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A PRELIMINARY REPORT ON THE SURVEILLANCE OF HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N1) AND NEWCASTLE DISEASE (ND) VIRUSES IN EDIBLE BIRD NEST SWIFTLET (Aerodramus fuciphagus and Aerodramus maximus)

LIM K.H.¹, KHOO C.K.², LAURENTIUS N.A.³ AND YEO B.K.¹
¹ Department Of Veterinary Services & Animal Industry (DVSAI), Kota Kinabalu
² Department of Veterinary Services, Putrajaya
³ Sabah Wildlife Department, Wisma Muis, Kota Kinabalu

ABSTRACT. The emergence of Highly Pathogenic Avian Influenza (H5N1) in 2004 draws attention to the safety of rearing edible bird nest swiftlets within the vicinity of human dwellings in urban areas. There is also concern on the safety of the product itself. Convenient sampling of edible bird nest swiftlets (A. fuciphagus and A. maximus) was conducted by the Department of Veterinary Services and Animal Industry from September 2004 until June 2011. A total of 137 samples were collected from four premises and two natural caves in four locations, namely Tawau, Kunak, Sandakan and Penampang. The samples were collected for Egg Innoculation Tests for H5N1 and Newcastle Disease (ND). All samples yielded negative results for both diseases.

Keywords: H5N1, ND, EBN swiftlets, caves, surveillance

INTRODUCTION

Sabah, “The Land Below The Wind”, is bestowed with many natural resources of flora and fauna, forests, streams, rivers and insects. These pristine factors provide a conducive and prime environment for the survival of edible bird nest (EBN) swiftlets. This EBN swiftlet industry contributed more than RM13.3 million to the economy of the state through the export of 8,876 kg of EBN products in 2009 (Anon, 2009). The main markets for EBN products are Hong Kong and China.

The major producers of EBN from natural caves are found in the Gomantong and Madai caves (Photos 1 and 2), respectively located in Sandakan and Kunak districts. Ambu (2009) estimated that the swiftlet population in both the Gomantong and Madai caves was 1.29 million and 0.8 million heads respectively. However, EBN production from ranched population is unknown. It is estimated that there are about 500 man-made EBN swiftlet houses (Photo 3) in Sabah. However this figure is dynamic and fast changing due to increasing interest in this high value industry.

The emergence of H5N1 in late 2004 around the world draws much concern in this industry, bringing doubts about
the safety and possible transmission of the disease from swiftlets to human population. Although studies on H5N1 in poultry populations have been extensively conducted by the DVSAI in Sabah which hitherto has been free of the disease, no studies on swiftlets have been attempted. This paper describes the results of the surveillance of H5N1 and ND in swiftlets over the period of September 2004 to June 2011.

MATERIALS AND METHODS

Live birds (Photo 4) were caught using mist nets from the Gomantong and Madai caves where EBN has been traditionally harvested for commercial purposes. Tracheal and cloacal swabs were subsequently taken from these live birds (Photo 5). Five to ten tracheal swabs were pooled and inoculated into Tryptose Phosphate Broth (TPB), maintained at 2°C to 8°C (Photo 6). This procedure was similarly done for the cloacal swabs collected. However in EBN ranches or premises where live birds are rarely allowed to be manipulated, faecal swabs were taken from the premises and either singly or pooled into TPB (Photo 7). Nevertheless where swiftlets were also available in these ranches or premises, tracheal and cloacal swabs were concurrently taken. The samples were later inoculated into fertilised eggs for viruses isolation as recommended by OIE (2004) for both the H5N1 and ND viruses (Photo 8).

A summary of samples collected for the surveillance is shown in Table 1.

Table 1. Distribution of swiftlet samples collected for the H5N1 and ND surveillance in Sabah from September 2004 till June 2011.

<table>
<thead>
<tr>
<th>PREMISE TYPE</th>
<th>LOCATION</th>
<th>SPECIES</th>
<th>TYPE OF SAMPLES [SWABS]</th>
<th>Total swabs for egg inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranched</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pertama Commercial Centre, Apas Road, Mile 1.5, Tawau. [shop lots]</td>
<td>A. fuciphagus</td>
<td>Tracheal</td>
<td>Cloacal</td>
</tr>
<tr>
<td></td>
<td>Jln Dunlop, Tawau. [Shop lots]</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Kg.Tindai Kolopis, Penampang. [Free standing buildings - 2 houses]</td>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Caves</td>
<td>Simud Putih, Gomantong Cave, Sukau, Sandakan</td>
<td>A. maximus</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Simud Putih, Gomantong Cave, Sukau, Sandakan</td>
<td></td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Madai Cave, Kunak</td>
<td></td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65</td>
</tr>
</tbody>
</table>
RESULTS

The result of 137 samples taken and tested for H5N1 and ND is shown in Table 2. All the samples collected in the study did not show the presence of either the Highly Pathogenic Avian Influenza (H5N1) or the Newcastle disease (ND) viruses.

DISCUSSION

This preliminary study is indeed an excellent indication that EBN swiflets in both cave and farmed environments collected in Sabah are free from both the H5N1 and ND viruses. Although only convenient samplings were taken, the localities viz. the Gomantong and Madai caves chosen are very representative since these sites cover a wide span of the total bird population in Sabah, and have traditionally remain the main sources of EBN harvests.

Swiflet farming continues to attract investments in the millions in Sabah (Ongkili, 2011) and hence the number of man-made or ranched swiflet farms will inevitably continue to increase in view of the lucrative income from this industry. This thus calls for further comprehensive studies on the presence of any other infectious agents in these birds and products. Caves and farming premises must continue to be monitored and good animal husbandry practices must be advocated to ensure the production of safe EBN.

<table>
<thead>
<tr>
<th>PREMISE TYPE</th>
<th>TYPE OF SAMPLES [SWABS]</th>
<th>NO. OF SAMPLES</th>
<th>RESULT OF EGG INOCULATION FOR H5N1 AND ND VIRUSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranched</td>
<td>Trachael</td>
<td>7</td>
<td>No virus isolated</td>
</tr>
<tr>
<td></td>
<td>Cloacal</td>
<td>3</td>
<td>No virus isolated</td>
</tr>
<tr>
<td></td>
<td>Faecal</td>
<td>11</td>
<td>No virus isolated</td>
</tr>
<tr>
<td>Caves</td>
<td>Trachael</td>
<td>58</td>
<td>No virus isolated</td>
</tr>
<tr>
<td></td>
<td>Cloacal</td>
<td>58</td>
<td>No virus isolated</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>137</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Result of egg inoculation for H5N1 and ND viruses from EBN swiflet samples.
REFERENCES


ACKNOWLEDGEMENTS. The authors would like to express sincere appreciation to Kamarudin Bin Md. Isa, Azri Bin Adzhar and Helen Mitin from the Department of Veterinary Services, Malaysia; Assis Kamu from Universiti Malaysia Sabah as well as Nasip Eli, Maria Kissey, Kennedy Juani and staff members of the Veterinary Diagnostic Laboratory at Kepayan and Tawau (DVSAI) for their guidance, advice and cooperation rendered during the whole period of the study. Thanks are also due to all the members of staff of the Wildlife Department of Sabah involved in assisting the collection of swiftlets samples.

Map of Sabah
A BEEF FATTENING DECISION SUPPORT SYSTEM

SHANMUGAVELU S.1, WAN ZAHARI M.2, WONG H.K.1 AND MARDHATI M.1
1 Malaysian Agricultural Research and Development Institute, MARDI, Serdang, Selangor.
2 Universiti Malaysia Kelantan, Pengkalan Chepa, Kota Bharu, Kelantan. shan@mardi.gov.my

ABSTRACT. A beef feedlot production decision support system (DSS) was developed based on Microsoft® Excel. The DSS comprises of three modules i) an ingredient database ii) a least-cost ration formulation module and iii) beef growth simulation module. The program uses empirical equations developed for tropical beef to simulate nutrient requirements and daily body weight gains based on the formulated feed ration. The formulated least cost ration can be pasted automatically into the growth model to evaluate performance and economic viability. The growth model calculates nutrient available and computes body weight gain on a daily basis, summates weight gain and stops at the targeted body weight. The data output include i) days to reach target body weight, ii) cumulative feed consumed, iii) anticipated average daily gain, iv) total cost of feed (concentrates and grass), and v) gross profit per cattle. If a portion of the feed is fed as grass, then the model also computes the pasture land required in hectares, based on the forage species chosen. It is anticipated that the developed model can assist cattle entrepreneurs and farmers in the development of the beef cattle industry in Malaysia.

INTRODUCTION

Beef is an important commodity in Malaysia with a per-capita consumption of 5.6 kg. However, only 28% of this requirement is produced locally (DVS, 2010). The main factor that contributes to the low self sufficiency level is the high cost of local beef production. For example, the average cost of local beef for 2010 was RM15.85 compared to RM9.20 for imported beef from India (DVS, 2010). The lack of cheap feed and the inefficient use of available feed resources contribute significantly to the higher cost of production as feed generally comprises 60-70% to the total production cost. Many local beef producers do not have access to information on nutrient values of available feed resources nor the ability to efficiently utilise the resources. This paper describes a beef fattening decision support system that can help improve the efficiency of a beef production enterprise.

MATERIALS & METHODS

A Microsoft® Excel based software was developed based on cited publications (NRC, 2000; Leonard, 1982) and beef growth data collected from research conducted in MARDI. The model comprises
of three modules i) an ingredient database ii) a least-cost ration formulation module and iii) beef growth simulation module. The ingredient data base comprises of nutrient content information of local feed resources. The least cost module utilizes the linear optimization module inbuilt in Microsoft® Excel 2007 for Windows. The beef growth simulation uses empirical equations developed for tropical beef based on research data to simulate nutrient requirements and daily body weight gains. The nutrient requirements for the beef production module are based on Department of Standards Malaysia (Standards Malaysia) (unpublished). Microsoft® Excel 2007 Visual Basic for Application was employed for the beef daily growth simulation module. The program algorithm is shown in Figure 1.

Figure 1. Model algorithm
Other features incorporated into the model include the options to evaluate two different feed formulations and their growth rate predictions.

RESULTS AND DISCUSSION

The captions of the beef fattening decision support system are shown in Figures 2, 3, 4, 5, 6, 7 and 8 with simple user friendly
modules. The model enables the user to alter feed ingredients and test different feed combinations based on costs and nutrient requirements and allows the comparison of two types of formulations (Figure 5). The user has the option to alter, add and make changes to the feed ingredient database including the use of grass as a proportion

![Figure 4. Ingredient database](image)

![Figure 5. Least cost formulation module](image)
of the diet. If grass is chosen as an option, the model computes the land required to cultivate the grass species chosen. The beef fattening decision support system was verified against actual beef cattle growth from studies conducted in MARDI.

