# EFFICACY OF SPLENIC AND CELL CULTURE HAEMORRHAGIC ENTERITIS VACCINES IN COMMERCIAL AND SPECIFIC-PATHOGEN-FREE TURKEYS

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Splenic and cell culture haemorrhagic enteritis (HE) vaccines were administered to separate groups of 4-week old commercial and specific-pathogen-free (SPF) poults. SPF poults receiving both vaccines seroconverted and were protected against virulent HEV challenge. In commercial birds, antibody response to splenic vaccine was significantly higher ($P<0.05$) than that to cell culture vaccine. Moreover, the protection provided by splenic vaccine was 97% as compared to 61% by cell culture vaccine. The results of this study indicate that in 4-week-old poults, splenic vaccine provided greater seroconversion and better protection than cell culture vaccine.

## INTRODUCTION

In a laboratory study, Fadly and Nazerian (1989) found that the efficacy of cell-culture-propagated HE vaccine was interfered with in turkey poults up to 5 weeks of age in commercial poults. The purpose of this study was to compare the efficacy of splenic and cell culture propagated HE vaccines in 4-week old commercial and specific-pathogen-free (SPF) turkeys for seroconversion and protection from challenge. Protection from challenge was based on absence of clinical signs and HEV antigen in the spleen of turkeys after challenge.

## MATERIALS AND METHODS

**Birds:** Fertile turkey eggs from an SPF source (National Animal Disease Laboratory, Ames, IA) were obtained and hatched. The poults were raised in an isolation facility under negative pressure. One-day-old poults were obtained from a commercial hatchery and were raised in similar facilities. At 4 weeks of age, before vaccination the poults were bled and their sera were tested for presence of maternal antibody.

**Vaccines:** Marble spleen disease virus, a vaccine strain used for HE, was obtained from Dr. C.H. Domermuth (Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061) and was intravenously inoculated into susceptible turkey poults. At 4 days post-inoculation, large mottled spleens were taken and homogenised with a stomacher homogeniser (Tekmar Scientific Co., Cicinatti, OH) and a 10% (w/v) suspension in PBS was made. This suspension was used as splenic vaccine. A commercial freeze-dried cell culture vaccine was obtained and reconstituted as per instructions of the manufacturer. Both vaccines were titrated in SPF poults as described by Domermuth *et al.* (1977) and stord at -70°C until used. The splenic vac-

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cine had a titre of $10^{5.7}$ mean turkey infective doses ($TID_{50}$) and the cell culture vaccine had $10^{4.8}$ mean turkey infective doses $ml^{-1}$. Both vaccines were given orally @ 100 mean turkey infective doses $poult^{-1}$.

**Challenge virus:** The challenge virus used in this study was originally isolated from an outbreak of HE in Minnesota. The characteristics of this isoalte have been described by Zhang and Nagaraja (1989). The challenge dose given $poult^{-1}$ was $3 \times 10^3$ mean turkey infectious doses.

**Experimental design:** One hundred and thirty, 4-week old commercial poults, positive for various titres of maternal antibody were randomly divided into 3 groups. Birds in groups 1 and 2 (60 each) were vaccinated with the splenic and a commercial cell culture vaccine, respectively. The birds in group 3 (10 birds) served as unvaccinated controls. Thirty, 4-week old SPF poults, negative for maternal antibody were also divided into 3 groups of 10 birds each. Birds in group 1 were vaccinated with splenic vaccine and those in group 2 were vaccinated with cell culture vaccine. The birds in the third group served as unvaccinated controls. At 2, 3 and 4 weeks post-vaccination, sera from all birds were collected and tested for HEV-specific antibody with ELISA. At 4 weeks post-vaccination all vaccinated and unvaccinated controls were challenged with a virulent HEV. Clinical signs and mortality were recorded daily for 7 days post-challenge. At 7th day post-challenge, all birds were sacrificed by inhalation of $CO_2$. Lesions specific for HE including swollen and mottled spleens and haemorrhages in the intestinal tract were recorded, splenic index calculated and HEV specific antigen detected in spleens by AGP test.

