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MOLECULAR IDENTIFICATION AND HISTOPATHOLOGICAL CHANGES IN LAYER HENS INFECTED WITH *SALMONELLA* SPECIES IN DIYALA GOVERNORATE

A Thesis Submitted to Council of College of Veterinary Medicine, University of Diyala in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Medicine/ Veterinary Microbiology

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ABSTRACT

This study was aimed to detect the presence of *Salmonella* SPP in layer chicken farms by conducting through bacteriological examination of different types of samples at different ages during laying stage. Then determine *Salmonella* SPP by PCR using inv A gene including biochemical tests, *Salmonella* serotypes by O antigen and H antigen, API 20 and viteck and examine the histopathological changes fallowing the observations of clinical signs and lesions in suspected birds.

For further characterizations between October 2023 and January 2024, a total of 585 samples were collected from 150 suspected birds flock acros Diyala governrate. The samples comprised (liver (150), ceca (50), heart (48), spleen (37), oviduct (150), ovary (150) were obtained from flocks.

The study identified *Salmonella* species in 55 out of 585 samples indicated in overall rate of (9.4%). The liver exhibited the higher infection rate at 22.66% with 34 isolates (25 *Salmonella ohio*, 4 *Salmonella kentucky* and 5 *Salmonella enteritidis*). The ovary had an infection rate of 10 % yielded 15 isolates (13 *S. ohio* and 2 *S. enteritidis*).

The oviduct showed 4% infection rate (6 isolates) divided into (5 *Salmonella ohio* and 1 *Salmonella enteritidis*). No Salmonella isolates were observed in heart, spleen and ceca.

PCR assay targeting the conserved gene (inv A) we conducted to approve the presence of Salmonella SPP at the genome level. DNA was extracted from 55 isolates that had previously tested positive through bacteriological methods. The inv A gene is commonly used as target in PCR for Salmonella detection due to highly specificity.

This study is the first research in Diyala province to confirm the presence of Salmonella species. However, not all these serotypes are in inhertenly pathogenic to poultry for instance *Salmonella enteritidis* is known to cause disease in chicken while *Salmonella ohio* and *Salmonella kentucky* are generally in low pathogenic in layer hense. The pathogencity of these serotypes can very based on factors such as the host immune status, and environmental conditions. The histopathological examinations revealed microscopic lesions detected in the liver and oviduct.

Chapter One
Introduction

Introduction

Among many diseases in poultry such as influenza, Gumboro, Marek, Newcastle, the *Salmonella* also consider as main cause of mortality and egg reduction in layers which lead to economic losses (Dar *et al*.2017). *Salmonella* according to Ryan *et al*., (2015), Salmon and Smith were the first to isolate *Salmonella* from pigs in 1885. A significant genus within the Eenterobacteriaceae family is *Salmonella*. The genus's *Salmonella* members live in both humans and animals' digestive tracts and are facultative anaerobes that are Gram-negative (Holt *et al.*, 1994; Gast *et al.*, 2024).They can be recovered from a variety of hosts, including humans, pigs, poultry, and food sources. *Salmonella* genus members can be harmful to wild or Humans with domesticated animals(Stathas *et al.*, 2024). Food-borne outbreaks have been linked to this disease, which is significant to the food sector. Enteric fever, gastroenteritis, and septicemia are among the human pathogenic conditions caused by *Salmonella*(Higuera-Ciapara *et al.*, 2024) .

Salmonellosis in poultry results in significant financial loss because of mortality and low manufacturing (Aragaw *et al.*, 2010). Salmonellosis has grown to be a major issue around the world as poultry farming has expanded (Hossain *et al.*, 2021). The fact that the disease's causative agents are passed vertically from parent to offsprig makes it especially important. The natural hosts of *S. pullorum* and *S. gallinarum* are chickens (Xiong *et al.*, 2020). *pullorum* illness often strikes between two and three weeks of age and can occasionally affect adults (Penha Filho *et al.*, 2016). It's common knowledge that fowl typhoid affects adult birds, but reports of significant mortality rates in young chicks also exist(Berhanu and Fulasa, 2020) , Farms with laying hens of varying ages, housing arrangements, and flock size are risk factors linked to salmonellosis infection in laying hens (Sasaki *et al.*, 2012).

According to reports, *S. livingstone* was isolated for the first time in the Kurdistan area of Iraq and for the first time in Iraq in recent decades. Saeed *et al*. (2013) identified *S.typhimurium* from hens in Iraq, while AL-Iedani *et al*. (2014) isolated *S.ohio*, *S.newport*, and *S.typhimurium* from chicken cloacal swabs. Four *Salmonella* serotypes were identified in a prior study conducted in Iraq: *S. ohio*, *S. livingstone*, *S. newport*, and *S. typhimurium*. According to Taha *et al.* (2015), *S. ohio* was the most prevalent serotype. This could have been caused by contamination that occurred during processing in the abattoir or could have been a result of the serotype typically found in the digestive tracts of chickens, which contaminated the carcasses during the slaughtering process. More recently, *S. kentucky* has become the most commonly detected serovar in chickens, while *S. typhimurium* remains the most common cause of human infections (Centers for Disease Control and Prevention, 2008). *S. kentucky* was less often identified in human salmonellosis averaging 62 cases per annum in the United States between 1996 to 2004, but rose to 123 cases in 2006; accordingly, the prevalence in chickens rose from 25% in 1997 to approximately 50% in 2007(Centers for Disease Control and Prevention, 2008).

