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# Field Evidence for Vertical Transmission of Avian Metapneumovirus Subtype B in Naturally Infected Broiler Breeder Chickens in Egypt

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**Abstract**

Avian metapneumovirus (aMPV) represents a serious respiratory pathogen of poultry and is associated with considerable economic losses in breeder flocks worldwide. Although horizontal transmission is well established, the contribution of vertical transmission remains poorly understood, especially under field conditions in chickens. In this study, we aimed to assess whether aMPV could be transmitted vertically in unvaccinated broiler breeder flocks that tested positive by PCR in Egypt. Therefore, 10 broiler breeder flocks (≥30 weeks) from seven Egyptian governorates were screened for aMPV subtypes A and B. From each flock, tracheal swabs were collected from breeder hens, along with 20 fertile eggs and 20 newly hatched chicks. All samples, including tracheal swabs, chicken tissues (trachea, lungs, reproductive tract, and spleen), eggshells, internal egg contents, and embryonic tissues were analyzed for aMPV RNA using subtype-specific RT-qPCR. All breeder flocks tested positive for aMPV subtype B, but not subtype A. No aMPV RNA was found in eggshells, internal egg contents, embryonic tissues, or tissues from newly hatched chicks. In conclusion, Despite PCR detection of aMPV in breeder hens, the absence of viral RNA in eggs and their progeny provides field evidence that vertical transmission of subtype B is unlikely to play a significant role in virus spread in commercial broiler breeder flocks. These results support horizontal transmission as the primary route of aMPV spread and highlight the need for further longitudinal and genomic studies to better elucidate aMPV transmission dynamics.

**Keywords:** avian metapneumovirus; broiler breeders; Egypt; qRT-PCR; vertical transmission

## 1. Introduction

Avian metapneumovirus (aMPV) is an enveloped, single-stranded RNA virus in the genus Metapneumovirus of the family Pneumoviridae. It causes turkey rhinotracheitis (TRT), an acute highly contagious upper respiratory disease, and is associated with swollen head syndrome (SHS) and reproductive disorders in chickens and other poultry species [1,2]. Since its initial description in South African turkeys in the late 1970s, aMPV has emerged as a global pathogen with significant economic impact on the poultry industry [3].

The aMPV genome is linear and consists of eight genes arranged in the following order: nucleoprotein (N), phosphoprotein (P), matrix (M), fusion (F), matrix 2 (M2), small hydrophobic (SH), attachment glycoprotein (G), and large polymerase (L) [4]. Based on the genetic variation within the G gene, at least four subtypes (A, B, C, and D) have been recognized [5]. Subtypes A and B are the most widely distributed and have been reported across Asia, Africa, Europe, and South America [6]. Subtype C includes two distinct lineages, with the North American lineage predominantly affecting

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turkeys and the Eurasian lineage primarily detected in ducks [7], whereas subtype D has only been identified once in France and has not been reported elsewhere [8].

Transmission of aMPV is predominantly horizontal, occurring via direct bird-to-bird contact, aerosols, and contaminated fomites [1,2]. While vertical transmission is a major concern for the poultry industry due to its potential for long-distance spread and early infection of progeny, evidence supporting this route for aMPV remains limited and inconsistent [9–11]. Experimental infections in laying hens have demonstrated reductions in egg production and eggshell quality but have largely failed to detect viral RNA in eggs or embryos [12]. Although viral RNA has occasionally been detected in the oviduct of infected turkeys, its absence in ovarian tissue suggests that, if vertical transmission occurs, it is likely transient and biologically inefficient [13].

In Egypt, aMPV circulation in poultry has been increasingly reported, with both subtypes A and B detected in chickens, turkeys, and ducks, often in conjunction with bacterial co-infections that exacerbate respiratory disease [14–16]. However, previous studies have been fragmented, geographically limited, and primarily focused on clinical outbreaks rather than systematic epidemiological investigations. Notably, no comprehensive studies have examined the potential role of vertical transmission in naturally infected broiler breeder hens, leaving critical gaps in understanding virus persistence and dissemination within breeder production systems.

