

# Anatomical spatial distribution of Influenza virus receptors in some poultry species raised in Egypt

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## ABSTRACT

**Background:** Avian influenza H5N1 has been distressing not only the poultry industry but also humans causing fatal infections in Egypt. Understanding the initial steps in the viral infection was proposed by many to be a key for solving the entire problem. Domestic healthy chicken, Pekin duck, Egyptian goose, Japanese quail, pigeon and turkey were purchased; three adult birds per each species. Lectin histochemistry was performed using fluorescein isothiocyanate labelled Sambucusnigra agglutinin specific for SA $\alpha$ 2,6-gal receptors, and FITC labelled Maackiaamurensis agglutinins specific for SA $\alpha$ 2,3-gal receptors. **Methods:** From each bird, three specimens per each trachea, lung, duodenum, colon, liver and brain were used. In chicken, duck, goose, Japanese quail, domestic pigeon and turkey, both SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors were expressed in at least one segment of respiratory and intestinal tracts except in pigeons where SA $\alpha$ 2,3-gal receptors were not expressed in the respiratory tract while in ducks were not expressed in lower respiratory tract and in turkey not expressed in small intestine. The human type receptors were not expressed in the lower trachea of goose, large intestine of chicken and intestinal tract and liver of turkey and pigeons. **Results:** The widespread detection of both SA $\alpha$ 2,6-gal and SA $\alpha$ 2,3-gal receptors in different tissues from each species suggests that these birds' organs may be potential targets for both avian and human influenza viruses, and can act as adaptive host for avian influenza viruses to change receptor specificity. This may indicate that different native bird species in Egypt could have participated equally or variably in the generation of H5N1 viruses that were able to extensively infect humans. All experimental procedures were approved by Damanshour university ethics committee. The widespread detection of both SA $\alpha$ 2,6-gal and SA $\alpha$ 2,3-gal receptors in different tissues from each species suggests that these birds' organs may be potential targets for both avian and human influenza viruses, and can act as adaptive host for avian influenza viruses to change receptor specificity. **Conclusion:** This may indicate that different native bird species in Egypt could have participated equally or variably in the generation of H5N1 viruses that were able to extensively infect humans.

**Keywords:** Influenza, Virus, Receptor, Egypt, poultry

## INTRODUCTION

Avian influenza has been distressing the poultry industry in Egypt ages ago. However, the highly pathogenic avian influenza H5N1 virus has been the one that massively affected this industry since 2006 (Aly et al., 2008). Despite the different regimes adopted by the Egyptian government to control this viral

infection, the virus became endemic in Egypt by 2008

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(OIE, 2008; FAO, 2013). Further, H5N1 avian influenza has been infecting humans in Egypt since its start in 2006 but strikingly fatal in 2015 with 39 deaths out of 136 infected cases (WHO, 2015). Adaptation of avian influenza viruses to humans has been the main concern regarding the pandemic threat to human health. And in Egypt, the increasing percentage of death among infected human cases raises many concerns regarding the species-crossing capabilities of avian influenza viruses in the country. Birds are reared in Egypt together in one place and in close proximity to human as well. This increases the chances of transfer of viral infections among different birds and humans. Adding to that the abundance of live bird markets all over the country that facilitates the mixing between birds and direct handling by man. Previous studies on the epidemiology of H5N1 infection in domestic birds in Egypt reported infections in backyard reared and farm reared chickens, ducks, turkeys and geese since 2006 (Hafez et al., 2010). The prevalence was more in mixed waterfowls and chickens than turkeys. Further, there was no pigeon infections early 2006-2007 even though the samples were from dead or clinically ill pigeons from the vicinity of infected poultry (Aly et al., 2007). Only till 2011, when Kayali et al., (2011) reported only 1 pigeon swab sample to be positive. On the contrary, ducks are known hosts of influenza viruses and once infected are able to asymptotically excrete quantities of the virus, thus acting as a silent reservoir facilitating transmission to other birds (Olsen et al., 2006). Watanabe et al., (2011a) reported that ducks are a possible reservoir for the emergence of H5N1 outbreaks, spread to domestic poultry and humans, and the ongoing epidemics in Egypt. Influenza viruses attach to host cells through the interaction of the viral hemagglutinin with sialic acid (SA) containing receptors on the cell surface and subsequently allowing the viral envelope and host cell endosomal membrane fusion, releasing the nucleocapsid into the cytoplasm (Skehel and Wiley, 2000). These interactions determine to a large extent the host range and the consequent successful interspecies circulation of influenza viruses (Matrosovich et al., 1999). Avian influenza viruses prefer  $\alpha$ 2,3SA-gal receptors whereas human and swine influenza viruses prefer  $\alpha$ 2,6SA-gal receptors (Suzuki et al., 2000; Gambaryan et al., 2005). Previous studies showed that ducks and geese abundantly express the avian-type receptors  $\alpha$ 2,3SA-gal in the tracheal epithelium (Kuchipudi et al., 2009; Kimble et al., 2010; Yu et al., 2011), while chickens, turkeys and quails, express both avian and human-type receptors in their

