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Biological Properties and Therapeutic Potential of Kayvirus vB_MboM_W17 Bacteriophage in Infectious Keratoconjunctivitis in Cattle

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ABSTRACT

Infectious keratoconjunctivitis in cattle caused by *Moraxella bovis* and related species remains a significant problem in animal husbandry. The increasing resistance of *Moraxella* spp. to antibiotics limits the effectiveness of traditional therapy and requires the search for alternative remedies. Isolation and complex characterization of a lytic bacteriophage active against *Moraxella* spp., with an assessment of its potential for use in infectious keratoconjunctivitis phage therapy. The bacteriophage *Kayvirus* vB_MboM_W17 was isolated from wastewater, characterized by electron microscopy, and studied for its host spectrum, thermal stability, and pH resistance; whole-genome sequencing and bioinformatics analysis were then performed. The phage formed transparent plaques with halos and showed lytic activity against all tested isolates of *M. bovis, M. bovoculi, and M. ovis*. It remained stable at 4-40°C and pH 6-9. The genome of double-stranded DNA, with a length of 140.6 thousand base pairs, did not contain genes for virulence, lysogeny, or antibiotic resistance. The results confirm the safety and efficacy of the bacteriophage *Kayvirus* vB_MboM_W17, making it a promising candidate for the development of phage therapy for infectious keratoconjunctivitis in cattle.

Keywords: Bacteriophage, *Moraxella bovis*, Phage therapy, *Kayvirus*, Keratoconjunctivitis, Cattle.

INTRODUCTION

Infectious keratoconjunctivitis (IKC) in cattle, also known as pink eye, remains one of the most widespread and economically significant problems in animal husbandry worldwide (Gupta et al. 2023; Bilbao et al. 2024; Kilama et al. 2025). Current studies consistently confirm that IKC poses a considerable economic and welfare challenge for cattle producers (Sheedy et al. 2021; Gupta et al. 2023; Kilama et al. 2025). The disease causes substantial economic damage through multiple mechanisms that include reduced weight gain in calves and decreased milk production (Sheedy et al. 2021). Ocular pain and impaired vision reduce feed intake in affected cattle, directly leading to stunted growth (Angelos 2015; Sheedy et al. 2021). Beyond these direct impacts, producers incur significant additional expenses related to veterinary care, labor for treatment, and preventive actions (Sheedy et al. 2021). The financial and time investment required for treating infected animals further contributes to these economic burdens (Seid 2019). The pervasive economic and health effects of IKC are recognized globally, underscoring the ongoing and critical need for effective control measures in the livestock industry (Bilbao et al. 2024; Kilama et al. 2025).

Moraxella bovis is traditionally considered the primary etiological agent of IKC (Bilbao et al. 2024; Kilama et al. 2025). However, *Moraxella bovoculi* also plays a significant role, with both Gram-negative bacteria recognized as the main etiological agents of the disease (Loy et al. 2021a, b; Bilbao et al. 2024). Research also indicates that various other bacteria may contribute to the development of IKC, confirming its multifactorial nature (Loy et al. 2021a, b; Gafen et al. 2023). Despite decades of intensive research, IKC remains a serious issue in veterinary medicine and cattle breeding, highlighting the complexity of its pathogenesis and the difficulties in developing universally effective control strategies (Loy and Brodersen 2014; Loy et al. 2021a, b; Wynn et al. 2022; Pimenov et al. 2024).

Traditional therapy for IKC in cattle is typically based on the systemic or topical administration of antibacterial agents, often combined with anti-inflammatory drugs (Sheedy et al. 2021). However, a major and growing

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obstacle is the increasing problem of antimicrobial resistance in *Moraxella* species (Neupane et al. 2023; Pimenov et al. 2024; Kilama et al. 2025). In recent years, *M. bovis* and *M. bovoculi* isolates have repeatedly demonstrated resistance to commonly used antibiotics, including tetracyclines, macrolides, and fluoroquinolones, which considerably reduces the clinical effectiveness of conventional antibiotic therapy (Bengtsson and Greko 2014; Maboni et al. 2015; Bokma et al. 2020; American Veterinary Medical Association Committee on Antimicrobials 2020; Pimenov et al. 2024). The increasing prevalence of resistant strains complicates pathogen eradication and elevates the risk of disease recurrence, emphasizing the urgent need for effective alternative strategies for managing antibiotic-resistant *Moraxella* spp.

