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Diagnostic Examination of Infectious Bursal Disease on Broiler Chicken Farming In Majalengka. West Java

PEMERIKSAAN DIAGNOSTIK PENYAKIT GUMBORO MENULAR PADA AYAM PEDAGING PADA PETERNAKAN AYAM DI MAJALENGKA. JAWA BARAT

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ABSTRACT

Broiler chicken is a type of chicken that grow fast. The presence of a disease cause the diminished of body weight. A case study was found on broiler chicken farms in Majalengka with a high mortality and clinical symptoms appeared of lethargy, tremor, diarrhea, white, bloody and green feces, fever, standing hair, and paleness. Supporting examinations were carried out include necropsy, PCR, and ELISA. The results of pathological findings were hemorrhage of bursa of Fabricius, thigh and chest muscle. There were also found nephrosis, hydropericardium, and enteritis. PCR resulted positive for Infectious Bursal Disease (IBD) Virus and the mean of antibody titer was 2,767 of ELISA. Based on anamnesis, indications, cage and environmental observations, clinical symptoms, physical examination, and supporting diagnostics, the broilers from the farms in Majalengka were affected by IBD. IBD is a disease caused by the RNA virus of the Birnaviridae family which has a mortality of 56.09% in broilers. The main predisposing factor found in the farm was the excess of chlorine in the drinking water. Treatments were given to maintain and increase the body's immunity of the chickens to reduce the secondary infection was the Hexamine and vitamins. The administration of chlorine in the drinking water were stopped. The treatments were successful proved the decrease of the depletion rate after the incubation period.

Keywords: Broiler; ELISA; Infectious Bursal Disease; PCR.

ABSTRAK

Ayam pedaging merupakan tipe ayam yang dapat tumbuh lebih cepat. Adanya suatu agen penyakit yang menyerang ayam broiler akan membuat bobot badan turun. Ditemukan studi kasus pada peternakan ayam broiler di Kota Majalengka dengan insiden kematian yang tinggi dan menunjukkan gejala klinis berupa letargi, tremor, diare feses putih, berdarah, dan kehijauan, demam, bulu berdiri, dan pucat. Dilakukan pemeriksaan penunjang berupa nekropsi, PCR, dan ELISA. Didapatkan hasil nekropsi berupa adanya temuan

patologi anatomi hemoragi bursa fabricius, hemoragi otot paha dan dada, ginjal nefrosis, hidroperikardium, dan enteritis. Untuk hasil PCR positif dan hasil ELISA didapatkan *mean titer* 2.767. Berdasarkan anamnesis, sinyalemen, observasi kandang dan lingkungan, gejala klinis, pemeriksaan fisik, dan pemeriksaan penunjang ayam broiler pada peternakan di Kota Majalengka tersebut terinfeksi IBD. *Infectious Bursal Disease* (IBD) merupakan penyakit yang disebabkan oleh virus RNA famili Birnaviridae yang memiliki mortalitas 56,09% pada ayam broiler. Faktor predisposisi utama yang menyebabkan turunnya imun tubuh pada peternakan ini adalah pemberian *chlorine* berlebih pada air minum ayam. Pengobatan dan penanganan yang diberikan untuk menjaga dan menaikan imun tubuh ayam agar tidak adanya infeksi sekunder yang menyertai penyakit IBD adalah pemberian obat Hexamine dan vitamin serta pemberhentian pemberian *chlorine* pada air minum ayam. Hal ini terbukti dapat menjaga imun tubuh ayam sehingga angka deplesi setelah masa inkubasi turun.

Kata-kata kunci: Broiler; ELISA; *Infectious Bursal Disease*; PCR.

INTRODUCTION

According to Amrullah (2004), broiler chickens are a sort of heavy meat bird that is smaller and younger than other varieties. They grow swiftly, allowing for a harvest at four weeks of age. It has been demonstrated that broiler chickens produce meat, and raising them is profitable. When compared to other types of birds, broiler chicks have the benefit of growing quickly, allowing them to be sold before they are five weeks old and weight an average of 1.5 kg. The conversion of feed into meat by broiler chickens is highly efficient (Situmorang *et al.,* 2013). Feed conversion, body weight gain, and feed intake all affect broiler production (Nurkholis *et al.,* 2014). The body weight of broiler chickens can be influenced by a variety of factors, such as housing management, diet, and genetics.

