Animal Health Research Reviews

cambridge.org/ahr

Efficacy of experimental phage therapies in livestock

Marta Dec, Andrzej Wernicki and Renata Urban-Chmiel

Sub-Department of Veterinary Prevention and Avian Diseases, Institute of Biological Basis of Animal Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland

Review

Cite this article: Dec M, Wernicki A, Urban-Chmiel R (2020). Efficacy of experimental phage therapies in livestock. *Animal Health Research Reviews* **21**, 69–83. https://doi.org/10.1017/S1466252319000161

Received: 6 August 2019
Revised: 18 November 2019
Accepted: 19 November 2019
First published online: 19 June 2020

Key words:
Bacteriophages; experimental therapies; livestock; pathogens

Author for correspondence:
Renata Urban-Chmiel, Sub-Department of Veterinary Prevention and Avian Diseases, Institute of Biological Basis of Animal Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland.
E-mail: renata.urban@up.lublin.pl

Abstract

Bacteriophages are the most abundant form of life on earth and are present everywhere. The total number of bacteriophages has been estimated to be 10^{32} virions. The main division of bacteriophages is based on the type of nucleic acid (DNA or RNA) and on the structure of the capsid. Due to the significant increase in the number of multi-drug-resistant bacteria, bacteriophages could be a useful tool as an alternative to antibiotics in experimental therapies to prevent and to control bacterial infections in people and animals. The aim of this review was to discuss the history of phage therapy as a replacement for antibiotics, in response to EU regulations prohibiting the use of antibiotics in livestock, and to present current examples and results of experimental phage treatments in comparison to antibiotics. The use of bacteriophages to control human infections has had a high success rate, especially in mixed infections caused mainly by *Staphylococcus*, *Pseudomonas*, *Enterobacter*, and *Enterococcus*. Bacteriophages have also proven to be an effective tool in experimental treatments for combating diseases in livestock.

Introduction

Bacteriophages are the most abundant form of life on earth, present wherever there is a potential host – a bacterium. An important factor facilitating the acquisition and characterization of bacteriophages, in terms of their suitability for combating bacterial infections, is their common occurrence in diverse environments (e.g. wastewater, water bodies, soil, forest undergrowth, and food products). Their presence has also been confirmed in commercial sera, human vaccines, the human mouth (dental plaque and saliva), and the gastrointestinal tracts of human beings and other animals. The presence of bacteriophages is a natural phenomenon that has existed for billions of years, resulting in the balance of various bacteria in the natural environment (Batinovic *et al.*, 2019).

The total number of bacteriophages on Earth has been estimated at 10^{32} virions, which is 10 times the number of characterized bacteria. The phage population in water bodies has been determined to range from 10^4 to 10^8 virions mL^{-1}, while in the soil it reaches about 10^9 virions g^{-1} (Weinbauer, 2004; Wittebole *et al.*, 2014). Currently, more than 25,000 bacteriophage nucleotide sequences have been deposited in INSDC databases (Adriaenssens *et al.*, 2017).

The mechanisms of activity of bacteriophages

Bacteriophages are characterized by specific mechanisms of action against host cells:

- Replication takes place exclusively in live bacteria that are susceptible to a given phage. The means of replication has similarities to eukaryotic viruses.
- In both lytic and lysogenic cycles, adsorption, penetration, replication of nucleic acids, formation of virions, and their release from the host cell occur.
- Phages are specifically associated with a specific bacterial strain.
- Phages can transmit new genes to microorganisms, which contributes to the genetic diversity of bacteria and the emergence of pathogens enriched with new virulence factors, such as adhesins or toxins.
- Bacteriophages show a specific affinity for individual types of bacteria.
- The specificity and spectrum of activity of phages are determined by the presence of bacterial cell surface receptors, i.e. LPS, envelopes, fimbriae, and other proteins (Weinbauer, 2004; Skurnik and Strauch, 2006; Rakhuba *et al.*, 2010).

Taxonomy and classification of bacteriophages

The criteria of bacteriophage taxonomy applied by the ICTV (International Committee on Taxonomy of Viruses, EC 48, Budapest, Hungary, August 2016) are based mainly on genome type and virion morphology, including genomic and proteomic methods. Today, bacteriophages

are usually classified into more than 870 species, 14 families, over 204 genera, and more than 6000 types of phages, including 6196 bacterial and 88 archaeal viruses (Ackermann and Prangishvili, 2012; Krupovic *et al.*, 2016; Adriaenssens and Brister, 2017; Adriaenssens *et al.*, 2017). However, the classification of viruses (including bacterial viruses) is still in progress, and many changes were made in 2018. Consequentially, there are now 142 families, 81 subfamilies, and about 4978 species (ICTV, 2018).

Bacteriophages can be distinguished by shape, structure, and capsid symmetry – isometric (polyhedral) and helical (spiral), nucleic acid, and interaction with the microbial host. Phages are also distinguished by size – small, medium, or large; shape – filiform or spherical; and the presence or absence of a head and/or tail. The tailed phages are a large group of viruses which account for 96% of phages. They are grouped into three families: *Myoviridae*, *Siphoviridae*, and *Podoviridae* (Karthik *et al.*, 2014; Urban-Chmiel *et al.*, 2015; Wernicki *et al.*, 2017).

The main division and characterization of bacteriophages is based on the type of nucleic acid (DNA or RNA) and on the structure of the capsid, which is built of structural proteins. Numerous scientific reports (Karthik *et al.*, 2014; Adriaenssens and Brister, 2017) confirm that bacteriophages have only one type of nucleic acid and that the vast majority of them have double-stranded, or less often single-stranded, DNA. There are also species with single- or double-stranded RNA. The detailed unofficial classification of bacteriophages proposed by the ICTV, taking into account the nature of the genomic nucleic acid and virion morphology, is presented in Table 1.

According to the classification proposed by Goyal *et al.* (1987), who classified phages based on their receptors on the host, phages may be classified as follows:

- Somatic phages – with receptors present on the cell wall.
- Capsular phages – with receptors on the capsular polysaccharide.
- Appendage phages – with receptors localized on bacterial virulence factors, such as flagella, pili, or fimbriae.

According to Wittebole *et al.* (2014), bacteriophages can also be classified on the basis of the specific target bacterial host, e.g. the staphylococcal phage family (Deghorain and Van Melderen, 2012) or the *Pseudomonas* phage family (Ceyssens and Lavigne, 2010); the environment of the phage, e.g. marine virus or land virus; and its life cycle – lytic or lysogenic, pseudo-lysogenic, or chronic infection (Ackermann, 2011; Wernicki *et al.*, 2017).

Hence there are a number of criteria for classifying bacteriophages, according to need and their possible uses in measures taken to eliminate bacteria.

The history of bacteriophages

Phages were first discovered more than 100 years ago by the English bacteriologist Frederick Twort and the French-Canadian microbiologist Felix d'Herelle (Twort, 1915; d'Herelle, 1917). Twort demonstrated the presence of an antibacterial element with a lytic effect in cultures of micrococci, and also confirmed that the transparent substance tested could pass through filters that were able to retain larger microorganisms, such as bacteria. Twort described this material, which is not capable of growth in the absence of bacteria, as a ferment secreted by the microorganism, the reason for which was not entirely transparent. It is also worth noting that the first reports on bacteriophages had been presented by the British bacteriologist Ernest Hanbury Hankin, who as early as 1896 had discovered an unknown 'biological suspension' obtained from the water of the Ganges

Table 1. Classification of bacteriophages proposed by the International Committee on Taxonomy of Viruses (ICTV), taking into account the nature of the genomic nucleic acid and virion morphology (Baj *et al.*, 2015)

Order	Family	Morphology	Kind of Nucleic acid
Caudovirales	*Myoviridae*	Non-enveloped, short contractile tail	Linear dsDNA
	Siphoviridae	Non-enveloped, long non-contractile tail	
	Podoviridae	Non-enveloped, short non-contractile tail	
	Lipothrixviridae	Enveloped, rod-shaped	
Ligamenvirales	*Rudeiviridae*	Non-enveloped, rod-shaped	Linear dsDNA
Unclassified	*Ampullaviridae*	Enveloped, bottle-shaped	Linear dsDNA
	Bicaudaviridae	Non-enveloped, lemon-shaped	Spherical dsDNA
	Clavaviridae	Non-enveloped, rod-shaped	Spherical dsDNA
	Corticoviridae	Non-enveloped, isometric	Spherical dsDNA
	Fuselloviridae	Non-enveloped, lemon-shaped	Spherical dsDNA
	Cystoviridae	Enveloped, spherical	Segmented dsDNA
	Globuloviridae	Enveloped, isometric	Linear dsDNA
	Guttaviridae	Non-enveloped, ovoid	Spherical dsDNA
	Inoviridae	Non-enveloped, filamentous	Spherical ssDNA
	Leviviridae	Non-enveloped, isometric	Linear ssRNA
	Microviridae	Non-enveloped, isometric	Spherical ssDNA
	Plasmaviridae	Enveloped, pleomorphic	Spherical dsDNA
	Tectiviridae	Non-enveloped, isometric	Linear dsDNA

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

and Yamuna Rivers, which caused lysis of the cholera bacteria *Vibrio cholerae* (Hankin, 1896).

However, the first microbiologist to isolate and describe phages, and to develop the first phage therapy, was Felix d'Herelle, who is still credited by many scientists with the discovery of bacteriophages and the therapeutic implications he proposed, known as "phage therapy." Félix d'Herelle described his observations while examining patients suffering from or cured of 'shigellosis' caused by infection with *Shigella* spp. By treating *Shigella* bacteria obtained from sick patients with an 18-h active filtrate from feces, d'Herelle achieved the arrest of bacterial growth and their destruction by lysis. He also demonstrated the antibacterial activity of this 'anti-Shiga-microbe' by applying a phage suspension in laboratory animals as an effective treatment for shigellosis, and thereby introduced the use of bacteriophages to clinical medicine. This was also a precursor to the use of intravenous phage therapy in sick patients (Wittebole *et al.*, 2014).

Both scientists (Twort and d'Herelle) called these agents bacteriophages, and d'Herelle suggested that there was only one phage, *Bacteriophagum intestinale*, of which all phages were various 'races'. However, d'Herelle emphasized that 'the history of phage is still older than what has been documented, which is extracted from Greek word "phagein" which means "eat" – to eat or devour the bacteria'.