Therefore, the present study was conducted to investigate the potential for vertical transmission of aMPV subtypes A and B by screening for the virus in eggs, eggshell surfaces, embryonic tissues, and newly hatched chicks under commercial field conditions in Egypt. This work aims to provide a clearer understanding of aMPV persistence and its impact on the breeder production system, potentially informing more effective control and biosecurity strategies.

## 2. Materials and Methods

### *2.1. Flock selection and sample collection*

During 20225, a total of 10 unvaccinated broiler breeder flocks (34–40 weeks of age) across seven Egyptian governorates (Kafr El-Sheikh, Gharbia, Sharqia, Menoufia, Qalyubia, Giza, and Fayoum) were investigated (Figure1A). All flocks exhibited clinical respiratory signs, including nasal discharge, conjunctivitis, and mild facial swelling (Figure 1B). The flocks also showed a reduction in egg production ranging from 16% to 18% at the time of sampling.

Ten female birds from each flock were sampled (n = 100 birds total). Tracheal swab and tissue samples (trachea, lungs, reproductive tract, and spleen) were collected from each bird. In addition, 20 fertile eggs and 20 newly hatched chicks were obtained from each flock (Table 1). Fertile eggs incubated to day 18 of embryonation, were aseptically opened to collect the embryonic tissues (Figure 1C), including the trachea, lungs, reproductive tract, and yolk sac. Eggshell surfaces were swabbed circumferentially along the long axis, with two passes at different radial positions to maximize viral recovery. All swabs and tissue samples were immediately transported in ice boxes to the Virology Laboratory, Faculty of Veterinary Medicine, Cairo University, for further analysis. Samples were prepared according to the standard methods accordingly.

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**Table 1.** Summary of the sampling scheme from ten backyard broiler breeder flocks across seven Egyptian governorates, detailing the number of birds sampled, types and numbers of tissue and tracheal swab samples collected, total fertile eggs obtained, internal embryonic tissues examined, and eggshell swabs analyzed for viral RNA extraction and RT-qPCR detection.

| Flock No. | Governorate | Age (week) | No. of Birds | Tissue Samples | Nasal/Tracheal Swabs | No. of Eggs | No of Newley hatched chicks | Egg Internal Samples | Eggshell Swabs |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Kafr El-Sheikh | 34 | 10 per each | 4 × bird (Trachea, Lung, Ovary, Spleen) | 10 per each flock | 20 per each flock | 20 per each flock | 4 ×Embryo (trachea, lung, reproductive tract, egg sac) | 2 per Egg |
| 2 | Gharbia | 35 | | | | | | | |
| 3 | Sharqia | 35 | | | | | | | |
| 4 | Qalyubia | 36 | | | | | | | |
| 5 | Menoufia | 37 | | | | | | | |
| 6 | Giza | 38 | | | | | | | |
| 7 | Giza | 38 | | | | | | | |
| 8 | Fayoum | 39 | | | | | | | |
| 9 | | 40 | | | | | | | |
| 10 | | 40 | | | | | | | |
| Total | - | - | 100 | 400 | 100 | 200 | 200 | 400 | 400 |