Infectious bursal disease/IBD can only spread horizontally (Alkie and Rautenschlein, 2016). A number of risk factors, including weakened chicken body immunity, housing management, disease-transmission vectors, and staff, animal, tool, and livestock contamination, can also contribute to the disease's spread (Teshome *et al.,* 2015).

Infected broiler chickens can be diagnosed with IBD by taking into account the post-mortem lesions, clinical signs, and history of livestock illness. Viral antigen can be detected using the Enzyme-Linked Immunosorbent Assay (ELISA) or an agar gel precipitation test. ELISA testing makes it possible to identify the IBD virus and show that IBDV-specific antibodies are present. In addition, ELISA measures the amount of antibodies against IBD, makes immune status monitoring easier in the flock, aids in timing vaccinations, and can identify the virus's origin from the vaccine or challenge field based on the chicken antibody titer. The gold standard test for IBD at the moment is PCR, which works on the basis of multiplying DNA segments using two primers that are positioned to flank the desired DNA area. According to Teshome *et al.* (2015), RT-PCR (a two-step process) in conjunction with restriction enzyme analysis is a better method for diagnosing IBD since it can swiftly and directly identify IBDV variations and determine the virus's origin, whether it originated in a vaccine or challenge area. IBD is still uncommon in Indonesia (Wiedosari and Wahyuwardani, 2015). In order to aid in the identification of IBD disease, which damages the bursa

of Fabricius in broiler chickens raised in Majalengka farms, the author created this case study. This study was aimed to provide light on the diagnosis of IBD in broiler chickens, a condition that is linked to many diagnostic techniques such as post-mortem examination, PCR, ELISA, and clinical symptom assessment.

RESEARCH METHODS

A Medical Exam of Chickens

The clinical examination consists of the following procedures: the chickens were examined and palpated, and its body temperature was then measured. A review and assessment of the farm's cage management practices was done as part of another inspection. An emometer, a chlorine test kit, and a thermometer are the instruments and supplies utilized.

Necropsy

Using gloves and a harsh, sharp scissor, necropsies were performed on broiler chickens suspected of having IBD.

Polymerase Chain Reaction Examination

In this instance, the bursa fabricius, intestines, heart, and kidneys were the organ samples that were extracted and subjected to PCR testing. Conventional one-step PCR is the kind of PCR that is employed. Micropipettes, blenders, centrifuges, tips, heat cyclers, mixmaster fluids, computers, vortexes, collection tubes, recovery tubes, gel imagers, spatulas, agar molds, Bunsen molds, organ samples, lysate fluids, PE liquid, wash buffer liquid, free water liquid, microwave, and agarose gel are some of the equipment and materials used.

ELISA Testing

Using the IDEXX kit, ELISA testing was performed on blood serum from the chickens that had received IBD treatment and were 28 weeks old. Micropipettes, tips, microplates, plate shakers, test plates, blood serum samples, ELISA kits, and ELISA readers are among the equipment and supplies utilized.

RESULTS AND DISCUSSION

Findings from the Anamnesis, Symptoms, and Physical Assessment

A total of 13,700 commercial broiler chickens live in a 23-day-old cage on a farm in Majalengka City. The birds have a history of vaccination against Newcastle Disease at 7 days of age, Avian Influenza H5 at DOC, and IBD at DOC. The Immune Complex Vaccine is the kind of IBD vaccine that is administered. The previous course of treatment included 19–22 days of Paragin, 1–5 days of Tyloflox, and 8–13 days of Intertrim. On April 1, 2023, the first case was reported. Lethargy, tremors, diarrhea with white, red, and greenish feces, fever, standing hair, and paleness were the clinical signs (Figure 1).

Figure 1. Case study of a chicken exhibiting clinical signs of lethargy.

