Avian Influenza

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Avian Influenza Outbreak Investigations

Virological Investigation Group : SD Pawar, AK Chakrabarty, K. Ray, SS Koratkar, SS Kode, B. Pal, S. Raut, VV Thite, MR Khude, D. Hangekar.

Molecular Detection and Characterization Group :

- **West Bengal Outbreak :** AK Chakrabarti, SD Pawar, S Raut, B Pal
- **Manipur Outbreak :** K Ray, AK Chakrabarti, SD Pawar B Pal, S Raut

Along with AI group, staff members from the following groups were also involved in the outbreak investigation. Processing of bird specimens: DR Patil, P Barde, Sandeep, RS Jadi, and Dinesh Singh. RT-PCR & Sequencing: Hepatitis, Japanese Encephalitis, Measles, Rotavirus, Dengue

Introduction

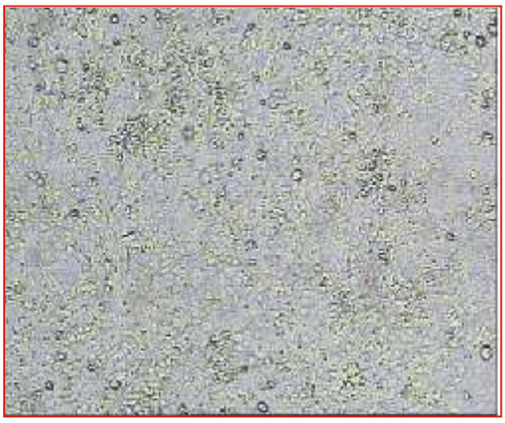
The recent emergence of Avian Influenza and specifically the subtype H5N1 pose a major problem and a biggest threat to public health globally as well as in India. H5N1 virus is known to cause widespread infection in birds and also to human respiratory tract. It is very important to identify this lethal virus if it is present in any outbreak. We established a combination of molecular diagnostics, serological tests and virus isolation to identify and confirm the presence of highly pathogenic Avian Influenza viruses from field/clinical specimens. During the last year India experienced outbreak of H5N1 in poultry in several occasions Manipur (July 2007), West Bengal (January to April 2008). We received samples from the entire above outbreak area and investigated to confirm the presence of highly pathogenic avian influenza viruses (H5N1) in these samples.

Objectives

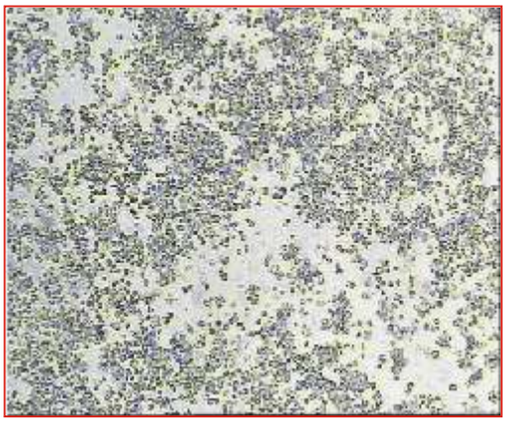
- Virus isolation and characterization of H5N1 isolates.
- Molecular detection and characterization of H5N1 strains.

Work done

Specimens received from outbreak areas were processed in high containment laboratory for virus isolation in SPF eggs and MDCK cell line. We performed one step reverse transcription-polymerase chain reaction (RT-PCR) using WHO sets of diagnostics primers specific for influenza A, HA (H5) and NA (N1) and Real time RT-PCR using ABI influenza A/H5 and N1 kits. Surface glycoproteins (Hemagglutinin and Neuraminidase) were sequenced from all the isolates. The specimens which were positive for H5N1 were further processed for virus isolation in 10-day-old embryonated chicken eggs and in MDCK cell line. Chick embryos died within 24 hours post-infection. Madin-Darby Canine Kidney (MDCK) cultures with confluent monolayer were prepared. These bottles were inoculated and observed for cytopathic effect (CPE) (**Fig. 1**). CPE was evident within 48 hours post-infection. When +++ CPE was evident, TCFs from these bottles were harvested and tested by HA test for virus titration. Characterization of the genome of Manipur and West-Bengal strains have been performed for all the 8 gene segmentsof representative isolates of Manipur and West-Bengal. Full genome sequencing revealed the presence of unique virus, which belongs to clade 2.2 of Z genotype with few unique mutations. Additionally, molecular marker analyses also depicted that this virus is sensitive to drugs Amantadine and Oseltamivir. All the above studies confirm the virus to be of avian origin.



