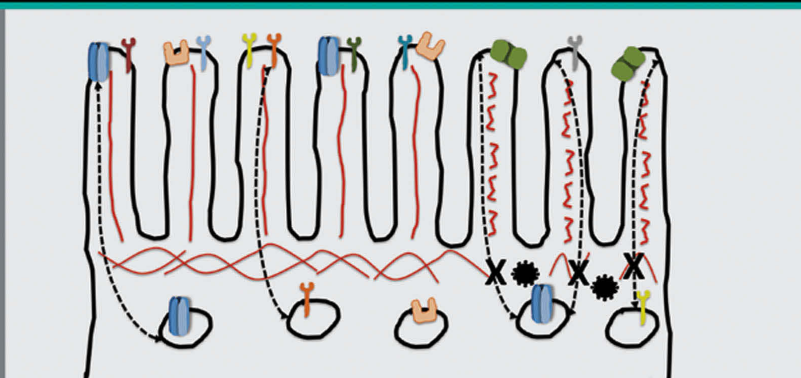
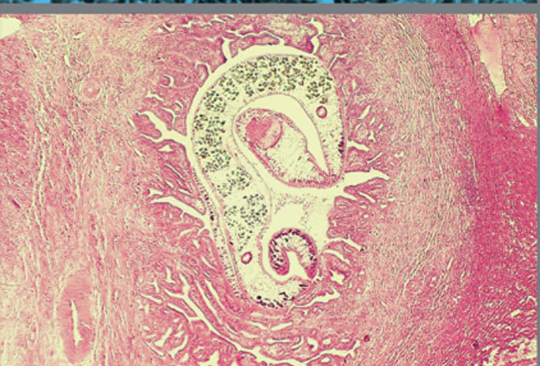
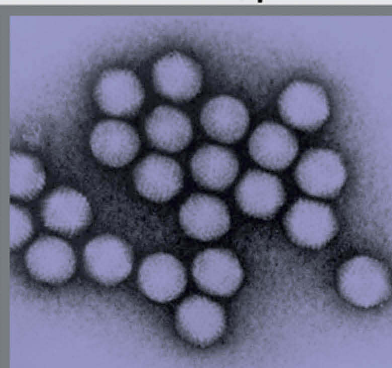
Food Microbiology Series

Laboratory Models for Foodborne Infections







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Dongyou Liu

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Laboratory Models for Foodborne Infections

Food Microbiology Series

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Dongyou Liu

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Laboratory Models for Foodborne Infections

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Preface for Food Microbiology Series

Microorganisms (including viruses, bacteria, molds, yeasts, protozoa, and helminths) represent abundant and diverse forms of life that occupy various ecological niches of earth. Those utilizing food and food products for growth and maintenance are important to human society due not only to their positive and negative impacts on food supply, but also to their potential pathogenicity to human and animal hosts.

On one hand, foodborne microorganisms are known to play a critical role in fermentation and modification of foods, leading to a variety of nutritious food products (e.g., bread, beverage, yogurt, cheese, etc.) that have helped sustain the human civilization from time immemorial. On the other hand, foodborne microorganisms may be responsible for food spoilage, which, albeit a necessary step in keeping up ecological balance, reduces the quality and quantity of foods for human and animal consumption. Furthermore, some foodborne microorganisms are pathogenic to humans and animals, which, besides creating havoc on human health and animal welfare, decrease the availability of meat and other animal-related products.

Food microbiology is a continuously evolving field of biological sciences that addresses issues arising from the interactions between food-/waterborne microorganisms and foods. Topics of relevance to food microbiology include, but are not limited to, adoption of innovative fermentation and other techniques to improve food production; optimization of effective preservation procedures to reduce food spoilage; development of rapid, sensitive, and specific methods to identify and monitor foodborne microbes and toxins, helping alleviate food safety concerns among consumers; use of *-omic* approaches to unravel the pathogenicity of foodborne microbes and toxins; selection of nonpathogenic foodborne microbes as probiotics to inhibit and eliminate pathogenic viruses, bacteria, fungi, and parasites; design and implementation of novel control and prevention strategies against foodborne diseases in human and animal populations.

The *Food Microbiology Series* aims to present a state-of-art coverage on topics central to the understanding of the interactions between food-/waterborne microorganisms and foods. The series consists of individual volumes, each of which focuses on a particular aspect/group of foodborne microbes and toxins, in relation to their biology, ecology, epidemiology, immunology, clinical features, pathogenesis, diagnosis, antibiotic resistance, stress responses, treatment and prevention, etc. The volume editors and the authors are professionals with expertise in their respective fields of food microbiology, and the chapter contributors are scientists directly involved in foodborne microbe and toxin research.

Extending the contents of classical textbooks on food microbiology, this series serves as an indispensable tool for food microbiology researchers, industry food microbiologists, and food regulation authorities wishing to keep abreast with latest developments in food microbiology. In addition, the series offers a reliable reference for undergraduate and graduate students in their pursuit to becoming competent and consummate future food microbiologists. Moreover, the series provides a trustworthy source of information to the general public interested in food safety and other related issues.

Preface

Foodborne infections result from the ingestion of foods and beverages (including drinking water) that are contaminated by pathogenic microorganisms (including viruses, bacteria, fungi, and parasites). While some microbial pathogens stay in the gastrointestinal system and produce toxins (e.g., enterotoxins, exotoxins, and mycotoxins) that are absorbed into the bloodstream, others may directly invade deeper body tissues. Although foodborne infections generally tend to induce mild clinical symptoms (e.g., nausea, vomiting, fever, abdominal cramps, and diarrhea) in immunocompetent individuals, they may have serious consequences in young children and people with suppressed immune functions.

With the increasing consumption of manufactured foods and beverages, foodborne infections are becoming a common and expensive public health problem worldwide. The World Health Organization (WHO) estimates that food-/waterborne diarrheal diseases kill about 2.2 million people (mostly children) annually. Based on FoodNet data collected between 2000 and 2007 by the Centers for Disease Control and Prevention (CDC), 48 million foodborne illness cases (16,000 cases for 100,000 inhabitants) occur in the United States every year, including 128,000 hospitalizations and 3,000 deaths. Interestingly, 31 foodborne pathogens have been implicated in 9.4 of the 48 million foodborne illness cases, with 7 (*Salmonella*, norovirus, *Campylobacter*, *Toxoplasma*, *Escherichia coli* O157:H7, *Listeria*, and *Clostridium perfringens*) accounting for 90% of these illnesses alone. Similarly, an estimated 4.1 million cases of foodborne gastroenteritis were documented in Australia in 2010, with norovirus, pathogenic *E. coli*, *Campylobacter* spp., and nontyphoidal *Salmonella* spp. being the main culprits.