Unlike conventional therapies, bacteriophages—viruses that specifically target and lyse bacteria—offer a promising alternative (Kutter 2009; Letarov and Kulikov 2009; Abedon et al. 2011; Ferriol-González and Domingo-Calap 2021; Danis-Wlodarczyk et al. 2021; Bianchessi et al. 2024; Choi et al. 2024). Phage therapy has gained renewed interest due to its capacity to combat antibiotic-resistant bacterial infections in both human and veterinary medicine (Mourenza et al. 2020; Ferriol-González and Domingo-Calap 2021; Li et al. 2023; Nale and McEwan 2023; Bianchessi et al. 2024; Choi et al. 2024). The advantages of bacteriophages include their high host specificity, self-replicating ability at the site of infection, and activity against antibiotic-resistant strains without contributing to generalized antimicrobial resistance (Ferriol-González and Domingo-Calap 2021; Pimenov et al. 2024; Choi et al. 2024).

This work aimed to isolate and comprehensively characterize the lytic bacteriophage *Kayvirus* vB_MboM_W17, which exhibits activity against *M. bovis*, *M. bovoculi*, and *M. ovis*. The study evaluates its biological properties, genomic organization, and potential for use in the phage therapy of IKC in cattle, addressing a critical need for novel and effective treatments amid rising antimicrobial resistance.

MATERIALS AND METHODS

Scope and timing of the study

The work was performed at the Immunology and Biotechnology Laboratory of the K.I. Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology – Moscow Veterinary Academy (MGAVMiB – MVA) (Moscow, Russia) from January 2024 to March 2025. Biological material was sampled in the Moscow region and the Rutulsky district of the Republic of Dagestan.

In this study, a new bacteriophage vB_MboM_W17 was isolated from wastewater and characterized. Its biological properties were determined, genome sequencing was performed, and a comparative analysis was performed with closely related phages of the *Kayvirus* genus. The bacteriophage vB_MboM_W17 effectively lysates various *Moraxella* spp., highlighting its therapeutic potential and making it a promising candidate for further use in phage therapy.

Bacterial strain and cultivation conditions

Bacterial isolates were isolated from conjunctival smears of animals showing clinical signs of IKC. Conjunctival swabs were taken with sterile cotton swabs, then inoculated with 10% blood agar and incubated at 37°C for 24 hours. For species identification, single colonies were analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (the BactoSCREEN system, NPF LITEKH LLC) and polymerase chain reaction (PCR) amplification of the 16S rRNA gene using universal primers (343F, 798R). The confirmed strain was cultured in Brain Heart Infusion (BHI) broth.

Bacteriophage isolation, purification, amplification, and quantification

The study used 35 wastewater samples and 30 clinical samples (eye swabs and saliva from sheep and goats). Bacterial isolates of *M. bovis, M. bovoculi*, and *M. ovis* were obtained from animals with clinical signs of IKC. *P. aeruginosa, K. pneumoniae, E. coli, Streptococcus* spp., and *E. cloacae* strains were used for control (Table 1).

Table 1: Material for the isolation of bacteriophages

Material	Number of samples	Sample collection sites
Wastewater	35	Moscow region
Eye swabs, sheep and goat saliva	30	Rutulsky district, Republic of Dagestan

Before phage enrichment, wastewater was centrifuged at 12,000×g for 5 min, and the supernatant was filtered through a 0.22μm polyethersulfone (PES) syringe filter. Bacterial colony swabs were resuspended in 1mL of 0.9% NaCl solution, shaken intensively, and filtered through a 0.22mm membrane. The resulting filtrate was transferred to a sterile 15mL tube, and 100μL of a logarithmic bacterial host culture and 5mL of 2× concentrated BHI agar were added. The mixture was incubated at 37°C for 24 hours. The incubation mixture was centrifuged at 10,000×g for 10 min; then the supernatant containing candidate phages was filtered again (0.22μm) to remove residual cells. The filtrate was mixed with the bacterial culture in BHI supplemented with 0.6% agar and applied to plates containing 1.5% BHI agar. After 18 hours of incubation, individual plaques were removed with a sterile needle and eluted in 0.9% NaCl.

A 0.22μm filter was used to filter the eluted bacterial lysate the bacterial lysate was mixed with a bacterial culture it was seeded and a phage plaque was selected. These steps were repeated three times to obtain one strain of phage. The bacterial lysate from the last elution step was filtered again into the 0.22μm filter. Then, 100μL of the filtrate adds to 10mL of bacterial suspension with about 0.6 OD600 and incubates for 16h at 37°C, resulting in the clear culture solution containing the phage. This was filtered again through 0.22μm filters and stored at 4°C.

Electron microscopy

For transmission electron microscopy (TEM), the phage lysate was preliminarily adjusted to a titer of 10^8 plaque-forming units (PFU)/mL in Luria-Bertani (LB) broth. The samples were concentrated by ultracentrifugation at 25,000×g, 4°C, for 60 min; the supernatant was removed, and the precipitate was resuspended in 0.1M ammonium acetate ($C_2H_7NO_2$) and re-centrifuged under the same conditions. Then, one drop of

phage lysate was placed on a copper grid, 5µL (2%) uracyl acetate was added for contrast, the mixture was allowed to dry, and the sample was examined using a JEOL JEM-1011 transmission electron microscope. The electronic magnification was 200nm. The obtained micrographs were used to measure the capsid and tail sizes and to classify the phage morphologically.

