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Amine Mernizi [1,2,*], Abdeljelil Ghram [3], Hicham Fathi [1], Enrique Carceller [1], Juan-Luis Criado [1], Martina Dardi [1], O. Arbani[2], Mohammed Bouslikhane [4], Mohammed Mouahid [5], Caterina Lupini [6], Elena Catelli [6], Siham Fellahi [2] and Saadia Nassik [2]

[1] HIPRA Laboratorios S.A., Av. Selva, Amer, 17170, Spain ; hicham.fathi@hipra.com; enrique.carceller@hipra.com; juan.criado@hipra.com; martina.dardi@hipra.com
[2] Department of Pathology and Veterinary Public Health, Avian Pathology Unit, Agronomic and Veterinary Institute Hassan II, PO Box 6202, Rabat, 10000, Morocco; s.fellahi@iav.ac.ma; s.nassik@iav.ac.ma
[3] Epidemiology and Veterinary Microbiology Laboratory, Pasteur Institute of Tunis, University Tunis El Manar, PO Box 74, Tunis, 1002, Tunisia; abdeljelil.ghram@pasteur.tn
[4] Department of Pathology and Veterinary Public Health, Microbiology, Immunology and Contagious Diseases Unit, IAV Hassan II, Rabat, 10000, Morocco; m.bouslikhane@iav.ac.ma
[5] Mouahid Veterinary Clinic, Bd. Hassan I Lot20 Appt1 Hay Abbadi, Témara, 12010, Morocco; mohamedmouahid@gmail.com
[6] Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di sopra 50, Ozzano dell'Emilia (BO), 40064, Italy; caterina.lupini@unibo.it; elena.catelli@unibo.it
* Correspondence: amine.mernizi@hipra.com; Tel.: +34.680.569.936

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 understand better 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, were 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 three distinct clades of the same aMPV/B strain, two of which originated from used vaccines and one from field recombination, 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

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

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Metapneumovirus avis, it belongs to the Pneumoviridae family, specifically 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, with often more severe clinical symptoms displayed [6,7] and documented susceptibility 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 diverse pathogens' circulation. 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, broilers in Morocco are generally not vaccinated against aMPV, except in some cases, where vaccination is adopted but not 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 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, 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.

It should be pointed out that 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 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

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

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

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 kit, following the manufacturer's instructions (AniCon Labor GmbH, Emstek, Germany).

2.2. Reverse-Transcriptase and Double Conventional PCR Amplification

Using the kit Applied Biosystems® Reverse-Transcription Kit (ThermoFisher Scientific Waltham, Massachusetts, USA), 10µL of RNA from a 100µL dilution was mixed with 10µL of RT mix, made of MM RT Buffer (2µL), RT random primers (2µL), dNTP (0.8µL), multi-scribe RT (1µL) and H2O (4.2µL), 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. The sequences of these primers, used for a nested conventional PCR and designed in the attachment protein G gene [17,18], are reported in Table 2.

Table 2. Sequences of G gene primers used for nested conventional PCR.

Primers	Sequence (5′-3′)	References

 doi:10.20944/preprints202409.0892.v1

G Start+	CAAGTATCCAGATGGGGTC	[17]
G5-	CAAAGAA/G CCAATAAGCCCA	[18]
G6-	CTGACAAATTGGTCCTGATT	[18]
G8+A	CACTCACTGTTAGCGTCATA	[18]
G9+B	TAGTCCTCAAGCAAGTCCTC	[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.

Table 3 illustrates the steps of the nested PCR for aMPV cDNA amplification.

Table 3. Protocol of the aMPV Nested PCR and reagent details.

