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Revisiting Avian Metapneumovirus Subtype B in Broiler Chickens and Turkeys in Morocco: First Molecular Characterization †

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Abstract: Avian Metapneumovirus (aMPV) is a significant poultry pathogen with a global presence, primarily causing respiratory issues in turkeys. It also affects chickens, although the severity of its impact is often lessened in this species. In Morocco, aMPV has been detected in broiler flocks, prompting the need to deeply analyze circulating strains to better understand the epidemiology and develop control measures accordingly. This research focuses on the sequencing and molecular characterization of aMPV in these flocks. Additionally, aMPV isolated from turkeys displaying Turkey Rhinotracheitis (TRT) signs was included in the study to compare the findings. RNA extracted from positive swabs was subjected to nested PCR, targeting the attachment protein of the G gene, followed by gel electrophoresis. Amplicons were purified and sequenced using the Sanger method. Bioinformatics tools facilitated sequence analyses, including BLAST for similarity searches and Mega® for phylogenetic analysis using the maximum likelihood method with 1000 bootstrap replicates. The investigation unveiled the existence of two distinct clades of the aMPV/B isolates, which originated from used vaccines, all circulating in broilers and turkeys and indicating potential virus transmission between both poultry species. This article presents the first-ever molecular characterization of aMPV isolated from Moroccan broilers and turkeys, encompassing comprehensive investigations on its presence and subtype, and genetic characterization.

Keywords: Avian Metapneumovirus; subtype B; Morocco; characterization; sequencing; phylogenetic analysis

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1. Introduction

Avian Metapneumovirus (aMPV) is a globally recognized pathogen causing respiratory diseases, leading to substantial economic losses in the poultry industry. Also known as *Metapneumovirus avis*, it belongs to the family *Pneumoviridae* and the genus Metapneumovirus [1].

So far, aMPV has been classified into four subtypes: A, B, C, and D [2]. These subtypes have been shown to exhibit varying effects on their avian hosts [3]. The recent disclosure of two new divergent viruses has also raised the possibility of further subtype candidates [4,5]. Traditionally, turkeys have been considered the most susceptible hosts to aMPV, often displaying more severe clinical symptoms [6,7], and are documented to be susceptible to all four subtypes described [8,9]. However, recent reports have emphasized the infection's impact on broilers, highlighting the role of aMPV as a primary infectious agent [10].

While aMPV's global presence is established [11], specific epidemiological data at the local level are essential to understanding the virus dissemination, particularly in regions like North Africa, with high poultry density and the circulation of diverse pathogens. Unfortunately, background data on avian Metapneumovirus in this area is scarce and limited to only subtype B detection [12–14].

In Morocco, poultry production is diversified, with broiler farms established in various regions with different farm densities. Unlike turkeys, which are routinely vaccinated against aMPV, broilers in Morocco are generally not, except in some cases where vaccination is adopted but not performed regularly. Despite frequent field reports of swollen head syndrome (SHS), often attributed to aMPV, its real prevalence and impact remain unclear. Recent studies on Moroccan broiler flocks with varying health status have detected aMPV; thus it is considered a potential etiological agent of clinical respiratory cases [15,16].

Consequently, our study aimed to comprehensively investigate the molecular epidemiology of aMPV in Moroccan broiler farms. Additionally, the potential circulation of aMPV amongst turkeys was evaluated by sampling flocks displaying suggestive symptoms of Turkey Rhinotracheitis (TRT). This research represents the first-ever report on the molecular characterization of aMPV in Morocco.

2. Materials and Methods

2.1. Sample Collection and RNA Extraction

Twenty-two samples (n = 22) of aMPV RNA were extracted from field swabs, collected between March and June 2022 from six different broiler chicken flocks, and all of them were confirmed to be positive for aMPV by real-time RT-PCR [16]. Eighteen of them were obtained from a longitudinal field study [16], and four from broiler flocks affected by SHS.

