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Full Research Article

FIRST SEROEPIDEMIOLOGICAL AND RISK FACTOR SURVEY OF AVIAN METAPNEUMOVIRUS CIRCULATION IN MOROCCAN BROILER FARMS

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Abstract

Avian metapneumovirus (aMPV) is a highly contagious pathogen for broilers. Its epidemiological aspects remain poorly understood in Morocco. Given that, we carried out a pilot seroepidemiological survey from December 2020 to June 2021 to define aMPV seroprevalence in Moroccan broiler flocks in different bioclimatic zones (humid to sub-humid, semi-arid, arid) during winter and spring. According to these zones, 48 flocks not vaccinated against aMPV were selected using a stratified random sampling model. With a simple random sampling strategy, fifteen to thirty sera per flock were collected then analysed using a commercial indirect ELISA kit (CIVTEST AVI TRT®, HIPRA S.A., Amer, Spain) that was able to titre antibodies against aMPV subtypes A

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and B. Furthermore, questionnaires were shared with veterinarians in charge of flocks to collect data and analyse it with multivariable logistic regression models to identify risk factors associated with virus circulation. From 1142 sera, 912 tested positive with the ELISA used, showing an overall aMPV seroprevalence of 79.86% in the broilers. The arid zone had the highest seroprevalences: 94.16% during winter and 84.82% during spring. Bioclimate and season seemed to contribute to aMPV seropositivity. Likewise, high stocking densities and poor hygiene scores on farms were proven to predispose birds to seropositivity. Hereby, we show the circulation of aMPV in Moroccan broiler farms is influenced by bioclimate, season, density, and hygiene conditions. The present study is the first serological evidence of aMPV in broilers in the Maghreb region.

Key Words: avian metapneumovirus, broilers, risk factors, seroprevalence.

INTRODUCTION

Avian metapneumovirus (aMPV) is the causative agent of turkey rhinotracheitis in turkeys, while it is considered in broilers a predisposing factor for swollen head syndrome, to date present in most areas of the world (Suarez et al., 2019). Although it is believed that aMPV always triggers swollen head syndrome in chickens, the appearance of aMPV in mono-infected flocks may not be accompanied by clinical symptoms (Owoade et al., 2006), or paradoxically, can cause severe respiratory distress (Tucciarone et al., 2018).

Four different subtypes of aMPV have been identified so far, named A, B, C, and D (Cook and Cavanagh, 2002). The subtypes A and B are more widespread globally, but field evidence points to the higher circulation of aMPV-B viruses over aMPV-A for poorly understood reasons (Jones, 2010). Serology is a useful tool for aMPV diagnosis. ELISA seems to be the most common method (Cook, 2000), particularly in unvaccinated flocks where seroconversion is a clear indicator of field virus contact. Besides, many commercial ELISA kits detect subtypes A and B without differentiation, as both belong to a common serotype (Collins et al., 1993).

Chicken meat production in Morocco has undergone tremendous growth over time, contributing significantly to the national animal-protein demand but struggling with many handicaps. The live market dominates the sector, with only 10% of birds submitted to slaughterhouse processing. Besides, due to lack of land and restrictive policies of the law 49-99 that regulated the poultry industry, many producers had to look far away from traditional areas, known for their favourable climate and proximity to large consumption centres, and implement production in other, less convenient regions. Furthermore, the broiler segment suffers from the unavailability of highly qualified staff, which makes the production particularly vulnerable to infectious diseases.

Since the first description of aMPV in broilers in Morocco during the 1980s, no studies have been published on this issue, and field cases have often been reported without data on circulating subtypes. Thus, the present study was to investigate the presence of aMPV antibodies in serologically naïve chickens and to demonstrate the circulation of aMPV in broiler farms in Morocco for the first time. Moreover, each flock's clinical, field and biosecurity data were collected by interviewing poultry field veterinarians involved in the study. Data were analysed, along with aMPV serological results, bioclimatic zones where flocks were located and seasons, to identify the main risk factors for aMPV occurrence.

