Various Paramphistomes have been incriminated as the aetiological agents of paramphistomiasis which is a leading cause of heavy morbidity and mortality in young ruminants. Extensive studies have been made on * Paramphistomum microbothrium* in Africa and Israel, *Paramphistomum ichikawi* in Australia and *Cotylophoron cotylophorum* in India, with little information on *Paramphistomum cervi*.

However, Fischoeder (1901) changed the name Amphistoma as it was preoccupied and coined the new name Paramphistomum for it. Accordingly, the name of the family Amphistomidae Monticelli (1888) was changed to Paramphistomidae by him and divided the family Paramphistopmidae into two sub-families, namely Paramphistominae and Cladorchinae. Paramphistominae included three genera viz. Paramphistomum Fischoeder (1901); Stephanopharynx Fischoeder (1901) and Gastrothylax Poirier (1883). Under the Sub-family cladorchinae, included the genus cladorchis Fischoeder (1901).

Stiles and Goldberger (1910) did not follow the scheme of classification as given by Fischoeder (1901) and have a revised taxonomic account of all the amphistome known at that time. They have formed a super family under the Paramphistomidea to include the various features like position of acetabulum (terminal or subterminal, rarely ventral near posterior extremity). Ventral pouch present or absent. Testes usually double, may be single exceptionally. Male genital duct usually differentiated into tubular seminal vesicle, tubular pars musculosa and elongate ejaculatory duct. Cirrus
pouch present or absent. Genital Pore opening on mid ventral. Vitellaria usually extending in lateral fields of body.

Stiles and Goldberger (1910) created two more new families, namely Gastrothylacidae and Gastrodiscidae. The family Gastrothylacidae includes all the amphistomes possessing ventral pouch whereas the family Gastrodiscidae includes the amphistomes in which body is divided into cephalic and caudal portions. Although Maplestone (1923) has followed the classification of Stiles and Goldberger (1910) but suppressed the sub-family Deplodiscinae Cohn (1904).

Stunkard (1925) disagrees with Stiles and Goldberger (1910) and seems to have followed Fischoeder (1901) in recognizing only one family i.e., Paramphistomidae to include all the amphistomes. He does not recognize the families Gastrothylcidae and Gastrodiscidae of Stiles and Goldberger (1910). The division of the family Paramphistomidae into sub-families as given by:

- Paramphistominae Fischoeder (19010); Deplodiscinae Cohn (1904); Schizamphistominae Looss (1912); Cladorchinae Fischoeder (1901); Gastrodiscinae Monticelli (1892); Gastrothylacinae Stiles and Goldberger (1901); Zygocotylinae stunkard (1916); Balanochinae Stunkard (1917) and Travassos (1934) followed the concept of raising a superfamily Paramphistomidae by Stiles and Goldberger (1910) but he has suggested the inclusion of six families under the super family given as under.

- Paramphistomidae Fischoeder (1901); Gylianchenidae Travassos (1934); Gastrodiscidae Stiles and Goldberger (1910); Opistholebetidae Travosses (1934); Cephaloporidae Travassos (1934) and Microscaphididae Travassos (1934).

In the family Paramphistomidae, nine sub families have been included by Travassos (1934). This arrangement is similar to that of stunkard (1925)
except that Schizamphistominae has been suppressed whereas Kalitrematinae has been added.

Nasmark (1937) revised the family Paramphistomidae and his classification was based on the anatomy of pharynx, acetabulum and genital organs. He modified Stunkard’s (1925) classification but did not disturb the status of the family Paramphistomidae.

Thapar (1944) worked out the morphology of a newly created genus Olveria collected from the rumen of cattle in the United Provinces. Emett and Meintosh (1953) described two new trematodes of the genus Cotylophoron collected from American sheep.

Price and Meintosh (1953) described two new species of the genus Cotylophoron, namely *Cotylophoron noveboracens* and *Cotylophoron panamense* collected from American sheep.

Dawes (1956) has given an account of the family Paramphistomidae whereas Ershov (1956) has given a general account of the pathogenicity of Paramphistomes.

Rodonaya (1960) studied the biology of *Paramphistomum skrjabini* Popowa (1937). Yamaguti (1958) described some new genera of amphistomes like Glyptamphistoma and Pseudoparamphistoma. Dinnik (1964) studied some species of Paramphistomum Fischoeder (1901) collected from sheep in the Sukumaland area of the Lake Region.

Petrov and Davidova (1963) studied some species of Paramphistomidae of ruminants in USSR.

Sehad *et al.* (1964) observed some amphistomes from domestic ruminants in Malaysia. Vujic (1965) worked out the morphology of the various Paramphistomes like *Paramphistomum cervi* and *Paramphistomum ichikawai*. 
Petrov and Davidova (1963) studied some of the species belonging to the genera *Paramphistomum calicophoron*, *Cotylophoron* and *Ceylonocotyle*. They have followed Nasmarks (1937) classification.

Strydonck (1965) described some new species of amphistomes, viz *Paramphistomum dollfusi* and *Paramphistomum chafaudi*. Kongol (1965) studied Paramphistomum infection in the cattle in Pinsk area. Borko (1970) studied the distribution of Paramhistomes of cattle in Czechoslovakia.

Ruziev (1973) gave some account of life cycle, snail hosts and pathogenicity of *Gastrothylax crumnifer*.


Bali and Fotedar (1972) worked on a new amphistome *Olveria thapari* collected from the rumen of crossbred sheep in Kashmir.

Paramphistomes are commonly occuring trematodes, infecting various domestic ruminants in India (Dutt, 1980; Varma *et al*., 1989 and Gupta, 1993). Several species associated with paramphistomiasis have been reported (Harshey, 1934; Bhalerao, 1935; Alwar, 1949; Varma, 1957; Bhattacharyulu and Pandey, 1969 and Ghafoor, 1993). Various species which were reported earlier are not encountered now and a large number of Indian forms of Paramphistomes reported earlier have been synonymised (Sey, 1982 and Eduardo, 1982).

### 2.1. Incidence

Prasad and Varma (1999) assessed the prevalence of rumen Paramphistomes in domestic ruminants slaughtered at various abattoirs in Bareilly, U.P. revealed maximum rate of infection during post monsoon
(24%) as compared to summer (23%), winter (20%) and monsoon (19.67%) from buffaloes, eight species from sheep and nine from goats. *Paramphistomum epiclitum, Gastrothylax crumnifer* and *Cotylophoron* was recorded by Prasad and Varma (1999).

