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Isolation, molecular detection, and sequence analysis of *Avibacterium paragallinarum* from suspected cases of infectious coryza infected chickens from different areas of Ethiopia, 2022–2024

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Abstract

Background *Avibacterium paragallinarum* is a causative agent of infectious coryza (IC), a disease that affects the upper respiratory tracts and paranasal sinuses of chickens, resulting significant economic losses in the poultry industry. The objective of this study was to isolate and identify *Av. paragallinarum* using bacteriological and molecular methods between February 2022 and April 2024. A total of 74 swab samples were collected from chickens showing ocular and nasal discharges and swelling of the infraorbital sinuses.

Method Clinical samples were collected from chickens showing symptoms of IC from six locations of Ethiopia for the isolation and identification of the causative agent. Swab samples from the nasal cavity and cheesy material from the infraorbital sinus were screened using conventional PCR and inoculated onto chocolate agar enriched with 5% sheep blood. Colonies suspected of being *Av. paragallinarum* were transferred to brain heart agar supplemented with horse serum. Gram staining was used to examine the morphology of bacteria in pure colonies grown on chocolate and brain heart infusion agar.

Results The isolation of *Av. paragallinarum* on chocolate and brain heart infusion agar resulted in the observation of small, translucent, dewdrop-shaped colonies after 24 h of incubation at 37 °C in a 5% CO_2 incubator. A smear prepared from a single colony of revealed Gram-negative, short rod-shaped or coccobacilli *Av. paragallinarum* bacteria. Biochemical tests conducted on this isolate yielded negative results for catalase, oxidase, urease, indole,

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methyl red, and Voges-Proskauer tests. However, the bacterium exhibited positive fermentative activity with glucose, sucrose, and maltose. Biochemical assay revealed the presence of *Av. Paragallinarum*. The bacterial colonies confirmed a 511 bp PCR product. The partial HPG-2 gene nucleotide sequences of eleven isolates were sequenced and deposited in GenBank with the accession number PQ565862-72. A phylogenetic tree was constructed to determine the genetic relatedness of Ethiopian isolates with isolates from other African countries and elsewhere.

Conclusion The current investigation confirmed that the outbreaks were caused by *Avibacterium paragallinarum* and provided scientific evidence on the presence of different strains of *Av. paragallinarum* in Ethiopia. This is the first study in Ethiopia to detect and identify *Av. paragallinarum* from diseased chickens using molecular approaches. Further molecular characterization of locally circulating *Av. paragallinarum* isolates is recommended to be used as a vaccine strain for the prevention and control of infectious coryza.

Keywords *Avibacterium paragallinarum*, Infectious coryza, HPG-2 gene, PCR, Sequencing, Chicken

Introduction

Avibacterium paragallinarum, previously known as *Haemophilus paragallinarum*, is responsible for causing avian infectious coryza, an acute upper respiratory tract infection in chickens (*Gallus gallus*). This disease is characterized by the presence of ocular and nasal discharge, conjunctivitis, sneezing, and facial swelling due to the accumulation of purulent material in infra-orbital sinuses. The name "infectious coryza" is used to describe a contagious disease that affects the nasal passages [1].

Infectious coryza can affect chickens of any age, but older chickens tend to exhibit more severe symptoms. This disease is characterized by its rapid spread, high rate of infection, and low mortality. It can be transmitted through contaminated drinking water, nasal discharge, aerosols, and direct contact with infected or carrier chickens. The disease is prevalent worldwide, wherever chickens are present. Infectious coryza has a severe economic impacts on the poultry industry, resulting in increased culling, stunted growth, decreased feed and water intake, weight loss, and reduced egg production in layers [2, 3].

The bacterium *Avibacterium* (*Haemophilus*) belongs to the family *Pasteurellaceae*. This bacterium is Gram-negative, non-motile, and non-spore-forming, with a capsule and polar staining. It appears as small rod-shaped or coccobacilli. *Av. paragallinarum* is a non-invasive organism that strongly prefers ciliated cells. Its pathogenic mechanism involves the production of a capsule associated with virulence and a variety of haemagglutinins [4]. The bacterium is a fastidious microorganism that often requires nicotinamide adenine dinucleotide (NAD, V-factor) to grow in laboratory culture media [5]. However, NAD-independent strains have been identified in Mexico and South Africa [6, 7]. Isolating and preserving *Av. paragallinarum* cultures can be challenging due to the organism's fragility and tendency to degenerate easily, resulting in rapid inactivation outside of the host [4, 8, 9].

Confirmation of the disease requires the isolation and accurate identification of *Av. paragallinarum*. Traditional diagnostic methods require isolating the bacteria on enriched media, such as chocolate agar, followed by extensive biochemical characterization [10]. *Av. paragallinarum* isolates are serotyping using two different serological approaches. Page categorized *Av. paragallinarum* into three serogroups (A, B, and C) based on the agglutination reaction or hemagglutination (HA) test using chicken antisera. Subsequently, these classification systems were then further subdivided into nine Kume hemagglutinin serovars: A1–A4, B–1, and C1–C4 using hemagglutination inhibition [11].

The PCR method is a sensitive, effective, and dependable diagnostic technique for detecting *Av. paragallinarum* field isolates in clinical samples [12, 13]. *Av. paragallinarum* is frequently identified in nasal swabs and confirmed in laboratories using the species-specific HPG-2 PCR developed by Chen et al. [14]. A specific real-time PCR for the detection of *Av. paragallinarum* was successfully developed by Corney et al. [15]. More recently, *Av. paragallinarum* serotypes have been determined using multiplex PCR of the HMTp210 hypervariable region gene, which encodes the *Av. paragallinarum* HA antigen [16].