tracheal epithelium (Wan and Perez, 2006; Kuchipudi et al., 2009; Kimble et al., 2010; Yu et al., 2011; Yamada et al., 2012) suggesting the role of these species in the propagation and dissemination of viruses with human-receptor type binding abilities. Further, the analysis of a virus expressing the HA of H5N1 virus derived from geese in Egypt showed that the virus had an increased affinity to bind  $\alpha$ 2,6 SA-gal receptors, while retaining its  $\alpha$ 2,3 SA-gal binding properties (Watanabe et al. 2011b). Knowing that in Egypt, geese are mostly confined to house backyard rearing increases the concern of possible outcome of the closer and continuous contact with human with potential asymptomatic hosting of avian influenza viruses capable of inducing human serious illness. In addition, Elmasry et al., (2015) showed that ducks and geese in live bird markets in Egypt had higher positive probabilities of being infected with HPAI H5N1 virus as compared to chickens. They added that they can as well act as a silent carrier spreading the infection unnoticed among other poultry species. So, these species can represent a potential receptor switching host. Understanding the initial steps in the viral infection was proposed by many to be a key for protection protocols. The first step in the virus infection cycle is its interaction with the cell surface receptors, thus developing drugs that target this interaction would greatly help controlling the infection. Watanabe et al. (2011b) confirmed that the viral HA of human isolates from Egypt have changed their receptor specificity from  $\alpha$ 2,3 SA-gal to  $\alpha$ 2,6 SA-gal which may cause a subsequent increase in human H5N1 influenza virus infections in Egypt. However, studies on the type and distribution of avian influenza receptors in the different tissues of domestic poultry in Egypt are still lacking. In the present study, we aim to investigate the anatomical distribution patterns of H5N1 SA receptors in different organs of native bird species (chicken, ducks, turkeys, geese, quail and pigeons) that are intensively reared in Egypt in order to evaluate the potential of these species to support the virus infections with tropism for SA- $\alpha$ -2,6 and/or SA- $\alpha$ 2,3 terminal saccharides and therefore act as "mixing vessels" or potential receptor-switching hosts.

## MATERIALS AND METHODS

### Animals and tissue preparations:

Three adult healthy birds of the following six species were purchased from the Egyptian market: chicken, Pekin duck, Egyptian goose, Japanese quail, domestic

pigeon and turkey. The birds were handled in accordance with legislation and regulations of the Egyptian Veterinary authorities on the use of laboratory animals for research. Animals were sacrificed and samples were freshly collected from the trachea, lung, duodenum, colon, liver and brain. All samples were rinsed in phosphate buffered saline (PBS) then immersed in PBS containing 4 % paraformaldehyde for subsequent processing. The tissue samples were processed for paraffin sectioning (Bankroft and Gamble, 2008). Paraffin sections (5µm) were prepared using a rotary microtome (Leica RM 2255) and mounted on poly-L-lysine coated slides. Detection of SAα2,3-gal and SAα2,6-gal receptors by lectin histochemistry: The distribution and expression level of SAα2,3-gal and SAα2,6-gal receptors was analyzed in paraffin- embedded tissue sections. The sections were deparaffinized in xylene followed by hydration in decreasing alcohol concentrations. Fluorescein isothiocyanate (FITC) labelled Sambucusnigra agglutinin (SNA) specific for SAα2,6-gal, and FITC labelled Maackiaamurensis agglutinins (MAA II) specific for SAα2,3- gal were used. All lectins were provided by Vector Laboratories, Burlingame, CA. Lectin histochemistry was carried out as described previously (Konami et al., 1994, Shinya et al., 2006). The tissue sections were washed with 0.05M Tris buffer saline (TBS) pH7.6. Sections were blocked using a carbon free blocking solution (Vector Laboratories) according to manufacturer's instructions, followed by 4°C overnight incubation with FITC labeled SNA at a concentration of 5µg/ ml and FITC labeled MAA II at a concentration of 10µg/ml. Following incubation, the slides were washed with TBS then mounted with VECTASHIELDER Hard + set TM Mounting medium (Vector Laboratories) and examined. Relative intensity of the receptors expression was based on the percentage of cells in at least three sections. Reactivity was graded as: negative (-), low (+; > 1% - ≤ 10%), moderate (++; > 10% - ≤ 50%), strong (+++; > 50%).