Un-inoculated MDCK cells.



CPE on MDCK cell culture, induced by influenza H5N1 Manipur (MNI 1900)

Fig. 1 : Cytopathic effect of Manipur H5N1 virus in MDCK cell line

Allantoic fluids and MDCK supernatants were positive in hemagglutination (HA) tests with fowl and horse RBCs. Representative isolates were tested in hemagglutination inhibition (HAI) test for identification of viruses. A panel of antisera used for identification included antibodies against influenza A(H5N1)-Navapur A/Ck/India/33487/2006-H5N1, (H5N2), A(H7N3), A(H9N2), NDV and normal ferret and sheep sera. These isolates were identified as influenza H5N1.

AI surveillance during avian migratory season 2007-2008

SD Pawar, SA Pande, AK Chakrabarti, SS Koratkar, SS Kode, VV Thite, MB Nanavare, B. Pal, S. Raut, MR Khude, AV Jamgaonkar, P. Salunke, DS Hangekar and ELA foundation team.

Introduction

India reported outbreaks of HPAI H5N1 in poultry in Navapur, Maharashtra; Gujarat; Madhya Pradesh (February 2006), Manipur (July 2007), West Bengal (January 2008). The role of migratory birds in the transmission of H5N1 viruses is still unclear. In this scenario Avian Influenza (AI) surveillance in wild migratory, wild resident, domestic birds and poultry was undertaken by NIV jointly with ELA Foundation, Pune, India during 2006-2007.

Objectives

- Surveillance of H5N1 or any avian influenza in migratory and wild birds, and poultry
- Characterization of the strain found in the surveillance.

Work done

AI surveillance in migratory and wild birds was undertaken during avian migratory season 2007-2008 in collaboration with ELA foundation, Pune. During this season, 678 specimens (Fecal droppings: 3300, Cloacal swabs: 22, Tracheal swab: 12, Blood sample: 5) were collected from sites covering Maharashtra and Karnataka state. Screening of these specimens using molecular and virological diagnostics such as one-step RT-PCR, Real-Time PCR and virus isolation was performed. All these specimens were inoculated in embryonated chicken eggs for virus isolation.

A total of six fecal specimens from Eurasian Spoonbill, Shoveller, purple moorhen, Asian Openbill, Great Thick Knee or Greater Stone Plover and Greater Flamingo were positive in hemagglutination (HA) assay. These HA

positive allantoic fluids were tested in quick tests for influenza A, H5, NDV and IBDV. Specimen from Eurasian Spoonbill collected from Rui Chatrapati, Ahemednagar district was positive for influenza A. Further analysis of this specimen by RT-PCR and sequencing showed that this virus is of influenza A (H11N1) subtype. This is the first ever influenza A (H11N1) virus from the Indian subcontinent.

All other specimens were negative for influenza A, H5, NDV and IBDV. Electron microscope analysis of two representative specimens showed presence of Reovirus-like particles. Further analysis of these specimens is in progress.

Seroprevalence of antibodies to influenza A viruses using Microneutralization (MN) & Hemagglutination inhibition (HAI) assays in human sera from Nandurbar & Jalgaon districts, India

(Work done at Centers for Disease Control and Prevention (CDC), Atlanta, USA)

Personnel involved in the investigation: SD Pawar, Scientist B, Avian Influenza visited and worked as a "Guest Researcher" at CDC, Atlanta, USA.

Field study & sampling by: NS Wairagkar, MS Chadha, CG Raut.

Supervisor: Jacqueline Katz, Chief, Immunology & Pathogenesis Branch, Influenza Division, CDC. Work was done with Jenna Achenbach and Veguilla Vic.

