THE DETECTION OF *Staphylococcus aureus* IN SWIFTLETS' NEST USING IMMUNOHISTOCHEMISTRY (STREPTAVIDIN BIOTIN)

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ABSTRACT

A study to detect the presence of Staphylococcus aureus *in swiftlets' nest using immunohistochemistry (Streptavidin biotin Complex) has been successfully done. Tissue and supernatant were made from the nest, and the presence of the bacteria* Staphylococcus aureus *was detected by means of immunohistochemical method. As positive control, we used* Staphylococcus aureus *culture, while for negative control we replaced* Staphylococcus aureus *monoclonal antibody with Phospat Buffer Saline (PBS). The result showed that staining with* Staphylococcus aureus *monoclonal antibody in swiftlets' nest tissue revealed the presence of* Staphylococcus aureus *as a brownish group or cluster, resulting from the reaction of enzymes and chromogen in Streptavidin Biotin Complex. Based on this study, it can be concluded that immunohistochemical method (Streptavidin Biotin Complex) can be used to detect the presence of* Staphylococcus aureus *in swiftlets' nest.*

Keywords: *swiftlets' nest, S. aureus, immunohistochemistry*

INTRODUCTION

A major challenge for Indonesia is to produce animal food products which are safe for consumers' health. Animal food safety is not only the world's issue (Anonym, 2000), but also every indivual's concern. It is a consumer's right to have safe animal food. Indonesia is the largest producer and supplier of swiftlets' nest, with Hongkong, USA, Singapore, Malaysia, China, Japan, and UK as the major export destinations (Iswonto, 2002). Playing the role as largest producer, Indonesia should maintain the aspect of food quality as the main consideration in trade. Market requirement and product suitability for consumers should be met by increasing product acceptability and competitiveness in global market (Anonym, 2000).

Swiftlets' nest is an exotic or delicate food. In addition as a delicious serving, it can also be used as material for medications that improves physical strength (Winarno, 1994; Budiman, 2002; Iswanto, 2002). As in other food materials, swiftlets' nest may subject to damage resulting from pesticide residuals, animal drugs, heavy metals, other contaminants, as well as the growth of microbes, such as bacteria, virus, yeast, and fungi, which may cause food-borne disease.

To support the availability of safe food products as the basic consideration in trade, we need microbial detection method for swiftlets' nest. In a preliminary study, Animal Quarantine Board (Balai Karantina Hewan) in cooperation with Airlangga University School of Pharmacy had undergone a test on Microbiological Quality Control for export swiftlets' nest in Animal Quarantine Juanda (unpublished data). The result of this preliminary study, obtained using rapid test (Oxoid, United Kingdom) and followed with fertilization in agar media, showed that Staphylococcus spp was identified in five samples of swiftlets' nest, while Escherichia coli was identified in one sample. *Salmonella spp* and *Pseudomonas spp* were not found.

Staphylococcus spp is a group of bacteria that plays an important role in food microbiology, and *Staphylococcus aureus* is the prominent bacteria in food because during its growth the organism can produce enterotoxin. Ecologically, *Staphylococcus aureus* is closely related with human beings. In largest amount of cooked or salted foods, *Staphylococcus aureus* can unceasingly grow until reaching a hazardous level (Buckle, 1987). Based on this preliminary study, a fast and accurate method to detect *Staphylococcus aureus* in swiftlets' nest was needed. The method that is recently developing is the use of immunohistochemistry by means of the principle of specific binding between antigen and antibody which was visualized through enzymes and substrates. This method used basic principles of immunology in tissue or cells.

