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Spill-Over of Avian and Human Influenza a Viruses to Swine in Egypt and Lebanon

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Simple Summary

Influenza A viruses circulate between birds, humans, and pigs, creating opportunities for the emergence of new strains with potential public health impact. In avian influenza (AI)-endemic countries such as Egypt and Lebanon, pigs may be repeatedly exposed to viruses from multiple sources, yet systematic swine surveillance remains limited. This study investigated influenza A virus exposure in pigs using both molecular testing to detect active infection and serologic assays to identify prior exposure. Samples were collected from farms, piggeries, and abattoirs between September 2023 and August 2024. Active infection was detected more frequently in Egypt than in Lebanon, including human-origin H1 and avian-origin H5 viruses. Antibody testing revealed broader and more frequent exposure to avian, swine, and human influenza viruses in pigs from both countries. Serologic evidence consistently exceeded molecular detection, indicating that many infections were missed by PCR alone. Exposure patterns varied by housing type and age, with abattoirs representing important high-contact interfaces. These findings show that pigs in avian influenza–endemic settings experience repeated multisource viral exposure, creating conditions that may facilitate viral reassortment. Integrated surveillance combining virologic and serologic approaches is essential to strengthen animal and human health security.

Abstract

Pigs play a key role in the ecology of influenza A viruses (IAVs), particularly in avian influenza (AI)-endemic regions where co-circulation of viruses from different hosts increases reassortment risk. Between September 2023 and August 2024, we surveyed pigs from Lebanon and Egypt to study IAV ecology in AI-endemic countries. Nasal swabs and sera were collected and tested using real-time RT-PCR and hemagglutination inhibition assays against avian, swine, and human seasonal IAVs. Molecular analyses identified IAV-infections in both countries, including human H1 and avian H5 subtypes, which may

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reflect potential cross-species transmission from humans and birds. Serologic analyses revealed prior exposure to avian, swine, and human IAVs. Avian virus seropositivity reached 4.6% (H5N1) and 15.2% (H9N2) in Egypt and 8.6% (H5N1) and 4.3% (H9N2) in Lebanon. Antibodies against human H1N1 and H3N2 were prevalent in both countries. Serologic evidence exceeded molecular detection, indicating frequent past or transient infections not captured by PCR alone. Antibody responses were significantly associated with host-level factors such as housing type, age, shaping exposure risk. These findings demonstrate repeated multisource exposure of pigs to genetically distinct IAVs in AI–endemic countries, supporting the need for integrated virologic and serologic surveillance within a One Health framework.

Keywords: swine influenza a virus; avian influenza; Zoonoses; Reverse Zoonoses; surveillance

1. Introduction

Influenza A virus (IAV) is a zoonotic pathogen capable of crossing species barriers and causing widespread outbreaks in humans and animals. In humans, IAV contributes to annual influenza surges, resulting in considerable morbidity and mortality worldwide. The emergence of novel IAVs from animal reservoirs remains a persistent global health concern, underscoring the need for sustained monitoring of interspecies transmission [1,2].

Among animal hosts, pigs play a pivotal role in the ecology of influenza viruses due to their susceptibility to swine, avian, and human IAVs [3]. Swine influenza is a contagious respiratory disease that regularly causes outbreaks in pig populations, particularly during colder months [4]. Although associated with high morbidity, its mortality rate remains low [5]. Like human and avian IAVs, swine IAVs (SwIAVs) undergo continuous genetic drift and reassortment. Co-infection with multiple influenza viruses in pigs can lead to gene segment exchange, generating novel reassortant strains with zoonotic and pandemic potential [2]. The classical H1N1 (cH1N1), derived from the 1918 human pandemic strain, had circulated endemically in pigs for decades. New lineages, including H3N2 and H1N2, began to appear in swine in the late 1990s, expanding the viral diversity. Currently, three main IAV subtypes (H1N1, H1N2, and H3N2) are established in swine herds globally [4]. In North America, surveillance had identified at least seven distinct clades of H1 and four clusters of H3 viruses co-circulating in swine, complicating vaccine development [6]. In Europe, reassortment between endemic strains and the 2009 pandemic H1N1 virus had expanded the range of genotypes [6]. Likewise, in China, at least five reassortant genotypes of Eurasian avian-like H1N1 viruses had been detected, reflecting the ongoing genetic evolution of IAVs in swine and the continuous risk of cross-species transmission [6].

While the genetic and antigenic diversity of SwIAVs is well-documented in North America, Europe, and Asia, data from other regions remain limited, despite the presence of pig farming in several countries [7]. An overview of the current literature revealed a significant gap in swine influenza surveillance and research in the Middle East, with only a limited number of peer-reviewed studies available, primarily from Egypt. Between 1979 and 1980, swine H1N1 antibodies were detected in pig sera from Egypt [8]. In 2017, molecular and serological analysis of pigs sampled from backyard farms and slaughterhouses in Cairo tested positive for avian H5 and H9 viruses, and the human-origin 2009 pandemic H1N1 virus [9].

In the Middle East, zoonotic avian influenza viruses such as H5N1 and H9N2 are endemic and continue to pose a major threat to animal and public health [10]. H9N2 had circulated endemically in the Middle East and North Africa since the late 1990s [11].

