Characterization of *Pasteurella multocida* Isolated from Broiler Farm Suspected Fowl Cholera in Rhee Sub-District, Sumbawa District

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Abstract

This research aims to characterize Pasteurella multocida isolated from broiler farm suspected fowl cholera in a broiler farm in Rhee Sub-District, Sumbawa District. Pasteurella multocida was taken from a broiler farm suspected of fowl cholera within the clinical signs and pathological anatomic changes. Broiler within the clinical sign and pathological anatomic changes within necropsy for collecting the liver and heart for isolating the bacterium. The isolation bacterium was first inoculated on Blood Agar and incubated for 24 hours at 37ºC. Characterization of Pasteurella multocida with Gram staining, Catalase test, growth on MCA, and Biochemical tests such as TSIA, SIM, SCA, Urease, and Carbohydrate test included glucose, sucrose, maltose, mannose, and lactose. The bacterial colonies were examined for their morphological and biochemical characteristics of P. multocida. The result within positive one of P. multocida from 30 samples broiler showed similar characteristics.

Keywords: *Fowl Cholera, Pasteurella multocida, Broiler*

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1. Introduction

Broiler farming is one type of livestock that contributes quite a lot to meeting the needs of animal-origin protein for the people of Indonesia [1]. Broiler farming has excellent prospects to be developed on both a large and a small scale [2]. The main problem that is the most formidable challenge in poultry farming is the emergence of disease, so management needs to improve to be efficient and professional. Diseases that attack broilers are many, and the clinical signs are often almost the same [3]. One of them is fowl cholera. Some diseases can also be confused with fowl cholera because they have the same clinical signs and pathological anatomic changes, including Newcastle Disease (ND), fowl typhoid, and colibacillosis [4].

Pasteurella multocida can cause disease in a wide range of animal species and is the causative agent of numerous economically significant diseases, including avian fowl cholera, bovine hemorrhagic septicemia, zoonotic pneumonia, and swine atrophic rhinitis [5]. Fowl cholera can be found in various regions on broiler and layer farms in Indonesia. Sri Pornomo first reported the incidence of fowl cholera in Indonesia in 1972 [6]. Fowl cholera is a severe poultry disease and can present in either acute or chronic forms. Most acute fowl cholera cases are caused by serogroup A strains of *P. multocida*. Obvious clinical signs of acute fowl cholera may not occur until very late in the infection, including depression, ruffled feathers, fever, anorexia, mucous discharge from the mouth, diarrhea, and an increased respiratory rate [5]. Priadi and Natalia observed the characteristics of *P. multocida* isolated from broilers in West Java. The report of clinical signs on the farm includes fever, unwillingness to eat, feathers, discharge from the mouth, greenish diarrhea, respiratory distress, and cyanosis of the comb and wattle, accompanied by swelling. The mortality rate can be up to 3% per week in the most severe period. Decreased production is also observed in these cages and lasted 3-4 weeks. On pathological anatomic changes, it was found that congestion in all organs was due to septicemia. Hemorrhages occur in the lung organs, heart, fat, and intestines. The orbital sinus is hemorrhagic, and the trachea has much mucus. Isolation results from all five samples were confirmed as *P. multocida* serotype A:4 [7]. Zainuddin conducted a case study on fowl cholera based on the pathology on smallholder farms in Banda Aceh. The clinical signs include weakness, lethargy, anorexia, face, comb, and swollen wattle, accompanied by pathological anatomic changes resulting in pale hepatic color with necrotic lesions and soft hepatic consistency. These observations concluded that all samples were positive for fowl cholera [8]. The incidence rate of fowl cholera is currently more common in broiler farms due to maintenance management and contact with disease-carrying animals such as paddy field birds, rats, and insects (fleas) [7,9]. Until now, there has been no research on fowl cholera cases in broiler farms in the Rhee Sub-District, Sumbawa District.

The development of fowl cholera cases in broiler farms in Indonesia from 2017-2019 has decreased. However, the incidence rate of the disease is still less than 5% and is included in the list of 20th diseases that often infect broilers [10]. Losses due to fowl cholera can consist of death, weight loss, and decreased egg production. Natural transmission can result in 10-20% mortality in poultry [11].

