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Multidrug-Resistant Fluoroquinolone Resistance Genes and ESBL -Producing *Escherichia coli* and *Salmonella* sp. in a Broiler Breeder Farm in Ibadan: A Case Report

Oluwaseun Olanrewaju Esan[1], Foluke Olajumoke Jemilehin[1*], Adelekan Oluseyi Okunlade[1], Veronica Eyihuri Adetunji[2], Temitayo Ayodeji Esho-Oluborode[3], Temitope Olaotan Olodun-Coker[4]

1. Department of Veterinary Medicine, University of Ibadan, 200005, Ibadan, Nigeria
2. Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria
3. Faculty of Veterinary Medicine, University of Ibadan, 200005, Ibadan, Nigeria
4. Department of Veterinary Microbiology, University of Ibadan, Ibadan, Nigeria

*** Corresponding author email address:** foljem@yahoo.com

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A B S T R A C T

The poultry industry is a critical reservoir of antimicrobial-resistant bacteria, with *Escherichia coli* and *Salmonella* sp. *as* major disease-causing organisms that disseminate high-priority resistance genes into the food chain. This case report details the molecular detection and characterization of plasmid-mediated quinolone resistance (PMQR), carbapenemase, and extended-spectrum β-lactamase (ESBL) genes in isolates of multidrug-resistant *E. coli* and *Salmonella* sp. obtained from a broiler breeder farm in Ibadan, Oyo State, Nigeria. Post-mortem examination was conducted on 12 broiler breeders presenting with septicemia. One representative *Escherichia coli* isolate and one *Salmonella* sp. *isolate* from pooled liver, kidney, and gallbladder samples with obvious gross post-mortem lesions were identified based on colony morphology on differential media (MacConkey agar), Gram's reaction, and biochemical tests using the Analytical Profile Index. The isolates were tested for susceptibility to antibacterial agents with the Kirby-Bauer method. The isolates were also screened for ESBL (*blaSHV*, *blaTEM*, *blaOXA-1*, *blaCTX-M*), carbapenemase (*blaNDM*, *blaKPC*, *blaIMP*), and PMQR (*qnrA*, *qnrB*, *qnrS*) genes using PCR. The *E. coli* and *Salmonella* sp. isolates were resistant to all 12 tested antibiotics across six classes. The *E. coli* isolate had the *blaTEM*, *blaOXA-1*, *blaNDM*, *qnrA*, and *qnrS* genes; the *Salmonella* sp. isolate carried *blaOXA-1* and *qnrS*. This report confirms that poultry serve as a reservoir for bacteria co-harboring critical ESBL, carbapenemase, and PMQR genes, thereby exhibiting severe multidrug resistance. The findings point to a serious public health concern and underscore the need for improved antimicrobial stewardship and surveillance in the food animal industries.

Keywords: *Poultry, Antimicrobial Resistance, ESBL, Fluoroquinolones, Salmonella sp., Escherichia coli*

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1 Introduction

Antimicrobial resistance (AMR) is a significant global health problem, affecting animals, the environment, and humans (Ahmed et al., 2024). The menace of multidrug resistance has further worsened the problem, restricting treatment options, particularly in humans and animals (Rathored & Budhbaware, 2025). Recently, enteric bacteria such as *Salmonella* sp. and *Escherichia coli* have increasingly been recognized for their high resistance to first-line antibiotics and even to antibiotics regarded as a last resort in the management of life-threatening bacterial diseases in both animals and humans (Kumar et al., 2025; Poirel et al., 2018).

The poultry sector is an essential source of animal protein in many developed and developing countries, including Nigeria. It plays an essential part in meeting humans' daily protein needs through egg and meat consumption (Attia et al., 2022). However, this industry is a significant contributor to antimicrobial resistance due to the misuse of antibiotics for disease prevention and management, as well as to boost growth (Jemilehin et al., 2024; Mulchandani et al., 2023).