Previous studies in Ethiopia focused on identifying the causative agent of infectious coryza using clinical assessments and bacteriological techniques. While *Av. paragallinarum* was successfully isolated from backyard chickens in Jimma [17, 18], molecular confirmation of this pathogen has yet to be accomplished. The objective of this study was to isolate *Av. paragallinarum* from chickens suspected of having infectious coryza and to identify the pathogen using species-specific PCR and sequencing of the amplified products.

Materials and methods

Study area

The study was conducted in commercial poultry farms located in different parts of Ethiopia, specifically in areas where incidents of infectious coryza (IC) outbreaks have been reported. These areas include Haramaya, Mekele,

Metema, Addis Ababa (Akaki Kality sub-city), Legetafo-Legedadi, and Bishoftu town, from February 2022 to April 2024 (Fig. 1).

Clinical examination and sample collection

During clinical examinations, mucoid nasal discharge, conjunctivitis with closed eyes, facial edema, and bilateral swelling of the infraorbital sinuses were noted. A postmortem examination of the chickens' cranial areas was also conducted. To confirm the presence of the organism, isolation and identification were performed. A total of 74 swab samples were collected from the nasal and infraorbital sinuses of chickens exhibiting symptoms of coryza, including facial swelling and discharge. The samples were meticulously transported under refrigerated conditions to the bacteriology laboratory of the National Veterinary Institute (NVI), Bishoftu, Ethiopia where they were examined for causal agent isolation and molecular identification.

Isolation and morphological detection of *Avibacterium paragallinarum*

Before the isolation procedure, all swab samples were subjected to screening through PCR, which led to 28 samples yielding positive results. Following this, each of the PCR-positive samples was cultured on chocolate agar and brain heart infusion agar to enable the isolation of the bacteria and to examine its bacteriological characteristics. PCR positive samples were cultured on chocolate and brain heart infusion agar to isolate the bacteria. Swab samples from the nasal cavity or cheesy material from the infraorbital sinus of chickens were inoculated onto chocolate agar enriched with 5% sheep blood, which provides both NADH (V-factor) and haemin (X-factor) to enhance the growth of *Av. Paragallinarum* [4]. Inoculated plates were incubated at 37 °C with 5% CO_2 for 24 to 48 h based on the procedures mention in previous studies [4].

Colonies suspected of being *Av. paragallinarum* were selected from chocolate agar (smooth, transparent, white, and circular colonies) and transferred to brain heart agar supplemented with horse serum. The plates were placed in a 5% CO_2 incubator at 37 °C for 24 to 48 h. Gram staining was used to examine the morphology of bacteria in pure colonies grown on chocolate and brain heart infusion agars, as described by various investigators and procedures [19, 20].

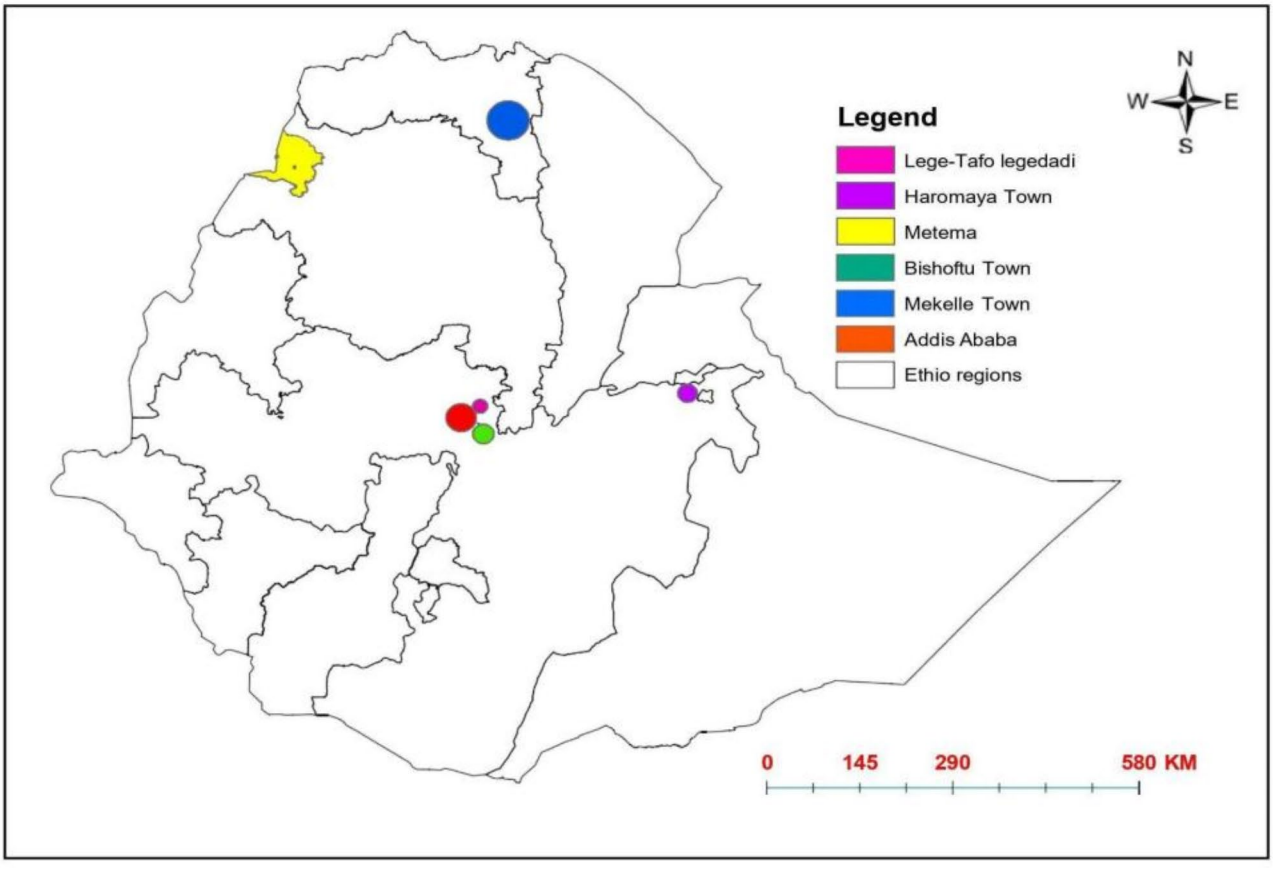

Fig. 1 A map of Ethiopia depicts the six study areas where suspected infectious coryza samples were collected. The map was created using ArcGIS 10.8.1

