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Short Communication: Detection Of The Serotype Of *Avibacterium Paragallinarum* From Indonesian Poultry Field Isolate Using Hemagglutination Inhibition Test

A.E.T.H. Wahyuni[1], Fadhli Nanda Putra[2], Low Kar Yee[3], Tan Yun Ru[3], Cheng Ern Wei[3], Yahya Pambudhi[3], Puteri Nur Natasha[3]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia;
[2]Student of Postgraduate Program of Veterinary Science, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia;
[3]Student of Undergraduate Program of Veterinary Science, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.
*Corresponding author : e-mail: wahyuni_aeth@ugm.ac.id
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ABSTRAK

Infectious coryza (IC) merupakan penyakit saluran pernapasan atas yang bersifat oportunistik dan menular pada unggas. Penyakit ini disebabkan oleh *Avibacterium paragallinarum*. Sifat infeksi penyakit ini dikenal memiliki morbiditas yang tinggi namun dengan mortalitas yang rendah. Kerugian utama yang disebabkan oleh IC adalah berkurangnya produksi telur dan peningkatan rasio konversi pakan (FCR). Ada tiga serovarian *Avibacterium paragallinarum* yang dikenal, yaitu serovarian A, B, dan C. Laporan mengenai serovarian *Avibacterium paragallinarum* di Indonesia masih sangat terbatas. Penelitian ini bertujuan untuk mendeteksi serovaran *Avibacterium paragallinarum* dari isolat peternakan unggas di Indonesia menggunakan uji hemaglutinasi inhibisi (HI). Sepuluh isolat lapang *Avibacterium paragallinarum* diidentifikasi ulang untuk kemudian diolah menjadi larutan antigen menggunakan teknik sonikasi yang selanjutnya digunakan untuk uji hemaglutinasi (HA). Setelah titer unit 4HA diperoleh, antigen diuji dengan HI menggunakan antisera referensi (Strain 221 serovar A, strain Spross serovar B, dan regangan Modesto, serovar C). Hasil penelitian menunjukkan bahwa satu isolat memiliki titer paling tinggi dengan antiserum serovarian A yaitu >5120 HI unit dan sembilan isolat memiliki titer tertinggi dengan antiserum serovarian B yaitu >5120 HI Unit. Oleh karena itu, dapat disimpulkan bahwa satu isolat lapang *Avibacterium paragallinarum* dari ayam petelur adalah serovarian A dan sembilan isolat lapang dari *Avibacterium paragallinarum* dari ayam petelur, ayam pedaging, ayam kampung, puyuh isolat lapangan merupakan serovarian B.

Kata kunci : *Avibacterium paragallinarum*, serotipe, penghambatan hemaglutinasi, isolat

ABSTRACT

Infectious coryza (IC) is an opportunistic and infectious upper respiratory tract disease in poultry, caused by *Avibacterium paragallinarum*. This disease has high morbidity but low mortality. The major losses caused by IC are the reduced egg production and increased feed conversion ratio (FCR). There are three recognized *Avibacterium paragallinarum* serovars, which are serovar A, B, and C. Limited reports regarding the serovars of *Avibacterium paragallinarum* in Indonesia are available. This research was done to detect the serovar of *Avibacterium paragallinarum* from Indonesian poultry field isolate using hemagglutination inhibiotion (HI) test. Ten field isolates of *Avibacterium paragallinarum* were re-identified, then processed into antigen solution using sonication, further on used for hemagglutination (HA) test. After the 4HA unit titer was obtained, the antigens were tested with HI using reference antisera (Strain 221 serovar A, strain Spross serovar B, and strain Modesto, serovar C). The results showed that one isolate had the highest titer with antiserum serovar A which was >5120 HI unit and nine isolates had the highest titer with antiserum serovar B which was >5120 HI Unit. Therefore, it can be concluded that one field isolates of *Avibacterium paragallinarum* from layer chicken is serovar A and nine field isolates of *Avibacterium paragallinarum* from layer, broiler, free range chicken, quail field isolates are serovar B.

Keywords : *Avibacterium paragallinarum,* serotyping, hemagglutination inhibition, poultry field isolates.

INTRODUCTION

Infectious coryza (IC), or snot, is an acute upper respiratory disease that often affects in poultry and is caused by *Avibacterium paragallinarum* (Blackall & Soriano, 2008). Some of the clinical signs that are commonly seen in IC are nasal discharge, conjunctivitis with swelling of the sinuses, face and wattles, lacrimation, diarrhea, decreased feed and water consumption, anorexia, and retarded growth in young chickens (Akter *et al*., 2013; Blackall, 1999).