## RESULTS

Detection of SAα2,3-gal and SAα2,6-gal receptors in the Respiratory tract There was a marked variation in the distribution and expression of influenza receptors among the different poultry species along the respiratory tract [Table 1 & Figure 1 a & b]

### Chicken

Low expression of SAα2,3-gal receptors was detected on ciliated epithelial cells of upper and lower trachea and bronchial epithelium, while there was no expression in the mucous glands along the respiratory tract as well as tracheal goblet cells and the alveolar lining. Moderate

expression of SAα2,6-gal was detected on ciliated epithelial cells of upper and lower trachea, while low in the mucous gland of the trachea, bronchial epithelium and alveolar lining. No expression was detected in goblet cells and the lung mucous glands.

### Duck

Strong expression of SAα2,3-gal receptors was observed in the ciliated epithelial cells of the upper trachea, while low in that of the lower trachea and the upper tracheal mucous gland epithelium. No expression could be detected in other parts of the respiratory tract. No expression for the SAα2,6-gal receptors could be detected in the upper trachea. the mucous glands and goblet cells of the lower trachea. The lower tracheal ciliated epithelium showed low expression for the SAα2,6-gal. Moderate expression for SAα2,6-gal receptors was detected in the bronchial epithelial cells, alveolar lining and mucous glands of the lung.

### Geese

Low expression of SAα2,3-gal receptors was detected in the upper tracheal ciliated epithelial cells, bronchial epithelium and mucous glands and alveoli of the lung. No expression could be detected in the mucous gland epithelium of lower trachea. Moderate expression was found in the lower tracheal ciliated epithelial cells and goblet cells as well as the upper tracheal mucous gland epithelium. In contrast, the expression of the SAα2,6-gal receptors showed moderate expression only in the upper tracheal ciliated epithelial cells. The other parts of the respiratory tract were either low; upper tracheal mucous glands, bronchial epithelium and mucous gland epithelium of the lung or negative; lower tracheal ciliated epithelial cells, goblet cells, mucous glands of lower trachea and alveolar lining.

### Turkey

Tracheal ciliated epithelial cells showed low expression for the SAα2,3-gal receptors, while moderate expression was detected in the bronchial epithelium and mucous gland epithelium of the lung. Upper tracheal mucous gland epithelium, goblet cells and alveolar lining didn't show any expression. In contrast, strong expression of SAα2,3-gal receptors was seen only in the mucous glands of the lower trachea. Strong signals of SAα2,6-gal receptors were detected in the upper tracheal ciliated epithelial cells and lower tracheal mucous glands. Low expression was present in lower tracheal ciliated epithelial cells and alveolar lining, while the upper tracheal mucous glands, goblet cells, bronchial epithelial cells and lung mucous glands were negative for SAα2,6-gal receptors.

### Pigeons

The respiratory tract of pigeons showed negative expression for SAα2,3-gal receptors. While the SAα2,6-

gal receptors were strongly expressed by the ciliated epithelial cells of the upper and lower trachea. Moderate and low expression for SA $\alpha$ 2,6-gal receptors were seen in the alveolar lining epithelium and goblet cells, respectively. Negative SA $\alpha$ 2,6-gal expression was detected in the other parts of the respiratory tract.

**Quails**

Expression of the SA $\alpha$ 2,3-gal receptors was negative in upper tracheal ciliated epithelial cells and alveolar lining, low in the upper tracheal and lung mucous gland epithelium, while moderate in the bronchial epithelium. Expression of SA $\alpha$ 2,6-gal receptors was low in the upper tracheal ciliated epithelial cells, moderate in the lower tracheal ciliated epithelial cells and strong in the bronchial epithelium, whereas other parts of the respiratory tract were negative.

Detection of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors in the Intestinal tract and Liver The distribution of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors varied between the different species along the intestinal tract (duodenum and colon) as well as the liver as described in details in Table 2 and Fig. 2 a & b.

**Chicken**

Low expression of SA $\alpha$ 2,3-gal receptors was observed in the duodenal columnar epithelial cells lining the villi and the epithelium of the crypts of Lieberkühn. The associated goblet cells were negative. The colon showed low expression of SA $\alpha$ 2,3-gal receptors except for the negative epithelial cells lining the villi. The liver, epithelial lining of the portal duct and goblet cells were moderately stained meanwhile, the hepatocytes were negative. On the contrary, SA $\alpha$ 2,6-gal receptors showed negative expression for all previous structure except for a low expression in the epithelial lining of the villi of the duodenum.

**Ducks**

The duodenal columnar epithelial cells lining the villus and the crypt of Lieberkühn showed negative expression for the SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors as well as the epithelial lining the villus of the colon. On the contrary, moderate expression was detected in the duodenal and colon goblet cells for SA $\alpha$ 2,3-gal receptors, while low for SA $\alpha$ 2,6-gal receptors. Colon crypts of Lieberkühn were moderately expressing the avian type receptors, while strong for the human type receptors. The liver had low SA $\alpha$ 2,3-gal expression for hepatocytes and goblet cells while moderate for the portal duct epithelium. In contrast, SA $\alpha$ 2,6-gal expression was strong in the hepatocytes, while no positive cells could be detected in the portal duct or goblet cells.

