economically useful information. However, owing to the cost of large clinical trials, a smaller sample might be contemplated. The savings from a smaller sample should nevertheless be weighed against the risk of finding that a useful vaccine is apparently worthless. Hence, economic analysis should be able to answer two questions when planning clinical or field trials; first what is the least required effect of the vaccine, and secondly, is the evidence from other trials sufficient to demonstrate that the vaccine is useless?

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Short Communications

Plasma vitamin E response in two black rhinoceroses following dietary supplementation

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Veterinary Record (1991) **128**, 185-186

CAPTIVE black rhinoceroses (*Diceros bicornis*) examined at several zoos have been found to have very low plasma vitamin E concentrations (Dierenfeld and others 1988, Ghembremeskel and others 1988, Lewis and Kirkwood 1990). It has been suggested that this may be a factor in the aetiology of the haemolytic anaemia that is an important cause of mortality in these animals (Miller and others 1986). Dietary supplementation with high doses of dl-α-tocopherol acetate or with dl-α-tocopherol have been found to have little effect on plasma vitamin E levels (Papas and others 1989, Lewis and Kirkwood 1990).

Recently, Papas and others (1989) have reported the reversal of vitamin E deficiency in several captive Asian and African elephants (*Elephas maximus* and *Loxodonta africanus*) at the Denver Zoo, and two black rhinoceroses at the Miami Metro Zoo, following dietary supplementation with d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS; Eastman Chemical, Kingsport, Tennessee). These authors found that plasma α-tocopherol concentrations in the rhinoceroses rose from baseline levels of 0·2 to 0·4 mg/litre to about 2·4 mg/litre after provision of TPGS at 2100 iu per day for 14 days. This communication reports on a comparable and marked plasma response to oral TPGS in two black rhinoceroses at the Zoological Society of London.

The animals treated with TPGS were adult males of similar size. The first, 'Jasper', was given TPGS once daily with food at a dose of about 12,000 iu per day and the second, 'Basha', was given about 7500 iu per day (approximately 8 and 5 iu/kg bodyweight daily, respectively). The TPGS, which is a waxy solid, was prepared by melting 20 g samples at above 40°C. When melted the liquid was poured slowly into 100 ml boiling water and stirred. On cooling the resulting clear fluid contained about 7000 iu/100 ml TPGS. The doses were provided by soaking the supplement into bread. Both animals were sufficiently tractable to allow blood samples to be regularly collected from their medial carpal veins without chemical or physical restraint. After withdrawal using a 19 gauge 1 inch needle, these samples were immediately transferred to tubes containing heparin for subsequent vitamin E and general biochemical analyses, and tubes containing EDTA for haematology. The plasma was separated by centrifugation and stored at -20°C before dispatch for assay. The samples from Jasper were analysed for vitamin E content by the nutritional biochemistry group at the Institute of Zoology (Ghembremeskel and Williams 1988), and those from Basha were analysed at the Shrewsbury MAFF Veterinary Investigation Centre.

The results are shown in Fig 1. Jasper's plasma vitamin E concentration increased from 0·6 mg/litre before TPGS supplementation to 3·9 mg/litre after 13 days. In Basha the plasma concentration increased from less than 0·1 mg/litre to 1·0 mg/litre after 14 days. The explanation for the difference in baseline levels is not clear but both animals showed a prompt surge in plasma vitamin E levels when TPGS supplementation began, to a greater than sixfold increase above their baseline levels. Samples collected from Jasper after supplementation ceased showed a quite rapid decline in plasma levels towards the baseline. Haematological parameters remained within normal limits throughout the period of study in both animals.

These results support the findings of Papas and others (1989)

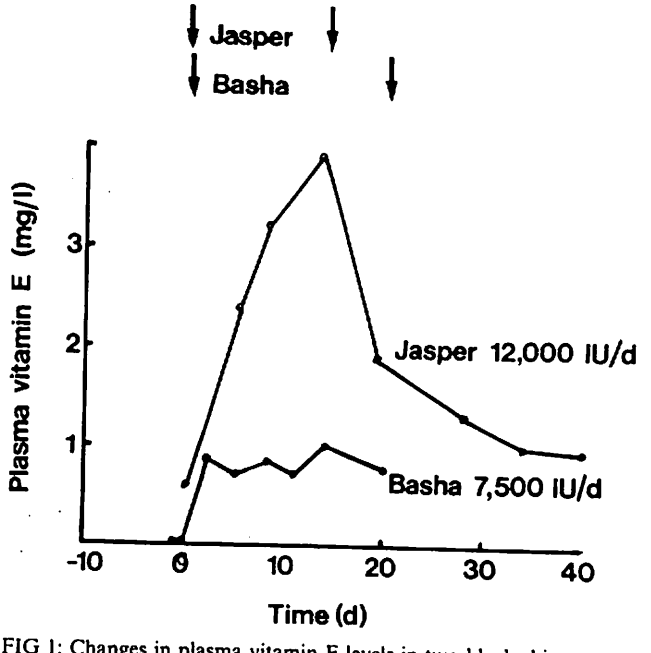

FIG 1: Changes in plasma vitamin E levels in two black rhinoceroses given oral supplements of d-α-tocopheryl polyethylene glycol 1000 succinate. The start and ends of the periods of supplementation are indicated by the arrows

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that TPGS is an effective source of vitamin E for black rhinoceroses.