Biochemical test of *Avibacterium paragallinarum*

To identify *Av. paragallinarum*, the suspected colonies underwent essential biochemical tests such as the Methyl Red test, Voges Proskauer test, Indole test, catalase test, motility test, and carbohydrate tests for glucose, sucrose, and lactose [21, 22].

Molecular detection of *Avibacterium paragallinarum*

The genomic DNA of *Av. paragallinarum* was extracted from swab samples and fresh bacterial cultures using the DNeasy® Blood and Tissue Kits (Qiagen, Germany) following the manufacturer's instructions [23].

The identification of *Av. paragallinarum* was conducted using a species-specific PCR known as HPG2-PCR, as described by Chen et al. [8]. This PCR used two primers derived from the bacterium conserved DNA region. The universal primers, N1: 5'-TGAGGGTAGTCTTGCA CGCGAAT-3' and R1: 5'-CAAGGTATCGATCGTCTCT CTACT-3', were used for this investigation [24]. In each PCR tube, a 20 µl reaction volume comprising 3 µl nuclease-free water, 10 µl IQ Super Mix, 2 µl of forward and reverse primer (10 µM), and 3 µl of extracted genomic DNA. Positive (previously known *A. paragallinarum*) and negative controls were included in all PCR assays within each set of reactions. The DNA amplification was carried out in a PCR thermal cycler (Applied Biosystems 2720). The HPG2-PCR conditions were based on previous studies with a minor modification: one cycle of 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min, and a final cycle of 72°C for 10 min [12]. The PCR product and 1 kb plus molecular ladder (GeneRuler™ 100 bp plus DNA Ladder, Thermo Scientific) were loaded and separated on a 1.5% agarose gel stained with GelRed® (Biotium, Fremont, CA, USA). The results were then observed and recorded using a UV transilluminator (UVI TEC, UK).

Sequencing and phylogenetic tree analysis

The PCR products of eleven representative *Av. paragallinarum* isolates were purified using the Wizard® SV Gel and PCR clean-up system kit (Promega, Germany) following the manufacturer instructions [25]. The concentration of the PCR purified product was quantified using a microvolume spectrophotometer (NanoDrop™2000c, USA). The concentration of the quantified purified PCR product was adjusted following the requirements set by the sequencing company. The purified PCR products were mixed with the sequencing primers and submitted for sequencing using Sanger Sequencing to a commercial sequencing company (LGC Genomics, Berlin, Germany). The raw sequence data obtained from the sequencing company were assembled using Staden Package software [26]. The sequences were aligned using BioEdit 7.1.3.0 [27]. The degree of sequence similarity search was conducted using the BLAST program (NCBI). All sequence analyses were conducted in MEGA11 [28]. Multiple sequence alignment was performed using ClustalW [28]. Phylogenetic relationships among the current *Av. paragallinarum* isolates and homologous sequences retrieved from the GenBank were determined based on phylogenetic tree constructed using the neighbour-joining algorithm.

Results

Clinical observations, detailed bacterial isolation results (such as colony characteristics, cell morphology, and biochemical tests), and molecular analyses (involving PCR, nucleotide sequence, and phylogenetic analysis) of *Av. paragallinarum* are presented separately.

Clinical and postmortem examination

During clinical assessments, the chickens displayed clear indications of infectious coryza, as depicted in Fig. 2. The observed symptoms included facial swelling, an increase in the size of the intraorbital sinus, conjunctivitis with a

Fig. 2 Photos of chickens taken during disease investigation displaying typical clinical signs of infectious coryza. (**A**) swollen infraorbital sinuses, (**B**) facial swelling around the eyes with a sticky eye, and (**C**) cheesy exudate in the chicken's sinus cavity (postmortem examination)

