haemagglutination test was done as described by Boyden (1951) with slight modification.

Sheep blood was collected in equal quantity of Alsever's solution and preserved at 4°C. The cells were washed thrice with PBS (pH 7.2) at 2,500 rpm for 5 minutes. 0.6 ml of the packed RBCs was taken in a test tube with 10 ml of PBS (pH 7.2) and 10 ml of freshly prepared Tannic acid solution (1 : 20,000) and the mixture was incubated at 37°C for 15 minutes in water bath. The tanned cells were centrifuged at 2,500 rpm for 7 minutes and to the packed cells, 20 ml of PBS was added. Ten ml of the cells were kept separately as control. To the remaining packed cells, 10 ml of PBS (pH 6.4) and 0.5 ml of the antigen (700 mcg protein) was added and incubated at 37°C for 30 minutes in the water bath. Later it was centrifuged for 5 minutes at 2,500 rpm and the supernatant was discarded. The packed cells coated with Tannic acid and antigen were washed 3 times with PBS (7.2) containing 1 per cent inactivated NRS.

Final concentration of 1 per cent RBCs suspension was made with PBS containing inactivated NRS.

Test proper:

The hyperimmune serum, suspected sera of horses and NRS were decomplemented at 56°C for half an hour before use in the test.

1. 0.25 ml of PBS (pH 7.2) containing inactivated NRS was added to all the wells of perspex plates.

2. 0.25 ml of hyperimmune serum/suspected sera of horses was added to the 1st well and double dilutions were made, commencing from 1 : 2.

3. 0.25 ml of tanned antigen coated RBC were added to all the wells.

4. Suitable controls of tanned sheep cells plus diluent, tanned sheep cells sensitized with antigen plus diluent, tanned sheep cells sensitized with antigen without diluent and positive known serum plus diluent were kept in each case.

5. The plates were shaken gently and the results were recorded after incubation overnight at 4°C.

CHAPTER IV

RESULTS

4.1 INCIDENCE

A total of 24 horses were examined on autopsy for the presence of Habronema nodules in the stomach, out of which 13 horses revealed the presence of *H. megastoma* nodules showing 54.16 per cent incidence. Remaining 11 horses did not reveal any Habronema tumours. The age group of horses examined ranged from 2 to 15 years. The Habronema infection was mostly seen in the age group of 3 to 8 years. The percentage incidence was more in 4 years aged horses. The nodules varied in their sizes and the number ranged from 1 to 7 per infected horse.

Out of 13 positive cases, seven horses had mixed infections. Out of the seven mixed infections recorded, two showed *H. muscae* and *Parascaris equorum*, two were infected with *H. muscae* and Gastrophilus bots and the remaining three had *H. muscae* infection.

4.2 IDENTIFICATION OF PARASITE

Habronema megastoma showed the characteristic head which was constricted off from the body and funnel shaped pharynx (Fig. 3). The posterior end of the male worm was curved and

possessed unequal spicules (Fig. 4). The observation of stomach contents revealed the presence of eggs containing larvae (Fig. 5).

4.3 IMPACT OF THE NODULES IN THE STOMACH

Out of 13 positive cases, six horses showed loss of appetite, high temperature and colicy symptoms. The autopsy of the above six horses revealed, rupture of stomach in one horse at the Habronema nodule region and in five horses *Ha. megastoma* tumours of varying sizes were found in the cardiac and fundic part of the stomach. Remaining seven horses did not exhibit any signs and colicy symptoms but on autopsy revealed nodules in the stomach.

4.4 GROSS PATHOLOGY

The nodules were of different sizes and projected into the lumen of the stomach (Fig. 1 and 2). The tumours varied from a marble size to cricket ball size. The minimum size noticed was 2 x 2.5 x 2.2 cm and maximum was 12 x 10.5 x 9.6 c

The nodules were located mostly in the cardiac and fundic glandular region of the stomach close to the margo-plicatus. In one case big nodule was seen at the pyloric end of the stomach. In no case the nodules were observed in the non-glandular region of stomach.

The tumours were very hard to cut and on opening showed worms in bundles rolled uptogether in regular cavities with greyish caseated debris. The cavities communicated with the lumen of the stomach and opened on the summit of the tumour. The openings on the tumours ranged from 1 to 3 in number.

In five cases the stomach showed tumours with abscess formation. The tumours possessed a lot of pus and necrotic material. Examination of the pyogenic material revealed the presence of Streptococcus pyogenus and Staphylococcus aureus.

The above observations are shown in Table 1.

4.5 HISTOPATHOLOGY

Histopathological changes in the parasitic nodules involving different layers of the glandular stomach.

4.5.1 Changes in the mucosa and lamina propria

The mucosa of the glandular stomach showed changes of chronic catarrhal gastritis characterised by congestion, mild to moderate infiltration of eosinophils and mononucleare. Hyperplastic changes in the glandular epithelium were of mild to moderate nature (Fig. 6a & b). In addition, adenomatous glandular hyperplasia infiltrating the lamina propria was seen. A few sections showed desquamation of the epithelial

layer (Fig. 7) and lamina propria was thickened with fibrous connective tissue and inflammatory cells chiefly eosinophils and mononuclears (Fig. 8).

4.5.2 Changes in the muscularis mucosa

Muscularis mucosa revealed inflammatory changes characterised by infiltration of eosinophils and degenerative changes of muscle bundles.

4.5.3 Changes in the submucosa

The changes in the submucosa were characterised by massive infiltration of eosinophils, mononuclears, polymorphs, proliferating capillaries and mild to moderate fibrous connective tissue proliferation (Fig. 9, 10 & 11). The cut section of the parasite surrounded by necrotic debris and inflammatory cells was noticed (Fig. 12). In some sections area of worm penetration showed cellular debris surrounded by heavy tissue reaction (Fig. 13). Further, some cells showed irregularity in shape with mitotic figures (Fig. 14).

4.5.4 Changes in the tunica muscularis and tunica serosa

Tunica muscularis showed degenerative changes, infiltration of inflammatory cells and connective tissue proliferation while the tunica serosa showed mild eosinophilic infiltration.

4.6 IMMUNOLOGICAL ASPECT

4.6.1 Protein estimation

The protein content of the whole worm saline extract of *H. megastoma* antigen was 1.4 mg/ml.