	1st amplification		**2nd amplification**	
Steps	Composition	Volume (µl)	Composition	Volume (µl)
Preparation of the PCR mix	Mastermix DreamTaq® Green PCR	25	Mastermix DreamTaq® Green PCR	25
	Forward G6-	2	Forward G5-	2
	Reverse G START+	2	Reverse G8+A	2
			Reverse G9+B	2
	H_2O	11	H_2O	9
	cDNA	10	cDNA	10
	Total	40	Total	40
Hybridization	57°C		58°C	

2.3. Gel Electrophoresis

After a double conventional PCR amplification, the products were separated by gel electrophoresis. Thus, 0.8g of Agarose Top Vision Agarose® (Thermo Fisher Scientific Waltham, Massachusetts, USA) was added to 50mL of an in-house prepared Tris-Acetate-EDTA (TAE) solution. Containing 242g of Tris base, 57.1mL of glacial acetic acid, and 100mL of EDTA for every 1L of the preparation, this solution was adjusted to a pH of 8 and diluted to a 2% concentration (10mL of the prepared solution mixed with 490mL of distilled water). Additionally, 5µL of cDNA was included in the mixture with a green stain Xpert Green DNA Stain® (20.000X) (GRiSP, Lda, Porto, Portugal); such 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 268bp and 361bp.

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

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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, Maryland, USA; https://blast.ncbi.nlm.nih.gov/Blast.cgi), 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, California, USA; https://bioedit.software.informer.com/7.2/), and phylogenetic analysis was conducted with Mega® 11, using the maximum likelihood (ML) method, with 1000 bootstrap replicates (Biodesign Institute, Arizona State University, USA; https://www.megasoftware.net/) and Figtree (Institute of Evolutionary Biology, University of Edinburgh, UK; http://tree.bio.ed.ac.uk/software/figtree/). 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 Supplemental Tables S1, S2, and S3.

Additionally, two unpublished sequences, referred to as aMPV/Morocco/SF1 and aMPV/Morocco/SF2 and provided by the Avian Pathology Unit of the Agronomic and Veterinary Institute Hassan II, were included for comparative analysis with the study outcomes of this. It should be highlighted that the aMPV/Morocco/SF2 strain did not exhibit significant similarity with the reference strains available in GenBank; and for this reason, it was not considered for further accession number assignment.

Herewith, eight nucleotide sequences were submitted to GenBank, representing the Moroccan, under accession numbers PQ202991-PQ202998, as per Table 4.

Table 4. Nucleotide sequences submitted with their respective accession numbers.

Code	Sequence name on the submission	Accession Number
727G	aMPV/B/Morocco/Ck/MA-1/2023	PQ202992
727P	aMPV/B/Morocco/Ck/MA-2/2023	PQ202991
731G	aMPV/B/Morocco/Ck/MA-3/2023	PQ202994
764P	aMPV/B/Morocco/Ty/MA-4/2023	PQ202998
766G	aMPV/B/Morocco/Ty/MA-5/2023	PQ202995
766P	aMPV/B/Morocco/Ty/MA-6/2023	PQ202996
P5	aMPV/B/Morocco/Ck/MA-7/2023	PQ202993
SF1	aMPV/B/Morocco/Ck/FS-1/2023	PQ202997

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 (aMPV/Morocco/5P) and 2 out of 5 samples from broilers with SHS of which one yielded two different sequences after extraction (aMPV/Morocco/727P and G, aMPV/Morocco/731G). Similarly, 2 out of 3 RNA extracts from turkeys showing TRT signs were identified, of which only one exhibited two sequences after extraction in gel and solution (aMPV/Morocco/764P, aMPV/Morocco/766P and aMPV/Morocco/766G).

No samples were found positive for aMPV subtype A.

3.2. Sequencing and Genetic Characterization

 doi:10.20944/preprints202409.0892.v1

The partial G gene sequences of the Moroccan isolates were 350 nucleotides in length. These isolates have a nucleotide sequence identity between 91.8% (aMPV/Morocco/727P and aMPV/Morocco/SF1, GenBank accession numbers PQ202991and PQ202997, respectively) and 99.7% (aMPV/Morocco/731G, and aMPV/Morocco/766P, GenBank accession numbers PQ202994 and PQ202996, respectively), and when compared to each other and from 78.2% (aMPV/Morocco/766G, GenBank accession number PQ202995, and aMPV-B/BR/1890/E1/19, GenBank accession number OP572408.1) to 90.76% (aMPV/Morocco/727G, GenBank accession number PQ202992 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 derived vaccine ones.