Determining the host range

M. bovis, M. bovoculi, and *M. ovis* (Table 2) were used to study the lytic ability of phage W17 using the point test method to assess its potential to create lysis zones on lawn cultures of various bacterial strains. The bacteriophage was also tested on strains of *P. aeruginosa, K. pneumoniae, E. coli, Streptococcus* spp. and *E. cloacae*. A purified suspension of phage W17 (50µL, 10^7PFU/mL) was applied directly to the surface of a bacterial lawn culture and incubated at 37°C overnight; the dishes were then examined for plaques.

Table 2: The vB_MboM_W17 phage host range

Bacterial strain	Isolation source	Spot test
Moraxella bovis (n=5)	Cattle	5/5
Moraxella bovoculi (n= 4)	Cattle	4/4
Moraxella ovis (n=3)	Sheep	3/3
Pseudomonas aeruginosa (n=3)	People	0/3
Escherichia coli (n=4)	Cattle	0/4
Streptococcus spp. (n=3)	Sheep	0/3
Enterobacter cloacae (n=2)	People	0/2
Klebsiella pneumonia (n=3)	People	0/3

Resistance to aggressive factors (temperature, pH, chloroform)

Sterile 1.5mL tubes containing 200µL of phage suspension (10^7PFU/ml) were incubated for 1 hour at various temperatures (-20, 4, 37, 40, 50, 60, 70, and 80°C) to assess the temperature stability of the phage. The phage suspension (200µL, 10^7PFU/mL) was incubated for one hour at room temperature in the same buffer solution at different pH values (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12). In all cases, the titer was calculated using the double agar layer method and expressed in PFU per milliliter.

Single-stage growth curve

A single-stage growth curve was constructed in three replicates to determine the latency period, eclipse period, and average burst size of the phage, as described in (Kutter and Sulakvelidze 2004).

Whole-genome sequencing

Bacteriophage DNA was extracted from a high-titer lysate using the ExtractDNA Blood & Cells kit (Eurogen, Russia) with modifications. During the procedure, 400µL of lysate sterilized by filtration was treated with 1µL of DNase I and 1µL of RNase A for 1.5 hours at 37°C to remove residual bacterial DNA and RNA. The enzymes were inactivated by adding 20µL of 0.5M ethylenediaminetetraacetate (EDTA), followed by 10µL of proteinase K, 100µL of sodium dodecyl sulphate (SDS), and 10µL of GAUSS, and incubating the mixture for 1.5 hours at 56°C to digest capsid proteins. Next, the DNA was isolated according to the manufacturer's instructions. DNA quality control included purity and concentration assessment using a NanoDrop spectrophotometer and a Qubit fluorimeter, as well as electrophoresis on a 1% agarose gel with 5µL of the sample. The purified DNA was stored at -20°C in 1.5-mL Safe-Lock tubes. Whole-genome sequencing was performed using the Illumina platform at Genome (Moscow, Russia).

Bioinformatics analysis

FastQC v0.11.5 was used to assess the quality of the sequencing data. For analysis, the adapter sequences were removed, and then the sequence was assembled with SPAdes using the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) (https://www.bv-brc.org/) (Olson et al. 2023). The phage genome was annotated using Pharokka v1.7.5 (Bouras et al. 2023) in the Conda 25.3.1 environment. Coding DNA sequences (CDS) were predicted using PHANOTATE v.1.5.1 (McNair et al. 2019), tRNAs were predicted using tRNAscan-SE 2.0.12 (Chan et al. 2021), and tmRNAs were predicted using Aragorn v.1.2.41 (Laslett and Canback 2004). A functional annotation was performed by comparing each CDS with the PHROGs (Terzian et al. 2021), VFDB (Chen et al. 2005), and CARD (Alcock et al. 2020) databases (Pharokka v1.4.0, https://zenodo.org/record/8276347) using MMseqs2 v.13.45111 (Steinegger and Söding 2017) and PyHMMER v.0.9 (Larralde and Zeller 2023). The graphs were created using pyCirclize v.0.3.1 (Shimoyama 2022). The ends of the genome and the packing mechanism were determined using PhageTerm v. 1.0.12 (Garneau et al. 2017).

