

INVESTIGATION OF SWINE INFLUENZA SUB-TYPES H1N1, H3N2, N1N2 IN PIGS POPULATION IN ISRAEL (2002-2009)

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ABSTRACT

The recent emergence of H1N1 influenza in humans generated concerns about cross-species infections between humans and swine, and the potential amplifying role of infected and densely populated pigs units. In Israel there are 24 swine breeding units, mainly localized in Northern Region and a single unit in the South; these units produce about 200,000 slaughtered pigs per year. While in the past, data were collected and examined for various swine respiratory pathogens, no data has been examined for the presence of influenza virus in swine populations in Israel.

This work retrospectively examines and summarizes the epidemiological data for influenza viruses subtypes H1N1; H3N2; H1N2 in Israeli swine population from 2002 to the present and describes the methods used for serological and virological determinations. 306 blood samples and 40 organs samples from 31 samplings out of 16 swine units were found to be negative to sub types H1N1; H3N2; H1N2 influenza virus. The consistency of samplings used allowed us to conclude that swine populations in Israel are negative to sub-type H1N1; H3N2; H1N2 influenza virus. Particular susceptibility of naïve swine populations to influenza viruses suggests that vaccination of workers involved in the pig industry is indicated in order to decrease the risk of cross-infection and the possibility of reassortant strain development.

INTRODUCTION

Influenza virus is an enveloped RNA virus, belonging to Orthomyxoviridae family (1), able to infect among others, humans, swine and avian species (2). Aquatic birds are considered its natural host (3). It is cause of annual epidemics and occasional pandemics that have caused millions of fatalities among humans (4).

Influenza viruses are classified by type (A, B, C), according to their matrix; by their nucleoproteins structural differences; by subtypes, according to changes in their hemagglutinin (H) and / or neuraminidase (N) surface's glycoproteins and by geno-types, according to their RNA genetic sequence (1). Hemagglutinins mediate infection of host cells by binding to sialic acid receptors on the cell surface, while neuraminidase prevents virions aggregating by cleavage of sialic acid from its sugar residues.

Among the types, influenza A viruses are able to infect between species and occasionally establishing a stable lineage in the infected species, which become a new host (3). Sixteen different H and 9 different N subtypes are known, the combinations which defines influenza virus sub-types, all of them have been recovered from aquatic birds (1) which shed the virus in the feces and contaminate water (5).

Swine have receptors for mammalian and avian influenza viruses, thus potentially increasing the exchange of genetic sequences and the production of new reassortant viruses (2).

Following interspecies transmission to pigs, some influenza viruses may result in genetic instability and initiate variants which could again breach the species barrier

In the last century, the emergence of antigenically different strains in human influenza viruses, has been documented in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1), each time resulting in a pandemic (6).

H1N1 remains the classical sub-type involved in swine influenza outbreaks since the '30s (7) to the '80s when a H1N1 "avian-like" virus, genetically (9) and antigenically (1) distinguishable from the original one begun to diffuse rapidly in swine populations.

In the 1970, a H3N2 "human like" sub-type was recovered in swine as a consequence of the human "Hong Kong" pandemic (9) and the first wholly human-like H3N2 (Hong Kong/68-like) virus was isolated from pigs in Taiwan (8)

In the 1980's, as a consequence of genetic reassortant between "human-like" H3N2 and "avian-like" H1N1, a new H3N2 genotype was demonstrated in swine (1, 9).

Sub-type H1N2 started appearing in the United Kingdom and Italy in swine in the '90s (9) which resulted from reassortant of H1N1 "avian-like", H1N1 "human-like" and H3N2 "human-like" (1, 9, 10).

Today, H1N1, H3N2, H1N2 sub-types and their antigenic and

genetically diverse strains, are circulating in swine populations throughout the world (2, 11) and are a cause of concern to the swine industry because of potential losses (2,3). Furthermore, the recent emergence of H1N1 influenza in humans has generated concerns relative to cross-species infections between humans and swine (12), and the amplifying role of infected confined, densely populated, pigs units (13).