Table 5 presents the highest percentage of similarities observed between the Moroccan isolates and the documented reference strains.

Table 5. Percentages of similarity between aMPV Moroccan isolates and reference strains.

Code	Documented GenBank strains	Similarity (%)	Accession Number	Ref.
727G	Avian Metapneumovirus strain aMPV/B/Romania/Ty/67/17 glycoprotein (G) gene, partial cds	90.76	MT432878.1	[19]
	Avian Metapneumovirus isolate 2018_0404_Chicken_Turkey_2018 attachment protein (G) gene, partial cds		MH352465.1	[20]
727P	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	[21]
731G	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	[22]
	Avian Metapneumovirus isolate 101/2011 attachment protein (G) gene, partial cds		KC954647.1	[19]
764P	Avian Metapneumovirus strain aMPV/B/France/GuineaFowl/1060/18 glycoprotein (G) gene, partial cds	98.96	MT432904.1	[19]
	Avian Metapneumovirus strain aMPV/B/Romania/Ty/85/17 glycoprotein (G) gene, partial cds	98.63	MT432883.1	[19]
766G	Avian Metapneumovirus isolate aMPV-B/BR/1890/E1/19, complete genome	99.70	OP572408.1	[22]
	Avian Metapneumovirus strain aMPV/B/Italy/Ty/742-01/17 glycoprotein (G) gene, partial cds		MT436229.1	
	Avian Metapneumovirus isolate 101/2011 attachment protein (G) gene, partial cds		KC954647.1	[19]
	Avian Metapneumovirus isolate aMPV-B/BR/1890/E1/19, complete genome		OP572408.1 MT436229.1	[22]

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766P	Avian Metapneumovirus strain aMPV/B/Italy/Ty/742-01/17 glycoprotein (G) gene, partial cds Avian Metapneumovirus isolate 101/2011 attachment protein (G) gene, partial cds	98.70	KC954647.1	[19]
P5	Avian Metapneumovirus isolate aMPV/Chicken/PCRLAB/HG/2010 attachment protein (G) gene, partial cds Avian Metapneumovirus isolate 101/2011 attachment protein (G) gene, partial cds	96.76	MN108496.1 KC954647.1	
SF1	Avian Metapneumovirus strain aMPV/B/Spain/Ty/4954-1/15 glycoprotein (G) gene, partial cds Avian Metapneumovirus strain aMPV/B/France/Ck/785/17 glycoprotein (G) gene, partial cds Avian Metapneumovirus isolate aMPV-B/turkey/VA/USA/ADRDL-5, complete genome	99.10	MT432835.1 MT432891.1 PP273460.1	[19] [19] [23]

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 referenced strains. The ML phylogenetic analysis revealed that Moroccan aMPV isolates were separated into three main clusters (Figure 2).