Figure 6. Least cost comparison module

Figure 7. Model evaluation module
Figure 8. Model analysis and economic evaluation module

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RUN 1</th>
<th>RUN 2</th>
<th>DIFF</th>
</tr>
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<tr>
<td>Production parameters</td>
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<tr>
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<td>141</td>
<td>0.00</td>
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<tr>
<td>Target body weight (kg)</td>
<td>180</td>
<td>180</td>
<td>0.00</td>
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<tr>
<td>Days to target wt</td>
<td>65</td>
<td>60</td>
<td>5.00</td>
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<tr>
<td>Cum feed cost (RM)</td>
<td>169.21</td>
<td>92.12</td>
<td>77.09</td>
</tr>
<tr>
<td>Proportion of ration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrates (%)</td>
<td>100</td>
<td>50</td>
<td>50.00</td>
</tr>
<tr>
<td>Fresh Grass/Forage (%)</td>
<td>0</td>
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<td>-50.00</td>
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<tr>
<td>Anticipated ADG (kg)</td>
<td>0.60</td>
<td>0.66</td>
<td>-0.06</td>
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<tr>
<td>Individual Animal Economic Data</td>
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<tr>
<td>Cattle purchase price (RM/kg)</td>
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<td>6.50</td>
<td>0.00</td>
</tr>
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<td>Sale price (RM/kg)</td>
<td>7.50</td>
<td>7.50</td>
<td>0.00</td>
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<tr>
<td>Cost of feeder cattle (RM)</td>
<td>147.17</td>
<td>147.17</td>
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<tr>
<td>Feed Cost (RM)</td>
<td>169.21</td>
<td>92.12</td>
<td>77.09</td>
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<tr>
<td>Total grass/animal (kg, DM)</td>
<td>0.00</td>
<td>142.19</td>
<td>-142.19</td>
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<tr>
<td>Gross profit/cattle (RM)</td>
<td>1,633.62</td>
<td>1,110.71</td>
<td>-522.91</td>
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<td>Herd Economic Data</td>
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<tr>
<td>Total number of cattle</td>
<td>50</td>
<td>50</td>
<td>0.00</td>
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<tr>
<td>Total Herd Gross Profit (RM)</td>
<td>51,681.22</td>
<td>56,536.44</td>
<td>-4,855.22</td>
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<tr>
<td>Gross profit/month (RM)</td>
<td>23,852.87</td>
<td>27,767.72</td>
<td>-3,914.85</td>
</tr>
<tr>
<td>Pasture land required (ha)</td>
<td>0.00</td>
<td>2.70</td>
<td>-2.70</td>
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<td>Feed Parameters</td>
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<tr>
<td>Cost of Concentrates</td>
<td>2.38</td>
<td>1.19</td>
<td>1.19</td>
</tr>
<tr>
<td>Cost of grass</td>
<td>0.00</td>
<td>0.21</td>
<td>-0.21</td>
</tr>
<tr>
<td>Total feed cost/day (RM)</td>
<td>2.38</td>
<td>1.40</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Figure 9. Actual vs Predicted body weight
A sample data of predicted against actual growth rate based on the feed formulated is shown in Figure 9 with an average prediction error of 0.8kg. The model developed can be used by the beef feedlot industry to make intelligent decisions and avoid losses in feedlot operations. It can also be used by extension agents and as a teaching tool especially in universities.

CONCLUSION

A beef feedlot production decision support system (DSS) developed based on Microsoft® Excel was observed to predict beef growth under Malaysian conditions within reasonable limits. Beef feedlot is a challenging enterprise especially with the high cost of feed ingredients and this DSS software can be utilized to optimize returns. The model can be used by feedlot operators, beef nutritionists, and also in universities as a teaching tool. However, it is emphasized that as with most DSS systems, there can be variations in any biological system. Nevertheless this software can be used as an intelligent tool to assess feedlot operations and improve economic returns.

REFERENCES

ABSTRACT. Rodents particularly those belonging to the Muridae family in Malaysia have been well studied because of their medical and economic importance. Much of the work on rodents has been focusing on the identification of endo and ecto parasites. Parasites in rats (Murids) particularly helminthes belonging to the Nematoda family have been described by many workers for more than a century. This paper is an attempt to compile 50 papers on rodent nematodes that has been published in various scientific journals over the last 100 years in Malaysia. It is hoped that this literature overview on rodent nematodes will come useful as a reference material for the budding parasitologist and biology scientist.

Keywords: rodents, Muridae, Malaysia, wild, urban, rats, parasites, zoonotic, helminths overview

INTRODUCTION

Rodents are a key mammalian group and found in many environments throughout the world. They constitute more than 42% of the known mammalian species [1]. The Order Rodentia is divided into three major groups: the Sciuromorpha (squirrel-shaped), Myomorpha (mouse-shaped) and Hystrichomorpha (porcupine-shaped).

Commensal rats and mice are members of the rodent family Muridae, part of the Myomorpha group. Rodents belonging to the family Muridae are extremely successful and dominant species in most regions of the world largely due to their ability to adapt and then exploit new situations rapidly [1].

The genus Rattus consists of nearly 200 sub-species that can be further grouped into 20 species in Malaysia. Most of the members of this genus are forest or island forms and the number of rodent species that can be found in urban and agricultural habitats may comprise of about 12 species.
**Rattus rattus diardii** and **Rattus norvegicus** are the two most successful species in adapting to all kinds of environment and found widespread in the world [2].

All Malayan rats are nocturnal [3]. They form an important and diverse group in towns, cultivated land and forest, extending from the shore to the mountain peaks and from ground level to the forest. Different species of rodents tend to be selective in their habitat, when these habitats are destroyed or disturbed either through agricultural intensification, deforestation, or urbanisation it can bring about changes in rodent species diversity [4, 5]. This will invariably facilitate the emergence and transmission of rodent-borne zoonotic pathogens.

The endoparasite fauna of wild terrestrial rats from primary forest habitats in Peninsular Malaysia has been well recorded. Prior studies have provided insights into the rats’ habitat and behaviour and changes to the rats surrounding are often reflected in the endoparasites diversity and population.

### AN OVERVIEW OF RODENT NEMATODES

The earliest reports of nematodes from Malaysian murids and other animals were provided by Alessandrini [6] and Adams [7], who described parasites which are mostly from domestic animals. The nematode parasites identified from the rodent host were *Syphacia obvelata*, *Cyclodontostomum purivisi* and *Heterakis* sp. from the large intestine of rats caught in Raub. They also recorded another species of *Ancylostoma malayenum* [6] from a rat in Seremban and an oxyurid (*Oxyurid syphacia obvelata*) in rats from Taiping and Pahang. There was a long lapse of interest following which a batch of worms from murids from Pulau Jarak were identified by Buckely (quoted in Audy *et al.* [8]) to contain a strongyloid. This worm was subsequently described by Yeh [9] as *Hepatojarakies malayae*, representing a new species and genus.

The nematodes *Capillaria hepatica*, *Rictularia tani* and *Nippostrongylus* sp. were also recognised in the same collection. A larger collection of parasites were examined from other parts of Malaysia around this time and it included worms from murids. These murid worms were examined by Sandosham [10, 11, 12]) under the four major helminth groups in which the incidence of infection was recorded. Subsequently, nematode parasites from unidentified house rats were identified by Hall [13]. They were *Strongyloides ratti*, *Angiostrongylus cantonensis*, *Nippostrongylus muris* and *Gongylonema neoplasticum*. Four other undetermined species of *Capillaria*, *Syphacia*, *Protospirura* and *Mastophorus* were also mentioned.

A number of unnamed filarial worms were reported from rats. The first was said to be a new genus of filarial heart worm belonging to the Subfamily Splendidofilariinae which was isolated from *Rattus sabanus*, *Rattus whitheadi*
and *Rattus muelleri* by Sivanandam et al. [14]. They also found another filarial worm from the heart, lung and the liver of *Rattus sabanus*. The fourth species was recovered by Sandosham and Sivanandam [15] also from the heart of *Rattus sabanus*. Several years later, additional two species, *Breinlia booliati* [16] and *Dunnifilaria ramachandranri* were recovered from *Rattus sabanus* [17].

In 1963, Balasingham [18] redescribed the hookworms of *Cyclodontomum purvasi* from Malayan giant rats. Very few people have described the major groups of helminths in Malaysia during this time. Miyazaki and Dunn [19] reported *Gnathostoma malaysiae* from *Rattus surifer*. First Sandosham [12] introduced to a major group without the species identification. Dunn et al. [20] later described helminths of rats from Pulau Tioman and Pulau Tikus islands. The groups are nematodes, cestodes and trematodes. The helminths identified were *Raillietina* sp., *Hymenolepis diminuta*, *Rodentolepis* sp. (*H. nana*), *Leipertrema* sp. *Zonorchis* sp., *Strongyloides ratti*, *Globocephalus* sp., *Cyclodontostomum purvisi*, *Nippostroglus brasiliensis*, *Angiostrongylus (cantonensis) malayasensis*, *Syphacia* sp., *Subulura Andersoni*, *H. malaya*, *Enterobius* sp., *Physaloptera* sp., *A. malayasensis*, *S. ratti*, *Subulura* sp., and *H. diminuta*, *Dipylidium caninum*, *Taenia taeniaeformis*, *Moniliformis dubius*, and *Armilifer moniliformis*.

In the 1970s many reports were on the same finding. Durette-Desset [28] has renamed (*N. muris*) to *N. brasiliensis* from Malaysian rats, this Nipposstrongylus is common among all kinds of Malayan rats. Bhaibulaya and Cross [25] redescribed *Angiostrongylus cantonensis* and called it *A. malaysiensis*, a new species. Lim [29] noted the presence of this nematode in three commensal and three forest rat species in Tuaran, Sabah. Ow-Yang [22] and Mulkit and Cheong [23] examined nematode parasites from feral rats which have potential for human transmission.

The following nematode parasites were examined from the above rodent host examination. *A. (cantonensis) malayensis*, *Breinlia* sp., *C. hepatica*, *C. purvesi*, *Globocephales connorfilii*, *G. neoplasticum*, *H. malayae*, *N. brazilliensis*, *Physaloptera* sp., *P. mastophorous* spp., *R. tani*, *S. ratti*, *S. andersoni*, *S. muris* and *T. crassicauda*. Subsequently several people carried out surveys and identified several groups of rodent endo-parasites but little work was done to confirm the morphology of the parasites. Varughese [30] was the first person to describe the complete life cycle of a rodent hookworm, *Cyclodontostomum purvisi* from Malaysian giant rats.

Quentin and Krishnasamy [31] have reported a new species *Spirura malayensis* from *Tupaia glis* from Ulu Gombak Forest Reserve. Lim et al. [32] and Sinniah et al. [33] reported the liver worm *Capillaria hepatica* as one of the common nematodes species among rodents in Malaysia. Subsequently, Khairul [34] reported that the Acantocephala, *Moniliformis moniliformis* to be common among house rats in Penang, Malaysia. Sinniah [35] found *Angiostrongylus cantonensis (malaysiensis)* followed by *Strongyloides ratti* being the most prevalent nematode infections in 8 rat species in Peninsular Malaysia. Krishnasamy et al. [36] studied the wood rat (*Rattus tiomanicus*) helminth fauna and described the common helminth groups – nematodes, cestodes and trematodes.

Following this Ambu et al. [37] carried out a survey in Pantai Bengkoka, and described several rodents’ nematodes and cestodes. At the same time Singh et al. [38] attempted to isolate *Trichinella* sp. from rodents caught in the wild but, was not successful. Lynda and Krishnasamy [39] reported a new genus and species of nematode *Malayometastrongylus diardinematus* from a house rat *Rattus r. diardi* near Ulu Yam, Rawang.

Singh et al. [40] during wildlife expedition to Ulu Endau, Johor recovered some parasites from four species of rats which belong to the following groups: nematodes, cestodes, trematodes and pentastomids. Ho and Krishnasamy [41] conducted a survey at Taman Negara National Park and reported infections of endo-parasites as common among the small mammals including the rodents. Later in 1993, Krishnasamy et al. [42] described a rare nematode parasite *Gnathostoma malayae* from a forest dwelling rodent *Rattus rajah*. Three years later, Ambu et al. [43] conducted a survey at an Orang Asli settlement in Selangor to assess the potential health risk of rodent parasites. In his findings he reported nematodes and cestodes as the dominant group of helminths and trematode as rare.

Mohd Zain and Arnez [44, 45], conducted extensive studies of the parasite fauna at Endau-Rompin National Park and recovered 8 rat species of which 5 were new records and a total of 20 new records of endoparasites including 9 plural species of Trichostrongyloidea parasites (*Heligmonoides bulbosus*, *Malaystrongylus odontospicularis*, *Macrostrongylus ratti*, *whiteheadi*).
Maxomystrongylus sp., Nippostrongylus brasiliensis, Orientostrongylus sp., Paraheligmonelloides sp., Rattustrongylus odontoconus and Rattustrongylus rotundoconus. Their study also noted an emergence of commensal rats and cosmopolitan endoparasites suggesting impact of increasing human activities in the park.