4.6.2 Agar gel diffusion test

The agar gel diffusion test was conducted employing *H. megastoma* antigen with hyperimmune serum raised in rabbits which indicated three sharp precipitation lines at the zone of coalescene (Fig. 15). Specific precipitation lines could be detected as early as 18 hours of incubation which became conspicuous in 24 hours. Further, the agar gel diffusion test revealed clear lines of identity between the antigen and hyperimmune serum. However, the control wells did not reveal any precipitation lines with the antigen.

Similar diffusion test was carried out with 60 sera of suspected horses with the known antigen. None of the sera samples showed precipitation lines with the antigen even after 94 hours of observation.

4.6.3 Electrophoresis

The electrophoretic studies on the *H. megastoma* whole worm saline extract antigen revealed three antigenic components (Fig. 16).

4.6.4 Immunoelectrophoresis

Immunoelectrophoretic studies were conducted with the antigen and hyperimmune serum. The antigenic fractions of *H. megastoma* showed a total of three arcs of precipitation, out of which one arc was at the point of origin nearer to the base of the well which later migrated towards cathode. Two arcs migrated towards anode (Fig. 17). The control did not reveal any precipitation arcs.

4.6.5 Counter current immunoelectrophoresis

The counter current immunoelectrophoretic study with *H. megastoma* antigen and hyperimmune serum raised in rabbits revealed sharp precipitation lines in one hour. A total of three precipitation lines were observed among which two precipitation lines were towards anode well and one precipitin line at the centre of the two wells (Fig. 18).

A similar CCIEP was conducted with 60 sera of suspected horses employing *H. megastoma* antigen. None of the sera samples revealed positive precipitin lines after one hour of the test.

4.6.6 Indirect haemagglutination test

The hyper immune serum raised in rabbit, when subjected to IHA test showed a titre of 1 : 128.

A total of 60 sera from suspected horses were subjected to IHA test, out of which nine samples had an antibody titre of 1 : 2 to 1 : 8.

One horse serum showed a titre of 1 : 2 whereas five samples had 1 : 4 and three had a titre of 1 : 8.

The remaining 51 sera samples showed button formation in the first well itself without giving any titre. The control wells revealed negative results in all the above tests.

The results of the immunological tests are shown in Table 2 and 3.

Table 1. Incidence of Habronema megastoma infection in horses

Source	Age (Years)	No. examined	No. positive	Location of the tumour	No. of tumours (range)	Size of the tumour (range) (cm)	No. of worms/horse (range)	Other parasites	Remarks
Secunderabad Race Club	2	3	1	Fundic glandular region	3	4x4x3 to 5x4x3	87	-	Pus was present
	3	3	2	Fundic glandular region	2-4	3x2x1.5 to 5x4x4	130 to 760	H.muscae in one	Parascaris equorum
	4	4	4	In one pyloric region, others cardiac & fundic	1-7	2x2.5x2.2 to 9.2x4.8x4.4	189 to 2100	H.muscae in two	Pus present in 2, Gastrophilus in 1.
	5	1	1	Fundic region	1	12x10.5x9.6	960	-	-
	6	1	0	--	-	-	-	-	-
	7	2	2	Cardiac & fundic region	3-5	2x3.5x2.5 to 5x3x3.5	450 to 2500	H.muscae in two	Parascaris equorum in 1.
	8	2	1	Fundic region	1	4x3x3	60	H.muscae	G. bots.
P. Riding Club	6	1	0	--	-	-	-	-	-
	8	1	0	--	-	-	-	-	-
National Police Academy (Hyd.)	8	1	1	Cardiac & fundic region	3	5x5x4 to 9x7x5	4600	H.muscae	Pus present
	14	1	0	--	-	-	-	-	-
S, R'nagar	15	1	0	--	-	-	-	-	-
Biological E. Limited, Hyd.	14	1	1	Fundic region	2	4x4.2x3.2 & 3x2.2x2	320	-	Pus present
	15	2	0	--	-	-	-	-	-

Table 2. Results of immunological observations of *H. megastoma* antigen with hyperimmune serum

Sl. No.	Name of the test	Medium used	Time	Results
1.	Agar gel diffusion	1% Agarose in NSS	24-48 hours	3 sharp precipitation lines were seen
2.	Immunoelectrophoresis	1% Agarose in Barbitol buffer (pH 8.6)	1½ hr. electrophoresis and incubated for 24-48 hours	3 arcs were seen (one arc towards cathode and two arcs towards anode)
3.	Counter current immunoelectrophoresis	1% Agarose in Barbitol buffer (pH 8.6)	One hour	3 precipitation lines seen (2 towards anode well and one in the centre).
4.	Electrophoresis (only with antigen)	1% Agarose in Barbitol buffer (pH 8.6)	1½ hour	3 bands were seen

Table 3. Results of immunological tests with *H. megastoma* antigen against hyperimmune serum/suspected horse sera

Sl. No.	Source	No. of samples tested	No. of samples positive	IHA test (titre)	Agar gel diffusion test	Counter current immunoelectrophoresis
	A. Hyperimmune serum					
1.	Hyperimmune serum raised in rabbit	1	1	1 : 128	Positive	Positive
	B. Suspected horse sera					
2.	Hyderabad Race Club	30	5	Two: 1:4 Three: 1:8	Negative	Negative
3.	N.C.C. (R'nagar)	15	2	1 : 4	Negative	Negative
4.	National Policy Academy (Hyderabad)	15	2	One: 1:2 One: 1:4	Negative	Negative

CHAPTER V

DISCUSSION AND CONCLUSIONS

In the present study, 24 autopsied horses were examined for the presence of parasitic nodules in the stomach. The nodules were recorded in 13 horses, representing the incidence rate of 54.16 per cent. The remaining 11 horses did not reveal any parasitic nodules. The percentage incidence has been noted by many workers which varied from place to place.

In India, incidence of *H. megastoma* infection has been reported by Rai (1960) and Gaur and Reddy (1978) as 70 and 20 per cent respectively. From other countries, Canard (1937), Desales and Jansen (1945), Waddell (1969), Alcaino *et al.* (1980), Lyons *et al.* (1983) and Shamsul Islam (1985) reported 90.00, 97.70, 41.00, 22.90, 62.00 and 28.57 per cent incidence respectively. However, Pandey *et al.* (1981) from Morocco and Bauer (1986) from Northern Germany reported the non-occurence of the *H. megastoma* infection in horses. The incidence of present study was within the range of incidence reported by various workers.

