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Comparative virulence of three *Trypanosoma evansi* isolates from water buffaloes in the Philippines

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ABSTRACT

The virulence of three *Trypanosoma evansi* isolates in Luzon, Visayas and Mindanao water buffaloes was compared determining the mortality rate, parasitemia level, clinical signs, and lesions on mice. A total of 51 inbred Balb/c mice (5–6 weeks old) were used and divided into two sets. Set A had three groups corresponding to three trypanosomes isolates (Luzon, Visayas, and Mindanao) with seven mice each whose parasitemia level, clinical signs, and lesions were noted at necropsy. Set B had three groups corresponding to the three isolates with ten mice each whose mortality was monitored. Each infected mouse was inoculated with 0.2 ml of *T. evansi* intraperitoneally and blood was examined under high power magnification. Their parasitemia level was determined using "Rapid Matching Method". Dead mice were subjected to necropsy and the lungs, liver, spleen, brain and heart were subjected to histopathological processing. Results showed that the mortality rate was highest at Day 3 for the Visayas isolates (70%), while at Day 5 for Luzon (90%) and Mindanao (70%) isolates. The parasitemia level of Visayas isolates ($1 \times 10^{8.7}$) reached the earliest peak at Day 4 while Luzon isolates (1×10^{9}) at Day 6 and Mindanao isolates ($1 \times 10^{8.7}$) at Day 8. Statistical analysis using Least significant difference (LSD) revealed significant difference among treatment means at Days 2 and 4. All of the affected mice showed rough hair coat, decreased body weight, and decreased packed cell volume. The most obvious gross lesions observed were pale liver with petechiations and pale muscles. Histopathological examination revealed depletion of the red pulp and extramedullary hematopoiesis in the spleen. Congestion, intralesional trypanosomes in blood vessel and extramedullary hematopoiesis were observed in the liver. In the lungs non-specific lesions observed were pulmonary edema, congestion and hemosiderosis.

1. Introduction

Trypanosoma evansi is a blood protozoan parasite that causes surra, locally known as "bayawak". It is primarily transmitted by horseflies (*Tabanus* sp.), locally known as "pikot" or "tapusok," and affects a wide range of hosts (Amante, 2008). It principally infects horses and camels and also affects donkey, mules, deer, llamas, dogs, cats, cattle and buffalo (OIE, 2002). Large ruminants such as cattle and water buffaloes serve as reservoir hosts (Manuel, 1998). Clinical signs in buffaloes and horses include fever, weakness and lethargy, petechial hemorrhages (eyelids, nostrils and anus), edematous swellings of the legs, briskets and abdomen, urticarial eruption of the skin, progressive loss of weight, anemia, jaundice, and death which may occur in two weeks to four months (OIE, 2002).

Surra is endemic in the Philippines and considered as one of the most economically important animal parasitic diseases (Dargantes et al., 2009). It occurs in different regions of the country, particularly Regions II, III and IV in Luzon, Regions VI and VIII in Visayas (Domingo, 2007 as cited by Venturina et al., 2008) and Regions IX, X, XI and XII in Mindanao (Manuel, 1998). Variation in the virulence of *T. evansi* occurs among the three isolates representing the three main islands of the country. Some claimed that isolates from Mindanao are more virulent than Luzon and Visayas isolates in water buffaloes. Some hosts, despite of having severe parasitemia, were able to survive for a longer period of time.

Inoculation of the three isolates of trypanosomes in Balb/c mice could shed some light on the variation in the virulence of the three isolates. This will lead to a better strategic treatment of surra in water buffaloes in the different island groups. The general objective of the study was to compare the virulence of the three trypanosome isolates in Balb/c mice. Specifically, to determine the mortality rate, parasitemia level, clinical signs, and lesions produced by the three isolates.

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2. Materials and methods

2.1. Experimental mice

Fifty-one inbred Balb/c mice (5–6 weeks old, weighing about 15–20 g) from stocks bred at the Philippine Carabao Center laboratory were divided into two sets. Set A has three groups corresponding to the three trypanosome isolates (Luzon, Visayas, and Mindanao). Each group had seven mice each whose clinical signs and gross lesions were noted. All mice that died in setup A were subjected to necropsy for histopathological examination. Set B had also three groups corresponding to the three isolates. There were ten mice in each group and their mortality was monitored. Each group of mice was placed in a cage measuring 6 × 12 inches. A total of six cages were used for 21 days. Cages were cleaned twice a week. The mice were given unlimited supply of commercial feeds and clean drinking water.

