Role of helminthes in canine pup mortality

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DOI: https://doi.org/10.22271/chemi.2020.v8.i4c.9884

Abstract
Parasitism was the most encountered disease condition in all over the world. Out of 77 pup post-mortem examination, three (3.89 per cent) died due to helminthiasis (Two toxocarosis, one toxocarosis and ancylostomiosis). These were a 40 days old male non-descript and two 30 days old non-descript. Pale mucous membranes, anorexia, dullness and depression in all three pups, ascites in one pup and severe emaciation in another pup were observed. Severe anaemia, leucocytosis with neutrophilia, hypoproteinemia and hypoalbuminaemia were also recorded.

Keywords: Pup mortality, helminthes, toxocarosis, ancylostomiosis, anaemia

1. Introduction
Kagira and Kanyari (2010) [4] found in a study of 2492 dogs were examined, of which 351 were diagnosed as dying from parasitic diseases. Thus parasitic conditions accounted for about 14% of all the mortalities. Of the 351 deaths, gastro-intestinal helminthiasis accounted for 68%. The proportion of the dogs killed by hookworms (Ancylostoma caninum) was the highest (41%). Other gastro-intestinal worms encountered included Toxocara canis and tapeworms (Dipylidium caninum). It was observed that helminthiasis mostly affected young puppies. Intestinal parasites were common diseases in young dogs category. Toxocarosis or ascaridosis of dogs was the most widespread parasitic disease in dogs, caused by Toxocara canis. (Stanković et al., 2012) [11]. Toxocara canis could infect pups intra-uterine causing digestive and respiratory manifestations. The untreated infected puppy usually dies after birth. Toxocara canis burdens considered one of the causes of FPS (Salib 2013) [2]. Neonatal deaths might caused by Toxocara canis infection as a result of the presence of large worms burden that obstruct gastrointestinal tract or migrate up the biliary tract and cause hepatitis

1.1 Clinical findings
Toxocara canis infected puppies showed fever (39.4 °C), coughing, dyspnea, pot belly, pale mucous membranes, diarrhea, laziness, emaciation and severe pain, which observed as arched back, tensed abdomen, frequent crying and abnormal gait (Salib 2013) [2]. The presence of intestinal parasites in dogs could lead to a wide spectrum of clinical manifestations, from mild or imperceptible, to very heavy, which can lead to death (Stanković et al., 2012) [11]. Pot belly was usually observed in Toxocara canis infected puppies and it might be caused by intestinal distension with worms burdens and ascities resulted from hypoproteinemia (Salib 2013) [2]. Toxocara canis infected pup showed pot belly appearance, poor digestibility, increased/reduced appetite, diarrhea and failure to grow or even death might be occurred if treatment was not given (Fisher, 1982).

1.2 Postmortem findings
There were gastroenteritis, pneumonia, liver congestion, enlarged mesenteric lymph node, obstruction of small intestine, intestinal rupture, peritonitis and presence of adult worms of T. canis in the small intestine (duodenum) (Salib 2013) [2]. Adult parasites reduce passage or even completely close intestinal lumen, bile duct, or ductus of the pancreas and damage the intestinal mucosa. All this causes a disturbance in the digestion and resorption of food, and the general state of disorder and contribute to the metabolic products of parasites that have toxic. Larvae damage and cause inflammation of the liver and pneumonia (Vanparijs, 1991) [1]. Toxocara canis infected puppies may die from obstruction of the gall bladder, bile duct and
pancreatic duct, rupture of the intestine and peritonitis, pneumonia, ascites, fatty degeneration of the liver, and may be due to myocarditis. The affected puppies may die with 3 week after birth (OIE, 2005) [7].

1.3 Ancylostomiasis
*A. caninum* and *A. tubaeforme* infections might be fatal, particularly in young animals, due to blood loss. (CFSPH, 2005). Ancylostomiasis was more often seen in young puppies or dogs under one year of age infected by transmammary route, and they were susceptible due to their iron reserves. Sometimes young puppies presented with sudden hemorrhagic shock (Lefkaditis, 2001) [5].