Bary *et al.* (2002) observed an epidemiological studies on gastrointestinal parasites was conducted in 102 Djallonke goats in Central Guinea. Six to nine goats were autopsied every month for one year. They revealed the presence of various helminth parasites in which *Paramphistomum* species was 12%. The infection intensity was medium in relation to parasite seasonal variations.

Fake (1990) studied the epidemiology of helminthiasis in small ruminants under the traditional husbandry system in Eastern Nigeria for 12 months (August, 1987 to July, 1988). The infections were observed due to *Haemonchus contortus* (87.1%), *Trichostrongylus* species (63.8%), metacestodes of *Taenia hydatigena* (30.2%), *Monezia expansa* (6.0%) and *Paramphistomum* (0.9%). Mixed infections were most prevalent. The prevalence of endemic parasitic gastroenteritis in the area was indicated by the high prevalence of the helminths irrespective of the season of the year.

The prevalence of Paramphistome infection in buffaloes, sheep and goats examined at abattoirs in Barielly (Uttar Pradesh) from March, 1986 to February, 1987 was 58.28%, 8.79% and 7.98% respectively (Varma *et al.*, 1989). The overall prevalence was 32.18% prevalence increased in the rainy and post monsoon seasons and decreased slightly in the winter and summer. Adult parasites were found predominantly in the oesophageal end, reticular end and pillars of the rumen. Immature parasites were predominant in dorsal and ventral sacs of the rumen. The commonest species found were *Paramphistomum cervi, Paramphistomum lobatum, Paramphistomum dutti, Gastrothylax crumnifer* and *Duttiella cephaloporous.*
Observation on the establishment of *Paramphistomum cervi* and its humoral immune response in lambs and kids, experimentally infected with 3500 metacercariae of *Paramphistomum cervi* showed clinical symptoms at 2 weeks of post-infection by Varma et al. (1991). The fluke recovery was higher in lambs (38.585%) than in kids (22.85%) during 2-6 weeks of post-infection. The migration of flukes to the rumen in lambs (9.85%) and kids (4.7%) was seen at 12 weeks of post-infection.

Gupta and Singh (1990) studied prevalence of Paramphistomes in ruminants of Kerala and Ambala districts of Haryana during one annual cycle. The prevalence of Paramphistomes in sheep and goats was highest from June to September. From October to May there was no evidence of infection. The lower incidence was recorded during August, September and October and was significantly higher in cattle than sheep and goats (0.01%).

Prevalence of *Paramphistomum* species in ruminants in the southern Marmara region Turkey was observed by Tinar et al. (1992), a large abattoir was visited 3 times a week between December 1989 and December 1990, Paramphistomes were found in the rumen of 5% sheeps, 1% of goats and 94% of buffaloes. The Paramphistomes obtained was *Calicophoron daubneyi* and *Paramphistomum cervi* in Dharampuri area of Tamil Nadu (Krishnappa et al. 1992). Faecal samples collected from 2340 local sheep from January 1989 to April 1991, examined for helminth eggs and the intensity of infection found was 37.1% with *Strongyloides* eggs 26%, *Paramphistomum* 8.6%, *Fasciola* (9.8%) and *Trichuris* 0.72%. In postmortem examination of gastrointestinal tract of 300 sheeps and 300 goats slaughtered at abattoir in Quetta Baluchistan, Pakistan (Azad et al., 1997) between April 1993 and March 1994 and reported various helminth species such as *Paramphistomum cervi* (16.35% of sheep and 16.6% of goats), *M. benedeni* (11.6% and 10.0%) and *Trichuris globulosa* (24% and 24.3%). The overall incidence of helminth
infection was 80% in sheep and 70% in goats. The prevalence of helminth parasites of sheep in Dakahlia Egypt was determined by faecal examination of 250 animals between January and June 1997. The overall infection rate was 56.8%. *Fasciola* and *Paramphistomum* had prevalence rates of 6.4% and 11.2% respectively.

Dorchies *et al.* (1989) reported that faecal examination was used to investigate the prevalence of *Paramphistomum* in France during winter 1997-1998. The infection was 36.8% of the sampled animals. The prevalence of helminth species in sheep was conducted by Oncel *et al.* (2000) in the Southern region of Marmara and reported forty three of the necropsied sheep (865) infected with various helminth species and among them *Paramphistomum cervi* infection was (4%).

Cavalcante *et al.* (2000) reported infection by *Paramphistomes* in goats from Sobral, Ceara and Brazil, the highest prevalence of Paramphistomes (2.33%) was recorded in a year (2.33%). The Paramphistomes identified was *Cotylophoron travassosi*, *Cotylophoron burcillensis* and *Cotylophoron fulleborni*.

Patel *et al.* (2001) observed the prevalence of gastro-intestinal tract parasite infection among goats maintained under semi-intensive and field management in and around central Gujrat region. The study was carried out between January and March 1999. Out of 142 samples, 2.11% was *Paramphistomum* species. Mixed infection representing coccidal cysts, *Trichuris* and *Trichostrongylus* species was observed as (13.38%).

Mazyad *et al.* (2002) examined 1023 samples from 939 sheep and 84 goats in four areas in Egypt and revealed an overall infection rate of 12.70% with *Fasciola* spp. and 11.8% with *Paramphistomum cervi*.

and 1121 animals, amphistome infection was (1.30%) and (12.36%), the species reported were Gastrothylax crumnifer, P. cervi, Cotylophoron cotylophorum and Gigantocotyl explanatum.

Szmidt-Adjide et al. (2000) reported prevalence of Paramphistomum daubneyi infection in cattle (1994 and 1995) was 0.11% in summer (July, August and September), in winter 0.30%, in spring 0.38% and in autumn 0.36%. The percentages of prevalence recorded was higher in May, October and January.

Madan and Sahai (1986) reported 8365 cattle and 8419 buffaloes for Paramphistomes parasites in randomly selected 15 districts of Bihar for a period of two years. Seasonal incidence revealed highest rate in winter (60-90%) and lowest in summer (20.3%). Calicophoron cauliorchis reported by Stiles and Goldberger (1910) from Bos indicus from Sanwar at Punjab was briefly described by Nasmark (1937).