The region's first H5N1 outbreak occurred in 2005–2006, primarily affecting poultry, and had since been reported in several Middle Eastern countries [12–15]. In countries such as Egypt and Lebanon, avian influenza viruses continue to circulate in poultry under heterogeneous control strategies and variable biosecurity practices, creating persistent opportunities for interspecies transmission. In Egypt, highly pathogenic avian influenza (HPAI) H5N1 remains endemic in poultry, with control efforts hindered by suboptimal vaccination strategies and limited preventive measures [16]. H9N2 is also widespread in Egypt, infecting various avian species including chickens, quails, ducks, turkeys, and pigeons in both commercial and backyard sectors [17–19]. In Lebanon, the last outbreak of H5N1 (clade 2.3.2.1c) in poultry was reported in 2016 [20], while H9N2 continues to circulate endemically [21]. In parallel, human seasonal influenza viruses are actively co-circulating in the region, including A(H1N1) pdm09 and A(H3N2) [22]. Despite this, systematic surveillance of influenza A viruses in swine populations remains limited in the region, and existing data are largely restricted to sporadic or geographically narrow studies.

Swine influenza surveillance in the region has relied predominantly on molecular detection of active infection, with limited integration of serologic data capable of capturing cumulative exposure and past spillover events. As a result, key questions remain unresolved: to what extent are pigs in avian influenza–endemic countries exposed to influenza A viruses of avian, swine, and human origin? Furthermore, how do production and slaughter interfaces, host characteristics, and country-specific contexts shape the observed ecology of influenza A viruses in swine?

To address these gaps, we conducted combined virologic and serologic surveillance of pigs in Egypt and Lebanon. By integrating molecular detection of active infection with serologic assessment of prior exposure to avian, swine, and human influenza A viruses, this study aimed to characterize the ecology of IAVs in swine populations.

2. Materials and Methods

2.1. Sample Collection

In Lebanon, 211 nasal swabs and 210 serum samples were collected from pigs in small low-biosecurity farms located in rural areas East and North of the country between October 2023 and March 2024. In Egypt, 535 nasal swabs and 525 serum samples were obtained from pigs at abattoirs and backyards in Cairo between September 2023 and August 2024. Sampling included pigs of different age and sex.

In both countries, nasal swab samples were collected by inserting dacron swabs deep into the nasal cavity and rotating for a few times, then placed in viral transport medium (VTM) consisting of Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Waltham, MA, USA) supplemented with 4% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) and 2% Antibiotic-Antimycotic solution (Thermo Fisher Scientific, Waltham, MA, USA). Sera were collected by obtaining a 3 mL blood sample from the jugular vein in serum vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Samples were kept on ice until receipt in the laboratory on the same day of collection. Blood samples were centrifuged at 1500 rpm for 10 min to separate the sera that were then kept at −20 °C. Swabs were stored at −80 °C.

2.2. PCR Detection of IAV

Viral RNA was extracted from nasal swabs using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Detection of IAV was performed by one-step real-time reverse transcription PCR (rRT-PCR) targeting the matrix (M) gene following standard protocol adapted from the World Health Organization (WHO) guidelines (WHO Manual on Animal Influenza Diagnosis and Surveillance, 2002) [23]

using the Verso 1-Step qRT-PCR kit (Thermo Fisher Scientific, Waltham, MA, USA). Each reaction was performed in a final volume of 25 µL containing 5 µL RNA template, 12.5 µL 2× reaction mix, 0.25 µL enzyme mix, and primers and probe at final concentrations of 10 µM. Positive and negative controls were included in each run, including a no-template control and a known IAV-positive control. Amplification was carried out under the following conditions: reverse transcription at 50 °C for 15 min, initial denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Samples with a cycle threshold (Ct) value below 36.5 were considered positive for IAV.

2.3. Subtype Identification

RNA extracted from the samples was analyzed by rRT-PCR using the AgPath One-Step RT-PCR kit (Applied Biosystems, Waltham, MA, USA), along with specific primers and TaqMan probes (listed in Table 1) targeting the HA and NA genes of H1N1 and H3N2 viruses [24]. Subsequently, a standard RT-PCR was performed for subtyping H9N2 and H5 clade 2.3.4.4b [25,26].

Table 1. List of oligonucleotide primers used for subtyping of avian influenza (AI) H1N1, H3N2, H9N2 and H5 clade 2.3.4.4b viruses.

Primer	Sequence
H1pdm-F	AGTTCAAGCCGGAAATAGCA
H1pdm-R	CCCGGCTCTACTAGTGTCCA
H1pdm-P	FAM-CCCAAAGTGAGGRATCAAGAAGGGAG-BHQ1
H3sw-F	TGATGGAGCAAATTGCACACTG
H3sw-R	CGTTCAATGAAAAGGTCCCATTTC
H3sw-P	FAM-CACAATGAGGGTCCCCTAATAGAGCGTCCA-BHQ1
H5.2344-1673F	TACCAAATAYTGTCAATTTATTCAAC
H5.2344-1749R	GTAAYGACCCRTTRGARCACATCC
H5.2344-1718P	FAM-CTGGCAATCATDRTGGCTGGTCT-BHQ1
BDH9–4F2	CAAGCGTGACAACAGAAAATTTGG
BDH9–2R2	CTCCTGAGAGAACGTGTCCATACC
H9PROB	FAM-CTTACTCGCAATGTCTGGCCTGGTTTTAG-BHQ1
N1.3_F	AGRCCTTGYTTCTGGGTTGA
N1.3_R	ACCGTCTGGCCAAGACCA
AIV_N1.3_FAM	FAM-ATYTGGACYAGTGGGAGCAGCAT-BHQ1
N2_1367F	AGTCTGGTGGACYTCAAAYAG
N2_1468R	TTGCGAAAGCTTATATAGVCATGA
AIV_N2_1444_HEX	HEX-CCATCAGGCCATGAGCCTGWWCCATA-BHQ1

The thermal cycling conditions for H1 and H3 were programmed as follows: an initial reverse transcription step at 50 °C for 15 min, followed by polymerase activation at 95 °C for 10 min. This was followed by 40 amplification cycles consisting of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 60 °C for 30 s.