The age of the horses examined varied from two to 15 years. The infection rate was found to be more in three to eight years aged horses with maximum incidence of infection in the age group of four years. These findings are in agreement with Lyons *et al.* (1984).

The number of nodules observed varied from one to seven per horse, whereas, Lyons et al. (1983) noticed one to 12 nodules in each infected horse.

Out of 13 positive horses, mixed infection with H. muscae was noticed in seven cases. Out of these seven, two horses had infection with Gastrophilus bots and other two horses with Parascaris equorum. Similar type of mixed infection was reported by Alcaino et al. (1980) and Macruz et al. (1981).

The total number of H. megastoma worms collected from each horse ranged from 60 to 4500. This type of variation in number of worms per horse was also reported by Scialdo (1977), Lyons et al. (1983) and Shamsul Islam (1985).

The probable reasons for such a variation in the incidence rate, in the number of tumours and in the total number of worms per horse might be due to different climatic conditions, management of the animals and treatment of manure as opined by Gorshkov (1958).

In six of the 13 positive cases examined, the clinical signs exhibited by the horses included loss of appetite, high temperature and colicy symptoms which on autopsy revealed nodules in the glandular stomach. Such a colicy pain and other signs due to Habronema nodules were described by Scott

(1932), Gorshkov (1946) and Hanna (1986). Heavy burden of Habronema worms might be the cause for digestive disturbance resulting in colic.

In one case there was rupture of the stomach near the site of Habronema nodule resulting in peritonitis and death of the horse which might be due to obstruction by the large tumour at the pyloric end. This was in accordance to the observation of Misra (1984). Out of the 13 positive cases, the remaining seven horses did not exhibit any clinical signs eventhough they had nodules in the stomach which is in agreement with the suggestions made by Jubb and Kennedy (1985).

The variation in the sizes of the nodules from a marble to cricket ball size encountered in the present investigation was in concurrance with Reddy et al. (1976), Radhakrishna Reddy et al. (1978-79) and Misra (1984). Most of the nodules encountered were in the cardiac and fundic region except one which was seen at the pyloric region. Similar findings were reported by Soulsby (1965) and Nieberle and Cohrs (1967). However, Morgan and Hawkins (1953) and Arnold (1959) reported the involvement of the non-glandular portion of the stomach with Habronema nodule which is contrary to our findings.

The tumours were sessile, hard to cut and on opening revealed worms bundled up together with caseated debris in different cavities. The cavities communicated with the lumen

of the stomach by one or more openings on the summit of the tumour. These observations are in accordance with those of Thomas (1951), Lapage (1956) and Mansmann et al. (1982).

In the present investigation five tumours showed pyemic infection which on examination indicated Streptococcus pyogenus and Staphylococcus aureus infection. However, Macruz et al. (1973) besides Streptococci noted other microbes.

The histopathological changes of the nodules in the glandular mucosa were of chronic inflammatory nature which showed desquamation of epithelial layer in a few sections. Congestion, oedema, hyperplastic changes of the glandular epithelium with mild to moderate infiltration of eosinophils and mononuclear cells was noticed which suggests a long standing catarrhal gastritis. However, Soulsby (1965) and Reddy et al. (1976) reported degenerative and necrotic changes in the glandular mucosa of the stomach with atrophy of the glandular tissue which could not be noticed in the present study.

The lamina propria was thickened with fibrous connective tissue and inflammatory cells. Hyperplastic changes of the glandular epithelium of adenomatous nature infiltrating the lamina propria was observed, which is in confirmity with that of Petit and Germain (1967), who noticed adenoma like chronic inflammation and heterotropic glandular proliferation in the

Muscularis mucosa showed inflammatory changes with infiltration of eosinophils and degenerative changes of muscle bundles. These changes were considered as a process of extension of inflammatory change from submucosa. Such histopathological changes involving muscularis mucosa were not described by earlier workers although Reddy et al. (1976) described oedema in the muscularis mucosa resulting in separation of mucosa and submucosa. On the contrary, Nieberle and Cohrs (1967) did not notice any change in the mucous membrane as well as muscularis mucosa in the region of the nodule.

The changes in the submucosa were of chronic inflammatory nature with infiltration of eosinophils, mononuclears, polymorphs, angioblasts and fibrous connective tissue proliferation. The cut section of the parasite was surrounded by necrotic tissue and inflammatory cells. Similar changes were also reported by Reddy et al. (1976), Radhakrishna Reddy et al. (1978-79) and Jubb and Kennedy (1985). However, in the present study cells showed irregularity in shape with mitotic figures simulating anaplasia which is a characteristic of fibrosarcoma and such findings were not reported by the earlier workers.

The tunica muscularis showed degenerative changes, infiltration of inflammatory cells and connective tissue

proliferation while the tunica serosa showed mild infiltration of eosinophils. The involvement of these layers might be due to the extension of inflammatory changes from submucosa. Similar findings were described by Reddy et al. (1976) who also noticed degenerative changes and leucocytic infiltration in tunica muscularis.

The parasitic nodules caused by H. megastoma was described as adenomatous tumourous growth (Descazeaux and Morel, 1937), tumourous growth (Arnold, 1959) and granulomatous growth (Reddy et al., 1976).

The overall histopathological picture of the present study revealed that the tumour constitutes inflammatory cells predominantly eosinophils, mononuclears and fibrous connective tissue indicating a mixed granulomatous inflammation, which is in confirmity to that of Reddy et al. (1976).

Agar gel diffusion studies by employing undiluted hyperimmune serum against saline extract antigen of H. megastoma revealed three precipitin lines as early as 18 hours, indicating three antigenic fractions. But none of the sera samples collected from suspected horses did reveal the precipitin lines, which might be due to low level of precipitating antibodies in the suspected sera of horses. Sadun (1949) and Gaur and Deo (1972) expressed similar opinion in respect of other parasitic infection.