MATERIALS AND METHODS

Sampling protocol

The survey covered the period from December 2020 to June 2021 and targeted broiler flocks not vaccinated against aMPV and older than five weeks, an age that allows evidence of any recent seroconversion to be obtained. The sampling protocol was based on two steps to achieve statistical representativity in the study: first, broiler farms were selected using stratified random sampling, where each stratum (sub-group) represented a bioclimatic zone, and secondly, birds were chosen by simple random selection from each farm. Given the lack of data about aMPV in Morocco, the sample size was calculated according to the number of flocks, and the number of sera by hypothesising a theoretical prevalence of 10%.

Flock selection. The farms were located in different geographical areas of Morocco and were sampled regardless of the presence of respiratory symptoms in the birds. The number of sites per bioclimate zone was proportional to farm density in each zone. Starting from an initial population of 7992 broiler farms (according to official national data for 2021), the number of flocks to be sampled was selected and calculated with a confidence interval of 95% (Pfeiffer, 2002). Based on that, the minimum number of flocks needed for the study was 29 flocks. Nonetheless, we oversized this number and investigated 48 herds to compare the two seasons and the different bioclimatic zones. According to the bioclimate zones present in Morocco, 48 flocks were randomly selected while respecting the proportion of authorised farms in each region, as reported in Table 1.

Table 1. Number of flocks selected for investigation according to bioclimatic zone

Bioclimatic zone	Identification	Number of farms authorised	Number of flocks to investigate	
			Winter	Spring
Humid to sub-humid	Zone 1	1945	5	5
Sub-arid	Zone 2	4200	11	11
Arid	Zone 3	1710	8	8
Hyper-arid*	Zone 4	137	0	0
Total		7992	24	24

(*) Due to logistic issues, we were unable to collect samples from farms in the hyper-arid zone.

Sample size. The number of blood sera collected per flock was defined and set to n = 29 (Pfeiffer, 2002). Notwithstanding, for several logistic parameters (predefined number of samples taken in the field by veterinarians, or eventual losses during transport or storage), we lessened the sample size in some cases to fifteen.

Sample collection

Samples of fresh blood were collected on-farm from birds' brachial wing veins by puncture of the alar veins, then stored in sterile tubes before transportation to the Avian Pathology Unit of the Hassan II Agronomic and Veterinary Institute, Rabat, at 4°C. During the time between finishing all the blood collection from one farm and refrigeration, the tubes were placed on a slanted surface at room temperature, to allow clotting and initial separation of serum from coagulated blood. Blood samples were transported at 4°C to the Avian Pathology Unit laboratory, where sera were fully extracted and stored in Eppendorf tubes at -20°C until the day of analysis. In addition, some sera were supplied to us as prepared frozen sera collected by field veterinarians for the flocks we could not visit. For confidentiality purposes, a unique alphanumeric code was assigned to each flock.

Serology

The sera were analysed by the commercial indirect ELISA kit CIVTEST AVI TRT® (HIPRA S.A., Amer, Spain) to detect aMPV-A and aMPV-B antibodies. The mean titres, validity tests, and coefficients of variation were automatically calculated by flock and sample series with the software HIPRASOFT® 5.0 (HIPRA S.A., Amer, Spain).

Field data collection

Field relevance of respiratory diseases in Moroccan broiler flocks. A survey based on a very short online form (two questions) was carried out because of the lack of data about swollen head syndrome in broilers in Morocco and to clarify the subjective hindsight by field poultry veterinarians regarding aMPV as an involved agent in respiratory syndromes, in the absence of laboratory investigations. We enrolled 34 poultry veterinarians, and aimed to identify and classify the most important infectious respiratory diseases according to the frequency of observation and their relevance in the field: Newcastle disease, infectious bronchitis, low pathogenic avian influenza due to H9N2 virus, swollen head syndrome and mycoplasmosis.

Field and biosecurity parameters. Using a questionnaire assigned to veterinarians on the farms, information on field data and biosecurity measures were collected for flocks included in the aMPV serosurvey, except when veterinarians were reluctant to share inputs for flocks we did not visit. The data accuracy of the survey was enhanced by conducting personal interviews on the farm whenever possible. The different parameters concerned were: farm location, age of the flock, stocking density, single-age or multi-age flock, ventilation system, swollen head syndrome antecedent outbreaks, vaccination program, general hygiene measures and biosecurity applied. Biosecurity was assessed using an adapted evaluation grid, to assign a biosecurity score to each flock, including litter quality. The geographic location of each visited farm was recorded by global positioning system to check the distances from neighbouring poultry farms.