**Serology:** All serum samples were tested for HEV specific antibody by an indirect ELISA as described by Ianconescue *et al.* (1984) with some modifications. Flat bottom 96 well plates (Nunc-Immunoplate MaxiSorp; Nunc Inc., Naperville, Ill) were coated with (12 ng $well^{-1}$) freon and polyethylene glycol purified HEV antigen prepared from HEV-infected spleen homogenate. The plates were washed with 100 $\mu$l of blocking buffer. Next, 197 $\mu$l $well^{-1}$ of blocking buffer were added followed by 3 $\mu$l of test serum in each well. The plates were incubated for 15 minutes at 37°C. After 4 washes with PBS, 100 $\mu$l of 1:1500 diluted goat anti-turkey IgG (Kirkeegaard and Perry Laboratories, Gaithersburg, MD) were added and plates incubated at 37°C for 15 minutes. The plates were washed 5 times with distilled water. After washing, 100 $\mu$l of substrate (0.7% para-nitrophenyl phosphate in diethanolamine buffer) were added and incubation was continued for 30 minutes at 37°C. The reaction was stopped by the addition of 50 $\mu$l of 3 M NaOH. Absorbance was read at 410 nm. HEV antigen in splenic homogenates was detected by AGP test as described by Domermuth *et al.* (1977). The data were thus analysed by using Student's t-test ($P<0.05$).

## RESULTS

**Antibody response:** Table 1 shows the seroconversion in SPF and commercial birds vaccinated with splenic and cell culture HE vaccines. Commercial birds vaccinated with splenic vaccine had significantly higher ($P<0.05$) antibody response as compared to birds vaccinated with cell culture HE vaccine. SPF birds vaccinated with splenic and cell culture vaccines had comparable antibody titre (Table 1).

**Protection:** Protection from HEV challenge is shown in Tables 2 and 3. Protection was calculated based on average of birds showing absence of HEV antigen and splenic index (spleen weight/body weight x 1000) less than 1.3. Splenic and cell culture HE vac-

**Table 1. Humoral antibody response of 4-week old poults to splenic (SV) and cell culture (CCV) HE vaccines**

| Source of birds | Vaccine type | HEV-specific ELISA absorbance at post-vaccination (weeks) | | |
|---|---|---|---|---|
| | | 2 | 3 | 4 |
| Commercial | SV | 0.8948* ± 0.3320 | 0.8132* ± 0.2141 | 0.7636* ± 0.2703 |
| | CCV | 0.6521 ± 0.1272 | 0.6112 ± 0.1069 | 0.5139 ± 0.1382 |
| | None | 0.2124 ± 0.1018 | 0.1401 ± 0.0133 | 0.0829 ± 0.0101 |
| SPF | SV | 0.9436 ± 0.1370 | 0.8623 ± 0.1202 | 0.7318 ± 0.1490 |
| | CCV | 0.8945 ± 0.1081 | 0.8438 ± 0.1520 | 0.7196 ± 0.1305 |
| | None | 0.0916 ± 0.0015 | 0.1062 ± 0.0129 | 0.1102 ± 0.0071 |

*Mean(s) with an astrick superscript is significantly different from the mean of CCV of the same week.

