Biosafety professionals have ever-expanding roles at their institutions. In this Beyond Traditional Biosafety column, we focus on topics that may fall outside the scope of the traditional biosafety role, but where the expertise of the biosafety professional may be called upon to provide a valuable contribution to his or her institution. Please e-mail any comments or suggestions to Ted Myatt, tmyatt@mail.uri.edu, Co-Editor Barbara Johnson at barbara_johnson@verizon.net, or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Influenza Virus—Controversy Over Modes of Transmission

Biological safety officers may be asked to weigh in on public health issues relevant to the workforce of their institutions. While biosafety officers surely have been following the controversy on H5N1 avian influenza and the moratorium on H5N1 influenza research, during flu season they may be asked more general questions regarding influenza, such as how influenza is transmitted and what they should do to protect the workforce and their families. This is particularly relevant during severe flu seasons. The focus of this article is the debate surrounding the route or routes of transmission of influenza virus and methods to control the spread of influenza.

Transmission

The direct routes of transmission for respiratory viruses include contact of mucosal surfaces with the virus and inhalation of aerosolized tiny droplets, referred to as droplet nuclei, from an infected individual. Indirect contact between oral, nasal, or ocular mucosal surfaces in a susceptible host and virus can also occur through autoinoculation (e.g., fingers of susceptible host touching mucosal surface) by contact with contaminated inanimate objects (fomites). Direct contact also includes an intermediate route whereby a mucosal surface of a susceptible individual is directly hit with a large droplet (>10 to 100 microns diameter) generated from the respiratory tract of an infected individual (Fabian, 2005). Large droplets have sufficient momentum to travel approximately 1 meter through the air and are therefore considered “direct contact” due to the ballistic nature of the particle and the close proximity of the infectious individual to the susceptible host. The act of sneezing, coughing, and even talking can release both large and small respiratory droplets. The smaller droplets of respiratory fluid evaporate quickly with most of their mass lost within seconds. These evaporated droplets are called droplet nuclei (Riley & O’Grady, 1961) and are responsible for the second type of transmission that may occur. This route is typically referred to as the airborne route as droplet nuclei (typically considered less than 1 micron) can be carried on air currents within and between rooms and act as a mechanism for transmission between the infectious individual and the susceptible host.

Important variables influence all routes of transmission, such as the ability of the virus to survive on inanimate object surfaces, survival in the air, and the rate of dilution ventilation in the case of droplet nuclei. Influenza virus survival is dependent on environmental conditions such as temperature, relative humidity, and the composition of surface materials (Cole & Cook, 1998; Roy & Milton, 2004). For example, influenza virus has been shown to survive longer at lower humidity levels (Harper, 1961). There are also important individual-based variables, such as the differences in the ability of an infected individual to shed virus (i.e., generation rate) and the difference in host susceptibility due to immune system function (Fabian, 2005).

Importance of Understanding the Route of Transmission

Understanding the route of transmission of individual organisms is important since knowing the route helps to tailor specific methods to prevent transmission. For example, if a specific virus cannot be transmitted through droplet nuclei, then barrier protection would be sufficiently effective (gloves, gowns, face shields). However, in such circumstances the use of an N95 respirator would not be useful. Unfortunately, definitive data on the primary route of transmission for influenza virus are limited. Additionally, respiratory viruses do not react to environmental conditions in the same way (e.g., influenza virus survives best at low humidity, rhinovirus survives best at high humidity). As the route of transmission is partially dependent on survival in the environment, the data available for one virus, are not applicable for influenza virus.

Evidence for Influenza Transmission

While there is a wealth of clinical data on influenza (Moscona, 2008), data specific to how influenza is transmitted are relatively limited and in many cases the studies were conducted decades ago. Data that inform our understanding of the route of transmission predominately include epidemiology studies and animal infection models. Human studies may be related to specific outbreaks in natural settings or, to a minor degree, laboratory-based experimental exposure studies.
Only one intervention study specifically designed to control airborne transmission of influenza in a natural environment has been reported (McLean, 1961). The intervention study was conducted during two waves of influenza starting in 1957 at a Veterans Administration Hospital. In one wing of the hospital, upper room germicidal ultraviolet radiation (UVC) was installed to prevent tuberculosis transmission; in the other wing, it was not. Upper-room UVC specifically affects transmission by the airborne route as it only impacts organisms in the airspace near the ceiling and has no effect on large particles or on contaminated surfaces in the lower (occupied) part of the room. Over the course of waves of illness, 18% of staff and 19% of patients in the wing without UVC developed a 4-fold rise in influenza titers. However, only 2% of patients in the wing with UVC seroconverted (McLean, 1961). While this study strongly supports the airborne route as the primary route of transmission, it is essentially the only study specifically designed to evaluate human-to-human transmission in a natural environment.