Exploitation of data. The inputs from questionnaires, the evaluation grid, and serology data were gathered into a Microsoft® Excel® (2016) workbook. Data from the field respiratory disease survey were summarised and processed separately, with the following attributions: the frequency of observation was converted to numeric values, from 1 to 4, representing respectively: never observed, rare, often, very frequent. The relevance in the field was also adapted to an ordinal score from 1 (the least relevant) to 5 (the most pertinent). Afterwards, the data were converted into numeric codes, as reported in Table 2. Thus, data exploitation, exportation and analysis was simplified.

Table 2. Factors and appropriate codes used to evaluate the risk of aMPV infection

Variables	Type	Categories	Code attributed
Serological status*	Nominal	Negative	0
		Positive	1
Bioclimatic zone	Nominal	Humid to sub-humid	1
		Sub-arid	2
		Arid	3
Season	Nominal	Winter	1
		Spring	2
Flock density	Ordinal	≤ 11 birds/m2	1
		11 – 14 birds/m2	2
		> 14 birds/m²	3
Single-age or multi-age	Nominal	Single-age	1
		Multi-age	2
Ventilation	Nominal	Static	1
		Dynamic	2
Litter quality	Ordinal	Poor	1
		Average	2
		Good	3
Hygiene and biosecurity score	Ordinal	Poor	1
		Average	2
		Good	3
Presence of wild birds**	Nominal	No	1
		Yes	2

(*) Serological status is an independent variable, unlike all the other variables. It refers to serology results
(**) The presence of wild birds was assessed both by observing their circulation inside houses or the integrity of fences and net installed to avoid the ingress of wild birds

Field and laboratory data analysis

Field relevance of respiratory diseases in Moroccan broiler flocks. The form results were combined into one file and compared using a synthetic Microsoft® Excel® (2016) radar chart.

Flock and total aMPV seroprevalence. A flock was considered seropositive when the geometric mean antibody titre exceeded 195, corresponding to the CIVTEST AVI TRT® cut-off. Then, flock seroprevalence was calculated. Besides, the percentage of flocks seropositive to aMPV was also calculated along with total seroprevalence. Seroprevalences were also investigated by bioclimatic zone and by season.

Association between aMPV seropositivity and presence of clinical symptoms. By considering clinical respiratory symptoms, whether they exist or not, and the seropositivity, flocks were classified into different groups based on those abovementioned "categorical" variables. Since the Chi-square (χ^2) test of independence is a statistical hypothesis test used to determine whether two categorical or nominal variables are likely to be related, this method was used to demonstrate if an association between seropositivity and respiratory symptoms existed or not. Therefore, groups of sick birds and healthy birds were compared according to the presence of antibodies.

Statistical analysis of questionnaires data and comparison with serology. Multivariate logistic regression models were applied to study the effect of bioclimatic zone, season, flock density, single-age or multi-age, ventilation, litter quality, hygiene and biosecurity score and presence of wild birds as variables (independent variables) on the aMPV serological status (dependant variable) and to identify eventual risk factors for aMPV seropositivity after calculating both χ^2 and odds-ratio values. All the statistical parameters, including the χ^2 tests, were calculated with the software IBM SPSS® Statistics 22 (SPSS Inc., Chicago, IL, USA), and the level of significance was set at $p<0.05$.

RESULTS

Field relevance of respiratory diseases in Moroccan broiler flocks

Figure 1 shows a graphical representation of the dominant respiratory diseases in Moroccan broiler flocks, according to field poultry veterinarians' feedback. These diseases were Newcastle disease, infectious bronchitis, low pathogenic avian influenza due to H9N2 virus, swollen head syndrome and mycoplasmosis.

The field relevance of diseases seemed to be associated with the frequency of reports, based on veterinarians' feedback (Figure 1) and how often they observe clinical cases in their practice. This could explain the juxtaposition of data in Figure 1, where data points almost come together.

Low pathogenic avian influenza was the most important and frequent disease reported by veterinarians, followed by infectious bronchitis, then mycoplasmosis. aMPV infection, which is usually associated in the field with swollen head syndrome, was neither highly relevant nor frequently observed by veterinarians, as only 10/34 (29.41%) veterinarians reported it as a frequent disease.