For H5 and H9, the thermal cycling conditions were as follows: an initial reverse transcription step at 50 °C for 15 min followed by polymerase activation at 95 °C for 10 min. This was followed by 40 amplification cycles of denaturation at 95 °C for 15 s and a combined annealing and extension step at 60 °C for 30 s.

The thermocycling protocol for N1 and N2 consisted of an initial reverse transcription step at 50 °C for 15 min, followed by polymerase activation at 95 °C for 10 min. This was followed by 40 amplification cycles of denaturation at 95 °C for 15 s and a combined annealing and extension step at 60 °C for 30 s.

2.4. Hemagglutination Inhibition (HI) Assay

HI assays were conducted following standard protocol adapted from the WHO guidelines [23] to detect antibodies against avian (A/Muscovy duck/Egypt/RA20838OP/2022 H5N1 clade 2.3.4.4b and A/chicken/Egypt/BA19894OP/2021 G1-like H9N2), swine [A/swine/North Carolina/18161/2002 H1N1 (classical lineage clade 1A.3.3) and A/swine/Texas/4199-2/1998 H3N2 (TRIG lineage)], and human seasonal [A/California/04/2009 H1N1 (applied to Lebanese sera only); A/Egypt/807/2022 H1N1 (applied to Egyptian sera only) and A/Brisbane/07/10 H3N2] IAVs. The selected H9N2 virus is related to viruses detected in both countries and the H5N1 is related to viruses circulating in Egypt and globally. In total, 50 µL of each serum sample were treated with 150 µL receptor-destroying enzyme (RDE) (Denka Seiken, Tokyo, Japan) and incubated overnight at 37 °C, followed by heat inactivation at 56 °C for 30 min. After cooling to room temperature, 300 µL of phosphate-buffered saline (PBS) were added to each sample to reach a volume of 500 µL, resulting in a starting dilution of 1:10. A twofold serial dilution was then performed in 96-well U-bottom plates. Then, 25 µL of sera were incubated with 25 µL of the antigen containing 4 hemagglutination units for 15 min at room temperature. A 0.5% suspension of chicken red blood cells (RBCs) was added to each well and incubated for 45 min at room temperature. Positive sera for antibodies were considered positive if hemagglutination was inhibited. HI titers were determined as the reciprocal of the highest serum dilution that inhibited hemagglutination. An HI antibody titer of 1:80 was considered positive. We opted to use a conservatively high cut-off titer to reduce known limitations of the HI assay, especially potential cross-reactivity between viral species. Positive and negative sera controls were used in all assays.

2.5. Statistical Analysis

Pearson's Chi-square test and Fisher's exact test were used to compare categorical variables. Chi-square test was applied when expected cell counts were sufficient, while Fisher's exact test was used for analyses with small sample size.

Kruskal–Wallis test was used to compare non-normally distributed continuous variables, including HI antibody geometric mean titers (GMTs). GMTs were calculated by multiplying all observations together and taking the nth root of the product where n is the count of observations.

All analyses were performed using IBM® SPSS® Statistics version 23, and a p-value of <0.05 was considered to indicate statistical significance.

3. Results

In Lebanon, IAV was detected in 4 out of 211 collected swab samples, corresponding to an overall positivity rate of 1.9%. As illustrated in Figure 1, two positive samples were identified in October and two in November. All four positive samples were obtained from healthy juvenile pigs, three males and one female. No additional detections were observed outside this two-month period, indicating limited molecular evidence of active IAV circulation during the sampling timeframe. In Egypt, IAV was detected in 217 of 535 swab samples, an overall positivity rate of 40.6%.

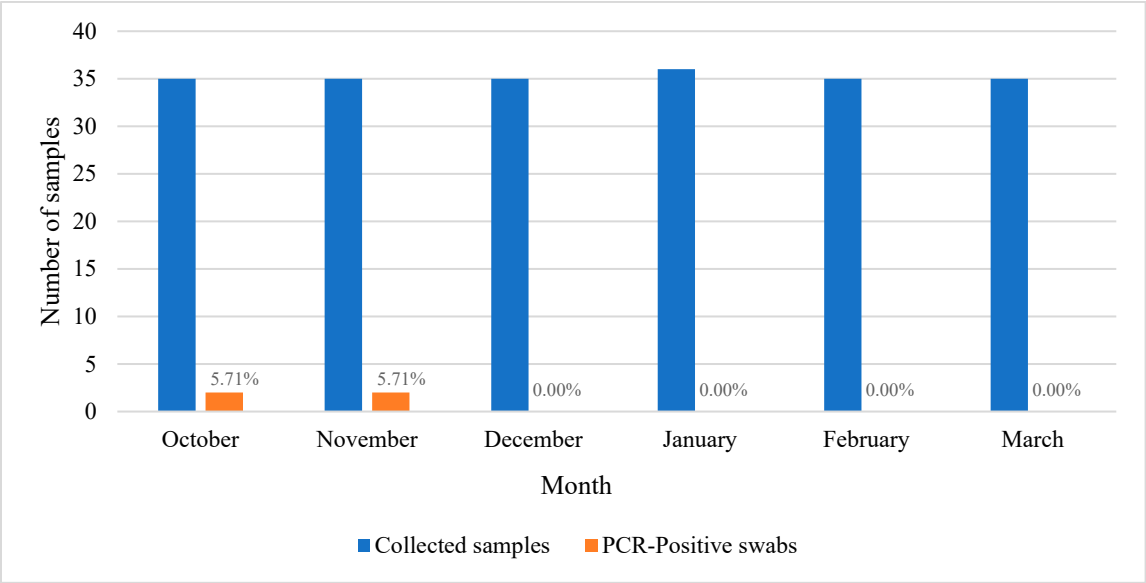

Figure 1. Monthly distribution of collected samples and influenza A PCR-positive swabs in Lebanon.