Immunoelectrophoresis with H. megastoma antigen against the hyperimmune serum raised in rabbit showed three precipitation arcs. These observations indicated that the antigen had both negatively and positively charged protein molecules as the migration of the charged particles was towards cathode and anode. Immunoelectrophoretic technique was utilized by several other workers (Biguet et al., 1962; Geyer, 1967 and Choi and Lee, 1979) in respect of other parasites and noticed different precipitin arcs.

Counter current immunoelectrophoresis for the rapid serodiagnosis was tried with hyperimmune serum which indicated three precipitation lines in one hour. Pathak et al. (1980) employed similar techniques for the early detection of cysticercous cellulosae infection in pigs by utilizing Barbitol buffer (pH 8.6). So also in the present study Barbitol buffer at pH 8.6 showed optimal migration ratio. Further, these findings corroborate with the observations of Geerts et al. (1981) who reported that the sensitivity of immunoprecipitation reaction in gel was influenced not only by the union of antigen and antibody in optimum proportion to provoke a visible reaction but also by the delicate balance of two opposing forces driving the reactants to unite in the gel. In the present investigation none of the sera samples from the suspected horses showed any visible precipitin lines indicating low level of antibodies.

Indirect haemagglutination test indicated a titre of 1 : 128 with hyperimmune serum raised in rabbit. However, suspected sera samples showed the range of titre from 1 : 2 to 1 : 8 which indicated the very low level of antibody in the suspected horse sera. The IHA test has been claimed to be superior over other tests by different workers (Sood et al., 1972; and Dinanath Kulkarni et al., 1975) in other parasitic infections. Further, it was noticed that the sera samples showed lower levels of antibody compared to hyperimmune serum (Sadun, 1949).

Paucity of information on various immunological techniques utilized for the detection of gastric habronemiasis necessiated comparison of results with other parasitic infections. Elaborate studies on humoral and cell mediated immune responses need to be explored to throw more light on the immunodiagnosis of this infection in horses, so that earlier chemotherapeutic measures could be adopted.

CHAPTER VI

SUMMARY

Examination of 24 horses on autopsy revealed the presence of Habronema megastoma nodules in 13 horses, representing 54.16 per cent incidence. The remaining 11 horses did not show the presence of any tumours. The infection was mostly noticed in the age group of three to eight years old horses.

There were several evaginated large and small granulomatous growths on the mucosal surface of the stomach with varied number of H. megastoma worms (60 - 1,500). The size of the nodules varied from 2 x 2.5 x 2.2 cm to 12 x 10.5 x 9.6 cm. The number of nodules ranged from one to seven per horse. Mixed infection of H. muscae, Parascaris equorum and Gastrophilus bots along with H. megastoma was noticed in some horses.

Symptoms of colicy pain and other digestive disturbance were noticed in horses, which on autopsy revealed H. megastoma tumours in the stomach. However, a few horses although had tumours in the stomach did not manifest any characteristic symptoms. The tumours were mostly located in the fundic as well as cardiac region of the stomach. In one case rupture of the stomach was noticed, which had a big tumour at the pyloric region. In five cases tumours showed

suppurative infection which on examination revealed Streptococcus pyogenus and Staphylococcus aureus organisms.

The histopathological changes in the mucosa of stomach showed congestion, oedema, mild to moderate infiltration of eosinophils and mononuclears with hyperplasia of the glandular epithelium. Desquamation of the epithelial layer was noticed in a few sections. The lamina propria was thickened with fibrous connective tissue and inflammatory cells chiefly eosinophils. Muscularis mucosa showed degenerative changes of muscle bundles and infiltration of eosinophils. The submucosa showed maximum changes. There was fibrous connective tissue proliferation with infiltration of eosinophils, mononuclears, polymorphs and proliferating capillaries. The cut section of the parasite was surrounded by necrotic debris and inflammatory cells. A few cells showed irregularity in shape with mitotic figures simulating anaplasia which is a characteristic of fibrosarcoma. Tunica muscularis showed degenerative changes of muscle bundles with inflammatory cells while tunica serosa showed eosinophilic infiltration.

The immunological studies with H. megastoma whole worm saline extract antigen against hyperimmune serum raised in rabbit revealed three antigenic fractions in the agar gel diffusion and counter current immunoelectrophoresis tests.

But none of the 60 sera samples from suspected horses showed any visible reaction in these tests. Immunoelectrophoresis, with the antigen and hyperimmune serum showed three precipitin arcs. Further, it was noticed that the *H. megastoma* antigen had both negatively and positively charged particles as shown by its migration. Indirect haemagglutination test showed the titre of 1 : 128 with hyperimmune serum raised in rabbit. Out of 60 suspected sera of horses, one showed a titre of 1 : 2, five had 1 : 4 and three had the titre of 1 : 8. The remaining 51 sera samples did not reveal any reaction.

Fig. 1. : Tumour in the fundic glandular region of the stomach of horse with necrotic material in the opening

Fig. 2 : Nodules in the glandular mucosa of stomach close to the margoplicatus

Fig.3 : Anterior end of H. megastoma showing funnel shaped pharynx and head constricted off from the body. X 160

Fig.4 : Posterior end of male H. megastoma showing characteristic unequal spicules X 160

Fig.5 : Characteristic egg of H. megastoma with larva X 300

Fig.6a : Section of the tumour in the stomach of horse showing hyperplasia, edema and infiltration of eosinophils H & E X 10(