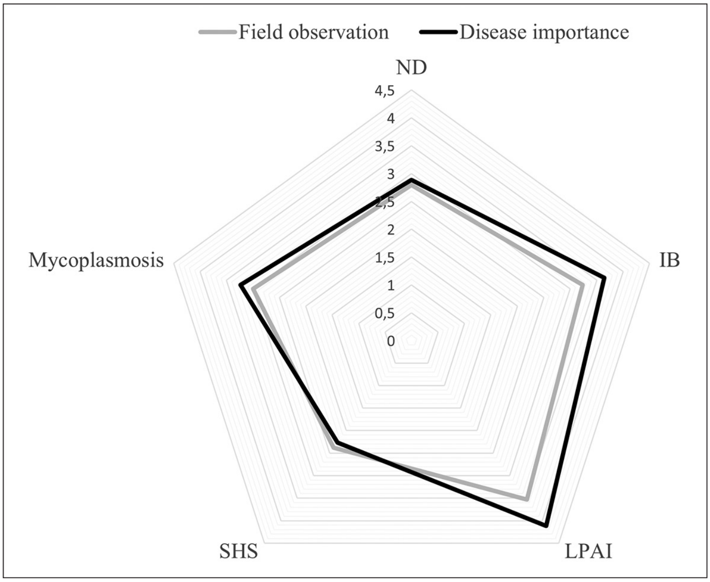

Figure 1. Respiratory infectious diseases reported in Moroccan broiler farms according to field poultry veterinarians' feedback

Flock and total aMPV seroprevalence

From the 48 broiler flocks, only two showed mean antibody titres less than 195 (and so were considered seronegative). Therefore, 46 (95.83%) flocks had been exposed to circulating aMPV.

Table 3. Seroprevalence of broilers according to bioclimatic zone and season

Bioclimatic zone	Winter				Spring			
	Sera	(+)	SP* (%)	GMT**	Sera	(+)	SP* (%)	GMT**
1	128	116	90.63 [84.20 – 95.06]	519.16 ± 124.30	130	88	67.69 [58.93 – 75.62]	315.25 ± 186.25
2	293	183	62.46 [56.64 – 68.02]	364.28 ± 348.06	246	218	88.62 [83.97 – 92.30]	867.21 ± 742.90
3	154	145	94.16 [89.20 – 97.29]	1043.58 ± 665.82	191	162	84.82 [78.93 – 89.59]	662.18 ± 301.94
Total	575	444	77.22 [73.57 – 80.58]	622.98 ± 300.63	567	468	82.54 [79.16 – 85.58]	697.62 ± 570.09

Sera = total number of sera analysed; (+) = positive sera; SP (%) = seroprevalence; GMT = Geometric mean antibody titre; *SP are shown with confidence intervals of 95%; **GMT are shown with standard deviations (GMT ± standard deviation)

Also, from the 1142 sera analysed, 912 were positive to aMPV with the ELISA test, which corresponded to a broiler seroprevalence of 79.86% with an interval of confidence of 95% [77.41% – 82.15%].

In all the bioclimatic zones studied, broiler seroprevalences measured were quite high. Table 3 summarises the broiler seroprevalences obtained by zone and season.

During winter, the highest seroprevalence was measured in broilers in the arid bioclimate zone (zone 3), with 94.16% [89.20% – 97.29%] of examined birds being seropositive, while the lowest seroprevalence (62.46% [56.64% – 68.02%]), still during the same season, was measured in broilers in the sub-arid zone (zone 2).

Association between aMPV seropositivity and presence of clinical symptoms

Eleven flocks had birds that exhibited various respiratory symptoms before or during the sampling, including sneezing, coughing, or dyspnoea, but which were unlikely to refer to aMPV infection. Among these farms, one (1-TS59) had broilers that showed typical symptoms of swollen head syndrome during the visit, including loud coughing, extended head position and swollen sinuses, as shown in Figure 2.

Figure 2. Broilers from farm 1-TS59 showed swollen heads and were coughing during the visit

Overall, there was a statistically significant association between respiratory symptoms and seropositivity to aMPV, with a χ^2 value of 14.087 and p-value <0.005.

Statistical analysis of questionnaire data and comparison with serology

According to the data reported in Table 4, bioclimate zone, season, flock density and hygiene/biosecurity score were identified as risk factors for the aMPV seroprevalence, with asymptotic significance of the relation between the variables and seropositivity lower than 0.05, P<0.05.