As shown in Figure 2, positive samples were identified throughout the year, except October, with peak detection in December and January. All positive samples came from healthy adult pigs. Of these, 198 were sampled at abattoirs and 19 on pig farms. Of the 217 positive pigs, 213 were male and 4 were female. Molecular detection was consistently higher in abattoir-sampled pigs compared to farm-sampled pigs, reflecting potential differences in sampling interfaces and exposure risk. During the influenza season, positivity rates were 61.9% in September, 98.5% in December, 55.0% in January, and 70% in March. Notably, substantial IAV detection persisted outside the typical influenza season, with May showing a detection rate of 42.5%. This sustained off-season detection suggests ongoing viral circulation or repeated introductions rather than strictly seasonal transmission dynamics. The difference in the M-gene PCR-positivity rate between Lebanon and Egypt was statistically significant (Pearson's Chi-square test, p-value of <0.05), indicating heterogeneity in virologic detection between the two countries, which may reflect differences in exposure interfaces, surveillance context and conditions, and animal movement patterns, rather than host health status, as all PCR-positive pigs were clinically healthy. However, these differences may also be influenced by variations in sample size and sampling context, including the predominance of abattoir-based sampling in Egypt compared to farm-based sampling in Lebanon.

Table 2 summarizes the epizootiologic data of swine IAV-positive pigs identified in Lebanon and Egypt between September 2023 and August 2024. The data were stratified by housing type, age group, health status, and sex of the sampled pigs, along with the corresponding percentages of influenza A-positive samples within each category. Across both countries, PCR positivity was observed primarily in clinically healthy pigs, indicating that active IAV infection frequently occurred in the absence of other diseases. In both countries, none of the tested host characteristics (age group, health status, or sex) were significantly associated with PCR positivity. This lack of association may suggest that molecular detection of active infection was not strongly driven by intrinsic host factors but may instead reflect exposure at specific interfaces rather than differential susceptibility among host subgroups.

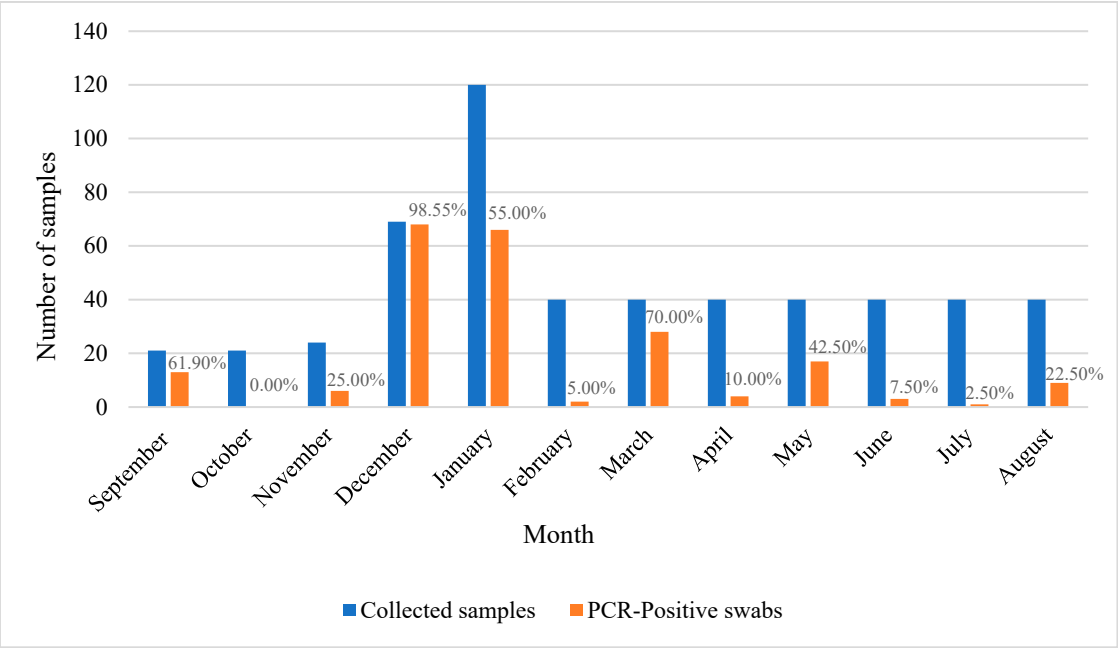

Figure 2. Monthly distribution of collected samples and influenza A PCR-positive swabs in Egypt.

Table 2. Epizootiologic data on swine AIVs (SwIAVs) (all subtypes), Lebanon and Egypt, September 2023–August 2024.

		Collected Samples (Lebanon)		Influenza A-Positive Samples (Lebanon)			Collected Samples (Egypt)		Influenza A-Positive Samples (Egypt)		
Variable	**Category**	**No.**	**(%) ***	**No.**	**(%) ****	***p*-Value**	**No.**	**(%) ***	**No.**	**(%) ****	***p*-Value**
Housing	Farm indoor	211	100	4	1.9	NA	0	0	0	0	N.S.
	Piggery	0	0	0	0		66	12.3	19	28.8	
	Abattoirs	0	0	0	0		469	87.7	198	42.2	
Age group	Juvenile	173	82	4	2.3	N.S.	24	1.7	1	11.1	N.S.
	Adult	38	18	0	0		511	98.3	216	41.1	
Health Status	Healthy	203	96.2	4	2	N.S.	535	100	217	40.6	NA
	Sick	8	3.8	0	0		0	0	0	0	
Sex	Male	102	51.7	3	2.9	N.S.	525	98.1	213	40.6	N.S.
	Female	109	48.3	1	0.9		10	1.9	4	40	

N.S.: Non-Statistically Significant, NA: Not Applicable. * Calculated by dividing number of samples per category by total number of samples collected. ** Calculated by dividing the number of positive samples by number of samples collected per category.