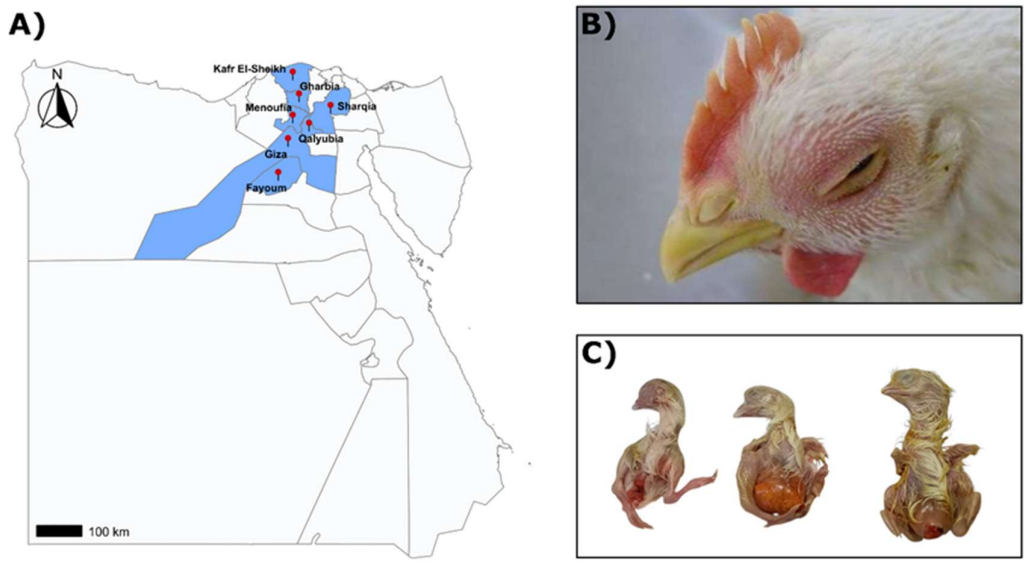


**Figure 1.** Field sampling and specimen collection for avian metapneumovirus (aMPV) surveillance in Egyptian broiler breeder flocks. (A) Map of Egypt with dropped red pins highlighting the seven sampled governorates (Kafr El-Sheikh, Gharbia, Sharqia, Qalyubia, Menoufia, Giza, and Fayoum). (B) Clinically affected broiler breeder hen showing respiratory signs, peri-orbital swelling, and facial edema.(C) Embryos and their contents collected at day 15 of incubation from infected flocks for laboratory investigation.

All studied breeder flocks were screened for avian metapneumovirus subtype-specific RT-qPCR assays. In parallel, flocks were systematically examined for selected co-infections, limited to Mycoplasma gallisepticum (MG) and Escherichia coli (E. coli) bacteria, using molecular and bacteriological methods [17].

Flocks 1–4 (34–36 weeks of age), located in Kafr El-Sheikh, Gharbia, Sharqia, and Qalyubia. Clinical signs included coughing, nasal discharge, conjunctivitis, and mild facial swelling. Co-infection with Mycoplasma gallisepticum was detected, and severe concurrent Escherichia coli

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infections were identified in several birds. Egg production was reduced by approximately 18% across these flocks.

Flocks 5–7 (37–38 weeks of age) from Menoufia and Giza. Affected birds initially showed mild respiratory distress and nasal discharge, which progressed to conjunctivitis, periocular swelling, and depression. Co-infection with E. coli was confirmed, and a sustained reduction in egg production of approximately 9-12% was observed.

Flocks 8–10 (39–40 weeks of age) from Fayoum exhibited more pronounced respiratory disease, characterized by nasal and ocular discharge, facial edema, ruffled feathers, and reduced activity. Secondary bacterial infections were consistently detected. Cumulative mortality percentages were tracked and calculated.

All experimental procedures and sample collection protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo University (Approval No. Vet CU 301220251281). All procedures were carried out in accordance with institutional and national guidelines for animal welfare.

### *2.2. Molecular detection of aMPV by RT-qPCR*

Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions and immediately stored at –80 °C until analysis. Detection of aMPV subtypes A and B was performed using a subtype-specific RT-qPCR assay with the Kylt® aMPV A&B real-time RT-PCR kit (AniCon Labor GmbH, Emstek, Germany) on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA). The assay was conducted following the manufacturer's instructions and previously described protocols [18]. Positive (pneumovac® and Poulvac® vaccine strains) and negative (Nuclease free water) controls were included in each run. Interpretation of results followed the manufacturer's guidelines for FAM/Cy5-labeled probes, where samples with Ct values < 37 were considered positive.