Table 4. Estimation of the risk due to some variables of aMPV-infection in broilers

Variables	Sig	χ^2	Odds-ratio[(a)]	CI 95% for odds-ratio	
				Inferior	Superior
Bioclimatic zone	0.000*	30.48	N/A	N/A	N/A
Season	0.016*	5.75	1.43	1.07	1.92
Flock density	0.000*	32.57	N/A	N/A	N/A
Single-age or multi-age	0.302[NS]	0.30	0.92	0.68	1.24
Ventilation	0.659[NS]	0.195	1.12	0.68	1.84
Litter quality	0.075[NS]	3.02	1.29	0.93	1.90
Hygiene and biosecurity score	0.000*	15.37	N/A	N/A	N/A

Sig = significance; χ^2 = chi-square value and CI 95% = Confidence interval of 95%;
[(a)]Calculated only for *2x2* pivot tables; *Significant value (P<0.05); [NS] Non-significant value

Following these results, logistic regression models were established for every variable to define the effect of each factor on seropositivity, with the outcome reported in Table 5.

Table 5. Binary logistic regression models of variables associated with aMPV seroprevalence in broiler farms

Factors	Exp(B)	CI 95%	Sig
Bioclimatic zone			
Humid to sub-humid	0.45	0.28 – 0.71	0.000*
Sub-arid	0.35	0.23 – 0.52	0.001*
Arid		Ref	
Season			
Winter	0.74	0.55 – 0.99	0.049*
Spring		Ref	
Density			
≤ 11 birds/m²	0.33	0.22 – 0.51	0.000*
11 – 14 birds/m²	0.74	0.49 – 1.14	0.172 NS
> 14 birds/m²		Ref	
Hygiene			
Poor	3.11	1.73 – 5.58	0.000*
Average	1.21	0.86 – 1.72	0.277 NS
Good		Ref	

*Significant value (P<0.05); [NS] Non-significant value; CI 95% = Confidence interval of 95%; Sig= significance; Ref = reference and Exp(B) = the chi-square value

Based on these findings, it was concluded that zone 3 (arid zone) presented 55% more risk of aMPV seropositivity than zone 1 (humid zone) and 65% more risk than area 2 (sub-humid zone). Also, in winter, the flocks were at 26% less risk of infection than in spring. Moreover, higher stocking densities were predisposing to aMPV infection. In fact, densities of > 14 birds/m² resulted in 67% more risk of flock seropositivity than densities of ≤ 11 birds/m². Finally, the farms with poor levels of hygiene (score 1) were three times more susceptible (risk 300% greater) to being seropositive than farms with good biosecurity levels.

DISCUSSION

According to poultry veterinarians, swollen head syndrome is not frequently observed in Morocco and has minor relevance in the field. One of the possible explanations is the regular use of antimicrobials in the broiler production in Morocco as a common measure for therapeutic or preventive purposes (Rahmatallah et al., 2018). That use would mitigate the chronic respiratory syndromes and the typical bacterial complications following an aMPV-infection, a *sine qua none* condition for the onset of swollen head syndrome, since the role of aMPV as a primary agent is always neglected in broilers.

Another suggestion is the overestimated impact of low pathogenic avian influenza due to H9N2 viruses since their introduction in Morocco in 2016, particularly as these are often reported with simultaneous detection of infectious bronchitis virus. This suggests that such co-infection could be the main possible cause of respiratory problems encountered in the field (Belkasmi et al., 2020), in the absence of extensive investigations for other respiratory viruses such as aMPV.

With the high aMPV seroprevalence in broilers of 79.86% found, it is possible to consider this virus as endemic, circulating in broiler farms regardless of the bioclimatic zone or the season. Similar seroprevalences were found in Bangladesh (Ali et al., 2019) and South Korea (Park et al., 2011), respectively, 72.30% and 73.10%. In the Middle East, aMPV serological investigations reported a relatively low prevalence of 21.7% in broilers flocks among 47.7% of poultry farms in Jordan (Gharaibeh and Algharaibeh, 2007). It should be pointed out that the broiler slaughter age in these countries is usually around thirty days, younger compared to the slaughter age in Morocco, which may explain the results obtained. In fact, the aMPV tends to affect birds older than 26 days of age (Franzo et al., 2020), and ELISA antibodies are not detectable before seven days post-infection (Jirjis et al., 2002). It would be extremely interesting to know the regional context of aMPV in North Africa. However, the seroprevalence in the surrounding countries is still unknown, unfortunately. In the case of Algeria, the only study regarding aMPV highlights the circulation of subtype-B in turkey flocks (Sid et al., 2015).