In Lebanon, subtyping by PCR was not conducted due to high Ct values, which were considered insufficient for reliable downstream analysis. This limitation indicates low viral RNA loads at the time of sampling, reflecting limited detectable viral material in the collected samples.

In Egypt, all 217 influenza A PCR-positive samples were subjected to subtyping. In total, 28 samples tested positive for H1pdm09 (including one in November, 17 in December, and 10 in January), of which 10 were subtyped as N1. Three samples were subtyped as H5 although their neuraminidase subtype was not determined. Additionally, seven samples tested positive for N1 and three for N2. The detection of both avian-origin (H5) and human-origin (H1pdm09) markers in swine suggests potential multisource viral exposure at the human–animal interface. H9 and H3 subtypes were not detected.

HI testing of pig serum samples from Lebanon and Egypt revealed the presence of antibodies against all tested avian, swine, and human viruses. In contrast to the limited molecular detection observed in Lebanon, serologic analysis demonstrated measurable

prior exposure to multiple influenza A virus lineages. In Lebanon, the highest antibody titers were observed against human seasonal H1N1 and H3N2. Seropositivity percentages (based on a cutoff of 1:80) were: avH5N1 (8.57%), avH9N2 (4.28%), swH1N1 (2.38%), swH3N2 (1.42%), huH1N1 (14.28%), and huH3N2 (43.33%) (Figure 3A, Table 3). Similarly in Egypt, the highest titers were observed for huH1N1 and huH3N2. Seropositivity rates (based on a cutoff value of 1:80) were: avH5N1 (4.60%), avH9N2 (15.20%), swH1N1 (30.28%), swH3N2 (3.23%), huH1N1 (59.8%), and huH3N2 (28.38%) (Figure 3B, Table 3).

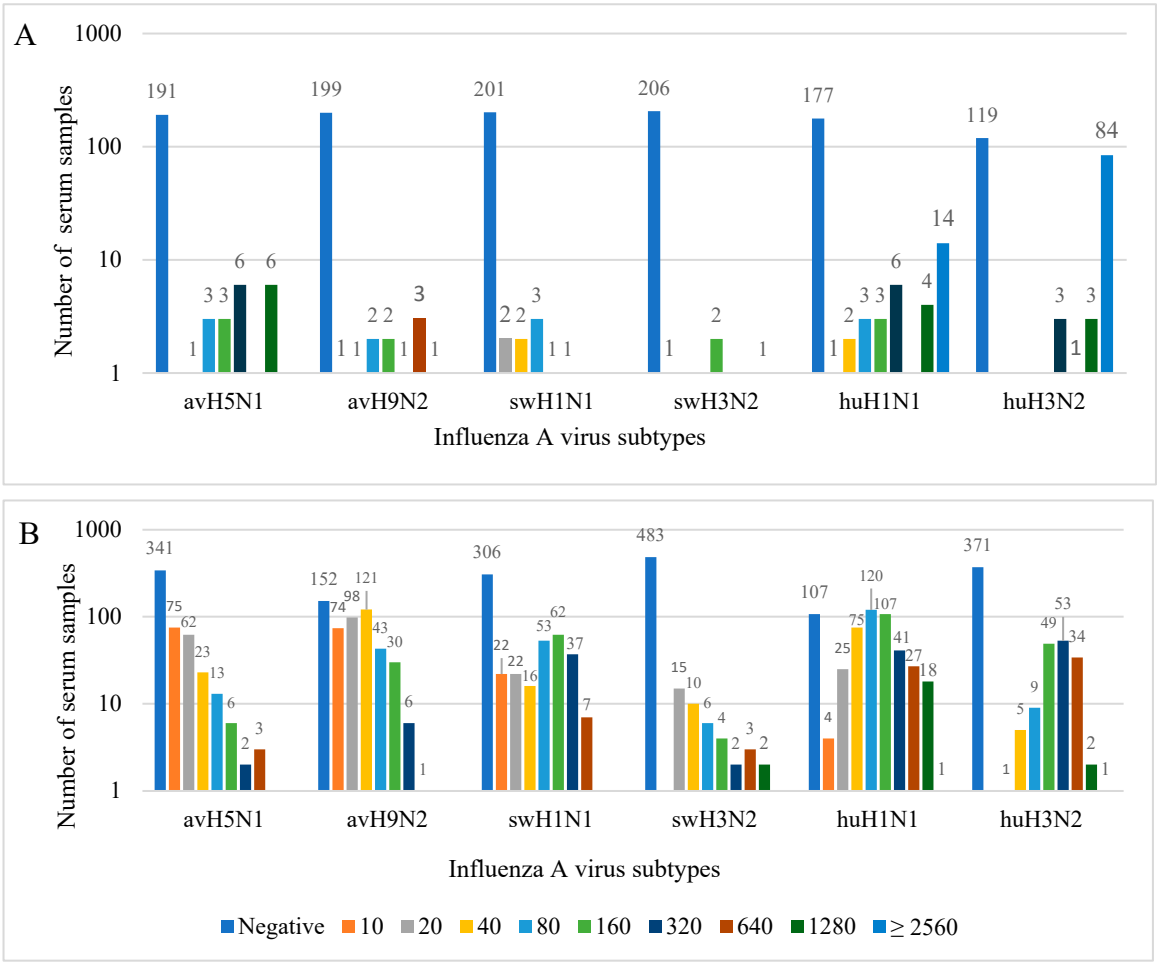

Figure 3. Distribution of antibody titers for avian (H5N1 and H9N2), swine (H1N1 and H3N2), and human seasonal (H1N1 and H3N2) IAV for (**A**) Lebanon and (**B**) Egypt.