Although proper storage and refrigeration of food play a vital role in the prevention of foodborne infections, other good food safety practices (handwashing, preventing cross-contamination, and maintaining cooking temperatures in the kitchen) are also valuable. In addition, accurate diagnosis and prompt medical intervention are crucial in reducing the mortality due to foodborne infections. However, thorough understanding of host–pathogen interactions and elucidation of molecular mechanisms of pathogenesis are critical for the development of effective vaccines that will lead to ultimate elimination of foodborne infections in human population. Toward this goal, application of laboratory models (including both *in vivo* and *in vitro* models) is essential.

As a part of the *Food Microbiology Series*, this book focuses on the value and utility of various animal and cellular systems (ranging from mice, rats, hamsters, guinea pigs, rabbits, nonhuman primates, birds, zebrafish, frogs, chicken embryo, fruit fly, nematode, and waxworm to established and nonestablished cell lines) in the study of foodborne infections. Written by experts involved in foodborne pathogen research, each chapter presents a state-of-the-art review of laboratory models in the study of a particular foodborne pathogen (of viral, bacterial, fungal, or parasitic origin) in relation to its life cycle, host–pathogen interaction, pathogenesis, immunity, and other related aspects. Besides providing a reliable reference for undergraduates and postgraduates of food microbiology, this book is a valuable guide for scientists using laboratory models in their investigation of foodborne infections.

Given the diversity of foodborne pathogens, a comprehensive book such as this is clearly beyond an individual's capacity. I am fortunate and honored to have a large group of scientists as chapter contributors, whose in-depth knowledge and technical insights on foodborne pathogens have greatly enriched this book. Additionally, the professionalism and dedication of the senior editor, Stephen Zollo, have enhanced its presentation. Finally, the understanding and support from my family—Liling Ma, Brenda, and Cathy—have helped me keep focused during the compilation of this all-inclusive volume.

Dongyou Liu

Editor

Dongyou Liu, PhD, studied veterinary science at Hunan Agricultural University, China, and completed his postgraduate training at the University of Melbourne, Victoria, Australia. Over the past two decades, he has worked at several research and clinical laboratories in Australia and the United States of America, focusing on molecular characterization and virulence determination of microbial pathogens such as ovine footrot bacterium (*Dichelobacter nodosus*), dermatophyte fungi (*Trichophyton*, *Microsporum*, and *Epidermophyton*), and listeriae (*Listeria* spp.), as well as development of nucleic-acid-based quality assurance models for security-sensitive and emerging viral pathogens. He is the author of over 50 original research and review articles in various international journals, a contributor of 165 book chapters, and the editor of *Handbook of Listeria monocytogenes* (2008), *Handbook of Nucleic Acid Purification* (2009), *Molecular Detection of Foodborne Pathogens* (2009), *Molecular Detection of Human Viral Pathogens* (2010), *Molecular Detection of Human Bacterial Pathogens* (2011), *Molecular Detection of Human Fungal Pathogens* (2011), *Molecular Detection of Human Parasitic Pathogens* (2012), *Manual of Security Sensitive Microbes and Toxins* (2014), and *Molecular Detection of Animal Viral Pathogens* (2016), all of which are published by CRC Press. He is also a coeditor of *Molecular Medical Microbiology*, 2nd edition (2014), which was published by Elsevier.

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1

Introductory Remarks

Dongyou Liu

CONTENTS

1.1 Preamble

Foodborne disease (also known as foodborne illness or colloquially as foodborne poisoning) is largely attributable to microbial pathogens and their toxins contained in food and food products that are inappropriately prepared or stored before consumption. Once inside the host, these pathogens establish in their predilection sites and cause damages to the host either through direct physical/mechanical destruction or through secretion of toxins and antigens that provoke host innate and acquired immune responses, leading to a range of clinical symptoms (e.g., diarrhea, abdominal cramps, nausea, fever, joint/back aches, and fatigue).

Although foodborne disease is a current buzzword that appears in various popular media outlets with alarming frequency, it has a long and tortuous history. Our awareness of as well as our struggle against foodborne disease goes hand in hand with our attempts to survive and prosper in a constantly changing, and challenging, world, with significant milestones marked by the use of fire, the development of crop cultivation, the luxury of food storage, the evolution of culinary art, the sophistication of sewage system, the observation of disease-causing microbes, the application of refrigeration, and the discovery of antibiotics [1,2].

From scavengers who searched for the scraps left by other predators for survival, humans have made enormous technological advances that overcome the barrier of seasonality for food supply, that reduce the proliferation of foodborne disease, and that enable rapid identification and tracking of foodborne pathogens implicated in any food-related disease outbreaks. Nonetheless, it is still a long way before we can call foodborne pathogens the genie in the bottle, foodborne disease a memory of the past, and foodborne outbreak an absolute nonevent.

1.2 Foodborne Pathogens and Diseases

1.2.1 Foodborne Pathogens

In a narrow sense, foodborne pathogens refer to microbes that contaminate the foods and related products (e.g., pasteurized carrot juice, peanut butter, broccoli powder on a children's snack food, frozen pot pies, canned chili sauce, hot peppers, white and black pepper, raw cookie dough, hazelnuts, fenugreek sprouts, papayas, pine nuts, raw frozen scraped ground tuna, etc.), the consumption of which by humans leads to infections and diseases. In a broader sense, foodborne pathogens include microbes that occur in animals (in farm/zoo animals and pets), the environment (soil, water, and air), and foods, the ingestion, inhalation, and contact of which by humans result in discomfort and illness. Based on the latter premise, this book covers not only microbial pathogens that come along with foods and food products (foodborne), but also those that may occasionally enter into human host via water (water-borne), air (airborne), or direct contact (skin wound), as well as those that cause diseases not through infection, but through production of toxins and antigens that disturb/upset/confuse the gut, neurological, and immune systems of the human host.