To find phages similar to vB_MboM_W17, a search was performed against the standard publicly available NCBI nucleotide (nr/nt, accessed 20.05.2025) database using the Basic Local Alignment Search Tool (BLASTn) (National Institutes of Health (NIH) NCBI, USA). The genome sequence of the intrinsic vB_MboM_W17 phage was compared with those of the closest relatives using the Virus Intergenomic Distance Calculator (VIRIDIC) (Moraru et al. 2020). Manual predictions of the phage's lifestyle based on genome annotation were performed with the Bacphlip tool (Hockenberry and Wilke 2021).

Statistical analysis

Experimental data were processed in GraphPad Prism v.10.0 (GraphPad Software, USA). The average values and standard deviations were calculated from three independent experiments. The significance of the differences was assessed using the Student's t-test at the $P<0.05$ level.

RESULTS

Morphological features

The vB_MboM_W17 bacteriophage showed high lytic activity against strains of *Moraxella* spp. On 0.6% agar, the phage forms plaques with a diameter of about 2mm, surrounded by a pronounced halo zone after overnight incubation (Fig. 1A).

For morphological characterization, the phage suspension was negatively stained and examined by transmission electron microscopy (Fig. 1B). The particles have an icosahedral capsid with a diameter of 82nm and a contractile tail with a length of 184nm with spiral symmetry, which is typical for phages of the myovirus-like morphotype group (Fig. . The vB_MboM_W17 nomenclature was assigned in accordance with the International Committee on Taxonomy of Viruses (ICTV) recommendations (Kropinski et al. 2009).

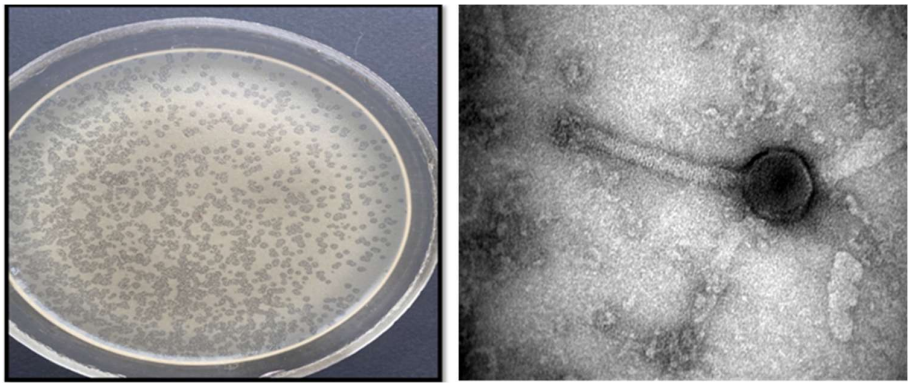

Fig. 1: Morphology of negative vB_Mbo_W17 colonies after incubation for 24 hours at 37°C on a lawn (A) and image of vB_Mbo_W17 phage obtained using a transmission electron microscope (B).

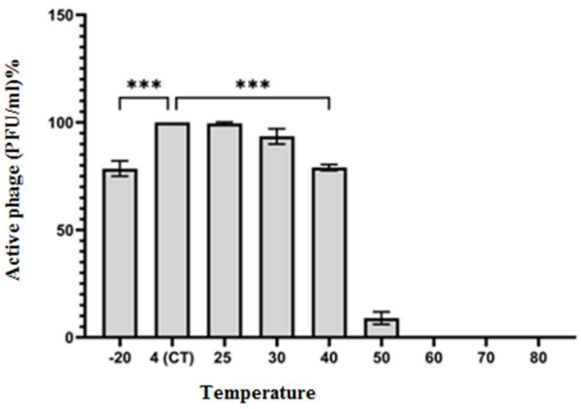

Fig. 2: The thermal stability of vB_MboM_W17 phage incubated at various temperatures for one hour.

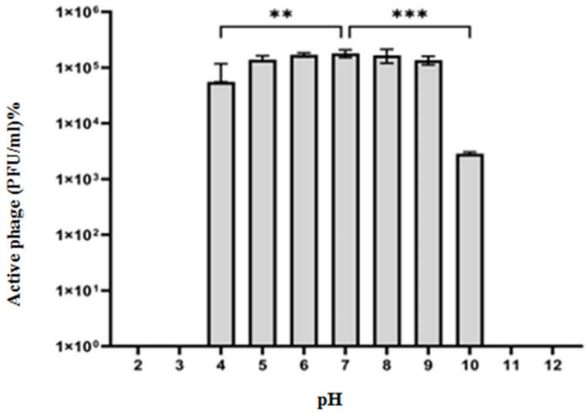

Fig. 3: The pH stability of vB_MboM_W17 phage incubated at various pH values for one hour.

Biological characteristics of vB_MboM_W17 and host range

The standard titration method in a double-layer agar plate was used to evaluate the thermo- and acid-base stability of the vB_MboM_W17 phage.