Other epidemiologic literature relevant to the route of influenza transmission consists largely of outbreak reports and, therefore, is not specifically designed to determine how influenza is spread (Drinka, 1996; Moser et al., 1979). The most often cited is an influenza outbreak reported by Moser et al. (1979) among passengers on an airplane grounded for 4.5 hours with its ventilation system shut-off. One passenger became ill within 15 minutes of boarding. Of 54 other passengers, 25 of 29 who remained on board throughout the delay and 7 of 25 who deplaned for part of the delay became infected with influenza. The infected passenger had not been in close contact with most of the passengers, so that airborne droplet nuclei appeared to be the most plausible explanation. A study based on influenza surveillance data from a multi-building nursing home demonstrated that one building with attributes that would likely reduce the risk of airborne transmission, such as higher ventilation rates and higher square footage per resident, had lower influenza attack rates than other buildings. These results suggested that influenza may have been transmitted via the airborne route (Drinka et al., 1996). However, further studies did not confirm this trend (Drinka, 2002), and the authors later concluded the results from the early study were not very robust (Drinka et al., 2004). In a reanalysis of the data from the 1996 study, Rudnick and Milton (2003) concluded that very high ventilation rates would have been needed to control the spread of influenza (Rudnick & Milton, 2003). In a recent example of an experimental study, small groups of participants were exposed to aerosolized influenza while wearing varying degrees of personal protective equipment (Bischoff, 2011). The results indicated that ocular transmission occurs as infection occurred in participants with N95 respirators without eye protection. Bischoff and colleagues collected air samples near infectious patients in a hospital setting. They were able to recover influenza in small aerosol particles via PCR at distance of 6 feet (Bischoff, 2013). These results provide suggestive evidence that aerosol transmission is possible.

A larger body of animal experimental studies exists compared to human studies, and it demonstrates infection transmission by droplet nuclei, both from experimental aerosols and in animal-to-animal transmission experiments (Andrewes, 1941; Edward, 1943a; Edward, 1943b; Koster, 2012; Schulman, 1962; Schulman, 1967; Schulman, 1968; Steel, 2011). For example, Edward et al. (1943) infected mice via an influenza aerosol generator (Edward, 1943a) and also showed that mice exposed to UVC-treated airborne influenza virus did not develop the infection, while 100% of the mice exposed to untreated airborne influenza either died or developed significant disease. These results indicate that aerosolized influenza virus is capable of causing infection and that UVC is capable of inactivating the aerosolized virus (Edward, 1943b). Several studies have evaluated the potential for influenza to be transmitted animal-to-animal via the airborne route in both ferrets and mice. For example, in one experiment (Andrewes, 1941), influenza virus transmission from sick to healthy ferrets was achieved through ventilated ducts that separated the two sets of animals. The ducts were designed to eliminate the potential for large droplet transmission. Ferrets became sick in all experiments, supporting the hypothesis that the influenza virus was transmitted via droplet nuclei. Schulman and Kibbourne (1962) developed a mouse model for influenza and found an inverse correlation between air exchange and infection rate regardless of whether mice were able to mingle in one cage or were separated by two layers of wire mesh, indicating that droplet nuclei were the vehicle of transmission. In subsequent experiments (Schulman, 1967; Schulman, 1968), Schulman showed that the ability to detect influenza in exhaust air from the cage of infected mice correlated with the transmissibility of the virus strain. More recently, animal experimental research in the area of influenza transmission has expanded. For example, in studies of guinea pigs, aerosol transmission between animals was modulated by the environmental conditions (e.g., air temperature and relative humidity) (Steel, 2011). In a ferret animal model, animals were intranasally infected with 2009 H1N1 pandemic strains of influenza. All three strains (swine-origin pandemic 2009 H1N1 viruses A/California/04/2009 [Cal/04], A/Mexico/4482/2009 [Mex/4482], and A/California/07/2009 [Cal/07]) were transmitted to susceptible animals (Koster, 2012).