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MATERIALS AND METHODS

This study used swiftlets' nest samples ready to be exported through Juanda Airport. The samples were processed to make preparations in Veterinary Disease Inspection Bureau (Balai Penyidikan Penyakit Veteriner, BPPV) Regional IV Yogyakarta in accordance with standard procedure of BPPV laboratory. The swiftlets' nest preparation in paraffin embedded tissue section was put onto Poly-L-lysin (SIGMA)-coated glass object. The immunohistochemical staining used Streptavidin Biotin, with stages as recommended by Wasito (1997). The swiftlets' nest preparation was paraffinized by giving (a) xylene, three times each for 2 minutes, (b) 100% ethanol, twice each for 2 minutes, (c) 95% ethanol, once each for 2 minutes, (d) 50% ethanol, each for 2 minutes, and (e) distilled water, twice each for 2 minutes, and Phosphate Buffer Saline (PBS) of 0.01 m with pH 7.1 for 5 - 10 minutes. Subsequently, the preparation was immersed in H2O2 to remove endogeneous peroxidase, and incubated in a microwave. The preparation was then washed with PBS for 10 minutes, and incubated with blocking serum (V Block) solution for 10 minutes. The excessive serum was removed from the preparation and the latter was directly given with primer antibody, i.e. *Staphylococcus aureus* monoclonal antibody and incubated at room temperature for 45 minutes, and washed with PBS for 10 minutes. Antigen retrieval was done using citric acid and microwaved for 10 minutes (Shan et al, 1997). The preparation was incubated with Biotynilated Secondary Antibody (Lab Vision, USA) at room temperature for 10 minutes, incubated with chromogen substrate (Lab Vision, USA) at room temperature for 15 minutes, washed with distilled water, and mounted with glycerol to be observed under the microscope.

For culture preparation of the isolates of *Staphylococcus aureus* and supernatant from swiftlets' nest sample, the stages of immunohistochemical staining were the same as those in paraffin-embedded tissue section. The difference was that in supernatant preparation, the swiftlets' nest should be paraffinized and washed directly for 5 minutes, while the rest of the procedures were all the same. To obtain supernatant preparation, 5 grams of swiftlets' nest sample were finely ground, added with physiologic NaCl and left overnight. Subsequently, the preparation was dripped on Poli-L-lysine-coated glass object, and incubated in microwave for 12 hours and subjected to immunohistochemical staining using Streptavidin Biotin method (Wasito, 1997). As positive control for this staining method, we used *Staphylococcus aureus* colony. Negative control was made by replacing primary antibody with Phosphate Buffer Saline (PBS).

RESULTS AND DISCUSSION

The objective of this study was to apply immunohistochemical method by using Streptavidin Biotin Complex. This technique is a modification of indirect method, in which one antigen from swiftlets' nest is bound by antibody in two stages. First, the primary antibody is directly bound to antigen. Afterwards, the antibody will be bound to biotinilyzed-primary antibody. The binding between antigen and antibody would be visualized by the change of enzymes and substrates into brownish color (Hoffman, 1996; Wasito, 1997; Harkow F and Lane, 1999). The application of immunohistochemical method is immunohistochemical staining to culture, supernatant, and swiftlets' net tissue. Immunohistochemically-stained *Staphylococcus aureus* culture from the nest revealed brown precipitation, indicating the binding between antigen and antibody as visualized through the reaction of peroxidase and 3,3 diaminobenzidine tetrahydrochloride (Figure 1). The *Staphylococcus aureus* looks grouped or clustered.

Immunohistochemically-stained swiftlets' nest supernatant showed the presence of antigen (*Staphylococcus aureus*) and antibody binding, which was visualized by the presence of brown precipitation (Figure 2). This was in line with the basic principle of chromogen, a marker that can visualize marker substance at immunocomplex binding in immunohistochemical staining. In this principle, the binding between chromogen and peroxide (marker substance) is visualized brown by using 3,3 diamonobenzidine tetrahydrochloride chromogen (Baroff and Cook, 1994; Wasito, 1997). The paraffin embedded tissue section of swiftlets' nest using Streptavidin Biotin method (Lab Vision, USA) showed the result of antigen (*Staphylococcus aureus*) and antibody binding visualized as having brown color (Figure 3).

