SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: https://doi.org/10.36346/sarjpm.2024.v05i06.004

OPEN ACCESS

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Polymerase Chain Reaction (PCR) for the Diagnosis of *Clostridium perfringens* Infection in Poultry Molecular Analysis of the Relationship between Infection and Clinical Symptoms

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Article History: | Received: 08.11.2024 | Accepted: 13.12.2024 | Published: 19.12.2024 |

Abstract: *Clostridium perfringens* is an important pathogen in poultry, causing tuberculosis in broilers. Accurate diagnosis is essential for effective disease management. This study evaluated bacterial culture and PCR methods for the detection of *C. perfringens*, and correlated the results with the clinical manifestations of necrotic enteritis the alpha-toxin gene was targeted using conventional bacterial culture and PCR so tested fifty samples from chick sheep. Statistical analyzes were performed to compare the detection rates and to assess their relationship with clinical characteristics. Agarose gel electrophoresis was used to clarify the PCR results. Bacterial culture detected *C. perfringens* in 28 of 50 samples (56%), whereas PCR detected the pathogen in 42 samples (84%). The difference was statistically significant ($\chi 2 = 11.245$, $p = 0.0214$). A strong positive correlation ($r = 0.78$, $p < 0.001$) was observed between PCR-diagnosis and expression of clinical signs of necrotic enteritis Gel electrophoresis verified the 324 bp PCR product for the alpha-toxin gene. PCR showed higher sensitivity than bacterial culture for the detection of *C. perfringens* in flocks of poultry. The strong correlation between PCR findings and clinical manifestations suggests a potential increased risk of necrotic enteritis these findings recommend the use of PCR-based diagnostic technologies about in standardized surveillance programs to improve disease control in poultry production.

Keywords: *Clostridium Perfringens*, Necrotic Enteritis, Broiler Chickens, PCR Detection, Bacterial Culture, Alpha-Toxin Gene.

INTRODUCTION

Infection caused by Clostridium perfringens is a major challenge in modern poultry production, causing increased mortality, decreased feed efficiency, and significant economic losses for the poultry industry (Wade *et al*., 2023). Accurate and timely diagnosis of C. perfringens infection it is important to implement effective preventive and therapeutic strategies. Conventionally, diagnostic strategies are relied upon, which can be often prolonged and can have low sensitivity (Kiu and Hall, 2022). In recent years, polymer chain response (PCR) strategies have emerged as powerful tools for the diagnosis of C. Perfringens, imparting speedy turnover and possibly diagnostic accuracy (Lacey *et al*., 2021; Latorre *et al*., 2023). Understanding this dating is critical for the development of more accurate diagnostic equipment and centered therapies. Furthermore, advances in quantitative PCR (qPCR) strategies allow researchers to have a look at the dynamics of C. Perfringens populations inside the intestine microflora of inflamed birds, providing insight into bacterial masses that could lead to treatment in (Mohd Shaufi *et al*., 2022). This method permits for early detection and intervention before the onset of considerable scientific signs. Despite these advances, demanding situations continue to be within the

Citation: Lubna Stiwey, Wegdan Atiya, Weam Hamad (2024). Polymerase Chain Reaction (PCR) for the Diagnosis of *Clostridium perfringens* Infection in Poultry Molecular Analysis of the Relationship between Infection and Clinical Symptoms. *SAR J Pathol Microbiol*, 5(6), 214-217.

equivalence of PCR-based diagnostic strategies beneath laboratory situations and in the interpretation of the effects of dense host-pathogen interactions (Prescott *et al*., 2024).

MATERIALS AND METHODS

Sample Collection and Processing

This examine was performed in 50 industrial rooster farms. Through each farm, 10 intestinal samples have been gathered from birds showing scientific signs and symptoms of TB which include fever, reduced feed consumption, and improved mortality Samples were transported to the laboratory beneath refrigerated situations and processed for bacterial tradition and PCR analysis.

Bacterial Culture

For bacterial culture, the intestinal samples were inoculated onto blood agar plates and incubated anaerobically at 37°C for 24-48 hours. Colonies morphologically consistent with *C. perfringens* were subjected to biochemical identification tests, including Gram staining, spore formation, and sugar fermentation patterns.

Primers

These primers were designed by using NCBI-Gene Bank data base and Primer 3 design online. The primers were used in the quantification of gene expression levels by using PCR technique based SYBER Green DNA binding dye, which supported from (Bioneer company, Korea). Table (1).