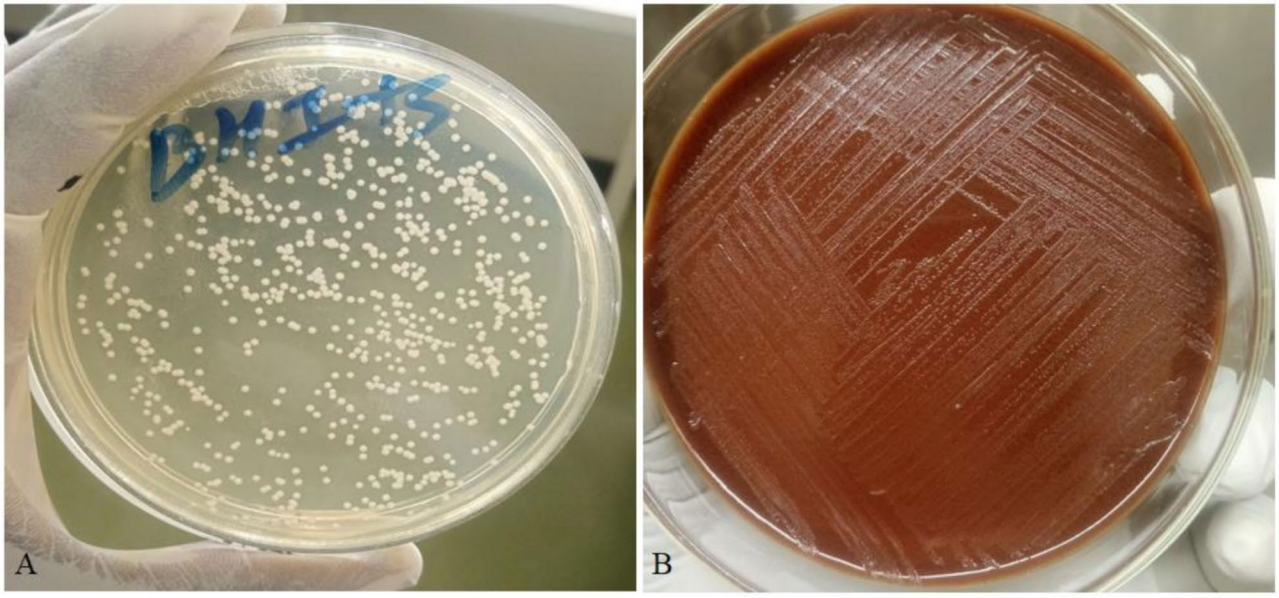

Fig. 3 Fine dewdrop-like colonies of *Av. paragallinarum* on brain heart infusion agar (**A**) and chocolate agar (**B**)

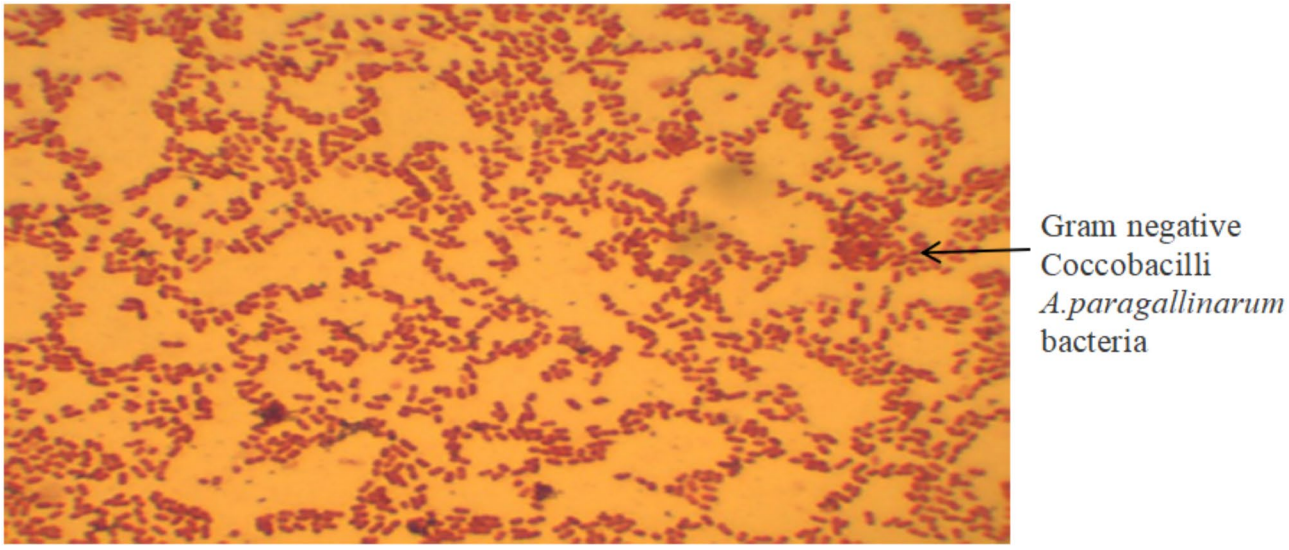

Fig. 4 A microscopic image of *Av. Paragallinarum* showing Gram negative coccobacilli

thick discharge in the conjunctival sac, as well as nasal and ocular discharge. Postmortem examination of the sinuses revealed the presence of exudates in the infraorbital sinus. In some cases, the condition was chronic, manifesting as fibrinous bilateral infraorbital sinusitis, leading to the closure of both eyes in the affected birds. Due to reagents and facility limitations, histopathology examination was not conducted.

Isolation and morphological detection *Av. paragallinarum*

Av. paragallinarum was isolated from outbreak samples by culturing the samples on chocolate and Brain Heart Infusion (BHI) agars. *Av. paragallinarum* colonies appeared as small, translucent structures resembling dewdrops on both chocolate and Brain Heart Infusion (BHI) agars after 24 h of incubation at 37 °C in a 5% CO_2 incubator (Fig. . 3). The growth characteristics of the bacteria obtained in this study were similar to those reported in other studies [4, 29].

A smear prepared from a single colony of *Av. paragallinarum* revealed Gram-negative, short rod-shaped bacteria or coccobacilli arranged singly or in pairs, as shown in Fig. 4. This morphological observation is consistent with previous investigations conducted by other researchers [30, 31].

Biochemical identification of *Av. paragallinarum*

The biochemical identification of *Av. paragallinarum* revealed negative reactions to catalase (does not produce the enzyme catalase), oxidase, urease, indole, methyl red, and Voges Proskauer tests. The bacterium showed positive fermentative ability with glucose, sucrose and maltose. The biochemical test results obtained from this study were similar to those from previous investigations [4, 32].

Molecular detection of *Av. paragallinarum*

All collected swab samples were screened for infectious coryza using conventional PCR, which specifically targeted the HPG2 gene. Out of the 74 clinical samples collected from chicken farms, 28 (38%) were positive for *Av. paragallinarum* using a specific, sensitive, and reliable HPG-2 PCR method. All PCR-positive samples were further cultured for bacterial isolation.