 doi:10.20944/preprints202409.0892.v1

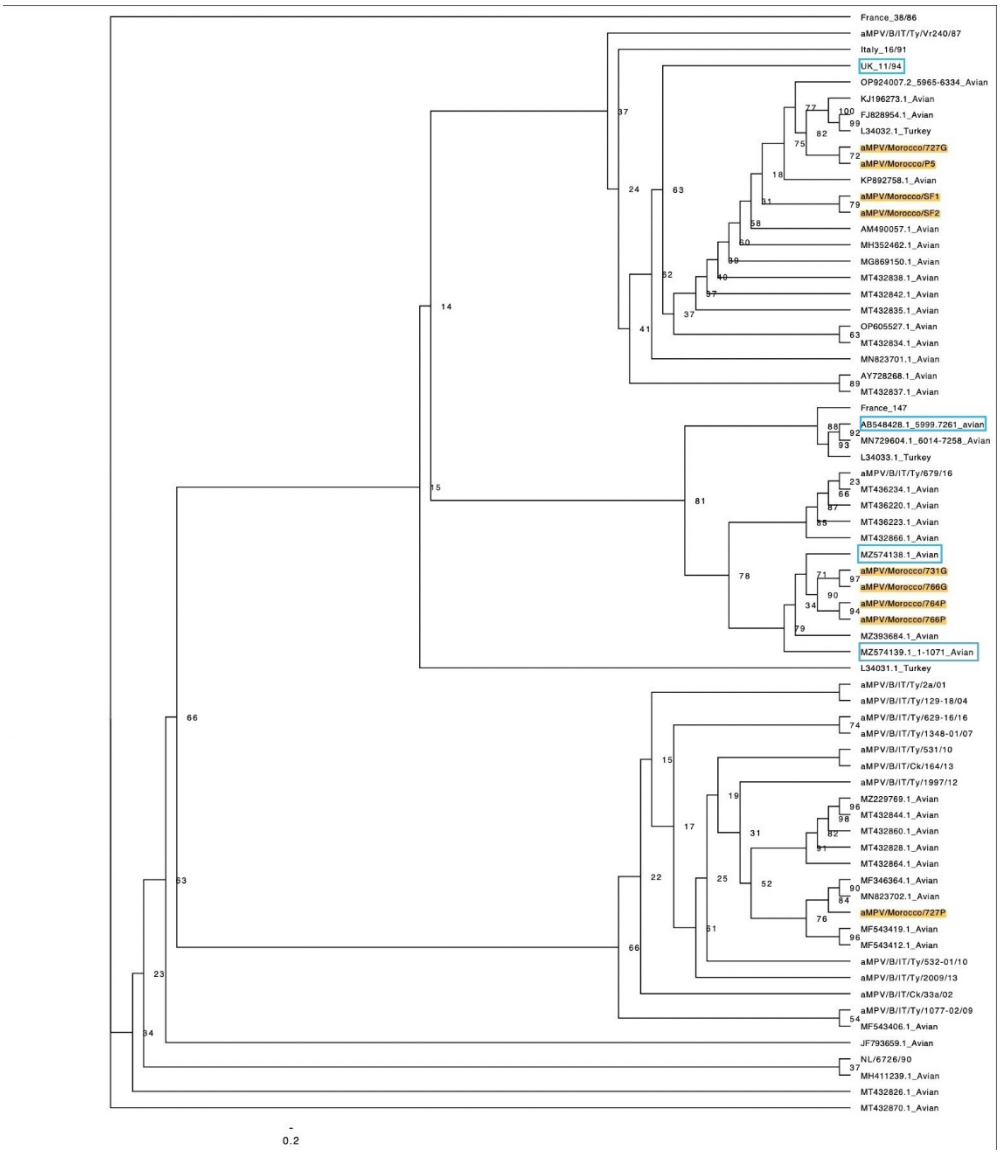

Figure 2. Phylogenetic analysis of the partial nucleic acid sequence of the G gene. Detections of aMPV/B from Morocco are shaded in yellow whereas vaccine strains are framed in blue. Data were analyzed using the maximum likelihood method.

The first cluster, related to documented 11/94 derived strains, included four Moroccan isolates: aMPV/Morocco/727G, aMPV/Morocco/P5, aMPV/Morocco/SF1 (GenBank accession numbers PQ202992, PQ202993 and PQ202997, respectively), besides the omitted isolate previously referred as aMPV/Morocco/SF2. These viruses grouped as well with chicken and turkey Nigerian and Algerian strains (aMPV/chicken/Nigeria/NIR89/2006; GenBank accession number AM490057.1, and aMPV/B/turkey/Algeria/26; GenBank accession number KP892758.1), respectively.

The second cluster, is represented by four viruses: aMPV/Morocco/731G, aMPV/Morocco/764P, aMPV/Morocco/766G, and 766P (GenBank accession numbers PQ202994, PQ202998, PQ202995 and PQ202996, respectively). It is grouped with vaccine strain pl21 (GenBank accession number MZ574139.1) and its derived strains.