2.2. Preparation of Trypanosome concentration

Preserved blood culture of *T. evansi* isolate from Visayas water buffaloes contained in cryogenic vial was taken out from the deep freezer (−88 °C) while the Luzon and Mindanao isolates were taken out from the liquid nitrogen tank (−196 °C). The sample was thawed in a water bath with a temperature of 37 °C for 15 min. A drop of blood was collected from the cryogenic vial using tuberculin syringe, placed on a glass slide and then covered with a cover slip. The motility of the trypanosomes was examined using high power magnification. If they were motile, 0.2 ml of the sample was inoculated intraperitoneally in each mouse per isolate as described by Villareal et al. (unpublished). After three days post-inoculation, a small drop of blood was collected from the tail of the inoculated mouse and placed on a glass slide and examined under high power magnification. If the parasitemia level of the inoculated mouse attained a log of 9.0, the mouse was sacrificed and 1 ml of blood was collected intracardiac using tuberculin syringe. The collected blood was placed in a microcentrifuge tube and was diluted. Dilution of the sample was done by adding a drop of phosphate buffered saline (PBS) in the blood, with gentle mixing, for every addition of PBS. The rate of dilution and the dilution was stopped only when 32 trypanosomes per field were attained.

2.3. Experimental challenge of mice

Experimental challenge was done on Day 1. In Set A, the 21 mice were grouped into three. In each group, five were inoculated with trypanosomes and the two served as the controls. In Set B, all mice in each group were infected. Using tuberculin syringe, the experimental mice were inoculated intraperitoneally with 0.2 ml diluted blood (Manahan et al., unpublished) with a log of 8.1 equivalents to 32 trypanosomes per field obtained from the isolates expanded by one passage in reference to the study of Herbert and Lumsden (1976). The experimental mice were marked based on the procedure done at the Philippine Carabao Center Laboratory. The clinical signs of the disease were investigated at Day 2. Coat conditions and body weight were recorded daily and these were compared to the control mice. Monitoring was done twice daily at 7:00 in the morning and 7:00 in the evening. Weighing of mice was done using digital weighing machine.

A capillary tube was filled with blood by placing its tip to the bleed tail. Bleeding the tail was done by placing the mouse on ventral recumbency and a portion of the tail was cut using scissors. It was centrifuged at 300 rpm for 5 min. After centrifuging, it was placed carefully on a reader to determine the percent of packed red blood cells. Collection was started at Day 2 and repeated daily.

2.4. Determination of parasitemia level

Parasitemia was observed by bleeding the tail of mice and using "Rapid Matching Method" by Herbert and Lumsden (1976). Bleeding the tail was done by placing the mouse on ventral recumbency and a portion of the tail was cut using scissors. A drop of blood was placed on a microscope slide and covered with cover slip. The technique of "Rapid Matching Method" depends upon matching the microscopic appearance of a wet film of infected blood with one of the series of eight pictures of microscopic fields. The pictures represent the concentrations of trypanosomes in decreasing number by halving steps from 256 to 2 organisms per field. The circles in Fig. 3 were used for matching when more than one organism per microscope field was present (Herbert and Lumsden, 1976). The measurement was started at Day 2 and was repeated daily in all mice in Set A.

2.5. Histopathological examination

All mice that died after the challenge of the different isolates of trypanosomes were subjected to necropsy to observe for possible internal organ lesions. A systematic approach was used in performing the necropsy. External surface was evaluated and the general condition of the mice was noted. Lesions on the skin, eyes, oral cavity, and anus were also observed. The lungs, heart, brain, liver, spleen and kidney from the dead mice were collected and fixed in a jar containing 10% formalin and then sent to Dr. Paulino J. Garcia Memorial Hospital Laboratory for tissue processing. Processed slides were examined under high power magnification.

2.6. Data gathering and statistical analysis

The parasitemia levels and packed cell volume (PCV) were compared using analysis of variance. Least significant difference (LSD) was further used to compare the treatments' PCV mean values. The level of significance was set at 5%. All analyses were done using SAS 9.1 (SAS Institute, Cary, NC).

3. Results and discussion

A total of 51 mice were used to compare the virulence of *T. evansi* isolates representing the three main island groups of the Philippines.

3.1. Mortality rate

Fig. 1 shows the mortality rate of the mice in Setup B. The earliest mortality was observed from the mice inoculated with the

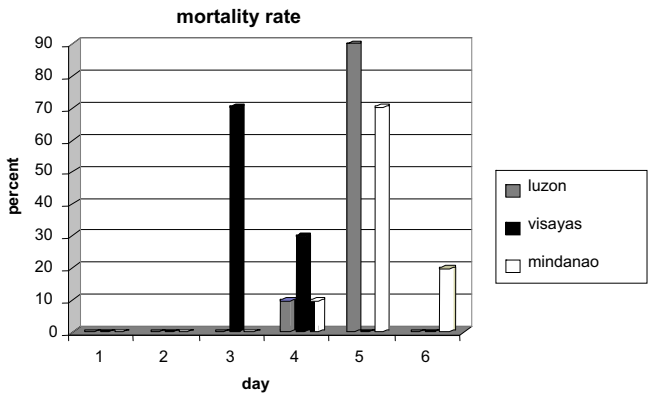

Fig. 1. Evaluation of mortality rate at Day 3 up to Day 6.