Mohd Zain [46] surveyed 2 islands on the Straits of Malacca to understand the effects of island biogeography on the diversity of rodent host and their parasites. However, both islands were disturbed habitats with only commensal rats recovered and low helminth community.

Paramasvaran et al. [47, 48, 49] reported several species of nematodes recovered from rodents from urban, rice field, forest and coastal habitats in the states of Selangor and Negeri Sembilan. It was shown for the first time statistically there was significant association of helminth infections and the habitats in which the rodents live.

As for the filarial parasites there were not many reports. Singh and Cheng [16], Mak and Lim [49] described the filarial worm Breinia booliati from rat. In the same year Mullin and Balasingam [17] reported Dunnifilaria ramachandrani from Rattus sabanus which is at present suspected to be zoonotic.

CONCLUSION

There have been no major changes in the fauna of the nematodes described over the last 100 years in the rodents. However with the global climatic changes that we are experiencing now, the rodent ecology may be altered giving rise to the proliferation and formation of new rodent foci and its parasites. If the rodent population increases there will be concomitant increased risk of maintaining zoonotic infection. Considering the rat-man proximity this situation may pose considerable threat to human and animal health in the future.

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ABSTRACT. Parasitic helminth infections in small ruminants are prevalent in South East Asia (SEA), limiting productivity and causing major economic loss for farmers. The hot, wet, tropical climate all year round favours trichostrongylid infections, predominantly haemonchosis in sheep and goats. Commercial large scale farms, with more than 300 animals, as well as small holders or backyard farmers with less than 50 animals face the debilitating effects of haemonchosis when they graze their animals as effective worm control is often hampered by anthelmintic resistance. In Malaysia, frequent and indiscriminate use of anthelmintics in the past has resulted in the majority of the small ruminant population facing resistance to one or more anthelmintics. Several alternative methods of worm control are being employed by farmers; the most important and effective being cut and carry or zero grazing, where the animals are kept in pens and grass is cut and fed. In Cambodia and Myanmar, ruminants are still tethered or stall fed with minimal drug use. In Indonesia and Thailand, commercial goat and sheep farms are fast expanding to produce breeder stock for the SEA market. However, up to 75% of the small ruminant population is still traditionally managed by small scale farmers.

In most of SEA, the McMaster method for faecal worm egg counts is the only diagnostic test used to assess helminthosis in ruminants. There is an urgent need to increase awareness and information on the need for testing faecal samples regularly before drenching, conducting faecal egg count reduction tests on a yearly basis, use of the FAMACHA technique to enable selective treatment of individual animals. The use of alternative worm control methods to manage helminthosis will help promote effective ruminant production with reduced drug use and encourage “green” farming methods. Extension of research on local bioactive plants which may have the potential to control helminthosis may also be beneficial in the longer term.

Keywords: helminth parasites, control, Malaysia, South East Asia

INTRODUCTION

The current trend in South East Asian livestock industries is towards greater commercialisation whereby smallholders and backyard farmers are finding it financially lucrative to expand and
commercialise their herds and flocks. Small farms of 10-20 animals traditionally kept as a backyard enterprise are fast disappearing especially in Thailand, Malaysia and the Philippines with Indonesia and Vietnam not far behind. Sheep and goats are especially important livestock commodities in Indonesia (11.0 million), the Philippines (2.1 million), Thailand (1.5 million) and Myanmar (1.1 million) as compared to Malaysia (DVS statistic, Vietnam Cambodia and Laos which have less than 300,00 head in each country (FAO, 2005)). The common breeds of small ruminants vary with locality and include the native breeds such as Siamese long tails in Thailand, Katjang goats in Indonesia and other hardy breeds that can tolerate the tropical climate and poor quality feeds. Imported breeds such as Commercial Merino Border Leicester crosses, Barbados Black belly and St Croix have found their way into the local populations with importations to upgrade the local breeds. Helminth parasites such as *Haemonchus contortus* and *Trichostrongylus* spp., followed in prevalence by *Strongyloides papillosus*, *Oesophagostomum* spp., *Moniezia* spp, *Trichuris* spp., *Cooperia* spp., *Bunostomum* spp., *Fasciola* spp. and rumen and pancreatic flukes, are commonly found in the Asian small ruminant production systems (Sani *et al.* 2004).

Studies by Chandrawathani *et al.* (1994 and 1995) evaluated the importance of helminth infections in small ruminants in Malaysia by estimating production increases after treatment with anthelmintics. Small ruminant farming is being constantly promoted by Malaysian government agencies in an effort to increase food production for the country, thereby opening up opportunities for goat and sheep farming under oil palm and rubber plantations. Several studies were conducted to support this effort such as epidemiological and production data, nutritional analyses, methods of pasture maintainance and worm control strategies for long term implementation at the smallholder level. The data generated was also very useful for the rest of South East Asia as the climate and geography in these countries had many similarities. This paper looks at the various methods of worm control practised in these Asian countries over the past decade and gives an insight into the future expectations and options for worm control in a changing environment.

**WORM CONTROL METHODS FOR SHEEP AND GOATS PRACTISED IN ASIA**

Strongyle infections, namely *Haemonchus* spp., is the major cause of helminth disease in South East Asia causing severe losses in terms of mortality and morbidity. In Malaysia, over the past 20 years, small ruminant helminths have primarily been controlled by anthelmintics which are used frequently up to 12 times a year. This has resulted in severe anthelmintic resistance in worm populations for the 4 drug groups namely benzimidazoles, avermectins,
levamisoles and salicylanilides (Chandrawathani, 1999, 2003; Dorny et al., 1993, Sivaraj et al.) Consequently, an investigation into the total anthelmintic failure in small ruminants revealed the need to seriously look at alternative methods of helminth control in East Malaysian farms (Chandrawathani, 2004). Several methods were introduced to farmers facing anthelmintic resistance in their flocks as follows:-

1. Rapid rotational grazing: Following the success achieved by Barger et al. (1994), this method was introduced to Malaysian government farms with large acreage in pasture whereby a flock of sheep or goats is allowed to graze in a paddock for 3-4 days only after which the flock is moved to the next paddock. A series of 10 paddocks are utilised. The grazed paddock is left empty for at least 30 days to reduce survival of any existing larvae. Experimental work showed that this method was highly successful in maintaining low worm burdens (Chandrawathani et al., 2004) and that the period without animals is sufficient for the grass to regrow and be ready for grazing with grass quality maintained between 10-15% crude protein. Service cutting was also done when necessary to maintain the pastures. Initial treatment with an anthelmintic was necessary to reduce pasture contamination during the first rotation, after which the faecal egg counts will be seen to be decreasing over time. This system needs strict management discipline to ensure effectiveness as any slackening in the implementation of the programme will cause a breakdown and increase in worm burdens (Barger, 1999).

2. Cut and carry or Zero grazing: This method is especially effective in smallholders who do frequent trading of animals. As sheep and goats are a source of ready cash, smallholders generally sell animals when they need cash and this leads to a lot of animal movement between smallholder farms. Traded animals may have strongyle infections and if grazed on common pastures after purchase, this can increase the infections at the destination. Farmers seldom quarantine or treat animals when acquiring new stock and this allows for regular mixing of helminth populations and leads to heavy infection levels. Thus with small numbers of animals, a cut and carry system is a workable measure to prevent infection as the animals are kept penned at all times and fed on cut grass with minimum exposure to pastures (Khadijah, 2006).

3. Improved nutrition: As improved nutrition has been implicated to positively affect worm infections, farmers are encouraged to improve the quantity and quality of feeding through the provision of supplements, concentrates, agricultural by-products which have been improved or silage, urea molasses blocks as well as planting
improved pastures for their animals (Knox and Wan Zahari, 1998).

4. Herbal remedies: The use of herbal remedies has been traditionally practised all over Asia and each country has its unique herbs and plants for use to control worms. However, the amounts and species of herbs used can vary according to region and this creates a dilemma for farmers who want to follow these practices as uniform guidelines for their use do not exist. In Malaysia, neem and cassava leaves have been shown to reduce worm burdens by 30% to 40% (Chandrawathani et al., 2002 and 2006, Nurulaini et al., 2009). Recently, the use of effective microbes has become popular and economical for worm control. Increased awareness on the need to reduce drug residues in food animals and promotions of healthy, green farming methods has sprouted several newage farmers keen on practising this.

5. Tethering: In many parts of Indonesia and Indochina where farmers have less than 5 animals, tethering has been found to be the management style of choice (http://www.fao.org/ag/AGP/AGPC/doc/Counprof/Philippines/Philipp.htm)

6. Biological control: This method has been proven to be effective. Reduced larval availability/ Reinfection rates by 80 to 90% (Chandrawathani et al., 2003) but the product is unavailable at the moment. However, for the Asian market, issues relating to storage is critical as the hot wet tropical climate may pose a limitation to storage of the spores in feed or blocks, with the risk of sporulation. Trials done using this product was maintained and stored in refrigerators and is fed as a supplement added to concentrate, for grazing animals (Chandrawathani et al., 1998 and 2002).

**DIAGNOSIS AND ASSESSMENT OF HELMINTH INFECTIONS**

In most of Asia, diagnostic tools for helminth infections are lacking. In Malaysia, the government-run laboratories conduct faecal egg counts and larvae culture as a routine procedure to help farmers identify potential problems and make recommendations of possible solutions. Extension services are responsible for assisting the development of new farmers and give free personalised service especially to farmers who are keen to commercialise their farming enterprise (Rajamanickam et al., 1990).

The faecal egg count reduction test is a routine test conducted to encourage farmers to be aware about anthelmintic resistance issues and to resort to alternative methods of control (Khadijah, 2006). The Malaysian scenario for anthelmintic resistance is extremely grave mainly due to the abuse of anthelmintics over the past 2 decades. The tropical climate aggravated the problem of anthelmintic resistance by providing the perfect microenvironment for larvae development and survival all the year round and this was exacerbated
by a policy of indiscriminate grazing in communal grazing areas. Many times, treatments were then given without proper faecal egg count screening to diagnose infections.

In Cambodia, the faecal egg count technique is conducted in only one laboratory. Farmers seldom screen the animals for worm eggs and it is even more rare that treatments are given due to the economics. In this instance, it is highly probable that anthelmintic resistance is not prevalent.

In the Philippines and Thailand, commercialisation of the small ruminant sector for export and breeding has seen the development of various highly commercial enterprises that use an intensive management system, which does not necessitate grazing, thus reducing worm infections and the occurrence of anthelmintic resistance. Parasitic diseases in these commercial facilities are minimal however, for smallholders who have 30-100 animals and graze their animals, parasitic disease is still a major limiting factor in small ruminant production (Barcelona, 1994).

One of the unique tools for assessing helminthiosis and currently gaining popularity is the use of FAMACHA which is an anemia guide, indicating a pale eye mucous membrane has a high probability of worm infections (reference needed). This is especially important in areas such as South East Asia where the main strongyle infection is *Haemonchus contortus*. Use of FAMACHA reduces the necessity of doing faecal egg counts to estimate worm burdens and is especially useful for smallholder farmers with few animals as they can monitor the helminth status of their animals regularly.

**CONCLUSION**

The Asian scenario for helminth infections appears to be seeing an upward trend in the severity of infections but there is also a marked improvement in awareness of diagnostic tests for helminth infection, knowledge of faecal egg count reduction test and anthelmintic resistance as well as a healthy view to reduced drug use and towards green farming (Chandrawathani et al., 2009). Frequently, Asian farmers combine the various worm control methods to get the best possible advantage in raising their flock. As finances and facilities are the limitation, disease tends to take a back seat. However, with the increasing trend towards commercialization of livestock production, helminthosis is facing a new challenge from farmers who are aware and proactive to combating this problem.