Due to their specificity for bacterial target hosts, bacteriophages have been used since their very discovery in various types of targeted human therapies, particularly the treatment of acute and chronic dermatological, ophthalmological, urological, oral, pediatric, otolaryngological, and surgical infections. It should be emphasized that significant therapeutic successes were achieved in the initial period of use of phage therapy, which constituted a major contribution to the development of phage therapy to treat bacterial diseases, especially in the pre-antibiotic era. According to sources from that time, the only treatment available in the 1920s and most of the 1930s was serum therapy for selected pathogens, such as pneumococci and the diphtheria bacterium, so the introduction of bacteriophages came to significantly dominate human medicine (d'Herelle, 1931; Abedon *et al.*, 2011).

According to studies on the use of bacteriophages in clinical treatments, the first article was published in Belgium by Bruynoghe and Maisin (1921), who used bacteriophages to treat skin necrosis caused by staphylococci, resulting in a significant improvement in the patients' clinical condition (i.e., reduction of pain, swelling and fever) within 48 h.

During the interwar period, the molecular structure of bacteriophages was not yet known in detail, so many scientists held the view that these microorganisms were derived from protein alone and acquired their antimicrobial properties as a result of various reactions (Northrop, 1938). It was not until the 1940s that the structure and shape of bacteriophages were first described and documented using an electron microscope (Rusca, 1940). Various types of phages were presented as photograms and their common structure was described as a non-uniform round head with a thin tail (Luria and Anderson, 1942). The appearance of electron microscopy, which remains a widely used technique, also enabled recognition of the stages of multiplication of bacteriophages involved in bacterial lysis, e.g. adsorption, penetration, and proliferation, and the release of daughter phages following lysis. Confirmation of the 'viral' nature of phages as well as the physicochemical properties of phage particles enabling their replication and lysogeny was made possible by advances in science, including the discovery of the structure of DNA and RNA molecules in the 1950s (Sankaran, 2010).

Modern, rapidly advancing methods for the identification of microorganisms are to a large extent based on comprehensive genetic analysis of individual fragments of microorganisms, including bacterial viruses. This has made it possible to sequence an entire phage genome (Sanger *et al.*, 1982) or selected subunits. This in turn has led to the possibility of restriction analysis of phages in their identification (Luria and Human, 1952; Bertani and Weigle, 1953). The last decade has seen the rapid development of mass spectrometry (e.g. MALDI TOF) and proteomics, which enables highly detailed identification of bacteriophages at the genus level by means of analysis of selected amino acid fragments of protein structures, as confirmed in our previous work (Urban-Chmiel *et al.*, 2018*b*).

The appearance of chemotherapeutics in the 20th century, with the introduction of sulfonamides and of penicillin (discovered by Fleming in 1928) in the 1940s, resulted in a significant decline in research into the use of bacteriophages to fight bacteria. In Western Europe, phage therapies were completely eliminated from medical research, although they remained an active area of research and development in Eastern Europe, including in the republics of the Soviet Union, mainly Georgia (the Eliava Institute in Tbilisi), as well as in Poland (the Phage Therapy Unit of the Hirszfeld Institute in Wrocław) and to a lesser extent in India. It is worth noting that during the last decade, the emergence of multi-drug-resistant bacteria has led scientists to reconsider this century-old approach and to have a fresh look at phage therapy as a 'new' and potentially effective treatment option for difficult-to-treat bacterial pathogens (Weber-Dąbrowska *et al.*, 2000).

Currently, in the era of increasing widespread drug resistance among microorganisms and the lack of effective methods for combating infections, phage therapies are beginning to experience a renaissance. There is practically no scientific center in the world where such research is not conducted, as confirmed by numerous publications and scientific conferences. The most important problem in combating many infections is the high multi-drug resistance of strains. This is the result of widespread and uncontrolled use of antibiotics, e.g. as growth stimulants in the form of feed additives and in treatment of bacterial infections. In human bacteria, most antibiotic resistance genes have emerged due to direct contact with strains derived from animals. For this reason, in many cases, the therapeutic effect is negligible, and the threat of infection caused by the increase in pathogens in the environment is an important factor necessitating the search for alternative methods to eliminate chemotherapeutic-resistant microorganisms.

According to numerous sources (Gardette and Tomasz, 2014; McGuinness *et al.*, 2017), methicillin-resistant *S. aureus* (MRSA) strains, responsible for serious nosocomial infections, have been recognized as the most dangerous type of bacteria. The significant spread of strains that are often susceptible to only one group of drugs (glycopeptides, e.g. vancomycin) is a serious problem. This phenomenon should be considered very dangerous, especially as the first vancomycin-resistant strains have already appeared in the world (including Poland), posing serious risks to the health and life of patients while causing a huge increase in health care costs (Gardette and Tomasz, 2014). Infections in the USA in 2011 caused 80,000 severe cases and 11,000 deaths. Asymptomatic colonization of the nasal cavity in the general population is estimated to range from 1.5% for MRSA to 30% for other *S. aureus* strains.

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Phage experimental therapies in animals

The high efficacy and safety of phage therapy in comparison with antibiotics is due in part to their specificity for selected bacteria, which is manifested by the ability to infect only one species, serotype, or strain. Such a mechanism does not cause destruction of commensal gut microflora, and due to the self-replication of bacteriophages during therapy, repeated applications are often unnecessary. It is also worth noting that the mechanisms of bacterial resistance against phages and antibiotics show significant species differences (Scott *et al.*, 2007*a*; Sultan *et al.*, 2018), Therefore, the use of phages in human medicine, veterinary medicine, or the agricultural industry does not significantly affect the susceptibility of bacteria to antibiotics used in human treatment, which is a crucial issue in the use of antibiotics in the agricultural industry. Some studies have confirmed that a single application of bacteriophages completely eliminates pathogenic bacteria, in contrast to some antibiotics, which must be administered multiple times (Lee and Harris, 2001; Bach *et al.*, 2003; Brüssow and Kutter, 2005; Rivas *et al.*, 2010). Another important advantage is the lack of species barrier in the antibacterial activity of phages, which means that the same bacteriophages can be used to combat infections in human and animal hosts, including pathogens such as *Staphylococcus* spp., various serotypes of *Escherichia coli*, or other species (Alomari *et al.*, 2016).

Many studies (Lee and Harris, 2001; Sheng *et al.*, 2006; Wall *et al.*, 2010) have confirmed that bacteriophages used in targeted experimental therapies can be used to prevent and treat various bacterial infections in livestock. A reduction of up to 99% or in some cases even 100% in bacterial pathogens, including zoonotic pathogens, has been shown to significantly improve clinical outcomes in many experimental treatments of infections in cattle or pigs.

As bacteriophages are ubiquitous in the environment, their use in veterinary medicine or animal and plant production is one of the most environmentally friendly antibacterial treatments available today. This means that they have no negative effect on the environment, as in the case of antibiotics or other chemotherapeutics.

Phages have several important advantages over antibiotics that make their use in various livestock industries potentially very appealing. Some examples of experimental bacteriophage therapy include treatments for *Salmonella* and *E. coli* infections in mice, poultry, calves, piglets, and lambs; for *Clostridium* spp. and *Pseudomonas aeruginosa* infections in mice or other laboratory animals, such as hamsters and rats; and for *Staphylococcus aureus* infections in mice, cows, and other livestock. The advantages and potential disadvantages of the use of phage therapies are presented in Table 2.

In the case of phage therapy in livestock intended for consumption, many experiments have dealt with combating infections caused by zoonotic microorganisms that pose a threat to human health, particularly pathogenic strains of *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *Listeria* spp., which are foodborne pathogens. As cattle, swine, sheep, goats, and poultry are raised as livestock for food, these bacteria have a significant impact on the safety of health and life of people. Proposals for replacing antibiotics as supplements result in part from current legal regulations in the European Union prohibiting the routine use of antibiotics in farm animals (Dibner and Richards, 2005) and limiting the chemical treatment of carcasses during processing (Atterbury, 2009).

The use of bacteriophages to treat human infections has had a high success rate (about 85% or even more), especially in the case of mixed infections caused mainly by *S. aureus*, *Klebsiella*, *E. coli*, *Proteus*, *Pseudomonas*, *Enterobacter*, and vancomycin-resistant *Enterococcus* (Smith and Huggins, 1983; Smith *et al.*, 1987*a*, 1987*b*; Weber-Dąbrowska *et al.*, 2000; Morozova *et al.*, 2018). In experimental treatments in livestock, bacteriophages have proven to be an effective tool in combating diseases in poultry, cattle, sheep, pigs, and fish. For example, the effectiveness of phage therapy has been confirmed in necrotic enteritis induced by anaerobic bacteria of the species *Clostridium perfringens* in poultry. The types and effectiveness of phage therapies used in poultry have been the subject of numerous publications (Loc Carrillo *et al.*, 2005; Scott *et al.*, 2007*b*; Atterbury, 2009). Phage preparations in the form of cocktails have been used in poultry in experimental therapies against infections caused by pathogens such as *Salmonella* spp., *E. coli*, and *Campylobacter* spp. A detailed description of the use of bacteriophages to control bacterial infections in poultry, including zoonotic infections, has previously been described in our review article (Wernicki *et al.*, 2017).

Research on phage therapy in large and medium-sized farm animals, such as cattle, pigs, sheep, and goats, plays an important role. The use of bacteriophages in cattle and other livestock species has proven an effective tool in reducing bacterial colonization in the course of chronic skin ulcers, caused mainly by staphylococci and streptococci, as well as respiratory diseases. In many cases, the number of bacteria has been limited to 2–4 $\log_{10}$ CFU, which was reflected in the course of the disease process as mitigation of disease symptoms (Tiwari *et al.*, 2014).

In ruminants, experimental phage therapies have been tested to combat infections in newborn calves and lambs, caused mainly by enterotoxigenic *E. coli* strains. The studies have confirmed the effectiveness of these therapies, based on mitigation of disease symptoms (diarrhea and fever) and a reduction in mortality ranging from 15% to about 67%. In addition, the phages remained in the gut of the animals for as long as pathogenic *E. coli* strains were present.

Experimental phage therapy in ruminants

Because cattle and other ruminants are the main reservoirs of pathogenic and zoonotic strains of *E. coli*, including O157:H7, a great deal of research concerns the use of bacteriophage treatment to eliminate these pathogens (Goodridge and Bisha, 2011).

The effectiveness of phage therapy against infections caused by enterotoxigenic *E. coli* strains in newborn calves has been varied, and researchers (Johnson *et al.*, 2008) have shown that it depends on a number of factors, including the following:

- the experimental design of the infection
- the form of phage application
- the quantitative and qualitative composition of the dose of bacteriophages

In an early study, Smith and Huggins (1983) used a phage cocktail containing a mixture of two phages, B44/1 and B44/2, at a titer of 10^{11} PFU mL^{-1}, against the enterotoxigenic *E. coli* strain O9:K30.99, inducing enteritis in calves. The authors demonstrated high (nearly 93%) efficacy of the experimental treatment in calves that were not fed colostrum but treated with phages, even when clinical symptoms were present. The results confirmed that a phage cocktail can significantly reduce morbidity and mortality, even when applied in the case of significant clinical symptoms.