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Coagulation induced by challenge with various doses of *A pleuropneumoniae* in piglets

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PLEUROPNEUMONIA in swine, caused by *Actinobacillus pleuropneumoniae* is an economically important disease worldwide. The bacteria induces acute fibrohaemorrhagic pleuropneumonia and often causes death or localised necrotising lung lesions. The pathogenesis of such lesions involves activation of circulating blood platelets or coagulation pathways (Bertram 1988). In the present report, the authors describe the changes in some circulating blood constituents which result from experimental challenge with various doses of *A pleuropneumoniae*.

Fifteen weaned Large White piglets weighing 20 to 25 kg were randomly allocated to three equal groups and placed in a temperature controlled isolation unit. They had negligible antibody titres against *A pleuropneumoniae* as measured by enzyme-linked immunosorbent assay. An aerosol of *A pleuropneumoniae* was generated from a bacterial suspension and blown into a chamber using a nebuliser as previously described (Osborne and others 1985). A strain of *A pleuropneumoniae* serotype 1 was used for the challenge (Wilson and Osborne 1985); piglets from each group were aerosolised with 5×10^5, 5×10^6 and 5×10^7 colony forming units (cfu) respectively. Blood samples were taken from a jugular vein at days 0, 1, 2, 3 and 6 after inoculation into sodium citrate or EDTA vacutainer tubes (Becton and Dickinson, Basle, Switzerland). Blood collected in sodium citrate tubes was centrifuged at 5°C for 15 minutes at 2000 *g* and plasma harvested for measurement of clotting parameters.

Total white blood cell counts (wbc) were determined using an electronic cell counter (Sysmex E 5000 Digitana TOA counter; Digitana, Lausanne, Switzerland). Activated partial thromboplastin time (aPTT), prothrombin time (PT) and fibrinogen levels were measured using a Cobas fibro thermoblock and reagent kits (Roche Diagnostic, Basle, Switzerland); examinations were carried out according to the manufacturers' instructions. The effect of bacterial challenge on wbc, PT, aPTT and fibrinogen levels were determined using student's *t* test. The increases in aPTT, PT or fibrinogen levels were compared with bacterial doses using analysis of covariance (Complete Statistical System – Anova; Ancova, StatSoft, Tulsa, USA).

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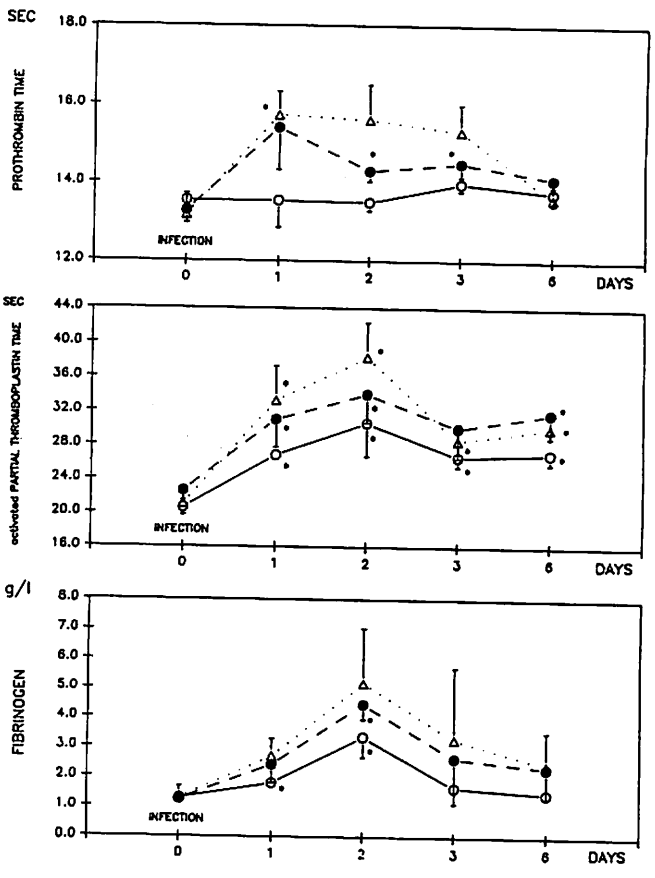

FIG 1: Mean prothrombin time (PT), activated partial thromboplastin time (aPTT) and plasma fibrinogen concentrations in piglets given various doses of *Actinobacillus pleuropneumoniae*.○ – ○ 5×10^5 colony forming units (cfu), ● – ● 5×10^6 cfu, △ . . . △ 5×10^7 cfu,Values with (*) are significantly different from baseline (day 0; $P < 0.05$)

Mortality and rectal temperatures were monitored daily. The mortality was dose dependent: three out of five piglets for the group challenged with 5×10^7 cfu died during the experiment compared with one out of five in the group receiving 5×10^6 cfu. There was no mortality in the third group; likewise piglets from this group showed no increase in body temperature. In piglets given the two highest doses of bacteria, body temperatures increased within 24 hours and returned to normal after 72 hours. Pigs that died during the experiment developed typical haemorrhagic necrotising pleuropneumonia.

A leucocytosis (from 13,000 to 22,000 wbc/mm^3 was found only in the group challenged with 5×10^7 cfu after 24 hours. No correlation was found between increased wbc and doses of bacteria.

Fig 1 shows data from the coagulation tests. Plasma fibrinogen levels and aPTT changed significantly from the base line in all groups after the bacterial challenge. An increase in PT was observed only for the piglets exposed to the highest doses of *A pleuropneumoniae*. Changes in aPTT, PT and fibrinogen levels correlate with the doses of bacteria given to the piglets.

The aPTT and PT measure, respectively, the intrinsic and extrinsic system of blood coagulation. The activation of the common pathway is monitored by plasma levels of fibrinogen. PT and aPTT were prolonged in the piglets of the present study