The thermal stability of the vB_MboM_W17 phage was assessed by incubating its suspension at -20, 4, 25, 37, 40, and 50°C, followed by titer determination. No significant changes in viability were detected in the range of 4-40°C, whereas at -20°C and 50°C, the titer decreased by more than an order of magnitude ($P<0.001$).

In the analysis of acid-base stability, the phage maintained infectious activity in the pH range of 6-9. At pH 4 and 10, there was a statistically significant decrease in titer ($P<0.001$), and at pH 3 and 11, the particles were completely inactivated.

According to the host spectrum definition, the bacteriophage vB_MboM_W17 exhibits lytic activity against all tested *Moraxella spp.* strains (isolates from cattle and sheep). At the same time, lysis was not observed in any bacterial strains of other genera, including *P. aeruginosa, E. coli, Streptococcus spp., E. cloacae,* and *K. pneumoniae*. These results indicate a high species specificity of the phage.

Single-stage growth curve

An experiment with a single-stage growth curve showed that the vB_MboM_W17 phage had an eclipse period of 30 minutes, a latency period of 70 minutes, and an average burst size 39±4 PFU per infected cell (Fig. 4).

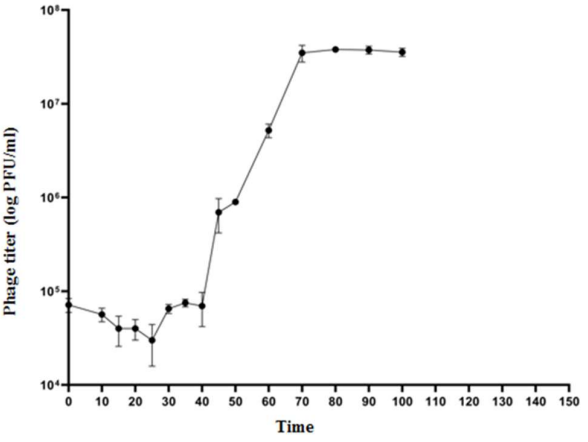

Fig. 4: Single-stage growth curve of the vB_MboM_W17 bacteriophage.

Genome sequencing and comparative analysis

Sequencing was done on the Illumina platform. The bacteriophage is a virus containing double-stranded DNA with a genome length of 140,676 bp. The final average reading coverage was 146X, and PhageTerm analysis of the genome indicated that there existed redundant ends and the phage genome is expected to be cyclic with a P1 packing type. Of the 249 protein genes in the genome, 92 had predicted functions based upon amino acid similarity to other proteins with known functions. The other 157 (~67%) were hypothetical proteins without assigned functional annotations. The phage genome tRNA genes existed for

methionine Met, phenylalanine Phe and aspartate Asp. The phage genome included no genes for antibiotic resistance or toxin encoding, did undergo lysogeny, or did express virulence. Guanine and cytosine make up 30.3% of it and Fig. 5 shows how its genome is organized. The Pharokka software tools were used to classify genes and create a genomic map.

A total of 30 genes are responsible for virion morphogenesis, including 17 head genes and 13 tail genes. The presence of both small and large terminase subunits is characteristic of many double-stranded DNA phages, where the small subunit is involved in DNA recognition, and the large one has ATPase and nuclease activity for DNA translocation and cleavage. Thus, DNA packaging into the phage head is carried out by the large terminase subunit (CDS_0178) and the small terminase subunit (CDS_0175).

The phage encodes its own set of key enzymes for DNA replication and repair. The presence of its own DNA polymerases (CDS_0237, CDS_0239, CDS_0243), DNA helicases (CDS_0218, CDS_0220), and DNA primase/helicase (CDS_0225) indicates that the phage probably largely controls the replication of its DNA. This may allow the phage to amplify the genome regardless of the host status rapidly.

The combination of phage-encoded RNA polymerase sigma factor (CDS_0004), which can redirect host RNA polymerase to phage promoters, with the presence of transcription factor (CDS_0043), RinB-like transcriptional activator (CDS_0126), and transcriptional repressor, as well as the presence of its own RNA polymerase beta subunit (CDS_0202), indicates that a phage can use several mechanisms to control gene expression.

The identified tail-associated lysines, like the tail-associated lysin (CDS_0203), the tail protein with lysin activity (CDS_0204), and the tail protein with lysin activity (CDS_0205), differ from endolysins involved in host lysis at the end of the infection cycle. They are commonly used to improve adsorption and may be responsible for the halo effect (Fig. 1A) observed in the resulting plaques.