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Table 2. Advantages and disadvantages of phage therapies.

Advantages	Disadvantages
• Obligatorily lytic bacteriophages destroy bacteria, whereas antibiotics often act only as bacteriostatic agents (Kutter *et al*., 2010) • They replicate at the site of infection, so they do not have to be applied in large quantities or in repeated doses (Loc-Carrillo and Abedon, 2011; Borie *et al*., 2014) • Some bacteriophages can cause lysis of bacteria that are resistant to antibiotic therapy, including those living in a biofilm (Ul Haq *et al*., 2011; Khalifa *et al*., 2015) • They exhibit limited specificity for bacterial hosts and may be active against antibiotic resistant Gram-positive and Gram-negative bacteria, sometimes infecting only one species or even one strain, so they do not affect commensal and symbiotic flora, which reduces the risk of secondary infection by pathogenic bacteria (Kutter *et al*., 2010; Loc-Carrillo and Abedon, 2011) • Bacteriophages (except those whose virions have a lipid component) are composed solely of proteins and nucleic acids, which limits their potential mechanisms of toxicity (Loc-Carrillo and Abedon, 2011) • They leave no residue in tissues and there is no withdrawal period • Bacterial mutations towards resistance to a specific phage are less common than in the case of antibiotics; moreover, if bacteria resistant to a phage do appear, it is much easier to find another phage than to discover or synthesize a new antibiotic (Elbreki *et al*., 2014) • The specificity of bacteriophages limits the possibility of the emergence of resistance, because they do not exert selective pressure on bacteria that are not their hosts (Loc-Carrillo and Abedon, 2011) • Phages can be used in combination with other antibacterial agents, including other phages, which significantly expands their spectrum of antibacterial activity (Kutter *et al*., 2010) • Waste from the production of phage preparations and treatment with them is mostly biodegradable, and their ecotoxicity only affects the phage's potential hosts • Production of phage preparations is simple and economical (Skurnik *et al*., 2007) • People are in constant contact with bacteriophages. They are present in the water, air, and food, and no negative reactions to phage particles have yet been reported (Wright *et al*., 2009; Kutter *et al*., 2010)	• Only strongly lytic phages are suitable in phage therapy (Pirnay *et al*., 2015) • Gram-negative bacteria lysed by phages release endotoxins or other toxic proteins that cause fever and potentially fatal toxic shock in living organisms (Pirnay *et al*., 2015) • Phage particles are larger than most drugs. This may prevent their use in some illnesses because they will be unable to reach the infected tissues. • Bacteriophage proteins can induce an immune response because they contain foreign proteins, which are potential epitopes for antibodies (Loc-Carrillo and Abedon, 2011, Górski *et al*., 2012, Dąbrowska *et al*., 2014) • The induction of an immune response against bacteriophage proteins could potentially reduce the effectiveness of the therapy, or cause death as a consequence of anaphylactic shock (Skurnik and Strauch, 2006; Loc-Carrillo and Abedon, 2011) • Phages are cleared by the reticuloendothelial system. Their half-life in animals is short, but can be extended by subculturing phages in infected animals and isolating mutants that are less easily cleared (Loc-Carrillo and Abedon, 2011) • Limited knowledge of the kinetics of phages is a major problem, as several parameters of critical therapies must be considered, including the degree of adsorption, the number of replication cycles, the latent period in the initial dose of phages, and their elimination by the reticuloendothelial system (Abedon and Thomas-Abedon, 2010; Pirnay *et al*., 2015) • Due to the specificity of bacteriophages, one phage cannot be used to treat a broad spectrum of infections, as in the case of antibiotics. In many cases, the etiological agent may have to be precisely identified and a suitable cocktail of phages selected (Loc-Carrillo and Abedon, 2011) • Not all bacteriophages can be used in treatment. In particular, phages capable of entering a lysogenic cycle are not suitable, because replication of the genome of such a phage does not kill the host and its presence often protects the bacterial cell against infection by other phages (Wernicki *et al*., 2017) • During lysis of bacterial cells, the endotoxins released (mainly LPS and components of the bacterial cell wall) can induce fever and lead to toxic shock (Krylov *et al*., 1993, Wernicki *et al*., 2017) • Phage preparations easily lose their activity if they are stored improperly or for too long (Pirnay *et al*., 2015; Wernicki *et al*., 2017) • There are problems with consumer perceptions, associated with the use of phage preparations to combat pathogens transferred onto food (food preservation). The presence of bacterial viruses in food may discourage consumers from eating food treated with phages. However, there are solutions that may in the future enable the use of new endolysins derived from phage particles or purified lysozyme (Moye *et al*., 2018)

In research by Callaway *et al*. (2008), oral application of a phage cocktail obtained for an *E. coli* reference strain, i.e. O157: H7 strain 933 (ATCC 43895), resulted in a significant reduction in bacteria in individual segments of the gastrointestinal tract: the rectum, caecum, and, in two cases, the rumen. The number of pathogenic bacteria isolated from animals after application of the phage cocktail ranged from 10^2 to 10^3 PFU g^{-1} of caecal and rectal contents in all samples tested, but only in two samples in the case of the rumen.

A significant reduction in strains of this human pathogenic serotype was also observed by Sheng *et al*. (2006) following oral administration of a bacteriophage suspension to young calves. The authors observed a significant reduction in colonization up to complete elimination of enterotoxigenic *E. coli* strains of serotype O157:H7 from the gastrointestinal tract up to day 16 after application.

High efficacy in reducing diarrhea and mortality has been obtained after a single *per os* administration of a phage mixture at a titer of 10^5 PFU mL^{-1} before or at the onset of diarrhea and simultaneous infection *per os* with various pathogenic strains of *E. coli*. Different doses of bacteriophages at titers of 10^2 and 10^5 PFU mL^{-1} in the period from 6 h to 10 min before challenge and up to 10–12 h after challenge resulted in a reduction in disease symptoms (fever and diarrhea). Repeated administration of bacteriophages with milk or colostrum at 10^5 PFU mL^{-1} resulted in high antibacterial efficacy in the case of administration of phages from 4 to 10–12 h after infection. The authors confirmed high phage titers of 10^{11} PFU mL^{-1} *in vivo* just 5 h after application, and they also reported that the application of small doses of phages with titers up to 10^2 PFU mL^{-1} immediately after the onset of diarrhea significantly alleviated disease symptoms (Brüssow and Kutter, 2005). Based on the diverse results obtained in experimental phage therapies in calves, it has been hypothesized that specific doses of phages can be used to 'control the course of the disease', because diarrhea was prevented even when the suspension was administered up to 8 h after experimental infection of calves with enterotoxigenic *E. coli*, and this was correlated with a simultaneous decrease in the number of pathogens.

An important element in phage therapies is their capacity for self-replication in target cells, and thus in the body of the infected individual. Waddell *et al*. (2000) treated weaned 7- to 8-week-old

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

calves with a six-phage cocktail up to 7 days before challenge with *E. coli* O157:H7 and obtained a significant increase in the number of phages excreted in feces after day 8 of application and a reduction in the number of *E. coli* excreted by the animals in comparison to the control animals. The increase in the number of bacteriophages was determined to be the result of their replication in bacterial cells and their release into the intestinal lumen.

Research conducted by Chase *et al.* (2005), in which the authors used a cocktail of 37 phages specific to *E. coli* O157:H7 strains in weaned Black Angus calves aged 4–6 months, showed a significant reduction in the bacterial concentration up to 16 or 24 h, depending on the number of applications. Moreover, the *E. coli* O157:H7 strains did not acquire resistance to any of the 37 bacteriophages used in the experiment, even after re-infection, which should be considered a highly beneficial therapeutic effect.

An important unfavorable phenomenon is the limited duration of an effective antibacterial titer of bacteriophages in ruminants, which is significantly linked to the sensitivity of bacteriophages to the gut environment. The viability of bacteriophages administered *per os* can significantly decrease due to the acidic environment of gastric acid, the presence of rumen microflora, and the activity of enzymes and other digestive compounds, such as bile (Goodridge and Bisha, 2011).

The effectiveness of phage therapies in calves in their first week of life following experimental infection with various ETEC strains has also been found to be determined by physiological factors in the newborn, such as the inactivating effect of gastric pH ≤ 3 and a body temperature raised to $\geq$40 °C due to fever, which may have a significant impact on bacteriophage virulence (Smith *et al.*, 1987*b*).

Some bacteriophages cannot survive in an environment with pH 2–3 or 7, which significantly reduces their titer, but sensitivity to specific pH values may also depend on the type of bacteriophage. Some research confirms (Dąbrowska *et al.*, 2005) that certain bacteriophages have a high survival rate in a pH range from 2 to 7. Furthermore, the problem of inactivation of phages by low gastric pH can be resolved by administering the phage suspension together with milk or directly after the calf feeds on milk, as confirmed by Barrow *et al.* (1998). The administration of substances neutralizing gastric acid, mainly saline solutions, immediately prior to administration of the bacteriophage suspension also appears to be an important element of effective phage therapy.

Taking into account the above assumptions, special attention has been paid to the possibility of rectal application of a phage suspension in order to bypass gastric juice and rumen microflora (Bielke *et al.*, 2012). Research by Sheng *et al.* (2006) based on previous *in vitro* experiments (Naylor *et al.*, 2003) has shown that application of a KH1 and SH1 phage cocktail (10^{10} PFU mL^{-1}) to young beef cattle in the form of a suspension *per rectum* with simultaneous administration of the same lytic phages (10^{6} PFU mL^{-1}) with drinking water caused a significant reduction in Shiga toxin-producing strains of *E. coli* O157:H7 in experimentally infected calves up to the detectable limit in most of the calves. However, this procedure did not completely eliminate pathogens from the herd.

The combination of various methods of bacteriophage application in the form of a suspension in cattle to eliminate pathogenic *E. coli* O157:H7 strains has also been the subject of research presented by Rozema *et al.* (2009). The authors also used simultaneous oral and rectal administration of a 10^{11} PFU mL^{-1} suspension of phages specific for nalidixic acid-resistant strains of *E. coli* O157:H7. However, the treatment did not completely eliminate pathogens from the body after experimental infection of the animals. Despite the reduction observed in the number of excreted *E. coli* bacteria, the values were not statistically significant compared to animals not treated with phages. Moreover, fecal excretion of bacteriophages specific to the *E. coli* O157:H7 strain used in the study was also observed in the young beef cattle from the control group, which the authors suggest may have been due to acquisition of phages *per os* from the farm environment.