As steadfast survivors, microbes (e.g., viruses, bacteria, fungi, and parasites) utilize animals (including humans), foods, beverages, and water as growth or maintenance media. Some microbes remain in humans (e.g., *Salmonella* Typhi and norovirus) or animal reservoirs and contaminate the food supply via excreta, meat, milk, or eggs. Others persist in the environment and contaminate the ecosystems that are fundamental to food production. Some microbes demonstrate the unusual ability to endure extreme temperature, pH, and osmolarity, to sustain for long periods on dry surfaces, food processing plants, and to exploit any temporary weakness in human innate and acquired immune defense networks (as seen in pregnant women, infants, the elderly, and individuals under immune suppressing therapies).

Although a large number of foodborne microbes are known to infect humans and cause diseases of varying severity, those having the most significant impact on human health in terms of prevalence, morbidity, and mortality include *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Salmonella enteritidis* (e.g., serotypes Typhi and Typhimurium), *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Streptococcus pyogenes*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Shigella*, hepatitis A virus, hepatitis E virus, norovirus, rotavirus, *Cryptosporidium*, *Cyclospora cayetanensis*, and *Toxoplasma gondii* [3]. It is notable that a majority of these high-impact foodborne pathogens are bacteria, and the remainder are viruses and parasites. Interestingly, some of these pathogens have emerged only in the past 30 years presumably due to the increased consumption of processed food products, globalization of food trade, and population ageing. For instance, *E. coli* O157:H7 is a shiga-toxin-producing bacterial strain that was first recognized as a human pathogen in foodborne outbreaks associated with ground beef in 1982, producing symptoms ranging from simple diarrhea and hemorrhagic colitis to hemolytic-uremic syndrome (which is characterized by hemolytic anemia, thrombocytopenia, and renal injury) [4,5]. Subsequently, lettuce grown in close proximity to a dairy farm from which wastewater contaminated with animal feces was used to irrigate the plant was linked to a 2006 outbreak of *E. coli* O157:H7 infection in Iowa and Minnesota (see Chapter 21 in this book). Another recently emerged foodborne pathogen of note is *Aeromonas* (mainly *A. hydrophila*, *A. caviae*, and *A. veronii*), which is responsible for both intestinal and extraintestinal diseases in humans (see Chapter 15 in this book). There is no doubt that new foodborne pathogens will likely emerge or reemerge in the future.

Apart from infections with foodborne viruses, bacteria, fungi, and parasites, another important cause of foodborne diseases is toxins or toxic chemicals produced by foodborne bacteria and fungi as well as those associated with shellfish and plants [6]. Toxins originated from foodborne bacteria can be separated into exotoxins (which remain part of the bacteria, and are secreted, or, similar to endotoxins, released during bacterial lysis) and endotoxins (which form part of the bacterial outer membrane, and are released during bacterial lysis). Some well-known foodborne bacterial exotoxins include superantigens from *S. aureus* and *Streptococcus pyogenes*; pore-forming toxins (PFTs) from *E. coli, L. monocytogenes*, and *Streptococcus pneumoniae*; heat-stable enterotoxins (ST, exotoxins targeting the intestine) from pathogenic strains of *E. coli*; and botulinum neurotoxin (BoNT) from *C. botulinum*. A notable foodborne bacterial endotoxin is lipopolysaccharide (LPS, which is made up of O antigen, core oligosaccharide, and lipid A) from Gram-negative bacteria. As water-soluble proteins, PFTs induce host membrane damages as amphiphilic surfactants and phospholipases. On the other hand, endotoxins (e.g., LPS) cause severe inflammation, endotoxemia (septic shock), and autoimmune disease. Being the by-products of foodborne fungi, mycotoxins are responsible for alimentary mycotoxicoses in humans through food consumption. The most common foodborne mycotoxins consist of aflatoxins (from *Aspergillus parasiticus* and *Aspergillus flavus*), altertoxins (from *Alternaria*), fumonisins (from *Fusarium moniliforme*), ochratoxins (from *Aspergillus ochraceus*, *Aspergillus carbonarius*, *Penicillium verrucosum*), patulin (from *Aspergillus*, *Penicillium*), and trichothecenes (from *Fusarium*).

1.2.2 Foodborne Diseases

Foodborne diseases usually arise from consumption of improperly handled, prepared, or stored foods that are contaminated with foodborne pathogens and/or toxins. With incubation period of several hours to 1 week, the initial symptoms of foodborne disease consist of diarrhea, vomiting, abdominal cramps, nausea, fever, joint/back aches, and fatigue, which may last for a week or so. However, some foodborne pathogens (e.g., *Streptococcus pyogenes*) may cause a spectrum of clinical diseases, including (1) localized inflammatory lesions; (2) both local and systemic diseases; and (3) immune dysfunction.

In localized inflammatory lesions, inflammation linked to foodborne pathogens is responsible for lesions in various locations, accompanied by other symptoms. As in the case of *Streptococcus pyogenes* (group A *Streptococcus* or GAS) infection of the pharynx (i.e., pharyngitis, or strep sore throat), inflammation in the pharynx and tonsils leads to sore throat, along with sudden-onset fever, headache, nausea, abdominal pain, vomiting, and patchy exudates. Similarly, GAS infection of the skin (i.e., impetigo) results in the formation of pustules that enlarge and rupture to become thick, honey-colored scabs (see Chapter 14 in this book) [7].

In local and systemic diseases, toxins (e.g., streptococcal pyogenic exotoxins) produced by foodborne pathogens (e.g., GAS) induce a local disease with a deep red, finely papular, erythematous rash (strawberry tongue) and exudates in the pharynx (scarlet fever), or cause soft tissue infection at a surgical site (surgical scarlet fever). Additionally, following minor nonpenetrating trauma, suction lipectomy, hysterectomy, vaginal delivery, bunionectomy, and bone pinning, GAS invades and produces streptococcal toxins that contribute to streptococcal toxic shock syndrome (STSS) (see Chapter 14 in this book) [7].