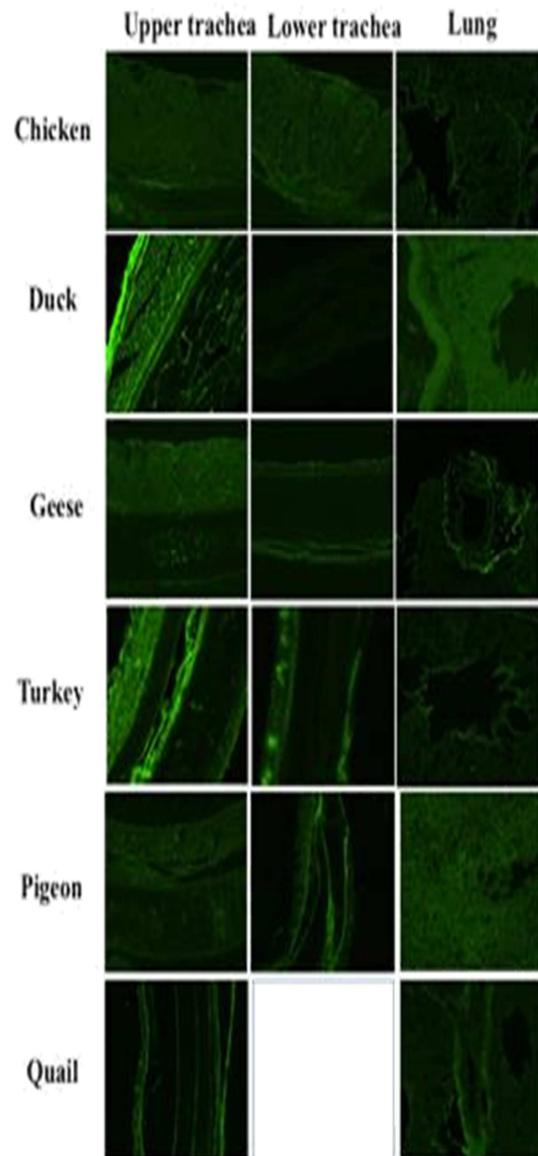
**Geese**

Duodenal epithelial lining of the villi showed negative expression of both receptors. In contrast, the goblet cells

showed moderate positivity for SA $\alpha$ 2,3-gal receptors and low for SA $\alpha$ 2,6-gal receptors. The Expression of SA $\alpha$ 2,3-gal receptors in the intestinal glands of duodenum was strong, in contrast to negative SA $\alpha$ 2,6-gal. The epithelial lining of the colon villi, goblet cells and intestinal glands showed low, strong and moderate expression for SA $\alpha$ 2,3-gal receptors respectively. Meanwhile, SA $\alpha$ 2,6-gal receptors in the colon showed moderate, low and no expression in the intestinal gland, goblet cells and the epithelial lining of the villi, respectively.

Fig. 1a

$\alpha$ 2,3



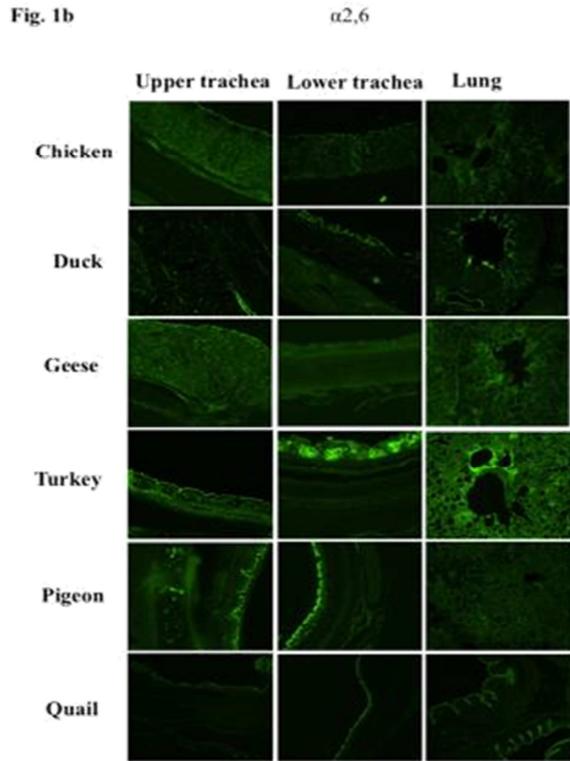


Figure 1: Expression patterns of Influenza receptors in the respiratory tract of different bird species. Sections were stained with FITC- MAAII for SA- $\alpha 2,3$  receptors (a) and were stained with FITC- SNA for SA- $\alpha 2,6$  receptors (b). (Magnification x20 and x40).

