

Characterization of *Avibacterium paragallinarum* Caused Infectious coryza/Snot: Satellite Colony Phenomenon

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INTRODUCTION

Infectious coryza (IC) is an acute upper respiratory disease of poultry that can appear in all ages. Some of clinical signs that are commonly seen in IC are rhinitis, facial swelling or edema, lacrimation, anorexia, and retarded growth in young poultry [1,2,3]. The disease can be found worldwide, especially in tropical countries [4]. Infectious coryza is very important in the chicken farm industry in developed and developing countries, including Indonesia [5]. The large economic losses due to IC such as increased number of culling, decreased egg production (10-40%), decreased body weight, stunting growth, and some mortality (2-10%) [4].

Avibacterium paragallinarum which was previously classified as *Haemophilus paragallinarum* is a causative agent of infectious coryza in laying and broiler chickens, quail, pearl chicken, turkey, and peacocks [4,6,7,8]. The bacteria is commensal in the mucous membrane of the upper respiratory system, is sensitive to preservation and does not last long outside the host body [8]. Factors X and V are needed for the growth of several types of *A. paragallinarum*. According to *in vitro* growth requirements, *A. paragallinarum* can be either nicotinamide adenine dinucleotide (NAD) independent or NAD-dependent. The reduced form of NAD (NADH; 1.56-25 µg/ml) and the oxidized form (20-100 µg/ml) is required for *in vitro* growth in most isolates *A. paragallinarum* that show satellitic colony on a medium [9]. The description of the need for V factor of field isolates *A. paragallinarum* has been few reported. The aim of this research is to find out the phenomenon of satellite colonies from a variety of poultry isolates.

MATERIALS AND METHODS

Sample collection

The samples were collected from layers, broilers, and quails with typical facial edema and discharges from nasal and infraorbital sinuses. The

quails were from commercial farm in Indonesia. The study was done in Microbiology Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Isolation and identification

The initial inoculation was performed onto chocolate agar plate and then incubated in the 5% CO₂ incubator at 37°C for 24-48 h. The suspected colony of *A. paragallinarum* was then stained using Gram staining method, tested for catalase test, oxidase test, motility test, urease test, indole test, and carbohydrate fermentation test [10,11].

Satellite colony test

Satellite colonies test with isolates *A. paragallinarum*, which was cultured using the S-streak method to the blood agar plate medium and then cross-streaked with a bacterial feeder that was stiffly perpendicular to the scratch *A. paragallinarum* [7]. *Staphylococcus hyicus* were used as bacteria feeder. The medium incubated in the 5% CO₂ incubator at 37°C for 24-48 h. The phenomenon of satellite colony is showed the growth of *A. paragallinarum* around *S. hyicus* (NAD-dependent).

RESULT AND DISCUSSION

A number of *A. paragallinarum* have been isolated from layers, broilers, and quails. Morphology colony of *A. paragallinarum* on chocolate agar plate medium is circular, transparent, and smooth dewdrops ([12]. Gram stain of *A. paragallinarum* showed that bacteria were coccobacilli morphology and red color (Gram-negative) [13,1]. Colonies with morphological characteristics leading to *A. paragallinarum* colonies were re-cultured until got pure colony.

All isolates showed biochemical characteristics of *A. paragallinarum*, i.e., negative catalase, negative oxidase, negative urease, negative in indole test, and capable to fermenting

all carbohydrate that used, which are also reported by Blackall and Soriano [7]. In this study, two of five isolates from quails showed low fermentation of lactose, while one of four isolates from layer showed negative fermentation of sorbitol. The same results were reported by Akhter *et al.* [1] and Akhter *et al.* [14], that ability of fermenting lactose and sorbitol is variable.

