

# Particle size and pathogenicity in the respiratory tract

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Particle size dictates where aerosolized pathogens deposit in the respiratory tract, thereafter the pathogens potential to cause disease is influenced by tissue tropism, clearance kinetics and the host immunological response. This interplay brings pathogens into contact with a range of tissues spanning the respiratory tract and associated anatomical structures. In animal models, differential deposition within the respiratory tract influences infection kinetics for numerous select agents. Greater numbers of pathogens are required to infect the upper (URT) compared with the lower respiratory tract (LRT), and in comparison the URT infections are protracted with reduced mortality. Pathogenesis in the URT is characterized by infection of the URT lymphoid tissues, cervical lymphadenopathy and septicemia, closely resembling reported human infections of the URT. The olfactory, gastrointestinal, and ophthalmic systems are also infected in a pathogen-dependent manner. The relevant literature is reviewed with respect to particle size and infection of the URT in animal models and humans.

## Introduction: The Ubiquitous Nature of Bioaerosols

Bioaerosols are defined as a collection of particles suspended on a column of air derived from or incorporating material of biological origin. Individual particles within the bioaerosol can vary markedly in aerodynamic diameter from submicron (e.g., viruses) to many millimeters (e.g., pet dander). Importantly, bioaerosols can affect human health due to the presence of pathogens or allergens. The sources of bioaerosols containing pathogens are varied and individual aerosol particles may vary widely from submicron to several millimeters dependent on the method of aerosol generation and attachment to larger particulates, for example skin cells (Table 1); although only those <100  $\mu\text{m}$  are within the inhalable range for humans, it should be noted that the initial particles will rapidly evaporate depending on the humidity of the local environment.<sup>4,33,34</sup> The final inhaled particle size is dependent on a number of factors including the solid organic content of the initial particle (including pathogens) and location of an individual to the aerosol source. It is evident that even pollen grains that represent comparatively larger bioaerosols

can travel large distances in conducive meteorological conditions.<sup>15,35</sup> Airborne dissemination whether outdoors or indoors is influenced by a number of inter-relating factors that impact on air mass movement, turbulence, and thermal convection including meteorology, vehicle/human activity, and ventilation that may far outweigh the terminal velocity of particles that are generally calculated in very still air.<sup>4,34</sup> This review will concentrate on aerosols containing pathogens and the inter-relationship between factors governing particle size, deposition site, clearance, and inhalational infection.

## Aerosol Transmission: Relation to Mechanism of Generation

The mechanism of generation influences the particle size of the resultant bioaerosol (Table 1), and these may be biotic (e.g., sneeze or pollen), or abiotic where the aerosol is produced by a non-living system (e.g., water cooling towers). Irrespective, all aerosols will be generated with an initial mass median aerodynamic diameter (MMAD) that will decrease with increased distance from the source due to evaporation and settling dependent on environmental parameters such as relative humidity and turbulence.

All mechanisms of human oro-nasal activity such as breathing, talking, laughing, coughing and sneezing produce particles within the inhalable range for humans of <1 to >100  $\mu\text{m}$  (Table 1). Significant variation occurs between studies regarding number of particles expelled, size range of the particles and the number of pathogens incorporated within the particles, attributable to differences in methodology and human factors where standardization is difficult.<sup>21,26,28,36,37,39</sup> Comparatively, coughing and sneezing produce greater quantities of particles<sup>28,36,37</sup> that travel further due to the velocity of expulsion from the nose or mouth.<sup>40</sup> The majority of these particles reside in the inhalable fraction for humans (i.e., <100  $\mu\text{m}$ ) at 78.6–96.0% and 98.9% for coughing and sneezing respectively; while of this inhalable fraction, 7.1–46.7% and 18.8% produced by coughing and sneezing were less than 4  $\mu\text{m}$ , evaporating to droplet nuclei and deposit in the bronchoalveolar region of the lung.<sup>28,36,37</sup>

The situation in relation to deposition is more complex due to evaporation. Atmospheric relative humidity (RH) and temperature are generally lower than that of the body. Once the particles are in the atmosphere evaporation occurs at rates according to their original size and composition of the particle to reach equilibrium with atmospheric conditions. Hence, the aerosol

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**Table 1.** Sources of bioaerosol