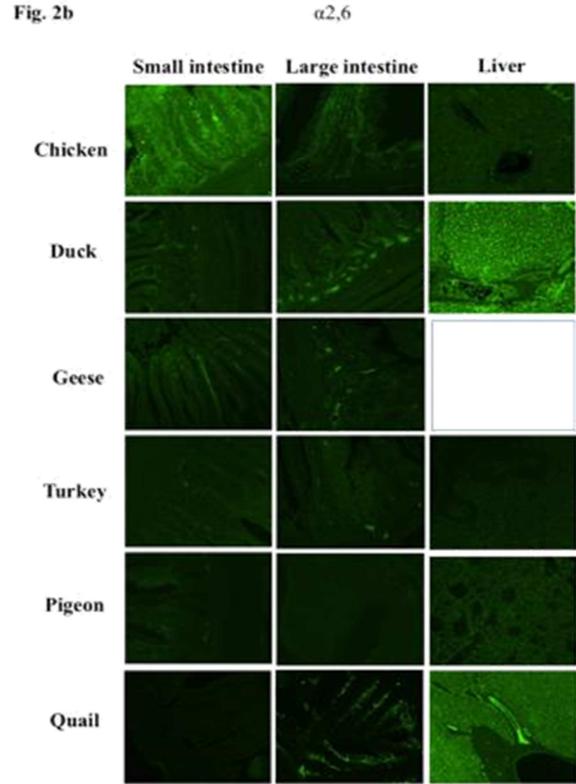


Figure 2: Expression patterns of Influenza receptors in the digestive tract of different bird species. Sections were stained with FITC- MAAII for SA- $\alpha 2,3$  receptors (a) and were stained with FITC- SNA for SA- $\alpha 2,6$  receptors (b). (Magnification x20 and x40).

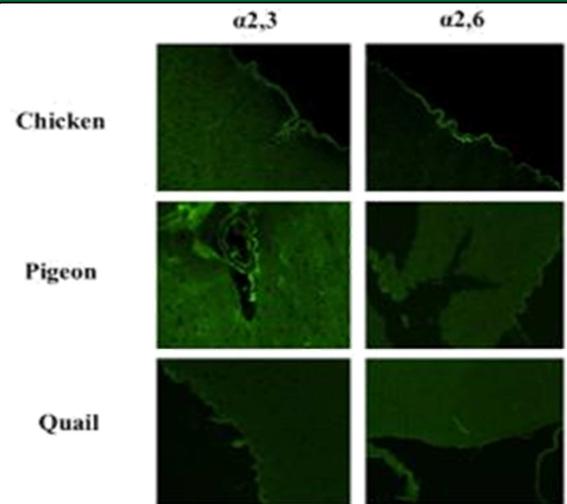
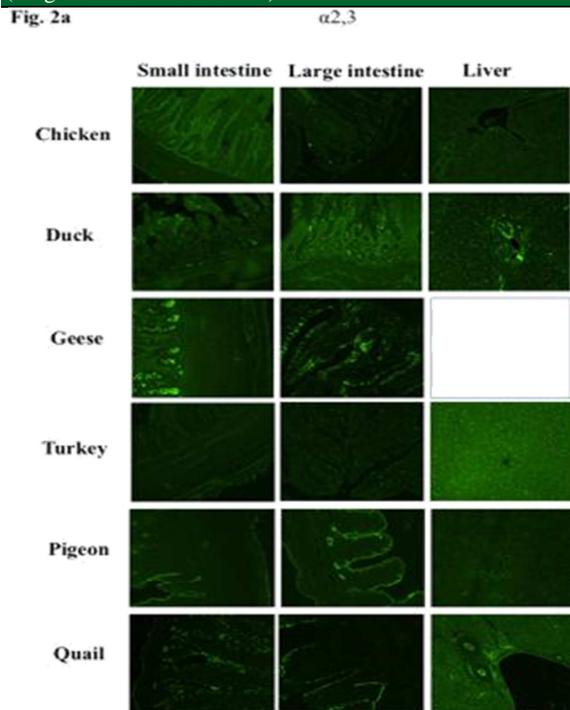


Figure 3: Expression patterns of Influenza receptors in the brain of different bird species. Sections were stained with FITC- MAAII for SA- $\alpha 2,3$  receptors (a) and were stained with FITC- SNA for SA- $\alpha 2,6$  receptors (b). The species with positive expression were only shown. (Magnification x20 and x40).

**Turkey**

No positive expression could be observed for either types of the receptors; avian or human in the intestinal tract except for a low expression in the colon for SA $\alpha$ 2,3-gal receptors was observed in the epithelial lining of the villi and goblet cells in addition to hepatocytes.