A total of ten representative cultures that successfully isolated *Av. paragallinarum* were selected and subsequently confirmed through HPG-2 PCR for sequencing. Eight of these cultures yielded positive PCR results, demonstrating more efficiency than direct PCR results from clinical samples. Electrophoresis of the PCR products on a 1.5% agarose gel stained with GelRed® yielded 511 bp DNA band for the positive cultures. Two cultures, one from Kality and the other from Bishoftu, produced negative results for *Av. paragallinarum*, as indicated in lanes 8 and 9 of the gel image in Fig. 5. The organism's extreme fragility, challenges in maintaining appropriate storage conditions, and difficulties in isolation due to the presence of contaminating bacteria may all contributed to the negative results of the PCR confirmatory test for these cultures. Furthermore, research has shown that direct PCR is a more sensitive approach than culture PCR [4, 14, 33, 34]. Previous studies have reported comparable results [35].

DNA sequencing and phylogenetic tree analysis

DNA sequencing of the HPG-2 gene was performed on eleven Ethiopian isolates (representative from each outbreak areas). Out of the eleven samples sequenced, two samples from Haramaya and one sample from Bishoftu were original samples; the remaining eight isolates were bacterial isolates (cultures), with one from Bishoftu and Kality and two from each of the three farms (Mekele, Metema, and Legetafo). The samples collected from Haramaya were not successfully cultured in vitro after being inoculated on culture media, mainly because of the extended storage period before the culturing process.

The partial hpg-2 gene nucleotide sequences of eleven Ethiopian *Av. paragallinarum* isolates (samples) were analyzed using bioinformatics software. The sequences were submitted to the GenBank database, and accession numbers assigned (Table 1). Samples collected in this investigation from disease-suspected farms, as well as those tested by PCR and sequenced are shown in Table 2.

Nucleotide differences observed at five locations between isolates obtained from central Ethiopia and isolates from northern and southern areas of the country (Fig. 6A). Similarly, the amino acid sequence alignment revealed variations at two residues (Fig. 6B).

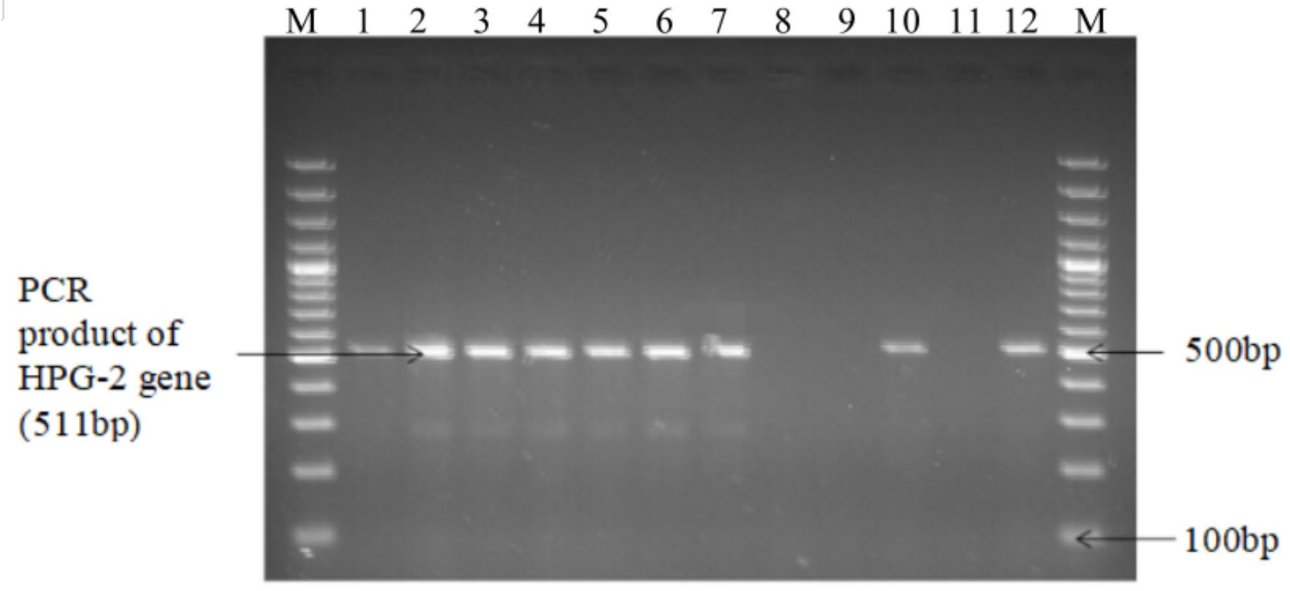

Fig. 5 Detection of HPG2 gene in *Av. paragallinarum* from culture samples. Lane M: GeneRuler 100 bp plus DNA ladder (Thermo Scientific), lanes 1 and 2: DNA extracts from culture (Mekele), lanes 3 and 4: DNA extracts from culture (Metema), lanes 5 and 6: DNA extracts from culture (Legetafo), lanes 7 and 8: DNA extracts from culture (Kality), lanes 9 and 10: DNA extracts from culture (Bishoftu), lane 11: negative control, and lane 12: positive control

Table 1 Avibactrium paragallinarum isolate name and HPG-2 gene partial sequence GenBank accession numbers