Additionally, five (n = 5) freshly collected heads from one broiler flock showing SHS symptoms and three (n = 3) from one flock of turkeys displaying clinical signs suggestive of TRT were analyzed. After sectioning the heads, a mixture of different tissues collected from skin, sub-cutis, and bones was crushed and blended, using a homogenizer to ensure proper mixing and uniform distribution of the viral antigen. The aim was to detect aMPV of viruses obtained in turkey tissues and compare their sequences with those already isolated and characterized from broilers.

All the studied broilers of this study were strictly unvaccinated against SHS.

Table 1 outlines the diverse RNA samples and their respective sources, indicating whether they originated from broilers or turkeys' flocks. These flocks were located in different and densely bird-populated geographic regions of Morocco, as shown in Figure 1.

Table 1. RNA samples taken between March 2022 and April 2023, and subject to PCR and sequencing.

Sample Identification	Number of Samples	Origin	Nature of RNA Samples
P1 to P22	22	Broilers	Extracted from aMPV-positive swabs [16]
727 to 731	05	Broilers	Extracted from birds with SHS
764 to 766	03	Turkeys	Extracted from birds with TRT

Figure 1. Map of Morocco, outlining the origins of the different samples studied. The sample types are indicated with symbols as follows: △: longitudinal study sampling; ○: one-off sampling; *: fresh broiler head sampling; ☆: fresh turkey head sampling from a slaughterhouse.

These flocks were located in different geographic regions of Morocco, as per Figure 1.

A live subtype B vaccine, the 1062 strain of chicken origin, was used as a positive reference control (Laboratorios HIPRA S.A., Amer, Spain). Its RNA was thus extracted, using the Kylt® RNA/DNA purification, following the manufacturer's instructions (AniCon Labor GmbH, Emstek, Germany).

2.2. Reverse-Transcriptase and Double Conventional PCR Amplification

Using the Applied Biosystems® Reverse-Transcription Kit (ThermoFisher Scientific, Waltham, MA, USA), 10 µL of RNA from a 100 µL dilution was mixed with 10 µL of RT mix,

made of MM RT Buffer (2 µL), RT random primers (2 µL), dNTP (0.8 µL), multi-scribe RT (1 µL), and H_2O (4.2 µL), with all the steps followed in compliance with the manufacturer's instructions. The obtained cDNA was subjected to a double conventional PCR amplification protocol, often referred to as "nested PCR" or "nested amplification" to detect and subtype aMPV. This technique involved using two sets of primers in two successive and separate PCR reactions, where the second set of primers is located within the region amplified by the first set of primers. These primers, namely G Start+, G5−, G6−, G8 + A, and G9 + B, were used for a nested conventional PCR and designed in the attachment protein G gene following previous studies [17,18].

The primary reason for using this nested PCR method is to increase the specificity and sensitivity of cDNA amplifications. Specific primers, targeting the conserved regions of aMPV subtypes A and B, were used in separate reactions for each tested sample.

The protocol of the nested PCR involved two amplification steps for aMPV cDNA, with a 40 µL reaction mix. Both used DreamTaq® Green PCR mastermix (25 µL) (ThermoFisher Scientific, Waltham, MA, USA) and cDNA (10 µL), whereas the primers and water volumes differed.

For the 1st amplification, forward primer G6− (2 µL), reverse primer G START+ (2 µL), and H_2O (11 µL) were used, with hybridization at 57 °C.

For the 2nd amplification, forward primer G5− (2 µL), reverse primers G8 + A (2 µL) and G9 + B (2 µL), and H_2O (9 µL) were used, with hybridization at 58 °C.

2.3. Gel Electrophoresis

After a double conventional PCR amplification, the products were separated by gel electrophoresis. Thus, 0.8 g of Agarose Top Vision Agarose® (Thermo Fisher Scientific, Waltham, MA, USA) was added to 50 mL of an in-house prepared Tris-Acetate-EDTA (TAE) solution. Containing 242 g of Tris base, 57.1 mL of glacial acetic acid, and 100 mL of EDTA for every 1 L of the preparation, this solution was adjusted to a pH of 8 and diluted to a 2% concentration (10 mL of the prepared solution mixed with 490 mL of distilled water). Additionally, 5 µL of cDNA was included in the mixture with a green stain Xpert Green DNA Stain® (20,000×) (GRiSP, Lda, Porto, Portugal); this fluorescent dye allowed DNA visualization within the gel after electrophoresis.