**Pigeons**

Moderate expression of SA $\alpha$ 2,3-gal receptors was observed on epithelial lining of the villi in the duodenum. Low expression of SA $\alpha$ 2,3-gal receptors was observed in the goblet cells and intestinal glands of the duodenum and goblet cells of the colon. In contrast, there was a strong expression in the epithelial lining of the villi and intestinal glands of the colon. Pigeon liver showed low expression of SA $\alpha$ 2,3-gal receptors. Regarding the expression of SA $\alpha$ 2,6-gal receptors, a low level was visualized in the duodenal goblet cells only, while other parts of the intestinal tract and the liver were negative.

**Quails**

The duodenum showed low expression of SA $\alpha$ 2,3-gal in the epithelial lining of the villi while strong expression in the goblet cells and intestinal glands. The SA $\alpha$ 2,3-gal in the colon was only strongly detected in the epithelial lining of the villi. The liver showed moderate expression in the portal duct and the goblet cells but not the hepatocytes. The SA $\alpha$ 2,6-gal expression was only detected in the intestinal glands of the duodenum with low expression. In contrast, SA $\alpha$ 2,6-gal expression was moderate and strong in the colon in epithelial lining of the villi and goblet cells, respectively. Further, the liver showed SA $\alpha$ 2,6-gal low and strong expression in the hepatocytes and the portal duct, respectively with no expression in the goblet cells.

**Detection of SA $\alpha$ 2,3-gal and  $\alpha$ 2,6-SA receptors in the brain**

No expression could be detected for SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors in the neuronal tissue of the brain of any of the tested bird species. Further, the SA $\alpha$ 2,3-gal expression was low in the brain meninges of chicken, pigeons and quails but negative in turkey. The expression of SA $\alpha$ 2,6-gal receptors was moderate in chicken and low in pigeons and quails (Table.3, Fig.3).

Table 1: Distribution of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors in the upper and lower respiratory tracts of poultry species.

Tissue, cell type	Species and lectin binding											
	Chicken		Duck		Geese		Turkey		Pigeons		Quails	
	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6
Upper trachea												
Ciliated epithelial cells	+	++	+++	-	+	++	+	+++	-	+++	-	+
Mucous glands	-	+	+	-	++	+	-	-	-	-	+	-
Lower trachea												
Ciliated epithelial cell	+	++	+	+	++	-	+	+	-	+++	ND	++
Goblet cells	-	-	-	-	++	-	-	-	-	+	ND	-
Mucous glands	-	+	-	-	-	-	+++	+++	-	-	ND	-
Lung												
Bronchial epithelium	+	+	-	++	+	+	++	-	-	-	++	+++
Mucous glands	-	-	-	++	+	+	++	-	-	-	+	-
Alveolar Lining	-	+	-	++	+	+	-	+	-	++	-	-

Distribution of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors was evaluated using lectin histochemistry. -: negative; +: low; ++: moderate; +++: strong; ND: not determined.

Table 2. Distribution of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors in the intestinal tract and liver of poultry species.

Tissue, cell type	Species and lectin binding											
	Chicken		Duck		Geese		Turkey		Pigeons		Quails	
	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6
Duodenum												
Epithelial lining of villi	+	+	-	-	-	-	-	-	++	-	+	-
Goblet cells	-	-	++	+	++	+	-	-	+	+	+++	-
Crypts of Lieberkühn (Intestinal glands)	+	-	-	-	+++	-	-	-	+	-	+++	+
Colon												
Epithelial lining of villi	-	-	-	-	+	-	+	-	+++	-	+++	++
Goblet cells	+	-	++	+	+++	+	+	-	+	-	-	+++
Crypts of Lieberkühn (Intestinal glands)	+	-	++	+++	++	++	-	-	+++	-	-	-
Liver												
Hepatocytes	-	-	+	+++			+	-	+	-	-	+
Epithelial lining of portal ducts	++	-	++	-	ND	ND	-	-	+	-	++	+++
Goblet cells	++	-	+	-			-	-	+	-	++	-

The distribution of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors was evaluated using lectin histochemistry. -: negative; +: low; ++: moderate; +++: strong; ND: not determined

Table 3. Distribution of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors in the brain of poultry species.

Tissue, cell type	Species and receptor type											
	Chicken			Duck	Geese				Turkey	Pigeons		Quails
	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6	$\alpha$ 2,3	$\alpha$ 2,6
Meninges	+	++		ND		ND	-	ND	+	+	+	+
Neuronal tissue	-	-					-		-	-	-	-

The distribution of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors was evaluated using lectin histochemistry. -: negative; +: low; ++: moderate; +++: strong; ND: not determined.

## DISCUSSION & CONCLUSION

In this study the distribution of influenza virus receptors was studied in a range of tissues from poultry species raised intensively in Egypt; chicken, Pekin duck, Egyptian goose, Japanese quail, domestic pigeon and turkey. The tested species are commonly reared in very close contact with human, mainly in backyards and even more directly in live bird markets, representing a potential risk of circulating avian influenza viruses. Using lectin immunohistochemistry, we found widespread and variable expression of both human (SA $\alpha$ 2,6-gal) and avian (SA $\alpha$ 2,3-gal) influenza virus receptors in a range of tissues from each species, suggesting that these species may be likely targets for both avian as well as human influenza viruses.