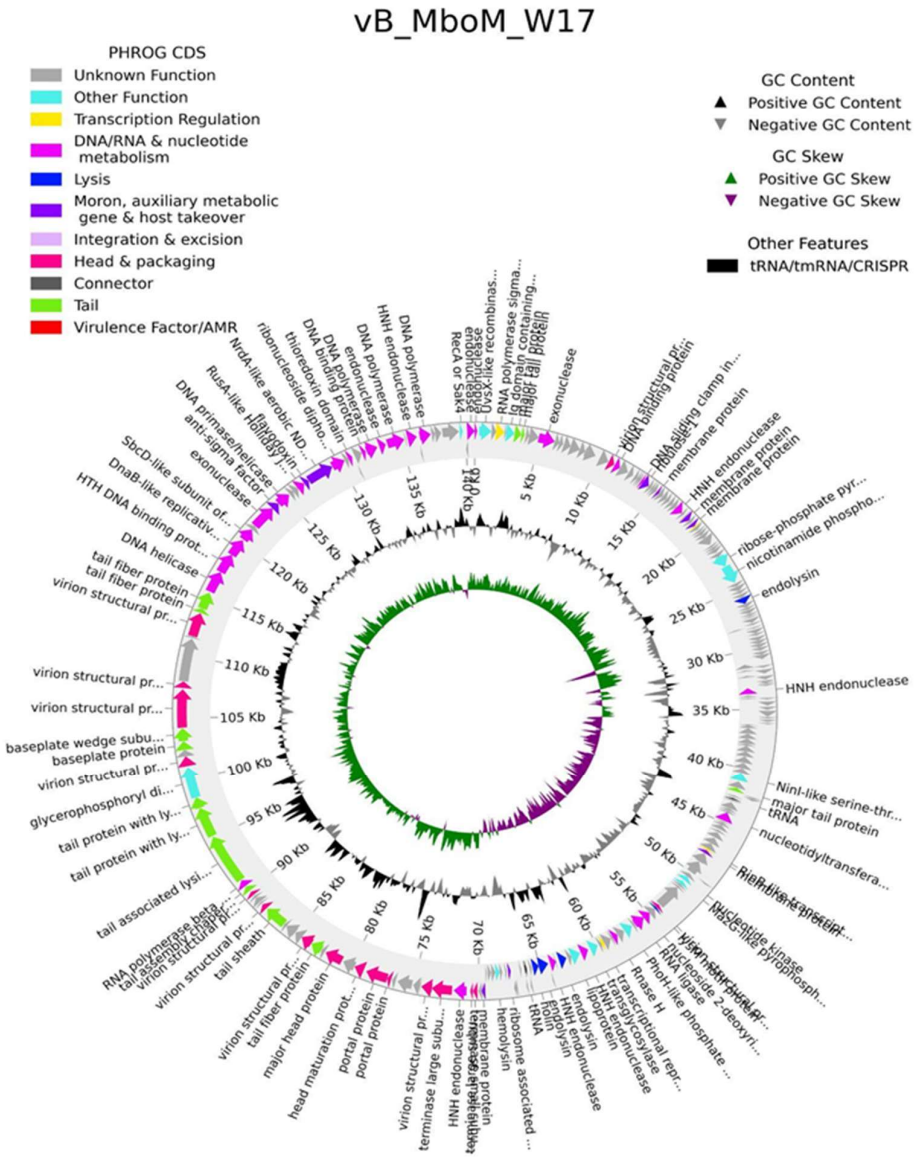

Fig. 5: The vB_MboM_W17 genomic map. The legend shows symbols and colors for predicted and classified coding sequences, tRNAs, GC content, and GC skew.

The presence of the holin gene (CDS_0159) in combination with endolysin genes (CDS_0154, CDS_0158) and an additional LysM motif protein (CDS_0138), which is associated with lysis, suggests that the phage may have an effective mechanism for lysing the host cell.

A comparative analysis of the genome sequence of the vB_MboM_W17 phage using the BLASTn algorithm showed that it was a close relative of *Staphylococcus* phages related to *Kayvirus*. Bacteriophages with 100% query coverage and an identity > 99.9% were selected for comparison using the VIRIDIC calculator.

The vB_MboM_W17 phage demonstrates a high degree of intergenomic similarity with representatives of the Herelleviridae family, the Twortvirinae subfamily, and the *Kayvirus* genus. Bacteriophages of the *Kayvirus* genus specifically infect *Staphylococcus spp.* strains, which constitute the predominant group of therapeutic phages used against *S. aureus* (10.1038/s41598-025-92032-6). Comparative genomic analysis showed that vB_MboM_W17 was most similar to the phages of the *Kayvirus* G1 species (*Staphylococcus phage* IME-SA1 (NC_047729.1), MSA6 (NC_047726.1), IME-SA2 (NC_047730.1), Team1 (NC_025417.1), vBSM-A1 (MK584893.1)). It also belonged to several not yet classified representatives of the *Kayvirus* genus (Fig. 6). According to our data, vB_MboM_W17 is the first described phage of the *Kayvirus* genus isolated using *M. bovis* as a bacterial host.