Srivastava (1945) carried out a general survey of helminthic infection of domestic ruminants in Northwest Punjab, Pramphistomes reported were Cotylophoron cotylophorum, Paramphistomum cervi, Paramphistomum explanatum and Gastrothylax crumnifer in varying degrees of incidence in different hosts.

A massive infection of sheep with adult amphistomes (trematodes) was reported by Mukherjee and Sharma (1961) and the incidence of different amphistomes recorded was 86%. The species recovered were Cotylophoron scoliocoelium (13%), Olvaria indica (0.06%), Olvaria bosii (0.04%) and Calicophoron species (0.15%).

Prasad et al. (1991) reported that experimental infection of Indoplanorbis exustus with meracidia of Paramphistomum epiclittum resulted in emergence of cercariae between 21-24 days of post infection from 84 (18.66%) out of 450 snails at 27-29°C.
Talukdar (1996) reported prevalence of helminthic infections of goats in Assam, in which 17 species of nematodes, 7 species of trematodes and 6 species of cestodes were recorded. The percentage of prevalence in trematodes was 80.50% (Paramphistomum cervi), 32.50% (Cotylophoron cotylophorum) and 58% (Gastrothylax crumnifer). Krishnappa et al. (1992) reported that out of 870 faecal samples, 37.17% were positive for eggs of different helminth parasites such as strongylus spp. (26.4%), Paramphistomum spp. (8.67%), Fasciola spp. (0.98%) and Trichuris (0.72%).

Shamsul (1988) reported 6.08% of Trichostrongylus spp. 1.28% of Paramphistomum cervi, 2.08% of Fasciola gigantica and 0.64% of Ascaris ovis infestation among sheep population of Zambia.

Dutt et al. (1995) studied the paramphistomiasis in cattle and other domestic ruminants from many states in India. The incidence percentage of parasites were (Cotylophoron calciphorum) 86%, (Gastrothylax crumnifer) 62%, (Olvaria scoliocioelium) 44%, (Olvaria indica) 36% and Gastrothylax explanatum 2%. Infection rate in rumen and reticulum was 58.30%. They concluded the incidence of infection was highest during the month of March and April.

Veena and Maitra (1981) reported the dried specimens of Gastrothylax crumnifer Creplin (1847); Poirier (1883) and Paramphistomum epiclitum Fischeoder (1904).

Observation on the seasonal incidence, severity of immature paramphistomiasis in sheep and goat have been described by Nath (1971) and Sharma et al. (1997). Mortality rate upto 80-90% in sheep by parasites has been reported (Bawa, 1949).

Prasad and Varma (1999) reported that out of 811 buffaloes, 744 sheep and 1028 goats examined, 52.03%, 8.06% and 7.97% respectively were found harbouring Paramphistomomes. They reported maximum prevalence during post-
monsoon seasons in the three hosts but the infection was prevalent throughout the year.

Hafeez and Rao (1978) reported *Paramphistomum epiclitum* to be the most predominant species (70.9%) in sheep and (64.9%) goats followed by *Gastrothylax crumnifer* (55.5%) in sheep and in goats (59.9%).

Manna *et al.* (1994) recorded three species of *Paramphistomes* (*Gastrothylax crumnifer, Cotylophoron indicum* and *Olvaria scoliocoelium*) from sheep and goats and five species from buffaloes (*Gastrothylax crumnifer, Cotylophoron indicum, Cotylophoron calicophorum, Fasciola elongatus* and *Olvaria scoliocoelium*). Galdhar and Roy (2005) examined in Durg district, 1108 grazing bovines. The percentage of prevalence of paramphistomiasis in cattle, and buffaloes were 26.27% and 22.86% respectively. In Raipur District, the prevalence of Paramphistomes was between 23.57% and 20.04%, recovered from rumen of sheep and were studied by Scanning electron microscopy at magnification ranging from 10 to 1000x to reveal the structural differences of the tegument between the two species.

Thapar (1956) recorded the occurrence of *Fischoederi us cobboldi, Fasciola elongatus, Cotylophoron cotylophorum, Gastrothylax crumnifer, Paramphistomum explanatum* and *Paramphistomum orthocoelium* in sheep and goats in U.P. Katyar Varshney (1961) observed amphistomiasis in sheep and goats in U.P. and recovered *Gastrothylax crumnifer, Cotylophoron cotylophorum, Paramphistomum cervi, Fischoederus elongatus* and *Paramphistomum explanatum*.

Dube *et al.* (2003) reported four species of the genus *cotylophoron* Fischoeder (1901), namely *Cotylophoron cotylophorum, Cotylophoron fuelliborni, Cotylophoron indicum* and *Cotylophoron jacksoni* were recovered from the inner walls of stomach of cattle slaughtered in the North and South
of Nigeria. Parasite loads ranged between 20 and 700 parasites in the infected animals with a prevalence of 35% for *Cotylophoron cotylophorum*, 2% for *Cotylophoron fuelliborni*, 10% for *Cotylophoron indicum* and 0.2% for *Cotylophoron Jacksoni*.

Ruiz *et al.* (2003) reported seasonal trends of *Paramphistomum cervi* in Mexico, the percentage of incidence 39.10% was through out the year. They observed livestock infection with *Paramphistomum cervi* occurred more frequently during the rainy (July-October) and windy season from (Nov. to Feb.).

Christian *et al.* (2002) observed over a 10-12 years of period, the changes in prevalence of natural fascioliasis and paramphistomiasis among cattle and snails in Central France. The prevalence of natural fascioliasis in cattle increased from 1990-1993 (13.6% to 25.2%) and diminished afterwards upto 1999 (12.6%). The natural infection of paramphistomiasis showed a progressive increase between 1990 and 1999 from (5.2% to 44.7%).

*Gastrothylax Poirier* (1883) was reported from Kashmir sheep by Bambroo (1970) who described a new species, *Gastrothylax orientalis* and *Gastrothylax glandiformis*.

