THE EFFECTS OF MIXED INFECTION OF STRONGYLES IN EXPERIMENTAL ANIMALS IN THE VETERINARY RESEARCH INSTITUTE

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Abstract. This paper reports the relationship between mixed infections of strongyles with fecal egg count (FEC), packed cell volume (PCV) of the blood and FAMACHA in experimental sheep in VRI. The third larval stage (L3) of strongylid nematode were fed orally to two experimental sheep. Each animal was given 3 ml L3 or equivalent to 3000 larvae. Faecal samples were then collected weekly for a 10 week duration. The FAMACHA scale for anemia estimation was used and blood collection through ear tip was also done twice a week to measure the packed cell volume (PCV) of the blood. The results show that the PCV decreases while faecal egg counts increase over the study period. The FAMACHA scale also increases with time indicating a progressive anemia with the decreasing PCV. As helminth infections are common in small ruminants, the effects of anemia are critical parameters to be monitored in any flock as this will determine its productivity in terms of mortality and morbidity.

Keyword: strongyles, third larval stage

INTRODUCTION

Diseases are the major constraints hindering the expansion of livestock industry in Malaysia. Among the domesticated animals in Malaysia, sheep and goats are more susceptible to gastrointestinal nematode infestations as shown from Annual reports of the Veterinary Research Institute (VRI) over the past 8 decades (Chandrawathani *et al.*, 2013). In most small ruminant farming areas in Southeast Asia, strongyle infestation is one of the major disease issues contributing to heavy morbidity and mortality, mainly caused by *Haemonchus contortus, Trichostrongylus* spp. and *Oesophagostomum* spp. (Sani and Gray, 2004). Infections with gastro-intestinal nematodes can have a detrimental effect on animal health leading to clinical and sub-clinical diseases that may result in financial loss and overall decreased productivity (Lüscher *et al.*, 2005). This infection mainly causes a collection of conditions and syndrome named parasitic gastroenteritis (PGE) which is characterized by dull, depressed, diarrhea, inappetance, anaemic

and emaciated animals (Cringoli *et al.*, 2004; Soulsby, 1982).

It is important to know the life cycle of strongyles in order to treat and manage the infected animals. According to Paddock (2010), the life cycle of strongyles involves the infective third stage larvae (L3) which are ingested by the host from pasture and later develops into adults in the abomasums. Adult worms produce eggs that are passed in the feces. The eggs hatch in the feces into first-stage larvae (L1) which in turn moult into second-stage larvae (L2) shedding their protective cuticle in the process. The L2 moult into third-stage larvae (L3) but retain the cuticle from the previous moult. These larvae which can migrate up blades of grass in drops of moisture are the infective stage for strongyles. Under suitable conditions, it takes only 3-4 days for an egg deposited in feces to develop to the L3 stage. Depending on temperature and moisture, L3 larvae can survive on pasture up to 6 months. Adult worms only survive several months in the host (Paddock, 2010; Soulsby, 1982). The period of time required for the hatching of the egg and development of the larvae is dependent on weather conditions, and larvae develop and survive best under warm, wet conditions. This explains why parasitism is a much greater problem in moist climates than in dry, arid climates (Whittier *et al.*, 2009). Once the L3 is ingested, it moults to the fourth stage (L_4) and remains in the mucous membrane of abomasum (or in the gastric glands) for about 10 to 14 days. They then emerge and moult into a young adult stage (L_5).

Haemonchus spp, found in the abomasum, is the most pathogenic of the blood suckers and infections with large numbers of this parasite often result in severe anaemia in the host (Fao.org, n.d.). Transformation of L4 to L5 leads to loss of architecture of parietal cells and thus causes reduction in hydrochloric acid (HCl) production. The pH within the stomach increases due to decrease in HCl which will then disrupt enzymatic reaction and causes protein maldigestion (Sani, 2011). *Trichostrongylus* spp which is found in the small intestines causes villous atrophy which will cause malabsorption and diarrhea. The L3 of *Oesophagostomum* spp migrates deep into the mucosa of large intestines and will provoke an inflammatory response with the formation of nodules (Taylor and Coop *et al.*, 2007) which will also lead to diarrhea. These symptoms result in the syndrome called parasitic gastroenteritis (PGE) which has brought about a surge of information to help farmers and veterinarians solve the related problems. In Malaysia, several studies conducted over the last 20 years has shed light on the various methods of helminth control including rapid rotational grazing of pastures, herbal remedies for worm control, biological control using *Duddingtonia flagrans* as well as zero grazing or cut and carry method (Chandrawathani *et al.*, 1999 & 2002).