Bioaerosol source	Mechanisms	Particle size distribution <sup>a,b</sup>	Reference(s)
Healthcare	Surgical or dental procedures	Up to 50 µm <sup>c</sup>	1 and 2
	Hospital air	<2 µm (22%), 2 to 6 µm (30%), >5 µm (48%)	3
	Mechanical ventilators, bed making, and resuspension on dust or skin squamae	0.3 to >5 µm	4 and 5
Water industry	Cooling towers	<5 up to >100 µm (bimodal peaks at <5 µm and 20–40 µm)	6
	Wastewater irrigation sites	1.0 to 5.9 µm	7
Agricultural/forestry industries	Grain harvesting, food processing, dust, and/or feces from animal housing and farming activities	0.9 to 18.9 µm 0.5 to >5 µm (increase in 2 to >5 µm range)	8 and 9
	Insecticidal crop spraying	4.6 to 39 µm <sup>d</sup>	10 and 11
		12.3 to 37.1 µm <sup>c</sup>	12
		15 to 45 µm <sup>c</sup>	13
	Genetic dispersion	60 to 100 µm (kromecote card); 5.3 to 7.3 µm (Anderson cascade impactor)	14
		Pollen grains: 10 to 100 µm <sup>e</sup> Fungal spores: 1 to 50 µm <sup>e</sup>	15 16
Postal and shopping industry	Mail sorting and opening	0.3 to >5 µm; 19.6-fold increase in particles >5 µm	17
	Mist machine	"between 40 and 70 µm"	18
Leisure activities	Marine activities, e.g., surfing	24 to 44 µm, median 34 µm	19
	Whirlpools	<1 to 15 µm dependent on turbulence	20
Human activity	Breathing	<0.8 to 2 µm	21
	Speaking	16 to 125 µm	22 and 23
		<0.8 to 7 µm	21
	Shouting	0.5 to 10 µm (mean = 1.0 µm)	24
	Coughing	0.62 to 15.9 µm	21, 22, and 25–27
		40 to 125 µm	23 and 28
	Sneezing	7 to 125 µm	28 and 29
	Vuvuzela playing	0.5 to >10 µm (mean = 1.3 µm)	24
Showering	Hot water (5.2 to 7.5 µm)	30	
	Cold water (2.5 to 3.1 µm)		
Miscellaneous	Pulp waste water treatment plant	2.4 to 3.5 µm (median); 99.9% of particles were below 15 µm	31
	Building tower (sweeping dust containing pigeon feces)	1.1 to 11.0 µm	32

<sup>a</sup>Aerodynamic diameter; <sup>b</sup>distributions should be viewed with caution as often experiments used samplers with cut off limits less than 15 µm and therefore were preferentially selective for particles smaller than this size; <sup>c</sup>spray-dried *Bacillus thuringiensis* produced at different homogenization speeds; <sup>d</sup>non-biological aerosols for vector control; <sup>e</sup>procedure- or species-dependent.

produced is dynamic, changing with distance from the initial point of generation. Particles produced from sneezing and coughing will contain varying amounts of saliva and mucus comprising inorganic and organic ions plus glycoproteins.<sup>33,38</sup> Many aerosol transmission modeling studies are based on assumptions e.g., settling velocities and/or evaporation parameters of pure water droplets within a vacuum and droplet distributions from healthy volunteers.<sup>21,22</sup> It is probable that irrespective of composition due to the small droplet sizes originating from a cough or sneeze that evaporation will be rapid unless the presence of solutes greatly retards evaporation, for example, a 5 µm water droplet will

evaporate within 0.8 s in 97% RH.<sup>21</sup> However, extrapolating to natural situations where the droplet composition will be very different and turbulence will exert a large effect on how rapidly particles deposit requires care. Indeed, one recent study demonstrated that infected individuals generated larger aerosol particles than healthy counterparts.<sup>41</sup> This could be attributable to differences in mucus (composition, quantity, and viscosity) produced during infection affecting evaporation and the location of the infection.<sup>33,42</sup> The closer an individual is situated to an aerosol source then the greater the likelihood of large particles being inhaled prior to complete evaporation.

**Table 2.** Tissues that may represent the primary site of deposition, clearance and infection during exposure to a bioaerosol

Anatomical region	Tissue(s)	Function	Epithelium
Nasopharyngeal/ Oropharyngeal	Nares to nasopharynx	Inhalation, filtration, and mucociliary clearance of foreign particles; warming/humidification of air	Ciliated
	Paranasal sinuses	Humidification of air; mucociliary clearance of foreign particles	Ciliated
	Olfactory epithelium	Detection of odors	Sensory
	Mouth to oropharynx	Mastication and ingestion; inhalation	Non-ciliated
	Tonsils, adenoids <sup>a</sup>	Defense against inhaled/ingested pathogens; URT mucosal immunity	Lymphoepithelial
Tracheobronchial	Larynx	Sound generation; mucociliary clearance	Ciliated/non-ciliated
	Trachea	Air conduction; mucociliary clearance	Ciliated
	Bronchi to bronchioles	Air conduction and particulate filtration; mucociliary clearance	Ciliated
Pulmonary	Respiratory bronchioles to alveoli	Air conduction; gaseous exchange; pulmonary clearance and immunity	Non-ciliated
Ocular	Ocular conjunctiva	Lubrication of ocular region	Non-ciliated
	Nasolacrimal duct	Drainage of excess tear fluid	Non-ciliated
Gastrointestinal	Esophageal, stomach and intestinal epithelium	Digestion and absorption of ingested material, including inhaled particles trapped in the mucociliary escalator	Non-ciliated
	Peyer patches	Defense against ingested pathogens	Lymphoepithelial

