

Swollen head syndrome in Taiwan-isolation of an avian pneumovirus and serological survey

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SUMMARY Outbreaks of swollen head syndrome (SHS) were observed in two broiler and two broiler-breeder farms in Taiwan. The disease was characterized by oedematous swelling of the head, especially surrounding the eyelids, the neck and wattles. Avian pneumovirus and *Escherichia coli* were isolated from birds in all four farms. In addition, *Staphylococcus aureus* and infectious bursal disease virus were each isolated from one farm. A serological survey of 398 birds from 11 broiler breeder farms showed 86.4 % (344) of them had ELIA antibodies against turkey rhinotracheitis virus.

Key words: Swollen head syndrome, SHS, Avian pneumovirus

INTRODUCTION

Swollen head syndrome (SHS) of chickens is characterized by swelling of the periorbital and infraorbital sinuses (Alexander, 1991). The avian pneumovirus, turkey rhinotracheitis virus (TRTV), which causes turkey rhinotracheitis (TRT), is considered as a predisposing factor triggering SHS (Picault, *et al.*, 1987; Wyeth *et al.*, 1987). Although TRTV has been isolated from turkeys in many countries (Alexander, 1991), its isolation from chickens has only been reported in France (Picault *et al.*, 1987), South Africa (Buys *et al.*, 1989), and England (Jones *et al.*, 1991).

In Taiwan, the turkey population is small and no clinical TRT case has been observed. However, four cases of SHS were observed in chicken flocks in

northern Taiwan from February 1991 to July 1992 (Table 1). The clinical signs included nasal discharge, sneezing, coughing, facial swelling especially around the eye. In extreme cases the swelling gelatinous to purulent oedema in subcutaneous tissue and congestion of the mucosa of head, neck and wattles. The presence of increased amounts of mucus in the trachea; pneumonitis, perihepatitis and pericarditis were also observed. Microscopically, large amounts of fibrinous exudate, bacterial clumps and infiltration of mononuclear cells, plasma cells and heterophils were found in subcutaneous tissue of head lesions of acute cases. In more chronic cases, typical coliform granulomas characterized by coagulative necrotic foci surrounded by macrophages and giant cells of foreign body type were found. In the centre of the foci, bacteria colonies were noted. In case A, congestion and

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haemorrhage of the bursa of Fabricius and haemorrhage of the leg muscle were also noted. In case D, which had a history of infectious bursal disease (IBD), bursal atrophy was found.

Standard techniques were used for isolation of bacteria, mycoplasmas, and viruses. From all four cases, pure *E. coli* was cultured from subcutaneous lesions of the head, neck and wattle, and from heart and liver. Avian pneumovirus was isolated from tracheal samples from all four cases. In case A, IBDV was isolated from bursae of affected chickens. In case D, *Staphylococcus aureus* was isolated from lung, liver, spleen and kidney of affected chickens.

For avian pneumovirus isolation, tracheal samples collected from sick birds were made into suspension and the suspension inoculated onto tracheal organ culture (TOC) prepared from 19 to 20-day-old specific pathogen free (SPF) chick embryos. Each litre of TOC medium contained 1 g glucose, 30 ml 5.6 % bicarbonate solution, 0.05 g kanamycin, 0.05 g vancomycin and MEM. No ciliostasis was observed after the first passage, and supernatants were inoculated into further TOC. Ciliostasis was observed in all the samples 5 days after inoculation. The supernatant of the TOC showing ciliostasis were examined by electron microscopy. Virus particles with a morphology of the family Myxoviridae were seen. Tracheal emulsions were also inoculated into SPF eggs via the allantoic route in order to exclude viruses such as coronaviruses or other Myxoviridae. After five passages of 8 days each, no specific mortality, lesions or HA activity in the allantoic fluid were seen.

The third TOC passage of one isolate-strain LU was titrated alone and after neutralization with a monospecific TRTV antiserum. This serum was prepared in 2-week old SPF chickens inoculated with the attenuated TRTV strain, VOC₃, adapted to Vero cells (Rhone Merieux, AVIFFA™) and bled 5 weeks later. The virus titre without neutralization was 10^{6.6}, and after neutralization 10^{4.5}. Thus, the neutralization index (NI) was 10^{2.1}. The same antiserum had a NI of 10^{3.4} against a Vero cell-adapted chicken TRTV strain, PL₂₀.