Sharma *et al.* (1997) reported pathomorphological examination of 1208 goats and sheeps at Barielly and Delhi and revealed parasitic lesions in the alimentary tracts, sarcocysts in lips and tongues of 35 goats and 7 sheep and also in the oesophagus of 45 goats and 11 sheep. Mature amphistomes identified included *Paramphistomum epiclitum* which were present in the rumen of 110 goats and 75 sheeps.

EL-Sayed Hashem (1997) reported the prevalence of helminth parasites of sheep in Dakahlia Province Egypt that were determined by fecal examination of 250 animals between January and June 1997. The overall infection rate was 56.8%. *Fasciola* and *Paramphistomum* had prevalence
rate of 6.4% and 11.2% respectively and a mixed infection of *Paramphistomum* and *strongyloids* as in 7.2%.

Vatta *et al.* (2002) reported amphistome infection in goats farmed under resource poor conditions in South Africa, from December 1998 to April 2000. The amphistome faecal egg count followed a seasonal pattern, with an increase in the counts during the warmer months of the year (October to March).

Raina *et al.* (1987) reported eleven species of Paramphistomes from domestic ruminants of sheep namely *Paramphistomum epiclitum* (Fischoeder, 1904); *Paramphistomum gracile* (Fischoeder, 1901); *Calicophoron calicophorum* (Nasmark 1937); *C. papillosum* (Stiles and Goldberger, 1910); *Orthocoelium dicranocoelium* (Fischeder, 1901); *Gigantopharanyx* (Sehad, 1984); *O. indonesiense* (Eduardo, 1980); *Olveria indica* (Thaper and Sinha, 1945); *Gastrothylax crumnifer* (Creplin, 1847) and *G compressus* (Brandes, 1898).

Bali (1976) reported his checklist of Paramphistomes from Jammu and Kashmir namely, *Gastrothylax crumnifer* (Creplin, 1847), *Paramphistomum cervi* (Zeder, 1790) and *Cotylophoron cotylophorum* (Fischoeder, 1901).

Rieu *et al.* (2007) reported reliability of coprological diagnosis of Paramphistomum species infection in cows.

### 2.2. Histopathology

Misra *et al.* (1996) have reported that immature Paramphistomes cause marked catarrhal enteritis and hyperplasia of goblet cells in the mucosa associated with a thick mucin coat and numerous plasma cells and lymphocytes in the sub mucosa of the intestinal tract.
Dutt (1980) and Sahoo (1984) reported thick mucin coat may be due to the profuse secretion from the hyperplastic goblet cells.

Varma (1961) observed some worms reaching the muscosa, submucosa and even into the muscular layer.

Deorani et al. (1967) and Deorani and Jain (1969) observed more worms in the submucosa than in the mucosa.

Horak (1967) did not found any Paramphiostomum microbothruin embedded in the mucosa and not a single worm in Bruners gland.

Prasad et al. (1974) studied that the goats infected with Cotylophoron cotylophorum, contain more worms in the muscosa, although many were also found in submucosa.

Mukherjee and Deorani (1962) described proliferation of epithelium and occasionally mild hypertrophy of the corneum, rarely necrosis and sloughing of the mucosa but no cellular reaction.

Cankovic and Batistic (1963) also observed lymphocytic infiltration in the lamina propria, and sometimes in the epithelium and submucous layer of the rumen infected with Paramphiostomum cervi.

Graubmann et al. (1978) reported a proliferated papillary body with cellular infiltration at the point of adhesion.

Prasad et al. (1974) stated that the flukes caused damage to the duodenal wall not only by their embedding habit, but also they exert an additional pulling action and consequently increased tissue damage with the help of their powerful acetabulum.

Noble (1956) observed cellular infiltration mainly eosinophil in Paraphistomum cervi infection in cattle and sheep. Histopathological alteration in the duodenum and rumen of sheep and goats naturally and experimentally infected with various species of amphistomes have been
reported (Pande, 1935; Srivastava, 1938; Mudhaliar, 1945; Boray, 1969; Nath, 1970; Deorani and Jain, 1969).

Prasad et al. (1974) and Singh et al. (1984) were stated on the histological examination after experimental infection with *Paramphistomum cervi*, that tissue reactions in the duodenum were more pronounced during early stage of the infection (20th day post-infection). Immature parasites were seen migrating to the muscularis layer, and focal infiltration of macrophages and lymphocytes was observed in the lamina propria and in the intestinal tissue of Brunners gland. At places, the entire length of the intestine was involved.

Histological section showed necrosis of mucosal cells resulting in erosive lessions of the villi and mucosa, catarrhal duodenites, ileites and typhilitis were constant. Desquamation of the intestinal mucosa was usually the most significant finding.

Chhabra et al. (1972) observed immature paramphistomiasis caused desquamation in the lining of epithelium of the intestine, lamina propria and the submucosa layer and were markedly infiltrated with a large number of small and large mononuclear cells and also the plasma cells.

The number of flukes reported to produce clinical disease varies according to the species of fluke, species of host and environment of the host. It has been reported that in housed sheep, 40,000 *Paramphistomum microbothrium* were required (Horak and Clark, 1963), while 2000 *Cotylophoron calicophorum* may be sufficient in grazed sheep (Whitten, 1955).

Rolfe et al. (1994) reported that 20-25,000 *P. ichikawai* in the small intestine would be required to produce clinical disease in housed sheep.
Moqbel (1986) observed that the role of eosinophils in parasite expulsion is still unclear, after degradation at the site of infection potent mediators are released that have a wide variety of non-specific inflammatory roles as well as direct cytotoxic activity against the invading parasites.

Doy et al. (1978) reported that the eosinophil leucocytes have an important role in the cystic dilation of Bruners gland. They concluded that the immature forms of *Paramphistomum cervi* caused more severe damage in the duodenal tissue, where as the adult form inflicted mild tissue damage in the rumen of the experimental kids.

Dwivedi et al. (1997) reported pathological lesions were mostly confined to small intestine especially duodenum and jejunum, there was nodular thickening of mucosa at the site of mucosal invasion and the area was found congested with petechial haemorrhages. Histopathologically, there was sloughing, loss of villi, penetration of muscoa by the flukes, loss of glands in the area of invasion and mononuclear cell infiltration especially lymphoid cells.