Considerable work need to be done on the reference section, please pay attention to the Veterinary Parasitology guidelines (http://www.elsevier.com/wps/find/journaldescription.cws_home/503321/authorinstructions) in this regard.

**REFERENCES**


EFFECT OF STRENuous SUB-MAXIMAL RACE on HEART RATES OF ENDURANCE HORSES

LAwan A.1,3, noraniza m.a.1, rasedee a.2 and bashir a.1
1 Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2 Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
3 Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, PMB1069, Borno State, Nigeria
Corresponding Author: drlawan3758@yahoo.com

ABSTRACT. The study was carried out to investigate the effect of prolong submaximal exercise on heart rate reflecting the performance of endurance horses after covering distances of 40 and 80 km races. This study was conducted in Malaysia to determine the post-race heart rates of endurance horses based on distances for the eliminated horses and those that completed the races with good performance. Heart rates of competing endurance horses were examined at pre- and post-race. Thirty-four endurance horses were sampled; N = 9 (40 km) and N = 9 (80 km) were the good performance horses while, N = 3 (40 km) and N = 13 (80 km) were the poor performance horses eliminated from the race. The mean heart rate of the good performance horses in the 40 km category was 53 ± 8 bpm and that of the 80 km in the good performance category was 56 ± 7 bpm, while the eliminated horses of the 40 km group had 74 ± 17 bpm and those in the 80 km group had 78 ± 7 bpm. The study showed that eliminated horses in the 40 and 80 km categories both exhibited high heart rates compared to the good performance horses that were in the same racing category. Thus, the heart rates of horses during training may be used to predict performance based on distances covered in endurance races.

Keywords: endurance, heart rate, race category, performance.

INTRODUCTION

Measurement of the fitness or exercise tolerance of a horse is by assessment, through physical examination of heart rates and respiratory rates (Cottin et al., 2006; Bashir and Rasedee, 2009). The active working muscle of endurance horses depends on heart size and capacity to deliver large volumes of blood to the tissue and the splenic reserve supply (Kenneth et al., 2004; Lawan et al., 2010). Endurance horses require calcium for muscle contractions and low plasma levels of calcium during strenuous endurance rides can lead to metabolic problems and failures, including synchronous diaphragmatic flutter. However, high blood calcium concentration is needless because it may
increase the frequency of thumps during endurance competitions (Lewis, 1995).

The best known abnormality in horses at risk of developing metabolic problems and exhaustion is persistently elevated heart rates after the competition (Rose et al., 1977; Carlson, 1985; Schott and Charlton, 1996; Schott et al., 1997, 2006; Harold, 2010). During endurance rides, cardiac output increases in response to the metabolic demands of active skeletal muscle and also due to a demand of increased skin blood flow for thermoregulatory cooling (Rowell, 1986; Hodgson et al., 1994). Moreso, during a prolong endurance ride, a decrease in blood flow may compromise function of less well-perfused organs. Specifically, the barrier function of the mucosal lining of the intestinal tract may become compromised. An intact mucosal barrier prevents absorption of many toxins that are present in the bowel lumen, but a prolonged decrease in intestinal blood flow during endurance exercise can lead to both decreased intestinal motility, increased absorption of toxins and heart rate variability which is an effective measure of equine fitness, overtraining and metabolic derangements (Harold, 2010).

High heart rate is the predominant indicator of decrease performance in strenuous equine endurance. Thus, this study was conducted to investigate the effect of prolong sub maximal race on post-race heart rate as an indicator of performances in endurance horses after covering the distances of 40 and 80 km races.

**MATERIALS AND METHODS**

**Subjects**

Thirty-four endurance horses that participated in endurance competitions each consisting of 40 km (N=3) and 80 km (N=13) were eliminated from the race while the good performance horses that completed the race successfully are 40 km (N=9) and 80 km (N=9) were examined. Among these, 16 horses were eliminated and 18 horses completed the race successfully. The good performance horses are those that completed the race successfully while the poor performance horses are those that were eliminated due to high heart rate.

**Veterinary inspection**

Veterinary inspection was conducted after each loop of the race on all competing horses and the resting heart rate was recorded. The heart rate was re-evaluated and recorded each time the horses enter the vet-check after each loop of the race. The heart rate was evaluated as (44-64 = normal, 65-70 = high, 71-90 = very high). Good performance horses continue the race in the subsequent loop, while poor performance horses that were eliminated due to heart rate were sent to the clinic for treatments and further workout. Descriptive statistic mean ± SD was used to analyse the result at P < 0.05 using statistical software JMP 9, SAS.
RESULT

Thirty-four horses participated in the endurance ride. Sixteen horses were eliminated from the endurance competition. Eighteen horses managed to complete the race without metabolic signs and all were from the N=9 (40 km) and N=9 (80 km) category. All horses from the N=3 (40 km), and N=13 (80 km) category were eliminated because of high heart rates. Horses that completed the races with good performance showed a lower mean heart rate post-race (Table 1) and those eliminated from the race because of poor performance had elevated heart rates post-race as shown in (Table 2).

DISCUSSION

Recent studies showed that up to 40% of the horse populations in Malaysia are eliminated from endurance race due to cardiorespiratory derangement. High heart rates appears to affect cardiopulmonary performance and tissue oxygenation in the endurance horses, subjecting the endurance horses to higher risk of developing hyperthermia and thumps caused by alterations in fluid and electrolyte status resulting in a large percentage of these horses ultimately being eliminated from endurance races. The heart rates are also reliant on the oxygen carriage capacity of blood, which is dependent on erythrocyte number and hemoglobin concentrations (McKeever et al., 2000).

Table 1. Pre and post-ride heart rates of good performance horses

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-ride (km)</th>
<th>Pre-ride (km)</th>
<th>Post-ride distances (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>40</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td>(n=9)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>41 ± 5</td>
<td>37 ± 4</td>
<td>53 ± 8</td>
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<td></td>
<td></td>
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<td>56 ± 7</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± Std Dev.

a, b, within each row, means with different superscript are significantly different at P < 0.05, n = the number of horses.

Table 2. Pre and post-ride heart rates of poor performance horses

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-ride (km)</th>
<th>Pre-ride (km)</th>
<th>Post-ride distances (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>40</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td>(n=3)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>43 ± 8</td>
<td>45 ± 6</td>
<td>74 ± 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>78 ± 7</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± Std Dev.

a, b, within each row, means with different superscript are significantly different at P < 0.05, n = the number of horses
In the face of depletion of body fluid stores consequent to prolonged sweating, struggle for cardiac output may develop between active muscle and skin, resulting in a decrease in performance and heighten core temperature (Hodgson et al., 1994; Harold, 2010). Thus from the results of this study, it seems that the depletion of body fluid stores due to prolonged sweating and decrease in cardiac output to active muscles and skin could be the resultant decrease in performance of endurance horses (Hodgson et al., 1994). Thus, resulting in the persistent elevated heart rates in poor performance endurance horses.

CONCLUSION

The study indicates that eliminated horses have higher heart rates than the good performance endurance horses. Therefore, high heart rates may be used as an indicator of performance in endurance horses during the conditioning protocols and training.

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ACKNOWLEDGEMENTS. The authors appreciate the effort and assistance offered by Mr. Mohamed Halmi Othman, Mr. Abdullah Misron, the staff of Veterinary Teaching Hospital, Universiti Putra Malaysia especially Mr. Salehuddin and Drs Mohammad Fairuz Jamaluddin, Muhammad Munsiff Kamarudin and Mimi Armiladiana Mohamad for their assistance, advice and encouragement.
PREVALENCE OF LAMENESS AND METABOLIC DISORDERS IN ENDURANCE HORSES

LAWAN A.1,3, NORANIZA M.A.1, RASEDEE A.2 AND BASHIR A.1
1 Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2 Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
3 Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri,
PMB1069, Borno State, Nigeria
Corresponding Author: drlawan3758@yahoo.com

ABSTRACT. This study was carried out to investigate the prevalence of equine lameness and metabolic disorders in endurance horses during an endurance race. Out of 67 horses that participated in the race, 19 horses completed the race successfully without any derangement while 48 horses were eliminated from the race for various disorders. Fifty-three (53.73%) percent of these horses had metabolic disorders and 17.91% were eliminated due to lameness. The study showed that the highest number of endurance horses that were eliminated were due to metabolic disorders followed by lameness. These findings may assist veterinarians in designing laudable measures in the management and conditioning protocols of endurance horses during training and further prevent the morbidity and mortality during endurance races.

Keywords: endurance, horse, metabolic disorders, lameness and morbidity.

INTRODUCTION

The prominent causes of lameness in endurance horses are associated to wear and tear injuries due to concussion and the additional loading of the joints and tendons by continues long distance races. However, the cumulative effects of long distance races, specifically over rough and hard, concussive surfaces, aggravated by conformational weaknesses, poorly made shoes and nutritional imbalances, can escalate the incidence of bone, joint and tendon injuries, especially as a horse ages. The occurrences of lameness are lowered in properly conditioned horses that have adapted and strengthened their musculo-skeletal structures to withstand the rigors of endurance races (Whitney et al., 1996).

On examination before, during and after races revealed an association of heart rate, cardiac recovery index, abnormal gastrointestinal sounds and gait with elimination of endurance horses from race during competition (Fielding et al., 2011).

Assessment of the fitness of a horse is by thorough physical examination of heart
rates, respiratory rates and conformation (Cottin et al., 2006; Bashir and Rasedee, 2009). The active muscles of endurance horses depend on heart size and capacity to deliver large volumes of blood to the tissues and the splenic reserves supply (Persson and Lydin, 1973; Lawan, et al., 2010). Endurance horses need calcium for muscle fiber activities and decreased plasma levels of calcium during strenuous endurance rides can cause metabolic disorders, including synchronous diaphragmatic flutter. However, increased blood calcium concentration is unwanted because it may increase the odds of thumps during endurance competitions (Lewis, 1995).

The most dehydrated horses are at greatest risk of developing metabolic problems and exhaustion (Harold, 2010). Once the intensity of race reaches a certain threshold, energy is partially provided by anaerobic metabolism. Consequently, lactic acid efflux, from cells to the blood occurs, and blood lactate increases (Snow and Valberg, 1994).

During endurance races, the primary mechanism for heat removal is the evaporation of sweat. The water from sweating is derived from both extracellular and intracellular fluids, and indicates a loss of over 15% of total body water. Sweat is hypertonic in comparison to plasma (Rose et al., 1980). Therefore, its production is followed by a loss of electrolytes. This change in fluid and electrolytes levels impairs performance ability, and may even be life-threatening (McConaghy, 1994). Lameness and metabolic derangements are the major causes of reduced performance in endurance horses. Therefore, this study was conducted to investigate the prevalence of equine lameness and metabolic disorders in endurance horses during an endurance race.

MATERIALS AND METHODS

Subjects

Sixty-seven endurance horses participated in endurance competitions of 40, 80 and 120 km racing category. Among these, 48 horses were eliminated and 19 horses completed the race successfully.

Veterinary inspection

Veterinary inspection was conducted after each loop of the race on all competing horses and physical parameters recorded. The physical parameters evaluated were the resting heart rate, cardiac recovery index (CRI), the gut sound, dehydration status, capillary refill time, color of mucous membrane, the muscle and anal tone and the gait soundness. All these parameters were re-evaluated and recorded each time the horses enter the vet-check after each loop of the race. The horses were also observed for soreness or injuries on the back, withers, girth area, body or distal extremities. Good performance horses continue the race in the subsequent loop, while poor performance horses are eliminated either due to metabolic ailments.
or due to lameness and are sent to the clinic for treatments and further workout.