To further identify the strain, we tried to adapt it to cell culture. The third TOC harvest was passaged

on Vero cells. No cytopathic effect (CPE) was observed at first passage. The infected cell layer of this passage was trypsinized and reseeded into culture flasks. Typical syncytium-type CPE appeared on the third day after seeding. The virus was harvest on the 4th day when the titre was 10^{6.8} TCID₅₀/ml. The same monospecific serum used above had a NI of 10^{2.7} against the Vero cell-adapted LU virus. Further identification was done by the β serum neutralization test against 100 TCID₅₀ of virus. The VCO₃ antiserum, homologous titre 8 log₂, gave also a titre of 8 log₂ with the LU strain. The strain was studied in immunofluorescence by Rhone Merieux, Lyon, France and confirmed as a pneumovirus by recognition with 26 different monoclonal antibodies produced against a chicken TRTV isolate (Picault *et al.*, 1987); two of them being specific for the 20 KD_a protein, typical of the pneumovirus genus (Le Gros, personal communication).

Serum samples from case A were tested with a TRT ELISA kit supplied by Rhone Merieux, Lyon, France. The mean optical densities (OD) of the sera collected at onset of disease and 4 and 5 weeks later were 0.736, 1.082 and 1.171, respectively; thus, the ODS at 4 and 5 weeks were 0.346 and 0.435 higher than those of sera collected at onset of disease. A difference of greater than 0.2 has been considered to indicate exposure to TRTV (Wyeth *et al.*, 1987).

A serological survey for TRT antibodies was conducted on 398 birds from 11 broiler breeder farms during 1992. The mean OD value of 30 SPF chicken sera plus 3 standard deviations was used as the cut-off value. The results showed that 86.4 % (344) of the birds surveyed had ELISA antibodies against TRTV (Table 2).

Although TRTV has been considered to be the primary causal agent of SHS in chickens, several researchers have failed to reproduce typical SHS by inoculating chickens with TRTV alone (Morley & Thomson, 1984; Jones *et al.*, 1987; Picault *et al.*, 1987). Also, TRTV antibodies have been found in apparent healthy flocks (Cook *et al.*, 1988). The complication of secondary bacterial infections like *E. coli* is thought to be important in the development of typical SHS. This was true in the four cases we observed in Taiwan; *E.*

coli was isolated in pure culture from them. Also, SHS has been observed associated with Newcastle disease, infectious bronchitis, and IBD (Morley & Thomson, 1984). Two of the cases reported here were also

associated with IBD. The immunosuppressive effect of IBD might contribute to the increased susceptibility of chickens to SHS.

Table 1. Four outbreaks of SHS in Taiwan in 1991 and 1992

| Flock | Chickens type | Age at outbreak | Date of outbreak | Flock size | Mortality (%) |
|-------|-----------------|-----------------|------------------|------------|---------------|
| A | Broiler | 26 days | Feb. 1991 | 12,000 | 13.3 |
| B | Broiler | 6 weeks | Oct. 1991 | 10,000 | 2.0 |
| C | Broiler breeder | 12 weeks | May 1991 | 7,000 | 4.6 |
| D | Broiler breeder | 11 weeks | July 1992 | 8,000 | 8.0 |

Table 2. Serological survey for TRT antibodies in broiler breeder flocks Taiwan

| Flock | No. positive/no. tested (%) | | Optical density | | |
|-------|-----------------------------|--------|-----------------|-------|---------------|
| | | | Mean | SD | Range |
| A | 46 / 65 | (70.8) | 0.540 | 0.200 | 0.084 - 1.013 |
| B | 55 / 55 | (100) | 0.953 | 0.140 | 0.680 - 1.220 |
| C | 39 / 40 | (97.5) | 0.676 | 0.154 | 0.367 - 1.051 |
| D | 40 / 40 | (100) | 0.817 | 0.140 | 0.469 - 1.029 |
| E | 38 / 38 | (100) | 0.813 | 0.164 | 0.461 - 1.329 |
| F | 40 / 40 | (100) | 0.932 | 0.146 | 0.653 - 1.250 |
| G | 15 / 38 | (39.5) | 0.413 | 0.171 | 0.172 - 0.829 |
| H | 20 / 20 | (100) | 0.613 | 0.153 | 0.415 - 0.987 |
| I | 20 / 20 | (100) | 0.720 | 0.092 | 0.594 - 0.987 |
| J | 10 / 17 | (58.8) | 0.480 | 0.200 | 0.175 - 1.071 |
| K | 21 / 25 | (84) | 0.793 | 0.326 | 0.138 - 1.173 |
| Total | 344 / 398 | (86.4) | 0.717 | 0.326 | 0.084 - 1.329 |

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RESUME

Syndrome Infectieux du gonflement de la tete (GTI) a Taiwan-Isolament d'un pneumovirus aviaire et etude serologique.