In chicken, Pekin duck, Egyptian goose, Japanese quail, domestic pigeon and turkey, both SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors were expressed in at least one segment of the respiratory and intestinal tracts except in pigeons where the SA $\alpha$ 2,3-gal receptors were not expressed in the respiratory tract which may explain why they are not commonly naturally infected with avian influenza viruses. This was previously confirmed by Liu et al., 2009 who showed that little or no expression of SA $\alpha$ 2,3-gal could be detected in the respiratory tract of pigeon with minimal occasional alveolar expression. Contrary to that, Franca et al., (2013) reported strong expression of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors in the trachea and lungs of wild pigeons, which could be explained by differences in the lectins isoforms or bird breed used in individual studies. However, pigeons have been recently reported to be naturally infected with H5N1 in Egypt (Mansour et al, 2014). On the contrary, the SA $\alpha$ 2,6-gal receptors was abundantly expressed in the pigeon intestinal tract. This may suggest that either H5N1 virus can have altered receptor usage in the respiratory tract of these birds or that the infection is first established in the intestinal tract and spreads thereafter to other organs, explaining why it is not commonly a natural host for H5N1 virus. Considering that pigeons are intensively reared in Egypt as either a meat source or a fancy bird and in both cases they are in very direct and close contact to human, they

could represent a possible asymptomatic carrier and a silent transmitter of avian influenza viruses.

The avian-type receptors, SA $\alpha$ 2,3-gal, in ducks dominated in the upper tracheal epithelial cells, while in the lower tracheal ciliated epithelial cells of geese as reported (Franca et al., 2013). The tracheal expression in ducks and geese was reported before but as concerning the trachea in general and not in parts as in the current study and less in abundance as well (Kuchipudi et al., 2009; Kimble et al., 2010). While on the contrary, Costa et al., (2012) reported moderate expression on the tracheal ciliated epithelium in mallards.

Further, Yu et al., 2011 reported that only few cells expressed the SA $\alpha$ 2,3-gal in the upper and lower trachea in ducks No expression of SA $\alpha$ 2,3-gal receptors could be detected in the lower respiratory tract of ducks and turkey small intestine as well. Franca et al., (2013) reported strong expression of SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors in lungs and trachea, respectively, of wild ducks, which is opposite to our findings. Thus it is possible that a difference in the bird breed could be responsible for the different expression profiles among different studies.

The human type receptors, SA $\alpha$ 2,6-gal was not expressed in the lower trachea of goose, large intestine of chicken and the intestinal tract and liver of turkey and pigeons. The dominant SA $\alpha$ 2,6-gal receptors expression pattern was detected in the upper tracheal ciliated epithelial cells in chicken, geese, turkey, pigeons and quails and is consistent with previous reports (Gambaryan et al., 2002; Kim et al., 2005; Kuchipudi et al., 2009 and Pillai and Lee, 2010) in chicken, (Liu et al., 2009; Costa et al., 2012) in pigeons, while it was in contrast to Kimble et al., (2010) and Franca et al., (2013) in goose, Pillai and Lee, (2010) and Costa et al., (2012) in turkey. Wan and Perez, (2006) showed that the majority of the epithelial cells in chicken trachea expressed SA $\alpha$ 2,3-gal receptors, while few were positive for SA $\alpha$ 2,6-gal receptor, this is in contrast to our results were SA $\alpha$ 2,6-gal showed wider and denser expression in the upper and lower trachea as well as the alveolar lining of the lung. A possible explanation for the discrepancy in the receptor distribution in chicken trachea could be the chicken breed and/or the source of the lectin used. Lectins from

different suppliers or isoforms may show different binding specificities, in particular the source of MAA has been shown to significantly affect specificity (Nicholls et al, 2007; Pillai and Lee, 2010). The high levels of expression of SA $\alpha$ 2,6-gal receptors in the tracheal epithelium, especially in pigeons, suggests that these species may be more vulnerable to support the evolution of avian influenza viruses with higher affinity for human SA $\alpha$ 2,6-gal receptors.