Blood samples from the jugular vein were obtained from the eliminated horses with metabolic disturbances that are sent to the clinic for treatments and also from the good performance endurance horses using 21G needles in ethyldiaminotetra-acetic acid (EDTA) for whole blood analysis, lithium heparin for serum biochemistry. Other instruments were the hematocrit centrifuge machine to obtain plasma for biochemistry analysis, the hematocrit centrifuge for hemoglobin concentrations analysis, by (Hettich-Hematocrit 210 and micro hematocrit reader-Hawksley), spectrophotometer (UV/visible-Secomam-Anthelie Advanced) as well as the Automatic Hematology Analyzer (Abbot-cell Dyn 3700) for blood cells count. Descriptive statistic was used to analyse the results using statistical software JMP 9, SAS.

RESULT

Forty-eight (48) horses were eliminated from the endurance competition. Nineteen (19) horses managed to complete the race without metabolic signs. A total of 36 (53.73%) and 12 (17.91%) horses were eliminated due to metabolic disorders and lameness respectively as shown in Figure 1 and 2.

![Figure 1. Status of performance in endurance horses](image-url)
DISCUSSION

This study shows that up to 71.64% of the horse population in one of the endurance races in Malaysia were eliminated from the endurance race due to various derangements comprising of metabolic disorders and lameness. This may be due to the small number of horses exposed to inappropriate conditioning protocols that are continuously being circulated for endurance races.

The results show that approximately 28.36% of these horses managed to complete the races in good condition. Metabolic disorders seem to be the major contributory factor in elimination of horses from races. The metabolic disorders include high heart rates, dehydration, increase capillary refill time, severely congested mucus membrane and decrease gut motility. Endurance horses need calcium for muscle contractions. Low and very high plasma levels of calcium during strenuous endurance rides can lead to metabolic problems (Lewis, 1995).

The most dehydrated horses are at greatest risk of developing metabolic problems and exhaustion (Harold, 2010). When endurance race is prolonged in such situations, muscle strain may occur, giving way to muscle damage and myopathy (Arthur, 2005). Sweat production is accompanied by a loss of electrolytes. This change in fluid and electrolytes levels impairs performance ability, and may be life-threatening (McConaghy, 1994). The wear and tear injuries due to concussion and the additional loading of the joints and tendons by continues long distance races is one of the major causes of lameness during endurance ride (Whitney et al., 1996).
CONCLUSION

The study showed that the highest number of endurance horses that were eliminated were due to metabolic disorders followed by lameness. These findings may assist veterinarians in designing laudable measures in the management and of endurance horses during training and further prevent the morbidity and mortality during endurance races.

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ACKNOWLEDGEMENT. The authors appreciate the effort and assistance offered Mr. Mohamed Halmi Othman, Mr. Abdullah Misron, the staffs of Veterinary Teaching Hospital, Universiti Putra Malaysia especially Mr. Salehuddin and Drs Mohammad Fairuz Jamaluddin, Muhammad Munsiff Kamarudin and Mimi Armiladiana Mohamad for their assistance, advice and encouragement.
ABSTRACT. A number of studies have measured collagen fibers and collagen deposition in wound healing process with advances imaging techniques. However, these are performed by complicated methods and need specific tools. In search of the easier ways in routine histopathological laboratory, collagen measurement and staining pattern of wound healing process were observed in wounded skin of Sprague Dawley’s rat by using two different stains which are standard haematoxylin and eosin (H&E) and modified Masson’s trichrome staining (MT). The comparison between these staining in wounded tissues was made to evaluate the advantages and disadvantages of both staining in wound healing study for 21 days post-wounding. Tissues which stained with MT staining was then evaluated its collagen re-organization and density by using polarized light microscope with the aid of image analyzer software. Results showed that tissues stained with standard H&E could not be used to measure and differentiate the collagen deposition which is contradictory to MT staining. Wounded tissue stained with MT staining has showed a clear view of collagen fibers deposition and re-organisation compared to H&E staining. This finding could validate the using of modified MT staining which leads to accurate histopathological analysis and observation in wound healing study.

Keywords: wound healing, hematoxylin and eosin staining, Masson’s trichrome staining, collagen

INTRODUCTION

Histopathological study of wound healing process is normally used for evaluating the efficacy of pharmacological products which promote and accelerate dermal skin substitutes. The study is usually related to phases of cutaneous wound repairing which can be categorised into four phases such as homeostasis, inflammation (early and late), proliferation and remodeling phases. In histopathological study of wound healing, a number of criteria are considered to determine the level of histopathological change such as the depth and length of healed wound, epithelial stratification, leucocytes and macrophage infiltration, fibroblast, extent of elastin formation and...
the most important is collagen fiber as it plays a dominant role in maintaining the structural integrity of wound healing (Ukong et al., 2008).

However, by using a conventional staining method such as haematoxylin and eosin (H&E), the study of wound healing becomes more challenging as the stain is not able to differentiate important histopathological change in the wound healing process such as collagen deposition and scab formation which could later lead to misinterpretation in histopathological observations. Many studies have attempted to quantify the amount of collagen change and orientation in any stage of wound healing such as by using the epipolarisation microscope with picrosirius red-stained (Noorlander et al., 2002), computer vision analysis of collagen fiber bundles (Elbischger et al., 2005), Fourier transform infrared (FTIR) spectral imaging (Potter et al., 2001), and laser scanning confocal microscopy (Taylor et al., 2002). There was also a study made by Dallon et al., (2006) which used a mathematical approach and equation model to evaluate the alignment and arrangement of collagen fibers in wound healing process; however this method is difficult to be understood and interpreted by non-mathematicians. Conversely, all these methods and protocols are complicated steps which require some special technicians or equipment in the routine of histopathological laboratory.

To overcome this problem, an alternative staining such as modified Masson’s trichrome staining (MT) can be used in the histopathological study of wound healing. Differing from H&E staining the MT staining is able to differentiate clearly the important morphological keys for wound healing assessment such as keratin, haemoglobin, and muscle fiber (red colour), cytoplasm and adipose cells (light red or pink), cell nuclei (dark brown to black) and collagen fiber which stained blue in colour and later could be measured by using imaging analysis software. Clear differentiation of morphological and anatomical structure in the stained skin tissue are advantageous and provide further understanding in histopathological study of wound healing in future.

MATERIALS AND METHODS

Animals

Eighteen clinically healthy Sprague Dawley’s female rats weighing between 200 to 250 g were obtained from the Animal Laboratory of Universiti Sains Malaysia, Kubang Kerian, Kelantan for wound healing study. All animals were housed in standard environmental conditions with temperature of 25 ± 1°C with 12 hours light and 12 hours dark cycle. They were acclimatised to a hygienic laboratory condition for 7 days before the start of experiment and observed for any clinical sign such as diarrhoea, food and water intake, behavior and blood in urine (Tuffery, 1995). Animals were fed with standard commercial pellet diet (10% of
their body weight) and distilled water ad libitum.

**Experimental design for wound healing study**

A total of 18 clinically healthy male white rats were used in the wound healing study and divided into three groups with six rats per group for different interval days of 7, 14 and 21 of post-wounding. All animals in each group was anaesthetised with light ether prior to the wound creation and 70% of alcohol was applied as topical disinfection on a shaved area at the dorsal thoracic region. An 8 mm diameter of full thickness wound was created by using sterile wound biopsy punch. Each animal was wounded on the dorsal part individually to represent a duplicate. At the end of the interval days, samples of the skin were harvested and processed for histology examination with three samples of wounded skin from each group was subjected to Masson’s trichrome staining and another three rats were subjected to standard haematoxylin and eosin staining. The wounds were measured based on the percentage according to the healed wound area. The epithelisation time was measured from initial day (Singhai et al., 2006).

Percentage of wound contraction

\[
\text{Healed area (mm}^2\text{)} = \frac{\text{Healed area (mm}^2\text{)}}{\text{Total wound area (mm}^2\text{)}} \times 100
\]

**Histopathological studies**

Skin specimens for Masson’s trichrome staining was fixed in Bouin’s solution, meanwhile for standard haematoxylin and eosin staining the skin specimens was fixed in normal 10% buffered formalin. All the epithelial tissues which subjected to these staining were assessed under light microscope to evaluate fibroblast proliferation, collagen formation and re-epithelization and wound healing processes (Reddy et al., 2007).

**Haematoxylin and Eosin (H&E) Staining**

Slides were placed in staining jar and deparaffinized by submerging into three series of absolute xylene for 4 minutes followed by 100%, 100%, 95%, 90%, and 70% of ethanol for 4 minutes of each percentage. Next, slides were washed in running tap water for 2 minutes. Then, slides were submerged into Harris Hematoxylin (Sigma-Aldrich, GERMANY) for 2 minutes and then washed in running tap water for 2 minutes. The slides then were submerged into 1% acid alcohol for 3 dips to decolorize it and washed in running tap water for 2 minutes. The slides then were submerged into 2% potassium acetate for 3 minutes and again washed in running tap water for 2 minutes. After that, slides were submerged into Eosin for 2 minutes followed by washing in running tap water for 2 minutes. Stained slides were dried for 24 hours at 38°C. Before observation,
slides were dipped into absolute xylene for 1 minute and finally mounted with cover slip using DPX mounting.

**Collagen Special Stain (Modified Masson’s Trichrome Staining)**

Method was modified from Kiernan (2008). Granulation skin tissue slides were placed in staining jar and deparaffinised by submerging into three series of absolute xylene for 4 minutes each followed by 100%, 95%, 90%, 80% and 70% of ethanol for 4 minutes in each percentage. The slides then were submerged in warmed Bouin’s solution at 60°C for 45 minutes. Next, the slides were washed in running tap water until yellow colour in samples disappeared. To differentiate nuclei, slides then were immersed in modified Weigert’s haematoxylin for 8 minutes, after that washed in running water for 2 minutes. In order to stain cytoplasms and erythrocytes, slides were submerged in anionic dyes, acid fuschin (C.I. 42590, Merck, Germany) for 5 minutes; then again slides were washed with running tap water for 2 minutes. Next, slides were treated with phosphomolybdiacid solution for another 10 minutes as a mordant and immediately slides were submerged into methyl blue (C.I. 42780, Merck, Germany) solution for 5 minutes in order to stain fibroblast and collagen. After that, slides were washed in running water for 2 minutes and lastly treated with 1% acetic acid solution for 1 minute. Slides then were dehydrated into a series of alcohol of 70%, 80%, 95% and 100% for 1 minutes each percentage. Before observation, slides were dipped into absolute xylene for 1 minute and finally mounted with cover slip using DPX mounting.

**Collagen Density Evaluation**

Method was modified from Elizabeth et al., (1995) and Ukong et al., (2008). The slides stained with Masson’s trichrome stain were examined using polarised light microscope (Leica, Germany) and with the aid of a software image analyser (Video Test-Master 4.0 software), measurements were made at the intensity of blue colour which represent the collagen density. Collagen density was measured under the wound area compared to normal dermis at 100× magnification. The mean of the collagen values obtained for the normal dermis was accepted as the equivalent of 100. For each group, the mean of the collagen density under wound area was expressed in the ratio of percentage compared to collagen density of normal dermis during the post-wounding day.

\[
\text{Ratio} = \frac{\text{Average collagen intensity under wound}}{\text{Average collagen intensity of normal dermis}} \times 100
\]
RESULTS

Gross observation and wound contraction

The normal healing of full thickness wound has showed that at day 1 of post wounding (Figure 1), blood was accumulated at the wound area and followed by swelling which showed the inflammatory phase. At day 7, the blood became dry and turned into scab which initiates the proliferative phase of normal wound healing. The scab was detached from the skin on day 11 as the wound contraction become higher. Scar was formed on day 21 of normal wound healing which lead to remodeling phase.