Based on reports from local farmers, the farm often experiences disease incidence with clinical signs of lethargic chickens and standing feathers, difficulty moving, snoring, and greenish diarrhea, accompanied by a mortality rate of more than 12%, and in general, the farmer often performs treatment using the antibiotic enrofloxacin. However, the result was that the chickens did not experience any change and still showed clinical symptoms after treatment.

Rhee Sub-District is one of the 24th Sub-Districts in Sumbawa District. Badan Pusat Statistik Kabupaten Sumbawa data shows that the total broiler population in the Sumbawa District was 469,329 birds in 2018 [12]. The data broiler population 2016 in the Rhee Sub-District was 13,500 birds, and in 2017 was 10,500 birds [13]. Rhee Sub-District is ranked 5th with the largest broiler population in Sumbawa District to Alas Sub-District, West Alas Sub-District, Buer Sub-District, and Utan Sub-District. Broiler farming in the Rhee Sub-District applies an intensive rearing system with an open-house system.

Pasteurella multocida is a bacterium that causes fowl cholera in domestic and wild poultry [14]. This bacterium is a normal flora that lives in the nasopharyngeal organs of a bird [15]. However, it can infect when it moves due to several factors [5]. These factors include population density, seasonality, concurrent disease, stress due to lack of diseases that co-occur, stress due to lack of nutrition, and age [16]. Climate change due to global warming causes an increase in ambient temperature that can impact the world, such as increased heat stress [17]. As a result, a chicken's immune system can be decreased so that it can be infected [3]. Fowl cholera can occur at all ages and manifest in per-acute, acute, and chronic forms [18].

Inayatullah has conducted similar research for isolating and characterizing *P. multocida* based on clinical signs of fowl cholera in layer farms in Bojonegoro District. The study was based on clinical signs such as limpness, difficulty moving, greenish diarrhea mixed with mucus, snoring, and exudative from the mouth and nose—the result of this study was a positive one that was confirmed fowl cholera [19]. Subekti has conducted another research for isolated and characterized *P. multocida* based on clinical signs from several poultry farms in East Java. Clinical signs include death and swelling of the wattle and joints. As a result of this study, one bird was confirmed to have fowl cholera [20]. According to the WOAH, the best diagnostic technique for fowl cholera is isolating and characterizing *P. multocida* based on clinical signs or lesions caused by the disease [21].

This study aims to confirm fowl cholera's clinical signs and pathological anatomic changes by characterizing *P. multocida* from one of the broiler farms in the Rhee Sub-District, Sumbawa District.

2. Materials and Methods

This research design uses a purposive sampling method with a survey study form. The samples in this study are taken from a broiler farm in the Rhee Sub-District of Sumbawa District. The total sample size in this research is 30 broilers within the clinical signs and pathological anatomic changes suspected of cholera. Each sample was collected for the liver and heart. Samples for the liver were labeled "A," and for the heart, they were labeled "B." Sample criteria were based on clinical signs and pathological anatomic changes on broilers. The clinical signs include lethargy, fever, anorexia, feathers, whitish or greenish diarrhea, snoring, and local infection in the joints of the legs [21]. Characteristics of *Pasteurella multocida* observed within profile bacterium in Blood Agar, gram-staining, Catalase test, MacConkey Agar, Biochemical test including Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), Urease test, Simmon's Citrate Agar (SCA) and Carbohydrate test (Glucose, Sucrose, Mannitol, Mannose, and Lactose).