The colony which identified as *A. paragallinarum* in biochemist test was cultured to blood agar plate medium and added with *Staphyococcus hycus* as bacterial feeder [15,16]. The *A. paragallinarum* that needs V factor would grow alongside the bacteria feeder and form satellite colony [4,6,8,17], while some *A. paragallinarum* species could grow even without V factor. The addition of *Staphyococcus hycus* onto BAP medium in this study showed that *A. paragallinarum* isolates from layer were NAD-dependent (100%), while NAD-independent isolates of *A. paragallinarum* detected from broiler (80%) and quail (100%). Characteristic of NAD-independent colony had bigger size (1-2 mm) compared with NAD-dependent colony and did not show any satellite colony [18].

| Isolate /Host | C / O | M | U | I | L | M l | M n | S | N A D |
|---------------|-------|-----|---|---|---|-----|-----|---|-------|
| 1/Layer | - /- | N M | - | - | + | + | + | + | D |
| 2/Layer | - /- | N M | - | - | + | + | + | - | D |
| 3/Layer | - /- | N M | - | - | + | + | + | + | D |
| 4/Layer | - /- | N M | - | - | + | + | + | + | D |
| 5/Broiler | - /- | N M | - | - | + | + | + | + | D |
| 6/Broiler | - /- | N M | - | - | + | + | + | + | ID |
| 7/Broiler | - /- | N M | - | - | + | + | + | + | ID |
| 8/Broiler | - /- | N M | - | - | + | + | + | + | ID |
| 9/Quail | - /- | N M | - | - | + | + | + | + | ID |
| 10/Quail | - /- | N M | - | - | + | + | + | + | ID |
| 11/Quail | - /- | N M | - | - | + | + | + | + | ID |
| 12/Quail | - /- | N M | - | - | + | + | + | + | ID |
| 13/Quail | - /- | N M | - | - | + | + | + | + | ID |

C=catalase; O=oxidase; M=motility; U=urease; I=Indole; L=lactose, Ml=maltose; Mn=mannitol; S=sorbitol; NM=non-motil; D=NAD-dependent; ID=NAD-independent; NAD= Requirement for factor V (NAD); *low fermentation.

Previous study report that NAD-independent isolates have more virulence level compared to NAD-dependent, because that isolates may have an ability to avoid host immune responses. It may also cause vaccine failure if the vaccine based on NAD-dependent *A. paragallinarum* strain [16,19].

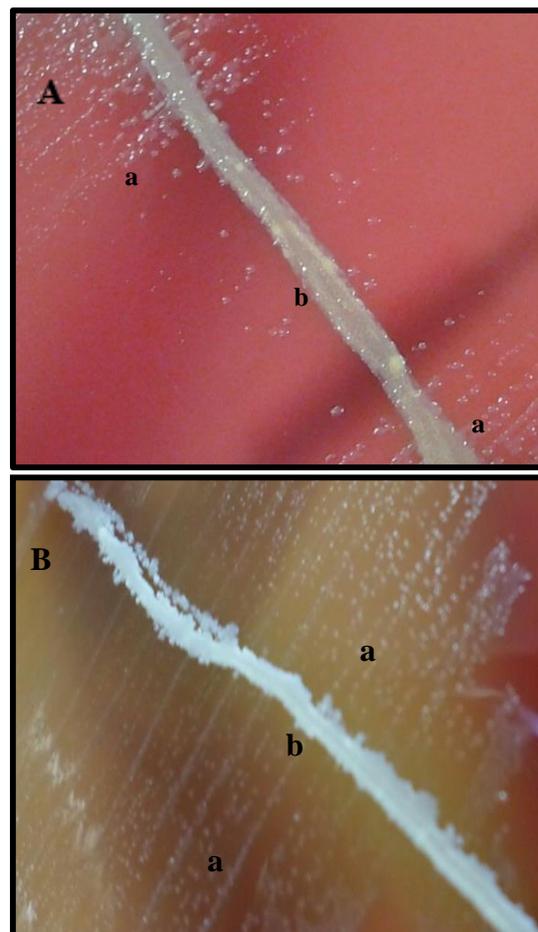


Figure 1. (A) Isolate *A. paragallinarum* (a) origin from layer grow alongside the *S. hycus* (b) and form satellite colony. (B) Isolate *A. paragallinarum* (a) origin from broiler growing spread not only alongside the *S. hycus* (b), but also on the medium surface.

CONCLUSION

In this study showed that *A. paragallinarum* isolates from layer were NAD-dependent (100%), isolates from broiler were NAD-independent (80%), and isolates from quail were NAD-independent (100%). Determination of NAD dependency properties is very important for the determination vaccine isolates used for prevention of infectious coryza.

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