Sl No	Isolate name	Area of collection	GenBank accession number
1	Avibacterium paragallinarum strain ETH/Haramaya/01/2023 hpg2 gene, partial cds	Haramaya	PQ565862
2	Avibacterium paragallinarum strain ETH/Haramaya/02/2023 hpg2 gene, partial cds	Haramaya	PQ565863
3	Avibacterium paragallinarum strain ETH/Bishoftu/01/2024 hpg2 gene, partial cds	Bishoftu	PQ565864
4	Avibacterium paragallinarum strain ETH/Bishoftu/02/2024 hpg2 gene, partial cds	Bishoftu	PQ565865
5	Avibacterium paragallinarum strain ETH/Kality/01/2024 hpg2 gene, partial cds	Kality	PQ565866
6	Avibacterium paragallinarum strain ETH/Legetafo/01/2024 hpg2 gene, partial cds	Legetafo	PQ565867
7	Avibacterium paragallinarum strain ETH/Legetafo/02/2024 hpg2 gene, partial cds	Legetafo	PQ565868
8	Avibacterium paragallinarum strain ETH/Mekele/01/2024 hpg2 gene, partial cds	Mekele	PQ565869
9	Avibacterium paragallinarum strain ETH/Mekele/02/2024 hpg2 gene, partial cds	Mekele	PQ565870
10	Avibacterium paragallinarum strain ETH/Metema/01/2024 hpg2 gene, partial cds	Metema	PQ565871
11	Avibacterium paragallinarum strain ETH/Metema/02/2024 hpg2 gene, partial cds	Metema	PQ565872

The phylogenetic tree built using the current eleven Ethiopian *Av. paragallinarum* isolates and homologous sequences obtained from GenBank including isolates from African countries revealed that the isolates under the current investigation clustered into two groups based on geographical origin of the samples, as shown in Fig. 7. The tree shows the bootstrap values that supported the isolates' genetic relatedness.

Discussion

Infectious coryza is recognized as one of the most economically impactful respiratory diseases in the poultry sector, owing to significant decreases in egg production among laying hens and breeders, weight loss in broilers, and an increase in the rejection rates of chickens destined for slaughter. To confirm and manage the disease, isolation and accurate identification of *Av. paragallinarum* are essential. A variety of diagnostic methods, including bacteriological tests, can be used to isolate and identify *Av. paragallinarum*. Nonetheless, due to numerous factors that may impede the successful culturing of *Av. paragallinarum*, polymerase chain reaction (PCR) serves as a reliable diagnostic alternative [36].

Traditionally, bacteriological techniques have been used to detect and characterize *Av. paragallinarum* from samples collected from infectious coryza suspected chickens in Ethiopia. This study is the first to verify the presence of *Av. paragallinarum* in Ethiopia, using PCR and DNA sequencing of the HPG-2 gene. A total of 74 field samples were collected from commercial chicken farms located in six areas of Ethiopia where disease outbreaks had been reported, and these samples were subjected to HPG-2 PCR analysis. All samples that showed positive by PCR were then cultured on chocolate agar for isolation. The resulting dewdrop colonies of *Av. paragallinarum* were cultured on chocolate agar and subsequently transferred to Brain Heart Infusion (BHI) agar enriched with horse serum. On both types of culture media, the isolates exhibited small, round, transparent colonies that looked like dewdrops [37].

DNA extractions were carried out from both direct clinical samples and bacterial cultures to detect *Av. paragallinarum* at the molecular level. The PCR amplification of the HPG-2 gene from *Av. paragallinarum* produced a 511 bp amplicon, confirmed the identity of the

Table 2 Samples collected and sequenced for this study

Farm location	Field samples collected from each farm	No. of positive field samples screened by PCR	No. of culture samples tested by PCR	No. of positive isolates confirmed by PCR	No. of isolates sequenced
Mekele	12	2	2	2	2
Metema	10	6	2	2	2
Legetafo	4	2	2	2	2
Kality	18	6	2	1	1
Bishoftu	24	6	2	1	2*
Haramaya	6	6	-	-	2*
Total	74	28	10	8	11

*The original samples sequenced in this investigation were two from Haramaya and one from Bishoftu