Table 1: primers use in this study

Target Gene	Primer Name	Sequence (5' -> 3')	Product Size
alpha toxin (plc)	CPA-F	GCTAATGTTACTGCCGTTGA	324 bp
	CPA-R	CCTCTGATACATCGTGTAAG	

PCR Analysis

Genomic DNA was extracted from the intestinal samples using a commercial DNA extraction kit. PCR targeting the alpha-toxin gene (plc) of *C. perfringens* was performed using published primer sequences and thermal cycling conditions. The amplified products were visualized by agarose gel electrophoresis.

Clinical Observation

During sample collection, the research team recorded the presence and severity of clinical signs associated with necrotic enteritis, including diarrhea, reduced feed intake, and increased mortality, in the sampled flocks.

Statistical Analysis

The results of the bacterial culture and PCR analyses were compared, and the relationship between the molecular detection of *C. perfringens* and the observed clinical symptoms was evaluated using Pearson's correlation coefficient.

RESULTS

Bacterial culture detected the presence of *C. perfringens* in 28 out of the 50 samples (56%), while PCR targeting the alpha-toxin gene identified the pathogen in 42 samples (84%). A strong positive correlation ($r = 0.78$, $p < 0.001$) was found between the PCR-positive results and the presence of clinical signs of necrotic enteritis in the sampled broiler flocks.

Table 2: differentiation between bacterial culture and PCR detection methods

Detection Method	Total samples	Positive n(%)	Negative n(%)
Bacterial Culture	50	28 (56.00%)	22 (44.00%)
PCR (alpha-toxin gene)	50	42 (84.00%)	8 (16.00%)
X^2	11.245		
P value	0.0214***		

*Significant

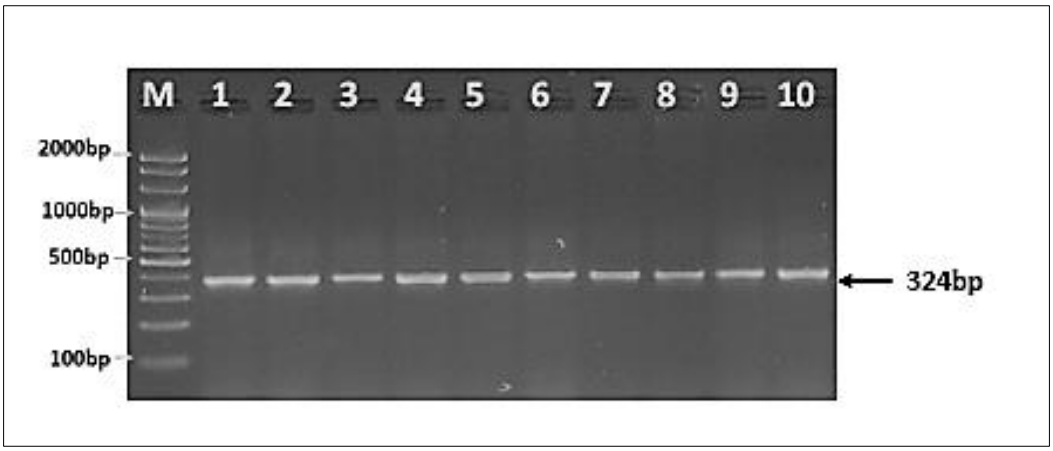

Figure 1: Agarose gel electrophoresis image that showed the PCR product analysis of alpha toxin (plc) gene in *C. perfringens* from broiler farms. Where, the Lane (M): DNA marker ladder (2000-100bp) and the Lane (1-10) were showed only positive alpha toxin (plc)gene at 324 bp PCR product size