Fig.6b : Section of the tumour showing glandular hyperplasia H & E X 200

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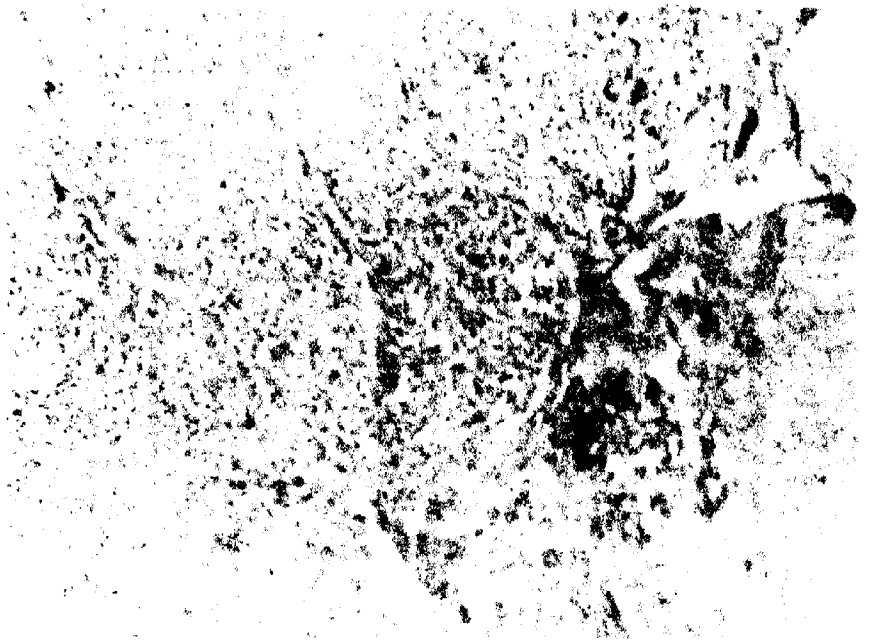


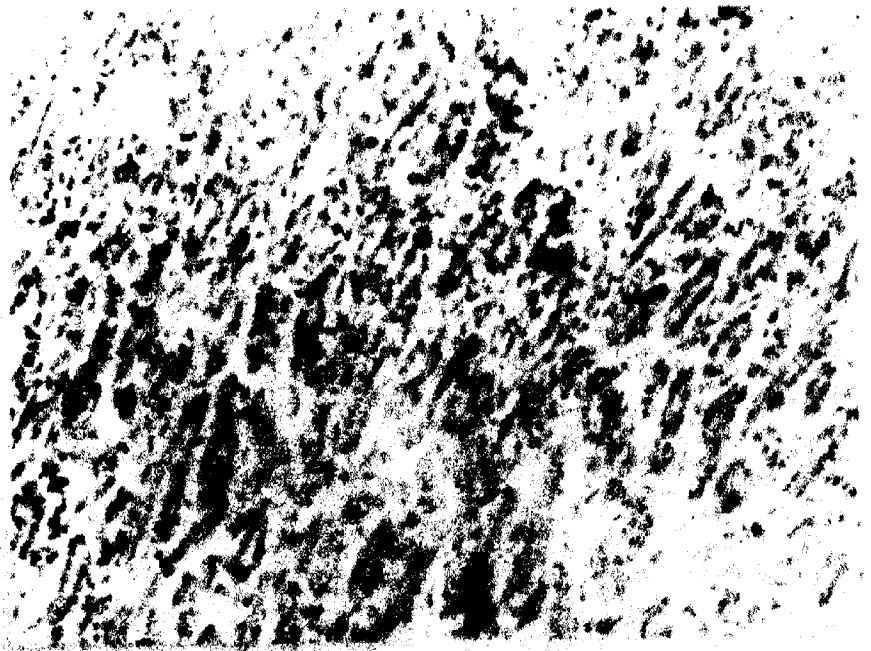


Fig.7 : Section of the nodule in the stomach show bare mucosa without epithelial lining
H & E X 100

Fig.8 : Section of the tumour showing thickening of lamina propria with fibrous tissue proliferation and cellular infiltration
H & E X 200