The expression of SA $\alpha$ 2,6-gal receptors in the lower and upper tracheal ciliated epithelial cells was the same in chicken and pigeons, while lower in the upper as compared to the lower trachea in quails. Meanwhile, human type receptors showed low levels of staining in duck's upper trachea and turkey's lower trachea. To our knowledge, only few reports discussed the expression of influenza receptors in different parts of the trachea and only in chicken, ducks and quails (Yu et al., 2011). The expression of both receptors in the bronchial epithelial cells in chicken, geese and quails was in agreement with Costa et al., (2012) in chicken and quails, (Yu et al., 2011) in common quails, and Wan and Perez (2006) in Japanese quails in which both SA $\alpha$ 2,3-gal and SA $\alpha$ 2,6-gal receptors were observed with a slight difference in the expression level. This difference in receptor expression could be related to interspecies differences. On the other hand, as reported by Kimble et al., (2010), there was no expression of SA $\alpha$ 2,6-gal receptors in older geese trachea and lung, which is opposite to the strong expression reported by Franca et al., (2013), however, age differences could be a factor. Negative expression for both receptors was recorded in pigeon bronchial epithelial cells in contrast to Liu et al., (2009) who recorded high expression of SA $\alpha$ 2,6-gal receptors in pigeons. Moderate expression of SA $\alpha$ 2,6-gal receptors in bronchial and alveolar lining epithelium of duck was in agreement with Pillai and Lee (2010) and in contrast to Kuchipudi et al., (2009) and Costa et al., (2012). The moderate expression of SA $\alpha$ 2,3-gal receptors in turkey is as recorded by Costa et al., (2012) and Kimble et al., (2010) but in contrast to Pillai and Lee (2010). Such difference could be related to the lectin's isoform as they used MAA not MAII.

The human type receptors were not expressed in the epithelial lining the villus in the duodenum and colon and also in the intestinal gland of the small intestine of ducks, geese, turkey and pigeons consistent with Pillai and Lee (2010) and Costa et al., (2012) in ducks and turkey, Kimble et al., (2010) in turkey, Kuchipudi et al., (2009) in ducks, Liu et al., (2009) in pigeons and Franca et al., (2013) in ducks and geese duodenum. The absence of SA $\alpha$ 2,6-gal receptors in the intestinal tract of these species did not prevent the infection, even low, with the human-origin H1N1 virus in the small intestine of ducks and turkey (Costa et al., 2012). In contrast, Franca et al., (2013) reported strong expression of

SA $\alpha$ 2,6-gal receptors in the large intestine in ducks and geese. In chicken, we observed a low level of staining for both receptors on the epithelial cell of duodenum, whereas Liu et al., (2009) and Costa et al., (2012) did not detect SA $\alpha$ 2,6-gal receptors in the intestinal tract of chickens, and Kuchipudi et al., (2009) only detected this receptor in the large intestine. These differences could be attributed to the differences in the bird breed used.

The avian type receptors had strong expression in the colon of quails and low level of staining in the duodenum which is in agreement with Costa et al., (2012) but in contrast to Kimble et al., (2010) who showed low level of expression in the colon. A recent study, however, reported a very minimal expression of SA $\alpha$ 2,3-gal receptors in the large intestine of duck and geese Kimble et al., (2010), which is opposite to the strong expression reported by Franca et al., (2013) in the large intestine. In agreement with Wan and Perez (2006) and Guo et al., (2007) abundant SA $\alpha$ 2,3-gal receptors were detected in the colon of quails.

The isolation of avian influenza viruses from other organs such as the liver and the brain has been reported before (Watanabe et al., 2011a). The avian type receptors was moderately expressed in the liver of chicken, ducks, turkey and pigeons in the portal duct and goblet cells in contrast to SA $\alpha$ 2,6-gal receptors, which were expressed only in the duck hepatic cells. In contrast, quails had strong expression of SA $\alpha$ 2,6-gal receptors in the epithelial lining of the portal duct while moderate expression of the SA $\alpha$ 2,3-gal receptors. Even though, no data is available regarding the receptor expression pattern in the liver, the wide and abundant expression of SA $\alpha$ 2,6-gal receptors in quails support those reported before by Wan and Perez (2006) and Guo et al., (2007) that quails could be more likely to be an intermediate host for the generation of influenza viruses with adaptive mutations with pandemic potential. Noting that several avian influenza viruses has been successfully recovered from the brain of naturally infected birds (Watanabe et al., 2011a), low expression of both receptors was detected in the meningeal layer in pigeons as reported before Pillai and Lee (2010), and also in chicken and quails, while there was no expression detected in the neuronal tissue. The two interesting species in our results are the pigeons and geese, both having human type receptor expression in the upper respiratory tracts that can possibly modulate the replication of SA $\alpha$ 2,6-gal-using influenza viruses. Such viruses that could possibly replicate in the upper respiratory tract of such birds may acquire the human type receptor binding capabilities and be a potential pandemic risk. Even though, influenza receptors are one of the essential requirements for host specificity of influenza type infection, the distribution patterns of the receptors detected here indicate that there could be other determinants that are utilized by influenza viruses to

overcome the host barriers. The current findings supplied a map for the distribution of avian and human influenza viruses receptors in the different domestic birds reared in Egypt. This might be considered in vaccination and protection programs, and help in pathological and immunological investigations.

**Ethical statements:**

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