<sup>a</sup>Nasal-associated lymphoid tissue (NALT) represents the rodent equivalent.

Similar principles can be extrapolated to any aerosol present in Table 1, simply the mechanism of generation, the solute type and concentrations (organic and inorganic) plus the surrounding environment will differ and therefore the processes of evaporation and dissemination will accordingly vary. Irrespective of whether aerosol particles are generated by abiotic or biotic processes (e.g., Table 1), inhalation of particles into the warm humid respiratory tract will prompt rehydration and affect deposition due to particle growth.<sup>43</sup> It is evident that there is significant potential for URT deposition depending on how close an individual is to the source.

### Initial Site of Deposition and Infection is Dependent on Particle Size

The respiratory tract is complex, comprising a collection of specialized organs, tissues, and cells ranging from the nares to the alveoli.<sup>44</sup> These tissues convey a range of physiological functions connected to breathing (i.e., air conditioning, air conductance, and gaseous exchange) and defense against foreign particulates (i.e., immune function and mucociliary or phagocytic clearance). After exposure to a bioaerosol containing pathogens, the initial site of deposition where infection may ensue is likely to be the respiratory epithelium. However, due to the interconnecting anatomical features and clearance mechanisms within the mammalian body, the ocular conjunctiva, olfactory epithelium, or URT immunological tissues may represent further sites where infection could initiate after inhalational exposure to a pathogenic aerosol (Table 2; Fig. 1). Various viruses with dual tropism for both ocular and respiratory tissues utilize the nasolacrimal duct to produce URT infection (e.g., influenza virus, respiratory syncytial virus, and adenovirus).<sup>45</sup> Depending on clearance mechanisms and the sensitivity of the pathogen to stomach acidity,

gastrointestinal (GI) tissues may represent a further portal. The enteric Norwalk-like virus has been demonstrated to be transmitted by aerosol, presumably via droplets produced during diarrhea and vomiting.<sup>46,47</sup> Similarly, aerosol dissemination of *Clostridium difficile* spores has been observed in hospital wards<sup>48</sup>; however this has not been conclusively linked to infection via inhalation.

The aerodynamic diameter of inhaled particles determine where within the respiratory tract pathogens incorporated within the particles deposit and interact with host tissues. A number of mechanisms determine deposition of particles within the respiratory tract including inertial impaction, Brownian diffusion, gravitational sedimentation, and electrostatic effects.<sup>44</sup> Small particles (<1–3  $\mu\text{m}$ ) diffuse deep into the lung tissue, depositing in the alveoli by a number of mechanisms including diffusion, sedimentation, and electrostatic effects. In contrast, larger particles (>8  $\mu\text{m}$ ) impact further up the respiratory airways due to greater inertia, depositing in a size-dependent manner from the nasal passages to the larger bronchioles. This relationship is extant across mammalian species albeit differences in respiratory anatomy and physiology dictate the penetration of a particular particle size into the respiratory tract.<sup>49–54</sup> The size and body shape of the species determines the morphometry of the nasal cavity and respiratory airways influencing the size of the particles that may deposit in corresponding anatomical regions.<sup>51,55</sup> Even within species, variables including age, body weight, breathing mode (oro-nasal/nasal), sex, strain (or ethnicity), activity (e.g., sleeping, exercise), and disease state (e.g., asthma, pneumonia, emphysema, and chronic obstructive pulmonary disorder) influence biometry or affect respiratory physiology and hence deposition profiles.<sup>49,56,57</sup> The propensity of humans to revert to oro-nasal breathing during exertion significantly increases the size of particles that may be inhaled into the respiratory tract due to the comparative size of the oral cavity and bypassing the



**Figure 1.** Schematic of the interplay between deposition sites and clearance mechanisms in the respiratory tract. BALT, bronchial-associated lymphoid tissue; LALT, lung-associated lymphoid tissue; LNs, lymph nodes; NALT, nasal-associated lymphoid tissue.

filtration of the nasal cavity.<sup>49</sup> Differences in deposition can then affect clearance mechanisms and rates and ultimately infection kinetics for an inhaled pathogen (Fig. 1).