Similar results in the elimination of pathogenic *E. coli* strains responsible for diarrhea in newborn calves have also been observed in our own research (Urban-Chmiel *et al.*, 2018*a*). Significant improvement in the health of newborn calves with symptoms of *E. coli*-induced diarrhea was achieved following six rectal applications (over 5 days) of a suppository containing a mixture of 10^{7}–10^{9} PFU ml^{-1} bacteriophages specific for pathogenic *E. coli*, in combination with probiotic strains isolated from cattle (patent application no. P.424314). The results indicated a significant therapeutic effect of the experimental suppositories, manifested by a decrease in rectal temperature and a reduction or complete elimination of diarrhea within 24 or 48 h after the first application of phages. A preventive effect of the experimental treatment was confirmed as well, manifested as stimulation of specific and non-specific mechanisms of the humoral immune response. It should also be emphasized that no pathogenic strains of *E. coli* were found in the calves for 3 weeks after application of suppositories.

Another example of a means of controlling infections in calves involves spraying of a bacteriophage suspension in the form of an aerosol cocktail to eliminate microbes. The use of phages obtained from uncleaned rooms where calves are kept (bedding, remains of feed, or feces) has also been found to result in highly effective protection in calves infected 3 h after being placed in the rooms. When a bacteriophage suspension was sprayed on litter in the amount of 10^{5}–10^{9} m^{-2} immediately before infection and up to 6 h before infection, bacteria were completely eliminated within 10 days after treatment. Spraying of a bacteriophage suspension in an aerosol form on the mucous membranes in the initial and terminal sections of the alimentary tract of calves within 24 h of the onset of diarrhea completely eliminated clinical symptoms within the next 20 h. Moreover, a high concentration of bacteriophages was found to persist until the end of infection, i.e. until *E. coli* was no longer isolated from the gastrointestinal tract, and sharply decreased after the animals had recovered, which resulted in protection of calves against diarrhea (Smith and Huggins, 1983).

According to Sheldon *et al.* (2006) and Machado *et al.* (2012), bacteriophages may also have beneficial effects in reducing the incidence of uterine infections induced by *E. coli*. However, Meira *et al.* (2013) were unsuccessful in treating cows with postpartum metritis caused by mixed flora, mainly pathogenic strains of *E. coli*, by intravaginal administration of a cocktail of 10 different phages at a titer of 10^{9} PFU mL^{-1}, specific for *E. coli* and without taking into account other etiological agents. Despite the reduction in the number of *E. coli* bacteria, there was no improvement in the health of the animals, whose disease symptoms were not alleviated. Furthermore, no preventive effect was obtained in the form of a reduction in the incidence of metritis. In addition, the incidence of retained placenta increased in cows after parturition, which the authors suggest may have been due to

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

suppression of localized cellular immune mechanisms, including inhibition of neutrophil migration, phagocytosis, and oxidative activity. In addition, the authors indicate that the problem of metritis in dairy cows is very often the result of mixed infections induced by pathogenic strains of species such as *E. coli*, *Trueperella pyogenes*, and *Fusobacterium necrophorum*, which makes the elimination of infections particularly difficult. A similar situation has been observed in attempts to combat subclinical mastitis caused by *S. aureus* in dairy cows. Five intramammary applications of a suspension of bacteriophage K at 10^{11} PFU mL^{-1} cured only about 16.7% of cases, and this percentage was not statistically significant compared to the control group. The authors suggested that such a low success rate may have been due to the application of the phage suspension during lactation, which was linked to the activity of enzymes contained in the milk that inactivated the bacteriophages (Gill *et al*., 2006*a*, 2006*b*).

Another example of the use of bacteriophages to combat bacterial infections in cattle was the development of a preparation using phage enzymes in combination with specific bacteriophages. This research resulted in a project to combat skin and mucous membrane infections caused by *F. necrophorum* in beef cattle (patent application no. WO 2004064732 A2).

In the case of experimental treatments in sheep, most research has concerned the control of infections caused by pathogenic strains of *E. coli*. For example, Raya *et al.* (2006) treated crossbred sheep with a single oral gavage application of a CEV1 phage suspension at ~10^{11} PFU mL^{-1} 3 days after experimental infection with pathogenic *E. coli* O157:H7 EDL 933 (10^{10} CFU per sheep), and reported a significant reduction in pathogenic strains from individual segments of the gastrointestinal tract (rumen, caecum, and rectum) 2 days after application of the phages. In another study (Smith and Huggins, 1983), application of a phage suspension to lambs 8 h after they were infected with an O8:K85,99 enteropathogenic strain of *E. coli* S13 reduced the numbers of pathogenic bacteria in the alimentary tract during the first 24 h after application. According to the authors, the experimental procedure also had a 'beneficial ameliorating effect' on the course of the disease.

On the other hand, a study by Bach *et al.* (2003), following oral administration in sheep a single phage (DC 22) at a titer of 10^5 PFU mL^{-1}, apart from a reduction in the number of *E. coli* O157:H7 up to 13 days after phage application, found no significant effect on fecal excretion of *E. coli* O157:H7 on successive days of the experiment (as of day 30). The authors suggest that this was probably due to non-specific binding of phages with food particles and other waste present in the rumen and gastrointestinal tract, which could ultimately have limited their effectiveness. Other authors (Sheng *et al*., 2006) have found that four *per os* applications of a suspension of phage KH1 specific for *E. coli* ATCC 43894 at 1.3×10^{11} PFU mL^{-1} in 7-month-old sheep caused no significant reduction in these *E. coli* strains in the gut.

Phage therapy in pigs

In the case of pigs, experimental applications of bacteriophages to eliminate foodborne pathogens have mostly been focused on controlling infections caused by pathogenic strains of *E. coli* and *Salmonella* spp.

The results of experimental phage therapies used in pigs to combat infections caused by pathogenic strains of *E. coli* have also been promising in many cases. For example, in an early experiment carried out by Smith and Huggins (1983), oral application of a cocktail of two phages, P433/1 and P433/2, or phage P433/1 alone in piglets with diarrhea caused by pathogenic *E. coli* P433 O20:K101, 987P significantly reduced clinical signs of diarrhea and the numbers of *E. coli* strains excreted by the animals.

In other experiments (Lee and Harris, 2001), simultaneous oral and intramuscular application of phage Felix 01 specific for pathogenic *Salmonella* Typhimurium strains at 10^{10} PFU mL^{-1} in 3-week-old piglets caused a significant reduction in the bacteria in the tonsils and caecum. In contrast, one of these author's previous study in 2000, assessing the effectiveness of a cocktail containing 26 phages specific to *Salmonella* spp. strains, had shown no significant reduction of bacteria in animals treated with the phage cocktail (Harris, 2000).

In another study, two applications of a suspension of two phages targeting *Salmonella enterica* strains at 3×10^9 PFU mL^{-1} at 24 and 48 h after challenge with *S. enterica* serotype Typhimurium caused a reduction in bacteria of >1.4 $\log_{10}$ CFU in the caecum, but a significant ($p < 0.05$) reduction in the pathogens was observed only in the rectum (Callaway *et al*., 2011). These authors also emphasized that several phages should be combined in the form of a cocktail and that several applications were necessary to exclude the potential risk of resistance in the pathogens and to prolong their exposure to phages.

The research results indicate that phage therapies directed against bacteria of the family *Enterobacteriaceae*, particularly pathogenic strains of *E. coli*, have proven to be the most effective in eliminating pathogens. For example, a study on the elimination of infections caused by pathogenic strains of *E. coli* in piglets using phage T4 of the *Myoviridae* family at 10^5 PFU mL^{-1} achieved up to 100% protection against infection. The optimal concentration of bacteriophages guaranteeing complete protection in 1-month-old piglets against experimental infection with *E. coli* O157:H7 strains was found to be 10^9 PFU phages, applied three times (Skoblikow and Zimin, 2013), but the authors suggest the need to individually adjust the concentration of bacteriophages and the individual therapy regimen.

Dietary supplementation with phage cocktails specific for mixed pathogens such as *Salmonella* spp., *E. coli*, *S. aureus*, and *C. perfringens* as an alternative to antibiotic growth stimulants has also been found to significantly improve growth performance in growing pigs (Kim *et al*., 2014; Svircev *et al*., 2018). For example, in a study by Gebru *et al.* (2010), in which weaned piglets were given a feed supplement of bacteriophages specific for *S.* Typhimurium at 3×10^9 PFU kg^{-1} of feed; *Lactobacillus plantarum* CJLP56 at 6.5×10^8 CFU kg^{-1} of feed (LP); 0.2% microencapsulated organic acids; or 5% fermented soybean meal. Bacteriophage supplementation together with probiotic strains was carried out for 2 weeks before and 2 weeks after oral challenge with *S. enterica* serotype Typhimurium. The results confirmed that a diet with the phage + probiotic supplement had a similar beneficial effect on growing pigs as an antibiotic-supplemented diet, especially after bacterial challenge.

In other research conducted in weaned piglets aged 3–4 weeks old, administration of an anti-*Salmonella* phage cocktail at the time of inoculation with *S. enterica* serotype Typhimurium significantly reduced *Salmonella* colonization by 2- to 3-log in the tonsils, ileum, and caecum, which was 99.0–99.9% of pathogens. The phage cocktail also showed lytic activity *in vitro* against *Salmonella* strains not belonging to the Typhimurium serotype. These include the Dublin, Enteriditis, Indiana, Kentucky, Litchfield, and Schwarzengrund serotypes of *S. enterica* (Wall *et al*., 2010). The results reported by the authors

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Table 3. Summary of studies of experimental phage treatment in livestock