Histological Analysis

Histological analysis showed the wounded skin stained with standard haematoxylin

![Figure 1. Normal healing of full thickness wound from day 1 to day 21 post-wounding in rats.](image1)

![Figure 2. Photomicrograph of the normal wounded skin tissue stained with standard haematoxylin and eosin, 40×. Note that poor visible of fine and coarse collagen fiber and its arrangement throughout the wound healing processes. CCF: Coarse Collagen Fibers, FCF: Fine Collagen Fibers, HF: Hair Follicles, SC: Scab](image2)
and eosin has poor tissue reconstruction and collagen deposition toward the wound area. Skin stained with H&E only showed the angiogenesis and scab formation with less visible of other organelles and structure such as fine and coarse collagen fibers (Figure 2). However, by using the modified Masson’s trichrome staining, it showed clear visible fine and coarse collagen deposition and its arrangement in the wounded skin together with visible differentiation of angiogenesis, scab formation, collagen fibers, adipose tissue and hair follicle (Figure 3). Even though the gross observation showed complete epithelisation at day 17 (Figure 1), the collagen was still less deposited (97.82%) in the wounded skin even at day 21 post-wounding as presented in Table 1.

**Collagen Density Evaluation in Wound**

Computerised quantification of collagen deposition in normal wounded tissue stained with Masson’s trichrome staining evaluated that collagen deposition and density was significantly (p<0.05) different for every week of post-wounding as presented in Table 1. Even though the gross observation showed complete epithelisation at day 17 (Figure 1), the collagen was less deposited (97.82%) in the wounded skin even at day 21 post-wounding as presented in Table 1.

**DISCUSSION**

In histopathological study of wound healing, various characteristics are considered to determine the stage of histopathological change such as the depth and length of healed wound, epithelialisation period, white blood cells infiltration, elastin...

---

**Figure 3.** Photomicrograph of the normal wounded skin tissue stained with modified Masson’s Trichrome staining, 40×. Note that clear visible and differentiation of fine and coarse collagen fiber and its arrangement, scab formation and angiogenesis throughout the wound healing processes. **CCF:** Coarse Collagen Fibers, **FCF:** Fine Collagen Fibers, **HF:** Hair Follicles, **SC:** Scab
formation, fibroblast aggregation and the most important is collagen fiber as it plays a dominant role in preserving the anatomic integrity of wound healing. When tissues are disrupted following injury, collagen is needed to repair the defect in order to restore anatomic structure and function. This unique protein with three alpha chains that intertwine into a triple helix is very important in all stages of the wound healing process as it provides strength and integrity to all tissues (Mayer & Willemsteijin, 2008). For example, during the proliferative phase of the wound healing mechanism, secretion of collagen subtype within the injury site increases to replace necrotic tissue (Kondo, 2007). Meanwhile in the remodeling phase of the wound healing process, the collagen is cross-linked into a more organised structure to produce greater wound tensile strength (Enoch and Leaper, 2007).

Fibroblast fibers is a connective tissue cell which is responsible for production and synthesis of the collagen protein in skin (Diegelmann and Evans, 2004). It would be advantageous if the collagen fibers could be evaluated or measured in order to deeply understand how the collagen is synthesised and re-organised in the wound healing process. As mentioned, plenty of studies has been done to measure the collagen in wound healing, but somehow all the methods used were not practically applied to some of histopathological laboratory as it needed special equipment and protocols. The most used method by researchers all around the world in histopathological study of wound healing process is by using the standard haematoxylin and eosin (H&E) staining. This standard staining could only give a basic study of anatomical and morphological changes in the wounded tissues but not for the collagen re-organisation and deposition.

This study attempted to develop and modify the usage of a known special stain that is the Masson’s trichrome (MT) staining in wound healing. The MT staining is widely used in medical pathology laboratories to differentiate between collagen and smooth muscle in tumours, determine the increase of collagen in disease such as cirrhosis and it is also a routine stain for liver and kidney (Sheehan and Hrapcahk, 1980). However, there has been not many studies of using MT staining in wound healing. The results

<table>
<thead>
<tr>
<th>Group</th>
<th>Absolute Average Values</th>
<th>Normal Dermis Collagen Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>Control</td>
<td>202.64 ± 27.8 (80.44%)*</td>
<td>244.96 ± 2.04 (97.24%)*</td>
</tr>
</tbody>
</table>

n=18 values are expressed in means ± S.D.  *p<0.05 statistically difference in comparison between each day post-wounding.
of this study showed that wounded skin stained with MT provides an understanding of the wound healing process as it illustrates the migration and re-organisation of collagen fibers in the healed skin at every post-wound stage (Figure 3). By using the MT staining we can differentiate the fine and coarse collagen fiber (Figure 3) which usually appear in the remodeling phase of wound healing that influence scar formation. Collagen remodeling and degradation occurs simultaneously to provide the tensile strength and reduce scar formation (Enoch and Leaper, 2007). Other than collagen, MT staining also helps histopathologists differentiate other anatomical structures and organelles in the healed skin such as scab, fine and coarse collagen fibers, hair follicle and adipose tissue (Noorlander et al., 2002).

The difference between H&E and MT staining were more related to the steps and dyes used in the staining preparation. Standard H&E staining involves two types of dyes which are haematoxylin and eosin Y. This staining method involves application of haemalum, which is a complex formed from aluminium ions and oxidised haematoxylin. The dye stains nucleus of cells and a few other objects, such as keratohyalin granules dark blue or purple in colour. The nuclear staining is followed by counterstaining with an aqueous or alcoholic solution of eosin Y, which colours eosinophilic and other structures in various shades of red, pink and orange (Junqueira and Carneiro, 2007).

However, the modified MT staining involves three colours of staining dyes, as the name implies, “trichrome”. The principle of trichrome staining is that the less porous tissues are coloured by the smallest dye molecule and followed by the larger molecule (Sheehan and Hrapcak, 1980). Theoretically, most of the stains are based on the attraction of the opposite charges in the tissues to the dyes applied. In MT staining the sections are first stained with an acidic dye such as acid Fucshin, C.I. 42590. In this step, all acidophilic tissue elements such as cytoplasm, muscle and collagen will bind to the acid dyes. The section is then treated with phosphomolybic acid to decolourise the collagen but not to the cytoplasm. The decolorised collagen is then stained with fiber stain such as methyl blue, anilline blue or fast green dye (Masson, 1929). The blue colour of collagen can be enhanced by preliminary treatment of the section in hot Bouin’s solution which is absent in the H&E staining protocol. The blue colour intensity of the collagen can be measured by a computerised imaging analyser with the aid of software. Several studies used this computerised method to measure collagen content in order to quantify dermal wound recovering for application to pharmacological products such as toxicity and efficacy tests in wound healing (Truong et al., 2005; Bae et al., 2005 and Rao et al., 2007).

The abilities of modified Masson’s trichrome (MT) staining to differentiate the collagen fibers in skin tissue gives an
alternative method for histopathologists to have a better understanding of the wound healing process as it could give clearly visible differentiations of morphological and anatomical structures of the skin. Moreover, by using this special staining, collagen fibers which were not differentiable using haematoxylin and eosin (H&E) staining could be clearly seen using MT staining represented by its blue colour.

CONCLUSION

Collagen measurement and arrangement is very essential in wound healing studies as the collagen restores the integrity of the skin. Alternative staining methods such as the modified Masson’s trichrome staining and the use of computerised software to measure the collagen colour intensity helps histopathologists make accurate interpretations and have a better understanding of the histopathological study of wound healing which is absent in standard haematoxylin and eosin staining.

REFERENCES

ABSTRACT. A biogas plant is an anaerobic digester that produces biogas from organic materials such as animal waste, sewage slurry, vegetable waste and others. The Department of Veterinary Services had developed a small-scale biogas plant at a commercial dairy farm in Semenyih, Selangor. The objectives of this project were to promote green technology and zero waste concepts on animal farming as well as to mitigate adverse effects on the environment due to unsystematic management of animal waste disposal. The biogas produced was used as fuel for biogas lamp, biogas stove, biogas water heater, biogas rice cooker and 1 kW biogas generator. The generator was connected to the distribution board in order to supply electricity to a few switch sockets and lighting system for farm use. A biogas plant in an animal farm is one of the green technology applications because it not only produces renewable gas and generates electricity but also minimises greenhouse gas emissions and environmental pollution. Furthermore, the residual solid waste produced at the end of the process can be dried to be used as organic fertiliser.

Keywords: biogas, biogas plant, animal waste, green technology

INTRODUCTION

The livestock industry is a part of the agricultural sector which is a growth sustaining industry in Malaysia. However, the unsystematic management of animal waste disposal is one of the causes of the greenhouse effect and serious environmental pollution especially smells and water pollution. Therefore, the initiative undertaken to mitigate its adverse effects to the environment is by the application of biogas technology for livestock farms in Malaysia. Biogas is a source of green technology, a renewable energy and has high potential to be expanded. Besides, biogas is an inexpensive source of energy compared to other sources like petrol, diesel and coal (Aashish, 2002). However, the implementation of biogas plants is still in its infancy among farmers in Malaysia due to high costs of investment in the construction of the plant and lack of knowledge related to biogas technology.

Generally, a biogas plant is built for the purpose of producing biogas as fuel for cooking, fuel for vehicle and to generate electricity and heat. The production of biogas at each plant depends on the amount of waste materials available for processing and the capacity of the biogas digester.
Biogas can be produced from anaerobic digestion of biological waste by anaerobic bacteria with the absence of oxygen inside the biogas digester.

The composition of biogas is shown in Table 1. The major composition in biogas is methane at 50-70% followed by carbon dioxide at 30-50% while nitrogen, hydrogen, hydrogen sulfide and water vapour are other compositions found in small amounts (Biogas China, 2006). Methane is a colourless and odourless gas but it is 21 times more harmful than carbon dioxide. The uncontrolled emission of methane gas tends to trap heat in the atmosphere and lead to the greenhouse effect or global warming. (Agung and Tekun, 2005). Therefore, the implementation of a biogas plant in an animal farm is one of the initiatives taken by the Department of Veterinary Services to mitigate adverse effect on the environment.

**DESIGN AND CONSTRUCTION**

Biogas plants can be designed and constructed as big or small units as required depending on the amount of waste available and the amount of gas needed. (Jatinder K. and Sarbjit, 2003). Figure 1 shows the basic diagram of a biogas plant that has been constructed at a commercial dairy farm in Semenyih, Selangor. The biogas digester was designed and erected based on the technology acquired from Germany. A 50 m³ concrete biogas digester was constructed below ground with a diameter of 6 meters and height of 2 meters. There are numerous materials that can be used to construct the biogas digester, namely bricks and cement, stainless steel and fiberglass, whereby each of them have their advantages and disadvantages in terms of price and quality. Basically, a biogas plant consists from the same principle components which are biogas digester, gas holder or dome, collection sump, inlet chamber, outlet chamber and storage balloon as shown in Figure 2, Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7 respectively. Technically, each part has its function in the operation of the plant.

The participating commercial dairy farm has 150 heads of cattle including 45 young cattle. In initiating the operation of the biogas plant, the dairy farm was

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Formula</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>CH₄</td>
<td>50-70</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>CO₂</td>
<td>30-50</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>N₂</td>
<td>0-2</td>
</tr>
<tr>
<td>Hydrogen Sulfide</td>
<td>H₂S</td>
<td>0-1</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>H₂</td>
<td>0-1</td>
</tr>
<tr>
<td>Water Vapor</td>
<td>H₂O</td>
<td>0-1</td>
</tr>
</tbody>
</table>
Figure 1. Basic diagram of a biogas plant

Figure 2. The construction of below ground biogas digester

Figure 3. Biogas holder or dome

Figure 4. Collection sump

Figure 5. Inlet chamber
cleaned twice a day. The slurry was then channeled directly from the cow shed into the collection sump through an underground drainage piping system. A bar screen was installed at the collection sump to prevent the unwanted impurities from entering the inlet chamber that may clog the pump. After that the slurry was channeled into the inlet chamber from the collection sump and left to settle for a specific time until the sediment slurry formed at the base of the inlet chamber. The slurry was pumped into the biogas digester once daily until the biogas digester reached its capacity. The slurry was pumped from the top of the biogas digester in order to avoid the formation of a hard crust that will disturb the process of producing biogas and possibly trap biogas in the slurry. This technique is quite significant because the maintenance cost of the biogas digester can be reduced if there is no formation of a hard crust. This minimises capital cost and the use of electricity as there is no need for a stirrer to operate the plant.