Bands of the expected size would confirm aMPV detection and its subtype. The differentiation between the A and B subtypes was possible based on their respective base sizes of 268 bp and 361 bp.

2.4. Pre-Sequencing Samples Purification and Sequencing

Following gel electrophoresis, amplicons displaying the most robust band intensity were chosen for purification and sequencing. Gel extraction was performed using a solution extraction kit and a gel to ensure optimal retrieval. The resulting sequence products from solution extraction and gel were subsequently denoted at the end of the sequence, named "P" and "G", respectively. The purification process was carried out using the NucleoSpin® Gel and PCR Clean-up XS kit (Macherey Nagel, Düren, Germany), following the manufacturer's guidelines.

For sequencing, the purified DNA fragments were sent to Plateau de Génomique Get-Purpan, UDEAR UMR 5165 CNRS/UPS, CHU PURPAN, Toulouse, France. Sanger sequencing was performed, using the forward and reverse primers used for PCR amplification. This allowed us to obtain the nucleotide sequence of the aMPV isolates.

2.5. Bioinformatics Analysis and Genetic Characterization

The obtained sequences were analyzed using bioinformatics tools to determine the genetic characteristics and relationships of the Moroccan aMPV isolates. Using the BLAST

program (National Center for Biotechnology Information, Bethesda, MD, USA) https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 20 September 2023), sequence similarities were sought with documented reference for aMPV strains in the database from North Africa, Europe, and the Mediterranean basin. Subsequently, multiple sequence alignment was performed using BioEdit® 7.2 (Ibis Therapeutics, Carlsbad, CA, USA) https://bioedit.software.informer.com/7.2/ (accessed on 27 September 2023), and phylogenetic analysis was conducted with Mega® 11, using the maximum likelihood (ML) method, with 1000 bootstrap replicates (Biodesign Institute, Arizona State University, Tempe, AZ, USA) https://www.megasoftware.net/ (accessed on 27 September 2023) and Figtree v1.4.4 (Institute of Evolutionary Biology, University of Edinburgh, UK) http://tree.bio.ed.ac.uk/software/figtree (accessed on 27 September 2023). Data were then analyzed using the neighbor-joining method.

Partial nucleotide sequences of the G gene of the Moroccan aMPV viruses were compared with published aMPV sequences, using a BLAST search within the EMBL/GenBank database. Sequences were investigated through phylogenetic analysis, comparing partial G sequences of the Moroccan aMPV subtype B detections with reference strains in the GenBank. Notably, the inclusion of reference strains in the phylogenetic tree was delimited to one strain per country, per year, and per bird type to mitigate excessive condensation. These data are shown in Supplementary Tables S1–S3.

Additionally, one unpublished sequence provided by the Avian Pathology Unit of the Agronomic and Veterinary Institute Hassan II was included for comparative analysis with the study outcomes of this study.

Herewith, eight nucleotide sequences were submitted to GenBank, representing the Moroccan viruses, with accession numbers: PQ202991, PQ202992, PQ202993, PQ202994, PQ202995, PQ202996, PQ202997, and PQ202998.

3. Results

3.1. Double Conventional PCR Amplification

Although aMPV subtype B was detected in all analyzed samples, sequencing analysis was specifically conducted only on those displaying highly intense bands during gel electrophoresis. Thus, only one sequence of aMPV RNA extracts was identified (PQ202993), and two out of five samples from broilers with SHS were identified, of which one yielded two different sequences after extraction (PQ202991, PQ202992, and PQ202994). Similarly, two out of three RNA extracts from turkeys showing TRT signs were identified, of which only one exhibited two sequences after extraction in gel and solution (PQ202995, PQ202996, and PQ202998).

No samples were found positive for aMPV subtype A.