Animals	Objective	Challenge	Phage application	Observations	Reference
Growing pigs (barrows) ABW 50.9 kg; breed (Landrace × Yorkshire × Duroc)	Determine the effects of dietary supplementation with bacteriophages alone or in combination with probiotics on growth performance and serum immunoglobulins in pigs	No challenge	Feed application (35 days) of commercial phage product containing a cocktail of bacteriophages specific to *Salmonella* (Typhimurium, Enteritidis, Derby), *S. aureus*, *E. coli* (k88, k99 and f41) and *C. perfringens* types A and C at 10^9 PFU kg^{-1}	1. Dietary supplementation with probiotics and bacteriophages or bacteriophages alone has the potential to improve performance of growing pigs 2. 3.5 log CFU reduction of *S.* Enteritidis PT4 per gram of caecal content	Kim *et al.* (2014)
Weaned pigs ABW 58.7 kg	Reduce morbidity, disease severity and mortality	Oral challenge with *S.* Typhimurium at 5×10^8 CFU mL^{-1}	Bacteriophages specific to *S.* Typhimurium as feed additive at 3×10^9 PFU kg^{-1} combined with probiotic *L. plantarum* CJLP56, 6.5×10^8 CFU kg^{-1} of feed	1. Significant influence on the growth of weaned pigs in comparison to pigs not treated with phages 2. Phage cocktail feed supplement has a comparable protective effect to that of antibiotics against infection by *S.* Typhimurium strains	Gebru *et al.* (2010)
Small pigs (3 to 4 weeks old; 14–18 kg)	Reduce morbidity, reduce *S.* Typhimurium colonization, disease severity and mortality	Oral challenge with *S. enterica* ser. Typhimurium *γ4232* by oral gavage (5 mL) at 5×10^8 CFU mL^{-1}	Microencapsulated phage cocktail with bacteriophages specific for *S. enterica* ser. Typhimurium strains – 5 ml by oral gavage; ~10^9 PFU mL^{-1}	1. Significant reduction (99%) or complete elimination (100%) of *S. enterica* ser. Typhimurium strains in ileum, tonsils and caecum samples within 48 h after the first administration. 2. Significant influence on growth and average weight of weaned pigs	Wall *et al.* (2010)
1-Month old piglets	Reduce morbidity, disease severity and mortality	Challenge with 1 ml of 8.0–log 10 CFU ml^{-1} suspension of *S.* Enteritidis	Application of phages as triple treatment at 10^9-bacteriophage 151 against *S.* Enteritidis, bacteriophage 25 against *S.* Hadar, bacteriophage 10 against *S.* Typhimurium. Bacteriophage suspensions administered by oral gavage at 10^9 PFU ml^{-1} and 10^{11} PFU ml^{-1}	1. Significant reduction in the concentration of 2 of 3 serovars (*S.* Enteritidis and Typhimurium) by 2–4 log CFU after administration of bacteriophage suspension at 10^{11} PFU	Skoblikow and Zimin (2013)
15 4-week-old pigs	Protection against experimental infection	10 ml of *S.* Typhimurium (ATCC 14028) culture at 10^8 CFU ml^{-1}.	5 ml of the *Salmonella*-specific bacteriophage cocktail C (SEP-1, SGP-1, STP-1, SS3eP-1, SalTP-2, SChP-1, SAP-1 and SAP-2) at 10^9 PFU ml^{-1} with feed	1. The 100% reduction of *Salmonella* ATCC 14028 reference strain and 92.5% of field isolates	Seo *et al.* (2018)
3-Week-old pigs	Reduce and eliminate *S. enterica* ser. Typhimurium strains	Oral challenge with *S. enterica* ser. Typhimurium at 10^8 CFU	Single *Salmonella*-lysing phage (Felix 01) at 10^9 PFU ml^{-1} both orally and intramuscularly 3 h after challenge	1. 100% efficacy in eliminating *S.* Enteritidis strains from tonsils 6 h after application of bacteriophage suspension 2. PSE phage more effective when administered prophylactically prior to *S.* Enteritidis infection than as a treatment for established *S.* Enteritidis infections	Lee and Harris (2001)

(*Continued*)

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Table 3. (*Continued.*)

Animals	Objective	Challenge	Phage application	Observations	Reference
6-Month-old weaned Holstein steers	Reduce the concentration of and eliminate *E. coli* O157:H7 strains	Single rectal application of 10^{10} CFU mixture of 4 strains of *E. coli* O157:H7 (ATCC 43894, WSU180, WSU400, 588)	Cocktail of phages SH1 and KH1 (25 ml of 10^{10} PFU mL^{-1}) by rectal application for 4 days. Administration of phages via drinking water at a final concentration of 1.8×10^6 to 5.4×10^6 PFU mL^{-1} from day 0 to end of experiment	1. Rectal application of phage significantly reduced number of *E. coli* O157:H7 2. Lack of complete elimination of pathogen from steers	Sheng *et al.* (2006)
1-Day-old chickens	Reduce morbidity, disease severity and mortality	Oral challenge with 2.95×10^5 CFU mL^{-1} *S.* Enteritidis	Cocktail of 3 phages by aerosol spray at 10^8 PFU ml^{-1} per dose for each phage at 6 days of age (2 daily doses) and probiotics administered at 1 day of age by coarse spray	1. Effective method for reducing *S.* Enteritidis colonization in the chicken intestine, leading to complete elimination of deaths in broiler chickens caused by infection with *S.* Enteritidis	Smith *et al.* (1987*a*, 1987*b*)
1-Day-old calves	Reduce morbidity, severity of clinical signs of disease, and mortality	Oral challenge with ETEC O9:K30.99; 3×10^9 CFU	Single oral application of bacteriophage cocktail B44/1 and B44/2, 10^{11} PFU mL^{-1}; 1 or 8 h after challenge with *E. coli*	1. Highest doses of bacteriophage significantly inhibited replication of pathogens in the digestive tract of calves	Johnson *et al.* (2008); Smith and Huggins (1983)
Cattle	Reduce morbidity, disease severity and mortality	Injection of 10^8 PFU mL^{-1} of *E. coli* O157:H7 by oral challenge	Phage cocktail (e11/2, e4/1c) applied directly by oral gavage at 10^8 PFU mL^{-1} Bacteriophage suspension applied via drinking water (10^3 or 10^4 PFU mL^{-1})	1. Reduction of mortality rates 2. Substantial reduction in the number of excreted bacteria (2 log units) within 24 to 48 h after phage administration 3. Reduced mortality rates to 5% and 25% depending on the titer of bacteriophage suspensions	Rivas *et al.* (2010)
Six 7-month-old Suffolk ewes	Reduce the concentration of and eliminate *E. coli* O157:H7 strains	Single oral dose of 3.5×10^{10} CFU *E. coli* O157:H7 ATCC 43894/animal	Four oral doses of 1.3×10^{11} PFU phage KH1 per animal per d	1. No reduction of intestinal *E. coli* O157:H7 in sheep	Sheng *et al.* (2006)
Young feedlot steers	Reduce or eliminate fecal shedding of *E. coli* O157:H7 strains	Oral challenge of nalidixic acid-resistant *E. coli* O157:H7	1. Oral application of phages (3.3×10^{11} PFU) or rectal application (1.5×10^{11} PFU), 2. Both oral and rectal application of phages (4.8×10^{11} PFU)	1. No significant differences in fecal shedding of *E.coli* O157:H7 strains 2. Presence of *E.coli* O157:H7 phages in feces of non-treated animals	Rozema *et al.* (2009)
Yorkshire/Duroc crossbred weaned swine ABW 10 kg	Reduce and eliminate *S.* Typhimurium strains	Inoculation with *S.* Typhimurium (2×1010 CFU per pig) via oral gavage (10 mL total volume per pig)	Application of phage cocktail via oral gavage (3×109 PFU mL^{-1}) at 24 and 48 h after challenge	1. Reduction in intestinal populations of inoculated *S.* Typhimurium in pigs 2. Reduction in *Salmonella* strains in fecal samples	Callaway *et al.* (2011)
Newborn calves	Reduce morbidity, disease severity and mortality, protection	*E. coli* O9:K30.99 10^6 CFU mL^{-1} by oral administration	Oral application of cocktail of 2 bacteriophages B44/1 and B44/2, (10^{11} PFU mL^{-1})	1. Good protection against lethal infection caused by enterotoxigenic strains of *E. coli* 2. Significantly reduced mortality (100%) and morbidity (93%) after challenge in comparison	Smith and Huggins (1983)

(*Continued*)

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Table 3. (*Continued.*)

Animals	Objective	Challenge	Phage application	Observations	Reference
				to control (untreated calves) 3. Significant protective effect even when clinical signs were present	
Suffolk sheep ABW 60 kg	Reduce *E.coli* O157:H7 numbers	Inoculation with *E. coli* O157:H7 933 (1×10^{10} CFU per sheep) or (2×10^{10} CFU per sheep) via oral gavage (10 mL total volume per sheep)	Inoculation of 21-phage cocktail via oral gavage at 48 and 72 h to obtain phage dosage of approx. 10^7 or 10^8 PFU mL^{-1}	1. Significant reduction of *E.coli* O157:H7 933 strains to 10^2–10^3 PFU g^{-1} in caecal and rectal contents, at 84 and 96 h after phage application	Callaway *et al.* (2008)
Lactating dairy cows	Reduce morbidity, and clinical signs of disease	Subclinical mastitis caused by *S. aureus*	10-ml intramammary infusions of 1.25×10^{11} PFU mL^{-1} of phage K for 5 days	Decrease in morbidity and clinical signs of mastitis of about 16.7%, not significant in comparison to control	Gill *et al.* (2006*a*, 2006*b*)
Lactating HF cows	Reduce morbidity, disease severity	Subclinical mastitis caused by *S. aureus*.	Cocktails of 3 phages K, CS1, DW2 (10^8 PFU ml^{-1}) applied as infusions into teats	1. No detectable increase in somatic cell counts in milk 2. No udder irritation. 3. 10,000-fold reduction in staphylococcal counts	O'Flaherty *et al.* (2005)
Lactating HF cows with metritis, weighing 650 kg and producing 45 kg of 3.5% FCM	Reduce morbidity, incidence of metritis, reduce bacterial count, the prophylactic effect	Metritis caused by *E. coli* pathogenic strains	Intravaginal application of 20 mL bacteriophage cocktail with 10 different phages at 10^9 PFU mL^{-1} on 230, 260 and 275 day of gestation	1. No prophylactic effect is shown as the absence of *E. coli* pathogenic strains or prevention of metritis 2. Increased incidence of retained placenta 3. Reduction in cellular immune response parameters (neutrophil migration, phagocytic and oxidative activity)	Meira *et al.* (2013)
Romanov wether lambs (4 months of age)	Reduce numbers of *E. coli* strains in fecal samples	Orally inoculated with 50 mL of PBS containing 10^8 CFU *E. coli* O157:H7 strain E318N	Oral application of bacteriophage DC22 at titer 10^{13} PFU mL^{-1} 2 days after *E. coli* challenge	1. Reduction in *E. coli* strains during the first 13 days after inoculation, except one lamb (increase in numbers of *E. coli* O157: H7 observed 13 and 16 days post-inoculation) 2. No changes in the reduction of *E. coli* O157:H7 strains at 30 days of experiment 3. Undetectable level of *E. coli* strains in feces until 27 days after phage application	Sheng *et al.* (2006)
Sheep, crossbred ewes	Reduce numbers of pathogenic *E. coli* strains	Oral dose of *E. coli* O157:H7 EDL 933 (10^{10} CFU per sheep)	Single oral administration of bacteriophage CEV1 at ~10^{11} PFU mL^{-1} 3 days after challenge	1. Significant reduction of *E. coli* strains from different parts of the alimentary tract (caecum, rectum) 2. Phage treatment prior to bacterial challenge does not prevent but could delay bacterial colonization	Raya *et al.*, (2006)