Introduction

India reported outbreaks of HPAI H5N1 in poultry in Navapur, Maharashtra; (February 2006) and NIV had sequenced H5N1 isolates. In order to understand the seroprevalence of antibodies to influenza A in the human sera two different assays were performed. The microneutralization (MN) assay is sensitive and detects H5-specific Ab in human serum specimens at low titers. The haemaglutination inhibition (HAI) assay is advantageous over the MN assay as the assay works well with inactivated H5 viruses, so that the A (H5N1) serology can be safely performed in a BSL-2 laboratory.

Objectives

- Detection of antibodies to AI A (by MN assay and HHAI assay)

Work done

A total of 100 test sera and 25 sera from unexposed population were tested in MN assay. It was found that all the sera were negative for the presence of antibodies against an Indian isolate of influenza A (H5N1) virus. However, these sera showed presence of Abs against human influenza A (H1N1) [62.4 % sera positive] & A (H3N2) [88.8 % sera positive] strains. Cut-off in the test was antibody titer >=80. Serum specimens with A (H5N1) viruses were tested using 1% horse RBCs and with human influenza A (H1N1) and A (H3N2) viruses were tested with 0.5 % turkey RBCs for HAI assays. All the tested sera were negative with Indian isolate of influenza A (H5N1) virus and with A/Whooper swan/Mongolia/244/2005 (H5N1) virus. However, these sera showed presence of Abs against human influenza A (H1N1) [20.8 % sera positive] & A(H3N2) [78.4 % sera positive] strains. Cut-off in the test was antibody titer >=40.

Generation of infectious influenza A virus particle and vaccine reference strain of highly pathogenic influenza viruses (H5N1) using reverse genetics

AK Chakrabarti, B. Pal, S. Raut and AC Mishra

Introduction

Reverse genetics is one of the most important and latest tools that have revolutionized the research in influenza virus to generate recombinant virus, vaccine virus strain and study pathogenicity.

Objectives

- Construct a pre-pandemic vaccine reference strain from highly pathogenic avian influenza virus (H5N1) of Indian origin.

Work done

Reverse genetics facility was set up for the rescue of influenza virus in collaboration with St. Jude Children's Hospital, Memphis, TN, USA. We have successfully rescued wild type A/WSN/33 and currently working to rescue wild type influenza A (H5N1) viruses. Cloning of different gene segment of highly pathogenic avian influenza viruses is underway.

The highly pathogenic AI strain A/Chicken/India/NIV-33498/06 (H5N1) was modified to a low pathogenic virus using recombinant DNA technology and reverse genetics. Recombinant virus generated possessed surface glycoproteins (modified HA and complete NA) of influenza A H5N1 in A-PR8 background and grew well in embryonated chicken eggs to a HA titer of 1256. This is a low pathogenic virus as per its dependency on trypsin for plaque formation and animal challenge study and can be used to generate inactivated vaccine against highly pathogenic avian influenza A (H5N1) viruses.

Modification of the above HPAI virus was performed at CDC, Atlanta. AK Chakrabarti[1], Y Matsuoka[2] and RB Donis.

Future Plan

- Characterization of the strains of H5N1.
- Initiate studies on animal models for H5N1.
- Avian influenza surveillence in India and study at the avian-human interface.
- Development of antiviral agent.

Publications

- Ray K, Potdar VA, Cherian SS, Pawar SD, Jadhav SM, Waregaonkar SR, et al. Characterization of the complete genome of influenza A (H5N1) virus isolated during the 2006 outbreak in poultry in India. **Virus Genes** 2008 Apr; 36(2): 345-53.

Book Chapter

- Shailesh Pawar "Avian Influenza", Lecture notes for Certificate Course in Basic Ornithology. Edtrs. S. Pande, S. Kharat, H. Ghate, A. Mahabal; Published by MES Abasaheb Garware College & ELA Foundation, Pune, 2008, p225 - 233.

Workshop/Conference/Meeting Organized

- AI Group organized an International Workshop in collaboration with CDC, USDH on Real-Time PCR for detection of AI viruses, 8-12, Oct. 2007.

