The Epidemiology, Pathogenesis And Diagnosis Of Eperythrozoonosis In Sheep

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Introduction:

Eperythrozoon ovis is a rickettsial organism that has been recorded world wide in may animal species. It is found both on and around erythrocytes. Affected sheep become unthrifty, weak, anaemic and icteric. Poor growth or loss of wool has also been recorded. Post mortem lesions include enlarged spleen, jaundice, pale musculature and pale mucous membranes. In pigs, the disease is more pathogenic and death is not uncommon. Infection may persist in animals for 10 years or more. Antibodies have been detected 1-2 weeks of infection, peak in 5-6 weeks and thereafter decline.

To eliminate *E.ovis*, macrophages phatocytised affected cells which are then carried to the spleen for destruction.

Giemsa staining of blood smears is the common technique used to diagnose the organism. This technique is feasible with several samples but is time consuming and labour intensive when sample numbers are large and infection subclinical. A more rapid technique is necessary, be it biochemical or molecular to identify an infected animal especially when the infection is subclinical. The objectives of the project are:

To define the clinical and clinical pathological responses of host animals to eperythrozoon infection

To determine the biochemical and molecular characteristics of eperythrozoon organisms

To develop a less tedious diagnostic technique to identify either the eperythrozoon or its antibodies for big scale screening

To determine the immunological response of infected animals

Materials and Methods

Blood were collected in EDTA and plain vacutainers tubes from 13 sheep naturally infected with *E.ovis* at regular intervals during the study. The sheep were clinically examined at sampling. Blood smears were made and stained with Giemsa to examine for the presence and score of *E. ovis*. The haematological and serum biochemical analysis were carried out using an automated haematology counter (Serono 9120, Biochem Immunosystem, USA) and automated clinical chemistry analyser (Cobas Mira Plus). Blood samples from both parasitaemic and non-parasitaemic periods were collected for the scanning and transmission electron microscopic studies.

Spleen and lymph nodes were collected during slaughter for histological and electron microscopic studies.

Attempts were made to maintain *E.ovis* invitro cultures using RPMI-1640 medium, Eagle's Minimum Essential Medium (EMEM) and 8-day old chicken embryonated eggs. Indirect immunofluorescent test (IFT) electron microscopyand confocal electron microscopy examination were tried out. Transmission of *E.ovis*-infected blood into mice or ratswas also carried out. Alternative diagnostic techniques which were either more sensitive, or less tedious or less time consuming than Giemsa staining were tested.

Results and Discussion

The clinical (TPR, colour of mucous membranes) and clinical pathological parameters, except triglycerides, in animals naturally-infected with *E.ovis* were generally within normal range, although there were fluctuations during parasitaemias and parasitaemia scores. Triglycerides were significantly different at different parasitaemia score. White (WBC) and red blood cell (RBC) counts, haemoglobin (Hb), packed cell volume (PCV) and plasma proteins were negatively correlated with parasitaemia after 8 days of parasitaemia whilst mean corpuscular haemoglobin concentration (MCHC) after 4 days of infection. Mean corpuscular volume (MCV) was positively correlated with parasitaemia after 4 days of parasitaemia. Glucose, gamma glutamyl transferase (GGT) and blood urea nitrogen (BUN) were negatively correlated with parasitaemia after 4 days, cholesterol after 2 days and triglycerides after 10 days. Erythrocyte osmotic fragility (EOF) was within normal range and not significantly different between parasitaemic scores. RBC, Hb, PCV, MCV, icterus index, glucose, triglycerides and GGT were positively correlated whereas MCHC, plasma proteins, cholesterol and BUN were negatively correlated with EOF.

Transmission studies in mice or rats showed that blood monocytes were predominantly involved in the active erythrophagocytosis. There was an increased in numbers of plasma cells and platelets at day 20 post infection. A moderate increased of Kupffer cells phagocytosing infected erytthrocytes, of hemosiderin in spleen and membrano-proliferative glomerulonephritis were also observed. These findings were similar to those found in sheep in an earlier study.

Protein profile of *E.ovis* isolated from sheep and goats showed common bands – 180, 172, 118, 95, 79.6, 40, 36 kDa. All but the last band were detected by polyclonal hyperimmune serum produced in rabbits in a Western blot hybridization test indicating antigenic homogeneity of isolates. Protein band 95kDa was dominant.
Parasitised erythrocytes were maintained up to 150 hours in RPMI-1640, 264 hours in EMEM and in all 3 passages in the embryonated eggs. The IFT and PCR were more sensitive, able to detect organisms in samples scored negative by Giemsa. SEM, TEM and confocal examination of E ovis from blood or in vitra culture product some morphological details but is laborious, time consuming, and expensive.
A latex agglutination technique to identify *E.ovis* and ELISA to detect antibodies in serum have been optimized but could not continue due to firstly difficulties in getting adequate antigens either from field isolates or cultures and the ending of the project time and funds. Similar limitations were encountered in the attempts to sequence the DNA fragments of the PCR product.

Conclusions

The haematological, clinical biochemical and erythrocyte osmotic fragility values were within normal range, although fluctuations occurred between parasitaemic episodes. Affected red blood cells were destroyed by macrophages, through phagocytosis, especially in the lymph nodes. *E.ovis* can be maintained in vitro for only a short time. *E ovis* isolated from sheep and goats are closely related if not the same strain. The rat or mouse may be used as the lab animal model to study *E.ovis* infection. IFT and PCR are more sensitive than Giemsa staining in detecting *E.ovis* although expensive. Alternative diagnostic techniques such as the latex agglutination and ELISA may be useful for screening large number of samples once the difficulty and challenge of producing large quantities of *E.ovis* antigen is overcome.

Benefits from the study

Establish knowledge about the micro organisms and epidemiology of disease, training of postgraduates

Patent(s), if applicable

Possibly a product if the problems of producing large quantity of *E.ovis* antigens are overcome

Stage of Commercialization, if applicable:
Nil

Project Publications in Refereed Journals:

Nil

Project Publications in Conference Proceedings

Ershaduzzaman, M., Fatimah C.T.N.I., Omar, A.R., Hair-Bejo, M., Chulan U., Saharee, A.A.(1999). Detection of *Eperythrozoon ovis* in sheep and goats by a modified indirect immunofluorescent antibody test (IFAT). In: Proceedings National Congress on Animal Health and Production on Environment Care in Animal Production. Malacca. p379-381.

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Marina, H. Fatimah, C.T.N.I. Rasedee, A. Ungku-Chulan, U. M, Hair, B (2002) Haematological and clinical biochemistry parameters and erythrocyte osmotic fragility in sheep infected by *Eperythrozoon ovis*. In: Proceedings 12th FAVA and 14th. VAM Congress, Subang Jaya p208.

Graduate Research

	Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year

	Ershaduzzaman M	Characterisation of *E.ovis* isolated from sheep and goats in Malaysia	Veterinary Immunology	Ph.D	2001
	Marina Hassan	Erythrocyte osmotic fragility in sheep affected by *E.ovis*	Internal Medicine	MVSc.	2002
	Shanker Ganesh a/l Kanabathy	Immunological response of ruminants to *E.ovis* infection	Internal Medicine	MVSc.	2003/4

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