The purpose of this work is to summarize the epidemiological data relative to the spread of influenza virus type A in the Israeli swine population.

MATERIALS AND METHODS

Israeli swine population.

In Israel, about 200,000 pigs are produced per year, from 24 swine breeding units. One unit is in the Southern Region and 23 in the Northern Region, of which 13 are concentrated in a relatively small area, representing a unique epidemiological entity. Countries on Israel's borders have a small to minimal swine population which has largely been recently eliminated it due to the emotional reaction generated by the recent H1N1 epidemic (Palestinian Authority eliminated 400 heads; Jordan 800; Egypt is eliminating 300,000). Furthermore, the extensive distance from Egypt and small populations in Jordan have contributed in keeping Israeli swine population rather isolated, even if rare contacts may have taken place with the local wild boar population.

Samples:

Over 7 years, from 2002 and 2009, 306 blood samples and 40 samples from internal organs (lungs, lymph nodes) (16) were collected in 31 samplings from 16 herds in northern and southern regions of Israel

Following restraint of pigs using a hog-snare, blood samples were collected from the jugular vein using "vacutainer" vials without anti-coagulant and a new needle for every subject. Samples sera were obtained by centrifugation performed at "Kimron Veterinary Institute" in Bet Dagan, Israel; then, frozen sera were shipped to IZSLER Animal Health Institute in Brescia (I) for serological investigation against Swine Influenza Viruses (SIV). Table 1 shows the number of herds sampled per region.

Table 1: Date (year) of collection and location

Year	Farms	Location	Samples examined
2002	12	11 North - 1 South	129
2005	2	North	40
2007	2	North	18
2007	1	North	5 organs
2008	4	3 North - 1 South	66
2008	3+1	North	10 + 5 organs
2008	4	3+1 North - 1 South	20 organs
2009	2	1 North - 1 South	53
Total samplings	31	Total samples	306 + 40 organs

Haemo-Agglutination-Inhibition test (HI)

Sera were examined by the haemo-agglutination-inhibition test (HI) against H1N1, H3N2 and H1N2 sub-types viruses. A pig was considered positive when it had an HI antibody titre of $\geq 1:20$. A herd was considered positive when at least 1 pig had an HI antibody titre $\geq 1:40$ or at least 2 pigs had an HI antibody titre of $\geq 1:20$. Positive and a negative reference controls were included in all the tests. Experimental infection trials in pigs have demonstrated that serological cross-reaction between European swine influenza virus sub-types in the HI test is extremely rare and the test is suitable to discriminate between H1N1; H3N2; H1N2 viruses. (17)

Influenza virus isolation

Samples from internal organs were submitted for SIV isolation, according to the techniques in use at IZSLER, Brescia. Samples were screened using the gene-matrix (M) Real Time RT-PCR technique to detect viral genomes of the type A influenza virus (18). Virus isolation methods included inoculating cell line monolayers cultures MDCK (Madin-Darby Canine Kidney) and inoculating allantoic fluid of four 10-11 day-old chicken embryonated eggs. Subsequently, the allantoic fluid and the cell supernatants were analysed with the haemagglutination (HA) test to evaluate haemagglutinating activity of the virus (19) as well as double ELISA sandwich enzyme immunoassay with monoclonal antibodies (Mab) anti-NPA (ATCC n. HB65 H16-L10-4R5) carried out as previously described by Siebinga and de Boer, 1988 (20). The latter was used to identify the viral subtype using HI and neuraminidase (NI) inhibition tests. When necessary, the viral strain was then subtyped with the aid of 4 RT-PCR which used 8 specific primers to amplify the portion of the genes coded for H1, H3, N1 and N2 (21). When several antigen detections were requested (16), samples were fractionated and every fraction destined to a specific PCR reaction test.