Across both countries, seroprevalence for human-origin influenza viruses suggested a potential contribution of reverse zoonotic transmission to swine influenza exposure in these settings.

A cross-country comparison of serologic responses revealed higher HI GMTs in pigs from Egypt compared to Lebanon for nearly all tested antigens. This pattern may indicate differences in cumulative exposure to influenza A viruses, although part of this variation may also be influenced by differences in sampling context, sample size, and antigen selection between the two countries.

For avian strains, GMTs for H5N1 and H9N2 were approximately 1.4-fold and 3-fold higher in Egypt (10.49 and 18.67) than in Lebanon (7.28 and 6.05), respectively. These differences suggested more frequent or sustained avian influenza virus exposure among pigs in Egypt. The swine-origin H1N1 GMT in Egypt (16.56) was nearly 3-fold greater than that in Lebanon (5.57), while swH3N2 showed a minor difference, with values of

6.12 and 5.34, respectively. The relatively modest swH3N2 difference contrasted with the pronounced disparities observed for other antigens, indicating heterogeneity in lineage-specific exposure between countries. A notable difference was observed for the human seasonal pdm09 H1N1, with GMT in Egypt reaching approximately 5-fold higher (60.01) than in Lebanon (11.61), which may be influenced by differences in antigen selection between countries, as different H1N1 strains were used in the HI assays, and may also reflect differences in exposure to human-origin influenza viruses. Conversely, an opposite pattern was identified for huH3N2, where GMTs in Lebanon were nearly 9.5-fold greater (150.90) than in Egypt (15.92), which may reflect differences in circulating strains or exposure patterns between countries (Table 3).

Table 3. Cross-country comparison of seroprevalence to avian, swine and human seasonal IAV in pigs from Lebanon and Egypt.

	Lebanon			Egypt				
Antigen	**No.**	**GMT**	**% Positive (80)**	**No.**	**GMT**	**% Positive (80)**	**Kruskal–Wallis**	**Pearson Chi-Square**
A/Muscovy duck/Egypt/RA20838OP/2022 H5N1 HI	210	7.28	8.6	525	10.49	4.6	<0.0001	0.035
A/chicken/Egypt/BA19894OP/2021 H9N2 HI	210	6.05	4.3	525	18.67	15.2	<0.0001	<0.0001
A/swine/North Carolina/18161/2002 H1N1 HI	210	5.57	2.4	525	16.56	30.3	<0.0001	<0.0001
A/swine/Texas/4199-2/1998 H3N2 HI	210	5.34	1.4	525	6.12	3.2	<0.0001	<0.0001
Human seasonal pdm09 H1N1 ‡ HI	210	11.61	14.3	525	60.01	59.8	<0.0001	<0.0001
A/Brisbane/07/10 H3N2 HI	210	150.90	43.3	524	15.92	28.2	<0.0001	<0.0001

Kruskal–Wallis was used to compare GMTs; Pearson Chi-square was used to compare % positive. ‡ Human seasonal pdm09 H1N1 denotes A/California/04/2009 H1N1 for Lebanon and A/Egypt/807/2022 H1N1 for Egypt.

Table 4 summarizes the seroepidemiologic findings of IAVs in pigs sampled in Lebanon and Egypt between September 2023 and August 2024 by HI assay. The data are stratified by viral antigen and host characteristics.

Statistically significant differences in serologic responses were associated with housing type and age group. These associations indicate that cumulative influenza A virus exposure in pigs may be shaped by both environmental interfaces and host demographic factors. In Egypt, pigs from piggeries had higher seropositivity to avH9N2 HI (60.6%) and avH5N1 HI (15.2%), while those sampled from abattoirs showed elevated responses to swH1N1 HI (34.4%) and huH1N1 HI (64.1%). This pattern suggested differential exposure pathways at distinct production and slaughter interfaces, with avian-origin exposure more prominent in piggeries while swine- and human-origin exposure were more pronounced in abattoirs. Age-related differences were also significant. In Egypt, adults exhibited higher seropositivity for huH1N1 HI (60.7%) compared to younger age groups, while in Lebanon, adults displayed significantly higher antibody responses to huH1N1 HI (43.2%) and huH3N2 HI (37.8%).

Table 4. Seroepidemiologic data of IAVs in pigs from Lebanon and Egypt (September 2023–August 2024), by antigen and host factors.

Variable			Collected Samples (Lebanon)	Serology-Positive Samples (Lebanon)			Collected Samples (Egypt)	Serology-Positive Samples (Egypt)		
Antigen	**Host Characteristics**	**Stratum**	**No.**	**No.**	**(%) ***	***p*-Value**	**No.**	**No.**	**(%) ***	***p*-Value**
A/Muscovy duck/Egypt/RA20838OP/2022 H5N1	Housing	Farm indoor Piggery Abattoirs	210 0 0	18 0 0	100 0 0	NA	0 66 459	0 10 14	0 15.2 3.1	<0.0001
	Age group	Juvenile Adult	173 37	15 3	8.7 8.1	N.S.	9 516	0 24	0 100	NA
	Health Status	Healthy Sick	202 8	19 0	100 0	NA	525 0	24 0	100 0	NA
	Sex	Male Female	102 108	10 8	9.8 7.4	N.S.	515 10	20 4	3.9 40	<0.0001
A/chicken/Egypt/BA19894OP/2021 H9N2	Housing	Farm indoor Piggery Abattoirs	210 0 0	9 0 0	100 0 0	NA	0 66 459	0 40 40	0 60.6 8.7	<0.0001
	Age group	Juvenile Adult	173 37	9 0	5.2 0	N.S.	9 516	2 78	22.2 15.1	N.S.
	Health Status	Healthy Sick	202 8	9 0	100 0	NA	525 0	80 0	100 0	NA
	Sex	Male Female	102 108	6 3	5.9 2.8	N.S.	515 10	75 5	14.6 50	0.01
A/swine/North Carolina/18161/2002H1N1	Housing	Farm indoor Piggery Abattoirs	210 0 0	5 0 0	100 0 0	NA	0 66 459	0 1 158	0 1.5 34.4	<0.0001
	Age group	Juvenile Adult	173 37	5 0	100 0	NA	9 516	0 159	0 100	NA
	Health Status	Healthy Sick	202 8	5 0	100 0	NA	525 0	159 0	100 0	NA
	Sex	Male Female	102 108	4 1	3.9 0.9	N.S.	515 10	159 0	100 0	NA