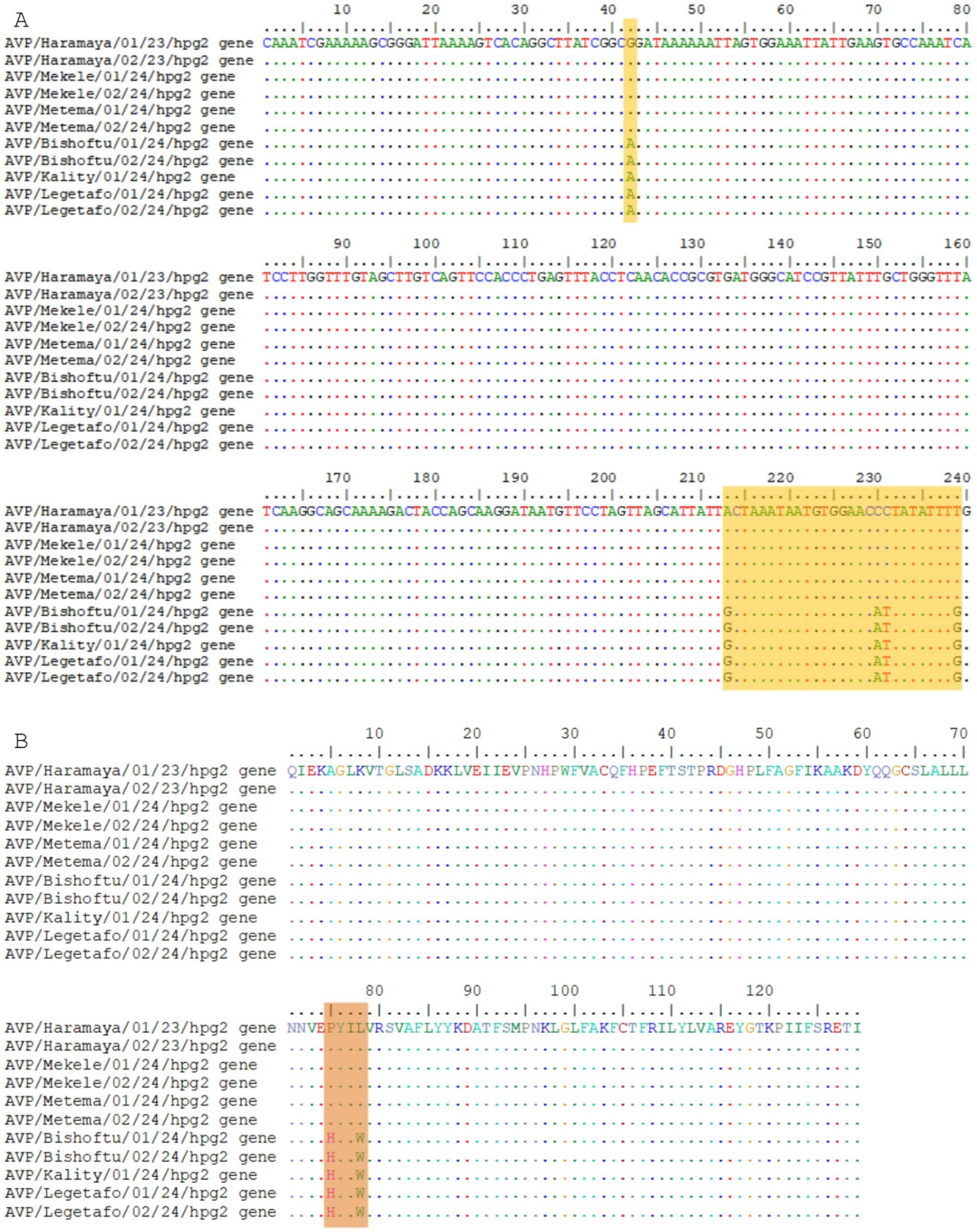

Fig. 6 The partial nucleotide sequences of the HPG-2 in eleven Ethiopian *Av. paragallinarum* isolates revealed differences in nucleotide (**A**) and amino acid (**B**) sequence

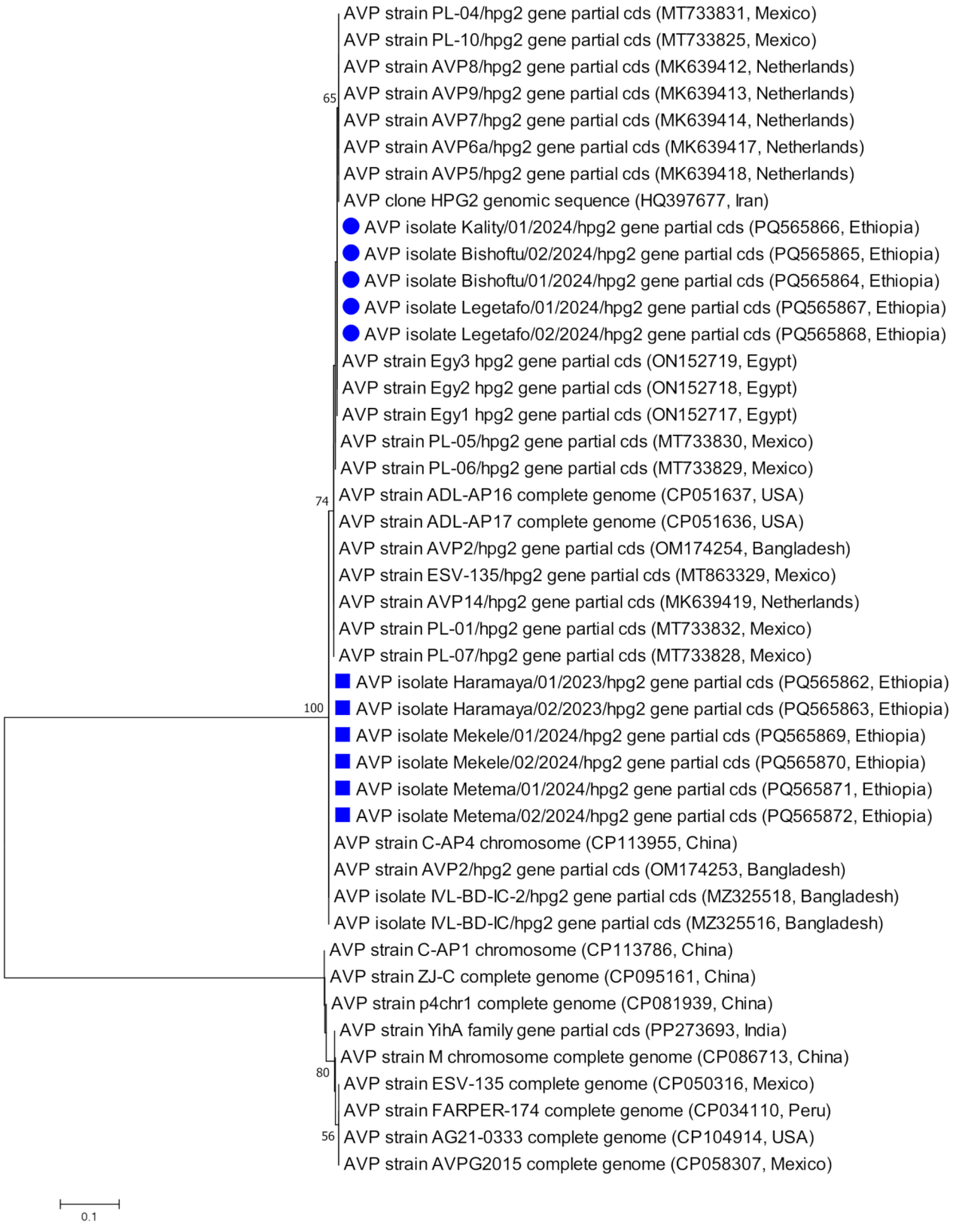

Fig. 7 (See legend on next page.)

