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Research note: Indirect evidence of avian Metapneumovirus circulation in broilers in Italy

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ABSTRACT The clinical relevance of avian metapneumovirus (**aMPV**) is growing in the poultry sector, especially in broiler farming, where no vaccination is administered in Italy. Given the naïve status of the birds, a serological survey was conducted in a densely populated area of Northern Italy, to evaluate aMPV circulation. Seven farms were selected and sampled in summer/fall, then sampling was repeated in the following season (winter/spring) to assess a possible seasonal effect. In each farm, fifteen birds were blood sampled towards the end of the cycle and sera were analyzed with an ELISA test. Clinical signs were reported in 5 out of 7 farms, although all farms were positive at both sampling points, except for one, which was negative at the first sampling. The seroprevalence within farm ranged from 26.6% to 100%, and antibody titres appear to increase with age. No seasonality effect was evidenced, whereas a farm effect was more distinct. aMPV circulation appears wide in Northern Italian farms, with different clinical outcomes that could be modulated by intrinsic characteristics of the farms. In absence of vaccination, serological monitoring can be a useful tool for viral entrance monitoring, although sampling timing should be evaluated in order to spot seroconversion after late infections.

Key words: broiler, avian metapneumovirus, serology, Italy, circulation

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INTRODUCTION

Avian metapneumovirus (**aMPV**) is a respiratory pathogen affecting poultry, primarily turkeys and chickens. It belongs to the family *Paramyxoviridae*, subfamily *Pneumovirinae*, genus *Metapneumovirus*, and it has been renamed *Metapneumovirus avis* by the latest the International Committee on Taxonomy of Viruses (**ICTV**).

aMPV infection causes mainly respiratory signs; when it affects turkeys, the disease is called turkey rhinotracheitis (**TRT**), whereas in chickens, the involvement of aMPV and other pathogens in the upper respiratory tract can cause a clinical form known as swollen head syndrome (**SHS**) (Suarez et al., 2020). aMPV can also contribute to reproductive disorders and predispose to secondary infections, supporting the worth of vaccination in turkeys (Kaboudi and Lachheb, 2021) and in long-life chickens such as layers and breeders (Cook et al., 2000), where killed vaccines are usually applied after live vaccine priming. On the other hand, live vaccination is mainly adopted in growing turkeys and administered via spray, drinking water or ocular drop (Kaboudi and Lachheb, 2021). Despite not preventing the infection, vaccination can effectively contain symptoms and losses and reduce viral circulation.

However, vaccination against aMPV presents some drawbacks related to sporadic vaccine reactions, vaccine strain circulation and reversion to virulence (Catelli et al., 2006). Suboptimal live vaccination procedures and coverage might generate incomplete protection and vaccine strain passages within the flock; consequent adverse reactions and disease episodes might follow vaccination, with vaccine strain persistence in the field (Catelli et al., 2006).

Over the years, different genetic subtypes of the virus have been recognized and classified as subtypes A to D, based on genetic and antigenic variations (Suarez et al., 2020). aMPV subtypes exhibit distinct geographical distribution and host tropism (Kaboudi and Lachheb,

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2021), influencing the epidemiological picture and diagnostic surveys.

In Europe, aMPV subtype A detections have decreased in recent years, in favor of a wider circulation of subtype B (Franzo et al., 2020), both in turkeys and chickens. Two additional subtypes have been identified in gulls and parakeets in North America, where, even more importantly, A and B subtypes have also been recently detected (Luqman et al., 2024) (personal communication and published sequences for subtype A): in fact, the spreading infection had a great impact on the naïve turkey and chicken populations and the absence of dedicated tests initially complicated the diagnosis. Previously, subtype C was the only subtype present in the US and the North American lineage of subtype C circulated in the turkey domestic population and in wild birds (Luqman et al., 2024).

Based on these epidemiological evidences, a subtype shift or the entrance of a new subtype in the domestic population could be hardly diagnosed without a steady monitoring of the susceptible populations and appropriate diagnostic tests, as it happened in the US for A and B subtypes. Similarly, subtype C circulation in Europe could be underestimated as most of the diagnostic tools rely on methods detecting only A and B subtypes. On the other hand, aMPV is problematic also in terms of detection timing, since its direct identification can be performed only within a short timeframe from the beginning of the symptoms (Suarez et al., 2020), possibly leading to an underestimation of its contribution due to delayed sampling.

