

From the State Veterinary Medical Institute, Helsinki, Finland.

INFECTIOUS LARYNGOTRACHEITIS IN FINLAND VIRUS ISOLATION

By

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Infectious laryngotracheitis (ILT) of fowls was first verified in the United States. *May & Tittler* (6) described the disease in Rhode Island in 1925. The viral etiology of the disease was established in 1930 by *Beach* (3) and by *Beaudette* (4). This disease is now quite widespread in Europe, e. g. *Bakos et al.* (1) verified it in Sweden in 1959.

In Finland there fairly often appear avian diseases that cause various degrees of symptoms in the upper respiratory organs. In some of the cases *Hemophilus gallinarum* can be isolated and in others some other bacteria (*Pasteurella*, *Pseudomonas* etc.); but in the majority neither a specific bacteria infection nor a specific fungus infection is found. For above a year this complex has been investigated in Finland by virological methods. Attention has been mainly directed to the following avian diseases that show respiratory symptoms: infectious laryngotracheitis (ILT), infectious bronchitis (IB), chronic respiratory disease (CRD) and Newcastle disease (ND).

The isolation of the ILT virus, which is presented in this report, may be regarded as being the first step in this investigation.

MATERIAL

The material, which is comprised of the lungs of a chicken, arrived in December 1962 for virological investigation from the Department of Pathology of the State Veterinary Medical Institute, to which a practising veterinarian had sent two chickens in

toto for examination. The clinical symptoms as well as the autopsy findings indicated an inflammation of the respiratory ducts probably caused by either a chemico-mechanical irritation or ILT virus.

METHODS

The propagation of the virus on chicken embryo chorioallantoic membrane (CAM) was carried out by a method described by *Burnet* (5). A 10^{-1} broth dilution was prepared from the lungs sent to be examined. 10,000 IE/ml of penicillin and 10 mg/ml of streptomycin were added to the suspension. 0.2 ml of the suspension was inoculated on the CAMs of 10 days old embryonated eggs using the dropped membrane technique. Half of the eggs were opened 3 days after inoculation and half 5 days after.

Neutralization tests were made as follows: To CAM supernatant (10^{-1} broth dilution) containing virus was added a like amount of serum, and the mixture was kept for an hour at room temperature, after which 0.1 ml of it was inoculated on the CAMs of 10 days old embryonated eggs. Opening of the eggs took place 5 days subsequently.

Agargel precipitation tests were made in Petri dishes by the method described by *Woernle & Brunner* (7). Homogenized CAM containing virus was used as antigen and chicken serum as antibody.

Test animals used in this investigation were a five-week old cock and two full-grown hens (White Leghorns), all of which got CAM suspension in 10^{-1} broth dilution 1.0 ml intratracheal.

As positive controls in the neutralization and agargel precipitation tests were used the ILT virus and the ILT serum obtained from Dr. *H. Woernle* of the Staatl. Tierärztl. Untersuchungsamt Stuttgart. The negative controls in these tests were corresponding normal CAM suspension and normal chicken serum.

RESULTS

Upon opening the eggs inoculated with a suspension prepared from the lungs sent to be examined, several greyish white opalescent pock-like lesions of 1—2 mm diameter were found on all the CAMs. The number of lesions increased constantly in the first three of the subsequent passages, of which 11 have been made up to now. On some CAMs there appeared around the place

of inoculation a greyish white disc formation of 20—30 mm diameter. At inoculation the eggs in various dilutions received $EID_{50} 0,2 \times 10^{-3,5}$ ml.

At the virus neutralization test the known ILT positive serum was found to neutralize the agent causing lesions on the CAM.

At the agargel precipitation test this agent also reacted with known ILT positive serum in forming a sharp precipitation line.

When the three test animals were inoculated with the agent to be investigated, the five-week old cock fell sick 3 days after inoculation, exhibiting severe respiratory troubles, and died in 6 days. Upon autopsy macroscopic hemorrhagic tracheitis was found. The agent mentioned was again isolated from the tracheal mucosa and propagated on the CAM of embryonated eggs.

There was no abnormal clinical findings in the two full-grown hens used in the test, but in blood samples taken 11 days after inoculation there were found in agargel precipitation tests antibodies against both the inoculated agent and the known ILT virus.

The normal bacteriologic control tests were negative at every stage of this investigation.

On the basis of all the tests mentioned above, the isolated agent which caused pock-like lesion on the CAMs of embryonated eggs was considered to be ILT virus, and for the first time the presence of this disease in Finland was verified with scientific certainty.

ACKNOWLEDGEMENT

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SUMMARY

The author has isolated from the lungs of a chicken, that died from a respiratory disease of unknown etiology, an agent forming pock-like lesions on the chicken embryo chorioallantoic membrane. The author has classified this agent as infectious laryngotracheitis virus. Thus, this virus and the disease caused by it have been verified also in Finland.

ZUSAMMENFASSUNG

Die infektiöse Laryngotracheitis in Finnland. Virusisolierung.

Der Autor hat aus den Lungen eines Huhnes, das an einer respiratorischen Krankheit unbekannter Aetiologie starb, ein Agens isoliert, welches auf der Chorioallantoismembran embryonierter Eier pocken-ähnliche Knötchen hervorruft. Das Agens wurde von dem Autor als das Laryngotracheitisvirus klassifiziert. Somit ist dieses Virus und die von diesem Virus verursachte Krankheit auch in Finnland festgestellt worden.

SAMMANFATTNING

Infektiös laryngotracheitis i Finland. Virusisolering.

Författaren har isolerat ett agens från lungorna av en höna, som dog i en respiratorisk sjukdom av okänd etiologi. Detta agens förorsakar pox-liknande lesioner på chorioallantoishinnan i embryonerade hönsägg, och har av författaren klassificierats såsom infektiöst laryngotracheitvirus. Sålunda har detta virus och av detsamma förorsakad sjukdom konstaterats även i Finland.

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