INFLUENCE OF MAREK'S DISEASE VIRUS INFECTION ON THE HAEMATOGRAM OF BROILIER CHICKENS

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Summary

Marek's disease virus (MDV) infection in chickens is very common. We report the effects of MDV infection with a very virulent Australian isolate on various haematological parameters of broiler chickens. Packed cell volume (PCV), total erythrocyte count and the haemoglobin concentration were reduced by MDV infection. PCV was reduced the most. HVT vaccination provided limited or no protection against these MDV-induced effects. These data confirm that MDV induces pathological effects on myeloid as well as lymphoid cells. As PCV is simple to measure and depression occurs early in infection, it may provide an additional diagnostic tool for evaluating MDV status.

I. INTRODUCTION

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by an α-herpesvirus. The disease is immunosuppressive, causing destruction of lymphoid and haematopoietic tissues of chickens (Jokowski et al., 1970; Gilka and Spencer, 1995; Islam et al., 2002). A significant decrease in erythrocyte (RBC) population (Neilsen and Anderson, 1971) and a marked decrease in packed cell volume (PCV) or haematocrit value after two to four weeks of MD virus (MDV) infection has been reported (Vickers et al., 1967; Jokowski et al., 1970; Gilka and Spencer, 1995; Spencer et al., 1996). The mechanism of pathology produced in the hematopoietic system by MDV infection is not clear but it may be due to reduced hematopoiesis (Jokowski et al., 1970) or due to erythropagocytosis by hyperactive reticuloendothelial cells of the liver and spleen of infected chickens leading to extravascular haemolytic anaemia (Gilka and Spencer, 1995).

However, reduction in RBC is a non-specific condition and has been reported in broiler chicken following stress such as feed restriction (Maxwell et al., 1991; Hocking et al., 1994). Reduced PCV was also observed in broiler chickens following physiological stressors such as feeding of mycotoxin-contaminated feed (Arunvind et al., 2003).

HVT vaccine is routinely used in the Australian poultry industry to vaccinate broiler chickens. Vaccination with herpesvirus of turkey (HVT), recombinant HVT and bivalent vaccines is reported to protect against reduction in PCV following MDV challenge (Spencer et al., 1996). HVT vaccination is also partially protective against immune organ damage due to MDV infection (Morimura et al., 1998; Islam et al., 2002). The primary objective of this study was to evaluate the effects of Australian MDV isolates on the hematological parameters of broiler chickens and the role of HVT vaccine in protecting against these effects.

II. MATERIALS & METHODS

Two experiments were conducted; experiment 1 included three treatments (Control, HVT and MDV infection) in a completely randomized block design with three replications of each treatment. A total of 378 chickens were randomly divided into three equal groups (126 per group). The first group (Control) was not inoculated with any virus. The second group

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(Vaccine) was vaccinated sub-cutaneously with 4000pfu of live cell-associated HVT vaccine and the third group (MDV) was infected intra-peritoneally with 50pfu of MDV.

Birds of each treatment were reared separately in nine positive pressure isolation units. At intervals of 3-7 d up to 35 d of age, five randomly selected chickens were removed from each isolator (replicate), weighed, blood sampled and euthanased for other studies.

Fig 1. Exp 1. Total RBC count, hemoglobin concentration and PCV in Control, HVT vaccinated and MDV infected chickens. Vaccination with 4000pfu of HVT vaccine subcutaneously and MDV infection with 50pfu intraperitoneally was performed at hatch. Columns not sharing a common letter within each time period are significantly different (P<0.05). Data presented as mean (±se of mean)

Experiment 2 utilized a 2x2 factorial design with two levels of vaccination with HVT (4000 or 0pfu) and two levels of MDV Challenge (100 or 0pfu) with four replicates of each treatment combination (N=360). Vaccination was performed sub-cutaneously at hatch and vaccinated chickens were permanently identified by foot web marks. Approximately equal numbers of vaccinated and unvaccinated chickens were placed in each of eight isolators (45/isolator). Chickens in four of the isolators were injected intra-peritoneally with 100pfu of MDV at three days of age while chickens in the remaining four isolators were sham challenged. At each of sample collection days 3, 7, 14, 21, 28 and 35 post-challenge, 12 randomly selected chickens from each treatment combination were weighed, blood sampled and euthanased for other studies.

Commercial feather-sexed female Cobb broiler chickens were used in both experiments. The parent flocks of the chickens were vaccinated against MD with serotype 1 MDV vaccine (Rispens CV1988), so the chickens had homologous maternal antibody to MDV. A cell-associated preparation of HVT strain FC126 (The Marek Company, Victoria, Australia) was used and the challenge virus was Australian very virulent strain of MDV (MPF 57) free of contaminants such as chicken anaemia virus.

