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THE FLIES' AS A MECHANICAL VECTOR OF AVIAN VIRAL PATHOGENS

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ABSTRACT

Flies are the most important insect pest associated with poultry, where the accumulated organic waste and favorable environmental conditions often promote rapid development of large populations. This study aims to evaluate flies as a vector for avian viral pathogens. A total of 90 flies were collected from campus of School of Veterinary Medicine, Shiraz University, Iran. Reverse transcription-polymerase chain reaction test using specific published primers for Newcastle Disease virus, Avian Influenza virus, Infectious Bronchitis virus and Infectious Bursal Disease virus was carried out to detect the viruses. The M1 gene of avian influenza and F gene of Newcastle disease virus genes were detected from 18 and 32 separated samples respectively by reverse transcription-polymerase chain reaction. Infectious Bronchitis and Infectious Bursal Disease viruses were not detected from all houseflies. These results suggest that it is possible that flies could become a mechanical transmitter of avian influenza and Newcastle disease viruses.

Keywords: Flies, Mechanical Vector, Avian Pathogens, Influenza, Newcastle disease

1. INTRODUCTION

The most viral avian pathogens are Avian Influenza, Infectious Bronchitis, Newcastle Disease and Infectious Bursal Disease that causing many economic losses to the poultry industry for many years and many studies have been done on the transmission of this virus. Flies are "pests" of great medical and veterinary significance and are one of the most important vectors of human and animal diseases worldwide. (Aberg-Cobo *et al.,* 1959). The behavior of this pest is typically synanthropic and, because of its high reproductive rate and ability to pros per in a wide range of environments, it pullulates throughout the entire year. In general, they are considered to be mechanical and/or biological vectors for a number of major pathogens such as bacteria (De Jesus *et al.*, 2004; Holt *et al;* 2007), protozoa (Forster *et al.*, 2007; Ugbogu *et al.,* 2006), viruses (Watson *et al.*, 2007) and helminthes eggs (Sulaiman *et al.,* 1988). Flies transmit pathogens via mouthparts, vomit droplets, feces and their body surface (Greenberg, 1973). Their ability to disperse contaminated pathogens correlates with the flying distance which was shown to be approximately 1–3 km per day (Herms *et al.,* 1969). Accordingly, flies are considered as an important vector for spreading disease in the poultry production system. For example, an outbreak of AI (H5N2) in Pennsylvania, USA, in 1983-84, leads to the death of countless birds. In more than one third of the adult Muscat samples (121 samples of three species), the virus of avian influenza could be identified (Wilson *et al.,* 1986).

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The purpose of this work was to determine if flies collected from campus of School of Veterinary Medicine of Shiraz University, carried the Newcastle Disease, Avian Influenza, Infectious Bronchitis and Infectious Bursal Disease viruses.

2. MATERIAL AND METHODS

Ninety flies were collected from campus of School of Veterinary Medicine, Shiraz University, Shiraz, Iran, on July 2015. All the collected flies were individually put into a 1.8 mL micro tube and stored in an icebox during transportation to the laboratory. All samples were kept in a freezer at –80°C in laboratory until used in virus detection.

The samples were subjected for RNA extraction. Then 1 mL of RNX solution (a commercial RNA extraction kit, Cinna Gen, Iran) was added to 50–100 mg of each homogenized sample, then 200 µL of chloroform was added to the mixture. After centrifugation of the samples at 12000 g for 15 min, the aqueous phases were transferred to another tube. The RNA was precipitated at 12,000 g for 15 min after the addition of an equal volume of isopropanol. The RNA pellet was washed with 75% ethanol, then eluted in 50 µL of distilled water after drying and stored at -70 °C until used. Virus detection was performed by reverse transcription polymerase chain reaction (RT-PCR) method using specific published primers. The cDNA was synthesized using an Accu Powder®RTPreMix kit (BioNeer Corporation, Daejeon, South Korea) according to the manufacturer's instruction. The primer pairs used in the cDNA synthesis and PCR for each genome-sequences of virus are shown in table 1.

Five µl of the cDNA was used for PCR amplification using AccuPower® PCR PreMix (Bioneer Co., Daejeon, South Korea). The PCR thermo cycling conditions for the each gene were as recommended previously Mosleh *et al.* (2013) for ND Nili *et al.* (2013) for AI, Shirzad *et al.* (2012) for IB and Rangbar *et al.* (2013) for IBD. Finally, 5µl of PCR product was subjected to 2% agarose gel electrophoresis containing ethidium bromide and visualized under ultra violet light.