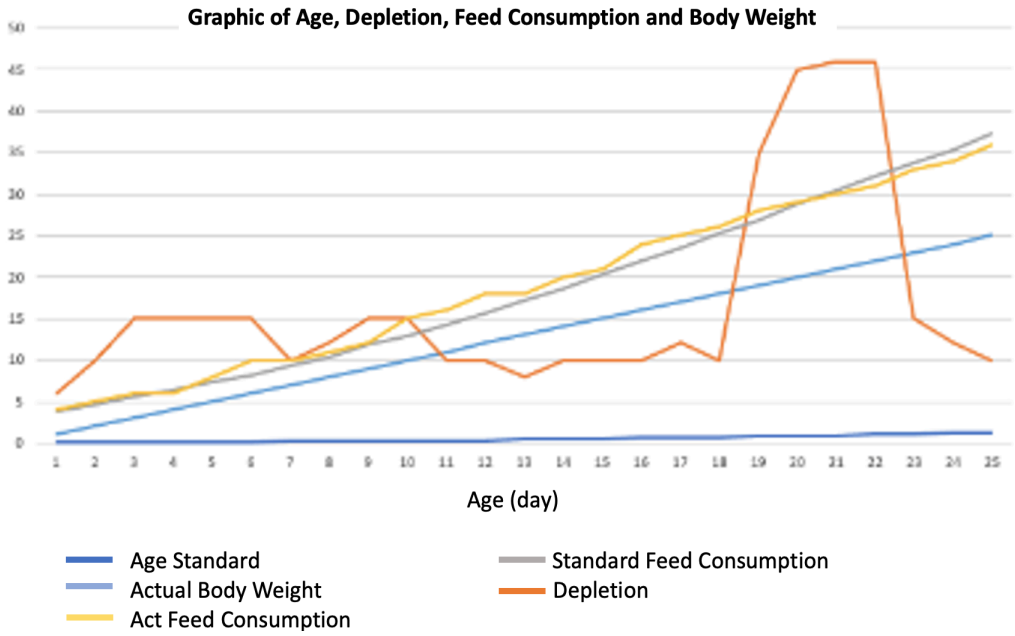

Figure 2. A graph showing the decline in chicken performance of the farm.

Breeders' anamnestic data revealed a significant rate of chicken mortality at the onset of clinical signs. Then, on April 4, 2023, there was another high fatality incident, this time with a greater number of fatalities. In addition, farmers frequently add excessive amounts of chlorine to the drinking water to sanitize the hens. Figure 2's depletion graph illustrates a rise in depletion followed by a 5-day peak of significant mortality. Following treatment, the depletion level dropped.

Foot dipping and disinfection spray were provided, but flowing water for soap and washing was absent, according to observations of the cage and surroundings. The temperature in the chicken coop is 29.7°C, and the humidity level is 73%. With four blowers, the enclosure's wind speed is 1.5 m/s. A test of the drinking water revealed 50 parts per million of chlorine (Figure 3). Feed places, filthy nipple regions replete with husks, and foul-smelling nipple water are among the additional amenities on the farm.

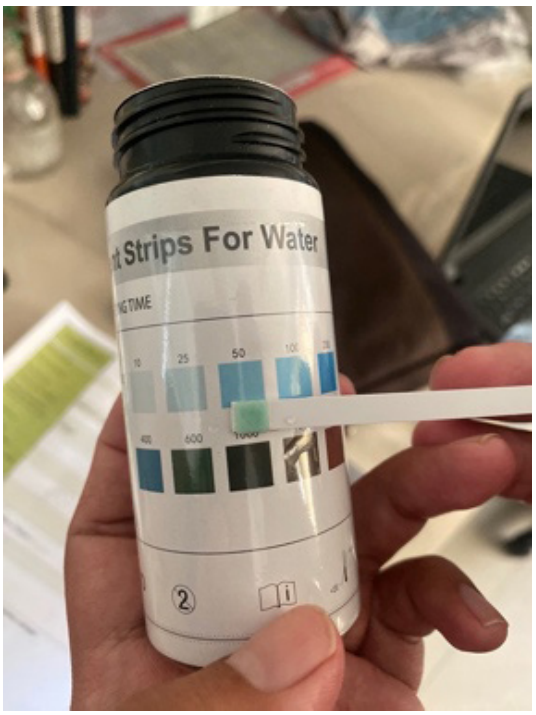

Figure 3. 50 parts per million of chlorine were detected in the drinking water of chickens.