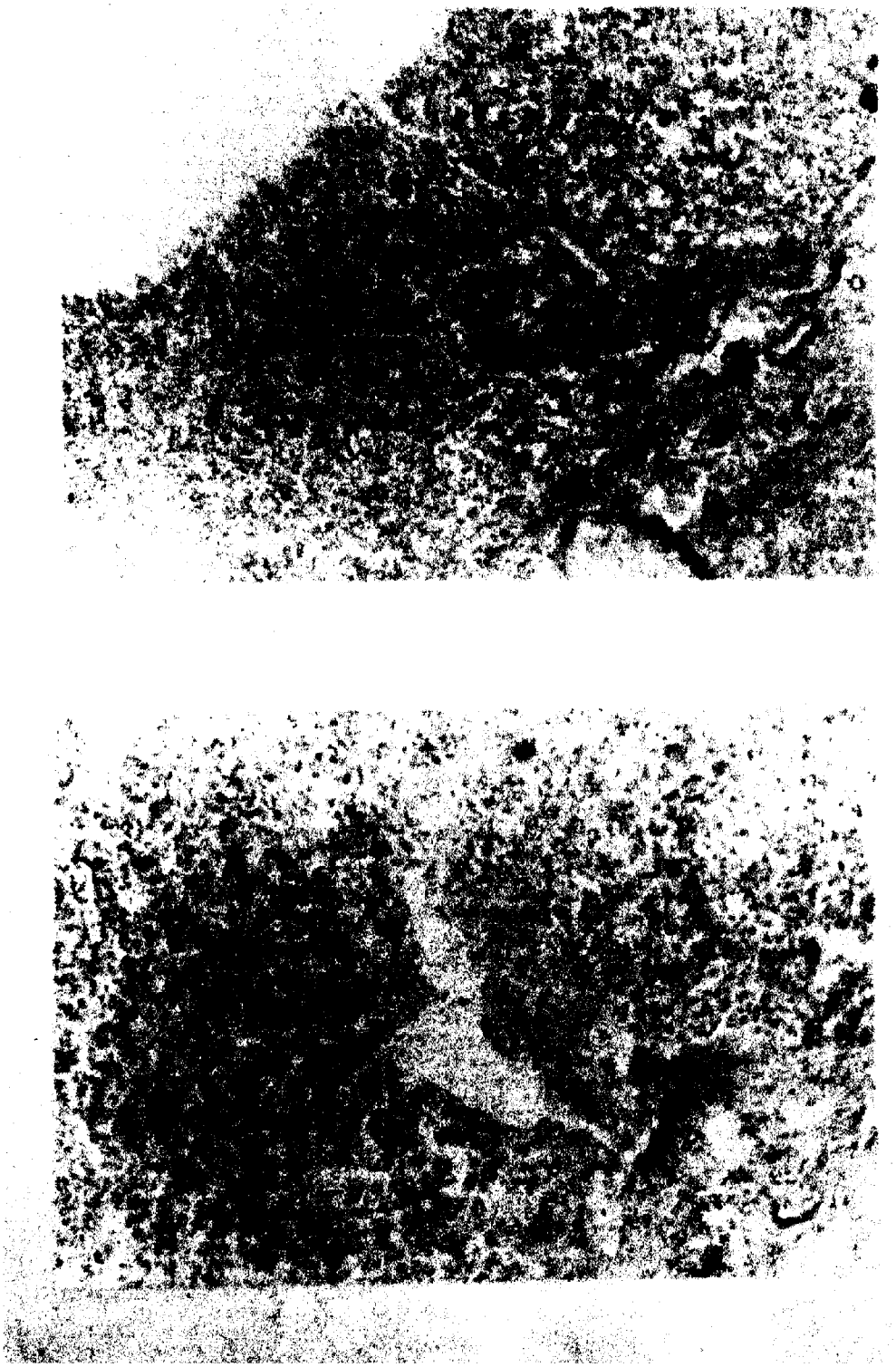


Fig.9 : Section of tumour showing marked infiltra
of eosinophils and plenty of angioblasts
the submucosa H & E X 100

Fig.10 : Section of the nodule showing marked fib
tissue proliferation besides cellular
infiltration H & E X 100

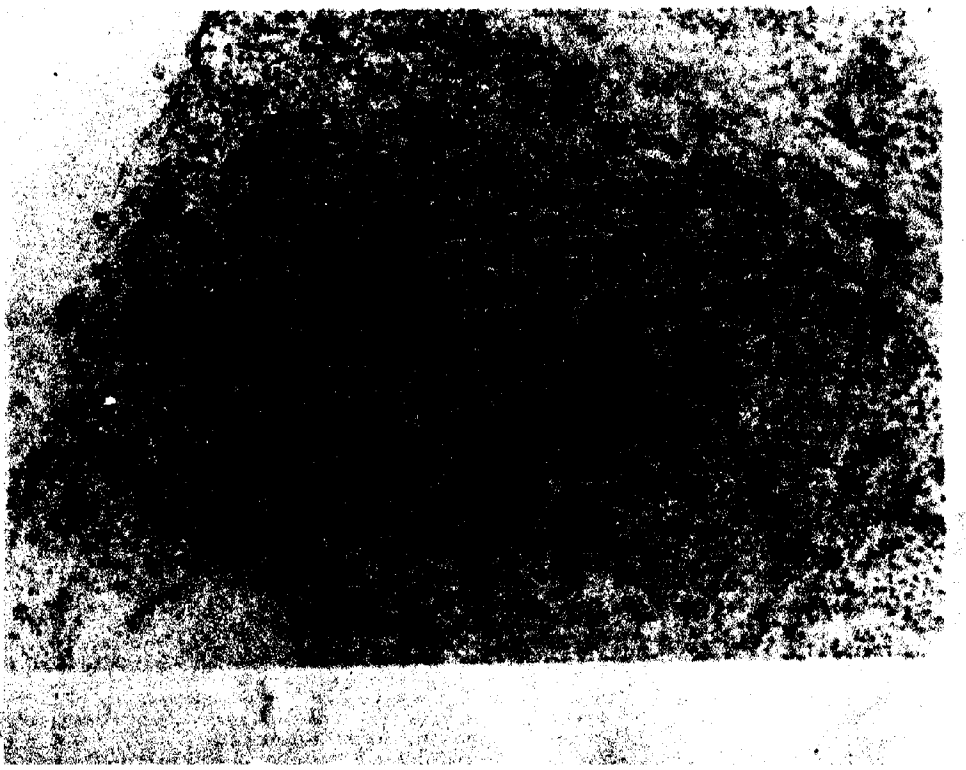


Fig. 11 : Section of the nodule showing marked infiltration of eosinophils and mononuclears separated by fibrous bands H & E X 200

Fig.12 : Section of the tumour showing the cut section of the parasite surrounded by necrotic debris and inflammatory cells H & E X 100

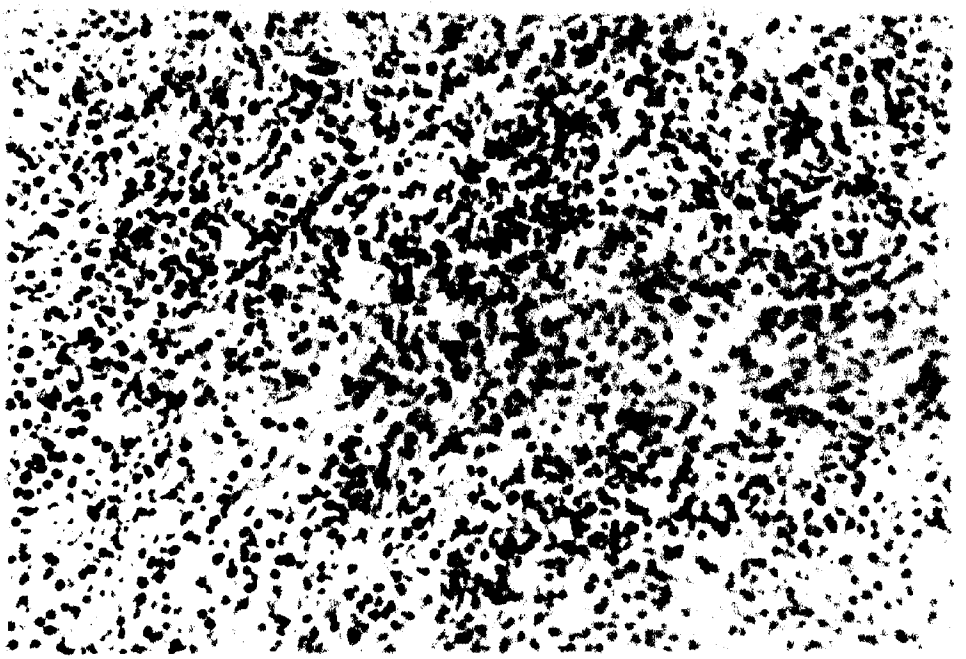
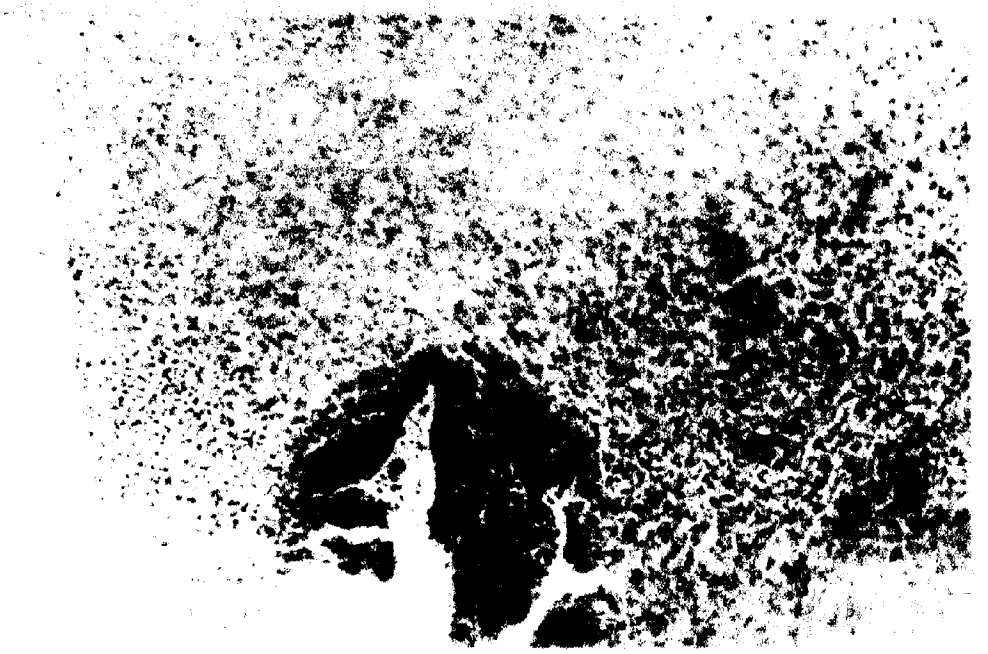