Thus, the aim of this study is to elucidate the effects of helminthiasis

caused by a mixed infection of strongyles which are sourced from a resistant farm; namely worms that are resistant to common anthelmintics and to observe and record symptoms of anemia through the PCV and FAMACHA scores. In the course of this study it is also envisaged that the use of faecal culture techniques for research and production of L3 for other tests such as Larval development test, and the simplicity of engaging the use of FAMACHA for the common farmer is assessed and can be promoted for regular use. In trying to convince farmers to use the FAMACHA technique for helminth diagnosis, it is hoped that helminth infections are more easily noted and treated quickly thereby preventing countless deaths in the field.

MATERIALS AND METHODS

This study was conducted in the Veterinary Research Institute (VRI), Ipoh. The general plan of this study involves the feeding of L3 obtained from faecal cultures to experimental sheep over a period of time and monitoring the PCV and faecal egg counts simultaneously with the FAMACHA scores. Deductions were made based on the data collected over 10 weeks.

Faecal Cultures

A large amount of faeces, approximately 2 kg, were collected per recta from several animals, from Small ruminant Unit, Infoternak Farm, Sungai Siput as this farm was identified to have resistant strain of strongyles to various drug groups (Chandrawathani *et al.,* 2004). This sheep in this farm were identified to have heavy gastrointestinal nematode infections, with faecal egg counts (FEC) in sheep in the range of 500-4,000 epg, by the modified McMaster technique (MAFF, 1979) before the study began. The faecal culture was performed in order to get the infective third stage larvae (L3). This method is a technique for culturing eggs of nematodes of the order Strongylida to infective L3 larvae and then recovering these larvae for identification. It involves a culture phase, whereby the eggs hatch and develop from L1 through L2 and L3. The larvae are identified according to the length of tail and sheaths (MAFF, 1979).

Animal Innoculation

Infective larvae were harvested from faecal culture by day seven. Three ml of infective L3 stock which contained approximately 3000 larvae was given two times by oral route to two experimental sheep (Sheep X and Y) on day 1 and day 2, which were housed in pens in VRI. The two experimental sheep were clean and did not have any FEC thus deemed to be free of any residual strongyle infection. No anthelmintics or medications such as antibiotics or supplements were given to the experimental sheep as it was required that the L3 be fully able to infect these sheep. The animals were put on basal feeding of grass at the rate of 10% body weight

daily with ad lib water. It was important that the animals were adequately fed but still able to take on the worm infection. As with helminth infections, the nutrition plays a significant role in manifesting the effects of helminthiasis (Sani and Gray, 2004). After introduction of the L3, faecal samples were collected throughout the study from the first week until Week 11.

Blood tests

Blood collection through the ear tip was also done twice a week to measure the PCV of the blood to measure the blood proportion of blood volume (MAFF, 1979). Blood collection was started on day 3 post-L3 introduction at three to five days interval for 11 weeks duration. The FAMACHA system was also being used to diagnose the level of anaemia and the presence of stomach worms in livestock (http://www.scsrpc.org). The FAMACHA system uses a 5-point scale to measure ocular mucous membrane colour which indicates the PCV in sheep or goat (Paddock, 2010). The FAMACHA is calibrated into five categories: 1 = red, non-anemic; 2 = red-pink, non-anemic; 3 = pink, mildly anemic; 4 = pink-white, anemic; 5 = white, severely anemic (Anderson and Rings, 2009). The system utilizes an eye anemia guide to evaluate the colour of mucous membrane of the eye of a sheep to determine the severity of parasite infection as evidenced by anemia. A bright red color indicates that the animal has few or no worms or that the sheep has the capacity to tolerate its worms. An almost white eyelid colors a warning sign of very severe anemia; the worms present in the sheep's gut are in such numbers they are draining the animal of blood (Pugh and Baird, 2011).