DISCUSSION

Infectious keratoconjunctivitis in cattle, primarily caused by *Moraxella bovis*, *M. bovoculi*, and *M. ovis*, represents a significant economic burden and animal welfare concern globally (Pimenov et al. 2024; Kilama et al. 2025). The effectiveness of conventional treatments is increasingly challenged by the rise of antimicrobial resistance in Moraxella species (Pimenov et al. 2024; Lubbers et al. 2025). This escalating resistance underscores an urgent need for alternative therapeutic strategies, with bacteriophage therapy emerging as a promising and sustainable solution in both human and veterinary medicine (Bianchessi et al. 2024; Choi et al. 2024; Banar et al. 2025; Ibrahim et al. 2025).

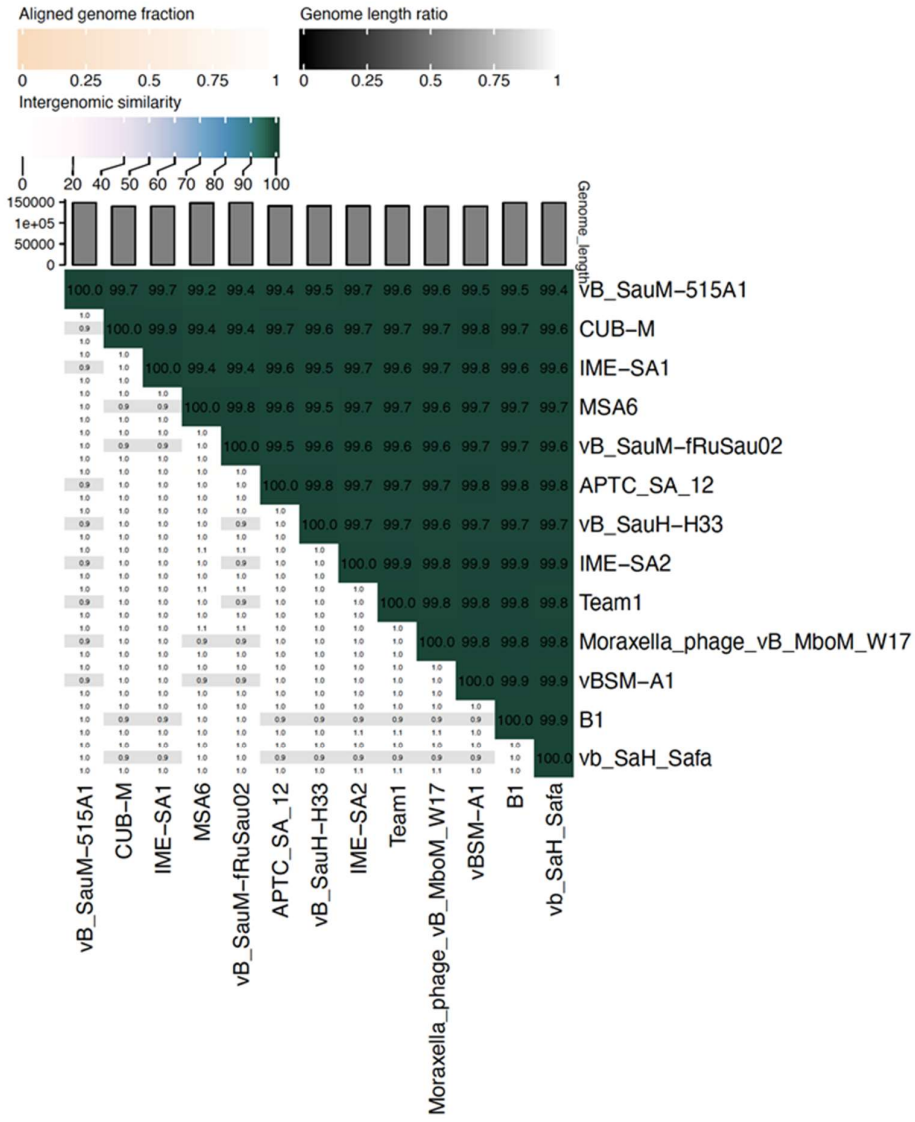

Fig. 6: A comparison of the entire genome and clustering of the vB_MboM_W17 phage with selected bacteriophages. The comparison was performed using VIRIDIC with the default BLASTn parameters. The values represent inter-genomic similarity.

