ENDOTOXAEMIA & HYPERMUNE

equine plasma products

Endotoxin is the outer coating of gram negative bacterial cell walls and comprises lipopolysaccharide and lipid-A components (LPS). In the horse, endotoxaemia arises when endotoxin invades the circulation, triggering the release of mediators. These cause the classic endotoxic cascade, resulting in fever, systemic hypotension, pulmonary hypertension, bronchoconstriction, leucopoenia, platelet aggregation, vascular permeability and haemoconcentration. Clinically this is seen as severe unrelenting abdominal pain, reduced tissue perfusion (as seen in the oral mucosa and the sclera,) increased heart rate, decreased intestinal motility, and haemo-concentration. In the severe case there is rapid onset of depression and shock, leading to death. It should be born in mind, however, that clinical signs are related to the levels of circulating endotoxin. Low levels (0.03ug/kg) produce symptoms such as mild depression with clinical "silence" but changes in the blood, medium levels (10 ug/kg) are clinically "noisy" and high levels (125ug/kg) will cause death. (1). The more common high risk situations for endotoxaemia to occur in the equine are colitis, retained placenta, proximal enteritis, neonatal septicaemia, intestinal ischaemia and pleuropneumonia.

Endotoxaemia is the number one cause of death in the horse (1). In the healthy animal the cells and secretions of the gut lining form a barrier to the normal bacteria and endotoxin present. When ischaemia occurs there is a loss of barrier function of the villi of the small intestine, which exposes the capillary network, thus allowing absorption of endotoxin. A similar sequel occurs in the colon where the mucosal layer sloughs off.

The treatment of endotoxaemia should be prompt, aggressive and complete, as there is such a narrow therapeutic window through which to achieve success. In the neonate especially, clinical signs can be very subtle. If there is a hint of septicaemia the assumption should be that it is endotoxic and treatment started.

Any underlying cause, surgical or medical, **should be identified and treated**. **Specific endotoxic therapy, however, has three main approaches:-**
The first is to try to prevent LPS-cell interaction and so arrest any further triggering of the cascade. This can be attempted by using an anti-endotoxin antibody and is the concept behind the use of J-5 plasma used extensively throughout the USA (2). *E. coli* J5 is a rough or mutant strain of *E. coli* which, when used as a vaccine stimulates the horse to produce antibodies to the core antigen (LPS), ie anti-endotoxin antibodies (3). Plasma harvested from these donors is used at a dose rate of 1-2ml per kg body weight. The use of this plasma is still a controversial subject, but success is reported (3) and it is recommended in colitis (4), colic (5,6) and endotoxaemia generally (7). In the United Kingdom, there is no J-5 vaccine licensed for use in the horse. Consequently, from 1994 in some of its donors, Veterinary Immunogenics Ltd used a gram negative core antigen vaccine specially prepared by a leading UK University Medical Microbiology unit. It was demonstrated in vitro that plasma produced from these horses contained anti-endotoxin antibodies with cross reactivity to a wide range of gram negative bacteria, including pathogenic *E. coli*, Salmonellae and *E. coli* J5. This plasma was designated HYPERMUNE-J and was indicated in any condition where endotoxaemia prevailed or was anticipated at dose rates of 2-4 ml/kg in the adult horse and 20ml/kg in the foal, the best results being seen when used early (8). It has been reported that in the United States infections in the neonate are primarily gram negative with *E. coli* being the most common agent and that early treatment is essential (9,12). Plasma itself also has added benefits as it contains

beneficial peptides such as the immunological mediators gamma interferon and interleukins. Its albumin content, too, is important in stabilizing the oncotic pressure of the blood. It is therefore excellent in the role of support therapy and does provide enormous benefits. (10,11) .

Veterinary Immunogenics Ltd has come under pressure from the UK Regulatory Authority, the Veterinary Medicines Directorate, to gather data and submit a dossier of application for a national Marketing Authorisation for Hypermune-J. In response, the company has conducted research work on its existing plasma products and held discussions with eminent equine veterinary surgeons who use the product.

