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Editorial

Avian influenza: Expect the best but prepare for the worst

Winter is drawing to a close and for that reason the scare of avian influenza is also fading for the season. What is in store for us in the coming years is not given to us to foresee. If it is not influenza, then surely something else will come. With severe acute respiratory syndrome (SARS) and with avian influenza, India was lucky in the past. Only so far. There was the jaywalker who, when warned, said he was doing it all his life without any mishap, only to be run over the next minute. With emerging infectious diseases what can be predicted is that the unpredictable will happen. A nation with such rich resources of science and technology as India, surely is expected to be prepared to face outbreaks, and also to help nations around us less endowed with the expertise we have.

Humans have lived with influenza viruses for centuries and we thought we knew all about their inter-host transmissions, antigenic shift, drift, epidemics, pandemics and vaccines. The scene has changed drastically since 1997 with the Hong Kong experience of fatal avian influenza H5N1in humans and the culling of close to two million chicken and ducks to intercept further transmission. During the winter of 2003-2004, H5N1 influenza virus turned up in chicken farms in South Korea, Taiwan, China, Hong Kong, Viet Nam, Cambodia and Thailand. We must anticipate its arrival and spread in India one day and every component in our response for its containment must be thought through and designed now, before and not after the event. They must include diagnostic virology, disease surveillance, epidemiology intelligence, veterinary virology and epizootology and most important, research into the biology, genetics and antigens of avian, mammalian and human influenza viruses. Research must be commissioned and funded by the public health wing of the Government of India. Now. It is not sufficient to be left simply to the investigative curiosity or inspiration of interested individuals. While such exploratory research is to be encouraged and supported, the public health leadership must articulate and ask their questions for our research agencies to answer on contractual commitment. Traditional research-promoting agencies like the ICMR already have their agenda and constraints of support systems for epidemics as these. Experience of researchers has been, in general, that the supply of research findings did not drive demand by the public health wing. The time has come for the Government to demand information and solutions for public health problems through research. The Government, on its part, should not be tardy or miserly to provide budget for such targeted research specifically addressing the problems of influenza viruses and SARS coronavirus, remembering that the spin-off will be a bonus of new and commercially exploitable intellectual property. Moreover, once we face these and other emerging pathogens through new R&D, we will be confident and competent to confront such epidemics in future with little lag time.

The functional properties of influenza viruses are controlled by its genome of segmented RNA in 8 parts. The two envelope glycoprotein antigens, the hemagglutinin (H) and the neuraminidase (N) determine host specificity and efficiency of infection and transmission on the background of the susceptible cells of the host species. The ligand for attachment to host-cell is the H antigen and post-replication budding of new virions at the cell surface requires the enzymatic release by the N antigen. Outbreaks and epidemics occur with influenza A viruses and B and C viruses are less frequent and generally less pathogenic. There are 15 H and 9 N antigen types among influenza A viruses.

The host species of influenza viruses may be considered in five groups. They are wild birds, domestic birds, large land mammals, sea mammals and humans. Wild birds include migratory water birds, shore birds, pheasants and quails. Domestic birds include chicken, ducks, turkeys and quails. Land mammals are swine and horses. Sea mammals are

seals and whales. H1, N1 and N2 are found in human, swine and bird viruses. H3 is shared among human, swine, horse and avian viruses. H2 is shared by human and bird viruses. Birds are susceptible to viruses with any H and any N antigens. The common human agent was at one time H1N1 and currently H3N2 virus, which occurs in regular annual winter epidemics in temperate regions. The 1957 pandemic was due to H2N2 virus. In 1988-89 in China and in 2002 in the UK and Middle East there were limited outbreaks due to H1N2, a reassortant between H1N1 and H2N2. Such 'shifts' are uncommon events, but point mutations alter the antigenic fit of the surface glycoproteins, thus causing antigenic 'drift'. Such variants seem to develop by virus interchange between wild and domestic birds and then between the latter and swine. So far evidence was that humans got new viruses only from swine, but not directly from birds. In 1997 in Hong Kong chicken/duck virus H5N1 infected humans directly. In 1998 and 99, there were small clusters of human cases due to H9N2, another avian influenza virus.

In 1997, there were 18 cases of human infection with H5N1 with 6 deaths, in acute respiratory distress syndrome, or multi-organ failure, mostly in young adults, not senior citizens. The source was an outbreak in chicken farms and live markets. With culling of all chicken and ducks on the island, the episode ended, without evidence of secondary human-to-human spread. The same virus reappeared on chicken farms in May 2001, February 2002 and February 2003. The next human episode was in January 2003, a family of 5 persons traveling from Fujian Province of China to Hong Kong, in which the father and two children died. This episode of avian influenza seems to have delayed the recognition of SARS as a new disease. The current winter season, from mid December 2003, saw avian influenza (called fowl plague or fowl pest) outbreaks due to H5N1 virus in Republic of Korea, Viet Nam, Japan, Thailand and Cambodia. In affected farms thousands of birds died and rapid culling of infected or exposed poultry resulted in virtual halt of the outbreaks. Human cases were documented in Viet Nam and Thailand, not elsewhere. Of the seven cases in Viet Nam, six died. In Thailand three cases were diagnosed and two, both children, died. The sudden appearance of H5N1 virus in several countries led to speculation that migratory birds might have carried the virus from somewhere. Southeast China, placed rather centrally for the semicircular distribution of the countries with outbreaks, became a prime suspect. Indeed, by the end of January, China confirmed the presence of the virus in a duck farm in Guangxi province. In addition, Ducks were found infected in Hunan and chicken in Hubei.