1.4 Clinical signs
Anemia (macrocytic hypochromic), pale membranes, bloody stool, weight loss, weakness, poor growth and pneumonia were observed in ancylostomiasis infested puppies (Sherding and Johnson, 1994). Turkar et al., (2009) [3] observed ascites associated with ancylostomiasis in a 2 month old German shepherd pup.

1.5 Gross pathology
With many hookworm species, the intestinal contents were bloodstained. Small, grayish-white to reddish-white, cylindrical nematodes (approximately 5-20 mm long) were found in the intestines. The intestinal mucosa might be congested and swollen, with many tiny hemorrhagic points, red marks or ulcers. Larvae in aberrant sites might be associated with necrotic and hemorrhagic tracks in the tissues, as well as hemorrhages and other signs of tissue damage (CFSPH, 2005). The liver and other organs appeared as icteric and regional lymphadenopathy, was identical to that seen in eosinophilic gastroenteritis. Additional, more specific features included pathological reactions centered on attached worms, mucusal alterations and ulcers considered to be hookworm bite sites, and submucosal and lymph node granulomas with central eosinophil degranulation and degradation products. Liver showed prominent pathological aberrations. The normal arrangement of hepatic cells was disturbed. Hepatocytes lost their texture. Vacuolization and dilation of central veins were distinct. Sinusoids and central vein were moderately occupied by cell debris. Moderate infiltration of cells near the dilated central vein was clearly evident. (Gollapudi, 2012) [6]

1.6 Histopathology
Walker et al., (1995) [9] stated that the essential pathology, i.e. edema and eosinophilic infiltration of the gut wall, ascites, and regional lymphadenopathy, was identical to that seen in eosinophilic gastroenteritis. Additional, more specific features included pathological reactions centered on attached worms, mucusal alterations and ulcers considered to be hookworm bite sites, and submucosal and lymph node granulomas with central eosinophil degranulation and degradation products. Liver showed prominent pathological aberrations. The normal arrangement of hepatic cells was disturbed. Hepatocytes lost their texture. Vacuolization and dilation of central veins were distinct. Sinusoids and central vein were moderately occupied by cell debris. Moderate infiltration of cells near the dilated central vein was clearly evident. (Gollapudi, 2012) [6]

1.7 Diagnosis
Peracute ancylostomiasis was more likely to be diagnosed during postmortem than on clinical examination. Hookworm infections were diagnosed by fecal flotation and detection of the eggs (Lefkaditis, 2001) [5]. Death might be resulted in young pups before eggs were passed in their feces, i.e., as early as 1–2 week of age (The Merck Veterinary Manual)

2. Materials and Method
Puppies less than 6 months old, admitted to the Small Animal Clinics were subjected to a thorough clinical examination. Laboratory examinations like complete blood count, serum biochemistry were applied. Detailed post-mortem examinations of the dead puppies were conducted. Tissue samples from various organs were collected in 10 per cent formalin for histopathology. Spleen impression smear was collected Peripheral and heart blood smears, impression smears from various lesions, heart blood swabs and swabs from lesions, thoracic fluid, pericardial fluid, peritoneal fluid and urine were also collected and examined.

3. Results
Out of 77 pups, three (3.89 per cent) pups died due to parasitic infections. These were a 40 days old male non-descript and two 30 days old male non-descript. Haematobiochemistry in two pup revealed severe anaemia, leucocytosis with neutrophilia, hypoproteinemia and hypoalbuminemia. Anorexia, dullness and depression were reported in all the three pups. Ascites was observed in one case.

3.1 Laboratory diagnosis
Peripheral and heart blood smears revealed anaemic changes in all the three pup. Examination of intestinal contents revealed numerous *T. canis* eggs in all the three pup. Out of three heart blood swabs, one revealed both *Staphylococcus* spp. and *Streptococcus* spp.