3.2. Sequencing and Genetic Characterization

The partial G gene sequences of the Moroccan isolates were 350 nucleotides in length. These isolates have a nucleotide sequence identity between 91.8% (PQ202991 and PQ202997) and 99.7% (PQ202994 and PQ202996) when compared to each other and from 78.2% (PQ202995, and aMPV-B/BR/1890/E1/19, GenBank accession number OP572408.1) to 90.76% (and aMPV/B/Romania/Ty/67/17, GenBank accession number MT432878.1), when compared to non-Moroccan aMPV isolates. The reference strains aforementioned are classified as strains derived from vaccines.

Table 2 presents the highest percentage of similarities observed between the Moroccan isolates and the documented reference strains.

Table 2. Percentages of similarity between aMPV Moroccan isolates and reference strains.

Isolate	Documented GenBank Strains	Similarity (%)	Accession Number	Ref.
PQ202991	Avian Metapneumovirus isolate Algeria/26/aMPVB/turkey attachment protein gene, partial cds	96.79	KP892758.1	[14]
	Avian Metapneumovirus partial mRNA for attachment protein (G gene), strain aMPV/chicken/Nigeria/NIR89/2006	96.09	AM490057.1	[17]
PQ202992	Avian Metapneumovirus strain aMPV/B/Romania/Ty/67/17 glycoprotein (G) gene, partial cds	90.76	MT432878.1	[18]
	Avian Metapneumovirus isolate 2018_0404_Chicken_Turkey_2018 attachment protein (G) gene, partial cds		MH352465.1	[19]
PQ202993	Avian Metapneumovirus isolate aMPV/Chicken/PCRLAB/HG/2010 attachment protein (G) gene, partial cds	96.76	MN108496.1	
	Avian Metapneumovirus isolate 101/2011 attachment protein (G) gene, partial cds		KC954647.1	
PQ202994	Avian Metapneumovirus isolate aMPV-B/BR/1890/E1/19, complete genome	99.05	OP572408.1	
	Avian Metapneumovirus strain aMPV/B/Italy/Ty/742-01/17 glycoprotein (G) gene, partial cds		MT436229.1	[20]
	Avian Metapneumovirus isolate 101/2011 attachment protein (G) gene, partial cds		KC954647.1	[18]
PQ202995	Avian Metapneumovirus isolate aMPV-B/BR/1890/E1/19, complete genome	99.70	OP572408.1	
	Avian Metapneumovirus strain aMPV/B/Italy/Ty/742-01/17 glycoprotein (G) gene, partial cds		MT436229.1	[20]
	Avian Metapneumovirus isolate 101/2011 attachment protein (G) gene, partial cds		KC954647.1	[18]
PQ202996	Avian Metapneumovirus isolate aMPV-B/BR/1890/E1/19, complete genome	98.70	OP572408.1	
	Avian Metapneumovirus strain aMPV/B/Italy/Ty/742-01/17 glycoprotein (G) gene, partial cds		MT436229.1	[20]
	Avian Metapneumovirus isolate 101/2011 attachment protein (G) gene, partial cds		KC954647.1	[18]
PQ202997	Avian Metapneumovirus strain aMPV/B/Spain/Ty/4954-1/15 glycoprotein (G) gene, partial cds	99.10	MT432835.1	[18]
	Avian Metapneumovirus strain aMPV/B/France/Ck/785/17 glycoprotein (G) gene, partial cds		MT432891.1	[18]
	Avian Metapneumovirus isolate aMPV-B/turkey/VA/USA/ADRDL-5, complete genome		PP273460.1	[21]

Table 2. *Cont.*

Isolate	Documented GenBank Strains	Similarity (%)	Accession Number	Ref.
PQ202998	Avian Metapneumovirus strain aMPV/B/France/GuineaFowl/1060/18 glycoprotein (G) gene, partial cds	98.96	MT432904.1	[18]
	Avian Metapneumovirus strain aMPV/B/Romania/Ty/85/17 glycoprotein (G) gene, partial cds	98.63	MT432883.1	[18]

All Moroccan isolates showed a substantial likelihood, ranging from 90.76% to 99.70%, to reference strains reported as vaccine-derived or associated with commercial subtype B vaccines.

Phylogenetic trees were constructed from the nucleotide and deduced amino acid sequences of the partial G glycoprotein genes of Moroccan aMPV strains and non-Moroccan aMPV reference strains. The ML phylogenetic analysis revealed that Moroccan aMPV isolates were separated into two main clusters (Figure 2).