Resistance to β-lactams (including cephalosporins, carbapenems, and penicillins) and fluoroquinolone antibiotics is of particular concern, as these antibiotics are critical for the management of life-threatening bacterial diseases (Foudraine et al., 2021; Kherroubi et al., 2024). Apart from phenotypic resistance exhibited by most bacteria to these classes of antibiotics, some bacteria carry plasmid-mediated quinolone resistance (PMQR) genes and Extended-spectrum β-lactamases (ESBLs), which confer resistance to these important and commonly used antimicrobials, thereby limiting therapeutic options in both animal and human medicine (Shaikh et al., 2015). Genes such as *bla*TEM, *bla*SHV, *bla*KPC, *bla*OXA-1, and *bla*NDM are frequently implicated in β-lactam resistance, while quinolone resistance is often associated with *qnr* genes (*qnr*A, *qnr*B, *qnr*S) (Jemilehin et al., 2024). The *qnr* genes are plasmid-borne and facilitate horizontal gene transfer, thereby enhancing their ability to spread across bacterial populations and making such bacteria a significant public health risk (Jemilehin et al., 2024; Poirel et al., 2016).

The detection of bacteria resistant to multiple antibiotics, with associated ESBL and quinolone resistance genes, within the food chain not only compromises animal health and production but could also be a source of resistant bacteria transmitted to people and the ecosystem (Ballash et al., 2024; Juraschek et al., 2022). This underscores the need for continuous screening and maintenance of strict biosecurity practices to protect the food chain, especially animals that are mostly consumed, such as poultry, from multidrug-resistant bacteria. This will help to make evidence-based interventions and promote the judicious use of antibiotics. This case reports the molecular detection and characterization of PMQR and ESBL in multidrug-resistant *Salmonella* sp. and *E. coli* isolates from poultry.

2 Case Detail Presentation

2.1 Case History and Flock Description (August 08, 2025)

Twelve (12) fresh carcasses of 9-week-old female Arbor Acre broiler breeders were presented at the poultry diseases clinic, Veterinary Teaching Hospital, University of Ibadan. History showed an average daily mortality of about 25 birds continuously for about 15 days before presentation. The birds were floor-reared, with a total population of 16,000, divided equally between two pens designated Houses 1 and 2. They were maintained on a self-compounded broiler breeder ration, and water was provided ad libitum through a nipple drinking system. The feather's appearance was dirty, with litter adhering to the claws. Yellowish-whitish diarrheic fecal pasting was observed around the vent area in the majority of the carcasses presented.

2.2 Postmortem Findings

There was marked thickening of the air sacs with whitish exudates, marked perihepatitis and pericarditis; the liver was observed to be darkish-brown in color with some areas of necrosis, while the gallbladder was observed to be markedly engorged, as shown in Figure 1. Mucoid enteritis was pervasive throughout the intestine, with yellowish pasting of the vent area. The kidneys were mildly swollen, and there was a hemorrhage presenting as a zebra-like appearance in the colon.