Resources allocated to the present study allowed serology testing with one kit only, the CIVTEST AVI TRT®. Thus, we encourage future studies using other kits, because results could differ, as much as the specificity of the kits used.

Considering that none of the flocks in the study were vaccinated against aMPV and broilers were at least 35 days old, the titres detected by ELISA presumably referred to an immune response following a field virus exposure. The titres cannot have been vaccine- or maternally-derived, the latter being supposed to decrease to their lowest level by the time broilers are two weeks old (Rubbenstroth and Rautenschlein, 2009).

These results align with a previous study confirming the association between seropositivity and clinical respiratory symptoms, emphasising the incidence of the swollen head syndrome (Al-Ankari et al., 2004). In the current study, though, almost all the flocks tested showed positive serological results, with 46 positive flocks among 48 examined, but only eleven flocks showed respiratory symptoms. Thus, it is important to cautiously consider the possible role of aMPV as a trigger of these symptoms, despite the association between respiratory problems and seropositivity demonstrated by the statistical analysis. In this regard, another study proved the synergistic effect between aMPV and *Escherichia coli* in co-infections, even without manifest swollen head syndrome (Al-Ankari et al., 2001). Besides, some external factors might contribute to the prevalence of aMPV-infection, like density, farm management, ventilation, general hygiene conditions and veterinary monitoring (Al-Ankari et al., 2004). This supports the suggestion that aMPV infection can occur without swollen head syndrome (Owoade et al., 2006), and this would explain the positive flocks without clinical symptoms that we found on 37 farms.

Furthermore, the sampling period and the waning of respiratory problems are important to consider and could explain the absence of symptoms in 79.86% of the birds that tested positive. In fact, the incubation period of aMPV is 2 to 3 days following contact with infected birds before their respiratory symptoms wane, and infection lasts 2 to 3 weeks in the absence of bacterial complications (Jones et al., 1987). However, seroconversion is detectable by ELISA between 13 and 28 days post-infection (Aung et al., 2008), which coincides with the period when clinical symptoms disappear.

In contrast, respiratory distress has been previously described in flocks infected exclusively with the aMPV (Tucciarone et al., 2018), with 68.80% of the birds showing respiratory symptoms positive to the aMPV when tested by RT-PCR. Those data (Tucciarone et al., 2018) confirmed that aMPV is not a minor pathogen in broilers.

Farms within the arid bioclimate zone seem to be at higher risk of aMPV infection than humid or sub-arid zones. The highest seroprevalence in zone (94.16%) was measured during winter. Focusing on the arid zone, a wide temperature range dominates this zone, varying from warm weather along the Atlantic coast to very cold in the Middle and Anti-Atlas Mountains chain edges. Broilers are sensitive to temperature variation, and heat adversely impacts performance (Lucas and Rostagno, 2013). At the same time, cold stress can increase the susceptibility of birds to infectious pathogens (Huff et al., 2007). In the case of aMPV, cold weather is likely to preserve the virus, and the stress induced would lower the chickens' immunity (Bakre, 2020).

Overall, seropositivity in the current study was higher in spring than winter, and surprisingly, our results contradict previous studies from different parts of the world that reported higher aMPV impact during the winter than in warmer seasons (Ali et al., 2019; Seifi and Booromand, 2015; Kwon et al., 2010). In this respect, it is important to highlight particularities of the weather and seasons in Morocco with its seasonal temperatures. During winter, for example, the difference between the lowest and highest temperature is less than 5 °C, while in spring, the difference is up to 11°C.

One other aspect to consider is the post-COVID19 economic impact. The crisis forced many farmers to stop activity temporarily or even definitively for some others, with subsequently up to 60% drop of placements until the beginning of 2021 according to official national data. In other words, the fact that many farms were empty with few chicks reared would be one of the reasons the lowest prevalence was observed in winter, by slowing down the virus transmission from one site to another. However, the possible role of the season as a risk factor should be investigated by extending the present study to other seasons.