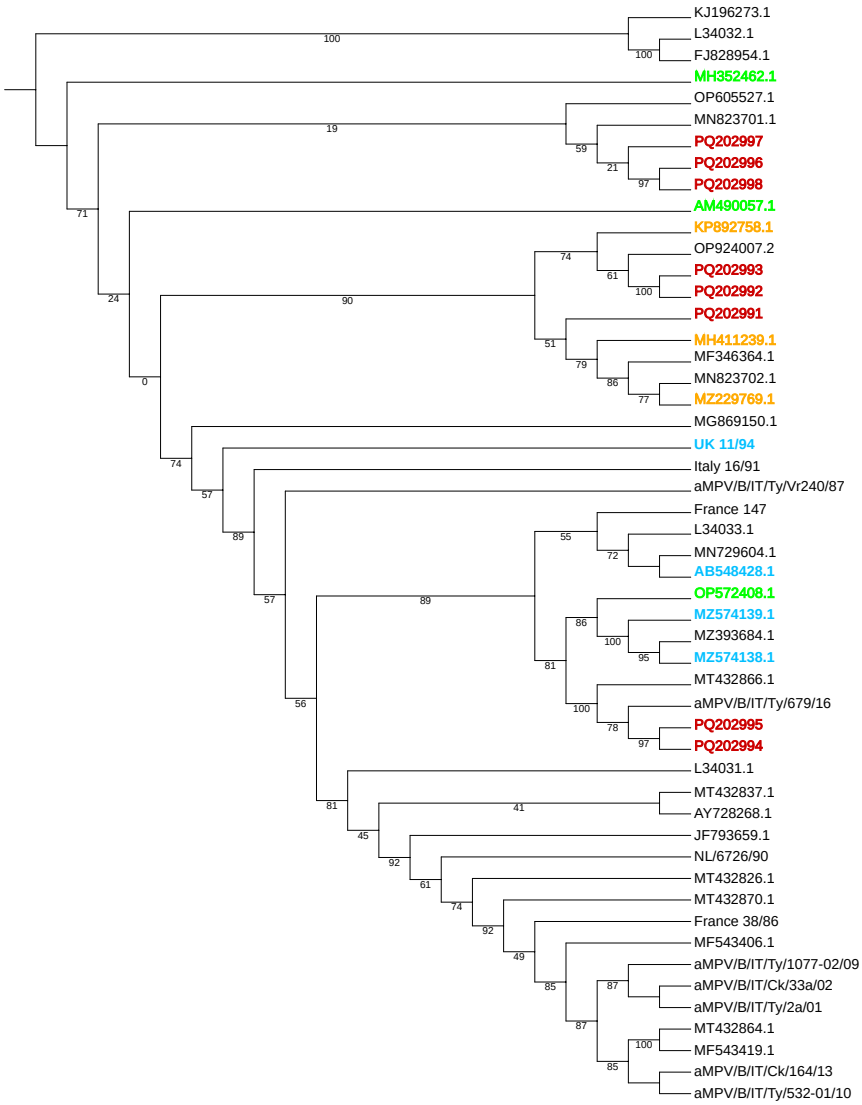

Figure 2. Phylogenetic analysis of the partial nucleic acid sequence of the G gene. The aMPV/B strains from Morocco are highlighted in red, and vaccine strains are highlighted in blue. North African strains are in orange, and reference strains with relevance are marked in green. Data were analyzed using the maximum likelihood method.

The first cluster, related to the 11/94 documented derived strains, included six Moroccan isolates: PQ202991, PQ202992, PQ202993, PQ202996, PQ202997, and PQ202998. These viruses were also grouped with Nigerian, Turkish, Algerian, and Tunisian strains (aMPV/chicken/Nigeria/NIR89/2006; GenBank accession number AM490057.1, Avian metapneumovirus isolate 80_Chicken_Turkey_2018; GenBank accession number MH352462.1, aMPV/B/turkey/Algeria/26; GenBank accession number KP892758.1, TN1015/17; GenBank accession number MH411239.1 and TN1000/19; GenBank accession number MZ229769.1, respectively).