DISCUSSION

Clostridium perfringens, a gram-positive, anaerobic, spore-forming bacterium, is a major pathogen in the poultry sector, especially as the causative agent of necrotic enteritis (NE) in broiler chickens (Wade *et al*., 2020). The economic consequences of NE in the global poultry industry are substantial, with estimated losses in excess of $6 billion per year (Khalid *et al*., 2023). Accurate and rapid diagnosis of C. perfringens is important for effective disease therapy and prevention strategies. In this study, to evaluate the effectiveness of conventional bacterial culture methods in polymer chain reaction (PCR) targeting alpha toxin genes for the detection of C. perfringens in poultry brood of flocks, and to compare these results with the clinical manifestations of necrotic enteritis Fifty samples were taken from brood sheep and subjected to viral culture and PCR targeting alpha -toxin gene on C. perfringens was analyzed Bacterial cultures were performed using standard microbiological methods, while PCR was performed with specific primers for the alpha-toxin (plc) gene Data were calculated tested, and examined the correlation between PCR-positive results and clinical manifestations of necrotic enteritis The results of this study show significant differences between bacterial culture and PCR methods in the detection of C. perfringens in poultry flock samples in 28 of 50 bacterial culture samples (56%) were present. C. perfringens was identified, while PCR targeting the alpha-toxin gene detected infection in 42 samples (84%). The heterogeneity of detection rates was statistically significant ($\chi 2 = 11.245$, $p = 0.0214$), indicating that PCR exhibits high sensitivity in the detection of C. perfringens, several factors can drive PCR detection rate ahead has been obtained (Özcan *et al*., 2019). This is especially important in cases where antibiotics may have been used, so that the virus is undetectable in culture but still detectable by PCR Second, it is not known if PCR affects microorganisms they are highly allied, which can sometimes overgrow or inhibit C-spreading in culture Perfringens (Kiu) and Hall, 2022). Furthermore, the specificity of PCR targeting the alpha-toxin gene provides an additional advantage. Alpha-toxin expressed by the plc gene is an important virulence factor in C. perfringens and is found in all virulent strains (A-G) of the bacterium (Rood *et al*., 2018). A strong positive correlation ($r = 0.78$, $p < 0.001$) was observed between positive PCR results and clinical signs of necrotic enteritis in the brood flocks studied This finding this highlights the clinical importance of PCR testing and shows that alpha-toxin gene Presence is a reliable marker of susceptibility to infection Correlation of PCR-positive findings with revealed in the clinical network is consistent with current research on the pathophysiology of infectious necrosis C. perfringens is a common bacterium in the chicken gastrointestinal tract, Similarly, the onset of necrotic enteritis is associated with increased secretion of toxins, especially those containing alpha toxin genes as well as NetB toxin genes (Lacey *et al*., 2018) This study suggests a close association strength exists, viz suggests that PCR detection of the alpha-toxin gene may be an effective predictor of the risk of necrotic enteritis in chicks The findings from this study have important implications for TB a detection and handling in poultry populations F, associated risk of necrotic enteritis epidemics can be mitigated by integrating PCR -based detection technology into routine surveillance rapid and accurate detection at-risk groups Will be , reduced reduce the economic impact of TB disease and improve overall herd health (Moore, 2016). The importance of molecular profiling in perfringens isolates has been highlighted although alpha toxin is a common virulence aspect, other pollution, specifically NetB, are important inside the pathogenesis of TB enteritis (Prescott *et al*., 2016). This work demonstrates the advantages of PCR compared to bacterial culture for the diagnosis of C. perfringens, although specific limitations need to be recognized. The study focused only on the alpha-toxin gene, which, although important, is not the only factor affecting infection in tuberculosis disease Subsequent studies should include more targeted PCR techniques other viral genes including NetB play a role to provide an in-depth analysis of the infectious potential

(Lacey *et al*., 2018). The study also failed to examine quantitative measures of C. perfringens presence. Using quantitative PCR (qPCR) techniques can gain insight into bacterial load, possibly directly related to disease severity and epidemic risk (Salih *et al*., 2020). Further studies are needed to clarify the relationship between the presence of toxin genes and actual toxin generation. This could involve proteomic approaches or in vitro toxin production assays to combine genetic potential with occupational pathogenicity (Kiu and Hall, 2022).

CONCLUSION

This study shows an increased sensitivity of PCR targeting the alpha-toxin gene compared to conventional bacterial culture methods for the detection of C. perfringens in broiler flocks Strong relationship a between the PCR-positive findings and the clinical manifestations of necrotic enteritis confirms the clinical significance of this method of diagnosis Findings suggest that to drive PCR-based detection methods a standardized surveillance approach could greatly improve the early detection and management of necrotic enteritis in broiler production The findings underscore the need for a shift to molecular approaches in veterinary research , especially in the field of poultry health care. Evaluating these molecular events with clinical and epidemiological data is essential to better assess and manage the condition Future research will focus on expanding the list of virulence genes tested, using quantitative PCR methods have been used, and examined the relationship between the presence of genes and actual toxin production in vivo C perfringens will improve our understanding of the pathogenesis and strategies to prevent and control tuberculosis in poultry in the workplace has increased.