Veen et al. (1977) reported that ovine enteric paramphistomiasis is relatively common in west Africa, infection of ruminants by adult parasites in the rumen is however, very common. Five species of *Paramphistomes* have been described of which *Paramphistomum microbothrium* is considered the most pathogenic.

Horak (1971) stated that the paramphistomiasis caused by juvenile *Paramphistomum microbothrium* has been reported from East and South Africa and from other Parts of the world where the disease is considered enzootic.

Bida and Veen (1977) observed small intestine of sheep containing *Paramphistomum microbothrium* that caused most striking changes in gastrointestinal tract such as submucosal oedema, erosion and corrugations in
which the mucosa of the intestine particularly of the duodenum was thickened with catarrhal exudates.

Rothwell and Dineen (1973) reported that the *Trichostrongylus colubriformis* in guinea pigs showed no differences in the number of mucosal mast cells in uninfected, infected or immune animals, although there was some difficulty in the identification of these cells. The origin and role of globule leucocytes in parasite rejection have been disputed for several years.

Murray *et al.* (1986) considered that these cells (globule leucocytes) to be amine producing cells derived from subepithelial mast cells, while others consider they are unrelated (Ruitenberg and Elgersma, 1979).

Boch, *et al.* (1983) found that the role of secretory antibody from plasma cells in expulsion of flukes was uncertain, however circulating IgG was present in sheep infested with *Paramphistomum cervi*. The role of mucin glycoprotein in protection and expulsion of parasites has been examined and reviewed by Douch *et al.* (1986) and Moqbel (1986).

Douch *et al.* (1986) found that the antiparasitic activity of mucin was correlated to the number of globule leucocytes rather than goblet cells.

Mapes (1951) suggested that some toxic substances released by *Dicrocoelium dendriticum* may have been partially responsible for fibrotic changes in the liver as had already been suggested for fascioliasis at the turn of the century.

Horak (1971) found that the heavier infections extend the time that immature flukes spend in the small intestines and crowding apparently delays physiological or physical maturation in *Paramphistomes*.

Barker (1973) recognized that unaffected areas of the lower small intestine have the ability to compensate for functional deficiencies in the proximal portion.
Rolfe et al. (1994) carried out the experimental studies on the pathogenesis of *Paramphistomum* infection in sheep, infected with various number of metacercariae. Microscopically there were degenerative changes in the parenchymal organs, the duodenum and the rumen as well as considerable reticulo-histocytic proliferation and eosinophilic infiltration with non-reactive necrotic foci in the rumen.

Guelhorn and Priouzean, (1945) reported subsequent recovery on migration of *Paramphistomum cervi* and *Cotylophoron cotylophorum* to the rumen and a large number of parasites caused chronic gastritis.

Mukherjee and Deorani (1961) observed a massive infection of sheep with adult amphistomes and the histopathology of the parasitized rumen was determined. In association with the worms at places, there was proliferation of the epithelium and only sometimes little hypertrophy of the corneum rarely necrosis and sloughing of the mucosa but no cellular reaction.

Pathological effects of helminth borne diseases of sheep in Kashmir has been studied by Bali et al. (1972).

Ershov (1956) remarked that *Paramphistomes* inflict damage to the rumen when attaching to it by means of their powerful acetabula. Severe traumatic effects are inflicted to mucosa of the intestine and other organs by immature Paramphistomes (Patnaik, 1964; Deorani et al., 1967; and Dwivedi et al., 1997). Extent of damage by Paramphistomum infection in cattle has been studied widely (Tsevetaevao, 1960; Reuhardt, 1969; Rimbaud et al., 1995).

Misra et al. (1996) studied the haematological and histological alteration in lambs caused by immature paramphistomiasis and reported decrease in Hb% of PCV values due to sucking of blood by immature *Paramphistomes*, marked catarrhal enteritis and hyperplastic goblet cells with profuse mucous secretion. In addition to it other reported complications
included weakness, diarrhoea, emaciation, oedema and fatty changes in the liver (Lapage, 1962).

Vatta and Krecek (2002) reported amphistome infection of goats farmed under resource poor conditions in South Africa.

Haridy et al. (2006) observed parasitic flukes infecting farm animals in AL–Santa Gharbia Egypt. The results showed they infected with *Paramphistiomum* species in cattle (7.3%), buffaloes 10%) and sheep (4%).

### 2.3. Immunodiagnosis

Analysis of parasite derived antigens and their application in immunological tests for the diagnosis of parasitic infections has been a subject of several recent reviews (Fife, 1971; Barbet, 1982; and Fox et al., 1986).

Ghosh and Gupta (1985) studied the effect of gamma irradiation on establishment, migration and growth of *Paramphistomum cervi*.

Hafeez and Avasthi (1986) reported simple invitro precipitation test for the detection of hepatic amphistomiasis due to *Gigantocotyl explanatum* and cross-reaction with ruminal amphistome *Paramphistomum epiclitum*. Several serodiagnostic tests have been employed by various workers for the diagnosis of infection at the early stage of the disease (Horak, 1971; Albay 1981; Hafeez and Rao 1983).

Katyar and Varshney (1963) performed intradermal allergic test by injected saline washed extract of Paramphistomes in infected sheep by intradermal route on the side of the neck and compared them with injected distilled water as control. There was no change in the body temperature or thickness of the skin among both the groups.

However, recently Saifullah et al. (2000) analysed excretory /secretory and somatic antigens of a rumen amphistome *Gastrothylax crumnifer* and
demonstrated a number of antigens in its excretory/secretory and somatic extracts by gel filtration chromatography, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western immunoblotting using rabbit hyperimmune sera but no attempt has been made to characterize antigen of *Giagantocotyl explanatum*.

Maji *et al.* (1997a) investigated antigenic cross reactivity among three species of Paramphistomes using the Ouchterlony double diffusion test.

In another study, Maji *et al.* (1997b) analyzed the ploypeptides of *Paramphistomum epiclitum, Gastrothylax crumnifer* and *Gigantocotyl explanatum*.

Santiago and Hillyer (1988) identified some prominent somatic antigens of the liver trematode *Fasciola hepatica* with an apparent molecular weight ranging from 30 to 38KDa with diffuse bands of 56, 64 and 69Kda.