The risk of infection with aMPV was also associated with high stocking densities, static ventilation, and poor farm hygiene scores. These results confirm a previous investigation in Saudi Arabia (Al-Ankari et al., 2004). It is, moreover, important to ensure proper stocking density rates for optimal results. In contrast, negative effects on growth performance and welfare conditions are observed in broilers reared with a density of more than ten birds/m² (Cai et al., 2019). Farmers can overcrowd birds to reduce fixed charges and optimise heating costs during cold seasons. Still, this practice is favourable for virus transmission. In this regard, it was demonstrated that the broiler's natural defences decrease with increasing stocking density (Wang et al., 2019), explaining the high level of infection.

Although litter quality was not a risk factor in the present study, it remains a factor highly impacted by stocking density, to the best of our knowledge. Overdensity raises the temperature of the litter, moisture, dust or ammonia and leads to respiratory distress (Meluzzi and Sirri, 2020). This situation is further complicated when ventilation is poor, and so air quality and relative humidity are not adequate. Unfortunately, most of the broiler farms in Morocco rely on static ventilation with curtain-side housing systems. Thus, the litter quality needs to be investigated more accurately, with emphasis on the possible interrelation with density and ventilation.

Our results align with those reported elsewhere (Al-Ankari et al., 2004), since good general hygiene, and particularly good biosecurity, together with good farm management procedures, ventilation, and a proper stocking density, help to decrease the load of environmental microorganisms. Additionally, a success story in Colorado, United States, showed eradication of the subtype-C aMPV thanks to biosecurity alongside other stringent sanitary measures was possible (Suarez et al., 2019).

The human factor and the subjective perception of hygiene are limiting factors for effective biosecurity. The lack of understanding of biosecurity principles leads to poor consistent application and compliance measures (Racicot and Vaillancourt, 2009). It is widely accepted that farm biosecurity and hygiene conditions are important risk factors for the occurrence of aMPV infection and swollen head syndrome.

Nontheless, in Morocco, a high level of monitoring is accorded to breeder and layer farms, whereas broiler are exploited on farms with high capacities and suffering from underskilled human resources. Thus, improving observation abilities and skills in management by training is crucial to improve hygiene and, subsequently, disease prevention (Collett et al., 2019). Still, the present study found that bioclimate, season, stocking density, and farm hygiene/biosecurity scores were the only risk factors associated with aMPV seropositivity.

Interestingly, while collecting field data from veterinarians and farmers during sampling visits, we realised that several farms used to integrate aMPV vaccination in their preventative programs in different ways: seasonally during cold seasons or periods of high challenge, for three to four consecutive flocks until the incidence of clinical symptoms ceased, or following other patterns. Notwithstanding, all of them ceased long ago to vaccinate against aMPV, either because the advantages of vaccination are not well perceived or to reduce production costs. It is a common belief that the cost/benefit impact of aMPV vaccination in broilers is usually underestimated (Bayraktar et al., 2018).

In the absence of a specific control strategy against aMPV in broilers, the virus is likely to contaminate birds, spread among farms and contaminate successive flocks within the same site. In this regard, vaccination is crucial in the protection against aMPV, particularly in endemic areas with high field virus pressure or co-existing factors (Tucciarone et al., 2018). It has been proved that vaccination of broilers would effectively lower the circulation of aMPV (Franzo et al., 2020). Because the number of broilers vaccinated in Morocco is negligible compared to the total national broiler production, it was not possible in the present study to evaluate the effect of previous aMPV vaccination on virus circulation.

The rapid growth of the avian industry in Morocco, particularly in broiler chickens, has led to densely populated poultry regions. Besides, Morocco is known for its very important production of meat turkeys, with more than 12.5 million turkey poults placed in 2020. The farms in proximity, especially turkeys, can be exposed to aMPV virus (Lupini et al., 2011), possibly enhancing the virus's spread and dissemination in the environment.

For instance, Souss-Massa and Draa (formerly one cluster Souss-Massa-Draa) is an important geographic area for broiler production in Morocco, with 1,015 farms authorised on these two territories according to official national data, corresponding to 12.70% of total farms. This area was also an important part of the present study, with 27% (13/48) of the farms studied.