Regarding immune dysfunction, some foodborne pathogens produce antigens that confuse host immune systems, leading to autoimmune diseases. For example, as a sequela of untreated GAS pharyngeal infection, acute rheumatic fever (ARF) results from the activity of antigens produced by GAS that cause inflammation in the joints (arthritis) and the heart (carditis, also known as rheumatic heart disease or RHD). Another sequela of GAS infection is acute poststreptococcal glomerulonephritis (APSGN), which is a disorder of the kidneys mediated by the immune complex, with symptoms ranging from edema, hypertension, and urinary sediment abnormalities to reduced serum complement components (see Chapter 14 in this book) [7].

While all people are at risk for foodborne illness and most recover without any lasting effects, some may show serious long-term consequences such as kidney failure, chronic arthritis, brain and nerve damage, and even death, especially infants and toddlers, the elderly, pregnant women, transplant recipients, and individuals with chronic illnesses (e.g., cancer, diabetes, or HIV/AIDS) and compromised immune systems.

1.3 Laboratory Models

1.3.1 Rationales for Using Laboratory Models

In spite of our unrelenting past efforts, including the implementation of procedures to reduce pre- and postharvest contamination, the introduction of best-practice in food products processing, package and storage, the education of the general public about the danger of and effective prevention measures against foodborne diseases, the application of antibiotic and antimicrobial therapies, and the redirection of public resources into research on the mechanisms of foodborne infections and diseases, the war against foodborne pathogens and diseases is far from being over [3]. Among many other things, we are still uncertain how foodborne pathogens sabotage host immune defense and manipulate host cell machinery for their own gain.

Since the best way to observe a battle is to get close to the battlefield, use of laboratory models (i.e., *in vivo* animal models and *in vitro* culture models) provides a unique opportunity to determine the infectivity, host specificity, and life cycle of foodborne pathogens; to compare the virulence potential of various microbial strains and serotypes; to generate large quantities of pathogenic microbes for detailed analysis;

to examine host immune responses to foodborne pathogens; to uncover the pathological and histological details resulting from foodborne infections; to validate the accuracy of clinical diagnostic techniques; and to evaluate the efficacy of newly developed antimicrobial and vaccine preparations against foodborne pathogens without harming human hosts. This is made possible by the common ancestry of all living organisms, the similarity of anatomical structures and functions (e.g., breathing, digestion, movement, sight, hearing, reproduction, immunity, etc.), the homology of genetic materials, the sharing of hundreds of illnesses, and the conservation of cell biological and developmental pathways among vertebrates as well as between vertebrates and invertebrates [8].

1.3.2 Milestones in the Use of Laboratory Models

Animals have long been employed as laboratory models for investigation of the anatomy, physiology, epidemiology, and disease mechanisms of vertebrates. The available records suggest that in the 6th century BCE, Alcmaeon of Croton examined dogs to demonstrate the brain as the seat of intelligence and sensory integration; Aristotle (384–322 BCE) studied embryogenesis and ontogeny in chickens; after analysis of the cardiovascular system in live animals, Erasistratus (304–258 BCE) postulated that the heart functions as a pump; in the 2nd century, Galen of Pergamum employed live animals for extensive studies of cardiovascular and neuroanatomy; in the 12th century, Avenzoar polished his tracheotomy skill on animals before applying to humans; in the mid-16th century, Servetus and Lusitano identified pulmonary and systemic circulation as two connected but distinct blood circuits in the body through animal experiments; in the 17th century, through comparison of the anatomic and functional properties of the heart and vasculature in eels, chicks, and pigeons, William Harvey provided accurate and detailed descriptions of the function of the cardiovascular and other systems; in the 18th century, Antoine Lavoisier used guinea pigs in a calorimeter to prove respiration as a form of combustion; in the 19th century, Louis Pasteur demonstrated the germ theory of disease using a sheep model of anthrax; in the late 19th century, Emil von Behring observed the effect of diphtheria toxin in guinea pigs that led to the development of an antitoxin against diphtheria in animals and humans.

Another significant milestone in the use of laboratory animals for microbial research was achieved in 1902, after William Castle and Abbie Lathrop generated the DBA ("dilute, brown, and non-agouti") inbred mouse strain and other inbred mice for genetic studies. Between 1910 and 1927, working with the fruit fly *Drosophila melanogaster*, Thomas Hunt Morgan pinpointed chromosomes as the vector of inheritance for genes. In the 1920s, Frederick Banting utilized the isolates of pancreatic secretion to treat dogs with diabetes; in the 1930s, Little and MacDowell produced the first fully inbred mouse (20 brother × sister matings); in the 1940s, John Cade discovered the anticonvulsant properties of lithium using guinea pigs, which helped replace lobotomy or electroconvulsive therapy for the treatment of bipolar disorder (manic depression); also in the 1940s, Jonas Salk isolated the most virulent forms of the polio virus from the rhesus monkey and created a highly effective polio vaccine; in the 1960s, Albert Sabin passed the polio virus through animal hosts (including monkeys) to improve the effectiveness of the Sabin vaccine for mass application; in 1976, Rudolf Jaenisch and colleagues developed the first transgenic mouse; in 1987, Capecchi, Evans, and Smithies developed the first knockout mouse; in 1997, Wilmut and Campbell obtained the first cloned animal (Dolly the sheep) from an adult somatic cell; in 2009, Aron Geurts and colleagues developed the first knockout rat [9].

1.3.3 Characteristics of Laboratory Models

Laboratory models used for the study of foodborne infections are of two main types: *in vivo* animal models, and *in vitro* culture models. The *in vivo* animal models involve vertebrates [nonhuman primates (e.g., rhesus monkey, cynomolgus monkey, chimpanzee, baboon), rodents (e.g., mice, rats, gerbils, hamsters, chinchillas, guinea pigs), rabbits, cats, dogs, pigs, sheep, cattle, chicken, zebrafish (*Danio rerio*), etc.] and invertebrates [fruit fly (*D. melanogaster*), silkworm (*Bombayx mori*), waxworm (*Galleria mellonella*), roundworm (*Caenorhabditis elegans*), protozoa (*Tetrahymena thermophila* or *Tetrahymena pyriformis*), etc.]; the listing order reflects the evolutionary relationship between these animals and humans, with nonhuman primates being most close and roundworm being least close to humans (Table 1.1) [10–14]. The *in vitro* culture models rely on the use various established and non-established cell lines (derived from epithelia, endothelia, macrophage, etc.), embryonated eggs, and organs and tissues from hosts (Table 1.1).