## 3. Results

RT-qPCR analysis of tracheal swabs and tissue samples from clinically affected chickens demonstrated the exclusive detection of aMPV subtype B in all flocks, whereas subtype A was not detected. Molecular screening of egg samples revealed no detectable aMPV RNA in eggshell swabs or embryonic tissues. All egg samples tested negative for both aMPV subtypes A and B by RT-qPCR.

## 4. Discussion

The present study documents the continuous circulation of aMPV subtype B in broiler breeder flocks across the surveyed Egyptian governorates during the study period. This persistence occurred despite the implementation of enhanced biosecurity practices primarily designed to control the spread of highly pathogenic avian influenza virus (HPAIV) [19]. These findings indicate that under the identified production conditions, aMPV can maintain transmission cycles even without apparent breakdowns in general biosecurity protocols.

The consistent molecular detection of aMPV subtype B in all examined flocks underscores its dominance in breeder populations during the study period. This is in alignment with several reports documenting the widespread circulation of this subtype in poultry populations across North Africa and several Asian countries [1,6,20]. The detection of virus in clinically affected breeder chickens, coupled with the absence of detectable viral RNA in eggs, embryonic tissues, eggshell surfaces, and newly hatched chicks, provides strong field-based evidence that vertical transmission did not occur at a detectable level in these flocks. This observation is concordant with previous experimental and field studies in chickens and turkeys, which reported limited or no vertical transmission despite confirmed breeder infection and the presence of the virus within the reproductive tract [12,13,21].

The absence of virus detection in eggs is biologically plausible and likely reflects transient or inefficient nature of aMPV replication within the reproductive tissues. Previous work indicated that

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viral presence in the reproductive tract, when it occurs, is short-lived and highly contingent upon infection timing and host-specific factors [21,22]. Accordingly, within the temporal framework of this study, vertical transmission does not appear to contribute meaningfully to aMPV persistence in backyard broiler breeder systems.

Conversely, these findings support horizontal transmission as the primary mechanism sustaining virus circulation in the investigated flocks. Direct contact, aerosols, contaminated fomites, personnel movements, and environmental contamination; specifically, the documented persistence of aMPV RNA in poultry litter, represent plausible pathways for continued virus detection across the studied governorates [20].

Moreover, the ecological context of Egypt, situated along major migratory bird flyways, introduces an additional but unconfirmed exposure pathway. While aMPV has been detected in wild birds globally [23,24], our data does not establish a causal link between migratory birds and virus introduction. Nonetheless, the open housing typical of backyard breeder systems may increase susceptibility to environmental exposure during seasonal migration periods, warranting further investigation.

## 5. Conclusion

In the present investigation, aMPV subtype B was molecularly confirmed in broiler breeder hens, while viral RNA was not detected in eggs or newly hatched chicks. These findings provide field-based evidence that vertical transmission of aMPV is unlikely under the conditions examined. Interpretation of the results is constrained by the cross-sectional study design, limited geographic representation, and the absence of genomic characterization, which may have limited the detection of transient viral presence within reproductive tissues. Longitudinal studies incorporating genomic analyses are therefore warranted to further elucidate aMPV transmission dynamics in breeder production systems.

**Author Contributions:** **O.S.S**.; Conceptualization, Study design, Sample collection, Data curation, Formal analysis, Writing original draft, **S.A.S**.; resources, review and language editing, **B.A.A**.; Supervision, manuscript review and editing, **A.H.D**.; Supervision, manuscript review and editing, **H.M.A**.; Supervision, manuscript review and editing. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The birds enrolled in this study were sourced Neighboring to commercial, privately owned broiler breeder farms and lakes and were neither owned nor managed by the authors or by any academic or governmental institution. Before sample collection, permission was formally obtained from the respective farm owners or authorized farm managers to access the farms and to collect samples from their birds for research purposes. All bird handling procedures, farm access, and sample collection from privately owned flocks were conducted in accordance with approved animal welfare guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo University (**Approval No. Vet CU-301220251281**).

**Informed Consent Statement:** The study did not involve any human experiments.

**Data Availability Statement:** The datasets generated and/or analyzed during the current study are included within the manuscript. Additional data is available from the corresponding author upon reasonable request.