Another region, Rabat-Salé-Kénitra, is also as important as the abovementioned region, having 10.24% of total national broiler farms. Farm 1-TS59, which housed broilers with the typical clinical form of swollen head syndrome aforementioned, is located in Rabat-Salé-Kénitra and is close to one commercial layer farm and four other poultry farms.

It should be clarified that several participants in the study were reluctant to share the geographic location of farms for different reasons. Since this data was required to identify surrounding sites and how close they are, we were unable to further explore the potential risk associated with geographic location and proximity to other poultry sites.

CONCLUSION

The high aMPV seroprevalence in Morocco obtained in our study, regardless of the region, was quite unexpected, particularly in farms without clinical symptoms, and shows the important circulation of aMPV in Moroccan broiler farms.

Bioclimate, season, density, and general farm hygiene were shown to contribute as risk factors to aMPV circulation and, ultimately, to respiratory problems.

Since the molecular tools offer rapid and reliable results with good sensitivity and specificity today, upcoming investigations must emphasise detecting the aMPV using RT-PCR. The results obtained would aim to identify the circulating subtype(s), and sequencing perspectives, coupled with our serological evidence, would help us to understand more epidemiological aspects of aMPV circulation.

Ethical statement

Committee on Animal Research and Ethics Hassan II Institute of Agronomy and Veterinary Medicine in Morocco guarantees that all animal procedures conducted in this research are in accordance with international ethical standards (European Union Directive 2010/63/EU) legislation and ARRIVE (Animal Research Reporting of *In Vivo* Experiments) guidelines.

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Authors' contributions

SB for serology and statistical analysis. HF for support on the design of the study. JLC for backing for ELISA kits. MB for review of the epidemiological study. AG for assessment of the manuscript. MM for access to farms and review of field data. EC for review of manuscript, both first and amended versions. SN for supervision of the project (Thesis Director).

Competing interests

The authors declare that they have no competing interests.

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PRVO ISPITIVANJE SEROEPIDEMIOLOŠKIH FAKTORA I FAKTORA RIZIKA OD INFEKCIJE PTIČJIM METAPNEUMOVIRUSOM NA MAROKANSKIM FARMAMA BROJLERA

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Kratak sadržaj

Ptičji metapneumovirus (aMPV) je veoma zarazan patogen za brojlere. Međutim, epidemiološki aspekti ove bolesti su nedovoljno istraženi u Maroku. Izvršili smo pilot seroepidemiološko ispitivanje, koje je trajalo od decembra 2020. do juna 2021. godine da bismo utvrdili seroprevalencu ptičjeg metapneumovirusa u jatima marokanskih brojlera u različitim bioklimatskim područjima (vlažnim do umereno vlažnim, polusušnim, sušnim) u toku zime i proleća. U ovim područjima, prilikom stratifikovanog nasumičnog uzimanja uzoraka, izabrano je 48 jata koja nisu vakcinisana protiv aMPV. Jednostavnim nasumičnim uzorkovanjem sakupljeno je 15-30 seruma po jatu korišćenjem komercijalnog indirektnog ELISA testa (CIVTEST AVI TRT®, HIPRA S.A., Amer, Spain) koji je mogao da titrira antitela na aMPV podtipove A i B. Dalje su u cilju prikupljanja podataka i njihove analize pomoću multivarijabilnih modela logističke regresije upitnici deljeni sa veterinarima zaduženim za jata kako bi se identifikovali faktori rizika koji su povezani sa cirkulacijom virusa. 1142 seruma, 912 je bilo pozitivno na ELISA testu, sa ukupnom seroprevalencom aMPV od 79,86%. Najviša seroprevalenca zabeležena je u sušnom području – 94,16% tokom zime i 84,82% tokom proleća. Čini se da bioklima i godišnje doba doprinose cirkulaciji aMPV. Isto tako, kao predispozicija su se pokazali visoka gustina i loši higijenski uslovi držanja. Ovde prikazujemo cirkulaciju ptičjeg metapneumovirusa na marokanskim farmama brojlera pod uticajem bioklime, godišnjeg doba, gustine i higijenskih uslova držanja.Ovo je prvi serološki dokaz ptičjeg metapneumovirusa u regionu Magreba.

Ključne reči: ptičji metapneumovirus, brojleri, faktori rizika, seroprevalenca