Among various *in vivo* animal models, nonhuman primates (NHPs, with genomes of 2.85–3.09 Gb dispersed in 21–24 chromosome pairs) are the closest relatives to humans (with a genome of 3.23 Gb dispersed in 23 chromosome pairs), and represent ideal models for investigation of foodborne infections and other human diseases, on the basis of biological, physiological, immunological, and genetic similarities. However, because of limited availability, prohibitive cost, and ethical concerns, NHPs are rarely used nowadays [15].

TABLE 1.1

Characteristics of Laboratory Models for Foodborne Infections

Model[a]	Common Species/ Cell Type	Characteristics	Exemplary Application
In Vivo			
Nonhuman primates (family Hominidae, order Primates)	Chimpanzee (*Pan troglodytes*), rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*), olive baboon (*Papio anubis*)	Chimpanzee has a genome of 3.02 Gb, rhesus monkey 3.09 Gb, cynomolgus monkey (crab-eating macaque, long-tailed macaque, or Java macaque) 2.85 Gb, olive baboon 2.94 Gb. Ideal models for foodborne infections and other human diseases, but limited by availability, cost, and ethical concerns	*Helicobacter pylori*, *L. monocytogenes*, *Mycobacterium*, hepatitis E virus
Mice (family Muridae, order Rodentia)	House mouse (*Mus musculus*) strains: BALB/c/(inbred, albino), C57BL/6/ (inbred, dark brown), athymic nude mice (outbred)	Mice (house mice) possess a genome of 2.67 Gb, are small, readily available, easy to handle, amenable to genetic manipulation, and reproduce quickly, representing an efficient, cost-effective, and widely applicable animal model for experimentation on foodborne infections and other human diseases	*L. monocytogenes*, *S. aureus*, *Salmonella*
Rats (family Muridae, order Rodentia)	Norway rat (*Rattus norvegicus*) (inbred), Wistar rat (outbred, albino), Lewis rat (inbred)	Norway rat (brown rat) has a genome of 2.61 Gb. Developed in 1906, Wistar rat (outbred albino) is the ancestor of most laboratory rats used today, including the Lewis rat. Wistar rat shows albino coloring, a docile behavior, and low fertility, and tolerates crowding	*Salmonella*, *S. aureus*, *Yersinia*, *Acanthamoeba*
Gerbils (family Muridae, order Rodentia)	Mongolian gerbil (*Meriones unguiculatus*) (outbred)	Mongolian gerbil (Mongolian jird) is easy to keep as it adapts to a new setting well	*H. pylori*, *L. monocytogenes*, *Giardia*
Hamsters (family Cricetidae, order Rodentia)	Syrian hamster (*Mesocricetus auratus*) (outbred), Chinese hamster (*Cricetulus griseus*)	Syrian hamster (golden hamster) possesses a genome of 2.50 Gb, Chinese hamster 2.36 Gb. Hamsters have a short life cycle and breed well in captivity; being relatively free from natural diseases, hamsters are susceptible to experimental pathogens	*Mycobacterium*, *Acanthamoeba*
Chinchillas (family Chinchillidae, order Rodentia)	Long-tailed chinchilla (*Chinchilla lanigera*)	Chinchilla has a genome of 2.39 Gb. Being crepuscular rodents, chinchilla is a robust host for experimental study	*L. monocytogenes*, *Yesinia*
Guinea pigs (family Caviidae, order Rodentia)	Hartley Guinea pig (*Cavia porcellus*) (outbred, albino)	Guinea pig has a genome of 2.72 Gb, and shows similarity to humans in disease symptoms, immune response, and pathogenesis	*L. monocytogenes*, *S. aureus*

(*Continued*)

TABLE 1.1 (*Continued*)

Characteristics of Laboratory Models for Foodborne Infections

Model[a]	Common Species/ Cell Type	Characteristics	Exemplary Application
Rabbits (family Leporidae, order Lagomorpha)	New Zealand white rabbit (*Oryctolagus cuniculus*) (outbred)	New Zealand white rabbit possesses a genome of 2.73 Gb, and represents a nonaggressive host for experimental work	*L. monocytogenes*, *Salmonella*
Cats (family Felidae, order Carnivora)	Domestic cat (*Felis catus*)	Domestic cat has a genome of 2.9 Gb, and is useful for modeling some foodborne infections	*S. aureus*
Dogs (family Canidae, order Carnivora)	Domestic dog (*Canis familiaris*)	Domestic dog possesses a genome of 2.25 Gb, and may be used experimentally for a number of foodborne infections	*H. pylori*
Pigs (family Suidae, order Artiodactyla)	Domestic pig (*Sus scrofa domesticus*)	Domestic pig has a genome of 2.5 Gb. Being truly omnivorous, pigs (piglets) show strikingly similar nutritional requirements to those of humans. Pigs practice coprophagy, and represent a useful model for a number of foodborne infections	*L. monocytogenes*, *S. aureus*, *Taenia solium*
Sheep (family Bovidae, order Artiodactyla)	Sheep (*Ovis aries*)	Sheep harbors a genome of 2.61 Gb, and is useful for modeling some foodborne infections	*L. monocytogenes*, bovine spongiform encephalopathy (BSE)
Cattle (family Bovidae, order Artiodactyla)	Cattle (*Bos taurus*)	Cattle possess a genome of 2.69 Gb, and may be used for a number of foodborne infections	*E. coli*, *Taenia saginata*
Chicken (family Phasianidae, order Galliformes)	Domestic chicken (*Gallus gallus domesticus*)	Domestic chickens have a genome of 1.23 Gb, are noted for their rapid growth rate, distinct anatomy, relatively small size, and low cost	*E. coli*, *Aspergillus fumigatus*
Zebrafish (family Cyprinidae, order Cypriniformes)	Zebrafish (*D. rerio*)	Zebrafish possess a genome of 1.4 Gb. Due to small size, zebrafish are easy to house and care for, easy to introduce genetic changes, and easy to observe the impact of any genetic mutation (with transparent early life stages)	*Mycobacterium*
Fruit fly (family Drosophilidae, order Diptera)	Common fruit fly (*D. melanogaster*)	Common fruit fly has a genome of 139 Mb, and shares 75% of known human disease genes. Due to its small size, simple anatomy, high fecundity, and short life cycle (about 30 days at 29°C), the fruit fly is easy and inexpensive to maintain. However, the fruit fly does not have an adaptive immune system and is not an appropriate model for the study of antibody- and lymphocyte-dependent adaptive immune defenses	*L. monocytogenes*, *S. aureus*
Silkworm (family Bombycidae, order Lepidoptera)	Domestic silkworm (*B. mori*)	Domestic silkworm possesses a genome of 397 Mb, and represents a low-cost model for some foodborne infection. It has a body size large enough for easy handling (e.g., injecting sample solution into the hemolymph)	*Pseudomonas aeruginosa*, *S. aureus*