### Clearance Mechanisms in the Respiratory Tract

Clearance kinetics are fundamental to determining the dose of deposited pathogens within the respiratory tract and ultimately systemically. A schematic of the interplay between deposition site, clearance mechanisms and pathogen dissemination from the respiratory tract is illustrated in Figure 1. The nose effectively filters foreign particles that enter the nasal cavity in a manner dependent on particle size and air flow rate with filtration efficiency decreasing with particle size.<sup>49</sup> Once deposited, the speed of nasal clearance depends on the deposition location in

the nasopharynx. In healthy humans, clearance from the ciliated anterior region is much more rapid than the non-ciliated poster region, ranging from 1.3 to 12.6 mm min<sup>-1</sup>.<sup>58</sup> These rates are comparable to reported tracheal and bronchial mucociliary rates that range from 0.8 to 12.4 mm min<sup>-1</sup>.<sup>59,60</sup> Similarities exist in animal models however clearance rates are generally more rapid due to the decreased distances required to reach the larynx.<sup>61</sup>

Both the nasal and tracheobronchial escalators comprise mucus that entraps deposited particulates and via the cumulative action of the cilia remove deposited material to the GI tract. Mucus composition is highly variable comprising glycoproteins (mucins), proteins, proteoglycans, and lipids. The quantities of these components present at any particular time govern the viscoelasticity, adhesiveness, and wettability of the mucus and can influence the size of particles emitted by coughing or

**Table 3.** Influence of aerosol particle size on the respiratory lethal dose values for respiratory pathogens

Particle size (µm)	<i>Bacillus anthracis</i> (cfu)		<i>Yersinia pestis</i> (cfu)		<i>Francisella tularensis</i> (cfu)		<i>Brucella suis</i> (cfu)	<i>Burkholderia pseudomallei</i> (cfu)	<i>Coxiella burnetii</i> (cfu)	VEEV (pfu)	EEEV <sup>a</sup> (pfu)		Ricin <sup>b</sup> (µg kg <sup>-1</sup> )
	Guinea pig	Mouse (A/J <sub>sd</sub> )	Guinea pig	Mouse (Balb/c)	Guinea pig	Rhesus macaque					Guinea pig	Mouse (Balb/c)	
1.0–3.0	0.23–3.4 × 10 <sup>5</sup>	2.43 × 10 <sup>3</sup>	0.3–1.0 × 10 <sup>5</sup>	6.01 × 10 <sup>2</sup>	2.5	14–17.3	ANNEX A 1.3–1.9 × 10 <sup>2</sup>	4.0–5.0	1.9 × 10 <sup>6</sup>	20	0.52–1.1 × 10 <sup>4</sup>	4.6–6.2 × 10 <sup>2</sup>	12.2
4.0–6.5	2.21 × 10 <sup>5</sup>	ND	ND	ND	6.5 × 10 <sup>3</sup>	2.4 × 10 <sup>2</sup>	ND	ND	5.27 × 10 <sup>7</sup>	1.9 × 10 <sup>4</sup>	ND	ND	ND
8.5–13.0	0.7–5.7 × 10 <sup>6</sup>	7.65 × 10 <sup>3</sup>	0.7–2.1 × 10 <sup>5</sup>	2.95 × 10 <sup>3</sup>	1.95 × 10 <sup>4</sup>	5.5–8.72 × 10 <sup>2</sup>	ANNEX B 0.88–1.6 × 10 <sup>6</sup>	12.0	> 2.4 × 10 <sup>9</sup>	2.8 × 10 <sup>5</sup>	3.0 × 10 <sup>4</sup>	2.9 × 10 <sup>5</sup>	No lethality
20.0–25.0	ND	ND	ND	ND	ND	3.75–4.45 × 10 <sup>3</sup>	ND	ND	ND	ND	ND	ND	ND
Method 1–3 µm	COLL	COLL	COLL	COLL	NK	Atomizer	COLL	NK	NK	NK	COLL	COLL	COLL
Method >4 µm	STAG	FFAG	STAG	FFAG	NK	STAG	STAG	NK	NK	NK	STAG	STAG	STAG
References	70 and 73	76	71	75	73	69 and 73	72	77	73	73	74	74	78

cfu, colony forming unit; COLL, Collision nebulizer; EEEV, eastern equine encephalitis virus; FFAG, flow-focusing aerosol generator; ND, not determined; NK, not known; pfu, plaque forming unit; STAG, spinning top aerosol generator; VEEV, Venezuelan Equine Encephalitis virus. a. values represent LD<sub>50</sub> values from two virus strains (ArgM and NJ1959); b. no lethality using 45 µg kg<sup>-1</sup>

sneezing.<sup>41,42,62</sup> The diversity of oligosaccharide chains present on respiratory mucins and proteoglycans adds complexity providing a mechanism for microbial interaction and clearance.<sup>63</sup> Factors such as underlying disease both infectious and non-infectious (e.g., cystic fibrosis, smoking, and diabetes) reduce mucociliary clearance rates,<sup>58,59,62,64</sup> hence increasing residence time for deposited pathogens within the respiratory tract.