The biogas digester with a volume of 50 m$^3$ was filled with slurry and it was left to ferment for at least 3 weeks to produce optimal high quality biogas. The temperature of the biogas digester must be maintained between 35°C to 40°C so that the anaerobic digestion process can progress to completion (Al Seadi et al., 2009). The biogas produced was accumulated at the gas holder or dome before going through 3 stages of filtration processes to remove water vapour and

![Outlet chamber](image)

**Figure 6.** Outlet chamber

![Storage balloon](image)

**Figure 7.** Storage balloon
hydrogen sulfide which is corrosive and other impurities that exist as constituents in biogas by using water, iron oxide and activated carbon respectively. The remaining gas was subsequently stored in the storage balloon. Since the anaerobic digestion process runs continuously inside the biogas digester, a pressure relief device was installed to prevent gas explosion caused by over pressurisation of the storage balloon.

The biogas produced was then available to be routed directly to the biogas rice cooker, biogas lamp, biogas water heater, biogas stove and 1 kW biogas generator. All biogas appliances are slightly different from normal appliances. The amount of gas supplied to biogas appliances can be controlled by using a control valve. The generator with the power capacity of 1 kW was connected to the distribution board that will supply electricity to a lighting system and switch sockets for farm use. Hence the farmers can save money on electric bills. Moreover, the use of methane gas for cooking produces very little odour and smoke, consequently reducing smell and air pollution. Finally, at the end of the anaerobic digestion process, the effluent produced was channeled into the outlet chamber and it can be dried and processed to be used as an organic fertiliser.

**SELECTION OF SIZE OF BIOGAS PLANT**

The Semenyih biogas plant was implemented for the purpose of producing biogas for cooking and generating electricity. It is one of the ways which could help save the environment from serious pollution and climate change. Methane is a greenhouse gas that is more damaging than carbon dioxide. Thus, the implementation of biogas plants is one of the methods in order to save the environment from serious pollution and preventing climate change.

The size (capacity) of a biogas plant is the quantity of biogas (m³), which it can supply 24-hourly. From literature reviews, one adult cattle produces about 25-50 kg of manure depending on their body weight and each ton of fresh cattle manure can yield 32 m³ of biogas (SP Multitech). A cubic meter of biogas can generate 100 W of electrical power and produce 2.4 kWh of electrical energy per day. Thus, the farmers can estimate the size and capacity required in order to build a biogas plant within their farms. Table 2 shows the correlation between the number of cows to

<table>
<thead>
<tr>
<th>Number of cows</th>
<th>Electrical Power Potential (kW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>400</td>
<td>80</td>
</tr>
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</table>

**Table 2. Electrical power potential**
the electrical power potential that can be produced for a cattle farm.

Even though there are a lot of biogas plants built in a few countries recently, the manure digester technology especially in livestock industry is still in its infancy in Malaysia. Moreover, it is very expensive and the payback period takes around two to ten years based on the size and capacity of the biogas digester. The construction cost of implementing biogas plant depends on the farm size, amount of animal waste available, location, management and energy needs. However, there are a lot of advantages in the implementation of the biogas plant because it is not only producing gas as cooking fuel and to generate electricity, but also can minimise manure odour and greenhouse emissions, improve air and water quality, save the cost of disposing of the waste and save fuel purchase like Liquefied Petroleum Gas (LPG). In addition, at the end of the process of producing biogas, the residual slurry can be processed to be used as organic fertiliser (Al Seadi et al., 2009).

CONCLUSION

In conclusion, biogas is an excellent source of energy and the implementation of biogas plant is an alternative method in order to mitigate environmental pollution and global warming due to unsystematic management of animal waste disposal. Furthermore, there are a lot of advantages of implementing biogas plant at animal farms. For instance, it can open rural economics with the incorporation of green technology, relieves cost through lesser dependence on paying for non-renewable fuels, save on electricity bills due to the production of electricity and circulation of knowledge and skills. The simplicity of implementing a biogas plant in an animal farm, makes it one of the most environmentally sound energy sources especially for rural needs.

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PIG GROWTH PERFORMANCE DATA USING THE LOUDONG BIO-FERMENTATION WASTE TREATMENT TECHNOLOGY IN CLOSED HOUSE SYSTEM

HELEN M.¹, KHOO C.K.¹, KHOR S.K.², YEOH N.N.², LIM Y.S.¹, SYED HUSSEIN S.A.¹, CHUI I.³ AND ABU HASSAN M.A.¹

¹ Department of Veterinary Services, Wisma Tani, Blok Podium, Lot 4G1, Precinct 4, 62630 Putrajaya
² Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak
³ ICW Biotechnology, No 15, Jalan 36A, Kampung Cheras Baru 56100 Kuala Lumpur
Corresponding author: helen@dvs.gov.my

ABSTRACT. A trial and pig performance data collection was conducted to evaluate the efficiency of Luodong Bio-Fermentation Treatment Technology with zero discharge effect in a pig close house system at Tanjung Sepat, Selangor. This trial was done to gather and monitor the pig growth performance and the pig acceptance of using the Loudong Bio-fermentation Waste Treatment Technology in close house environment. Growth performance of the pigs were monitored by an average daily gain, feed conversion ratio, behaviour and veterinary observations. Close house temperature, moisture measurement and bedding sampling were also conducted.

Keywords: pig, growth performance, waste treatment, closed house system, zero discharge, feed conversion ratio (FCR)

INTRODUCTION

The pig industry has been known to cause environmental pollution especially air and water pollution. Therefore, new technologies are developed which are more environmentally friendly and effective to address the problem arising from the pig farming area. The technology of using microorganism Fermentation Bedding in pig farming has been studied in Malaysia since the 1980s (Choo, 1988; Ong, 1988; Teoh, 1988) because of the importance of the waterway pollution and social problem. Common raw material used in the bedding include saw dust, rice straw and rice husk. These bedding material is mixed with useful microorganism to facilitate the breakdown of waste in the pig manure thus no washing is required to clean the pig and the pig pens. This subsequently will reduce water usage in the farming system and reduce environmental pollution since less effluent is being released to the common drainage.

The Loudong Bio-fermentation Waste Treatment Technology is a technology that uses microorganism and enzymes in their product which includes Bacillus natto, yeast, Amylase, Protease and other probiotics in the feed and bedding. This technology was developed in Japan by the Japan Rakuto Kasei Chemical Industry Co. This product can be mixed in the bedding and also fed
to the animal to improve food digestion, nutrient absorption and reduce odour and ammonia emission from pig manure (as claimed by the manufacturer). This system operates only in a closed house system. The whole waste treatment system consists of the closed house system, bedding and the Luodong enzymes. In order to evaluate the efficiency of this system, data collection of the pig performance using the Luodong Bio-Fermentation Treatment Technology in a close house system with zero discharge effect was proposed. This trial was done to monitor pig growth performance and pig acceptance of using the Loudong Bio-fermentation Waste Treatment Technology in the closed house farming system in Malaysia.

MATERIALS AND METHODS

A total of 40 weaners (17 males and 23 females) of Large White cross-breeds were allotted in a closed house farming system in Tanjung Sepat, Selangor (Figure 1). The experimental closed house used in this trial was situated within an open house pig farming system. This trial was done from weaners age (day 60) to market age (day 180). Weighing was done 3 times during the trial at age 60 days, 120 days and 180 days. The feed was mixed with Loudong enzyme at 1% for below 35 kg body, 1.5% for 35 kg and above and 2% for above 65kg body weight. Drinking water was given ad lib. Nipple drinkers were provided inside the pen with cemented collection base under them in order to avoid wetting the bedding. The feed was self-mixed in the farm, supplied by the farm owner and the feed used was the Grower feed and Finisher feed. Feed consumption was monitored for grower and finisher. Feeding was done ad lib at the beginning and then restricted after the weaners showed signs of the effect due to overfeeding. The feeding was done twice daily, once in the morning at 8 am and once in the afternoon at 4 pm.

The temperature and moisture inside the house were monitored and recorded 3 times daily at 9 am, 12:30 pm and 4 pm. The bio-fermentation bedding used was made from 50% rice husk and 50% sawdust at 0.6 m thickness where the rice bran and sawdust were at 5-7 kg/m³ and Loudong enzyme at 200-500 mg/m³ (Figure 2). The floor litter size for this study was 28.24 m × 3.1 m or 2.18 m² per pig (Figure 3). The bedding material was wetted with normal water every 2 days depending on the condition of the bedding. The bedding was turned and mixed every 2 weeks with a mini excavator (Figure 4). Random sampling of the bedding material was carried out at day 45 and 105. The bedding material was sent for laboratory testing of bacterial culture and identification for pathogenic bacteria.

Behaviour and veterinary observations of the growing-finishing pigs were carried out from day one of loading to marketable age and weight. Mortality rate and any clinical symptoms were observed and reported.
Figure 1: Closed House System in Tanjung Sepat, Selangor

Figure 2: The bedding material used

Figure 3: Weaners on the Bedding Material
Figure 4: Mini excavator used to mix and turn the bedding

Figure 5: Means weight (kg) distribution of the male and female pigs according to age (days)

Table 1: Weight of the pigs

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of animals</td>
<td>Total weight (kg)</td>
<td>Mean weight (kg)</td>
<td>No. of animals</td>
</tr>
<tr>
<td>60 days</td>
<td>17</td>
<td>245</td>
<td>14.41</td>
<td>23</td>
</tr>
<tr>
<td>120 days</td>
<td>15</td>
<td>531</td>
<td>35.40</td>
<td>21</td>
</tr>
<tr>
<td>180 days</td>
<td>15</td>
<td>1320</td>
<td>88.0</td>
<td>21</td>
</tr>
</tbody>
</table>
RESULTS

Growth and Mortality

At the growing stage, 4 (2 male and 2 female) out of 40 (10% mortality) experimental pigs died. Three (3) died due to overfeeding – pigs were fed *ad lib* in the early stage and then switched to restrict feeding after many of the piglets had diarrhoea and soft stool, 1 died due to suspected Swine Erysipelas however no post-mortem was conducted as the body was already disposed off.

From Table 1, the female and male pigs were almost at the same weight at the first and second weighing but at the final weighing (day 180) the females were slightly heavier than the male pigs. The weight of the piglets was better after day 120 as shown in Figure 5. The feed conversion ratio (FCR) was higher during the growing stage (day 60 -120) which was 3.32 and lower during the finishing stage (day 120 to 180) which was 2.11 (Table 2).

Close House Temperature, Moisture and the Bedding Material

The temperature and moisture reading inside the closed house was taken 3 times daily at 9 am, 3 pm and 6:30 pm. The 9 am temperature ranged between 25.3°C to 30.3 °C. The 3 pm temperature ranged between 29.3°C to 34.1°C and the 6:30 pm ranged between 28.5°C to 31.5°C. The 9 am moisture reading ranged between 56% to 78%. The 3 pm moisture reading ranged between 68% to 90% and the 6:30 pm

Table 2: Growth Performance

<table>
<thead>
<tr>
<th></th>
<th>Grower (60-120 days)</th>
<th>Finisher (120-180days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of pigs</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Average Daily gain, g</td>
<td>590</td>
<td>494</td>
</tr>
<tr>
<td>Average Daily feed, kg</td>
<td>1.96</td>
<td>1.04</td>
</tr>
<tr>
<td>Feed Conversion Ratio (feed/gain)</td>
<td>3.32</td>
<td>2.11</td>
</tr>
</tbody>
</table>

Table 3: Result of bacteria isolation and identification from bedding material sampled at Day 45 and Day 105 post loading of weaners

<table>
<thead>
<tr>
<th>Day (post-loading of weaners)</th>
<th>Organisms from bedding material sampled at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface</td>
</tr>
</tbody>
</table>
moisture reading ranged between 63% to 78%. There were few technical problems that contributed to the high temperature and fluctuation of moisture reading in the closed house. This included malfunction of one of the exhaust fans, cooling curtain and the water pump.