Fig.13 : Section showing area of worm penetration wi
necrotic debris and cellular reaction
H & E X 100

Fig.14 : Section of the nodule showing irregular sha
cells and mitotic figures in the submucosa
H & E X 200



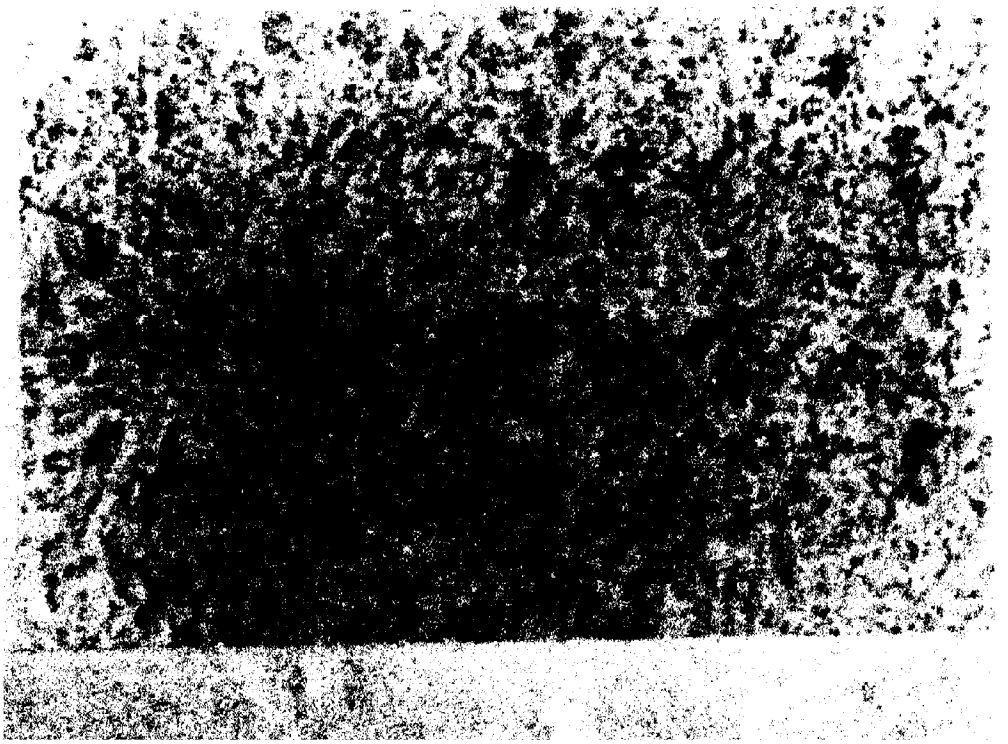
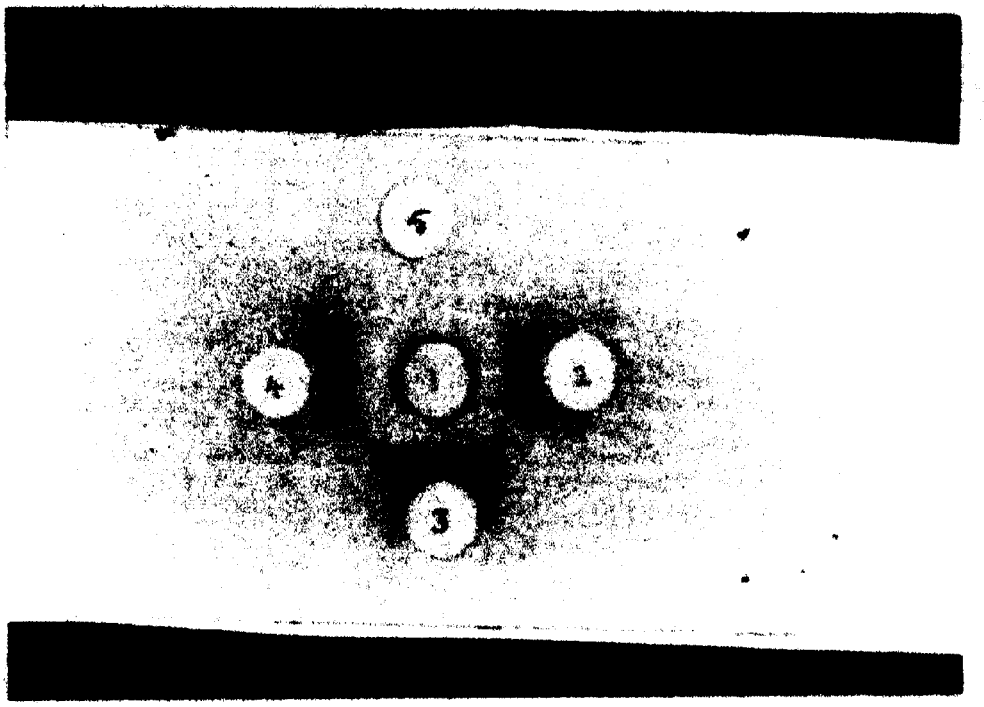