The pulmonary region is non-ciliated and clearance of foreign particulates is conducted by resident alveolar macrophages that phagocytose particles and transport to the local lung associated lymph nodes and play an important role in pulmonary immunity.<sup>65–67</sup> Across species, numbers of alveolar macrophages increase in response to deposited foreign particles, however inter-species variability exists with respect to the rate of chemotaxis and phagocytosis.<sup>68</sup>

### Pathogenesis as a Function of Particle Size in Animal Models

#### Lung and URT lymphoid tissue infections

No studies exist investigating the effects of particle size on respiratory infection in humans. However, comparative studies in animal models have been conducted using experimental systems that can differentially deposit pathogens in the LRT and URT within small or large particle aerosols.<sup>69–77</sup> The general theme running through studies investigating the effects of aerosol particle size on infectivity is that greater numbers of pathogens need to deposit in the URT to produce lethal infection compared with the LRT (Tables 3 and 4). This is likely a function of the mucociliary escalators present in the nasal cavity and tracheobronchial regions clearing material to the gastrointestinal tract. An increased time to death was observed in animals that inhaled large particles that in time-course studies could be related to differences in pathogenesis between infections initiating in the LRT and URT.<sup>69–77</sup>

The inhalation of pathogens within small particle aerosols results in the typical disease profile associated with deposition in the alveolar region across all the rodent and NHP models used. Bacterial pathogens such as *Francisella tularensis*, *Burkholderia pseudomallei*, *Brucella suis*, and *Yersinia pestis* proliferate within the alveolar spaces or alveolar macrophages causing an influx of neutrophils that contribute to a massive cytokine storm resulting in edema and pneumonic consolidation characteristic of primary pneumonia. Eventually tissue destruction leads to dissemination to visceral organs, septic shock and death.<sup>69–72,75,76,79–82</sup> *B. pseudomallei* is further characterized by the formation of abscesses throughout infected tissues and the potential to relapse after completion of antimicrobial therapy.<sup>77</sup> *B. anthracis* endospores are phagocytosed by alveolar macrophages and dendritic cells and trafficked to lung-associated lymph nodes where they germinate and replicate destroying the lymph node before eventually disseminating via the bloodstream resulting in septicemic shock and toxemia.<sup>76,83</sup>

Deposition of these pathogens within the nasal cavity produces a different disease profile with some similarities across the different bacterial pathogens. Degradation and ulceration of the

**Table 4.** Effect of aerosol particle size and deposition site on infection kinetics and pathology for selected pathogens in animal models of infection

Pathogen	<i>Yersinia pestis</i> (murine)		<i>Bacillus anthracis</i> (murine)		<i>Burkholderia pseudomallei</i> (murine)		<i>Francisella tularensis</i> (Rhesus macaque)	
	1	12	1	12	1	12	1	12
Deposition site	Lungs	Nasal mucosa	Lungs	Nasal mucosa	Lungs	Nasal mucosa	Lungs	Nasal mucosa
MLD (cfu)	$6.01 \times 10^2$	$2.95 \times 10^3$	$2.43 \times 10^3$	$7.65 \times 10^3$	4	12	17	$8.72 \times 10^2$
MTTD (h)	$72 \pm 0$	$90 \pm 11.5$	$101.6 \pm 10.4$	$161.0 \pm 16.1$	$73.8 \pm 11.3$	$174.7 \pm 14.9$	$138.9 \pm 8.8$	$224.5 \pm 9.6$
Mortality <sup>a</sup> (%)	100	80	78	56	100	90	100	83
Pathogenesis	1° pneumonia	Nasal ulceration	BALT infection	Nasal ulceration	1° pneumonia	Nasal ulceration	1° pneumonia	Nasal ulceration
	Splenitis	NALT infection	Mediastinal LN	NALT infection	Splenitis	NALT infection	Splenitis	Tonsillitis
	Septicemia	Cervical adenitis	Splenitis	Cervical adenitis	Septicemia	Cervical adenitis	Septicemia	Cervical adenitis
		Splenitis	Septicemia	Splenitis		Splenitis		Splenitis
		Septicemia				Septicemia		Septicemia
				Primary gastritis				
		2° pneumonia		Peyer patches		Olfactory neuritis		Conjunctivitis
				Mesenteric LN		Brain abscess		