RESULTS

Blood and organ sampling schedule and laboratory investigation results are illustrated in Table 2 and 3, respectively

Table 2: Blood samples: date (year) of collection; number of samplings; total samples from each Region and results

Year	sampling	Blood Samples examined			
		North Region	South Region	Total	Positive
2002	12	108	21	129	0
2005	2	40	0	40	0
2007	2	18	0	18	0
2008	4	18	48	66	0
2009	4	14	19	33	1
2009	2	0	20	20	0
Total	26	198	108	306	1

Table 3: Organ samples: date (year) of collection, total samples from each Region and results

Year	Organ Samples examined			
	North Region	South Region	Total	Positive
2007	5	0	5	0
2008*	16	4	20*	0
2008*	15	0	15*	0
Total	36	4	40*	0

Out of 306 sera samples tested, only one resulted positive at HI H1N1 antibody titre of 1:20 from one sampling (May 2009) from a farm in the southern region. The positive sera was not tested again, but taking into account its positivity at the lowest detectable level and that further samplings in the southern region (September 2009) were completely negative, we can assume the first result was a false positive. All other samples were negative to the three subtypes examined.

Samples from internal organs submitted for virological investigations for SIV detection, were all negative (16; partial results published).

DISCUSSION AND CONCLUSIONS

Serology and virology negative results in all samples in 31 samplings from 16 herds out of 24 total herds in Israel, allow us to conclude that H1N1, H3N2 and H1N2 viruses are not widespread in any region of Israel. The sampling size was enough to detect at least 1 positive sample in a population of 3,000 pigs, at an expected prevalence between 5% (at 90% confidence level) and 10% (at 95% confidence level) (22). Other seroprevalence studies in areas in which animals were not vaccinated against influenza viruses have been carried out with a lower number of blood sera from each herd (22)

Considering SIV is characterized by high morbidity (up to 100%) among animals of all ages in a very short period of time (1), the sampling used and the results obtained may be considered enough to assess Israeli swine population as negative for SIV. If SIV were present, like in densely swine populated regions of Europe, the seroprevalence would have been high with seropositive herds widely distributed (24). This concept also assists us in considering the above mentioned sole positive sample of 1:20, as a false positive result.

Two main risks can be forecasted in swine population negative to SIV related to swine and human health: In pig population SIV is mainly represented by acute respiratory disease in susceptible, seronegative pigs, from nursery to fattening units. In such a situation, complications with secondary bacterial infections (*P. multocida*, *A. pleuropneumoniae*, *M. hyopneumoniae*) (15) or presence of dual infection (Porcine Circovirus type 2; Porcine Coronavirus) (16) may enhance disease severity and increase losses. Occasionally reproductive signs, such as decreased fertility, abortions, stillbirths, and small and weak litters may be observed.

Recently the H1N1 strain has become an important issue between swine and human health, with concerns relative to exposure and susceptibility to human influenza viruses by workers in the swine sector (24) with possible generation of a novel reassortant influenza virus strain. While potential losses in production in swine industry are of interest to farmers and an accurate cost-benefit evaluation should be done in order to take into account stock vaccination, the zoonotic aspect of potential generation of new virus must be also considered. Studies in the USA demonstrated an increased "odd ratio" for influenza in adults occupationally in contact with swine (26) but also considered the "bridging role" of man in cross-species sharing and his role in introducing human influenza in poultry and swine (24, 27). On the other hand, confined animal operations can serve as amplifiers of (potentially novel) influenza virus (28).

Taking these issues into account, the swine and poultry industry in the USA has recommend the introduction of (swine and poultry) workers to vaccination schemes against pandemic influenza, aimed at limiting the "bridging role" of workers in introducing new influenza virus in susceptible animal population and re-exporting novel viruses to human population. We believe that same measures should be considered about personnel involved in swine industry in Israel.

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