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**Conflicts: of Interest** The authors declare that they have no competing financial or non-financial interests.

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## Abbreviations

The following abbreviations are used in this manuscript:

| Abbreviation | Full Term |
|---|---|
| aMPV | Avian metapneumovirus |
| TRT | Turkey rhinotracheitis |
| SHS | Swollen head syndrome |
| RNA | Ribonucleic acid |
| N | Nucleoprotein |
| P | Phosphoprotein |
| M | Matrix protein |
| F | Fusion protein |
| M2 | Matrix protein 2 |
| SH | Small hydrophobic protein |
| G | Attachment glycoprotein |
| L | Large polymerase protein |
| RT-qPCR | Reverse transcription quantitative polymerase chain reaction |
| PCR | Polymerase chain reaction |
| Ct | Cycle threshold |
| MG | Mycoplasma gallisepticum |
| E. coli | Escherichia coli |
| HPAIV | Highly pathogenic avian influenza virus |
| IACUC | Institutional Animal Care and Use Committee |

## References

1. Kaboudi K, Lachheb J. Avian metapneumovirus infection in turkeys: a review on turkey rhinotracheitis. J Appl Poult Res. 30:100211; 2021. doi:10.1016/j.japr.2021.100211.
2. Buys SB, du Preez JH, Els HJ. Swollen head syndrome in chickens: a preliminary report on the isolation of a possible aetiological agent. J S Afr Vet Assoc. 60:221–222; 1989.
3. Buys SB, du Preez JH, Els HJ. The isolation and attenuation of a virus causing rhinotracheitis in turkeys in South Africa. Onderstepoort J Vet Res. 56:87–98; 1989.
4. Afonso CL, Amarasinghe GK, Bányai K, Bào Y, Basler CF, Bavari S, Bejerman N, Blasdell KR, Briand F-X, Briese T, et al. Taxonomy of the order Mononegavirales: update 2016. Arch Virol. 161:2351–2360; 2016. doi:10.1007/s00705-016-2880-1.
5. Salles GBC, Pilati G, Muniz EC, Neto AJD, Vogt JR, Dahmer M, Savi BP, Padilha DA, Fongaro G. Trends and challenges in the surveillance and control of Avian Metapneumovirus. Viruses. 15:1960; 2023. doi:10.3390/v15091960.
6. Franzo G, Legnardi M, Mescolini G, Tucciarone CM, Lupini C, Quaglia G, Catelli E, Cecchinato M. Avian Metapneumovirus subtype B around Europe: a phylodynamic reconstruction. Vet Res. 51:88; 2020. doi:10.1186/s13567-020-00817-6.
7. Legnardi M, Allée C, Franzo G, Cecchinato M, Brown P. Research note: Detection of avian metapneumovirus subgroup C specific antibodies in a mallard flock in Italy. Poult Sci. 100:101186; 2021. doi:10.1016/j.psj.2021.101186.
8. Bäyon-Auboyer MH, Arnauld C, Toquin D, Eterradossi N. Nucleotide sequences of the F, L and G protein genes of two non-A/non-B avian pneumoviruses (APV) reveal a novel APV subgroup. J Gen Virol. 81:2723–2733; 2000. doi:10.1099/0022-1317-81-11-2723.
9. Li Y, Cui S, Li W, Wang Y, Cui Z, Zhao P, Chang S. Vertical transmission of avian leukosis virus subgroup J (ALV-J) from hens infected through artificial insemination with ALV-J infected semen. BMC Vet Res. 13:204; 2017. doi:10.1186/s12917-017-1122-4.
10. Grgic H, Philippe C, Ojkic D, Nagy É. Study of vertical transmission of fowl adenoviruses. Can J Vet Res. 70:230–236; 2006.