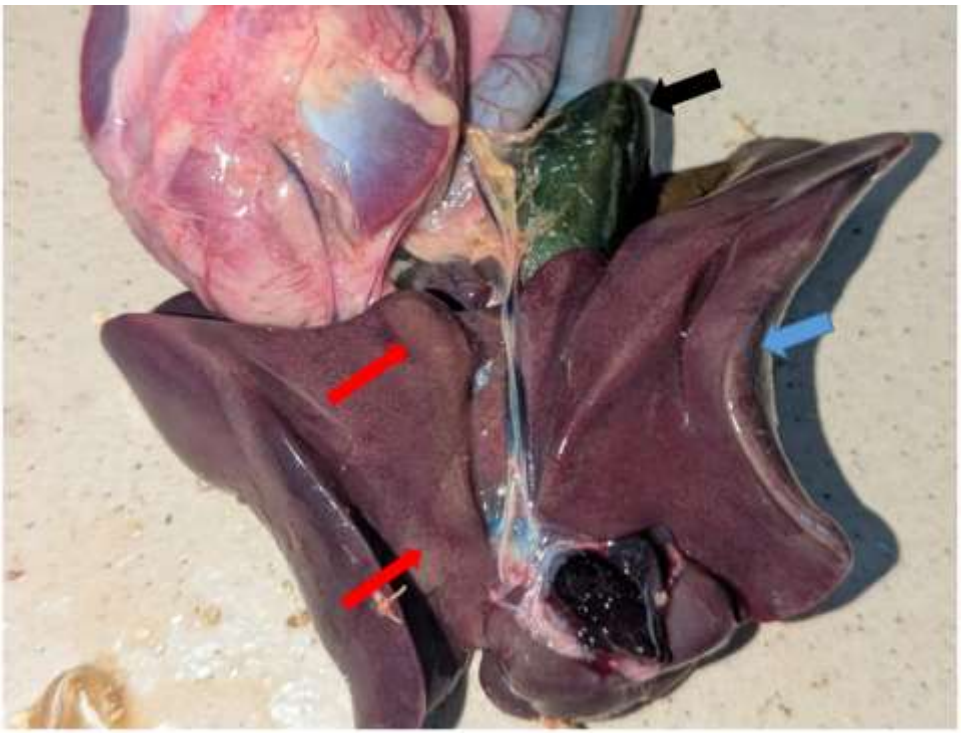

Figure 1. Visceral surface of the mildly enlarged liver of the examined broiler breeder displaying a markedly engorged gall bladder (black arrow) with multifocal areas of necrosis (red arrows) about 2cm lateral to the hilus of the biliary tract. The glistening capsule is markedly thickened by fibrinous exudate, indicative of marked fibrinous peri-hepatitis (blue arrow).

2.3 Sample Collection

Tissue samples from the kidneys, gall bladder, and liver with gross pathological lesions were aseptically harvested at post-mortem into sterile Petri dishes. Samples were submitted for microbiology culture and sensitivity within 2 hours of case presentation.

2.4 Microbial Culture and Identification

The samples were pooled and inoculated in Buffered peptone water for 24 hours at 37°C. To allow bacterial growth, the inoculum was subcultured onto MacConkey agar plates and incubated at 37°C for 24–48 h; discrete colonies were Gram-stained. The Lactose fermenter colonies on MacConkey agar were subcultured on eosin methylene blue agar at 37°C for twenty-four hours (Bedair et al., 2025; Chowdhury et al., 2020). A representative bacterial colony was selected for biochemical identification and antibiotic susceptibility testing. The API 20E kit and the APIWEB software were used to identify the non-lactose fermenter and the Gram-negative lactose fermenter colonies (bioMéieux, France).

2.5 Molecular identification

The Weerakkody and Witharana (2024) protocol was used to extract DNA. PCR was used to screen the isolates for the presence of *bla*TEM, *bla*SHV, *bla*KPC, *bla*IMP, *bla*NDM, *bla*OXA-1, *bla*CTX, *qnr*A, *qnr*B, and *qnr*S genes at 258bp, 319bp, 498bp, 568bp, 624bp, 190bp, 598bp, 516bp, 469bp, and 417bp, respectively, as previously described (Table 1) (Weerakkody & Witharana, 2024). Reaction cocktail used for all PCR per primer set included (Reagent Volume µl) - 5X PCR SYBR green buffer (2.5), MgCl2 (0.75), 10 pM DNTP (0.25), 10 pM of each forward and reverse primer (0.25), 8000U of Taq DNA polymerase (0.06), and made up to 10.5 with sterile distilled water to which 2 µl template was added. The recycling conditions consisted of an initial denaturation for 5 minutes at 94°C, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 40 seconds, extension at 72°C for 40 seconds, and a final extension at 72°C for 10 minutes. An ordinary buffer with no DNA was run alongside as a negative control. Electrophoresis was used to separate the amplicons on a 1.5% agarose gel in Tris-Borate EDTA buffer at 110 volts for an hour. A 100 bp DNA ladder was used, and the DNA

fragments were visualized using a Trans UV Illuminator after staining with ethidium bromide.