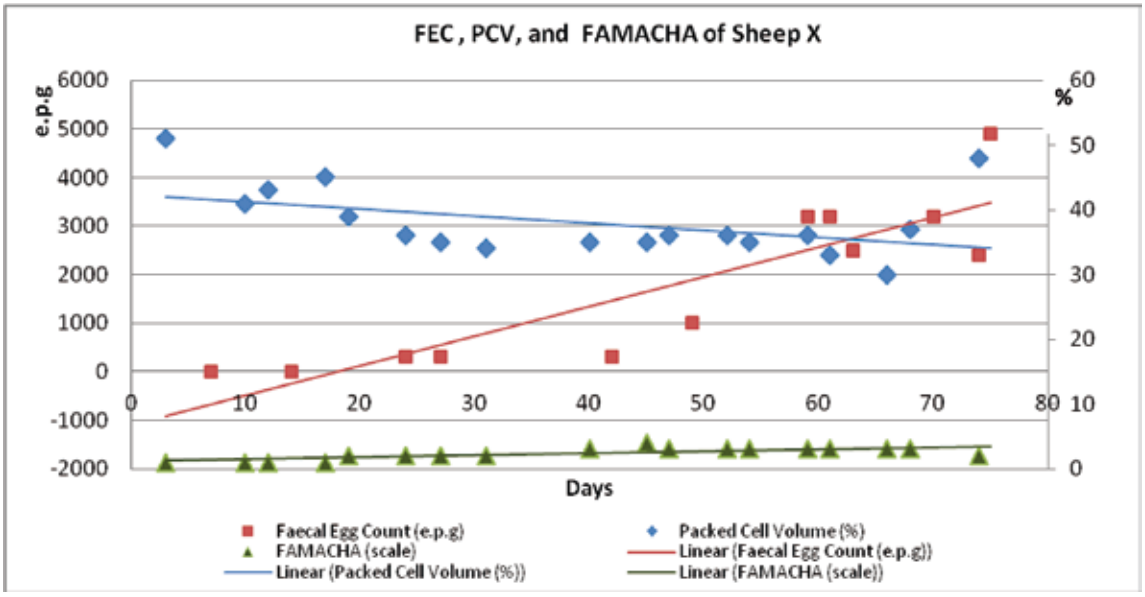
By the 11th week, faecal culture was done to identify worms to establish if the same species that were first inoculated were present. Throughout the study, the animals were observed for general body conditions and health to comply with the VRI Code of animal ethics in experimental animals.

RESULTS

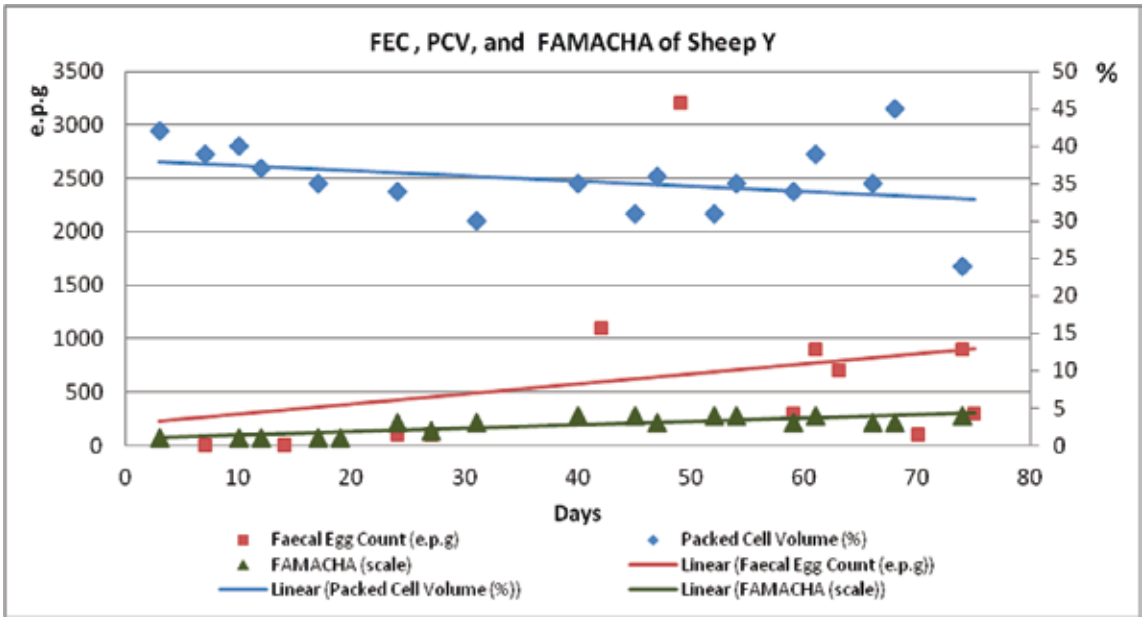
Results of this study are indicated as individual faecal egg count information for the two animals.