This study's most significant contribution is the isolation and comprehensive characterization of Kayvirus vB_MboM_W17, a novel lytic bacteriophage. Uniquely, this phage exhibits lytic activity against *M. bovis*, *M. bovoculi*, and *M. ovis*, making it the first described member of the Kayvirus genus to infect *Moraxella* species. This discovery expands our understanding of Kayvirus taxonomy and host range, as these phages have historically been associated with infecting *Staphylococcus* spp. (Finstrlová et al. 2022). The observed host specificity of vB_MboM_W17 to *Moraxella* species is a crucial advantage for targeted therapy, minimizing disruption to the bovine ocular microbiome, which plays a vital role in maintaining eye health (Kilama et al. 2025).

The biological properties of vB_MboM_W17 further support its therapeutic potential. Its high lytic efficiency, evidenced by clear plaque formation with halo zones, indicates a potent antibacterial effect. The stability of the phage across a broad range of temperatures (4-40°C) and pH values (6-9) is particularly important for its practical application in veterinary settings, suggesting robustness during storage and administration (Bianchessi et al. 2024; Pimenov et al. 2024). The rapid eclipse period (30 minutes) and latency period (70 minutes), coupled with an average burst size of 39±4 PFU per infected cell, demonstrate efficient replication kinetics, which is desirable for a therapeutic agent.

Genomic analysis confirmed the safety profile of vB_MboM_W17, revealing a double-stranded DNA genome of 140,676 base pairs that lacks genes associated with virulence, lysogeny, or antibiotic resistance. This adherence to modern safety criteria is paramount for any bacteriophage intended for therapeutic use (Bianchessi et al. 2024; Choi et al. 2024). The presence of genes encoding for virion morphogenesis, DNA replication, and repair machinery indicates a self-sufficient lytic cycle. Furthermore, the identification of tail-associated lysins alongside holin and endolysin genes explains the observed halo effect and suggests a highly effective mechanism for host cell lysis, contributing to its strong antibacterial action (Vander Elst 2024).

The placement of vB_MboM_W17 within the Kayvirus genus through comparative genomic analysis not only highlights its phylogenetic relatedness but also expands the known ecological breadth of this group of phages. The ability of this Kayvirus to infect *Moraxella* spp. represents a significant biological finding, underscoring the dynamic evolution of phage host ranges and the potential for new therapeutic applications from unexpected phage-host interactions (De Sousa et al. 2021; Jia et al. 2023). Research on phage-host interactions continues to elucidate the complex mechanisms determining host tropism (Kauffman et al. 2022; Beamud et al. 2023; Ferriol-González and Domingo-Calap 2025).

Given the growing concerns over antimicrobial resistance in *Moraxella* species (Bilbao et al. 2024; Pimenov et al. 2024), Kayvirus vB_MboM_W17 offers a promising alternative to traditional antibiotics for the treatment of IKC. The current state of phage therapy in livestock and companion animals shows a clear trend towards exploring these specific and self-replicating antimicrobial agents to combat resistant bacterial infections (Choi et al. 2024). Phage therapy in ocular infections, including microbial keratitis in humans, is also gaining traction as a precision-based antibacterial treatment (Marasini et al. 2023; Ibrahim et al. 2025).

Future studies should prioritize *in vivo* evaluations of vB_MboM_W17 to validate its therapeutic efficacy in cattle suffering from IKC. Additionally, exploring potential synergistic effects with antibiotics or other non-antimicrobial approaches could lead to more robust treatment protocols and help mitigate the development of phage resistance (Sheedy et al. 2021). The findings from this study lay a strong foundation for the development of innovative phage-based interventions to reduce economic losses and improve animal welfare in the cattle industry.

Conclusion

In this study, the lytic bacteriophage vB_MboM_W17 was isolated and characterized for the first time. It exhibits lytic activity against tested strains of *M. bovis*, *M. bovoculi*, and *M. ovis*, confirming its potential versatility for controlling various etiological agents of infectious keratoconjunctivitis. Notably, the phage genome lacks genes associated with lysogeny, virulence, or antibiotic resistance, thereby meeting modern safety criteria for phage therapy agents. Comparative genomic analysis places vB_MboM_W17 within the *Kayvirus* genus, making it the first described representative of this genus to infect *Moraxella* spp. and expanding our understanding of the taxonomic and host-range diversity of Kayviruses. Further research is recommended to evaluate its *in vivo* therapeutic efficacy and potential synergies with antibiotics, with the aim of minimizing economic losses in animal husbandry.

DECLARATIONS

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Conflict of Interest: None.

Data Availability: Data available by request from the corresponding author.

Ethics Statement: The research protocol was reviewed and approved by the local ethical commission of the K.I. Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology. All procedures involving animals were carried out in accordance with international guidelines for the humane treatment of animals in scientific research (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, ETS No. 123).

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purification, electron microscopy analysis, and contributed to data interpretation. SP: performed genomic sequencing, bioinformatics analysis, and comparative genomic assessments. MS: contributed to microbiological investigations, statistical analysis, and manuscript revision. All authors read and approved the final version of the manuscript.

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