Indrawati *et al.* (1991) studied partially purified polypeptides of *Paramphistomum heterotremus* using western blotting and identified a 35Kda antigen which showed 100% sensitivity and specificity for the detection of Paragonimiasis.

Ahmad *et al.* (2004) reported that soluble extracts of *Gigantocotyl explanatum*, isolated from the liver of buffalo were fractionated by Sephadex G-200 columns and nine major fractions were separated.

Yadav *et al.* (1999) reported an evaluation of *Fasciola gigantica* crude antigenic preparation viz., somatic antigen, excretory/secretory antigens and egg antigen in serodiagnosis of the diseases was undertaken. The referral antigenic preparations were evaluated against positive sera and control sera (negative) using immunodiffusion test (DID), counter-immunoelectrophoresis (CIEP) and ELISA. The performance of referral antigens as assessed from
sensitivity and specificity revealed an increasing trend from DID to CIEP followed by ELISA detecting higher number of sheep positive for fascioliasis.

Juyal et al. (2004) reported indirect Dot ELISA for anti-
*Paramphistomum epiclitum* antibodies detection using antigen concentration of 100ng/μl, and revealed antibody titre as high as 1:40,000.

Choi and Lee (1979) conducted immunoelectrophoretic test with rabbit antiserum and *Paramphistomum* species antigen, found that immunoelectrophoresis produces more specific and sensitive reaction than DID and was found to be more useful method for diagnosis.

Zharikov et al. (1976) studied the humoral immune response elicited by whole *Paramphistomum* antigen. They found 5 to 6 precipitation lines of varying mobility subjected to diffusion lines in IEP.

Osikovskii et al. (1977) conducted comparative electrophoretic study of the water soluble proteins of some representative genus of Paramphistomes like *Paramphistomum microbothrium* and *Paramphistomum cervi* respectively. Differences in mobility and intensity of staining were also detected in some of the fractions. The results confirm the validity of these species.

Hafeez et al. (1987) conducted immunoelectrophoresis with hyper-immunized rabbit serum which showed the presence of *Paramphistomum epiclitum* in experimentally infected lambs after 15 days and 45 days post infection but only faintly at 60 days post infection. Uninfected controls remained negative for paramphistomiasis using immunoelectrophoresis.

Rao et al. (1998) conducted Rocket immunoelectrophoretic analysis of various antigens from *Paramphistomum epiclitum*, *Gastrothylax crumnifer* and biliary Paramphistome, *Gigantocotyl explanatum* revealed partial immunochemical identity of the Paraphistomid antigens. The excretory
antigens of *Paramphistomum epiclitum* and *Gigantocotyl crumnifer* showed greater degree of homology (82 to 95%) Where as *G. crumnifer* and *Gastrothylax explanatum* exhibited nearly 90% antigens in the detergent solubilized fraction cross reactivity between the species.

Hillyer *et al.* (1980) conducted enzyme linked immunosorbertant assay, counter-immunoelectrophoresis and kato thick smear stool examinations to follow *Fasciola hepatica* infections in mice and rabbits given or not given chemotherapy. In both, mice and rabbit antibody levels were evident by ELISA within 2 weeks of infection, rising to high levels two weeks latter. Precipitin monitored by CIEP were also evident by 2 weeks of infection in mice but developed more slowly (4-6 weeks) in rabbits.

Yoshihara *et al.* (1981) conducted an application of agar gel diffusion test (AGDT) to the diagnosis in cattle and buffaloes in the Red River Delta of Vietnam. AGDT was positive for *Fasciola* antibody in about 50% of the sera from the cattle and buffaloes harbouring worms in their livers. The reaction was considered to be specific for *Fasciola* infection because the *Paramphistomum* antigen did not react with sera from the cattle infected with *Fasciola* species.

Bratnov *et al.* (1981) conducted an agar gel immuno-diffusion test of complete somatic antigens of *Paramphistomum* species and *Fasciola hepatica*, using the respective homologic and heterologic hyper immune rabbit sera. Differences were established in the spectrum and number of the produced precipitation lines 6 to *Paramphistomum* and 4 to *Fasciola*.

Choi and Lee (1979) investigated the antigen antibody reactions and the value of immunodiagnosis for several helminths using Ouchterlony tests and Immunoelectrophoresis.

Choi and Lee (1979) investigated the specific antigenic substance of *Fasciola hepatica* by Ouchterlony and Immunoelectrophorethic analysis.
Crude *fasciola* antigen was prepared and fractionated by Sephadex-200 column to antigen I, II and III according to protein content. Crude *Paramphistomum* species were prepared for control.

Morilla *et al.* (1989) conducted dot enzyme linked assay and compared it with the passive haemagglutination test and their layer immunoassay for the detection of antibodies against *Fasciola hepatica* in naturally and experimentally infected sheep. The infected animals gave titres from 1:2500 to 1:20400, while control animals gave titres from 1:100 to 1:800. In recent years, the ELISA has been used for the diagnosis of fascioliasis and paramphistomiasis in naturally or experimentally infected cattle (Farell *et al.*, 1981; Zimmerman *et al.*, 1982; Wescott *et al.*, 1984; Hillyer *et al.*, 1985; Juyal 2004).

Gupta and Yadav (1995) reported increase in antibody titre of rabbit to enhanced doses of *Fasciola gigantica* in experimental infection and found that the antibody response is predominantly dose dependent. Rabbits have proved very good responders as laboratory model for carrying out various investigations against *Fasciola gigantica* experimental infections (Gupta and Chandra, 1987).

Sharma *et al.* (1987) conducted agar gel precipitation test, counter-immunoelectrophoresis (CIEP) and indirect haemagglutination test (IHA) and evaluated these for the diagnosis of fascioliasis due to *Fasciola gigantica* in buffaloes. The sensitivity of tests varied with the intensity of infection, CIEP detected (76.06%) of infected sera and was most sensitive, followed by IHA detected (68.37%) of the infected sera. The AGPT was found least sensitive and detected (57.4%) of the infected sera.

Ogunrinade (1983) reported agar gel precipitation test as a simple and economical procedure and required no special equipment.
Deka and Gaur (1991) reported diagnosis of *Taenia hydatigena* and *cysticercosis* in goats by immunoelectrophoresis and showed 57.1% and 51.2% reactivity and its specificity 73.3% respectively.