There are three recognized serovars of Avibacterium paragallinarum according to the Page serotyping scheme, they are serovar A, serovar B, and serovar C. All three serovars are known to have distinct immune response since inactivated vaccine of one serovar does not provide protection to individuals infected with the other serovars. However, cross protection may occur within the same serovar (Blackall, 1999). Therefore, it is important to detect the serovar for suitable vaccination.

The hemagglutination inhibition (HI) test is most frequently used serological test in diagnosing and serotyping of Avibacterium paragallinarum (Gracia *et al.,* 2008). Glutaraldehyde-fixed chicken erythrocytes are used in HI test. It can be prepared by mixing 1-2% of washed chicken erythrocyte with 1% of glutaraldehyde solution which is then incubated at 40C for 30 minutes with occasional mixing (Blackall and Soriano, 2008). This study aimed to determine the serovar Avibacterium paragallinarum isolates from Indonesian poultry field isolates using HI test with reference antibodies of serovar A (211), serovar B (Spross), and serovar C (Modesto).

MATERIALS AND METHODS

Ethical approval

The samples were collected in accordance with standard collection procedure without hurting or necrotizing animals.

Study period and location

The research was conducted from December 2018 to July 2019 in the Microbiology Department, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Sample collection

Ten isolates *Avibacterium paragallinarum* were collected from various poultry (layer, broiler, free range chicken, and quail). The isolates were from several poultry farms in Indonesia.

Isolation and identification of A. paragallinarum

The nasal exudate samples were cultured on a chocolate agar plate (Oxoid™, Basingstoke, UK), added with 5% sheep blood at 80 °C, and incubated in an anaerobic jar for 24-48 h at 37 °C. Identifications were made based on bacterial colony and cell morphology with Gram staining and biochemical tests (catalase test, oxidase test, urease test, meavesotility test on semisolid media, indole test, and carbohydrate fermentation tests using lactose, maltose, mannitol, and sorbitol) [Akter *et al*., 2013; Jeong *et al*., 2017; Wahyuni *et al*., 2018].

Antigen preparation

Avibacterium paragallinarum is cultured in BHI broth, supplemented with NAD as supporting growth factor. The broth is incubated in 37 °C with 5-10% CO_2 for 18-24 h The broth is then washed twice with PBS, each time centrifuged at 3000 rpm for 10 minutes. The supernatant is removed and PBS is homogenized with sediment before it is sonicated for there minutes. The sonicated sediment is then added with adequate amount of NaCl 0.15 M so that the cloudiness of the solution matches with Mac Farland No. 5 standard solution (Swata *et al.,* 1982).

Hemagglutination test

A two-fold serial dilution of Avibacterium paragallinarum antigen is prepared in a *microplate,* from the first to the 8th well is used as control. The first well was filled with 80 µl of diluent, while the rest contained 40 ul. The volume of antigen in each well was 20 ul with increasing dilution. Forty ul of 1% glutaraldehyde-fixed chicken erythrocytes are added into each well, then incubated in room temperature until the erythrocytes in the 12th well forms a pinpoint shape (Eaves *et al*., 1989). The concentration of antigens is adjusted until a results of 4HA unit achieved.

Hemagglutination-inhibition test

A two-fold serial dilution of antisera is prepared in a microplate, each well contained 20 ul of antisera with increasing dilution, the 12th well is used as control. Fourty µl of 4HA unit *Avibacterium paragallinarum* antigen is added into each well, except the 12th well. The solution is allowed to sit and mix at room temperature for 15-30 minutes. Then, 40 ul of 1% glutaraldehyde-fixed chicken erythrocytes are added into each well and incubated at room temperature until the erythrocytes in the last well forms pinpoint

shape. All well are observed and the well that initiates hemagglutination (does not form pinpoint) is recorded as the HI unit (Chukiatsiri *et al.,* 2010).

RESULTS

Ten samples were found positive for *Avibacterium paragallinarum.* The isolates showed tiny, circular, transparent, dewdrop-like Gram negative coccobacilli colonies based on Gram staining. The isolates were non-motile, negative in catalase, oxidase, indole, and urease tests, and were able to ferment lactose, maltose, mannitol, and sorbitol based on biochemical tests (Table 1). The antigen used was sonicated. The hemagglutination test is necessary before hemagglutination inhibition (HI) test as 4HA unit of antigen concentration is required for HI test (Eaves *et al.,* 1989) and also to conform the ability of bacteria to agglutinate erythrocyte. To obtain 4HA unit, the first pinpoint should be formed in the fourth well of the microplate. The 4HA unit antigen was used to perform hemagglutination inhibition (HI) test immediately as further prolongation may cause alteration in concentration of antigen. Each isolate was tested with antibodies of all three serovars, which are serovar A strain 221, serovar B strain Spross and serovar C strain Modesto. in Table 2 shows the HI unit of each isolate sample towards antibody of each serovar.