**Table 2. Protection from HEV challenge[A] in commercial turkey poults vaccinated with splenic (SV) and cell culture (CCV) HE vaccine**

| Vaccine type | Number of birds negative for | | SI[B] | | Poults protected[F] (%) |
|---|---|---|---|---|---|
| | Clinical HE[C] | HEV antigen[D] | Mean ± SE | Number with SI ≤ 1.3[E] | |
| SV | 58/60 | 57/60 | 0.85 ± 0.28 | 60/60 | 97 |
| CCV | 38/60 | 36/60 | 1.21 ± 0.36 | 35/60 | 61 |
| No | 0/10 | 0/10 | 1.78 ± 0.19 | 0/10 | 0 |

[A]Poults in each group were challenged 4 weeks post-vaccination.
[B]Splenic index (SI) = Spleen/body weight ratio x 1000.
[C]Number of birds positive for clinical HE: intestinal bleeding and/or death.
[D]HEV antigen detected by agar gel precipitation test.
[E]An SI ≤ 1.3 indicated protection unless intestinal bleeding was present.
[F]Protection from HEV challenge is the average of C, D and E above.

cines protected 97% and 61% of the commercial birds, respectively (Table 2). However, the protection afforded in SPF poults was 100% and 97%, respectively by splenic and cell culture HE vaccines (Table 3).

Table 3. **Protection from HEV challenge[A] in SPF turkey poults vaccinated with splenic (SV) and cell culture (CCV) HE vaccine**

| Vaccine type | Number of birds negative for | | SI[B] | | Poults protected[F] (%) |
|---|---|---|---|---|---|
| | Clinical HE[C] | HEV antigen[D] | Mean ± SE | Number with SI ≤ 1.3[E] | |
| SV | 10/10 | 10/10 | 0.68 ± 0.19 | 10/10 | 100 |
| CCV | 10/10 | 9/10 | 1.11 ± 0.15 | 10/10 | 97 |
| No | 0/10 | 0/10 | 1.79 ± 0.20 | 0/10 | 0 |

[A]Poults in each group were challenged 4 weeks post-vaccination.
[B]Splenic index (SI) = Spleen/body weight ratio x 1000.
[C]Number of birds positive for clinical HE: intestinal bleeding and/or death.
[D]HEV antigen detected by agar gel precipitation test.
[E]An SI ≤ 1.3 indicated protection unless intestinal bleeding was present.
[F]Protection from HEV challenge is the average of C, D and E above.

## DISCUSSION

This study was conducted to determine the efficacy of a csplenic vaccine and a cell culture vaccine in commercial and SPF turkeys. Both cell culture and splenic HE vaccines provided comparable antibody production and protection in SPF poults. It has been shown that in commercial turkeys, cell culture HE vaccine was interfered with by the presence of maternal antibody (Fadly and Nazerian, 1989). The results of the present findings confirm these obsrvations and add to the existing knowledge that splenic HE vaccine can induce seroconversion and protection in commercial birds probably, vaccinated at 4 weeks of age. This is the first report showing that splenic vaccine is more efficacious than cell culture vaccine. From the results of the present study and those of Fadly and Nazerian (1989), it is evident that the efficacy of cell culture vaccine might be interfered with by the existence of maternal antibody. Better seroconversion and provision of protection with splenic vaccine in this study demonstrate that this vaccine was capable of breaking through effect of maternal antibody and inducing vaccinal infection in the face of maternal antibody. The basis of this difference in efficacy of cell culture and splenic vaccine is not known. One possibility may be that replication of the vaccine virus *in vivo* (turkeys) may lead to the production of more viral proteins (antigen) per infectious unit of the virus. Since, the same number of infectious units of both the vaccines were used, the higher immune response and protection afforded by splenic vaccine suggest that more antigen per infectious unit in splenic vaccine may have a role in neutralizing maternal antibody and enabling splenic vaccine to provide better immunity than cell culture vaccine.

This study shows that it is possible to confer immunity and protection through the use of splenic vaccine at 4 weeks of age. Since cell culture vaccines are routinely used

in the field, maternal antibody levels at hatch and the age at which poults should be vaccinated with cell culture HE vaccine is needed. In that case, vaccination of turkey poults, having various levels of maternal antibody, with splenic and cell culture vaccines may help determine the proper age for HE vaccination. Though, HE outbreaks are occasionlly reported in the field after vaccination with cell culture vaccine, there are no data available. Such a comparative study should be done in field situation.

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