<sup>a</sup>Mortality indicated for retained dose of  $10^3$  cfu in the lungs or nasal cavity; cfu, colony-forming unit. Data from references 69 and 75–77.

nasal epithelium precedes infection of the URT lymphoid tissues such as the nasal-associated lymphoid tissue (NALT) and tonsils. Eventually cervical lymphadenitis is observed prior to dissemination to other tissues and septic shock.<sup>69–72,75–77</sup> NALT is the rodent equivalent of the Waldemeyer rings in primates comprising the adenoids and tonsils. Lung infection is often observed despite primary deposition in the URT. There are two reasons for this, first a limitation of the aerosol device that produces a polydisperse aerosol comprising some 1–3  $\mu\text{m}$  particles in addition to the larger aerosol particle sizes. These will deposit in the alveoli producing dual infection of the LRT and URT in organisms such as *B. pseudomallei* that have a very low infectious dose of a few bacteria.<sup>77</sup> Second, secondary pneumonia may occur very late in infection in mice that inhaled *Y. pestis* aerosolized within 12  $\mu\text{m}$  particles resulting from hematogenous spread from the bloodstream into the alveolar spaces presumably after bacteraemic spread from the URT lymphoid tissues.<sup>71,75</sup>

#### Gastrointestinal, olfactory, and conjunctival infections from inhalational deposition

Intriguingly, differences in pathogenesis were observed between the bacterial pathogens upon deposition in the URT. In mice that inhaled 12  $\mu\text{m}$  particles containing *B. anthracis* endospores GI pathology was observed with primary gastritis (17%), and activation and degeneration of GI lymphoid tissues such as the Peyer patches (72%) and mesenteric lymph nodes (67%).<sup>76</sup> This pathology was not observed in other species, and is perhaps related to a combined effect of mucociliary clearance of the endospores to the stomach and the hardness of endospores to the harsh acidic environment of the stomach. Bacteria such as *Y. pestis*, *F. tularensis*, and *B. pseudomallei* that do not produce GI pathology after URT deposition are much more sensitive to low acidity.<sup>84–86</sup> Mice that inhaled *B. pseudomallei* within 12  $\mu\text{m}$

particles demonstrated tropism toward the olfactory epithelium with sequential infection and resultant inflammatory responses within the olfactory neurone and olfactory bulb (100%) culminating in brain abscessation (33%).<sup>77</sup> Similar observations were observed in an intranasal infection model.<sup>87</sup> Ocular infection characterized by severe conjunctivitis associated with “purulent discharge from the nose and eyes” was observed in 16% of rhesus macaques that inhaled *F. tularensis* within 12–24  $\mu\text{m}$  particles. In contrast, inhalation of 1–8  $\mu\text{m}$  particles did not produce this pathology.<sup>69</sup>

In contrast, for the encephalitic alphaviruses, the demarcation between LRT and URT infection is much less marked demonstrating the difficulties in employing rigid health-related demarcations to particle size penetration into the respiratory tract. Mice challenged with 1–3  $\mu\text{m}$  particle aerosols containing VEE virus or after intranasal deposition demonstrated neuroinvasion via trans-synaptic spread through the olfactory or trigeminal neuronal pathway to the brain. The terminal stages of infection were characterized by multifocal necrotising encephalitis.<sup>88–92</sup> Intranasal deposition resulted in the presence of higher viral titers in the nasal mucosa, NALT, and cervical lymph nodes.<sup>90,93</sup> Similarly, intranasal challenge in the Guinea pig resulted in targeted infection of the olfactory bulb prior to viremia.<sup>93</sup> Similar pathogenesis has been observed in the Rhesus macaque, however, the virus localized in the olfactory bulb apparently not progressing to the brain.<sup>94,95</sup> In eastern equine encephalitis virus (EEEV) the olfactory neuronal pathway is important for inhalational but not parenteral routes of infection in rodent models.<sup>74,92</sup> Utilization of the olfactory neurone to cross the cribriform plate into the central nervous system has also been observed in URT infection models for Nipah virus, Japanese encephalitis virus, Hendra virus, herpes simplex virus, influenza virus (H5N1 subtype),