Table 4. *Cont.*

Variable			Collected Samples (Lebanon)	Serology-Positive Samples (Lebanon)			Collected Samples (Egypt)	Serology-Positive Samples (Egypt)		
Antigen	**Host Characteristics**	**Stratum**	**No.**	**No.**	**(%) ***	***p*-Value**	**No.**	**No.**	**(%) ***	***p*-Value**
A/swine/Texas/4199-2/1998 H3N2	Housing	Farm indoor	210	3	100	NA	0	0	0	NA
		Piggery	0	0	0		66	0	0	
		Abattoirs	0	0	0		459	17	100	
	Age group	Juvenile	173	2	1.2	N.S.	9	0	0	NA
		Adult	37	1	2.7		516	17	100	
	Health Status	Healthy	202	3	100	NA	525	17	100	NA
		Sick	8	0	0		0	0	0	
	Sex	Male	102	2	2	N.S.	515	17	100	NA
		Female	108	1	0.9		10	0	0	
huH1N1‡ HI	Housing	Farm indoor	210	30	100	NA	0	0	0	<0.0001
		Piggery	0	0	0		66	20	30.3	
		Abattoirs	0	0	0		459	294	64.1	
	Age group	Juvenile	173	14	8.1	<0.0001	9	1	14.1	<0.0001
		Adult	37	16	43.2		516	313	60.7	
	Health Status	Healthy	202	30	100	NA	525	314	100	NA
		Sick	8	0	0		0	0	0	
	Sex	Male	102	15	14.7	N.S.	515	305	59.2	N.S.
		Female	108	15	13.9		10	9	90	
A/Brisbane/07/10 H3N2	Housing	Farm indoor	210	91	100	NA	0	0	0	NA
		Piggery	0	0	0		66	0	0	
		Abattoirs	0	0	0		459	100	100	
	Age group	Juvenile	173	77	44.5	N.S.	9	0	0	NA
		Adult	37	14	37.8		516	147	100	
	Health Status	Healthy	202	89	44.1	N.S.	525	147	100	NA
		Sick	8	2	25		0	0	0	
	Sex	Male	102	43	42.2	N.S.	515	147	100	NA
		Female	108	48	44.4		10	0	0	

N.S.: Non-Statistically Significant, NA: Not Applicable. ‡ Human seasonal pdm09 H1N1 denotes A/California/04/2009 H1N1 for Lebanon and A/Egypt/807/2022 H1N1 for Egypt.
* Calculated by dividing the number of positive samples by number of samples collected per category.

4. Discussion

This study presented data on the ecology of IAVs in pigs raised in avian influenza–endemic settings, underscoring the potential zoonotic and epidemiological risks associated with their circulation. By integrating molecular and serologic evidence, our findings revealed a clear disconnect between active infection detected by PCR and cumulative exposure captured through antibody responses. Antibodies against avian H5N1 and H9N2 viruses were detected in swine from both countries. In Lebanon, moderate seropositivity levels were observed for H9N2, despite limited PCR detection. This mismatch suggested that avian-origin IAV exposure in pigs may be episodic or transient and therefore underrepresented by virologic surveillance alone. Given the pig's susceptibility to both avian and human IAVs, such patterns of undetected exposure reinforce concerns regarding their role as a potential "mixing vessel" capable of facilitating viral adaptation and reassortment, raising the risk of emergence of novel zoonotic or pandemic strains [27]. In avian influenza–endemic settings, this role may be amplified by repeated exposure rather than sustained circulation of a single lineage. Such a role may be facilitated by avian-to-swine spillovers, potentially driven by shared environments and low biosecurity measures, as was the case in our sampling sites.

The marked disparity in PCR positivity between countries (1.9% in Lebanon versus 40.6% in Egypt) is likely reflected differences in sampling context, pig density, animal movement patterns, and surveillance intensity. These differences occurred despite all PCR-positive pigs being clinically healthy, suggesting that detection was driven by exposure interfaces rather than health status. In Egypt, the elevated detection rate, especially for the human H1N1 virus, may be attributed to the sampling of pigs at high-risk interfaces such as abattoirs, which represent convergence points for animals from multiple sources and increased opportunities for viral introduction and amplification through exposure to infected birds or contaminated environments in pigs held for processing. The increase in detection observed during December and January may also be linked to heightened slaughtering activities around the Christmas and New Year season. Such periods of intensified animal movement and congregation are known to increase the probability of influenza virus transmission.

In Egypt, subtyping of PCR-positive samples revealed a diverse range of circulating viruses, including H1, H5, N1, N2, and pandemic H1N1 (pdm09-like) strains. This diversity indicated exposure of swine populations to multiple IAVs lineages originating from both avian and human reservoirs. The detection of avian-origin H5 and human-origin pdm09 H1 viruses in pigs was particularly concerning, as it reflected exposure to genetically distinct lineages with zoonotic potential. Such concurrent exposure creates ecological conditions favorable for reassortment, even if active co-infection is not directly observed. Subtyping could not be performed for Lebanon due to high Ct values, limiting insights into the circulating strains. Additionally, the absence of H3 and H9 detection by PCR in Egypt, despite detectable H9N2 seropositivity, suggested temporal variation in virus activity or low viral shedding at the time of sampling.