Traditional bacteriological methods are recommended for identifying *salmonlla* species as out line by (Humphries and Linscott, 2015).The advent of molecular techniques such as PCR, has facilitated *Salmonella* surveillance(Bell *et al.*, 2016).

The external and internal contamination of eggs by *Salmonella* spp. during laying hen rearing is a serious problem. Therefore, it is exceedingly challenging to implement appropriate salmonellosis management methods (Mane and Khaire, 2022). Egg contamination can happen through either a vertical or horizontal path.

Horizontal transmission results from exterior egg shell contamination and may enter when the egg shell cracks, while vertical transmission is caused by bacterial agent colonisation in the female reproductive system and oviduct prior to egg creation of shells (Johnson *et al.*, 2018) , Salmonellosis in multilayer chicks as well as hens need to be assessed in order to carry out efficient disease control strategies (Gast and Porter Jr, 2020). Due to the lack of data on the prevalence of *Salmonella* spp. In the areas of Diyala Governorate and the extent of its pathological impact on internal organs, therefore :

Aims of the Study

1. Isolation and identification of *Salmonella* SPP. From laying hens in different regions of Diyala governorate were conducted using microbial and biochemical technique along with molecular methods as polymerase chain reaction PCR.
2. Investigate the serotyping of *Salmonella* spp. in layer chickens in Diyala governorate.
3. Detection of histolpathological changes in internal organs (liver and oviduct) in layers chickens that infected with *Salmonella* SPP bacteria.

الخلاصة

هدفت هذه الدراسة إلى الكشف عن وجود بكتيريا السالمونيلا السالمونيلا SPP في مزارع الدجاج البياض من خلال إجراء الفحص البكتريولوجي لأنواع مختلفة من العينات في أعمار مختلفة خلال مرحلة وضع البيض. ثم تحديد السالمونيلا SPP عن طريق تفاعل البوليميراز المتسلسل PCR باستخدام الجين (أ) بما في ذلك الاختبارات الكيميائية الحيوية، والأنماط المصلية للسالمونيلا بواسطة مستضد O ومستضدH ، و API 20 و Viteck وفحص التغيرات النسيجية المرضية التي تتبع ملاحظات العلامات والآفات السريرية في الطيور المشتبه بها.

لمزيد من التوصيفات بين أكتوبر 2023 ويناير 2024، تم جمع ما مجموعه 585 عينة من 150 طائر مشتبه به من قطيع الطيور المشتبه به في محافظة ديالى. وشملت العينات (150) عينة من الكبد ، والاعورين (50)، والقلب (48)، والطحال (37)، وقناة البيض (150)، والمبيض (150) تم الحصول عليها من قطعان الدجاج البياض.

وقد حددت الدراسة أنواع السالمونيلا في 55 عينة من أصل 585 عينة بنسبة إجمالية بلغت (9.4%). أظهر الكبد أعلى معدل إصابة بنسبة 22.66% مع 34 عزلة (25 من السالمونيلا أوهايو، 4 من السالمونيلا كنتاكي و5 من السالمونيلا المعوية). كان معدل الإصابة في المبيض 10٪ من 15 عزلة مقسمة (13 سالمونيلا أوهايو و2 سالمونيلا معوية). أظهرت قناة المبيض معدل إصابة بنسبة 4% (6 عزلات) مقسمة إلى (5 سالمونيلا أوهايو و1 سالمونيلا معوية). لم تلاحظ أي عزلات للسالمونيلا في القلب والطحال والأعورين.

تم إجراء فحص PCR الذي يستهدف الجين (inv A) الذي أجريناه للتأكد من وجود السالمونيلا SPP على مستوى الجينوم. تم استخلاص الحمض النووي من 10 عزل تم اختبارها سابقًا المعزولة التي ثبتت إصابتها سابقًا بالطرق البكتريولوجية. يشيع استخدام الجين "إنفو أ" كهدف في تفاعل البوليميراز المتسلسل للكشف عن السالمونيلا بسبب خصوصيته العالية.

هذه الدراسة هي أول بحث في محافظة ديالى لتأكيد وجود أنواع من السالمونيلا. ومع ذلك، ليست كل هذه الأنماط المصلية مسببة للأمراض للدواجن، فعلى سبيل المثال، من المعروف أن السالمونيلا المعوية تسبب المرض في الدجاج بينما السالمونيلا أوهايو والسالمونيلا كنتاكي بشكل عام قليلة الإمراض في الدجاج البياض. يمكن أن تعتمد الإمراضية لهذه الأنماط المصلية على عوامل مثل الحالة المناعية للمضيف والظروف البيئية. كشفت الفحوصات المرضية النسيجية عن وجود آفات مجهرية في الكبد وقناة البيض.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة ديالى
كلية الطب البيطري
فرع الاحياء المجهرية البيطرية

التشخيص الجزيئي والتغيرات النسيجية المرضية في الدجاج البياض المصاب بأنواع السالمونيلا في محافظة ديالى

رسالة مقدمة الى مجلس كلية الطب البيطري / جامعة ديالى ،
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