(*Continued*)

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Table 3. (*Continued.*)

Animals	Objective	Challenge	Phage application	Observations	Reference
1–14-Day old HF heifers	Reduce clinical signs of diarrhea and mortality, reduce numbers of pathogens and prophylactic efficacy of bacteriophages	Clinical signs of diarrhea caused by pathogenic *E. coli* strains	6 rectal applications of bacteriophage cocktail (φ26, 27, 29 at 10^7 to 10^9 PFU mL^{-1}) mixed with *Lactobacillus* spp. strains for 5 days	1. Significant reduction in clinical signs of diarrhea 2. Elimination of mortality in infected calves 3. Protection against re-colonization of the alimentary tract by *E. coli* strains for 3 weeks after application of phages 4. Reduction in the number of bacteria in intestines of infected calves to undetectable level	Urban-Chmiel *et al.* (2018*a*)
Angus feedlot cattle, AWG 391 kg		Challenge with 5-strain mixture of nalidixic acid-resistant *E. coli* O157:H7 strain (10^{11} CFU mL^{-1}) by oral gavage	Application of bacteriophage product (Ephage with es rV5, wV7, wV8, and wV11 phages) by oral bolus gavage or in feed at titers of 10^{10} and 10^{11} PFU mL^{-1}, 1 day before the challenge and 1, 3, 6, and 8 days after challenge	1. No reduction in resistance of *E. coli* strains to nalidixic acid by E phage 2. Reduction in numbers of *E. coli* strains for bolus and feed, averaging 1.82 and 1.13×10^9 PFU g^{-1}, respectively, in fecal samples	Stanford *et al.* (2010)
Weaned 7- to 8-week old calves	Reduce *E. coli* strains	Infection with *E. coli* O157:H7 strain (10^{10} CFU mL^{-1}) by oral gavage	6-Phage cocktail up to 7 days before challenge with *E. coli* O157:H7 at 10^7 PFU in feed or by oral gavage and 10^6 PFU oral dosage	1. Significant increase in phage concentration in feces 2. Reduction in *E. coli* O157:H7 after 8 days of the experiment	Waddell *et al.* (2000)
Weaned 4–6 months Black Angus calves	Reduce *E. coli* O157:H7 concentration	Oral challenge with *E. coli* O157:H7 in 2 separate trials	37-Phage cocktail of phages specific for *E. coli* O157:H7 strains by oral gavage in 2 separate trials	1. Significant reduction in *E. coli* O157:H7 concentration at 24 h after the second trial of phage application 2. No resistance of *E.coli* strains to phage cocktail	Chase *et al.* (2005)

confirm that bacteriophages have a broad spectrum of activity against microorganisms within one species but belonging to different serotypes. Another study by the same authors demonstrated that administration of a phage cocktail to young piglets immediately after experimental challenge with *S. enterica* serotype Typhimurium reduced bacterial counts to undetectable limits (by up to 95% in the tonsils and ileum, and up to 80% in the caecum).

Bacteriophages have also been observed to have antibacterial effects on *Salmonella* infection in weaned pigs experimentally challenged with *S.* Typhimurium. After treatment with a bacteriophage cocktail C containing eight phages (SEP-1, SGP-1, STP-1, SS3eP-1, STP-2, SChP-1, SAP-1, and SAP-2) with titers $\geq 10^9$ PFU ml^{-1}) (Seo *et al.*, 2018), the authors observed lytic activity against 100% of *Salmonella* ATCC 14028 reference strains and 92.5% of field isolates. The study confirmed that a bacteriophage cocktail is more effective than a single bacteriophage in controlling bacterial infections in pigs.

A summary of studies with experimental phage treatments in livestock and their results is shown in Table 3.

Commercial phage products in livestock production

Bacteriophages as components of commercial products are currently finding application in the elimination of pathogens from food products of animal origin (meat and meat products, milk and dairy products) or plant origin (fruits and vegetables). Most of these preparations have been officially approved in the USA, Canada, Israel, Australia, and some European countries, such as Sweden, Switzerland, or the Netherlands (BAG, Bundesamt für Gesundheit; CFR, Code of Federal Regulations; FSIS, Food Safety and Inspection Service; GRN, GRAS Notice; European Food Safety Authority EFSA; Standards Australia New Zealand FSANZ) (Moye *et al.*, 2018). It should be emphasized that the number of positive decisions around the world regarding the marketing authorization of phage preparations as substances generally recognized as safe (GRAS) is still rising, which is significantly linked to restrictions on the use of antibiotics in animal production.

For example, the United States Food and Drug Administration (FDA) has approved three phage preparations (ListShield™″, EcoShield™, and SalmoFresh™) as effective products for reducing bacterial contamination of various foods.

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Table 4. Selected commercial phage preparations with a list of administrative authorities allowing their use in veterinary medicine and food production (Moye *et al.*, 2018 with our own modification)

Name of phage preparation	Target bacterial species	Animal species or food products	Administrative organs and legal regulations permitting the product's use	Countries where the product is approved for use	Company website or references
SalmoLyse®	*Salmonella* spp.	Poultry (turkey, chicken), tuna, cantaloupe, lettuce, pets food products	FDA	USA	Soffer *et al.* (2016)
SalmoPro®	*Salmonella* spp.	Food products	FDA, GRN 603	China, Canada	Moye *et al.* (2018)
PLSV-1™	*Salmonella* spp.	Pets, livestock	FDA	USA	Moye *et al.* (2018)
SalmoFresh™	*S. enterica*	Poultry, red meat	FDA-2013 (GRAS) Notice No. GRN 000435 FDA, GRN 435; USDA, FSIS Directive 7120.1; Israel Ministry of Health; Health Canada	USA, Canada, Israel	Moye *et al.* (2018); Intralytix Corp. website
Salmonelex™, Phage Guard S	*Salmonella* spp.		FSANZ	USA, EU, Canada, Australia, New Zealand, Switzerland, Israel	Phage Guard Corp.
SalmoPro®	*S. enterica*	Food control	FDA, GRN 603	Canada	Phagelux Corp. website
INT-401™	*C. perfringens*	Poultry	FDA, FSIS	USA,	Intralytix Corp. Website;
Secure Shield E1	*E. coli* O157:H7	Human food application: beef products, turkey	FDA, GRN 724	USA	Woolston and Sulakvelidze (2015)
BAFASAL®	*S.* Enteritidis & *S.* Typhimurium	Feed supplement in poultry, other livestock	EU	European Union countries	Proteon Pharmaceuticals S.A. (PL)
ListShield™	*Listeria monocytogenes*	Beef and poultry meat products	GRAS Notice No. 000528; FSIS Directive 7120.1;	USA	Intralytix Corp. website; Moye *et al.* (2018)
Listex™	*L. monocytogenes*	Beef and turkey products	GRAS Notice No. 000218; FSIS Directive 7120.1	USA	Intralytix Corp. website; Moye *et al.* (2018)
PhageGuard Listex™	*L. monocytogenes*		FDA, GRN 198/218; FSANZ; EFSA; Swiss BAG; Israel Ministry of Health; Health Canada	USA, Canada, Switzerland, Israel	Moye *et al.* (2018)
EcoShield™	*E. coli* O157:H7	Various food including ground beef	FDA, FCN No. 1018; FSIS Directive 7120.1; Health Canada; National Food Service of Israel Ministry of Health Ref: 70275202; Health Canada	Canada, Israel, USA	Intralytix Corp.
Finalyse®	*E. coli* O157:H7 and other Shiga toxin-producing *E. coli*	Feed supplement or aerosol disinfection in cattle	Food Safety and Inspection Service	USA	West Des Moines, IA, USA
Ecolicide® (EcolicidePX™)	*E. coli* O157:H7		USDA, FSIS Directive 7120.1	USA	Moye *et al.* (2018)
ShigaShield™ (ShigActive™)	*Shigella* spp.	Ready-to-eat (RTE) meats, fish and shellfish, fruits, vegetables, dairy products	FSIS Directive 7120.1; GRAS notification	USA	Intralytix Corp. website
AgriPhage™	*Xanthomonas campestris* pv. Vesicatoria, *Pseudomonas syringae* pv. Tomato	Plant food products	EPA Reg. No. 67986-1	USA	Intralytix Corp. website

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

In addition, food safety guidelines in the USA recognize several phage preparations as safe and suitable ingredients for use in the production of meat, poultry, and egg products. For example, FSIS Directive 7120.1 permits the use of phages in livestock prior to slaughter (e.g. phages specific for pathogenic strains of *E. coli* O157:H7 for use on beef hides) and in foods (e.g. phages specific for *Salmonella* on poultry or meat products derived from livestock).

Other products, such as PhageGuard S™, containing phages specific to pathogenic strains of *Salmonella* spp. and *E. coli* O157:H7, have been recommended in Israel, Switzerland, and Canada. A detailed list of commercial phage preparations with their recommendations as products GRAS and approved for use in food production is presented in Table 4.

Conclusions

Summing up the scope of knowledge on the use of bacteriophage preparations, it should be emphasized that it is useful to test experimental therapies in animals to treat bacterial infections caused by antibiotic-resistant microorganisms, as indicated by the therapeutic success that has already been observed in combating selected infections. This is also evidenced by a significant increase in approvals and registrations of commercial phage preparations for controlling pathogens occurring in the food of animal and vegetable origin.

Author contributions. M.D. and A.W. contributed to the collection and revision of the literatures and wrote the manuscript. R.U.C. was involved in the concept of manuscript, collection and revision of the literatures, and wrote the manuscript.

Conflict of interest. The authors have declared no conflicts of interest for this article.

References

Abedon ST and Thomas-Abedon C (2010) Phage therapy pharmacology. *Current Pharmaceutical Biotechnology* **11**, 28–47.

Abedon ST, Kuhl SJ, Blasdel BG and Kutter EM (2011) Phage treatment of human infections. *Bacteriophage* **1**, 66–85.

Ackermann HW (2011) Bacteriophage taxonomy. *Microbiology Australia* **32**, 90–94.

Ackermann HW and Prangishvili D (2012) Prokaryote viruses studied by electron microscopy. *Archives of Virology* **157**, 1843–1849.

Adriaenssens EM and Brister JR (2017). How to name and classify your phage: an informal guide. *Viruses* **9**, 70. doi: 10.1101/111526.

Adriaenssens EM, Krupovic M, Knezevic P, Ackermann HW, Barylski J, Brister JR, Clokie MRC, Duffy S, Dutilh BE, Edwards RA, Enault F, Jang HB, Klumpp J, Kropinski AM, Lavigne R, Poranen MM, Prangishvili D, Rumnieks J, Sullivan MB, Wittmann J, Oksanen HM, Gillis A and Kuhn JH (2017) Taxonomy of prokaryotic viruses: 2016 update from the ICTV bacterial and archaeal viruses subcommittee. *Archives of Virology* **162**, 1153–1157.