(See figure on previous page.)

Fig. 7 Phylogenetic tree analysis of the 44 *Avibacterium paragallinarum* isolates based on the partial nucleotide sequences of the hpg2 gene. The tree was constructed using eleven field Ethiopian isolates from the current investigation (labeled with circle and rectangle) which are clustered into two groups and 33 homologous sequences retrieved from GenBank. MEGA11 software is used to compute the neighbor-joining method with the maximum composite likelihood nucleotide substitution model and the pairwise deletion option. The percentage bootstrap scores greater than 50% (out of 1000 replicates) are displayed next to each branch

bacterium. Using species-specific PCR primers, eleven *Av. paragallinarum* isolates were sequenced, that include eight cultures and three clinical samples. Following that, the sequences were compared to *Av. paragallinarum* isolates that had been added to the NCBI GenBank by other researchers. Partial nucleotide sequences of the species-specific HPG-2 gene from *Av. paragallinarum* were analyzed, and the results revealed that isolates from poultry farms in Mekele, Metema, and Haramaya shared comparable sequences. On the other hand, isolates from Bishoftu, Akaki Kality, and Legetafo have similar sequences. Interestingly, unlike the isolates from Haramaya, Mekele, and Metema, the isolates from Bishoftu, Akaki Kality, and Legetafo displayed genetic variations at nucleotide positions 42, 213, 230, 231, and 239. In Bishoftu, Akaki Kality, and Legetafo isolates, the nucleotides G, A, C, C, and T were substituted with A, G, A, T, and G, respectively, as shown in Fig. 6. Similarly, amino acid sequence differences were also observed between the two groups or serotypes at residue positions P75H and L78W. These differences in amino acid sequences necessitate further proteomic analysis to investigate the relationship with bacterial pathogenicity. These genetic differences among the isolates indicate the presence of at least two distinct serotypes of *Av. paragallinarum* in the country.

The six isolates from Mekele, Metema, and Haramaya in northern and eastern Ethiopia showed perfect hpg-2 gene sequence similarity (100%) to two *Av. paragallinarum* isolates from Iran (PP907193 and PP907194). Additionally, the six Ethiopian isolates exhibited 100% similarity with one isolate from China (CP113955) and three isolates from Bangladesh (OM174253, MZ325516, and MZ325518). In contrast, isolates from chicken farms in Bishoftu, Akaki Kality, and Legetafo shared a 98.79% sequence similarity of with the previously stated isolates from Iran, China, and Bangladesh. Furthermore, the *Av. paragallinarum* isolates from these farms exhibited a 100% sequence match with three field isolates from Egypt (ON152719, ON152718, and ON152717). The isolates from Mekele, Metema, and Haramaya, on the other hand, have 98.64% sequence similarity to the previously mentioned Egyptian isolates. Sequencing and BLAST analysis of the HPG-2 gene revealed a close relationship between Ethiopian *Av. paragallinarum* isolates and strains from the United States, Mexico, Peru, the Netherlands, and India as shown in Fig. 7. The present isolates are phylogenetically clustered into two groups, which suggest that two serotypes are circulating in the study areas; additional serotyping investigation of the causal agent is recommended. The genetic mean distance within the eleven Ethiopian *Av. paragallinarum* isolates is 0.008. Whereas, the genetic mean group distance between the two Ethiopian *Av. paragallinarum* isolates is 0.014. The genetic mean distance between the current eleven Ethiopian *Av. paragallinarum* isolates and the GenBank retrieved homologous sequences is 0.309. The overall mean distance among the 44 *Av. paragallinarum* isolates included in the phylogenetic tree analysis is 0.377.

Conclusion

This investigation successfully isolated and molecularly characterized *Avibacterium paragallinarum* from Ethiopia chickens, confirming its presence through bacterial culture, PCR, and genome sequencing techniques. The findings suggests that *Av. paragallinarum* is prevalent across various regions of Ethiopia, with isolates from the northern and eastern areas of Ethiopia showing significant genetic similarities to strains reported in Iran, China, and Bangladesh. In contrast, isolates from central Ethiopia are closely related to strains from Egypt. The observed genetic correlations between Ethiopian isolates and those from other countries provide valuable insights into the epidemiology of infectious coryza, suggesting potential cross-regional transmission pathways through different transmission mechanism. These results highlight the importance of continuous surveillance and monitoring of *Av. paragallinarum* in Ethiopia to effectively manage and control infectious coryza. Enhancing diagnostic capabilities and obtaining a better understanding of the pathogen's genetic variety will aid in the formulation of targeted control strategies and improve chicken health management practices in the country.

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Author contributions

G.D. and L.T. designed the study. G.D., E.A., L.Y., D.T. collected samples. G.D., E.A., L.Y., D.T., K.A., M.A., B.B., T.A., G.Z., A.L., K.B., A.A., M.J., A.M. conducted laboratory tests, analysis and data generate. E.G. performed sequence analysis. G.D. drafted the manuscript. G.D. and E.G. revised the manuscript. All authors have reviewed and agreed to the final version of the manuscript.

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Data availability
All data analyzed during this study are included in the manuscript. However, the raw data can be available from the corresponding author upon request.

Declarations

Ethical approval
Ethical approval for this study was obtained from the Animal Research Ethical Review Committee of the National Veterinary Institute (NVI) of Ethiopia. Verbal consent was obtained from the animal owners for their willingness to participate in the study. The sampling was performed following proper animal care and laboratory procedures.

Informed consent
Prior to collecting outbreak samples, each poultry farm owners provided written and verbal informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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