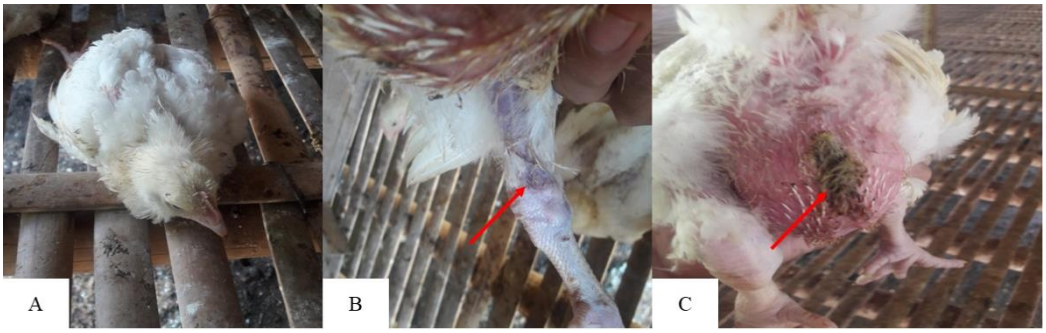

Figure 1. Clinical Signs in Broilers Include A. Poor Feathers, Lethargy, and Anorexia, B. Localized Infection in the Joint (Red Arrow), and C. Green Diarrhea (Red Arrow).

The pathological anatomic changes follow, including petechiae in the heart and intestines, an enlarged liver, the organ's color becoming striped, and multifocal necrosis spots [9].

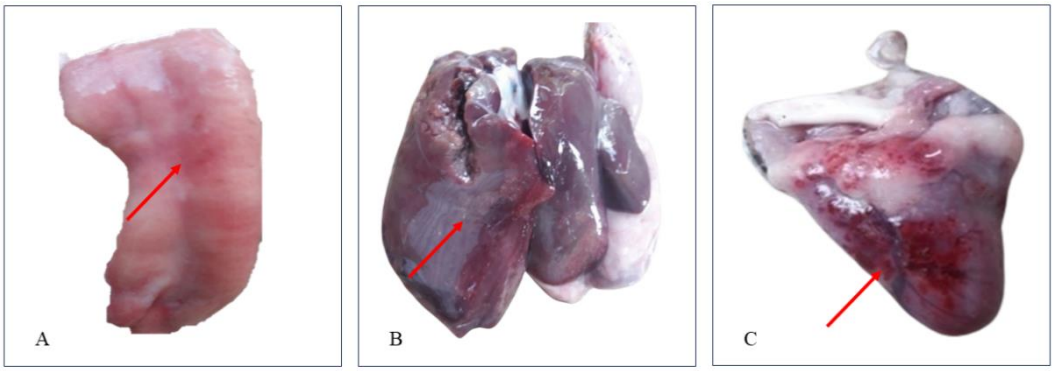

Figure 2. Pathological Anatomic Changes in Broilers Including A. Petechiae in Mucosal of Intestine (Red Arrow), B. Multifocal Necrose in Liver (Red Arrow), and C. Petechiae in Heart (Red Arrow).

Organ sampling is used aseptically using 70% alcohol. This is because contaminant bacteria can contaminate during selection [22].

3. Results

The first step was to perform primary isolation on all samples on Blood Agar. Observations on blood agar were characterized by no hemolysis and colonies 1-3 mm in size and round in shape [21], grey in color, and thick [23]. The primary isolation that has similarities with *P. multocida* is only in the sample labeled "A6". The model was then continued by performing gram staining and catalase tests. The results of gram staining are negative, short rod shape (coccobacillus), and bipolar [21]. The catalase test results are positive, characterized by the formation of bubbles [24]. Samples labeled "A6" were then re-cultured on Blood Agar and then continued for examination of biochemical tests and growth properties on MacConkey Agar (MCA). On MacConkey Agar (MCA), no bacterial colonies were found to grow [21]. In the Triple Sugar Iron Agar (TSIA) test, the results obtained on the slant (oblique) and butt (upright) are yellow, which indicates that it is acidic, does not produce gas, and does not produce H_2S [24]. In the Sulfide Indole Motility (SIM) test, the bacteria form indole, which is characterized by a red indole ring in the reagent layer but does not produce gas and is non-motile [24]. In Simmon's Citrate Agar (SCA) test, bacteria do not use citrate as a carbohydrate source, so the color of the medium remains green [24]. In the urease test, bacteria cannot produce the enzyme urease, or the reaction is adverse, so the medium is yellow [22].