The bedding material was taken at three levels, the outer surface, middle part and the bottom part of the bedding and also near the nipple drinker. The samples were sent for bacteria isolation and identification. Two sampling was done at day 45 and day 105 post-loading of weaners. Bacillus sp. and *E. coli* were the common bacteria isolated from the bedding (Table 3).

**Behaviour and Veterinary Observations of the Growing-Finishing Pigs**

In the first day of loading the weaners into the bio-fermented pen, they were not given food in the morning and afternoon, and most of them were sleeping on the concrete flooring near the feeding trough. Four (4) of them were heard coughing and most of the others were seen scratching their body due to the irritation made by the bedding materials. On day 4 post-loading, more piglets were coughing and one of them had diarrhoea. By day 9, the majority of the pigs had diarrhoea. They were treated with Clindamycin antibiotic (Clinda-plus®) for 3 days. The mortality case was recorded at day 18 (2 deaths with clinical signs of generalised weakness, coughing and diarrhoea), day 34 (1 death with clinical signs of generalised paralysis) and day 39 (1 death with clinical signs of generalised weakness, coughing and diarrhoea). No pig with severe skin problem was noticed during the whole trial.

**DISCUSSION**

When the pigs were just introduced to a different environment and management system, the adaptation process is very critical. After weaning (day 60 to 120) the pigs showed a much higher FCR as there were many factors that contributed to the result. As at the beginning of the trial, there were a lot of technical problems in the closed house system that caused the difficulty in maintaining or stabilising the temperature and moisture required. The fluctuation of the temperature and moisture in the closed house could be one of the factors that triggered stress on the piglets. The piglets were not used to the bedding material as from a day old to day 60 the piglets were living on cemented flooring with the sows. This explained the behaviour of the piglets munching and playing with the bedding material on the first week after loading onto the bio-fermented bedding. The dust, heat and the bedding material could have been the cause of coughing and diarrhoea at the growing stage but once the piglets were used to the different environment and improved management, the piglets progressed well and the FCR reduced to 2.11 at day 180. At day 180, both male and female pigs mean weight was still below the marketable weight (110 kg). The timing for the temperature and moisture reading was
not at the optimum (which was suppose to be at 8 am, 1 pm and 8 pm) but due to technical support constraints, this reading could not be performed.

There were no pathogenic bacteria isolated from the bedding material. The most common bacteria isolated from the bedding were *Bacillus* sp. as they were the main microorganism in the Loudong Bio-fermentation Waste Treatment Technology product. *E. coli* was the anaerobic bacteria commonly found in the manure and the gastro-intestinal tract. Since the experimental closed house was situated in the open housed pig farming area, the odour emission from the pig manure could not be appreciated. There were fewer odours in the house due to the usage of the exhaust fans and cooling pads but the odour in the surrounding area could not be determined. This trial should be supported with a more proper experimental design and location for better scientific comparison. The cost of production should be determined so that the farmers will not get discourage from using the Luodong Bio-Fermentation Treatment Technology in a close house system with zero discharge effect as one of the alternatives to control environmental pollution especially air and water pollution.

**CONCLUSION**

The behaviour of the pigs and herd health related to the bio-fermented bedding were greatly influenced by the management and adaptation of the pigs itself. The technical problem which occurred during the trial should be solved before starting to prevent negative effects to trial.

**REFERENCES**


ABSTRACT. This paper reports on the laboratory animal population in the Laboratory Animal Unit, Veterinary Research Institute (VRI), Ipoh from 2005 to 2010. Laboratory Animal Unit is a complementary unit which serves to supply laboratory animals to all diagnostic and research units in VRI. The objective of this unit is to breed and produce laboratory animals such as rabbits, mice, rats, guinea pigs and hamsters for research projects as well as diagnostic and biological activities. The unit also supplies laboratory animals to private agencies, universities and schools. There are 6 species of laboratory animals in VRI namely rabbit (New Zealand Breed), guinea pig (Hartlay Strain), hamster (Golden Syrian), white mice (Swiss Albino), BALB/c mice and Sprague Dawley Rat. Management of the animals includes breeding, fertility, production and disease monitoring. Generally, white mice bred well and had a high population from 2005 to 2010 compared to the other laboratory animals due to their short gestation period and large litter size. With the higher current demand for laboratory animals, the production of the unit is expected to increase.

Keywords: laboratory animal, population, production

INTRODUCTION

The Laboratory Animal unit in VRI functions as a complementary unit to produce laboratory animals for research purposes. There are 6 species of laboratory animals namely white mice (Swiss Albino), guinea pig (Hartlay Strain) and rabbit (New Zealand White), hamster (Golden Syrian), Sprague Dawley rat and BALB/c mice. This paper reports the general management and population of laboratory animals in VRI from 2005 to 2010. Data for this purpose was obtained from detailed daily and weekly records on management, feeding, breeding and production parameter of the various species. The annual population data is compiled based on the number of animals produced (born) plus total stock of laboratory animals in the past year.

Management of parent stock is of high priority to produce quality stock. Due to this, systematic feeding and cleaning of facilities with good sanitation is of utmost importance. The frequency and quantity of feed given to the animals is shown in Table 1. The feed given to all the species is
Table 1: Feeding and watering for laboratory animals in VRI, Ipoh.

<table>
<thead>
<tr>
<th>Species</th>
<th>Feeding rate</th>
<th>Watering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>40 g feed / 2 times per day + fresh vegetable</td>
<td>Ad lib</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>35 g feed / 2 times per day + fresh vegetable</td>
<td>Ad lib</td>
</tr>
<tr>
<td>Hamster</td>
<td>7 g / 2 times per day</td>
<td>Ad lib</td>
</tr>
<tr>
<td>White mice</td>
<td>6 g / 2 times per day</td>
<td>Ad lib</td>
</tr>
<tr>
<td>BALB/c mice</td>
<td>5-6 g / 2 times per day</td>
<td>Ad lib</td>
</tr>
<tr>
<td>Sprague Dawley rat</td>
<td>12 g / 2 times per day</td>
<td>Ad lib</td>
</tr>
</tbody>
</table>

a commercial feed pellet obtained locally and water is given *ad lib*. Fresh vegetables are also given to rabbits and guinea pigs as a feed supplement and as a source of Vitamin C.

Regular cleaning and changing of bedding of the laboratory animals is conducted as shown in Table 2. Their steel cages are washed once a week with disinfectant (Trigene 1%), air dried and stored in a cage store till used. For rabbits and guinea pigs, iron wire cages coated with aluminium paint is used to save cost. Good sanitisation standards such as changing footwear from outdoor to barrier point are maintained to control diseases and to maintain environment stability. Foot dips are provided at the entrance of the building.

Breeding management is monitored closely to maximise the productivity of each species. Table 3 illustrates the average litter size of each species from 2005 to 2010. Parent stock are evaluated based on the productivity and quality of progeny produced (Poole and Robinson, 1987). All laboratory animal parent stocks are renewed after five breeding session and culled. However, rabbits are kept as blood donors after the five breeding sessions. White mice show the highest litter size, hence their higher annual population. They also seem to have a higher survivability (about 95%) from birth to adulthood. Guinea pigs and rabbits have a 90% survivability, rat 88% and hamster 87% respectively.

Table 2: Type of bedding and frequency of bedding change for laboratory animals in VRI, Ipoh.

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of bedding</th>
<th>No. of times changed per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>No bedding</td>
<td>3</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>No bedding</td>
<td>3</td>
</tr>
<tr>
<td>Hamster</td>
<td>Sawdust ; dried &amp; disinfected</td>
<td>2</td>
</tr>
<tr>
<td>White mice</td>
<td>Sawdust ; dried &amp; disinfected</td>
<td>2</td>
</tr>
<tr>
<td>Balb-C mice</td>
<td>Sawdust ; dried &amp; disinfected</td>
<td>2</td>
</tr>
<tr>
<td>Sprague Dawley rat</td>
<td>Sawdust ; dried &amp; disinfected</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 3: Average litter size of laboratory animals from 2005 to 2010.

<table>
<thead>
<tr>
<th>Species</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Hamster</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>White mice</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>BALB/c mice</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Sprague Dawley rat</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 4: Total population and usage of laboratory animals in VRI, Ipoh annually from 2005 to 2010.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Population</th>
<th>Total usage</th>
<th>Total Population</th>
<th>Total usage</th>
<th>Total Population</th>
<th>Total usage</th>
<th>Total Population</th>
<th>Total usage</th>
<th>Total Population</th>
<th>Total usage</th>
<th>Total Population</th>
<th>Total usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>117</td>
<td>36</td>
<td>122</td>
<td>44</td>
<td>98</td>
<td>49</td>
<td>77</td>
<td>52</td>
<td>109</td>
<td>42</td>
<td>130</td>
<td>43</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>501</td>
<td>55</td>
<td>551</td>
<td>224</td>
<td>468</td>
<td>196</td>
<td>498</td>
<td>34</td>
<td>589</td>
<td>286</td>
<td>530</td>
<td>283</td>
</tr>
<tr>
<td>Hamster</td>
<td>57</td>
<td>20</td>
<td>37</td>
<td>0</td>
<td>51</td>
<td>22</td>
<td>87</td>
<td>9</td>
<td>106</td>
<td>8</td>
<td>68</td>
<td>10</td>
</tr>
<tr>
<td>White mice</td>
<td>2170</td>
<td>358</td>
<td>1842</td>
<td>803</td>
<td>1875</td>
<td>819</td>
<td>2728</td>
<td>1339</td>
<td>2329</td>
<td>752</td>
<td>2210</td>
<td>1164</td>
</tr>
<tr>
<td>BALB/c mice</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sprague Dawley rat</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>59</td>
<td>20</td>
<td>150</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 4 shows the population and usage of laboratory animals in VRI from 2005 until 2010. These laboratory animals were either sold or given to other VRI units, private agencies, universities and schools. Generally, white mice were highly productive with an annual population of 1,842-2,728. This was followed by guinea pigs, rabbits and hamsters. Sprague Dawley rats and BALB/c mice were introduced into the unit in 2009 and 2010 respectively. These were obtained from the Institute of Medical Research (IMR), Kuala Lumpur.

Laboratory animals in VRI are used for a variety of activities as shown in Table 5. More than 90% of them are used for diagnostic purposes, production of biologicals such as antiserum, in training and research. A small number of them are donated to schools, or used for exhibitions...
and recreational activities organised by the institute.

The health of the laboratory stock is maintained by careful daily observation on feeding and drinking as well as behaviour. Regular quarterly health screening is conducted by sacrificing 1% of the population to conduct postmortem and detailed laboratory diagnosis. Culling of some animals is conducted based on physical examination; for example on runts or weak animals. Approximately, 1% of the animals are culled annually.

Table 6 shows the mortality rate of the laboratory animals from 2005 to 2010. Hamster mortality rate was higher in 2009 (22%) and 2010 (31%). One of the reasons for this was because the breeders were old and had a decreased productivity. To overcome this, new hamster breeders were substituted in the year 2011.
With the high demand for laboratory animals, the laboratory unit in VRI is expected to increase its production and will continue to maintain a high standard of animal production in order to supply these animals for research and other activities in VRI and related agencies.

REFERENCES


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