Broilers are particularly exposed to aMPV, especially in contexts such as Northern Italy, where subtype B is prevalent and all productive types of chickens and turkeys are closely reared in a high densely populated area (Tucciarone et al., 2018; Franzo et al., 2020). Moreover, aMPV vaccination is not commonly administered to broilers (Tucciarone et al., 2018) and is usually reserved to long life birds for preventing reproductive diseases and production drops (Cook et al., 2000).

Given the particular context of a naïve Northern Italian broiler population, this study aimed at evaluating the circulation of aMPV in broiler farms in Italy in different seasons through a serological monitoring.

MATERIALS AND METHODS

Broiler farms were selected in densely populated areas in Northern Italy. Sampling was planned for animals older than 40 d of age (**doa**), towards the end of the production cycle in summer/fall 2022 for diagnostic purposes, then the same farms were sampled again in winter/spring 2023. Blood samples were collected from 15 birds per broiler flock, estimating a 35% seroprevalence with a population size of 10000 individuals per flock, assuming test specificity and sensitivity of 95% (https://epitools.ausvet.com.au/freecalctwo) to determine the presence/absence of viral circulation within the flock. Samples were centrifuged, serum was separated and stored at −20°C. Serum samples were transferred to the Laboratory of Infectious Diseases of Animal Medicine Production and Health (**MAPS**) Department (Legnaro, Italy) and were analyzed in 2 separate batches (summer/fall and winter/spring) using the ELISA Avian Rhinotracheitis Antibody test kit (Bio-Chek BV, The Netherlands), detecting total anti-aMPV antibodies for both A and B subtypes. Sample to positive (**S/P**) ratio was calculated with the following formula: S/P ratio = (sample mean optical density [**OD**] − mean negative control OD)/(mean positive control OD − mean negative control OD); then antibody titre was calculated as antilog of Log_{10} titre = 1 * Log_{10} (S/P) + 3.62, as recommended by the kit manual. Samples were considered positive with a titer equal to or higher than 1656. Results were organized in a Microsoft Excel® database including farm identification, shed number, bird age at sampling, collection date and the presence or absence of respiratory clinical signs along the cycle. The presence of farm or season effects on antibody titers was investigated by performing the Scheirer-Ray-Hare nonparametric test within the R environment (R Core Team, 2022), using the *rcompanion* package (Mangiafico and Mangiafico, 2017) and setting the significance level to $P < 0.05$. Posthoc Dunn's test with Bonferroni correction was then employed to investigate significant differences.

RESULTS AND DISCUSSION

Seven broiler farms were sampled firstly in the period from August to October 2022, then again in February-May 2023, and a total of 210 serum samples were collected. The mean age of the birds was 46.4 d (min = 41, max = 61, median = 45.5): at the summer/fall sampling, the mean age was 44 d (min = 41, max=49, median=43), whereas in winter/spring the mean age was 48.9 d (min = 44; max = 61; median = 47). All farms were positive at both sampling points, except for farm D, whose birds were negative at the first sampling and did not display clinical signs (Table 1, Figure 1A). Instead, respiratory clinical signs, including coughing, conjunctivitis, swollen sinuses and periorbital area were reported in 5 out of 7 farms (71.4%) and 9 out of 14 flocks (64.3%), even though birds seroconverted also in flocks where no signs were reported (farms A and E). Mortality was in line with expected rates at the end of the cycles in all farms (Table 1).

In 2 farms (A, B), summer/fall prevalence was higher than winter/spring prevalence, conversely in 3 farms (C, D, E) more birds were positive at the second sampling. Two farms (F, G) showed 100% seroprevalence at both sampling points. Antibody titers are reported in Table 1. Overall, titres slightly increased with age (Figure 1B), suggesting a more suitable time window for sampling towards the end of the cycle. A likely aMPV entrance around 30 to 35 doa, had been previously suggested (Tucciarone et al., 2018), and was supported by the high seroprevalence in birds sampled after 44 doa. However,

Table 1. Summary of data related to the birds and relative antibody titers.