**Table 5.** Upper respiratory tract symptoms in bacterial select agents

Symptoms	Percentage of patients with symptom (%)					
	Plague <sup>a</sup> (Pharyngeal)	Tularemia (Oropharyngeal)	Anthrax (Nasopharynx)	Anthrax (Oropharyngeal)	Anthrax (Laryngeal)	Melioidosis (Pharyngocervical)
Fever	92	96	83	97	50	60
Tonsillitis	75	-	-	63	-	15
Pharyngitis	92	81	33	72	-	30
Nasal/sinus complaints	-	-	83	3	0	10
Malaise/fatigue	92	54	33	-	25	-
Headache	92	62	17	6	0	-
Cervical/submandibular lymphadenitis	83	92	50	100	-	85
Arthralgia/myalgia	83	35	-	-	-	5
Abdominal pain	50	-	17	-	25	-
Vomiting/nausea	50	-	0	3	25	5
Cough	42	-	0	6	0	5
Dysphagia	17	-	17	-	0	-
Diarrhea	17	-	-	3	-	-
Hemoptysis/dyspnea	8	-	17	39	75	-
Anorexia	-	46	17	-	-	-
Depression	-	50	17	-	0	-
Concentration	-	42	-	-	-	-
Sleep disturbance	-	46	-	-	-	-
<b>Mortality</b>	<b>40%</b>	<b>&lt; 10%</b>	<b>50%</b>	<b>40%</b>	<b>50%</b>	<b>&lt; 10%</b>
<b>Treatable</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

<sup>a</sup>Also known as tonsillar plague. Data taken from references 107–113, 117, 118, 121, and 124–128.

Borna virus, *Balamuthia mandrillaris*, *Naegleria fowleri*, *B. pseudomallei*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, and *Neisseria meningitidis*.<sup>77,87,96–105</sup> Interestingly, recently differential phagocytosis of *Escherichia coli* and *Burkholderia thailandensis* was observed in vitro by olfactory sheathing and Schwann cells perhaps representing a mechanism for colonization, infection, or clearance.<sup>155</sup>

These studies demonstrate that deposition site can profoundly influence infection kinetics and pathogenesis within inhalational animal models. Depending on the initial site of deposition and clearance kinetics, aerosolized pathogens may come into contact with a range of tissues and organs through which infection may occur: nasal mucosa, nasal and URT lymphoid tissues, olfactory epithelium, bronchoalveolar epithelium, gastrointestinal tract, and ocular conjunctiva.

### Can CDC Select Agents Cause Infection of the URT in Humans?

In the United States, possession and transfer of biothreat agents is regulated under the Select Agent Program (SAP) administered by the Centers for Disease Control and Prevention (CDC). The pathogens and toxins controlled by the SAP are commonly referred to as select agents and represent a potential severe threat to the health and agriculture sectors. This review

specifically deals with those select agents that are harmful to humans.

It is difficult to extrapolate animal studies to humans directly because time-course data are not available in humans and therefore the intricacies of pathogenesis cannot be related to respiratory tract deposition. The reported pathology in human cases of inhalational infections is generally from terminal cases or during treatment regimens where the infection is already at a progressed stage and it is difficult to be certain where the infection initiated. It is further complicated by the high mortality, often approaching 90%, for the LRT infections caused by these pathogens.

However, cases of human infections caused by select agents occur that originate in the URT and demonstrate similar pathology to that described in inhalational animal infection models of the URT. Patients present with febrile illness, tonsillitis, pharyngitis, and cervical lymphadenitis prior to septicemia (Table 5). These presentations are generally termed pharyngeal or oropharyngeal infections and are predominantly associated with consumption of contaminated meats or water for *Y. pestis*,<sup>106–110</sup> *B. anthracis*,<sup>111–118</sup> and *F. tularensis*.<sup>119–123</sup> *B. pseudomallei* presents as a pharyngocervical infection predominantly in children, however there are adult cases.<sup>124–129</sup>

Two respiratory forms of plague have been observed from outbreaks of bubonic and pneumonic plague, one that results in primary pneumonia and a second that results in tonsillitis and cervical lymphadenopathy in the absence of pneumonia.<sup>130–133</sup>

Flexner<sup>131</sup> and Crowell<sup>130</sup> respectively stated that “of all the buccal structures, the tonsils seem to be most frequently the one attacked” and “tonsils have formed the portal of entrance for the bacilli and that the involvement of the cervical glands occurs secondarily through the lymph stream”. Meyer<sup>133</sup> further implied that “perhaps only the larger particles can lodge in the URT and give rise to tonsillar or septicemic plague”. The described URT pathology closely resembles that observed in NHPs, Guinea pigs, and mice (Table 4).<sup>71,75,133</sup> Interestingly, Guinea pigs suffering from pneumonic plague demonstrated 17% cross-infection to healthy control animals during cross-infection studies. The cross-infected animals all suffered from the URT infection.<sup>75</sup> It was implied that this may be a contributory factor toward the cessation of pneumonic plague outbreaks because the URT form is less infectious and has reduced mortality rates.<sup>133</sup> It is noteworthy that for *B. anthracis*,<sup>117,134</sup> *B. melitensis*,<sup>135</sup> *B. mallei*,<sup>136,137</sup> and *Coxiella burnetii*<sup>138</sup> there are also bona fide cases of inhalational infections that lack LRT involvement and present with the aforementioned pathology indicative of primary infection via the URT. Furthermore, in humans, VEE, WEE, and EEE viruses can infect via the inhalational route and cause neurological sequelae similar to that observed in animal models.<sup>139</sup> However it is not known in humans whether the olfactory neurone plays a role in the pathogenesis of these viruses or *B. pseudomallei*.