Des cas de syndrome infectieux de gonflement de la tete (GTI) se sont declares dans deux elevages de poulets de chair et deux elevages de reproducteurs de type chair a Taiwan. La maladie se caracterisait par un gonflement oedemateux de la tete, localise principalement autour des paupieres, du cou et des barbillons. De plus, la presence de *Escherichia coli* ont ete isolees parmi des sujets des quatre elevages. De plus, la presence de *Staphylococcus aureus* et du virus de la bursite infectieuse a ete mise en evidence dans un elevage. Une enqueteserologique portant sur 398 animaux de 11 elevages de reproducteurs de type chair a montre que 86.4 % d'entre eux presentaient des anticorps ELISA contre le virus de la rhinotracheite de la dinde.

ZUSAMMENFASSUNG

Swollen head-syndrom in Taowan-Isolierung eines aviaren pneumovirus und serologische erhebungen

Ausbrüche des Swollen Head-Syndroms wurden in zwei Mastkuken- und zwei Mastelertier-Herden in Taiwan festgestellt. Die Erkrankung war durch oedematöse Schwellung des Kopfes, hauptsächlich in der Umgebung der Augenlider, des Halses und der Kehllappen gekennzeichnet. Aviare Pneumovirus und *Escherichia coli* wurden von Tieren aller 4 Bestände isoliert. Außerdem wurden in einem Bestand *Staphylococcus aureus* und Bursitisvirus isoliert. Eine serologische Untersuchung von 398 Tieren aus 11 Mastelertierfarmen ergab, daß 86.4 % (344) ELISA-Antikörper gegen Puten-Rhinotracheitisvirus hatten.

RESUMEN

Síndrome de la cabeza hinchada en Taiwan. Aislamiento de un pneumovirus aviar y estudio serológico

Se observaron brotes del síndrome de la cabeza hinchada (SHS) en dos granjas de broiler y dos de reproductores de broiler en Taiwan. La enfermedad se caracterizó por una tumefacción edematosa de la

cabeza, especialmente alrededor de los párpados, el cuello y las barbillas. Se aisló un pneumovirus aviar y *Escherichia coli* en las aves de las cuatro granjas. Además se aisló *Staphylococcus aureus* y virus de la bursitis infecciosa en una granja. El estudio serológico de 398 aves procedentes de 11 granjas de reproductores de broiler mostró que el 86.4 % (344) de las mismas presentaban anticuerpos por el método de ELISA frente al virus de la rinotraqueítis del pavo.

台灣雞腫頭症候群研究：家禽肺病毒 之分離與血清學調查

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摘要 在本省共發現 4 個雞腫頭症候群病例，發生場之 2 戶為肉雞場，2 戶為肉種雞場，共飼有 37,000 隻雞，死亡 2,758 隻雞，平均死亡率 7.5 %。以病雞之氣管為病材，在 SPF 雞氣管環器官培養 (Tracheal ring organ culture) 分離病毒，結果由 4 個病例皆分離到家禽肺病毒 (Avian pneumovirus)。分離病毒以電子顯微鏡檢查呈現肺病毒之形態，並可馴化於 Vero 株化細胞，並形成特異性融合細胞性細胞病變，分離病毒並可被法國標準株 VCO₃ 株之抗血清中和，證明與法國所分離之家禽肺病毒株具相同之抗原性。發病雞群的配對血清有家禽肺病毒之抗體力價上升。另以一般的細菌及病毒分離培養技術，由病雞頭部皮下病灶處皆能分離到大腸桿菌，其中一病例並由華氏囊分離到雞傳染性華氏囊病病毒，另一病例由肺、肝、脾及腎分離到葡萄球菌。

初步調查本省 11 個肉種雞場 398 例血清，結果發現酵素結合免疫吸附法 (ELISA) 抗體十分普通，11 個肉種雞場皆為抗體陽性場，總陽性率高達 86.4 % (344/398)。

關鍵語：雞腫頭症候群，家禽肺病毒，氣管環器官培養

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