Farm Id	Date	Age	N. Birds/N. Sheds*	Clinical Signs[c]	Mortality†	Positive Birds	Gmt	Mean Titre	Median Titre	Min-Max	Cv[a]	Cv[b]
A	Sept. 2022	49	12,000/2	ABSENT	2.94%	100.0%	21,209.3	22,410.8	22,409.2	9,895.8–35,004.1	0.32	0.31
	Feb 2023	51		ABSENT	3.02%	33.3%	1,192.6	1,764.0	957.0	309.0–6,538.0	0.99	0.44
B	Oct 2022	43	16,000/1	PRESENT	3.12%	100.0%	13,883.7	15,568.9	15,626.0	2,394.4–26,784.3	0.39	0.39
	May 2023	44		PRESENT	4.26%	26.7%	1,135.9	1,227.4	1,129.1	534.0–2,240.0	0.39	0.11
C	Aug 2022	46	18,500/2	PRESENT	2.38%	86.7%	3,866.1	5,745.4	3,904.5	639.4–18,989.0	0.96	0.85
	Mar 2023	47		PRESENT	1.56%	100.0%	7,115.3	8,422.9	7,417.8	2,894.7–16,860.7	0.55	0.55
D	Sept. 2022	41	23600/2	ABSENT	4.36%	0.0%	715.6	761.8	655.7	443.5–1,325.1	0.37	-
	Mar 2023	46		PRESENT	3.17%	100.0%	7,946.9	8,902.6	7,219.4	2,722.8–17,449.3	0.46	0.46
E	Oct 2022	42	26000/2	ABSENT	1.98%	61.5%	1,995.6	2,332.2	1,945.4	598.6–6,549.2	0.63	0.52
	Mar 2023	44		ABSENT	2.21%	93.3%	4,898.8	5,799.5	4,693.4	1,525.9–13,402.3	0.57	0.53
F	Sept. 2022	45	28000/4	PRESENT	5.35%	100.0%	16,704.6	18,134.1	19,078.8	8,287.8–27,967.9	0.37	0.37
	Feb 2023	49		PRESENT	3.42%	100.0%	15,037.3	15,655.9	13,633.7	10,082.7–22,177.4	0.29	0.29
G	Sept. 2022	42	18000/1	PRESENT	3.87%	100.0%	7,024.1	8,347.0	7,928.6	1,684.2–18,580.9	0.53	0.53
	Feb 2023	61		PRESENT	2.96%	100.0%	10,321.3	11,759.2	11,484.6	3,893.2–27,136.9	0.50	0.50

Age is reported in days.
*Number of birds present in each shed/Number of sheds present in the farm.
†Mortality at the end of the cycle.
Abbreviations: GMT, geometric mean titer; CV, coefficient of variation.
CV[a] is calculated on all samples.
CV[b] is calculated on positive samples.
[c]Clinical signs, when present, included coughing, conjunctivitis, swollen sinuses and periorbital area.

some exceptions were identified; in 2 farms (A, B), a low percentage of positive birds was identified at 44 (farm B) and even at 41 doa (farm A), possibly indicating a later entrance of the virus and the ongoing seroconversion within the flock. Similarly, the negative flock (Farm D) was sampled around 41 doa and the infection onset could have been missed, even though the absence of clinical signs might corroborate the lack of aMPV circulation. In fact, sampling time and delay for seroconversion have been discussed in other situations as constraints for aMPV diagnosis by serological means, since antibodies are generally produced 10-14 d after infection (Rautenschlein et al., 2011), and the short broiler lifespan might set an early cut-off for serum sampling. On the other hand, the immune response against aMPV infection is also generally featured by a strong cell mediated component (Rautenschlein et al., 2011), that can intervene precociously and might compensate a weak seroconversion, masking a wider infection rate within the flock.

The percentage of seropositive birds within farm ranged from 26.6% to 100%; the coefficient of variation (**CV**%) was calculated on positive results and ranged from 0.11 to 0.85, whereas cumulative CV ranged from 0.32 to 0.99. Based on statistical analyses, seasonality was not found to play a significant role in determining antibody titers ($P = 0.108$), whereas the farm effect ($P < 0.001$) and the interaction between the 2 factors ($P < 0.001$) proved significant. According to posthoc tests, the titers measured in summer/fall and winter/spring differed significantly in farms A, B, D, and E (all with $P < 0.002$), but not in farms C ($P = 0.07$), F ($P = 0.37$) and G ($P = 0.09$). In addition to a clear farm effect, the titers measured in subsequent flocks of the same farm tended to also be significantly different. However, since the observed within-farm changes were not univocal, with some farms scoring higher and other lower in winter/spring compared to summer/fall, this finding can hardly be attributed to season alone and was likely determined by other factors, such as an inherent between-flock variability and the unaccounted time from the start of infection. Comparably, seroconversion was observed in both symptomatic and asymptomatic flocks, suggesting that other conditions (i.e., management, stress, concomitant infections...) might have contributed to determining the final infection outcome.