The second cluster is represented by two viruses: PQ202994 and PQ202995. It is grouped with vaccine strains VCO3, 1062, and pl21 (GenBank accession numbers AB548428.1, MZ574138.1, and MZ574139.1, respectively) as well as the Brazilian strain (Avian Metapneumovirus isolate aMPV-B/BR/1890/E1/19; GenBank accession number OP572408.1), with the pl21 strain being vaccine-derived.

4. Discussion

Avian Metapneumovirus has been implicated as the causal agent of swollen head syndrome since its first clinical description in the 1990s [22]. While the circulation of aMPV has been suspected in Morocco, it has often been considered a minor co-infecting agent in clinical respiratory problems, particularly in broiler flocks, for which its significance has been traditionally overlooked [15]. This has resulted in a lack of knowledge regarding its prevalence and incidence in broilers. This paper represents the first comprehensive report on the molecular characterization of aMPV strains circulating in Morocco, revealing two clusters of the aMPV/B subtype, which are vaccine-derived. The observed genetic diversity underscored the presence of different Metapneumovirus strains of subtype B among broiler and turkey farms in Morocco, possibly having diverse sources.

Following the prior research's focus on aMPV subtypes, our study has been limited to investigating aMPV/A and aMPV/B circulation, highlighting the widespread presence of the virus across diverse regions [15,16].

So far, only subtype B has been detected and sequenced from either stored or freshly collected samples. These findings align with previous investigations, emphasizing the dominance of subtype B, especially in broiler chickens [16]. The prevalence of aMPV/B has shown a substantial increase across different regions of the world, including a very recent description in the USA [21], aligning with previous research in neighboring countries from North Africa and the Mediterranean areas and emphasizing the dominance of subtype B [10,12–14,19,23–27]. It is worth noting that subtype B spreads more widely through respiratory routes, unlike subtype A, which is more limited to the oro-fecal route [3] and has low excretion in chickens [28].

The constructed phylogenetic tree and nucleotide likelihood identity highlighted distinct clades of aMPV subtype B in Morocco. In the first clade, Moroccan isolates formed clusters, with strains documented in various countries, notably those identified as vaccine-derived ones [17–19], and exhibited grouping with the vaccine strain 11/94. While previous reports pointed out the possibility of the vaccine spreading from nearby vaccinated flocks, presumably turkeys [18,19], the detection of aMPV in our case also resulted from flocks with clinical expression of the SHS, albeit far from any other poultry production types mandatorily vaccinated against aMPV [16]. This finding suggests the hypothesis of potential virus transmission from Europe to North Africa, possibly facilitated by migratory birds or trade [13]. It is crucial to acknowledge the limitations of our study due to the scarcity of research and data available from North African countries, except Tunisia. This limitation may introduce bias and distort the understanding of aMPV subtypes spread from wild birds [29]. Given the insufficient public databases, further investigations are

necessary to comprehensively elucidate the epidemiological patterns of aMPV infection and pathogenicity. Nevertheless, it is noteworthy that this hypothesis contradicts investigations from Italy that have downplayed the role of wild birds in the spread of aMPV subtypes A and B [30].

Notably, the Algerian strain (GenBank accession number KP892758.1) [14] and the two Tunisian isolates (GenBank accession numbers MH411239.1 and MZ229769.1) demonstrated substantial phylogenetic proximity to the Moroccan isolates within this clade, highlighting the consistent and comparable epidemiological context across North African countries. This finding is further supported where, interestingly, the Tunisian vaccine-derived strain MH411239.1 exhibits a 99% genetic similarity with the Algerian and Nigerian strains previously reported and also classified as vaccine-derived strains [13].