Alomari MMM, Nowaczek A, Dec M and Urban-Chmiel R (2016) Antibacterial activity of bacteriophages isolated from poultry against Shiga-toxic strains of *Escherichia coli* isolated from calves. *Medycyna Weterynaryjna* **72**, 699–703.

Atterbury JR (2009) Bacteriophage biocontrol in animals and meat products. *Microbial Biotechnology* **2**, 601–612.

Bach SJ, McAllister TA, Veira DM, Gannon VPJ and Holley RA (2003) Effect of bacteriophage DC22 on *Escherichia coli* O157:H7 in an artificial rumen system (Rusitec) and inoculated sheep. *Animal Research EDP Sciences* **52**, 89–101.

Baj J, Markiewicz Z and Piekarowicz A (2015) Biologia molekularna bakterii [Molecular biology of bacteria] chapt. 7, 2nd edn. pp. 359–403. ISBN 978-83-01-18183-3 Warszawa S.A.

Barrow P, Lovell M and Berchieri A Jr (1998) Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. *Clinical and Diagnostic Laboratory Immunology* **5**, 294–298.

Batinovic S, Wassef F, Knowler SA, Rice DTF, Stanton CR, Rose J, Tucci J, Nittami T, Vinh A, Drummond GR, Sobey CG, Chan HT, Seviour RJ, Petrovski S and Franks AE (2019) Bacteriophages in natural and artificial environments. *Pathogens* **8**, 100.

Bertani G and Weigle JJ (1953) Host controlled variation in bacterial viruses. *Journal of Bacteriology* **65**, 113–121.

Bielke LR, Tellez G and Hargis BM (2012) Successes and failures of bacteriophage treatment of enterobacteriaceae infections in the gastrointestinal tract of domestic animals. In *Bacteriophages*. Rijekas (Croatia): Ipek Kurtboke, IntechOpen, pp. 159–178. DOI: 10.5772/33407.

Borie C, Robeson J and Galarce N (2014) Lytic bacteriophages in veterinary medicine; a therapeutic option against bacterial pathogens. *Archivos de Medicina Veterinaria* **46**, 167–179.

Brüssow H and Kutter E 2005 Chapter 6 'phage ecology' in bacteriophages. In Kutter E and Sulakvelidze A (eds), *Bacteriophages Biology and Applications*. Boca Raton, Fla, USA: CRC Press, pp. 129–163.

Bruynoghe R and Maisin J (1921) Essais de thérapeutique au moyen du bacteriophage du Staphylocoque. *Compt Rend Soc Biology* **85**, 1120–1121.

Callaway TR, Edrington TS, Brabban AD, Anderson RC, Rossman ML, Engler MJ, Carr MA, Genovese KJ, Keen JE, Looper ML, Kutter EM and Nisbet DJ (2008) Bacteriophage isolated from feedlot cattle can reduce *Escherichia coli* O157:H7 populations in ruminant gastrointestinal tracts. *Foodborne Pathogens and Disease* **5**, 183–191.

Callaway TR, Edrington TS, Brabban A, Kutter B, Karriker L, Stahl Ch, Wagstrom E, Anderson R, Poole TL, Genovese K, Krueger N, Harvey R and Nisbet DJ (2011) Evaluation of phage treatment as a strategy to reduce *Salmonella* populations in growing swine. *Foodborne Pathogens and Disease* **8**, 261–266.

Ceyssens PJ and Lavigne R (2010) Bacteriophages of Pseudomonas. *Future Microbiology* **5**, 1041–1055.

Chase J, Kalchayanand N and Goodridge LD (2005) Use of bacteriophage therapy to reduce Escherichia coli O157:H7 concentrations in an anaerobic digestor that simulates the bovine gastrointestinal tract. Abstract 108-6. IFTAnnual Meeting and Food Expo, New Orleans, Louisiana.

Dąbrowska K, Switała-Jelen K, Opolski A, Weber-Dąbrowska B and Górski A (2005) Bacteriophage penetration in vertebrates. *Journal of Applied Microbiology* **98**, 7–13.

Dąbrowska K, Miernikiewicz P, Piotrowicz A, Hodyra K, Owczarek B, Lecion D, Kaźmierczak Z, Letarov A and Górski A (2014) Immunogenicity studies of proteins forming the T4 phage head surface. *Journal of Virology* **88**, 12551–12557.

Deghorain M and Van Melderen L (2012) The staphylococci phages family: an overview. *Viruses* **4**, 3316–3335.

d'Herelle F (1917) Sur un microbe invisible antagoniste des bacilles dysentériques. *Comptes rendus de l'Académie des Sciences Paris* **165**, 173–175.

d'Herelle F (1931) Bacteriophage as a treatment in acute medical and surgical infections. *Bulletin of the New York Academy of Medicine* **7**, 329–348.

Dibner JJ and Richards JD (2005) Antibiotic growth promoters in agriculture: history and mode of action. *Poultry Science* **84**, 634–643.

Elbreki M, Ross RP, Hill C, O'Mahony J, McAuliffe O and Coffey A (2014) Bacteriophages and their derivatives as biotherapeutic agents in disease prevention and treatment. *Journal of Viruses* **2014**, Article ID 382539.

Gardette S and Tomasz A (2014) Mechanisms of vancomycin resistance in *Staphylococcus aureus*. *Journal of Clinical Investigation* **124**, 2836–2840.

Gebru E, Lee JS, Son JC, Yang SY, Shin SA, Kim B, Kim MK and Park SC (2010) Effect of probiotic-, bacteriophage-, or organic acid-supplemented feeds or fermented soybean meal on the growth performance, acute-phase response, and bacterial shedding of grower pigs challenged with *Salmonella enterica* serotype typhimurium. *Journal of Animal Science* **88**, 3880–3886.

Gill JJ, Pacan JC, Carson ME, Leslie KE, Griffiths MW and Sabour PM (2006a) Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical *Staphylococcus aureus* mastitis in lactating dairy cattle. *Antimicrobial Agents and Chemotherapy* **50**, 2912–2918.

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Gill JJ, Sabour PM, Leslie KE and Griffiths MW (2006b) Bovine whey proteins inhibit the interaction of *Staphylococcus aureus* and bacteriophage K. *Journal of Applied Microbiology* **101**, 377–386.

Goodridge LD and Bisha B (2011) Phage-based biocontrol strategies to reduce foodborne pathogens in foods. *Bacteriophage* **1**, 130–137.

Górski A, Międzybrodzki R, Borysowski J, Dąbrowska K, Wierzbicki P, Ohams M, Korczak-Kowalska G, Olszowska-Zaremba N, Łusiak-Szelachowska M, Kłak M, Jończyk E, Kaniuga E, Gołaś A, Purchla S, Weber-Dąbrowska B, Letkiewicz S, Fortuna W, Szufnarowski K, Pawełczyk Z, Rogóż P and Kłosowska D (2012) Phage as a modulator of immune responses: practical implications for phage therapy. *Advances in Virus Research* **83**, 41–71.

Goyal SM, Gerba CP and Bitton G (1987) *Phage Ecology*. New York: John Wiley and Sons, p. 321.

Hankin EH (1986) L'action bactericide des eaux de la Jumna et du Gange sur le vibrion du cholera. *Annual Institut Pasteur* (*Paris*) **10**, 511–523.

Harris DL (2000) Reduction of *Salmonella* by bacteriophage treatment. Available at http://www.pork.org/PorkScience/Documents/REPORT%20 04-99-230-Harris-ISU.pdf. Accessed July 26, 2009.

International Committee on Taxonomy of Viruses ICTV, 2018 https://talk.ictvonline.org/taxonomy/.

Johnson RP, Gyles CL, Huff WE, Ojha S, Huff GR, Rath NC and Donoghue AM (2008) Bacteriophages for prophylaxis and therapy in cattle, poultry and pigs. *Animal Health Research Reviews* **9**, 201–215.

Karthik K, Muneeswaran NS, Manjunathachar HV, Gopi M, Elamurugan A and Kalaiyarasu S (2014) Bacteriophages: effective alternative to antibiotics. *Advances in Animal and Veterinary Sciences* **2**, 1–7.

Khalifa L, Brosh Y, Gelman D, Coppenhagen-Glazer S, Beyth S, Poradosu-Cohen R, Que YA, Beyth N and Hazan R (2015) Targeting *Enterococcus faecalis* biofilms with phage therapy. *Applied and Environmental Microbiology* **81**, 2696–2705.

Kim KH, Ingale SL, Kim JS, Lee SH, Lee JH, Kwon IK and Chae BJ (2014) Bacteriophage and probiotics both enhance the performance of growing pigs but bacteriophage are more effective. *Animal Feed Science and Technology* **196**, 88–95.

Krupovic M, Dutilh BE, Adriaenssens EM, Wittmann J, Vogensen FK, Sullivan MB, Rumnieks J, Prangishvili D, Lavigne R, Kropinski AM, Klumpp J, Gillis A, Enault F, Edwards RA, Duffy S, Clokie MRJ, Barylski J, Ackermann HW and Kuhn JH (2016) Taxonomy of prokaryotic viruses: update from the ICTV bacterial and archaeal viruses subcommittee. *Archives of Virology* **4**, 1095–1099.

Krylov VN, Tolmachova TO and Akhverdian VZ (1993) DNA homology in species of bacteriophages active on *Pseudomonas aeruginosa*. *Archives of Virology* **131**, 141–151.

Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S and Abedon ST (2010) Phage therapy in clinical practice: treatment of human infections. *Current Pharmaceutical Biotechnology* **11**, 69–86.

Lee N and Harris DL (2001) The effect of bacteriophage treatment as a preharvest intervention strategy to reduce the rapid dissemination of *Salmonella* Typhimurium in pigs. *Proc AASV American Association of Swine Veterinarians Perry, IA*, pp. 555–7.

Loc-Carrillo C and Abedon ST (2011) Pros and cons of phage therapy. *Bacteriophage* **1**, 111–114.

Loc Carrillo C, Atterbury RJ, El-Shibiny A, Connerton PL, Dillon E, Scott A and Connerton IF (2005) Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Applied and Environmental Microbiology* **71**, 6554–6563.

Luria SE and Anderson TF (1942) The identification and characterization of bacteriophages with the electron microscope. *Proceedings of the National Academy of Sciences of the USA* **28**, 127–130.

Luria SE and Human ML (1952) A nonhereditary, host-induced variation of bacteria viruses. *Journal of Bacteriology* **64**, 557–569.