Table 1. Oligonucleotide primers used in this study

Gene	Primer sequence 5'-3'	Band size (bp)	References
Bla$_{CTX}$	Forward: TTTGCGATGTGCAGTACCAGTAA Reverse: CGATATCGTTGGTGGTGCCATA	598	(Monstein et al., 2007)
bla$_{IMP}$	Forward: TCGTTTGAAGAAGTTAACG Reverse: ATGTAAGTTTCAAGAGTGATGC	568	(Mahmoud et al., 2020)
bla$_{KPC}$	Forward: CATTCAAGGGCTTTCTTGCTGC Reverse: ACGACGGCATAGTCATTTGC	498	(Mahmoud et al., 2020)
bla$_{NDM}$	Forward: GGTTTGGCGATCTGGTTTTC Reverse: CGGAATGGCTCATCACGATC	624	(Mahmoud et al., 2020)
bla$_{OXA}$	Forward: TTCTGTTGTTTGGGTTTCGC Reverse: ACGCAGGAATTGAATTTGTT	190	(Dallenne et al., 2010)
bla$_{Tem}$	Forward: GTCGCCGCATACACTATTCTCA Reverse: CGCTCGTCGTTTGGTATGG	258	(Cai et al., 2008)
bla$_{SHV}$	Forward: GCCTTGACCGCTGGGAAAC Reverse: GGCGTATCCCGCAGATAAAT	319	(Dallenne et al., 2010)
*qnr*A	Forward: ATTTCTCACGCCAGGATTTG Reverse: GATCGGCAAAGGTTAGGTCA	516	(Wang et al., 2008)
*qnr*B	Forward: GATCGTGAAAGCCAGAAAGG Reverse: ACGATGCCTGGTAGTTGTCC	469	(Wang et al., 2008)
*qnr*S	Forward: ACGACATTCGTCAACTGCAA Reverse: TAAATTGGCACCCTGTAGGC	417	(Wang et al., 2008)

2.6 Antibiotic Susceptibility Testing (AST)

The following antibacterial (Oxoid™) were used for AST according to the Kirby-Bauer disc diffusion method: ceftriaxone (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), ampicillin (10 µg), gentamicin (10 µg), azithromycin (15 µg), amoxicillin+clavulanic acid (20/10 µg), ciprofloxacin (5 µg), sparfloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg) and pefloxacin (5 µg). These antibacterial agents include widely used antibiotics for treating bacterial infections in poultry and antibiotics of last resort for treating life-threatening and complicated bacterial infections in humans. The results were interpreted according to the CLSI (2025) guidelines (Clinical Laboratory Standards Institute, 2025). (Table 2). Standard reference strain *Escherichia coli* ATCC 25922 was used for quality control to ensure the reliability of the results.

Table 2. Results of Antimicrobial Susceptibility Test of the Escherichia coli and Salmonella sp. isolates

Antimicrobial class	Antimicrobial tested	AST results			
		E. coli	*Zone of inhibition(mm)*	*Salmonella* sp.	*Zone of inhibition*
Beta Lactam Inhibitor	Amoxicillin+Clavulanic acid (20/10 µg)	Resistant	6	Resistant	6
Aminoglycoside	Gentamicin (10 µg)	Resistant	6	Resistant	11
Macrolide	Azithromycin (15 µg)	Resistant	6	Resistant	6
Cephalosporin	Cefuroxime (30 µg)	Resistant	6	Resistant	13
(2nd generation)	Cefoxitin (30 µg)	Resistant	6	Resistant	6
Cephalosporin (3rd generation)	Ceftriaxone (30 µg)	Resistant	15	Resistant	12
Fluoroquinolones	Ofloxacin (5 µg)	Resistant	6	Resistant	6
	Ciprofloxacin (5 µg)	Resistant	6	Resistant	6
	Pefloxacin (5 µg)	Resistant	6	Resistant	6
	Levofloxacin (5 µg)	Resistant	8	Resistant	6
	Sparfloxacin (5 µg)	Resistant	11	Resistant	13
Penicillin derivative	Ampicillin (10 µg)	Resistant	6	Resistant	10

3 Results

One *Salmonella* sp. (96.3%) *and* one *E. coli* (99.8%) were confirmed by colonial morphology and biochemical identification (API 20E).

The *E. coli* isolate was positive for *bla*TEM, *bla*OXA-1, *bla*NDM, *qnr*S, and *qnr*A, while the *Salmonella* sp.isolate was positive for *bla*OXA-1 and *qnr*S (Figures 2 and 3).

The bacteria showed complete resistance to all 12 antibiotics (Table 2).