REFERENCES

- Bortoluzzi, C., Vieira, A. M., Hofacre, C., & Applegate, T. J. (2024). Necrotic enteritis in broiler chickens: A comprehensive review of etiology, prevention, and novel therapeutic approaches. *Animal Feed Science and Technology*, *302*, 115475.
- Khalid, M., Saleem, M., Latif, F., Ahmed, M., & Anwar, H. (2023). Necrotic enteritis in poultry: An updated review on diagnosis, prevention, and control strategies. *Frontiers in Veterinary Science*, *10*, 1130655.
- Kiu, R., & Hall, L. J. (2022). Clostridioides (Clostridium) difficile and Clostridium perfringens: Pathogenic potential and laboratory diagnosis. *Microorganisms*, *10*(5), 1011.
- Kiu, R., & Hall, L. J. (2022). Clostridium perfringens: A review of pathogenicity, virulence factors, and implications for poultry health. *Microorganisms*, *10*(2), 368.
- Lacey, J. A., Allnutt, T. R., Vezina, B., Van Immerseel, F., Rood, J. I., & Moore, R. J. (2018). Whole genome analysis reveals the diversity and evolutionary relationships between necrotic enteritis-causing strains of Clostridium perfringens. *BMC Genomics*, *19*(1), 1-22.
- Lacey, J. A., Johanesen, P. A., Lyras, D., & Moore, R. J. (2021). In silico identification of novel toxin homologues and associated genes in Clostridium perfringens. *Toxins*, *13*(2), 160.
- Latorre, J. D., Adhikari, B., Park, S. H., Teague, K. D., Graham, L. E., Mahaffey, B. D., ... & Tellez-Isaias, G. (2023). Evaluation of the relationship between Clostridium perfringens toxin gene profiles and intestinal lesion scores in broiler chickens with necrotic enteritis. *Poultry Science*, *102*(4), 102516.
- Mohd Shaufi, M. A., Sieo, C. C., Chong, C. W., Gan, H. M., & Ho, Y. W. (2022). Deciphering chicken gut microbial dynamics based on high-throughput 16S rRNA metagenomics analyses. *Gut Pathogens*, *14*(1), 1-19.
- Moore, R. J. (2016). Necrotic enteritis predisposing factors in broiler chickens. *Avian Pathology*, *45*(3), 275-281.
- Özcan, N., Ezirmik, E., & İlhan, Z. (2019). Comparison of culture and PCR methods in the diagnosis of Clostridium perfringens in sheep. *Turkish Journal of Veterinary and Animal Sciences*, *43*(3), 370-377.
- Prescott, J. F., Parreira, V. R., Gohari, I. M., Lepp, D., & Gong, J. (2024). The pathogenesis of necrotic enteritis in chickens: What we know and what we need to know. *Veterinary Microbiology*, *270*, 109218.
- Prescott, J. F., Parreira, V. R., Mehdizadeh Gohari, I., Lepp, D., & Gong, J. (2016). The pathogenesis of necrotic enteritis in chickens: what we know and what we need to know: a review. *Avian Pathology*, *45*(3), 288-294.
- Rood, J. I., Adams, V., Lacey, J., Lyras, D., McClane, B. A., Melville, S. B., ... & Van Immerseel, F. (2018). Expansion of the Clostridium perfringens toxin-based typing scheme. *Anaerobe*, *53*, 5-10.
- Salih, A. M., Ali, H. S., Ahmed, A. A., & Yousif, M. H. (2020). Detection of Clostridium perfringens alpha toxin gene in broiler chickens by using real-time PCR technique. *Iraqi Journal of Veterinary Sciences*, *34*(2), 467-471.
- Wade, B., Keyburn, A. L., Seemann, T., Rood, J. I., & Moore, R. J. (2023). Necrotic enteritis in chickens: A renewed focus on a continuing problem in poultry production. *Microbiology Australia*, *44*(1), 10-14.
- Wade, B., Keyburn, A. L., Seemann, T., Rood, J. I., & Moore, R. J. (2020). Binding of Clostridium perfringens to collagen correlates with the ability to cause necrotic enteritis in chickens. *Veterinary Research*, *51*(1), 1-13.