Graph 1 shows the FEC of Sheep X, which increases gradually from the day of inoculation from 0 e.p.g in week 1, 2 and 3 to 300 e.p.g which was maintained throughout week 4 until week 7. In week 8, the FEC reached 1000 e.p.g and increased sharply to 3200 e.p.g in week 9. During the last week, the FEC rised up to 4900 e.p.g which is a fourfold increase in 3 weeks. In short, the FEC showed an increasing trend throughout the experimental period. As shown by several studies (Soulsby, 1982), the prepatent period of strongyles such as Haemonchus sp is three weeks which can be seen as egg counts appearing at 300 epg on the 3rd week after infection.

Meanwhile, PCV of the sheep showed a decreasing trend with FAMACHA grading showing an increasing trend which



Graph 1: The Faecal Egg Count , FAMACHA score and Packed cell volume observed in Sheep X over 11 week period.



Graph 2: The Faecal Egg Count , FAMACHA score and Packed cell volume observed in Sheep Y over 11 week period.

corresponded to the anaemic condition of the sheep from the day of L3 inoculation.

Graph 2 shows the FEC of the Sheep Y increased gradually since the day of inoculation from 0 e.p.g in week 1, 2 and 3 to 100 e.p.g in week 4. In week 6, the FEC increased substantially to 1100 e.p.g and then went up sharply to 3200 e.p.g in the week 8. In the same week, FEC dropped to 900 e.p.g and the drop continued to week 9 with only 300 e.p.g. In week 10, FEC increased again to around 800 e.p.g before it declined to 300 e.p.g in the last week. In short, FEC showed an increasing trend throughout the experimental period. As for Sheep X, a similar pattern of infection was observed.

Meanwhile, PCV of the sheep showed a decreasing trend with FAMACHA grading showing an increasing trend which corresponded to the anaemic condition of the sheep since the day of L3 inoculation.

From the fecal samples cultured on the 11th week, *Haemonchus contortus* was found to be most common (69%), followed by *Trichostrongylus* spp. (20%) and *Cooperia* spp. (11%).

DISCUSSION

The results of this study showed that strongylid nematode infestations causes an increase in the FEC. The FEC measures the number of strongyles egg per gram of faeces. The number of eggs in the faeces corresponds to the number of adult strongyles in the gastrointestinal tract. Thus, high number of FEC indicates high number of adult worms. Although the actual numbers of worms and faecal egg counts cannot be always correlated, it is generally accepted that the higher the number of worms, the more eggs are produced as seen in FEC. The production of eggs by the worms is also dependent on the immune status of the animal as the innate immunity can also suppress gee production if the animal is in good health, thus nutrition and the level of protein feeding plays an important role in helminth management (Sani and Gray, 2004). When the FEC increases, the PCV will eventually drop or decrease due to presence of active blood feeders. *Haemonchus spp.* worms have buccal cavity with conspicuous teeth (Kuchai *et al.*, 2012) that enable rapid blood sucking activities that will lead to massive blood loss of the host. The PCV of 22% and below is considered anaemic (Whitier *et al.*, 2009) for the small ruminants, however in this experiment, the PCV range of 25-45 was seen probably due to dehydration or sufficient nutrition which masked the anemia. However, the mucuos membrane colour showed paleness thereby giving a FAMACHA score of 4 to 5.

The associated blood loss may lead to anemia and causes a drop in PCV. Since anemia is the principle clinical problem, mucous membrane colour is one of the easiest ways to monitor the condition of the host and has proven effective as a diagnostic approach (Chandrawathani *et al.* 2013). Therefore, the FAMACHA system is an effective system to be used

for smallholders with a major problem of strongyle helminthiasis. From this study, the FAMACHA grading increases indicating that mucous membrane becomes paler due to blood loss that results in anaemia. The FAMACHA system contains five eye scores (1-5), which have been correlated with packed cell volumes; percentage of blood made up of red blood cells (http://www.sheep101.info).

In conclusion, this study shows inevitably the positive use of FAMACHA in recording the heminth infections as seen by the high egg counts (1,000-4,000 epg) and low PCV and FAMACHA (score of 4-5). It was fortunate that the 2 sheep did not show clinical symptoms of helminthiasis such as bottle jaw, inappettance and diarrhea as well as other concurrent infections that can occur such as pneumonia due to reduced immunity. Overall, the animals appeared normal and healthy and this is the fact that farmers need to realize in their efforts to control helminthiasis, that animals may buckle under very quickly if allowed to continuously have worm infections. It is hoped that this information can be used to encourage farmers to use the FAMACHA and bring awareness on the importance of helminth infections in apparently normal animals.

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