DISCUSSION

A total of 10 isolates from several poultry farms in Indonesia, were used as samples. The nasal exudates were cultured on a chocolate agar plate. The colony morphology of 24 isolates showed round, small, transparent, and dewdrop-like colonies, which is similar to the previous studies (Wahyuni *et al,* 2018; Tangkonda *et al.,* 2019; Akter *et al.,* 2014). Gram staining was done on 24 isolates and showed Gram negative coccobacilli. The results are similar to the findings of Patil *et al.* (2016) and Deshmukh *et al.* (2015) Then, the isolates underwent biochemical tests, such as catalase, oxidase, urease, indole, motility, and carbohydrate fermentation tests. A total of 10 isolates identified as *A. paragallinarum* showed negative results in catalase, oxidase, indole, motility, and urease tests, which were

Table 1 The biochemical test results of the suspected isolates of *Avibacterium paragallinarum.*

Test	Results	Confirmed isolates
Catalase	-	10
Oxidase	-	10
Urease	-	10
Indole	-	10
Motility	-	10
Lactose	+	10
Maltose	+	10
Mannitol	+	10
Sorbitol	+	10

Table 2 The hemagglutination inhibition (HI) unit of *Avibacterium paragallinarum* isolates towards antibody of serotype A, serotype B, and serotype C.

Isolate	Host	Serotype A (211)	Serotype B (SPROSS)	Serotype C (MODESTO)	Result
L.15	Layer	>5120	80	640	Serotype A
L.16	Layer	1280	>5120	1280	Serotype B
L.17	Broiler	5120	>5120	640	Serotype B
L.18	Broiler	1280	>5120	640	Serotype B
L.20	Broiler	640	>5120	1280	Serotype B
L.21	Free-range Chicken	160	>5120	1280	Serotype B
L.22	Free-range Chicken	5120	>5120	1280	Serotype B
L.23	Quail	160	>5120	1280	Serotype B
L.24	Quail	320	>5120	320	Serotype B
L.25	Quail	>5120	>5120	>5120	Serotype B

also reported in the previous studies (Akter *et al.,* 2016; Chukiatsiri *et al.,* 2010; Khatun *et al.*, 2016). All isolates were able to ferment lactose, maltose, mannitol, and sorbitol, and similar results were reported in other studies (Tangkonda *et al., 2019;* Akter *et al.,* 2014; Patil *et al*., 2016)

During the HI test, the first well to show hemagglutination indicates the minimum antibody titer needed to inhibit hemagglutination, also known as the HI unit. The higher the number of the first well to show hemagglutination, the higher the HI unit. When the isolate shows a highest HI titer towards antibody of a certain serovar, it indicates that the isolate has the highest titer of antigen of the same serovar as the antibody, from this the serovar of the isolate was assigned according to the absorbed antiserum giving the highest HI titer (Eaves *et al*., 1989).

The result show that the isolate sample L.15 had highest HI unit towards serovar A antibody and the isolates sample L.16, L.17, L.18, L.20, L.21, L.22, L.23, L.24, and L.25 had highst HI unit towards serovar B antibody. Furthermore, as is shown in Table-2, HI reaction occurred with all three serovars in each isolates sample. It may be possible that the host were infected wih *Avibacterium paragallinarum* of different serovars but the population of the other serovars was not as high as the one serovar that actually caused disease in the host. A similar case happened during a research conducted by Sun, *et al*., (2007), when a chicken experimentally challenged with serovar A *Avibacterium paragallinarum* tested positive in the serovar C HI at a six-week post challenge.

Out of the total samples, 10 isolates (100%) were identified as *A. paragallinarum*. The HI test results show that one isolate (L.15) of *A. paragallinarum* from layer chicken is serovar A and nine field isolates of *A. paragallinarum* from layer, broiler, free range chicken, quail field isolates are serovar B.

Authors' Contributions

AETHW: Planned and designed the study and contributed to the design of research and sampling. FNP: Conducted the research and analyzed the results. FNP: Prepared the manuscript under the guidance of AETHW. All authors have read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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