Machado VS, Bicalho ML, Pereira RV, Caixeta LS, Bittar JH, Oikonomou G, Gilbert RO and Bicalho RC (2012) The effect of intrauterine administration of mannose or bacteriophage on uterine health and fertility of dairy cows with special focus on *Escherichia coli* and *Arcanobacterium pyogenes*. *Journal of Dairy Science* **95**, 3100–3109.

McGuinness WA, Malachowa N and DeLeo FR (2017) Vancomycin resistance in *Staphylococcus aureus*. *Yale Journal of Biology and Medicine* **90**, 269–281.

Meira EBS, Rossi RS, Teixeira AG, Kaçar C, Oikonomou G, Gregory L and Bicalho RC (2013) The effect of prepartum intravaginal bacteriophage administration on the incidence of retained placenta and metritis. *Journal of Dairy Science* **96**, 7658–7665.

Morozova VV, Vlassov VV and Tikunova NV (2018) Applications of bacteriophages in the treatment of localized infections in humans. *Frontiers in Microbiology* **9**, 1696.

Moye ZD, Woolston J and Sulakvelidze A (2018) Bacteriophage applications for food production and processing. *Viruses* **10**, E205.

Naylor SW, Low JC, Besser TE, Mahajan A, Gunn GJ, Pearce MC, McKendrick J, Smith DGE and Gally DL (2003) Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infection and Immunity* **71**, 1505–1512.

Northrop JH (1938) Concentration and purification of bacteriophage. *Journal of General Physiology* **21**, 335–366.

O'Flaherty S, Coffey A, Meaney WJ, Fitzgerald GF and Ross RP (2005) Inhibition of bacteriophage K proliferation on *Staphylococcus aureus* in raw bovine milk. *Letters in Applied Microbiology* **41**, 274–279.

Pirnay JP, Blasdel BG, Bretaudeau L, Buckling A, Chanishvili N, Jason R, Corte-Real S, Debarbieux L, Dublanchet A, De VosJérôme D, Garcia GM, Goderdzishvili M, Górski A, Hardcastle J, Huys I, Kutter E, Lavigne R, Merabishvili M, Olchawa E, Parikka KJ, Patey O, Pouilot F, Resch G, Rohde C, Scheres J, Skurnik M, Vaneechoutte M, Parys LV, Verbeken G, Zizi M and Van den Eede G (2015). Quality and safety requirements for sustainable phage therapy products. *Pharmaceutical Research* **32**, 2173–2179.

Rakhuba DV, Kolomiets EI, Szwajcer-Dey E and Novik GI (2010) Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Polish Journal of Microbiology* **59**, 145–155.

Raya RR, Varey P, Cot RA, Dyen MR, Callaway TR, Edrington TS, Kutter EM and Brabban AD (2006) Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. *Applied and Environmental Microbiology* **72**, 6405–6410.

Rivas L, Coffey B, McAuliffe O, McDonnell MJ, Burgess CM, Coffey A, Paul Ross RP and Duffy G (2010) *In vivo* and *ex vivo* evaluations of bacteriophages e11/2 and e4/1c for use in the control of *Escherichia coli* O157:H7. *Applied and Environmental Microbiology* **76**, 7210–7216.

Rozema EA, Stephens TP, Bach SJ, Okine EK, Johnson RP, Stanford K and McAllister TA (2009) Oral and rectal administration of bacteriophages for control of *Escherichia coli* O157:H7 in feedlot cattle. *Journal of Food Protection* **72**, 241–250.

Rusca H (1940) Die Sichtbarmachung der bakteriophagen Lyse im Übermikroskop. *Naturwissenschaften* **28**, 45–46.

Sanger F, Coulson AR, Hong GF, Hill DF and Petersen GB (1982) Nucleotide sequence of bacteriophage lambda DNA. *Journal of Molecular Biology* **162**, 729–773.

Sankaran N (2010) The bacteriophage, its role in immunology: how Macfarlane Burnet's phage research shaped his scientific life. *Studies in History and Philosophy of Biological and Biomedical Sciences* **41**, 367–375.

Scott AE, Timms AR, Connerton PL, Carrillo CL, Radzum KA and Connerton IF (2007a) Genome dynamics of *Campylobacter jejuni* in response to bacteriophage predation. *PLoS Pathogens* **3**, e119.

Scott AE, Timms AR, Connerton PL, El-Shibiny A and Connerton IF (2007b) Bacteriophage influence *Campylobacter jejuni* types populating broiler chickens. *Environmental Microbiology* **9**, 2341–2353.

Seo B-J, Song E-T, Lee K, Kim J-W, Jeong C-G, Moon S-H, Son JS, Kang SH, Cho H-S, Jung BY and Kim WI (2018) Evaluation of the broad-spectrum lytic capability of bacteriophage cocktails against various *Salmonella* serovars and their effects on weaned pigs infected with *Salmonella* Typhimurium. *Journal of Veterinary Medical Science* **80**, 851–860.

Sheng H, Knecht HJ, Kudva IT and Hovde CJ (2006) Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. *Applied and Environmental Microbiology* **72**, 5359–5366.

Sheldon LM, Lewis GS, LeBlanc S and Gilbert RO (2006) Defining postpartum uterine disease in cattle. *Theriogenology* **65**, 1516–1530.

https://doi.org/10.1017/S1466252319000161 Published online by Cambridge University Press

Skoblikow N and Zimin A (2013) Experience of application of non-transducing bacteriophages for prophylaxy and therapy of intestinal coli-bacteriosis of pigs. *Proceed of International conference* '*Bacteriophages Theoretical and Practical aspects of medicine, veterinary and food industry*' ISBN 978-5-905970-14-6, Ulianowsk, Russia, т. I: 184 с.

Skurnik M and Strauch E (2006) Phage therapy: facts and fiction. *Journal of Medical Microbiology* **296**, 5–14.

Skurnik M, Pajunen M and Kiljunen S (2007) Biotechnological challenges of phage therapy. *Biotechnology Letters* **29**, 995–1003.

Smith HW and Huggins MB (1983) Effectiveness of phages in treating experimental *Escherichia coli* diarrhea in calves, piglets, and lambs. *Journal of General Microbiology* **129**, 2659–2675.

Smith HW, Huggins MB and Shaw KM (1987a) The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *Journal of General Microbiology* **133**, 1111–1126.

Smith HW, Huggins MB and Shaw KM (1987b) Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. *Journal of General Microbiology* **133**, 1127–1135.

Soffer N, Abuladze T, Woolston J, Li M, Hanna LF, Heyse S, Charbonneau D and Sulakvelidze A (2016) Bacteriophages safely reduce *Salmonella* contamination in pet food and raw pet food ingredients. *Bacteriophage* **6**, e1220347.

Stanford K, McAllister TA, Niu YD, Stephens TP, Mazzocco A, Waddell TE and Johnson RP (2010) Oral delivery systems for encapsulated bacteriophages targeted at *Escherichia coli* O157:H7 in feedlot cattle. *Journal of Food Protection* **73**, 1304–1312.

Sultan I, Rahman S, Jan AT, Siddiqui MT, Mondal AH and Haq QMR (2018) Antibiotics, resistome and resistance mechanisms: a bacterial perspective. *Frontiers in Microbiology* **9**, 2066.

Svircev A, Roach D and Castle A (2018) Framing the future with bacteriophages in agriculture. *Viruses* **10**, e218.

Tiwari R, Dhama K, Chakraborty S, Kumar A, Rahal A and Kapoor S (2014) Bacteriophage therapy for safeguarding animal and human health: a review. *Pakistan Journal of Veterinary Sciences* **17**, 301–315.

Twort FW (1915) An investigation on the nature of ultramicroscopic viruses. *Lancet* **186**, 1241–1243.

Ul Haq I, Chaudhry WN, Andleeb S and Qadr II (2011) Isolation and partial characterization of a virulent bacteriophage IHQ1 specific for *Aeromonas punctata* from stream water. *Microbial Ecology* **63**, 954–963.

Urban-Chmiel R, Wernicki A, Stęgierska D, Dec M, Dudzic, A and Puchalski A (2015) Isolation and characterization of lytic properties of bacteriophages specific for *M. haemolytica* strains. *PLoS ONE* **10**, e0140140.

Urban-Chmiel R, Alomari M, Dec M, Nowaczek A, Stęgierska D, Puchalski A, Wernicki A and Kowalski C (2018a) The experimental phage therapies of diarrhoea in newborn calves. *Proceed of XVIII Middle European Buiatric Congress*, 30 May–2 Jine 2018*a* Eger, Hungary 2018, *Magyar Allatorvosok Lapja* 140 (Suppl. 1), 179–185.

Urban-Chmiel R, Wernicki A, Wawrzykowski J, Puchalski A, Nowaczek A, Dec M, Stęgierska, D and Alomari M (2018b) Protein profiles of bacteriophages of the family *Myoviridae*-like induced on *M. haemolytica*. *AMB Express* **8**, 102.

Waddell T, Mazzocco A, Johnson RP, Pacan J, Campbell S, Perets S, MacKinnon A, Holtslander J, Pope B and Gyles C (2000) Control of *Escherichia coli* O157:H7 infection of calves by bacteriophages. *4th International Symposium and Workshop on Shiga toxin (verocytotoxin)-producing Escherichia coli infections*, Kyoto, Japan.

Wall SK, Zhang J, Rostagno MH and Ebner PD (2010) Phage therapy to reduce preprocessing *Salmonella* infections in market-weight swine. *Applied and Environmental Microbiology* **76**, 48–53.

Weber-Dąbrowska B, Mulczyk M and Górski A (2000) Bacteriophage therapy of bacterial infections: an update of our Institute's experience. *Archives Immunology and Therapy Experimentalis* **48**, 547–555.

Weinbauer MG (2004) Ecology of prokaryotic viruses. *FEMS Microbiology Reviews* **28**, 127–181.

Wernicki A, Nowaczek A and Urban-Chmiel R (2017) Bacteriophage therapy to combat bacterial infections in poultry. *Virology Journal* **14**, 179.

Wittebole X, De Roock SD and Opal SM (2014) A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence* **5**, 226–235.

WO 2004064732 A2 no. Appl. PCT/US2004/001077. The use of bacterial phage-associated lysing proteins for preventing and treating bacterial infections in humans, animals and fowl.

Woolston J and Sulakvelidze A (2015) Bacteriophages and food safety. In: eLS (ed.). *Hurdles in Phage therapy*. Chichester: John Wiley & Sons Ltd. Doi:10.1002/9780470015902.a0025962.

Wright A, Hawkins CH, Anggård EE and Harper DR (2009) A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibioticresistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clinical Otolaryngology* **34**, 349–357.