Preliminary studies on the development of vaccine against the ‘‘hydropericardium syndrome’’ of poultry

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Summary: A hydropericardium syndrome of broiler chicks, associated with an adenovirus, has caused considerable losses in Pakistan since August 1987.

Eight of ten chicks were protected from the disease by a vaccine prepared from infective liver suspension treated with formaldehyde. An oil-adjuvant vaccine protected only two of ten chicks, but challenge infection (after ten days) may have taken place before immunity could develop.

KEYWORDS: Aviadenovirus - Broilers - Hydropericardium - Pakistan - Poultry diseases - Vaccines - Viral diseases.

INTRODUCTION

Since August 1987, the “hydropericardium syndrome” has caused major economic losses in the nascent but fast developing broiler poultry industry of Pakistan. The syndrome is caused by a filtrable agent, and can be reproduced by subcutaneous inoculation of liver homogenate from the infected birds. We have detected an adenovirus in the liver homogenate from the infected birds (2).

The disease has been seen almost exclusively in broiler chicks 3-6 weeks old, although rare cases have been reported in layers and breeder pullets (1, 3). The disease appears suddenly at around twenty days of age. Illness usually lasts for 10-14 days, during which mortality is 30-60%. The most prominent gross lesion is the accumulation of clear, watery or jelly-like fluid in the pericardial sac. Other changes include petechial and ecchymotic haemorrhages in heart and other organs; swollen, pale and friable liver and pale, enlarged kidneys (1, 2, 3). Histologically, the basophilic, intranuclear inclusion bodies have been seen in hepatocytes (2).

This report describes a preliminary study to develop an inactivated vaccine against this syndrome.

MATERIALS AND METHODS

Birds

Forty one-day-old broiler chicks were procured from a local hatchery in Rawalpindi. These birds were reared under standard hygienic and management

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conditions and fed on a commercial feed. At the age of 10 days, they were divided into four groups, each of ten birds, and vaccinated.

**Preparation of vaccines**

Liver samples were collected aseptically from field cases of the hydropericardium syndrome. Liver (40 g) was cut into small pieces with sterile scissors and then ground in a glass tissue grinder with 30 ml of normal saline. Liver homogenate was centrifuged at 4000 rpm for one hour.

Supernatant fluid served as inoculum. For the preparation of vaccines, the virus present in the homogenate was inactivated by adding 0.5% formalin. The following four preparations were tested:

1. Oil-adjuvant vaccine was prepared by thoroughly mixing 6.2 ml of d-tocopheryl acetate with 0.8 ml Montanide 888 and 3 ml of liver homogenate.
2. Formaldehyde-inactivated vaccine was prepared as 30% liver homogenate in normal saline.
3. An oil-adjuvant control vaccine was prepared by using normal saline instead of liver homogenate.
4. Unvaccinated controls were inoculated with normal saline.

All preparations were injected subcutaneously in a dose of 0.5 ml.

**Challenge**

Ten days after vaccination, chicks of all groups were inoculated s.c. with 0.5 ml of infective liver homogenate in a dose equivalent to approx. 2000 LD$_{50}$. Dead birds were examined for hydropericardium and other lesions. All surviving birds were slaughtered three weeks after challenge.

**Histopathology**

Liver tissue was collected from the dead and slaughtered birds. Paraffin sections (5-7 µm thick) were cut and stained with haematoxylin and eosin. The sections were examined for inclusion bodies and other histological changes.

**RESULTS**

An oil-adjuvant vaccine and a formaldehyde-inactivated vaccine were prepared from liver homogenate obtained from naturally infected birds. Formaldehyde-inactivated vaccine gave good protection and 8 of 10 challenged birds were protected (Table I). In contrast, oil-adjuvant vaccine protected only 2 of 10 birds. Control birds (both oil-adjuvant and normal saline) died within five days of challenge, and typical lesions of hydropericardium were present.

Histologically, the birds vaccinated with formalinised liver homogenate did not have inclusion bodies in the hepatocytes. Prominent focal infiltration by heterophiles and lymphoid cells was seen in portal areas and elsewhere in the liver of three birds from this group. The livers from two slaughtered birds in the oil-adjuvant vaccine group that survived challenge showed mild to moderate fibrous tissue and bile-duct
TABLE I

Efficacy of different vaccine preparations against the hydropericardium syndrome

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. of birds vaccinated</th>
<th>Mortality after challenge</th>
<th>Birds showing hydropericardium</th>
<th>Percent protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver homogenate + oil-adjuvant</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Formalised liver homogenate</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>Oil-adjuvant control</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Normal saline control</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

proliferation spreading from portal areas into surrounding hepatic tissue. There was also mild infiltration by histiocytes. Megalocytes and inclusion bodies were not seen in these livers. No significant pathological change was observed in the liver of the surviving bird in the oil-adjuvant control group.

Although variable in intensity, uniform pathological changes were observed in the liver of birds with hydropericardium. There was moderate to severe fatty change, congestion and mononuclear cell infiltration in portal areas and around blood vessels. Megalocytes and basophilic intranuclear inclusion bodies were consistently present in all sections. Inclusion bodies were invariably seen when megalocytes were present in the sections.

DISCUSSION

The study indicates that formalinised liver extract can be used as a vaccine for protection against the hydropericardium syndrome in broiler poultry. Eight of ten birds vaccinated with formalinised liver homogenate survived a severe challenge. The two birds that died did not have gross or histological evidence of hydropericardium.

Birds vaccinated with the oil-adjuvant vaccine did not withstand the severe challenge of 2000 LD$_{50}$. The immune response to oil-adjuvant vaccines takes longer to develop, but then lasts longer. In this study, challenge occurred ten days after vaccination, at which time the immune response was probably not developed enough to withstand a severe challenge of 2000 LD$_{50}$.

Typical hydropericardium syndrome was reproduced in all but one control bird. Gross and histological changes were similar to the ones reported earlier by our laboratory (2). Basophilic inclusion bodies were always seen when sections contained megalocytes. It seems that these megalocytes are associated with viral infection and are either precursors of adenovirus inclusion bodies or associated with a co-infecting agent.
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Huit poulets sur dix ont été protégés contre la maladie par un vaccin préparé à partir d'une suspension de foie infectieux, traitée au formol. Un vaccin en excipient huileux n'a protégé que deux poulets sur dix, mais il est possible que l'épreuve virulente ait été réalisée trop peu de temps après la vaccination (dix jours) pour que l'immunité puisse s'installer.


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Resumen: Un sindrome del hidropericardio de los pollos de carne, asociado con un adenovirus, ha causado pérdidas considerables en Pakistán desde agosto de 1987.

Ocho de cada diez pollos se han protegido contra la enfermedad con una vacuna preparada a partir de una suspensión de hígado infectado tratada con formol. Una vacuna en excipiente de aceite sólo protegió a dos de cada diez pollos, pero es posible que la prueba virulenta se haya realizado demasiado pronto después de la vacunación (diez días) como para que la inmunidad haya podido instalarse.

PALABRAS CLAVE: Adenovirus aviar - Enfermedades aviares - Enfermedades virales - Hidropericardio - Pakistán - Pollos de carne - Vacunas.

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REFERENCES