Serologic analyses demonstrated exposure to a broad range of IAVs in both countries. The last reported detection of H5 in Lebanon was in 2016, but the presence of antibodies against clade 2.3.4.4 H5 viruses in pig sera [28] suggests that H5 may have been introduced but not detected. Such silent exposure events may therefore remain unrecognized in the absence of integrated serologic surveillance. Lebanon's wetlands are stopping grounds for migratory birds that potentially introduced H5N1 into the country. Although the sampling sites in Lebanon are not close to those wetlands, virus transmission could have likely occurred via resident wild birds that have access to the farms. In Egypt, high antibody prevalence across avian, swine, and human IAVs aligned with ongoing viral

circulation. H5N1 and H9N2 viruses are endemic and continuously circulate in poultry. Pigs contact with poultry in piggeries is the likely route of transmission rather than contact with migratory birds. The detection of antibodies to human H1N1 and H3N2 viruses in swine, from both countries, indicated potential reverse zoonotic transmission, as observed in North America, Europe, and China [24,29–32]. It is important to note that pigs in both countries do not receive any influenza vaccines; thus, our findings are related to virus spillover or transmission.

Host-level factors were found to significantly influence antibody responses in both countries. These findings indicated that cumulative influenza A virus exposure in swine is shaped not only by viral circulation but also by the environments in which pigs are housed and processed. Housing emerged as a strong determinant of exposure risk, particularly in Egypt, where pigs in abattoirs consistently exhibited broader and higher serologic responses across multiple antigens compared to those from piggeries. Abattoirs represented high-contact interfaces where animals from multiple sources converge, co-housed in tight housing conditions for few days, and where biosecurity is low, allowing for virus transmission and amplification to occur within abattoirs. Increase in influenza virus detection in abattoirs have been previously documented with studies in Brazil and Kenya reporting asymptomatic infections and sustained circulation of IAVs among pigs, underscoring the potential for silent viral maintenance and interspecies transmission at the animal–human interface [33,34]. Our findings were consistent with these observations and suggested that slaughter-associated interfaces may act as epidemiologically important amplification points. Previous research further indicated that IAV transmission in pigs accelerated upon leaving the farm during transport and within slaughter houses [35,36], hence our findings most likely reflect this scenario rather than viral infection that the pigs brought from the farm. Age also played a notable role, with adult pigs demonstrating higher antibody titers than juveniles across most tested antigens, likely reflecting cumulative exposure over time. Sex-based differences were apparent with female pigs demonstrating stronger antibody responses for several antigens, suggesting that immunological or behavioral factors may modulate susceptibility to infection [37].

This study has several limitations. Sampling periods differed between countries, with pigs in Lebanon sampled over a six-month timeframe, compared to a full year of surveillance in Egypt, potentially influencing seasonal representation and serologic patterns. However, this difference reflects real-world surveillance constraints and does not negate the comparative insights gained from integrated virologic and serologic analysis. Additionally, the sampling site types varied, pigs in Lebanon were exclusively sampled from indoor farms, whereas those in Egypt were collected from a broader range of settings, including piggeries and abattoirs, potentially introducing differences in exposure risk especially in abattoirs where virus amplification may be occurring. These differences in sampling context likely contributed to the observed heterogeneity in exposure profiles and highlighted the importance of interface-specific surveillance when interpreting swine influenza dynamics. The sampled cohort predominantly comprised healthy pigs, with very limited representation of visibly sick ones, which may underestimate associations between infection and health status. Additionally, obtaining symptom histories from farmers or obtaining post-mortem tissue samples from abattoir pigs may have provided more insights relevant to this study. Nonetheless, the detection of widespread serologic evidence of exposure among asymptomatic pigs underscored the potential for silent influenza A virus circulation in swine populations. The number of pigs sampled and the duration of sampling were limited potentially constraining the understanding of the full scope of IAV transmission dynamics among pigs in study countries. Despite these constraints, the study

provided valuable baseline data from avian influenza–endemic settings where systematic swine surveillance remains scarce.

These findings highlighted the critical importance of sustained influenza surveillance in swine populations, particularly in high-risk countries where other animal influenza viruses are endemic. By demonstrating a consistent mismatch between molecular detection and cumulative serologic exposure, this study showed that reliance on PCR-based surveillance alone is insufficient to capture the true ecology of influenza A viruses in pigs. The serologic and molecular evidence of co-circulation of avian, swine, and human IAVs supported the need for a One Health framework to monitor interspecies transmission and prevent potential emergence of reassortant viruses. Such frameworks must explicitly account for reverse zoonotic transmission and interface-driven exposure dynamics. Surveillance should prioritize human–animal interface and mixed-species settings and include both serologic and virologic testing to detect active and past infections. Together, these approaches are essential for improving early detection of zoonotic threats in avian influenza–endemic countries.

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Abbreviations

The following abbreviations are used in this manuscript:

AI	Avian Influenza
IAVs	Influenza A viruses
SwIAVs	Swine IAVs
cH1N1	classical H1N1
HPAI	Highly Pathogenic Avian Influenza
VTM	Viral Transport Medium
DMEM	Dulbecco's Modified Eagle Medium
BSA	Bovine Serum Albumin
rRT-PCR	real-time reverse transcription PCR
RDE	Receptor-Destroying Enzyme
PBS	Phosphate-Buffered Saline
RBCs	Red Blood Cells
GMTs	Geometric Mean Titers

HI	Haemagglutination Inhibition
WHO	World Health Organization

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