Fig.15 : Agar gel diffusion test showing three preci
lines with H. megastoma antigen(1) against
hyperimmune serum(2,3,4) and control showin
no precipitin reaction(5)

Fig.16 : Electrophoresis indicating three fractions
H. megastoma antigen


5
4
1
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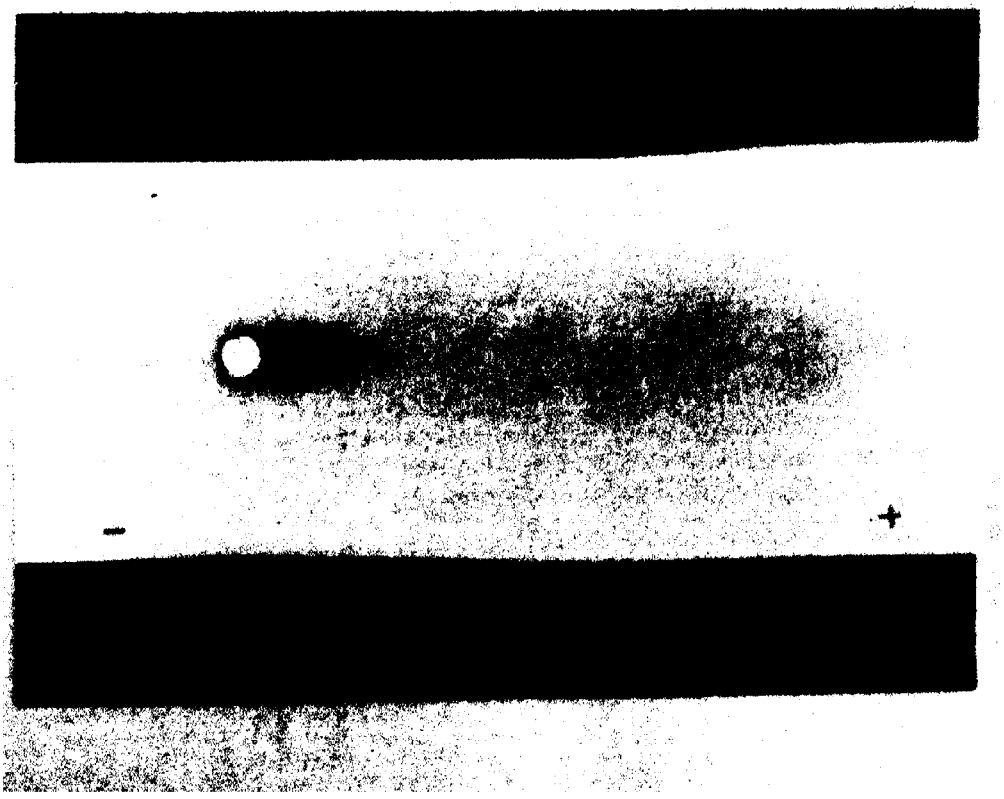
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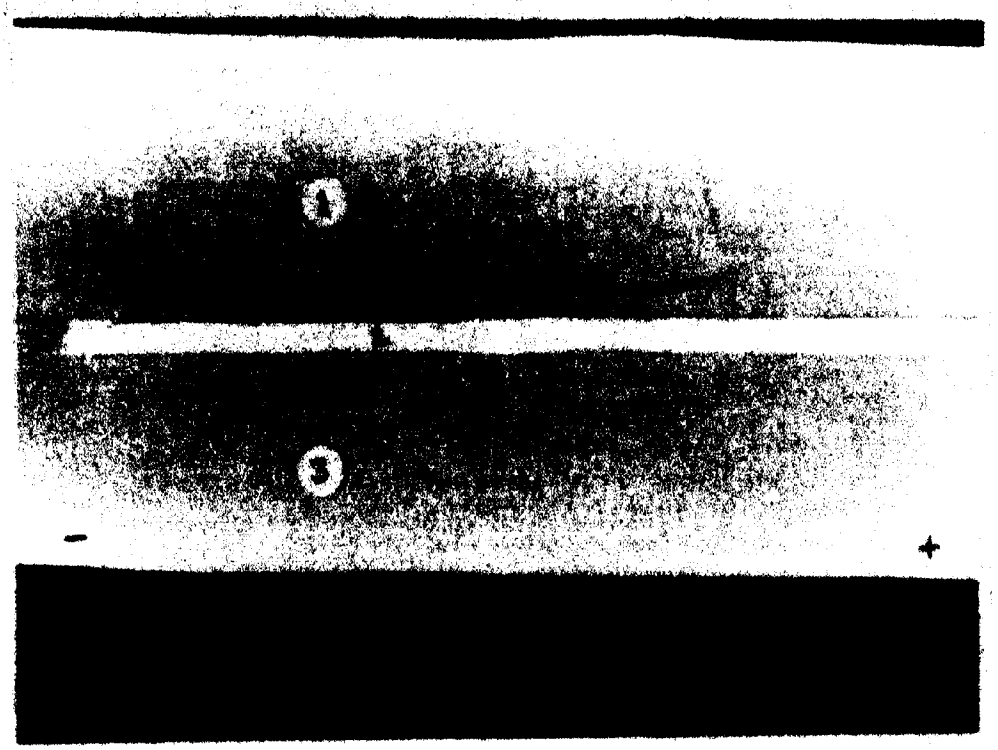
Fig.17 : Immunoelectrophoresis showing three precipitation arcs with *H. megastoma* antigen(1) against *H. megastoma* hyperimmune serum(2) with NRS as control(3)

Fig.18 : Counter current immunoelectrophoresis showing three precipitin bands with *H. megastoma* antigen(1) against hyperimmune serum(2)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