Blood samples were collected in acid citrate buffer tubes. Absolute (total) counts of red blood cells (RBC) were made using an automated Cell-DYN® 3500 Haematology Analyser (Abbott, Norfolk, VA USA) calibrated for chicken blood. The analyser also measured the PCV (hematocrit) and percent haemoglobin concentration (Hgb).
Variables were analysed by analysis of variance (ANOVA) using Statview (SAS Institute Inc USA) with mean separation by Duncan's New Multiple Range Test. The statistical model tested the main effects of Treatment (Control, Vaccine and MDV), Day (post challenge) and their interaction in Exp 1 and the main effects of Challenge (MDV+ or MDV -), Vaccination (HVT+ or HVT-) and Day with all interactions in Exp 2.

Fig 2. Exp 2. Total RBC, haemoglobin concentration and PCV of HVT vaccinated or unvaccinated and MDV challenged or unchallenged chickens. Vaccination with 4000pfu of cell-associated HVT vaccine subcutaneously at hatch, challenge with 100pfu of MDV occurred at day 3 post-vaccination. Columns not sharing a common letter within each time period differ significantly (P<0.05). Data presented as mean (±se mean)

III. RESULTS

(a) Experiment 1

There was a significant effect of Day (P<0.0001) and Treatment (P<0.001) on total RBC count with significant interaction between the effects of Day and Treatment (P<0.023). Overall, the RBC count increased with age (day) of chickens and was reduced by MDV infection. The interaction was significant because of the RBC number was reduced by MDV infection at days 14 and 22 but not at other time points (Fig 1A). There were significant effects of Day and Treatment (P<0.0001) and their interaction (P<0.0001) on Hgb concentration. Hgb increased with age of chickens up to day 14 and then remained relatively constant. MDV infection reduced Hgb from day 10 onwards but Vaccination had little effect on Hgb concentration (Fig 1B). here were significant effects of Day, Treatment (P<0.0001) and their interaction on the PCV (P<0.0001). There was an increase in PCV with age up to day 10 after which PCV remained steady. MDV infection reduced PCV from day 10 onwards but HVT vaccination did not affect it significantly (Fig 1C).

(b) Experiment 2

There was a significant effect of Day and Challenge (P<0.0001) but not Vaccination (P=0.06) on total RBC count. There was also significant interaction between the effects of Day and Challenge (P<0.005). There was a steady increase in RBC count to day 28.
Challenge with MDV decreased overall RBC count with HVT vaccination providing little protective effect. This effect was significant at all times other than day 21 (Fig 2A).

There was a significant effect of Day, Challenge (P<0.0001) and their interaction (P<0.006) on Hgb concentration. Vaccine had no effect on this variable. There was steady increase in Hgb with age up to day 28. Challenge reduced Hgb from days 14 to 35 (Fig 2B).

There was a significant effect of Day (P<0.0001), Challenge (P<0.0001) and Vaccine (P<0.01) on PCV with significant interaction between the effects of Day and Challenge (P<0.0001). Overall, PCV increased with age and MDV challenge reduced it from days 14-35. Vaccination provided partial protection against the reduction of PCV due to MDV challenge at days 14 and 35 only (Fig 2C).

IV. DISCUSSION

This study demonstrated that the Australian very virulent strain of MDV, MPF 57 markedly reduced the haematological parameters of total RBC count, haemoglobin concentration and PCV of commercial broiler chickens and that HVT vaccination provided very limited protection against this pathology. A 17% reduction in PCV due to MDV infection was reported in layer chickens (Spencer et al., 1996) but the reduction in our study was only about 10%. A very large reduction in RBC population (30% or more) has been reported following MDV challenge (Nielsen and Anderson, 1971) but reduction of both RBC and Hgb was about 6% in the current study. This variation might be due to strain differences in the viruses used or the chickens. In the report of Nielsen and Anderson (1971) freedom from chicken anaemia virus CAV was not stated and so CAV must be considered as a possible contributory factor. The mechanism of destruction of haemopoetic tissue was not studied here but it may be due to extravascular hemolysis as suggested before (Gilka and Spencer, 1995).

Vaccination with HVT and a recombinant HVT vaccine was found to be protective against anaemia produced following MDV infection in layer chickens (Spencer et al., 1996) but HVT was not protective against reduced RBC, PCV and haemoglobin concentration in the present study. Among the variables measured, PCV was the most affected due to MDV infection. Reduction of PCV was even more consistent than reduction in lymphoid organ weights in the same experiment (Islam et al., 2002). Therefore PCV may be used as an early indicator of MDV infection but the use of this indicator as a marker of vaccine protection may not be useful as has been suggested previously (Spencer et al., 1996).

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REFERENCES