The presumed third group contains only one Moroccan virus, aMPV/Morocco/727P (GenBank accession number PQ202991), clustering with field strains reported in Europe, but also the Tunisian vaccine-derived strain TN1015/17 (GenBank accession number MH411239.1).

4. Discussion

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Avian Metapneumovirus has been implicated as the causal agent of swollen head syndrome since its first clinical description in the 1990s [24]. 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 three clusters of the aMPV/B subtype, two of them being vaccine-derived. The observed genetic diversity underscored the presence of different Metapneumovirus strains of subtype B among broiler and turkey farmings in Morocco, possibly having diverse sources.

Per prior research's focus on aMPV subtypes, our study has been limited to investigating aMPV/A and aMPV/B circulation [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 [23], and aligning with previous research in neighboring countries from North Africa and the Mediterranean areas, emphasizing the dominance of subtype B [10,12–14,20,25–29]. 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 [30] and with low excretion in chickens [31].

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 [19–21], and exhibited grouping with the vaccine strain 11/94. While previous reports pointed out the possibility of the vaccine spread from nearby vaccinated flocks, presumably turkeys [19,20], the detection of aMPV in our case resulted as well 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 [32]. 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 [33].

Notably, the Algerian strain (GenBank accession number KP892758.1) [14] demonstrated substantial phylogenetic proximity to the Moroccan isolates within this clade, highlighting consistent and comparable epidemiological context across North African countries.

Within the second clade, which predominantly encompasses strains detected in turkeys, 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 aMPV/B vaccine, strains VCO3 or 1062, identified in vaccinated turkeys in Italy [19]. Previous reports on isolates with high phylogenetic proximity to our second cluster confirmed their unrelatedness to vaccine strains 1062 or pl21 [34]. However, the documented strains with similarity with the Moroccan isolates all showed closeness to vaccine-derived strain pl21 [22,23,31]., which can plausibly explain the observed similarities with the Tunisian isolate, linked to the vaccine strain pl21 [13], and that might have reverted to virulence. Interestingly, this Tunisian exhibits a 99% genetic similarity with the Algerian and Nigerian strains (GenBank accession numbers KP892758 and AM490058, respectively), classified as vaccine-derived strains [13]. Our isolates of the first group also clustered, on the phylogenetic tree, with these vaccine-derived strains. These observations reinforce the hypothesis that the North African isolates share a common origin.

 doi:10.20944/preprints202409.0892.v1

It is plausible that 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 still, of heterologous vaccines against aMPV/A for many years, exerting vaccine pressure and generating alternation of aMPV/B [35].

Remarkably, the cluster of isolates solely from turkey flocks affected by TRT also includes one aMPV strain detected in chickens (aMPV/Morocco/731G, GenBank accession number PQ202994), showing a high percentage of nucleotide identity to the Brazilian and Italian strains, aMPV-B/BR/1890/E1/19 (GenBank accession number OP572408.1) and aMPV/B/Italy/Ty/742-01/17 (GenBank accession number MT436229.1) respectively. The notable similarity existing between broiler and turkey aMPV/B local strains holds significance, 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 [36].

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. 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 [37].

The aMPV/Morocco/727P (GenBank accession number PQ202991), detected in broilers, presumably formed a distinct third clade with field strains. It displayed nucleotide sequence similarities with the Italian-related isolates, previously documented in chickens and various Euro-Mediterranean areas [10,25,27,29] and capable of escaping vaccine-induced immunity [38], just like the Tunisian strain TN1000/19 (GenBank accession number MZ229769.1) reported as field one and plausibly originating from Spain [13]. Nevertheless, performing nucleotide identity analysis with reference strains showed that aMPV/Morocco/727P disclosed high similarity with both the Nigerian and the Algerian strains, aMPV/chicken/Nigeria/NIR89/2006 (GenBank accession number AM490057.1) and Algeria/26/aMPVB/turkey (GenBank accession number KP892758.1), suggesting its clustering within the first group. This finding likely confirms that aMPV/Morocco/727P and aMPV/Morocco/727G could represent the same isolate.

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. Last but not least, 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 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 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 the website of this paper posted on Preprints.org. 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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