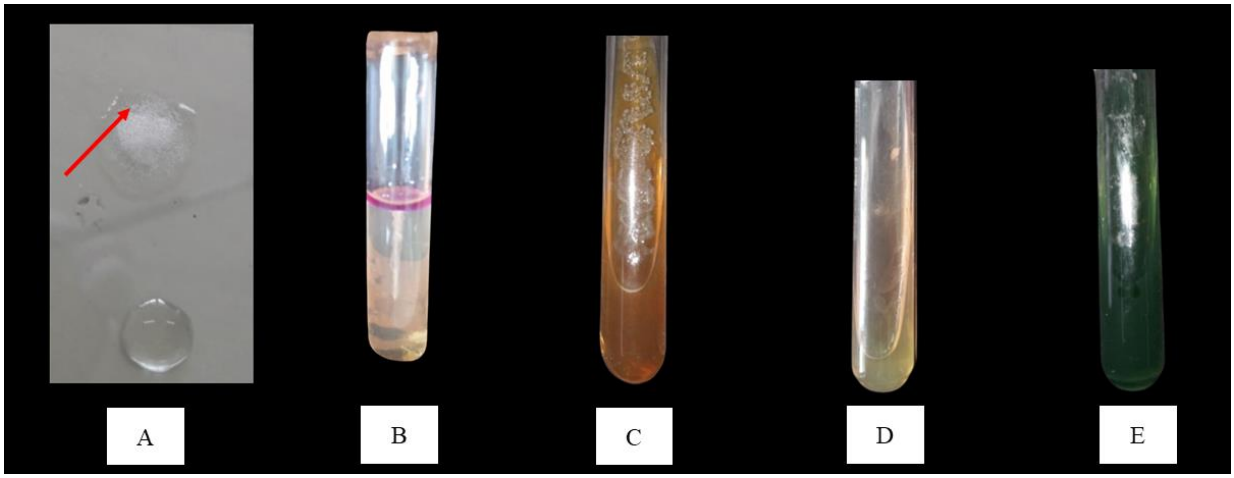

Figure 3. Characterisation Sample "A6" Including A. Catalase Test (Red Arrow), B. Sulfide Indole Motility, C. Triple Sugar Iron Agar, D. Urease Test, and E. Simmon's Citrate Agar.

The carbohydrates test found that the bacteria can ferment glucose, sucrose, and mannitol, which is indicated by a change in the color of the medium to yellow. Still, the bacteria cannot ferment lactose and maltose, so the media color remains red [22].

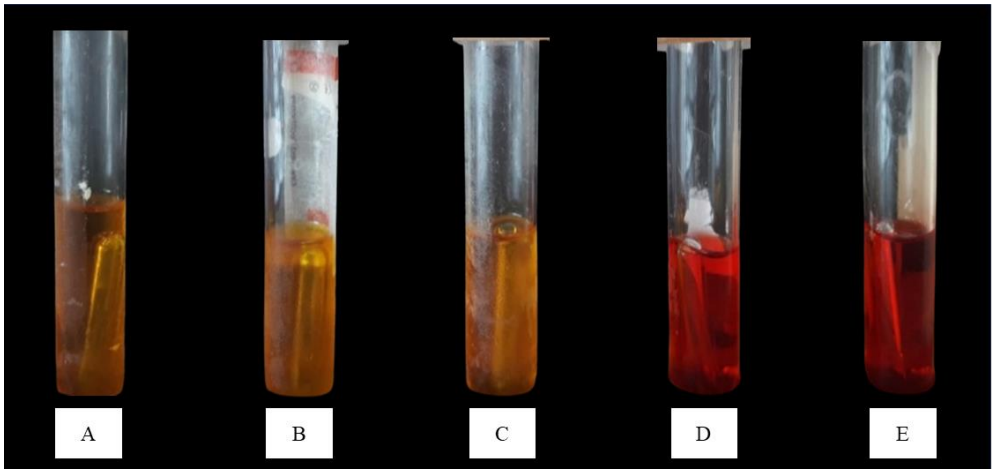

Figure 4. Characterisation Sample "A6" within the carbohydrate test, including A. Glucose, B. Sucrose, C. Mannitol, D. Mannose, and E. Lactose.

The characterization sample "A6" can be seen in Table 1 below.