Figure 4. The picture shows diarrhea with white stool.

It was determined that there was an issue with this farm based on the examination results of the signs, anamnesis, clinical symptoms, cage observations, and environment. The pathognomonic sign that was noticed was white diarrhea (Figure 4), which is a hallmark of IBD. This is consistent with the findings of Eladl *et al.* (2020), who state that diarrhea with white feces is one of the pathognomonic signs of IBD in hens. Aside from that, a number of issues with the cages are a contributing cause to the development of IBD on this farm. The temperature of the livestock cage is 29.7°C, which is above the standard, and that the humidity temperature of the cage is 73%, which is higher than the standard humidity for broiler cages between the ages of 21 and 28 days (Cobb, 2015). The cage's inadequate air circulation makes this issue worse. A kestrel was used to measure the wind, and the findings indicated that the recommended wind speed with four blowers was 2 m/s. Oloyo and Ojerinde (2019) state that 2.5–3.0 m/s is the ideal wind speed for raising broilers. The chicken may experience heat stress as a result of which its immune system may deteriorate. Moreover, oxidative stress-induced reduction in immunity will make infections more permeable to the broiler's body (Goel, 2021).

An additional risk factor that may impact this instance is the breeder's addition of excessive chlorine. The findings of testing high chlorine levels show that too much chlorine added to drinking water for sanitary reasons can irritate chicken intestines (Figure 3). According to a study by Corry and Atabay (2001), 25 ppm of chlorine should be added to drinking water tanks. Furthermore, it is simpler for IBD infections to spread to the case farm due to its location—less than 500 meters from neighboring farms.

Necropsy Results

A number of pathognomonic signs of IBD were shown by pathological findings in the chickens, one of which was hemorrhage in the bursa fabricius (Figure 5a). The bursa showed reddish in color and swollen.

Hemorrhage in the thigh and chest muscles of broiler chickens with IBD is evident in their pathognomonic gross pathology (Figure 5b). Wahyuwardani *et al.* (2011) revealed that muscular hemorrhage in the thighs and chest are the common findings of IBD.

Additional pathognomonic signs, such as kidney enlargement with uric acid accumulation and batik kidneys (nephrosis), suggest that the case chicken has IBD (Figure 5c). This is consistent with the 2014 Directorate General of Animal Husbandry and Animal Health statement, which states that kidney enlargement and uric acid deposits are common in IBD cases.

In addition to the physical condition that manifests as pathognomonic symptoms in case chickens, there are a number of predisposing variables that might weaken the chicken's immune system and make them more vulnerable to IBD attacks. Anatomical pathology in the form of hydropericardium (Figure 5d) and enteritis (Figure 5e) supports this. Stress that surpasses the heart's or lungs' ability to tolerate enough oxygen intake is the first step in the formation of hydropericardium

(Tabbu, 2002). The resulting enteritis will then result in a reduction in the body's ability to absorb nutrients, which will directly affect the chicken's immunity. The primary predisposing factor for pathogens (IBD viruses) to enter and infect case hens is the existence of enteritis in the chickens' bodies.