 doi:10.20944/preprints202605.0811.v1




11. Yu XH, Liu L, Wang F, et al. Serological evidence of avian encephalomyelitis virus infection associated with vertical transmission in chicks. Biologicals. 43:512–514; 2015. doi:10.1016/j.biologicals.2015.09.003.
12. Cook JKA, Chesher J, Orthel F, Woods MA, Orbell SJ, Baxendale W, Huggins MB. Avian pneumovirus infection of laying hens: experimental studies. Avian Pathol. 29:545–556; 2000. doi:10.1080/03079450020016788.
13. Jones RC, Williams RA, Baxter-Jones C, Savage CE, Wilding GP. Experimental infection of laying turkeys with rhinotracheitis virus: distribution of virus in the tissues and serological response. Avian Pathol. 17:841–850; 1988. doi:10.1080/03079458808436506.
14. Arafa AS, Mady W, Hussein A, Tamam S, Madbouly H. Molecular characterization of vaccine-derived mutants of avian metapneumoviruses isolated from turkeys in Egypt. Am J Virol. 4(1):1; 2015.
15. Saeed, O.S., Labib, M.A., Gamal, M. *et al.* Serologic and molecular evidence of avian metapneumovirus subtypes A and B in unvaccinated broiler breeder flocks in Egypt (2024–2025). *BMC Vet Res* **22**, 273 (2026). https://doi.org/10.1186/s12917-026-05459-y
16. Abdelmoez NS, Shawky MM, Abdelhady HA, Lebdah MA, Salama SS. Isolation and identification of some possible causative agents of Swollen Head Syndrome (SHS) in broiler chickens in Egypt. Int J Vet Sci. 2019:781–788.
17. Salles GB, Pilati GV, Savi BP, Muniz EC, Dahmer M, Vogt JR, de Lima Neto AJ, Fongaro G. Surveillance of avian metapneumovirus in non-vaccinated chickens and co-infection with avian pathogenic Escherichia coli. Microorganisms. 2023 Dec 28;12(1):56.
18. Mernizi A, Kadiri O, Rius JL, Bouslikhane M, Ghram A, Mohamed M, Catelli E, Nassik S. Detection of Avian Metapneumovirus Subtypes A and B in Moroccan Broiler Farms. Iranian Journal of Veterinary Medicine. 2024;18(4):479-88.
19. Abdelwhab EM, Hafez HM. An overview of the epidemic of highly pathogenic H5N1 avian influenza virus in Egypt: epidemiology and control challenges. Epidemiol Infect. 139:647–657; 2011. doi:10.1017/S0950268810003122.
20. Velayudhan BT, Lopes VC, Noll SL, Halvorson DA, Nagaraja KV. Avian pneumovirus and its survival in poultry litter. Avian diseases. 2003 Jul 1;47(3):764-8.
21. Mo J, Briggs K, Sweeney R, Parker M, Carlson J, Suarez DL, Kapczynski DR. Lack of avian metapneumovirus detection of subtypes A and B in eggs from virus-positive commercial turkey breeders supports the premise that vertical transmission does not contribute to virus spread. Avian Dis. 69; 2025.
22. Shin HJ, Jirjis FF, Kumar MC, Njenga MK, Shaw DP, Noll SL, Nagaraja KV, Halvorson DA. Neonatal avian pneumovirus infection in commercial turkeys. Avian Dis. 46:239–244; 2002. doi:10.1637/0005-2086(2002)046[0239:NAPIIC]2.0.CO;2.
23. Graziosi G, Lupini C, Catelli E. Disentangling the role of wild birds in avian metapneumovirus (aMPV) epidemiology: a systematic review and meta-analysis. Transbound Emerg Dis. 69:3285–3299; 2022. doi:10.1111/tbed.14680.
24. Tucciarone CM, Franzo G, Legnardi M, Pasotto D, Lupini C, Catelli E, Quaglia G, Graziosi G, Dal Molin E, Gobbo F, et al. Molecular survey on A, B, C and new avian metapneumovirus (aMPV) subtypes in wild birds of Northern-Central Italy. Vet Sci. 9:373; 2022. doi:10.3390/vetsci9070373.