Due to their multifactorial nature, respiratory problems are among the most common issues faced in intensive farming settings. The contribution of aMPV to the respiratory complex appears to increase, and the wide seropositivity of the investigated unvaccinated broiler flocks supports the perceived uprise of the virus in a relatively small geographic area (Northern Italy), where farms rearing various species and productive types of poultry are abundant and very close.

aMPV has generally been considered a minor problem for chicken farming compared with other pathogens; however, other than its primary pathogenetic role, its wide presence might predispose to the entrance of secondary pathogens, whose action might be enhanced and result in severe losses. In these circumstances, the

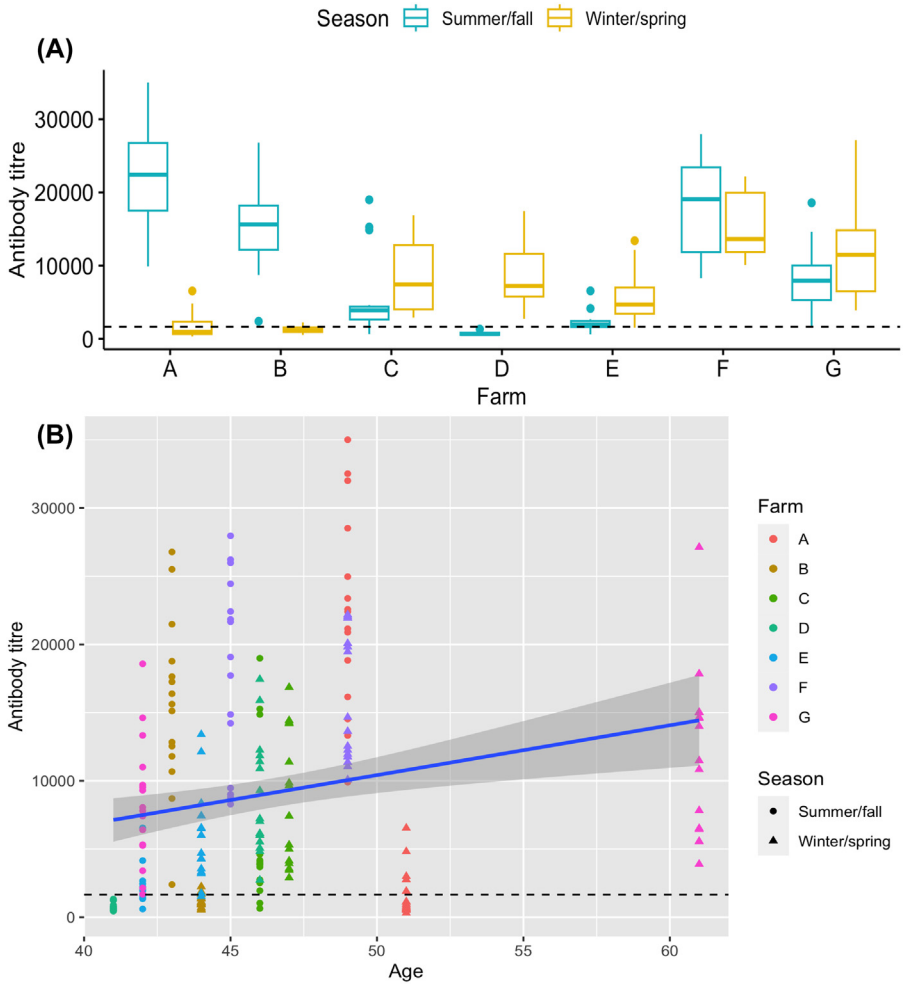

Figure 1. (A) Antibody titre distribution on the 2 sampling points for each farm. Samples below the dotted line (kit cut-off: 1,656) are negative; (B) Antibody titre distribution according to age at sampling, different farms are color-coded, samples collected in summer/fall are represented by circles, samples collected in winter/spring are represented by triangles.

Northern Italian productive context features an extreme density of turkey, broiler, layer, and breeder farms, setting the premises for an intense pathogen exchange, increasing the risks. Whereas aMPV-related issues are addressed in turkey and long-life bird farming by vaccination, broiler farming is more exposed to infection, since the cost-benefit ratio of vaccination has been considered unfavorable and other issues, such as timing and interactions with other vaccines, may play a role. However, the epidemiological scenario is constantly changing, and ongoing monitoring must be arranged and maintained in order to interpret new clinical episodes, update diagnostic tools and possibly re-evaluate control strategies.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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