While some animal studies suggest that airborne transmission occurs in humans, there are questions related to these studies. For example, differences between humans and animals may impact influenza transmission (e.g., guinea pigs lack clinical signs of infection that are observed in humans [Van Hoeven, 2009]). Additionally, none of the animal studies investigated if transmission occurs over long distances where the influenza virus may become noninfectious in the air and diluted via ventilation. In fact, in a number of early studies with infected and susceptible animals, the route of transmission was not controlled (e.g., the in-
fected and susceptible animals were housed in the same cage) and therefore these experiments provide no evidence for transmission via droplets (Andrewes, 1941; Schulman, 1963a; Schulman, 1963b). Finally, no studies were identified that specifically evaluated droplet or autoinoculation routes of transmission.

Clearly, overwhelming evidence does not exist showing that airborne transmission is the dominant or primary route of transmission for influenza. However, the data described above seem to support the notion of airborne transmission. Additionally, there is a lack of data contradicting the notion of airborne transmission or that indicate airborne transmission cannot occur. The lack of evidence does not prove anything; however, it has been taken into consideration when developing guidance on appropriate precautions to prevent transmission.

**Current Recommendations for Flu Prevention**

Vaccination is the best option for preventing transmission of influenza (CDC, 2010; WHO, 2009). In the absence of vaccination or in the case of a mismatch between the vaccine and the circulating strain of influenza virus, the intervention/control methods used to prevent influenza transmission should be suited to the route of transmission to be effective. For example, barrier methods such as gloves and gowns would be minimally effective against transmission by droplet nuclei or increased ventilation rates would have no impact on direct transmission of influenza via fomites. During the 2009 H1N1 influenza pandemic, the Institute of Medicine (IOM) recommended that healthcare workers use protection against all routes of transmission when in contact with infected patients (IOM, 2009). Similarly, the CDC recommends a combination of infection control strategies to prevent transmission in healthcare settings: administration of influenza vaccine, implementation of respiratory hygiene and cough etiquette, placing influenza patients in private rooms when possible, and having healthcare personnel wear masks for close patient contact (i.e., within 3 feet) and gowns and gloves if contact with respiratory secretions is likely. Recently, a study demonstrated that influenza in small particle aerosols could be detected beyond 3 feet of the infectious individual, calling into question the recommendation of wearing a mask only for close patient contact (Bischoff, 2013). The CDC acknowledges that no studies have definitively shown that mask use by either infectious patients or healthcare personnel prevents influenza transmission and masks are not usually recommended in non-healthcare settings (CDC, 2009; CDC, 2010a). For non-healthcare environments, vaccination continues to be the best option for preventing influenza infection, and prevention recommendations are similar to those for healthcare facilities (avoid close contact, stay at home when sick, cover your mouth and nose when coughing and sneezing, clean your hands, and avoid touching your eyes, nose, and mouth (CDC, 2010b). These recommendations are largely focused on preventing transmission via direct and indirect routes and are easily implemented. Strategies such as isolation would limit wide-scale transmission from all routes of transmission, but continue to leave family members at risk for airborne transmission. Prevention of airborne transmission is more problematic and relies on more technical interventions such as the use of respirators or air filtration or disinfection (i.e., UVC) and is likely not feasible for wide-scale implementation by the general public.

**References**


Centers for Disease Control and Prevention (CDC). (2010a). *Preventing the flu: Good health habits can help stop germs.* Atlanta: CDC.


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**Training Announcements**

**Advanced Biosafety Training Series Modules**

These modules are online offerings targeting intermediate to advanced biosafety professionals. Each module will address essential elements at a level appropriate for someone preparing to take the NRCM Biological Safety Specialist exam or interested in advanced training. The following modules are available on the ABSA web site at www.absa.org/educbsp.html.

- Risk Assessment and Hazard Identification: Infectious Agents, rDNA & Occupational Health Issues
- Regulatory Aspects Pertinent to Risk Assessment: NIH Guidelines, Select Agents & Animal Biosafety
- Facility Design & Large-scale Biosafety Issues
- Disinfection, Decontamination & Sterilization
- Work Practices, Equipment Biohazards and Personal Protective Equipment
- Equipment, Biological Safety Cabinets & Bioaerosols
- Regulatory Aspects, Standards, Guidelines, Institutional Biosafety Committees (IBCs) & Emergency Response

**Webinars**

The “Steam Sterilization and Gas Decontamination Systems for Laboratory Use” webinar will be held on May 15 and 22, 2013 from 1:00-3:00 p.m. Central Time. The “Basic Disinfection” webinar will be held on June 6 and 13, 2013 from 1:00-3:00 p.m. Central Time—additional information is currently available at: www.absa.org/edudisinfection.html. The “Q&A Infectious Substance Shipping” webinar will be held on July 11, 2013 from 1:00-3:00 p.m. Central Time.