(*Continued*)

TABLE 1.1 (*Continued*)

Characteristics of Laboratory Models for Foodborne Infections

Model[a]	Common Species/ Cell Type	Characteristics	Exemplary Application
Waxworm (family Pyralidae, order Lepidoptera)	Greater wax moth or honeycomb moth (*G. mellonella*)	Despite lacking an adaptive immune response, greater wax moth (waxworm) shows an innate immune response functionally similar to that of mammals, and provides a rapid, inexpensive, and reliable model for certain foodborne infections	*Streptococcus pyogenes*
Roundworm (family Rhabditidae, order Rhabditida)	Soil nematode (*C. elegans*)	Soil nematode possesses a genome of 100 Mb, and lacks an adaptive immune system. It has a short life cycle, simple anatomy, is easy to handle, and has low cost maintenance. *C. elegans* intestine is composed of cells that share striking similarities to human intestinal epithelial cells	*Enterococcus faecalis*, *E. coli*, *S. aureus*
Protozoa (family Tetrahymenidae, order Hymenostomatida)	Ciliated protozoan (*T. thermophila* or *T. pyriformis*)	Ciliated protozoan *T. thermophila* has a genome of 104 Mb. Being able to switch from commensalistic to pathogenic modes of survival, *Tetrahymena* offers a low-cost and easy to handle alternative for modeling foodborne infections	*Aeromonas*, *E. coli*, *Listeria*, *Vibrio*, *Yesinia*
In Vitro			
Epithelial cell lines	Human colorectal cells Caco-2 and HT29, human colonic cell T84, human cervical cell HeLa, African green monkey kidney cell Vero, Madin-Darby canine kidney cell (MDCK)	Easy and low-cost maintenance, high sensitivity, and broad spectrum	*E. coli* O157:H7, *L. monocytogenes*
Endothelial cell lines	Human umbilical vein endothelial cell (HUVEC), human glomerular microvascular endothelial cell (GMVEC)	Easy and low-cost maintenance, high sensitivity, and broad spectrum	*E. coli* O157:H7, *L. monocytogenes*
Macrophage cell lines	Mouse macrophage cell J774, human macrophage cell U937	Easy and low-cost maintenance, high sensitivity, and broad spectrum	*E. coli* O157:H7, *L. monocytogenes*
Embryonated eggs	Chicken eggs	Cost-effective and easy maintenance, ready availability, sterile, and wide ranging fluids and tissues	*C. perfringens*, *Aspergillus fumigatus*
In vivo grown organ cultures (IVOC)	Various organs or tissues	Close to native live tissue; IVOC usage is limited to several hours (when the tissue dies). Further, it is technically challenging and shows sample variability	*E. coli* O157:H7, *Salmonella*, norovirus
Ussing chamber	Epithelial tissues	Ussing chamber detects and quantifies transport and barrier functions of living tissue	*E. coli* O157:H7, *Salmonella*

[a] Animal models are listed (in descending order) according to their evolutionary closeness to humans, with nonhuman primates being the closest and roundworm being the most distant.

On the other hand, as the next in the order of closeness to humans, rodents (especially mice) are increasingly employed as preferred animal models for foodborne infections and other human diseases. Mice possess a genome of 2.67 Gb dispersed in 20 chromosome pairs with ~25,000 genes, 99% of which have human counterparts. Having a relatively small body size, 18-day gestation, 10–15 pups per litter, 7 weeks to sexual maturity, and a 2–3-year lifespan (1 mouse year equals about 30 human years), mice provide an efficient and cost-effective model for human disease research including foodborne infections. It should be noted that mice practice coprophagy, an aspect that may be considered in experimental design for certain disease types.

Mice are highly amenable to manipulation, and can be inbred to yield genetically identical strains, which allows for more accurate and repeatable experiments. Through practice of cesarian birth, flexible-film isolator cages, and irradiated food, mice (and other animal species) can be maintained in completely germ-free conditions or colonized with one or more defined bacterial species (gnotobiotics). In addition, use of genetic selection and manipulation technologies enables insertion of extra genetic materials into genome, creating a variety of transgenic mice (including knockout, knock-in, and humanized mice as well as mice with conditional gene modifications or chromosomal rearrangement) [8].

For example, athymic nude mice are selected for the nude spontaneous mutation (*Foxn1*^{nu}, formerly *Hfh11*^{nu}) (which results in abnormal hair growth) and in the defective development of the thymic epithelium (which abrogates a cell-mediated immunity, despite the presence of T-cell precursors). Homozygous nude mice show partial defect in B cell development probably due to the absence of functional T cells, and their responses to thymus-dependent antigens are primarily limited to IgM due to a defect in helper T-cell activity.

Knockout mice are created by inserting a specific mutation into the endogenous gene. This leads to inactivation/silencing of the gene of interest, suppressing its expression and function. Knock-in mice are created by inserting a transgene into an exact location for overexpression. Both knockout and knock-in animals rely on the use of embryonic stem (ES) cells containing null or point mutations and complex chromosomal rearrangements (e.g., large deletions, translocations, or inversions), which are injected into the host mouse embryo, and subsequently implanted into a foster mother.

Humanized mice are created by inserting human genes (more recently entire human systems) into mice for subsequent expression. For instance, mice with human "immune systems" were generated by implanting either fetal lymphoid tissue or peripheral blood leukocytes into mice with spontaneous severe combined immunodeficiency. Humanized mice are capable of accepting a variety of human cells (blood, immune, cancer, etc.) without rejection.