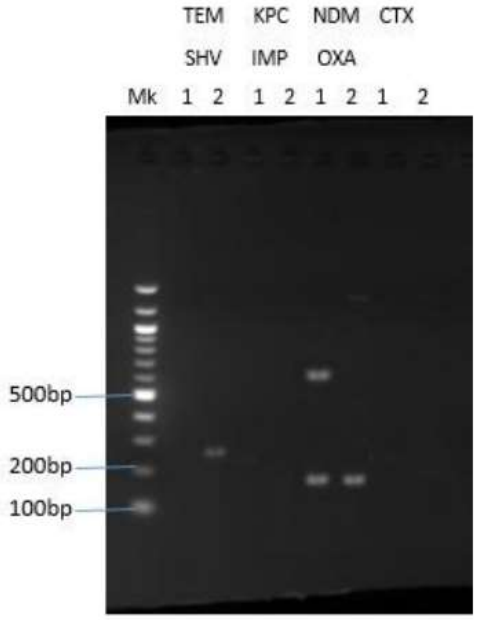

Figure 2. Agarose gel showing polymerase chain reaction amplification products of ESBL; blaTEM blaSHV blaKPC blaIMP blaNDM blaOXA and blaCTX gene amplified. (Band size approximately 258bp, 319bp, 498bp, 568bp, 624bp, 190bp and 598bp, respectively). MK: Molecular Marker; 1: E. coli; 2: Salmonella sp. Gel %: 1.5% Agarose gel; Ladder size: 100bp.

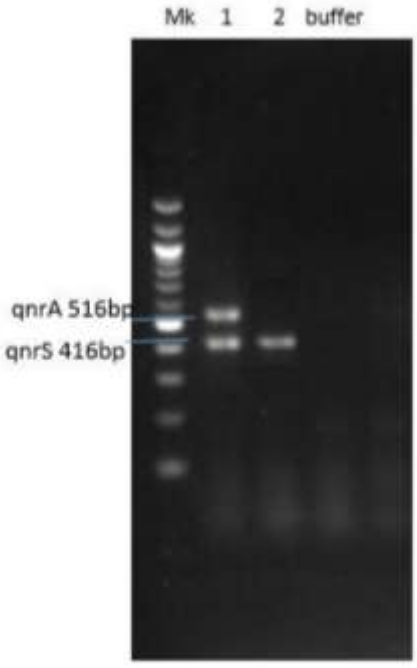

Figure 3. Agarose gel showing PCR screening for qnrA, qnrB, and qnrS genes. Positive amplification was detected for qnrA and qnrS in E. coli and qnrS in Salmonella sp.; qnrB was not detected. Expected band sizes were approximately 516 bp, 469 bp, and 417 bp, respectively. MK: Molecular Marker; 1: E. coli; 2: Salmonella sp.; Buffer: negative control; Gel: 1.5% agarose; Ladder size: 100 bp.

4 Discussion

This case reports the detection of *Salmonella* sp. *and E. coli* that were multidrug-resistant (MDR), demonstrating that food animals, especially poultry, are substantial reservoirs of clinically significant resistance genes. The detection of *bla*TEM and *bla*OXA-1 in the *Escherichia coli* isolate indicates that the bacterial isolate is a strain that produces ESBL. blaNDM, a carbapenemase gene, was detected in the *E coli* isolates. Carbapenemase genes, particularly NDM, are critical because they confer resistance to carbapenems, which are among the last-line antibiotics in human medicine (Song et al., 2025). Although *bla*CTX-M and *bla*SHV were not detected, the presence of multiple β-lactamase genes in an isolate indicates an increased likelihood of MDR and the potential for these resistance genes to spread within the ecosystem (Chen et al., 2022; Ruiz et al., 2025). The *Salmonella* sp. isolate also harbored blaOXA-1, a significant finding suggesting the potential presence of additional β-lactamase genes (Orole et al., 2024). This report is consistent with previous reports of OXA-type β-lactamases in *Salmonella* sp. isolates from poultry (Zhao et al., 2017).