Within the second clade, a convergence of viruses was observed, originating from widely employed commercial vaccines and Italian turkey field isolates. This pattern aligns with previous findings that grouped these viruses, whether vaccines, vaccine-derived, or old strains, with the aMPV/B vaccine, including strains VCO3 or 1062, identified in vaccinated turkeys in Italy [18]. Previous reports on isolates with high phylogenetic proximity to our second cluster confirmed their unrelatedness to vaccine strains 1062 or pl21 [31]. However, the documented strains with similarity to the Moroccan isolates all showed closeness to vaccine-derived strain pl21 [20,21,28]., which can plausibly explain the observed similarities with the Tunisian isolate in the first clade, which is linked to the vaccine strain pl21 [13] and might have reverted to virulence. These observations reinforce the hypothesis that the North African isolates share a common origin.

However, the rarity of findings from this geographic area limits the ability to generalize conclusions about the origin and spread of aMPV strains. For example, the detection of subtype B in Algeria was limited to just one case in turkeys [14], which makes it difficult to draw broad conclusions from such a limited data point. Furthermore, while the present study points out solely subtype B, our previous endeavor [16] has shown that both aMPV-A and aMPV-B are present in Morocco, which does not establish a definitive pattern, considering the emergent subtype C that was not considered in any of the North African countries.

Also, for Tunisia, it is unclear whether the introduction of aMPV is due to commercial trade or migratory bird flyways. These remain open questions that require further investigation before making any concrete conclusions.

It is plausible that the detection of aMPV/B in turkeys, looked up cautiously, given the limited number of samples tested (three individual birds originated from the same flock), might be related to the extensive use, in the past and potentially present, of heterologous vaccines against aMPV/A for many years, exerting vaccine pressure and generating the alternation of aMPV/B [32].

Remarkably, the two clusters showed notable similarity existing between broiler and turkey aMPV/B local strains, indicating the potential for aMPV transmission between these two species. This finding suggests that the aMPV strains, detected in Moroccan broilers, also circulate in turkeys and vice versa, implying a form of cross-species transmission [33].

This outcome is particularly pertinent for the specificities of farm complexes in Morocco, which are granted authorization for rearing either broiler chickens or fattening turkeys in the same complex, leading to the alternation of both production types throughout the year. This production switching between broilers and turkeys, due to market demands could facilitate cross-species transmission. On a larger scale, this holds for regions with high-density poultry, highlighting the need for vigilant biosecurity and disease management practices to curb the potential impact of diverse aMPV strains within poultry

populations. In high-risk areas, vaccination is an indispensable part of the strategic control of aMPV, alongside strict biosecurity measures [34].

Although our research coincided with a significant incidence of swollen head syndrome reported in the field, it does not rule out the possible circulation of either subtype B or subtype A or both in Morocco without prior documentation. Further research is required to enhance our comprehension of aMPV/B dissemination in Morocco through larger-scale sampling. Additionally, despite the description of virus isolation from apparently ill birds, the analysis could not necessarily provide definitive insights into the pathogenicity of the locally identified strains given the low sampling. Finally, it is essential to emphasize the significance of a surveillance program in other poultry industrial species to gain a more comprehensive understanding of the prevalence of the disease.

5. Conclusions

As the first endeavor of its kind, this research sheds light on Moroccan aMPV isolates, belonging to two clusters and cross-circulating between commercial broilers and turkeys. Hence, it sets the stage for future investigations and serves as a vital reference point for ongoing monitoring and surveillance efforts to better understand the dynamics of aMPV circulation in the Moroccan poultry population and to develop effective control strategies for preventing and managing aMPV infections, with an emphasis on broilers and turkeys. Ultimately, a comprehensive understanding of aMPV epidemiology is pivotal to mitigating its impact on poultry flocks and ensuring sustainable poultry production in Morocco.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/poultry4010003/s1, Table S1: North African aMPV/B strains obtained or retrieved from GenBank; Table S2: Mediterranean aMPV/B strains obtained or retrieved from GenBank; Table S3: Other aMPV/B strains obtained or retrieved from GenBank.

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Institutional Review Board Statement: All animal procedures were in accordance with the Hassan II and Agronomy and Veterinary Institute of Rabat and the Moroccan Ministry of Agriculture guidelines.

Informed Consent Statement: Not Applicable.

Data Availability Statement: The original data presented in the study are openly available in NCBI Blast at accession numbers: PQ202991 to PQ202998.

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