Table 1. Characterization sample "A6"

Medium Test	Characteristic
Blood Agar	Growth within the color of colonies is greyish, and non-hemolysis
Gram Staining	Gram-negative within, coccobacillus form and bipolar
Catalase	Positive
TSIA	Acid/Acid, gas (-), H_2S (-)
SCA	Negative
Urease	Negative
SIM H_2S Motility Indole	 Negative Non-motile Positive
Carbohydrates: Glucose Sucrose Mannitol Mannose Lactose	 Positive Positive Positive Negative Negative
MCA	No growth

4. Discussions

Fowl cholera has other names, such as Avian pasteurellosis and Avian Haemorrhagic septicemia, or in Indonesian, it is called fowl cholera. This disease is an infectious disease caused by *Pasteurella multocida* and can affect all types of poultry. In general, this disease can affect all ages of chickens. The condition can be manifested in per-acute, acute, and chronic forms [18]. Pasteurella is a commensal bacterium in chickens' respiratory tract or nasopharyngeal organs. However, it can infect when it moves due to several factors [5]. These factors include population density, seasonality, concurrent diseases, stress due to lack of nutrition, and age [16]. Climate change can also cause heat stress in livestock [17]. Fowl cholera mainly occurs during the rainy or early dry seasons [25].

The pattern of rainy and dry seasons has changed dramatically due to global warming. This increase in ambient temperature will bring various specific impacts, including increased heat stress in chickens [17], to the world of animal husbandry. This condition is exacerbated by the relatively high-temperature fluctuations between day (midday) and night (early morning). These conditions cause the stamina of the chicken body to decrease so that it will be easily infected with various diseases [3]. Several things affect the interpretation of *P. multocida*, such as: 1) Contaminant bacteria have overgrown the bacteria in the sample; 2) Microorganisms die in transit before arriving at the laboratory; 3) The animal has recovered naturally before the sample was taken; and 4) The animal has been treated with antibiotics [22].

Several things can cause the lack of fowl cholera cases in broiler chickens. First, the incidence of fowl cholera is more common in layers than in broilers due to the age factor [26-27]. In general, the rearing of layer chickens can last up to 96 weeks [28], while in broilers, it only lasts 5-6 weeks [29]. In addition, the age of chickens is related to antibodies originating from maternal antibodies. This maternal antibody can only protect chicks for a few weeks, depending on the parents' high and low immunity [30]. Based on data published by Medion, the incidence rate of fowl cholera in layer chickens is >5%, while broiler chickens are less than 5% [10]. So, fowl cholera in broilers is often ignored by farmers. According to WOAH, fowl cholera is more likely in old chickens or those older than 16 weeks [21]. Chickens become more sensitive to fowl cholera with age and in chickens in the egg-laying phase [7]. The results of sensitivity tests to antibiotics show that *P. multocida* is susceptible to oxytetracycline and doxycyclin antibiotics [7].

Many diseases attack chickens, and often the symptoms are similar. Therefore, farmers need general experience with the causes of illness to distinguish the appearance of sick chickens from healthy chickens [31]. The first thing to do in handling chicken disease cases is to analyze the cause. Approach through pathological diagnosis is a standard action in animal health management. Some chicken diseases have similar clinical symptoms, but with a surgical examination of the carcass supported by information on the history of the disease, the nature of the causative agent, the age of the chicken, and its epidemiological characteristics, the diagnosis can be directed to a more specific condition.

Lesions on organs characteristic of a particular disease can help make the correct diagnosis. In addition, it is necessary to consider several factors supporting the onset of illness, including climate, geographical location of the farm, management aspects, Day-Old Chick (DOC) quality, feed or water quality, and disease prevention systems [3].

Therefore, fowl cholera is still one of the bacterial diseases that still has the potential to infect the broiler. This potential is because fowl cholera is a highly infectious disease and causes high economic losses [32-33]. The main problem in chicken farming is the emergence of conditions, so farmers must manage their crops effectively and efficiently [3]. One of these diseases is fowl cholera.

5. Conclusion

Based on the research, one broiler sample tested positive for fowl cholera from a broiler farm in Rhee Sub-District, Sumbawa District. The sample is coded "A6", which means the sample comes from the heart, which is stated by isolating and characterizing the *Pasteurella multocida* bacteria.

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