3. RESULTS

N and VP2 genes of Infectious Bronchitis and Infectious Bursal Disease viruses were not detected from samples (table 2). From 90 collected flies, M1 and F genes of AI and ND viruses were detected respectively from 32 and 18 deferent cases. Remarkably, ND and AI viruses simultaneously were detected from 7 individual flies (table 2).

PCR fragments of 450 bp for AIV and 362 bp for NDV at the M1and F gene (AIV and NDV respectively) by RT-PCR are shown in Figure 1.

4. DISCUSSION

Flies at larval and mature stages are observed in poultry farms, garbage, and slaughter houses. Flies plays its role as a vector of diseases in humans, poultry and livestock from where it scatters to human habitats and activities. More than hundred different pathogens are reported on flies. These insects are a causative agent for the spread of various viral diseases. Usually transmission of pathogens occurs in three ways. First, pathogens may stick to their body parts especially at their legs and proboscis. Second, pathogens are deposited along the vomit drop onto the food

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because their mode of feeding is sucking the food after liquefaction in regurgitated saliva. Lastly, pathogens are deposited in their feces after passing through the gut (Iqbal *et al.*, 2014). Results of the present study are indicates that M1 gen of H9N2 Avian influenza and F gen of Newcastle disease viruses were isolated from flies. Mechanical transmission is a simple mechanism of pathogen transmission which, in itself, is considered to be the most important ‘‘indirect effect’’ of blood-sucking insects (Baldacchinol *et al.*, 2013). It has been known that *M. domestica* spp. are the most important fly species at poultry farms (Axtell, 1999), with regard to mechanical transmission of > 30 various pathogen (Greenberg, 1973). Chickens and many wild birds eat flies, even when they are flying. It is possible that chickens can take in the virus with fresh flies orally and/or contact with some contaminated feces excreted and vomited matter from infected flies. Viral diseases are one of the most severe clinical manifestations of *arbovirus* infection and may result in death or leave severe consequences in survivors, wherein mosquitoes (Sarwae, 2016). In additional of viruses, the potential of adult houseflies to transmit food borne pathogens such as Campylobacter, E. coli O157:H7, *Salmonella spp*. and *Shigella spp*. has been also reported (Barreiro *et al.*, 2013).

In regard to the results Sawabe *et al.* (2006) suggested it is possible that blow flies could become a mechanical transmitter of H5N1 influenza virus. They detected highly pathogenic H5N1 Avian Influenza A viruses from blow flies collected in vicinity of an infected poultry farm. The latter (Wanaratana *et al.,* 2013), demonstrated that houseflies could serve as vectors in highly pathogenic Avian Influenza H5N1 virus transmission in chickens under experimental conditions.

House flies (*Musca domestica*) and little house flies (*Fanniacanicularis*) were examined for their ability to take up and harbor a velogenic strain of exotic Newcastle disease virus (Chakrabarti *et al.,* 2008). They demonstrated that both fly species acquired viral titers greater than the infective dose for a susceptible chicken (10(3.0) EID_{50}-10(4.0) EID_{50}). Rogoff *et al.* (1977) clearly showed that virulent velogenic NDV can be transmitted to young chickens by *Fanniacanicularis*, either from a highly infective source or directly from infected birds. Exotic NDV was isolated from houseflies collected from backyard flocks during an outbreak in the U.S.A. (Chakrabarti *et al.*, 2007). Also, laboratory-reared flies that were experimentally exposed to NDV La Sota strain, the virus was detected in the dissected gastrointestinal tract of flies for up to 72 h post-exposure (Barin *et al.*, 2010) But, these infected flies could infect sensitive chickens under laboratory conditions the housefly did not carry sufficient quantities of NDV (Roakin strain) to cause disease in chickens (Watson *et al.*, 2007). The latter authors speculated that virus virulence factor plays a part in the ability of the housefly to mechanically transmit NDV.

CONCLUSION

This study discovered that the flies could transfer the avian viral pathogens. The results of this study and others have shown that the fly is capable of harbouring ND and AI viruses. The fly, inactively with its body surface and/or actively ingesting through droppings and secretions of infected chickens, could transmit the viruses.