Deka and Gaur (1991) studied on immunoelectrophoretic pattern in the hyperimmune antisera and sera of naturally infected buffaloes with hydatidosis. The sensitivity and specificity of the test in naturally infected buffalo was (47.55%) and (67.4%) respectively.

Enayat and Pezeshki (1977) studied the humoral response of guinea pigs infected with doses of *Toxocara canis*. Antibody titre were measured by counter-immunoelectrophoresis and indirect haemagglutination tests one to five weeks after infection. The antigens used in these measurements were prepared from adult *Toxocara canis*. Counter-immunoelectrophoresis showed lower titres than heamagglutination method but it was performed rapidly and with ease.

Muralidhara and Sastry (1997) conducted indirect haemagglutination test on 300 serum samples of cattle. Out of which 20 serum samples gave positive reaction with IHA test using sterile antigen where as none of 135 sheep serum samples gave positive reaction. De-Rosa and Puccini (1979) carried out indirect haemagglutination test in sheep. Werner and Keurim (1971) indicated IHA had specificity and sensitivity.

Tewari *et al.* (1972) studied a preliminary note on the passive haemagglutination test to detect circulating antibodies in sheep vaccinated with irradiated larvae of *Dictyocaulus filaria* and recommended IHA for immunodiagnosis of *Dictyocaulus filaria* infection in sheep.

Tsuji *et al.* (1975) comparatively studied the antigenic structure of several helminths by immunoelectrophoresis.
Hamal et al. (1982) used the cathodic antigen in the immunoelectrophoretic serodiagnosis of *Echinococcus granulosus* in sheep and concluded that the detection of cathodic antigen in sheep by immunoelectrophoresis was better tool for the diagnosis of Hydatidosis.

Gudlach and Sadzikowski (1985) studied the immunological aspect of fascioliasis in sheep.

Pathak et al. (1986) established the immunodiagnosis of fascioliasis by counter-immunoelectrophoresis in sheep and concluded that immunodiagnosis is the only specific method for the diagnosis of fascioliasis and paramphistomiasis, where as standard faecal examination gave false negative results due to low egg count or their complete absence in the faecal matter.

Yamaguchi et al. (1989) performed the immunoserological test in Japan for the diagnosis of *Trichinella* infection, examined the efficiency of CFT, ID, IE, CIE, IHA and ELISA for the diagnosis of disease.

Kassi (1989) studied the effect or mechanism of protective immunity induced by the intestinal helminths.

Hyndrickx (1990) studied the antigenic characterization of *Haemonchus contortus* and immune response in rabbits and sheep and reported that the parasitic antigens were having the cross reactivity.

Canals and Gasbarre (1990) practically characterized and isolated the somatic antigen and metabolic antigen of *Ostertagia ostertgi*. It was accomplished by SDS-PAGE and immunoblot technique.

Wedrychowicz and Bezubik (1990) observed the influence of adjuvant on immunity of rabbits vaccinated with infective larval somatic antigen of *Trichostrongyulus colubriformis* and reported that Freund’s Complete Adjuvant (FCA) treated rabbits developed a much higher serum
precipitating antibodies detectable by using Ouchterlony immuno-diffusion test than the beryllium hydroxide treated group.

Eddi et al. (1992) studied the defence mechanism of parasites to avoid the hosts immune response and discussed 11 evasive mechanisms of parasites including molecular mimicry, antigenic diagnose, production of proteinases, production of arachidonic acid, inhibition of the complements and modification of neutrophil activity.

Aris et al. (1999) characterized the Chabertia ovine by isoenzyme gel electrophoresis and compared it with Oesophagostomum venulosum. They concluded that isoenzyme pattern (G8PD) might be used as a diagnostic tool to identify these species.

Briton et al. (1993) characterized the secretory products of Dictyocaulus viviparous, according to their SDS- PAGE profile and a limited degree of the cross reactivity between nematode species.

Stankiewiez et al. (1994) studied the sheep small intestine mast cell/globule leucocyte activity and concluded that the number of the mucosal mast cell/globule leucocytes increase in intestinal mucosa in response to parasitic infection.

Katoch (1998) studied the effect of Adjuvant on the vaccination of goats against the adult worm Haemonchus contortus and concluded that the adult worm antigen provides only partial protection and FCA produces immunosuppressive effect.

Rao (1993) also applied IHA for the analysis of Gastrothylax explanatum antigen using rabbit immune sera and found good results.

Albay (1982) studied comparative diagnosis of Paramphistomum cervi infection in sheep with immunoperoxidase (ELISA) and IFAT techniques.
Avasthi et al. (1986) performed gel diffusion test for antigenic comparison of ruminal and hepatic *Paramphistomes*, precipitation bands were observed for all the 3 hyperimmunized sera of ruminal amphistome like *Paramphistomum epiclitum*, *Gatrothylax explanatum* and *Gastrothylax crumnifer* antigens and indicating the presence of common antigen.

Rao (1993) reported incidence of *Gigantocotyl explanatum* (59.2%) and immunodiagnostic studies with whole worm extract antigen against hyperimmune serum showed 3 precipitation bands while sera from naturally infected buffaloes did not showed precipitation lines. Electrophoretic study showed no shearing of antigen between *Gastrothylax explanatum* and *Fasciola gigantica*. Indirect haemagglutination test indicated titre of 2048 with hyperimmune sera but sera from naturally infected buffaloes did not showed diagnostic titre. The differences between the immunological responsiveness of these hosts may be due to variation in time course response to different components of the worm antigen (Doyle 1973; Santiago and Hillyer 1988).

Singh et al. (2004) study the cell mediated immune response in cattle experimentally infected with *Fasciola gigantica* employing somatic antigen.

Reichel (2002) studied performance characteristics of an enzyme linked immunosorbent assay for the detection of liver fluke infection in sheep and cattle.

Castro et al. (2000) reported serological responses of cattle after treatment and during natural reinfection with *Fasciola hepatica* as measured with a dot ELISA system.

Varma et al. (1990) reported the titres of cytotoxic antibodies in hyper immunized rabbit sera with its homologous antigen 0.282 % (*Gastrothylax*
crumnifer), 0.276% (Fasciola elongatus), 0.260% (D. cephaloporous, 0.239% (Paramphistomum cervi) and 0.200% (Paramphistomum lobatum) respectively.