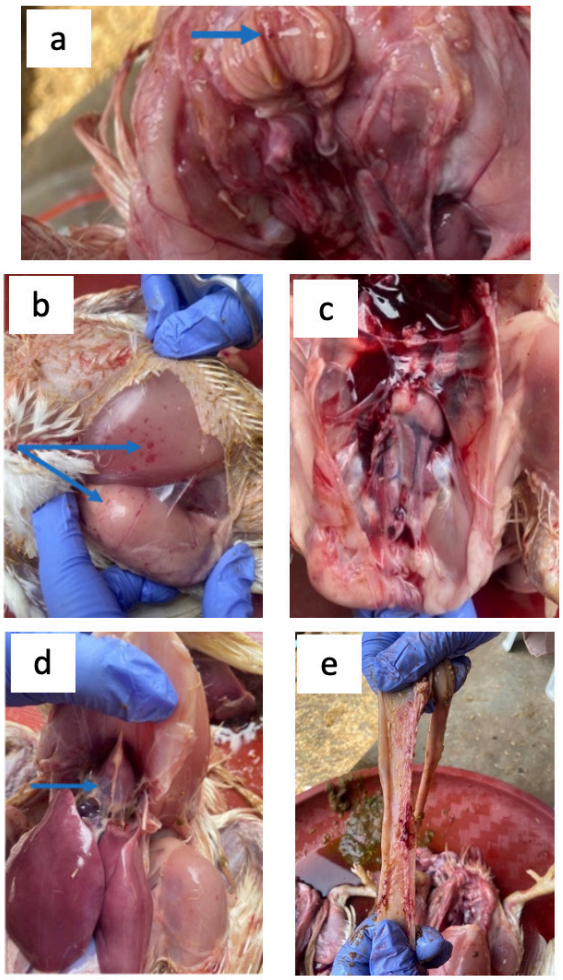

Figure 5. Shows bleeding in the bursa of the fabricius of the chickens (a). The chicken cases with petechiae-type bleeding in the thigh and chest muscles (b) The kidneys is swollen due to uric acid accumulation that are associated by edema and nephrosis (5c). Chicken instances with hydropericardium (5d). Enteritis in chicken intestines were shown from the chicken (5e).

Results of the PCR Test

A one-step polymerase chain reaction test result for IBD was positive (Figure 6). This is because, while not falling in the same line as the IBD positive control, a thick band has formed in the sample. Numerous things could be the source of this, such as the positive control strain that was employed (vaccine) not being the same as the test sample, or contamination of the positive control utilized. The optimal method for testing for IBD is two-step RT-PCR, which can be sent using FTA card medium. Wibowo *et al.* (2017) state that RT-PCR is a molecular method of detection that is known to be more sensitive for identifying the IBD virus.

Results of the ELISA test

Figure 7 displays the ELISA test results. A mean titer of 2,676 and a maximum and minimum individual titer of 3,764 and 618, respectively, are displayed. Next, the biocheck baselines and the vaccine baselines utilized at the facility were examined in order to analyze the mean titer. But in

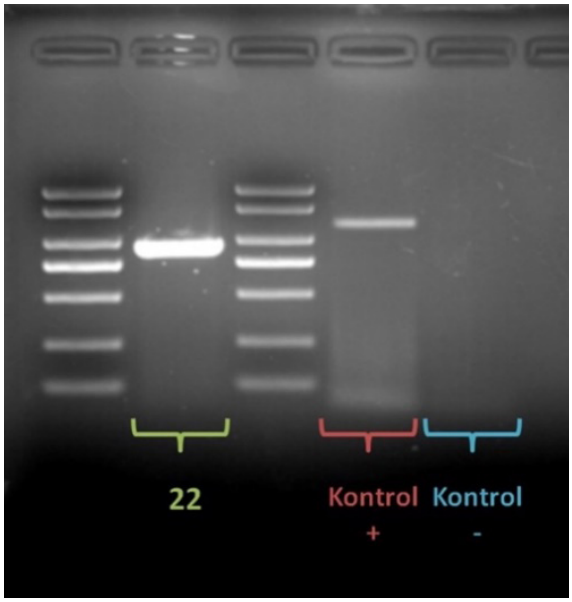

Figure 6. PCR test results showed positive marked by the presence of the band from the sample.

addition to the mean, the antibody titer of the identified subject must also be taken into consideration. Both the mean titer and individual titer findings indicate a field virus if they both have a titer greater than the baselines (Figure 7a and 7b).

The biocheck baselines for broiler chickens administered the Immune Complex Vaccine type for IBD showed a mean titer range of 5,000 – 14,000 at 35–45 days of age, according to the tests that were conducted. Given that the test sample was obtained when the chicken was 28 days old, it is understandable that the mean test titer obtained is below the mean titer range of the Biocheck baselines. The age at which Biocheck uses its baselines is too old; therefore, baselines that are more in line with the age at which ELISA sampling occurs on farms—28 days—are required. Immune Complex Vaccine, the typical vaccine supplier utilized in the hatchery, served as the baseline for testing this instance. On day 28, the Transmune IBD vaccine's mean titer was less than 1,000, although the test results showed a mean titer of 2,676, which was above 1,000. This suggests that the ELISA examination's mean titer was greater than the titer obtained by the vaccination. Thus, it may be said that the challenge field infection in conjunction with the vaccine response results in the formation of an IBD virus antibody titer.