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Table 1. RT-PCR primers and sequences

Virus	Gen	Primer	Sequence	base pair	References
Newcastle Disease	**F**	R	5′ GGA GGA TGT TGG CAG CAT T 3′	362 bp	MOSLEH et al., 2013
		F	5′ TTGATG GCA GGC CTC TTG C 3′		
Avian Influenza	**M1**	R	5′ GGG AAG AAC ACA GAT CTT GAG G 3′	450 bp	NILI et al., 2013
		F	5′ TGC TGG CTA GCA CCA TTC TG 3′		
Infectious Bronchitis	**N**	R	5' CAT TTC CCT GGC GAT AGA C 3'	76 bp	SHIRZAD et al., 2012
		F	5' GAG AGG AAC AAT GCA CAG C 3'		
Infectious Bursal Disease	**VP2**	R	5' CCG GAT TAT GTC TTT GAA GCC 3'	743 bp	RANJBAR et al., 2013
		F	5' GGC CCA GAG TCT ACA CCA TAA 3'		

Table 2: were positive for NDV and AIV by RT-PCR/total numbers of 90 collected flies.

Virus	base pair	Number of Positive
Newcastle Disease	362 bp	**32/90***
Avian Influenza	450 bp	**18/90***
Infectious Bronchitis	76 bp	**0/90**
Infectious Bursal Disease	743 bp	**0/90**

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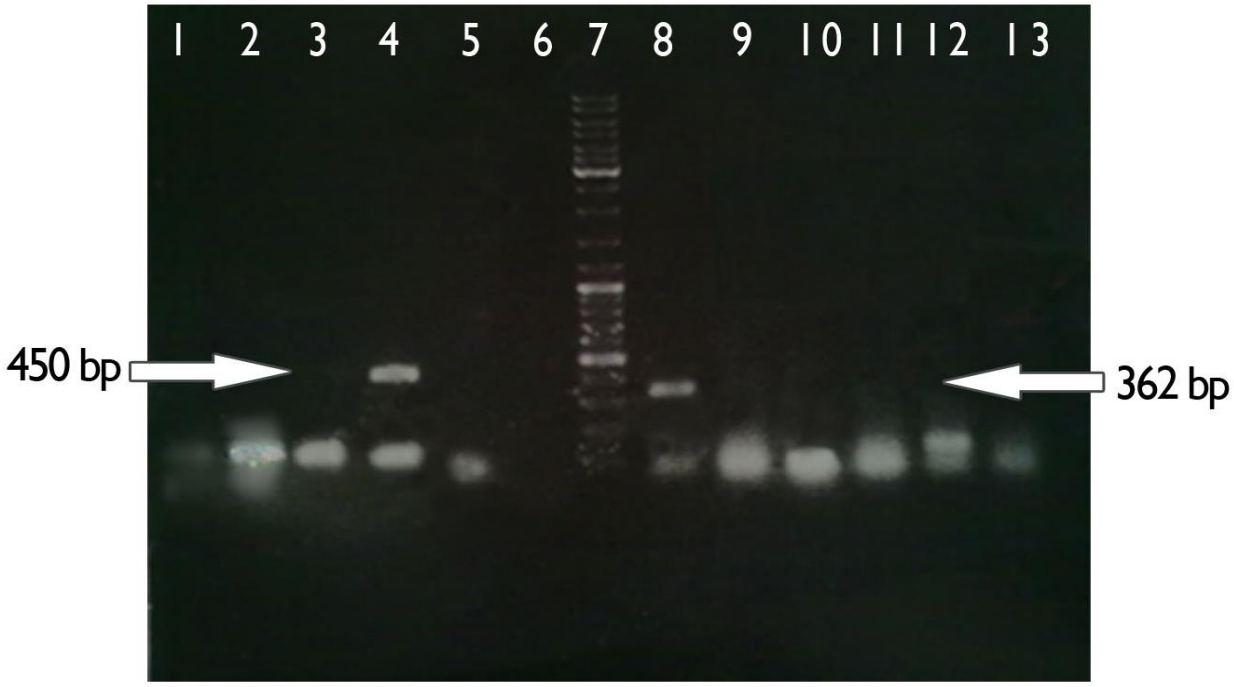

Figure 1. Agarose gel electrophoresis of Virus detection performed by reverse transcription polymerase chain reaction from purified RNA of known avian viruses. Lane 7 = molecular size marker. Lane 4 = H9N2 subtypes of avian influenza virus, 450 bp. Lane 6 = PCR reagent buffer as a negative control. Lane 8 = Newcastle Disease virus, 362 bp.