Fagbemi et al. (1995) studied time course analysis of antibody response by EITB and ELISA before and after chemotherapy in sheep infected with Fasciola gigantica.

Moreno et al. (1999) reported liver pathology and immune response in experimental Fasciola hepatica infection of goats.

Rao et al. (1990) studied the effect of Gamma radiation on metacercariae of Paramphistomum epiclitum.

Barbet (1982) revealed identification and analysis of surface antigens and parasitic induced antigens on the host cells.

Sandman and Howell (1981) conducted gel diffusion techniques to study antigen-antibody reaction in precipitates forming around juvenile, Semi-mature and adult Fasciola hepatica cultured in serum from infected sheep. The number of reactions were analyzed in both primary and challenge infections.

Tiggle and Over (1976) observed antibodies against Fasciola hepatica. These were detected in sera from experimentally infected sheep and calves. IHA, CIE and DID were compared.

Gonene et al. (2004) reported comparison of crude, excretory and secretory antigens for the diagnosis of Fasciola hepatica in sheep by western blotting and observed twenty three protein bands. These bands were detected between 6.5 and 205Kda in Polyacrylamide gel as separating and stacking gel in E/S antigen.

Rao (1997) reported the Polypeptide profile of ruminal and biliary Paramphistomes analyzed by sodium dodecyl sulphate polyacrylamide gel
electrophoresis. A maximum number of 21 ploypetides were identified in the molecular range of 19.9 to 125.8KDa in comassie blue stained gels.


Serrano and Hillyer (1986) reported fractionation of *Fasciola hepatica* tegument antigens and their application to the serodiagnosis of experimental fascioliasis by the enzyme linked immunosorbent assay (ELISA) with the antibody levels peaking by 10 to 12 weeks of infection.

Molly *et al.* (2005) reported evaluation of a commercially available enzyme – linked immunosorbent assay for detecting antibodies to *Fasciola hepatica* and *Fasciola gigantica* in sheep, cattle and buffalo in Australia. In sheep sensitivity and specificity was (96.9%) and (99.4%) while as in cattle (98.2%) and (98.3%) and buffalo was 60.2%.

In recent years a number of sensitive and specific serological tests for diagnosing parasitic infections have been described (Ibarra *et al.*, 1998 and Cornelissen *et al.*, 1999) availability of ELISA.

Martinez *et al.* (1996) reported an ELISA with excretory – secretory (ES) antigens has been evaluated as a technique for the early detection of specific antibodies in *Fasciola hepatica* infection in goats, The ELISA test was performed with ES antigens (10μg/ml) a single dilution of sera (1:800) and anti-goat IgG conjugate (1:1000), ES specific antigen were detected in all goats between 15 and 30 days post infection and maximum antibody levels were reached at 90 days post-infection.

Levieux (1994) reported a haemagglutination test for the early diagnosis of *Caprine fascioliasis*. The ELISA for sheep and cattle has been proved to be sensitive, specific, rapid and easy to perform as reported by (Zimmerman *et al.*, 1982). Improved sensitivity and specificity has been
obtained with purification of somatic antigens and the use of excretory-secretary (ES) products of *F. hepatica* as antigen (Santiago and Hillyer, 1988).

Hillyer and Galanes (1991) reported initial feasibility studies of the fast ELISA for the immunodiagnosis of fascioliasis with excretory-secretory antigens in various animal models.

Sarkar *et al.* (2003) assessed anti-fasciola antibodies in the buffalo sera collected from a local abattoir in Kolkata. DID and ELISA were performed using somatic and ES antigens of *Fasciola gigantica*. In DID out of 95 parasite positive sera samples, 8 samples produced precipitation bands against somatic antigen and 3 samples produced precipitation bands against ESAg but none with negative control.

Santiago Weil (1984) reported isolation of *Fasciola hepatica* antigens which induced antibody formation in acute fascioliasis were isolated by acid elution after reacting an *Fasciola hepatica* tegument antigen extract compared with IgG obtained from the serum of rabbits infected with fascioliasis for 6-10 weeks.

The rabbit antiserum obtained peak when tested by immunoelectrophoresis with a crude *F. hepatica* extract showed one main band identical to the main band observed with serum from acutely infected rabbits. Two main bands were observed in immunodiffusion with antigen eluting (pH range of 7.4 – 8.7).

Hillyer *et al.* (1981) reported serodiagnosis of experimental fascioliasis by immunoprecipitation test and counter immunoelectrophoresis was useful in detecting 100% of infections with fascioliasis in mice, rats and rabbits by 4-5 weeks of post infection and in most rats as early as 2 weeks of post-infection.
Swarup et al. (1987) reported serodiagnosis of fascioliasis in buffaloes by immunoprecipitation using adult *Fasciola gigantica* antigen.

Gupta et al. (1999) reported isolation of somatic and excretory antigens of *Fasciola gigantica* and used for immunization in rabbits. A protection percentage of (52.38%) and (73.65%) were obtained by double and triple inoculation of somatic antigens where as (57.77%) and (68.25%) protection was observed in excretory/secretory antigens.

Levieux et al. (1992) improved haemagglutination test using the purified specific f\textsubscript{2} antigen of *Fasciola hepatica* and has been evaluated with regard to its potential use for early diagnosis of infection.

Ibarra et al. (1998) reported comparison of three ELISA tests for seroepidemiology of bovine fascioliasis to compare sensitivity, specificity and usefulness of DIG-ELISA, DOT-ELISA and Indirect ELISA test. Sensitivity and specificity values for indirect ELISA, DIG-ELISA and DOT-ELISA were 96.5%, 98.8% and 97.5% respectively.

Hillyer and Santiago de Weil (1978) showed that antibody titres dropped rapidly when rabbits and rats were successfully treated with a fasciolicidal drug.

Moazeni et al. (2003) studied the comparison of excretory/secretory and somatic antigen of *Fasciola* species in gel diffusion test for preparation of antisera, the laboratory rabbits were immunized with both types of antigen.

Rinaldi et al. (2005) reported isolation of the rumen fluke *Calicophoron daubneyi* from various hosts of animals in Southern Italy that were characterized genetically by molecular techniques involving polymerase chain reaction (PCR).