In addition to the average and individual antibody titers of chickens, the percentage CV value is another indicator that must be taken into account. The vaccination's uniformity is shown by the %CV value. A CV value of 31.2% was found based on the ELISA test that was performed. The percentage CV in the Majalengka broiler farms can be regarded as having a good value because it is in the range of 40–60% when taking into account the biocheck baselines (Biocheck IBD CV percentage standard).

Treatment

The medication used in this instance contains hexamine, which is intended to lower the mortality risk in IBD patients with kidney enlargement. It also treats secondary urinary tract infections in IBD patients and acts as an antiseptic in the urinary system. According to the research done by Ahamad *et al.* (2018), birds with structural pathological abnormalities that manifest as kidney edema may benefit from the usage of medications containing the active compoundhexamine. In this situation,

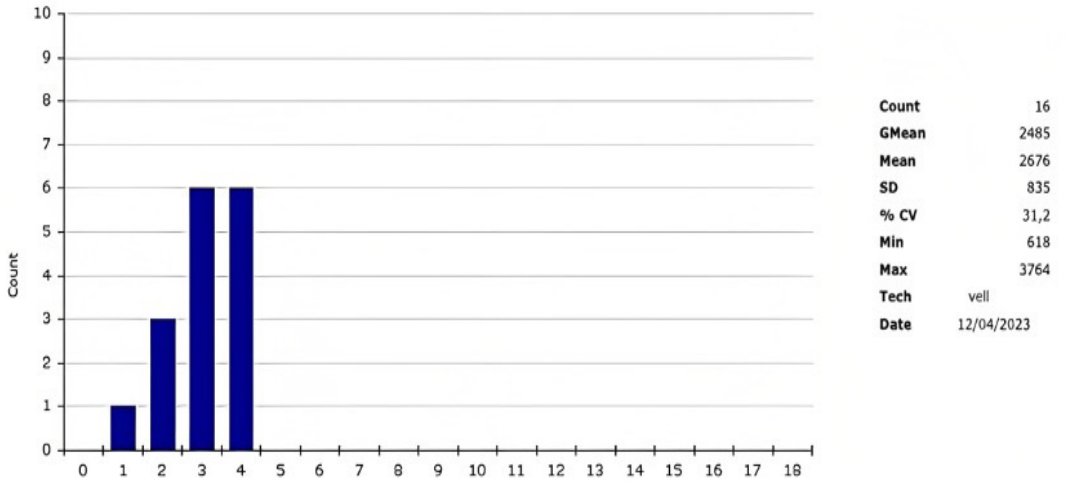

Figure 7. The mean titer of 2,676 was found in the ELISA test results.

vitamin supplementation was also advised to maintain the chicken's strong immune system and prevent secondary infections, which could exacerbate its physical condition. In addition, the supply of chlorine—which purports to sanitize drinking water—must be momentarily halted. The depletion rate has been successfully lowered by the implemented treatment (Figure 2).

CONCLUSION

High rates of mortality and clinical signs, such as lethargy, tremors, diarrhea with green, white, and red stools, fever, standing feathers, and paleness, were seen at broiler chicken farms in Majalengka. It was discovered that the temperature and humidity inside the cage were higher than usual, and the air circulation was lower than usual. The chickens were diagnosed with IBD based on necropsy, PCR, and ELISA. The presence of excessive chlorine in the drinking water was the primary risk factor contributing to the weakened immune system on this farm. It has been demonstrated that medication and therapy can sustain and strengthen the immune system of chickens, preventing secondary infections that coexist with IBD and reducing the rate of immune system depletion following the disease's incubation period.

SUGGESTION

Research can be carried out on more farms with various virus strains in order to assess the dynamics of IBD infection in poultry farm.

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