The detection of PMQR genes in the bacteria further highlights significant public health implications. Both isolates carried *qnr*S, while the *E. coli* isolate additionally carried *qnr*A, indicating multiple mechanisms that could contribute to fluoroquinolone resistance. The *qnr* genes are PMQR genes that confer quinolone resistance by protecting topoisomerase IV and DNA gyrase from quinolone inhibition, thereby facilitating the emergence of significant fluoroquinolone resistance under selective pressure (Amel et al., 2024). Similar studies in wild and domestic birds have suggested that avian populations contribute significantly to the dissemination of antibiotic-resistant genes, including PMQR genes, across ecological boundaries (Ghasabsaraei et al., 2026; Ong et al., 2020). The combined presence of *qnr*S in the two isolates, together with *qnr*A in the *E. coli* isolate, suggests a possible risk of plasmid transfer to other bacterial species within poultry production systems, among handlers and consumers of poultry products, and in the environment.

The detection of ESBL, PMQR, and carbapenemase genes in the isolates recovered from broiler breeders is of serious epidemiological concern, given the multiple pathways through which these resistance genes can be disseminated to animals, the environment, and people. Broiler breeders are long-lived birds, with their chicks often sold to several commercial broiler farms across different geographic locations worldwide (Ibayi et al., 2025; Riber & Wurtz, 2024). Invariably, resistance genes from such animals can be vertically transmitted to their eggs and chicks, which are then sold to other farms, thereby spreading these genes to other farms, regions, and the environment. In addition, these resistance genes could be transmitted via farm workers, poultry manure, slaughtering knives, equipment, and so on (Chen et al., 2025; Ren et al., 2025).

Phenotypically, the antimicrobial susceptibility test revealed complete resistance (100%) to all tested antibiotics, including fluoroquinolones, cephalosporins, β-lactams, aminoglycosides, and macrolides. This level of resistance is consistent with molecular detection of β-lactamase and PMQR genes. Multidrug-resistant poultry isolates have been previously shown to transmit resistance genes through direct contact, fecal contamination, or environmental pathways to animals, humans, and the environment (Scicchitano et al., 2024). The coexistence of ESBL, carbapenemase, and PMQR genes in the same bacteria underscores the serious threat posed by the misuse of antimicrobials in food-producing animals, especially poultry, and its contribution to increasing antimicrobial resistance.

This case report has several limitations. Only one representative *E. coli* isolate and one representative *Salmonella* sp. isolate were selected from pooled organ samples; therefore, the findings cannot be used to estimate prevalence, flock-level distribution, or farm-level burden of resistance. *Salmonella* sp. was not confirmed by serotyping or molecular confirmation, such as invA PCR. In addition, sequencing of resistance genes and positive controls for all PCR targets were not available. Therefore, the findings should be interpreted as the detection of resistance genes in representative isolates from a clinical case, and broader surveillance is required to confirm their epidemiological significance.

In conclusion, this case report is among the few reports demonstrating the concurrent presence of PMQR, ESBL, and carbapenemase genes in *Salmonella* sp. and *E. coli* from poultry in Nigeria. These findings suggest that poultry may be a significant reservoir of high-risk antibiotic-resistant genes, with serious public health implications. Further research is recommended to expand understanding of the potential for horizontal gene transmission, the spread of resistant strains in the environment, and the poultry industry's contribution to the menace of AMR.

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Use of Artificial Intelligence

An AI tool (Grammarly, Free version) was used for language editing during manuscript preparation.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

OOE carried out the post-mortem examination, managed the flock, and contributed to writing the manuscript. The laboratory analysis and manuscript preparation were completed by FOJ, AOO, and TOO. VEA and TAE oversaw the case and helped edit the manuscript. The final text was reviewed and approved by all authors.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Ethical Considerations

This case report was based on a routine postmortem diagnostic examination and laboratory investigations conducted at the Avian Unit of the Veterinary Medicine Department, University of Ibadan, Nigeria, following the submission of dead broiler breeder carcasses by the farm owner/custodian. No experimental procedures were performed on live birds for research purposes, and no birds were euthanized specifically for this study; therefore, formal institutional animal ethics approval was not required. Owner/custodian consent was obtained for necropsy, diagnostic sampling, laboratory testing, and publication of the case information and images. All postmortem procedures, sample collection, handling, and laboratory analyses were performed for diagnostic purposes and in accordance with institutional animal welfare principles, accepted veterinary ethical standards, and appropriate biosafety and biosecurity practices.

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