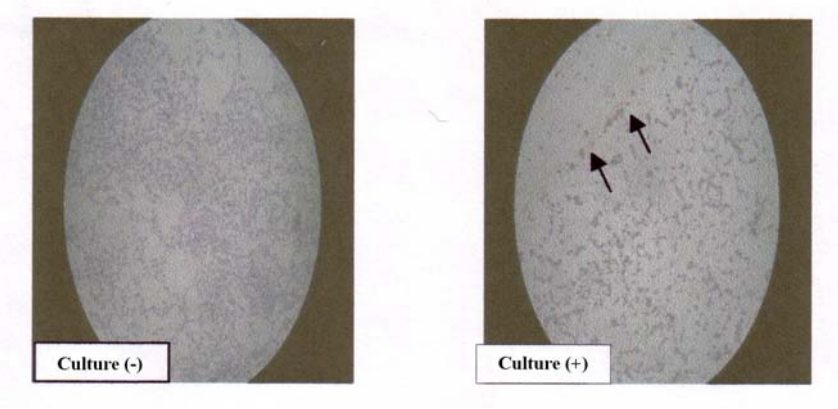

Figure 1. *Staphylococcus aureus* culture in swiflet's nest

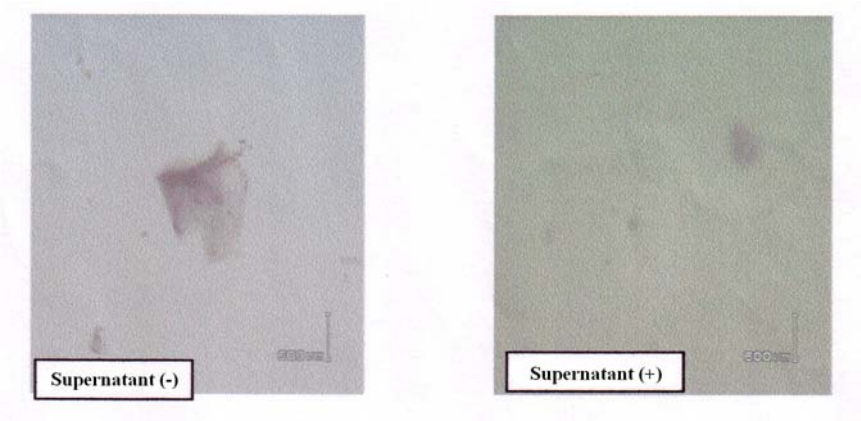

Figure 2. *Staphylococcus aureus* supernatant in swiflet's nest

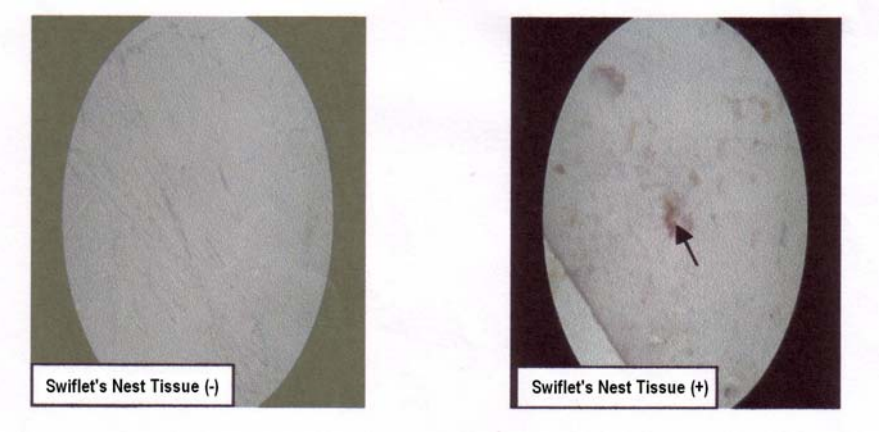

Figure 3. Swiflet's nest tissue

Those results showed that immunohistochemical method using Streptavidin Biotin can be used to detect *Staphylococcus aureus* in supernatant and swiftlets' nest tissue. Previous studies were reported by Cleary et al (2004), Priambodo (2004) and Tsusumi et al (1991) who detected bacteria in intestinal epithelium, blood and urine using immunohistochemical staining. In these studies the bacteria was apparent in the form of cluster or clump (bacterial coated antibody).

In culture preparation using immunohistochemical staining (Streptavidin biotin), the supernatant and preparation from swiftlets' nest tissue had a brown color, a result of binding between antigen (*Staphylococcus aureus*) and its monoclonal antibody which was visualized through chromogen substrate in the colony of the bacteria that formed a group or cluster (Duguid, 1989).

CONCLUSION

Immunohistochemical staining can be used to detect the presence of *Staphylococcus aureus* in swiftlet's nest.

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