CHAPTER - 6

SUMMARY
The present Ph.D. Thesis entitled "Incidence, Histopathology and Immunodiagnosis of Paramphistomiasis in Sheep" is mainly divided into three chapters in accordance with the approved topic. Besides, figures, plates, summary and bibliography are also added. The various observations and conclusions drawn from the study are summarized as under:

1. Out of 655 indigenous sheep and 600 exotic sheep only 450 and 440 were found infected with Paramphistomones respectively.

2. The peak infection was obtained in the month of May being 94.5% in indigenous sheep whereas in exotic sheep it was recorded in the month of April being 91.5%.

3. The parasites collected during the period (Jan. 2004 to Dec. 2004) were identified as *Paramphistomum cervi*, *Gastrothylax crumniiter* and *Cotylophron cotylophorum* from different regions of Kashmir Valley.

4. The overall infection rate of sheep with Paramphistomum species in indigenous sheep was 68% and in exotic sheep it was 73% throughout the year.

5. The incidence of parasites was found higher during the months March to May, because the harvesting of crops starts from the middle of October onwards and water recedes from inundated and low-lying areas. Animals start to graze in the swampy areas where the grasses and weeds may be harbouring large numbers of metacercariae. The metacercariae get entry in the host body for development.
6. The rate of infection and worm burden was found lowest during the winter months that may be due to minimum temperature and dry winter conditions which might inhibit larval development.

7. The breed wise analysis revealed that exotic sheep had higher prevalence rate than native breed because of low natural resistance and more consumption of less selective pasture grasses as compared to native breed.

8. The parasites were diagnosed through various characters like body length, body width, pharynx, acetabulum, ovary size and testes.

9. The mean number, mean intensity and index of invasion in indigenous sheep ranged between 2.6 to 9.8, 3.1 to 29.5 and 1.2 to 8.4 respectively where as in exotic sheep ranged between 2.7 to 9.0 and 3.7 to 20 and 1.25 to 8.26 respectively.

10. Large number of mature flukes were observed clinging to the ruminal mucosa especially between the ruminal papillae and only few cases revealed presence of immature flukes in the duodenum causing nodular thickening of mucosa with central depression at the parasite attachment.

11. Cases with immature flukes in the duodenum presented haemorrhages and oedematous thickening in the serosa of the pylorus and duodenum.

12. The histopathological changes observed in the present study varied among the infected animals as well as in different portions of rumen in some animals. However the general pathological changes included atrophy, widening, flattening globulation and desquamation of keratin layer of ruminal papillae.
13. In rumen increased cornification of stratum cornueum with increased number of cells in the stratum granulosum causing thickening of the mucosa was seen.

14. Infiltration at apical papillae and sparse infiltration in basal submucosal regions with lymphocytes, few eosinophils and globule leucocytes was seen in the submucosa.

15. Duodenal mucosa revealed sloughing, loss of villi, dilation and proliferation of submucosal glands and varied degree of oedema.

16. The most striking changes in the duodenum lumen was watery ingesta and catarrhal exudate in the submucosa of duodenal glands.

17. Changes varied from a localized enteritis and villous atrophy in the light infection to severe destruction of the mucosa extending into most of the jejunum in heavy infections.

18. Serological techniques used were Ouchterlony gel diffusion test, Electrophoresis, Immunoelectrophoresis, Indirect Haemagglutination Test and ELISA.

19. The sera of infected and uninfected sheep and immunized rabbits were tested. Ouchterlony test revealed the appearance of one precipitation line in naturally infected sheep sera and 2 to 3 precipitation lines in immunized rabbit sera against *Paramphistomum cervi* antigen. However, in uninfected sheep sera no such bands were observed.

20. The antibody titre of rabbit immunized sera, ranged from 1:2 to 1:16 dilution by Ouchterlony gel diffusion test.

21. Immunoelectrophoresis showed presence of three precipitation bands in undiluted immunized sera of the rabbit, where as naturally infected sheep sera showed the formation of one precipitation band against the antigen.
22. The simple horizontal electrophoresis of serum samples from normal, infected sheep and immunized rabbit sera was carried out that revealed proportion of β-globulin greater in sheep sera and γ-globulin in the hyperimmunized rabbit sera with paramphistomiasis.

23. The indirect haemagglutination test of naturally infected sheep sera showed antibody titre of 1:32 whereas hyperimmunized sera of rabbit against somatic antigen was 1:64. Negative control showed no reactions.

24. The serum samples from infected and uninfected sheep and hyperimmune sera of rabbits were also tested by ELISA by using 2-fold dilution. In sheep sera it ranged from 1:100 to 1:6400 whereas in hyperimmunized sera of rabbit ranged from 1:100 to 1:128,000 dilution.

25. Before ELISA antibody was detected by gel diffusion test during 2-3 weeks of post infection, only one precipitation band and after 3rd to 5th weeks of post infection two bands were observed. With the increasing serum dilution antibody absorbance value were found to be decreased sharply and could not be detected by gel diffusion test.

26. Out of the various techniques employed in the present study, ELISA was found the most effective, sensitive and specific test for paramphistomiasis.