A specific ELISA was used to assess the gram negative endotoxin antibodies in a number of randomly selected healthy adult horses in the United Kingdom which revealed a consistent base level as shown in Table 1 below. In contrast the levels in Hypermune-J were markedly higher as shown in Table 2.

TABLE 1

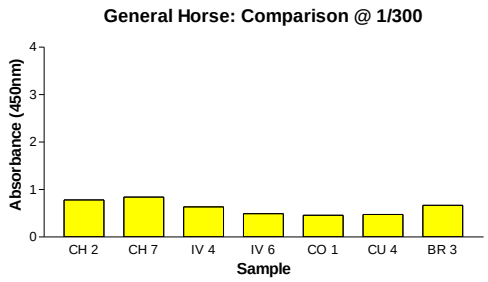
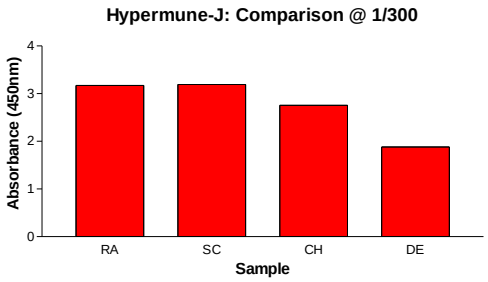


TABLE 2



These were then compared with the two existing plasma products which have UK National Marketing Authorisations and they too demonstrated a marked increase in gram negative endotoxin antibodies compared with the normal healthy adult population samples as shown in Table 3 and Table 4.

TABLE 3

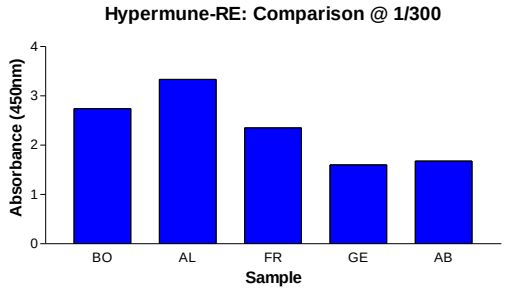
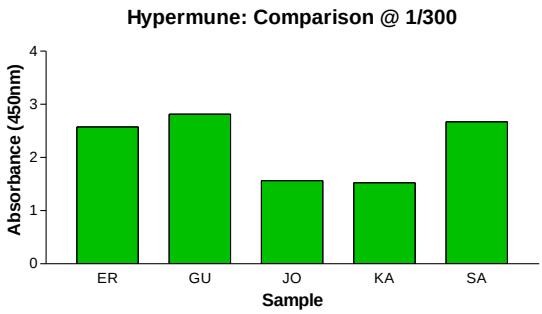


TABLE 4



In terms of gathering supportive efficacy data for a Dossier of Application for Hypermune-J the results indicated that it would be extremely difficult to prove a measurable difference between Hypermune J and both Hypermune and Hypermune-RE. This obstacle was further highlighted in discussions with eminent Newmarket equine veterinary surgeons who agreed that clinical efficacy would be difficult to prove.

Furthermore, recent publications have reported that ordinary equine plasma when administered to septic foals (13, 14) provides a measurable benefit in terms of prolonged opsonisation and improved neutrophil function. In addition, this was endorsed at Rossdales Foal Care Course in January 2008 when it was stated that all septic foals are given 1 litre of plasma irrespective of IgG status (15). These facts support the view that little extra benefit would be demonstrable arising from the small difference in the gram negative endotoxin antibodies between Hypermune-J and existing licensed Hypermune products.

These circumstances coupled with recent levels of demand for Hypermune-J compared with Hypermune-RE and Hypermune, place Veterinary Immunogenics Ltd in the position of being unable, at the present time, to justify a programme of studies and data collection required by the Regulatory Authority.

Consequently, from current knowledge of products and reports of their use by existing customers in septic and endotoxic situations Veterinary Immunogenics Ltd recommends the use of Hypermune.

It should be noted, however, that this is best used as part of an overall strategy when treating the endotoxic horse.

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