Mice with conditional gene modifications are created with two different types of genetic alterations: one contains a conditional vector [through inserting recognition sequences for the bacterial Cre recombinase (*loxP* sites) using homologous recombination in ES cells], which functions as an "on switch" for the mutation, and the other contains specific sites (called *loxP*) inserted on either side of a whole gene, or part of a gene, that encodes a certain component of a protein that will be deleted. Similarly, mice with chromosomal rearrangement are created using the Cre/*loxP* recombination system to induce site-specific mutations that display defects resembling those caused by human chromosomal rearrangements (e.g., chromosome deletions, duplications, inversions, translocations, and nested chromosome deletions) [8].

Depending on the levels of simulation to human disease, animal models may be separated into three types: homologous, isomorphic, and predictive. Homologous animals demonstrate identical causes, symptoms, and treatments relative to human diseases; isomorphic animals have identical symptoms and treatments; predictive models share only a couple of aspects of human disease with humans, but nevertheless provide useful predictions about mechanisms of particular disease features. Similarly, depending on the way in which animal disease is induced, animal models may be divided into four categories: experimental, spontaneous, negative, and orphan. Experimental disease models resemble human disease conditions in phenotype or response to treatment but are induced artificially in the laboratory. Spontaneous disease models are analogous to human disease conditions that occur naturally. Negative disease models are essentially control animals, and are used to validate an experimental result. Orphan disease models have no human analog and occur exclusively in the species studied. Furthermore, to examine a particular disease, various approaches may be used. For example, inflammation may be studied via Carrageenan footpad edema (CFE) model, collagen-induced arthritis (CIA) model, pristane-induced arthritis (PIA) model, adjuvant-induced arthritis (AIA) model, ovalbumin-induced arthritis (OIA) model, air pouch model, and delayed-type hypersensitivity (DTH) model [8].

The *in vitro* culture models provide an alternative to the *in vivo* animals for mechanistic studies, by preserving the physiology of the living cell, without the need to sacrifice an animal. The advantages of the *in vitro* culture models include low cost, easy maintenance, relatively high efficiency, and little ethical concern. For instance, Caco-2 cells (of human colonic origin) can differentiate in culture, form brush border membranes, demonstrate transport properties (similar to intestinal epithelia), and express abundant intestinal microvilli, enzymes, and differentiation markers (typical of human small intestinal enterocytes), offering a valuable model for investigation of vectorial epithelial passage by para- and transcellular routes. Apart from the established cell lines (of epithelial, endothelial, and macrophage origins), other cells, organs, and tissues may be obtained from animal and human hosts for *in vitro* modeling. These include enterocyte suspensions, brush border membranes and vesicles, perfused duodenal segment, everted gut sacs, lymphocytes, etc.

When selecting an animal model for research, considerations should include: (1) appropriateness as an analog, (2) transferability of information, (3) genetic uniformity of organisms, (4) background knowledge of biological properties, (5) cost and availability, (6) generalizability of the results, (7) ease of and adaptability to experimental manipulation, (8) ecological consequences, and (9) ethical implications. If possible, three basic principles should be applied: replacement, reduction, and refinement. Replacement aims to use alternatives [e.g., computer models, tissues and cells, "lower-order" animals (cold-blooded animals, invertebrates, bacteria) instead of "higher-order" animals (primates and mammals) for experimentation]. Reduction employs mathematical calculations of statistical power to minimize the number of animals used. For example, by using an alternative way to LD50 for result interpretation, the number of experimental mouse groups for assessing *L. monocytogenes* virulence may be reduced from four to two, with further advantage of obviating the necessity to perform colony forming unit (CFU) estimation [16]. Refinement aims to minimize the suffering of each animal subject through experimental design that is as painless and efficient as possible [8].

1.4 Future Perspective

Despite our nonstopping efforts in the past, foodborne disease continues to savage human society at random and cause particular misery to vulnerable population groups. Naturally, we can point our fingers to the fact that foodborne pathogens have uncanny ability to constantly evolve and develop phenotypic and genetic traits that enable their evasion of host innate and acquired immune defense mechanisms, and their sabotage of our every intervention attempt. However, this does not hide the reality that some obvious gaps exist in our knowledge about the molecular basis of pathogenicity of foodborne organisms. Use of laboratory models including animal and cell culture models has contributed greatly to our past understanding of foodborne pathogens and diseases, and more will have to be learned via this approach in combination with other emerging technologies. The documentation and summation of the existing findings in this area provide a platform from which new insights will be uncovered and innovative mitigation measures will be launched.

REFERENCES

1. Bell A. Foodborne illness: the history of an invisible enemy. Available at https://antiquitynow.org/2014/06/05/foodborne-illness-the-history-of-an-invisible-enemy/.
2. Satin M. History of foodborne disease—Part 1—ancient history. Encyclopedia of Food Safety (Internet). 2014. Available at http://tinyurl.com/kmzt56u.
3. Behravesh CB, Williams IT, Tauxe RV. Emerging foodborne pathogens and problems: expanding prevention efforts before slaughter and harvest. In: *Institute of Medicine (US). Improving Food Safety through a One Health Approach: Workshop Summary.* Washington, DC: National Academies Press (US); 2012. A14. Available at http://www.ncbi.nlm.nih.gov/books/NBK114501/.

4. Riley LW, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Engl J Med*. 1983;308(12):681, 684–685.
5. Rangel JM, et al. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg Infect Dis*. 2005;11(4):603.
6. Henkel JS, Baldwin MR, Barbieri JT. Toxins from bacteria. *Experienta Suppl*. 2010;100:1–29.
7. Walker MJ, et al. Disease manifestations and pathogenic mechanisms of group A *Streptococcus*. *Clin Microbiol Rev*. 2014;27(2):264–301.
8. McGonigle P, Ruggeri B. Animal models of human disease: challenges in enabling translation. *Biochem Pharmacol*. 2014;87(1):162–171.
9. Ericsson AC, Crim MJ, Franklin CL. A brief history of animal modeling. *Mo Med*. 2013;110(3):201–205.
10. Wolfgang MJ, Golos TG. Nonhuman primate transgenesis: progress and prospects. *Trends Biotechnol*. 2002;20:479–484.
11. Padilla-Carlin DJ, McMurray DN, Hickey AJ. The guinea pig as a model of infectious diseases. *Comp Med*. 2008;58(4):324–340.
12. Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V. The pig: a model for human infectious diseases. *Trends Microbiol*. 2012;20(1):50–57.
13. Bou Ghanem EN, Myers-Morales T, D'Orazio SE. A mouse model of foodborne *Listeria monocytogenes* infection. *Curr Protoc Microbiol*. 2013;31:9B.3.1–9B.3.16.
14. Disson O, Lecuit M. In vitro and in vivo models to study human listeriosis: mind the gap. *Microbes Infect*. 2013;15(14–15):971–980.
15. García Y, Díaz-Castro J. Advantages and disadvantages of the animal models v. in vitro studies in iron metabolism: a review. *Animal*. 2013;7(10):1651–1658.
16. Liu D. Listeria monocytogenes: comparative interpretation of mouse virulence assay. *FEMS Microbiol Lett*. 2004;233(1):159–164.