Much introspection is warranted in India. Will we diagnose the viral aetiology of influenza causing death in all major institutions? Will any outbreak in chicken or duck farms be rapidly investigated with adequate methods for detecting the causative infection, whether it be avian influenza virus or anything else? Will our local public health authorities disclose information or will they hide it? Are there sufficient linkages of information between veterinary disease surveillance and human outbreaks? We need to seek answers to these. Now.

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Commentary

Rapid diagnosis of rotavirus infection : key to prevent unnecessary use of antibiotics for treatment of childhood diarrhoea

Rotavirus is an important aetiological agent of acute diarrhoea below 4 years of age. It has been estimated that each year rotavirus infection is responsible for an estimated 111 million episodes of diarrhoea requiring only home care, 25 million clinic visits, 2 million hospitalizations, approximately 4,40,000 deaths in children < 5 years of age and most of it occurs in developing countries (1). An estimated 1,205 children die from rotavirus diseases each day and 82% of this death occurs in children in poorer countries of the world. A recent review of 27 prospective studies from 20 countries estimated the incidence of diarrhoea as 3.8 episodes per child per year for children $\leq$ 11 months of age and 2.1 episodes per child per year for children 1-4 years of age[1].

Since every child will be infected at least once in first five years of life and 82% of total death toll occurs in developing countries, control of rotaviral diarrhoea is an important proposition from public health point of view. The need to develop a safe and efficacious rotavirus vaccine to reduce disease burden and prevent deaths remains a very high priority for control of diarrhoeal diseases. Vaccine for control of rotavirus diarrhoea developed are either against a single serotype of animal or human origin or a quadrivalent vaccine comprising of 4 important serotypes (Serotypes 1-4) to reduce severity of diseases. The vaccines conferred protection to children in countries of their origin but failed during field trial in some developing countries. On the other hand, a vaccine with very high efficacy is yet to be developed. As an Indian initiative, two rotavirus vaccines were developed by using a nursery strain, 116E [2] and by using an asymptomatic naturally occurring human - bovine reassortant strain, I321[3] that are currently under phase I trial.

Though the ability of natural rotavirus infection and rotavirus vaccine to reduce the severity of rotavirus gastroenteritis has been proved, the lack of reliable laboratory marker that can predict immunity has hampered due to a clear understanding of the mechanism of protective immunity against rotavirus[4,5]. It is likely that mucosal immunity in small intestine is critical in the defense against rotavirus infection[6,7].

The segmented nature of rotavirus genome provides a unique mechanism for the generation of genetic diversity by the process of genetic reassortment that occurs during mixed infections *in vivo* as well as *in vitro*[8]. When this occurs between bovine and human strains, the progeny becomes asymptomatic[9]. On the other hand, one such reassortant strain between human and porcine has been implicated for its association with an outbreak of infantile diarrhoea[10,11]. Development of an efficacious vaccine to control rotavirus diarrhoea is not forthcoming and naturally occurring reassortment involving VP6 and NSP4 of porcine origin, two crucial proteins thought to be responsible for host range restriction and pathogenicity has been implicated in an epidemic of acute infantile gastroenteritis[11], therefore, the only option left is rehydration of the infants with ORS / or IV fluid therapy.

In developing countries, the treatment of diarrhoea cases starts with feeding ORS solution / IV fluid therapy followed by treatment with antibiotics. It is well known that administration of antibiotics will not be of any help in case of rotavirus diarrhoea or any other viral diarrhoea. Therefore, rapid diagnosis of rotavirus infection is the need of the day.

A number of diagnostic assays have been developed to detect the virus and / or to demonstrate the serological response induced by the viruses in the host. A number of diagnostic tests have been developed for detection of rotaviruses, however they are not routinely used due to low sensitivity and or specificity. On the other hand, detection of rotaviruses by reverse transcriptage polymerase chain reaction (RT-PCR) method for detection of rotaviruses is highly sensitive, however the system needs costly equipments, reagents and trained manpower and cannot be performed

as a routine laboratory diagnostic test for detection of rotaviruses in developing countries. The only diagnostic test, the enzyme linked immunosorbent assay (ELISA) is considered to be highly sensitive tool for screening of rotaviruses from hospital or field samples, because of its ability to detect positive isolates even at low concentrations. The pitfalls of ELISA test include failure to detect viral antigen in stool samples containing a high titer of the corresponding antibody and false positive results. However, regardless of its limitations, ELISA is still the method of choice in almost all laboratories for the identification of rotaviruses.

The article by SD Kelkar and colleagues in this issue of the Journal[12] addresses the need of a rapid diagnosis of rotaviruses by ELISA test. The investigators have developed an ELISA kit by using cell culture adapted semi purified simian rotavirus strain SA11 as antigen and compared the same with the ELISA kit (NIV-ELISA) developed by them previously. The sensitivity and the specificity of the short duration (< 4 hours) ELISA were as good as when compared with NIV-ELISA. On the other hand, the optical density values of positive samples were higher than NIV-ELISA. In addition, the plates pre-coated with antigen that are stable for over two weeks in a refrigerator, thereby substantially reducing the incubation period during the test. During development and evaluation of the ELISA kit, they have used only 155 stool samples, which are very small in number, and the kit needs to be tested in different laboratories to substantiate their claim. On the contrary, the new ELISA kit with short duration of incubation developed by NIV investigators seems to be very useful in providing diagnosis of rotaviruses in a short duration thereby helping the clinicians and saving unnecessary use of antibiotics and expense for treatment of diarrhoeal diseases.

Multi-drug antibiotic resistance was reported during an epidemic of Shigellosis in eastern India in the year 1984 due to indiscriminate use of antibiotics for treatment of Shigellosis. Therefore rapid diagnosis of rotavirus infection in children will reduce the unnecessary use of antibiotics and chances of development of antibiotic resistant strains.

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