Workshops / Conferences / Seminar / Meetings attended

SD Pawar

- Invited talk in International symposium on AI, New Delhi 28-31 Oct. 2007, "Neutralizing antibodies as a surveillance tool for AI".
- Invited Lecture on Avian influenza surveillance in migratory birds, International Workshop on Avian influenza surveillance, organized by BNHS-FAO-Wetlands International organization, 8th February 2008, Nashik.
- Attended meeting on Options for the control of influenza VI at Toronto, Ontario, Canada, June 17- 23, 2007. A discussion between Indian scientists and CDC Atlanta influenza group was arranged by the organizing committee of the "Option for the control of Influenza VI" Conference held in Toronto, Canada. Presented the work carried out by him at CDC Atlanta with respect to Microneutralization (MN) assay for detection of antibodies against avian influenza (AI) A (H5N1) virus and Haemagglutination (HAI) assay using horse RBCs.
- Presented data of the work performed at influenza branch, CDC to the staff of influenza division, CDC, June 11, 2007.
- Invited Lecture on Avian influenza, Ornithology course, Abasaheb Garware College, Pune, 14th January 2008.

Posters presented in an international ICVT conference, New Delhi, 11-14 Dec. 2007.

- Development of HA & HAI assays for detection & identification of AI viruses. Pawar SD, Koratkar SS, Thite VV, Kode SS, Khude MR, Nanaware MB, Mishra AC.
- AI surveillance in migratory and domestic birds during avian migratory season 2006-2007. Pawar SD, Pande SA, Koratkar SS, Kode SS, Thite VV, Nanavare MB, Khude MR, Randive SN, Jamgaonkar AV, Ray K, Mishra AC.

K Ray

- Attended meeting on Options for the control of influenza VI at Toronto, Ontario, Canada, June 17, 2007 to June 23, 2007

AK Chakrabarti

- Attended meeting on Options for the control of influenza VI at Toronto, Ontario, Canada, June 17, 2007 to June 23, 2007. Delivered a talk entitled "Development of PR8 reassortant vaccine strain against A/Chicken/India/NIV-33498/06 (H5N1)" that was carried out at CDC, Atlanta. Discussion session with Dr. Erich Hoffmann of St. Jude Children's Hospital, Memphis, TN, USA regarding future collaboration.
- Invited talk in an international symposium on AI, New Delhi 28-31 Oct. 2007, Title: "Reverse Genetics Modified Indian H5N1". Worked as a repertoire for a session in the same symposium.
- Guided our team in Real-Time PCR Training at Applied Biosystems authorized training center at Gurgaon (Lab India).
- Participated in CDC workshop "Real Time PCR detection of Avian Influenza virus" at NIV, 8-15 November 2007. Worked as a faculty to facilitate the workshop.

- Involved in teaching M.Sc. virology students.

Santosh Koratkar

- International Symposium on Avian Influenza: Epidemiological, Basic and Applied Research, Oct. 29-31, 2007.
- Training Attended workshop on Real-Time detection of Human and Avian Influenza, at NIV Pune, 8-15 November 2007.

Sadhana Kode

- Conference Attended and Presented Poster- International Conference on Emerging and Re- Emerging Viral Diseases of the Tropics and Sub- Tropics, December 11-14, 2007, New Delhi.

Biswajoy Pal

- Real- Time PCR Training at Applied Biosystems authorized training center at Gurgaon (Lab India).
- Participated in CDC workshop “Real Time PCR detection of Avian Influenza virus” at NIV, 8-15 November 2007.
- Attended international conference “ICVT' 07 at ICAR, New Delhi, presented principal authored poster at the conference.

Satish Raut

- Real- Time PCR Training at Applied Biosystems authorized training center at Gurgaon (Lab India).
- Participated in CDC workshop “Real Time PCR detection of Avian Influenza virus” at NIV, 8-15 November 2007.

Vishal Thite

- Conference Attended and Presented Poster- International Conference on Emerging and Re- Emerging Viral Diseases of the Tropics and Sub- Tropics, December 11-14, 2007, New Delhi.