Section I

Foodborne Infections due to Viruses

2

Adenoviruses

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CONTENTS

2.1 Introduction

Adenovirus (AdV) is an important human pathogen and is estimated to account for 8% of clinically relevant viral diseases globally.[1] First identified in 1953 as the cause for acute febrile respiratory disease, AdVs are endemic in the pediatric population worldwide, affecting children younger than 5 years old with mild symptoms and generally self-limiting illnesses.[2–4] Self-limiting infections may also occur in adults, but some serotypes have been associated with severe respiratory illness and potentially fatal outbreaks of pneumonia in residential facilities and military bases. The main etiologic agents for these outbreaks are serotype 4 and occasionally serotypes 3, 7, 14, and 21. It is possible that stress and crowding may contribute to AdV transmission and susceptibility.[5] AdVs have serious complications, impacting morbidity and mortality in immunocompromised individuals of any age.[6–8]

With more AdV serotypes being identified, it becomes clear that AdVs cause an array of clinical diseases, including epidemic keratoconjunctivitis (EKC), acute hemorrhagic cystitis, hepatitis, gastroenteritis, myocarditis, and pneumonia. Being one of the most prevalent enteropathogens causing infantile gastroenteritis, enteric AdVs are implicated in sporadic cases as well as in outbreaks of food-borne illness in kindergartens, schools, and hospitals.[9] Gastroenteritis due to AdVs often occurs in children younger than 5 years of age, accounting for ~12% of all enteropathogenic viruses identified, and is most commonly associated with serotypes 40 and 41; however, other types including 1, 2, 7, 9, 10, 18, 19, 22, 31, 42, 52, 58, 65, and 67 have also been reported as etiologic agents of viral gastroenteritis.[9–20] Serotypes 40 and 41 account for 5%–20% of hospital-admitted diarrhea cases in children under 2 years old. As children age, the incidence of AdV gastroenteritis decreases due to increasing levels of population immunity to AdV infection.

AdVs can be easily propagated in cell culture, and there are several cell lines that can be used as laboratory models for AdVs. The primary human embryonic kidney (HEK) cells are the best host for replicating various serotypes of AdVs. The lung epithelial cell line A549 and other epithelial cell lines, such as HEP-2, HeLa, and KB, are also good hosts for AdVs. For enteric AdVs, such as AdV40 and AdV41, the HEK 293 cell offers a convenient laboratory model.[2] In addition, *Sigmodon hispidus* cotton rats and mice, such as C57BL/6N, C57BL/lOScN, CBA/N, and C3H/N strains, were used as animal models to investigate the molecular pathogenesis of pneumonia caused by AdV infection.[21] AdVs have been used as models of virus–cell interaction. Decades of studies have contributed to the extensive understanding of the molecular biology, including life cycles, the host–pathogen interaction, genetics, epidemiology, and pathogenesis of AdVs, which are discussed in this chapter. AdVs continue to be studied as delivery vehicles for gene therapy, vaccination, and cancer treatment, which underscores the importance of understanding these viruses.

2.2 Classification and Morphology

AdVs constitute the Adenoviridae family, which is divided into the genera *Mastadenovirus* and *Aviadenovirus*. The genus *Mastadenovirus* covers viruses of several different animals, including bat, bovine, canine, equine, human, murine, ovine, porcine, simian, and so on, whereas the genus *Aviadenovirus* is limited to viruses of birds.[2] Currently, there are 68 reported human AdVs according to the National Center for Biotechnology Information Taxonomy Browser, representing seven different species or subgroups (A–G). The classification of AdVs was originally based on their hemagglutination patterns and serologic profiles. Recent advancements in sequencing capability have allowed the discovery and classification of new AdVs (types 52–68), where the differentiation of strains is based on bioinformatics analysis of their genomic sequencing (Table 2.1).[2,4,11–15,22–37] The majority of these newly discovered AdVs are products of homologous recombination, a common evolutionary adaptation of AdVs. Among the seven different species, species B can be further divided into B1 and B2 based on their organ tropisms.[22] There is a correlation between the species and their tissue tropisms, which determines the clinical manifestation of AdV infection. Species A, F, and G show tissue tropisms toward the gastrointestinal tract and induce gastroenteritis. Species B1, C, and E mainly cause respiratory illness; species B1, B2, D, and E produce ocular infection, whereas B2 AdVs cause kidney and urinary tract infection.[8,37]

AdVs are nonenveloped double-stranded DNA (ds DNA) viruses with icosahedral shells and nucleoprotein cores, ranging in size from 65 to 100 nm in diameter. The capsid of the viral particle is composed of seven proteins: hexon, three hexon-associated proteins, penton, a penton-associated protein, and fiber. The proteins form 252 capsomeres, which consist of 240 trimers of the major capsid protein hexon and 12 pentons. The fiber protein, which has a length that varies among the different serotypes, embeds in the penton base and projects out from the capsid. These 12 extensions out of the particle serve crucial

TABLE 2.1

Classification of Human Adenoviruses

Species	Hemagglutination	Serotype	References
A	IV	12, 18, 31, 61	2,12
B1	I	3, 7, 16, 21, 50, 66, 68	22–25
B2	I	11, 14, 34, 35, 55	22,26,27
C	III	1, 2, 5, 6, 57	2,28
D	II	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54, 56, 58, 59, 60a, 62–65, 67	2,13–15,25,29–36,120
E	III	4	2
F	III	40, 41	2
G		52	11

I, complete agglutination of monkey erythrocytes; II, complete agglutination of rat erythrocytes; III, partial agglutination of rat erythrocytes; IV, little or no agglutination.