P37 THE SEROPREVALENCE AND IDENTIFICATION OF ORNITHOBACTERIUM RHINOTRACHEALE (ORT) FROM BROILER AND BROILER BREEDER FLOCKS IN THAILAND

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Introduction and Objectives

A major problem for the poultry industry around the world is a respiratory tract infection which can cause major economic impact. ORT is a slow growing, gram negative bacterium. ORT infections have been reported in many countries around the world. The objective of this study was to determine the prevalence of ORT infection, its isolation and identification in broilers and broiler breeders in Thailand.

Materials and Methods

Broiler and broiler breeder serum samples. Serum samples were collected from 17 broiler farms and 23 broiler breeder farms from the major broiler producing companies of Thailand during the period Oct. 2004 to Sep. 2005. Samples were kept at -20°C until tested. ***ELISA***. Sera were analyzed by indirect ELISA with an ORT antibody test kit. ***Bacteria***. The bacteria were collected from chickens on the seropositive farms by swabbing from the air sacs of those with respiratory problems. The bacteria were cultured in a Columbia agar base with 5% sheep blood and 5 µg/ml gentamicin under microaerophilic condition, for 48-72 hr. Gram negative, catalase negative and oxidase positive colonies were tested by PCR. ***PCR analysis***. Primers used in this study were OR16S-F1 (5'-GAGAATTAA TTTACGGATTAAG-3') and OR16 S-R1 (5'-TTCGCTTGGTCTCCGAA GAT-3'), which flanked a 784-bp DNA sequence. PCR was conducted. A reaction volume of 20 µl contained 10XPCR buffer with $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 1.0 µM of each primer, 50-100 ng DNA Template, 1.5U Taq DNA Polymerase. Samples were subjected to 94°C, 90 sec followed by 35 cycles at 58°C, 60 sec and 72°C, 90 sec. PCR products were examined in 1.8% agarose gels with 0.5X TBE buffer, stained with 10 µl of ethidium bromide (20 mg/ml), and exposed to ultraviolet light.

Results and Discussion

The broiler breeder ELISA results were 12.2%, 38.0%, and 49.8%, neg., suspected and pos., respectively. The broiler ELISA results were 67.5%, 12.9% and 19.6%, neg., suspected and pos., respectively. ORT vaccines have not been used in Thailand so, the suspected results may be regarded as positive results. The suspected+positive results in the broiler breeder farms are 87.84% and the older birds were more positive than the younger birds. The suspected+positive results in the broiler farms were 32.5%. Nine isolates of the twelve PCR analysis samples revealed positive results to PCR analysis. Our studies are the first report confirming seropositive birds and the incidence of ORT in Thailand.

References

1. van Empel, P., van den Bosch, H., Loeffen, P. and Storm, P. 1997. Identification and serotyping of Ornithobacterium rhinotracheale. J. Clin. Microbiol. 35: 418-421.