3.2 Gross pathology
General body condition was poor in all the three pups. Severe emaciation was observed in one case. Blanched visible mucous membranes were observed in all the three pup. Subcutaneous tissue was poor in all the three pup and oedematous in one case. Sixty mL of clear serous fluid was observed in the abdominal cavity in one case. In all the three pup, stomach contents revealed numerous *T. canis*. Intestine was completely packed with numerous *T. canis* worms in the small intestine of all the three pup. In one case, large intestine showed focally diffuse areas of severe congestion and haemorrhage in the mucosa with *A. caninum* worms. In one case, multifocal areas of mild corrugation in the intestine were observed. liver was enlarged yellow-brown mottled in two pup with fibrin strands in one case and in another case, it was diffusely pale. Pancreas was slightly firm, grey-white, shrunken in appearance in one case. Heart was diffusely pale in all the pup. Lungs were oedematous and mildly congested in two pup and dark red-brown areas interspersed with pale pink areas in one case. Kidneys showed grey-brown cortical surface in two pup.

Severe congestion and haemorrhage in the mucosa with *A. caninum* & *T. canis* worms
3.3 Histopathology
Stomach showed mild degeneration and congestion of gastric mucosa in all the pup. Intestine showed blunting, necrosis of the mucosal epithelium with infiltration of admixture of eosinophils and neutrophils, dilated crypts completely packed with secretion and desquamation in all the pup. Many segments of T. canis were observed in one case. Liver showed diffuse micro- to macro- vesicular fatty degeneration of hepatocytes and infiltration of mild lymphocytes around the periportal areas in all the pup. Mild bile duct hyperplasia was observed in one case. Pancreas showed mild multifocal areas of congestion and fat necrosis in two pup. In one case, diffuse vacuolar degeneration of the parenchyma, severe, diffuse infiltration of neutrophils, occasional plasma cells in the parenchyma and interstitial proliferation and thickening with degenerating neutrophils were observed. Heart showed diffuse, mild vacuolar degeneration of myocytes in all the pup. Lungs revealed multifocal areas of oedema, emphysema, congestion, haemorrhage with interstitial proliferation and mild perivascular infiltration of lymphocytes in all the pup. Desquamation of bronchial epithelium and lumen containing debris were observed in one case. In another case there was hyperplasia of bronchial epithelium and goblet cells. Spleen and lymph nodes showed reactive hyperplastic changes in two pup and depletion of white pulp in one case. Kidneys showed diffuse vacuolar degeneration of proximal convoluted tubular epithelium in all the three pup.

4. Discussion
Out of 77 pup, three (3.89 per cent) died due to helminthiasis. These were a 40 days old male non-descript and two 30 days old non-descript. Whereas, Kagira and Kanyani (2000) observed 14 per cent of mortality from parasitic diseases. Failure of deworming could be attributed to death in all three pups as reported earlier observed by Salib (2013) [2]. Pale mucous membranes, anorexia, dullness and depression in all three pup, ascites in one case and severe emaciation observed in another case were in accordance with Salib (2013) [2]. Severe anaemia, leucocytosis with neutrophilia, hypoproteinemia and hypoalbuminaemia were also recorded (Turkar et al., 2009) [3]. General body condition was poor in all the three animals. Ascites, numerous Ancylostomes in GI tract in one case, corrugated intestine, mottled liver in two pup and pale yellow in one case noticed were also found by earlier workers (Vanparijs 1991; Salib, 2013) [1, 2]. Heart blood swab revealed Staphylococcus spp. and Streptococcus spp. in three pup. Micro-to-macrovesicular fatty degeneration, mild bile duct hyperplasia and mononuclear cell infiltration were observed in liver (Vanparijs, 1991) [1]. Pancreas revealed diffuse vacuolar degeneration of parenchyma, severe, diffuse infiltration of neutrophils, occasional plasma cells in the parenchyma and interstitial proliferation and thickening with degenerating neutrophils which were associated with bacterial sepsis caused by Staphylococcus spp., and Streptococcus spp.

5. Conclusion
Gastro-intestinal helminthiasis (Ancylostoma and Toxocara) plays a major role in pup mortality. Out of 77 pups, three (3.89 per cent) died due to helminthiasis (Two toxocarosis, one toxocarosis and ancylostomiosis). These were a 40 days old male non-descript and two 30 days old non-descript. Pale mucous membranes, anorexia, dullness and depression in all three pup, ascites in one case and severe emaciation in another case were observed. Severe anaemia, leucocytosis with neutrophilia, hypoproteinemia and hypoalbuminaemia were also recorded. Proper deworming could able to prevent pup mortality

6. References