These cases demonstrate that in humans these pathogens can produce infection via the URT and although mortality is reduced compared with the respective LRT infections, the rates are not insignificant if untreated (Table 5). Similar to the LRT infection, the URT infections caused by select agents are treatable by intervention with antimicrobial therapy, and prognosis is good if promptly administered. Furthermore, the window of opportunity is longer compared with the LRT infection due to the protracted course of infection. However, misdiagnosis is problematic resulting in incorrect treatment regimens because symptoms resemble common URT diseases. For example, similar URT pathogenesis characterized by adenotonsillar disease, pharyngitis and /or cervical adenitis has been observed for a range of common pathogens including respiratory viruses, measles virus, *Staphylococcus aureus*, *Hemophilus influenzae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Mycobacterium* spp.<sup>140-146</sup>

Gastrointestinal anthrax, different from the oropharyngeal form, is known to occur in humans due to consumption of contaminated meats with mortality rates reaching 29% if untreated. It presents typically as a febrile illness with severe abdominal pain, mesenteric lymphadenopathy, hemorrhagic ascites, hematemesis, and diarrhea.<sup>115</sup> Complications can include hemorrhagic gastritis.<sup>147</sup> Interestingly, a recent case of GI anthrax occurred after exposure to endospores aerosolized during drumming, the inference being that the deposited endospores were cleared from the respiratory tract to the GI tract.<sup>148</sup> This supports observations in mice where infection of the GI tract occurred in mice that inhaled 12  $\mu\text{m}$  particles containing *B. anthracis* endospores with pathology similar to that described for humans.<sup>76</sup>

Conjunctivitis was observed in NHPs exposed to 12–24  $\mu\text{m}$  particles containing *F. tularensis*.<sup>69</sup> In humans, *F. tularensis* can

cause a rare presentation known as oculoglandular syndrome characterized by conjunctivitis, ulceration and preauricular lymphadenitis,<sup>149</sup> although this appears to be a result of direct contact with fomites or vector-borne rather than airborne transmission.

## Conclusions

It is difficult to predict the particle size that an individual may inhale from a bioaerosol because any source comprises a particle distribution (Table 1) that changes with time and distance due to the local climate (e.g., meteorology, turbulent activity, ventilation, etc.) and removal from the air column. As such, from the view-point of inhalational infections the respiratory tract should be considered as a continuum with deposition occurring throughout and the probability of infection dependent on the interplay between respiratory physiology, regional dose, clearance kinetics, host-pathogen colonization mechanisms and immunological response. Therefore, a range of tissues that have perhaps not been thought of as routes of inhalational challenge are brought into consideration including the URT lymphoid tissues, olfactory system, GI tract and potentially the ophthalmic system.

The site of deposition after an inhalational event can affect disease kinetics and pathogenesis, however, the deposition of respiratory pathogens in the lungs will generally result in the more rapid aggressive infection with higher mortality rates. However, evidence exists in both animal models and humans for a number of select agents for URT infections involving the URT lymphoid tissues as the initial foci followed by dissemination via the cervical lymph nodes and bacteremic spread. In addition to LRT presentations, other presentations have been observed in a pathogen specific manner including neurological infection via the olfactory system, GI tract infection, and conjunctivitis.

Increased understanding of the pathogenesis and immunology of infections resulting from inhalation and resultant clearance will aid in the development of vaccine candidates and antimicrobial regimens. Research into host-pathogen interactions and the immunology of URT infections is still in its infancy compared with LRT and systemic infections.<sup>67,80,150</sup> However, recent years have seen an increased understanding of host-pathogen and pathogen-pathogen interactions throughout the URT and the function of immunological tissues.<sup>151-153</sup> In humans it is unknown whether the olfactory system is utilized as a direct pathway from the URT to the brain. However, it remains a potential route and only recently has the immune response within the olfactory system been researched for viruses<sup>154</sup> and the picture is even less clear for bacteria.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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