JOURNAL OF MEDICAL MICROBIOLOGY

JMM PROFILE

Egana-Labrin and Broadbent, *Journal of Medical Microbiology* 2023;72:001761

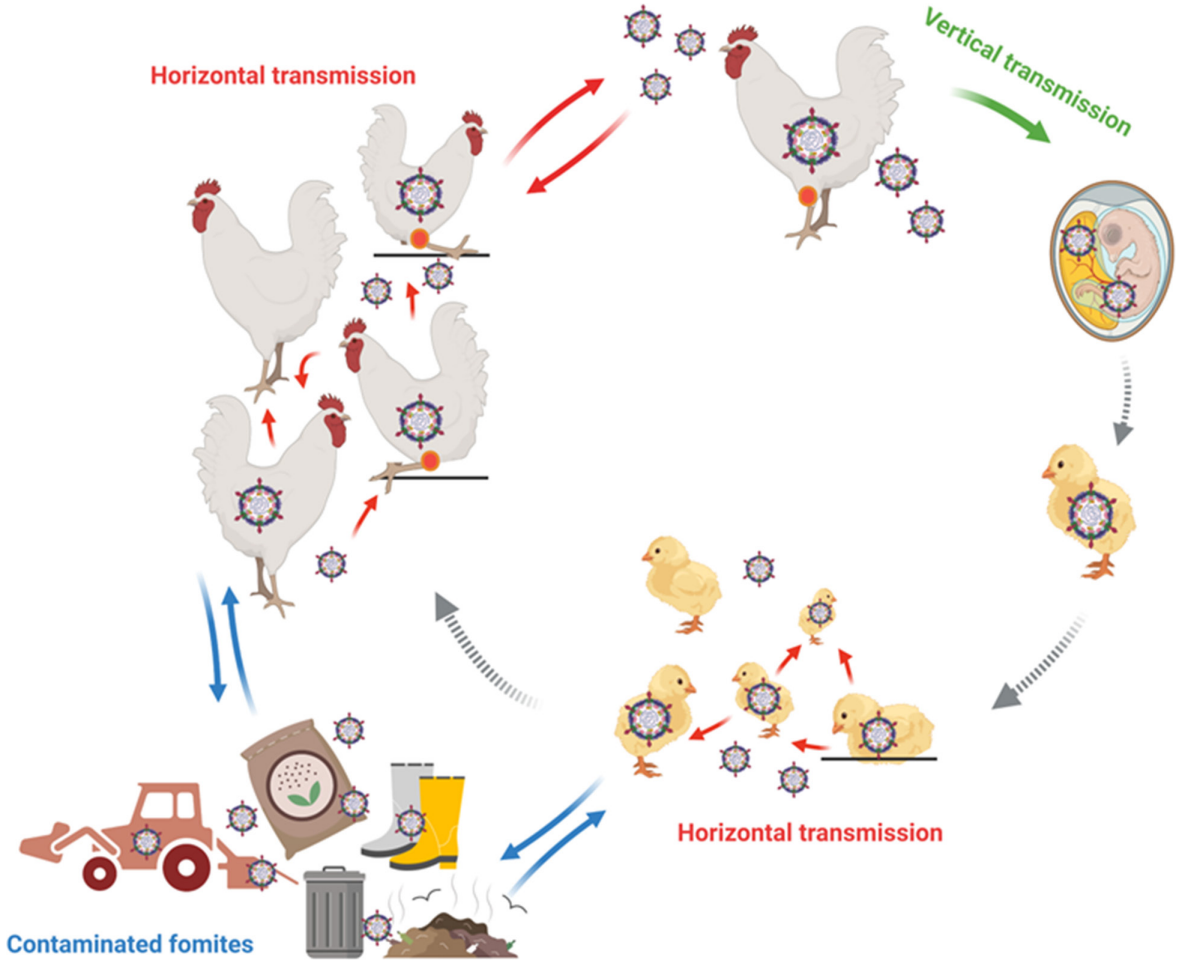
DOI 10.1099/jmm.0.001761

MICROBIOLOGY SOCIETY

OPEN ACCESS

Avian reovirus: a furious and fast evolving pathogen

Sofia Egana-Labrin and Andrew J. Broadbent*



Graphical abstract

Avian reovirus (ARV) can be transmitted vertically and horizontally. Pathogenic strains cause arthritis/tenosynovitis (depicted as red hock joints, and splayed legs), and can play a role in malabsorption syndrome in chickens. Contaminated fomites can be a dissemination source in poultry facilities. Figure generated with BioRender.

Abstract

Avian reoviruses (ARVs) have a significant economic impact on the poultry industry, affecting commercial and backyard flocks. Spread feco-orally, or vertically, many do not cause morbidity, but pathogenic strains can contribute to several diseases, including tenosynovitis/arthritis, which is clinically the most significant. The last decade has seen a surge in cases in the US, and due to ongoing evolution, seven genotypic clusters have now been identified. Control efforts include strict biosecurity and vaccination with commercial and autogenous vaccines. Research priorities include improving understanding of pathogenesis and developing new vaccines guided by ongoing molecular and serologic surveillance.

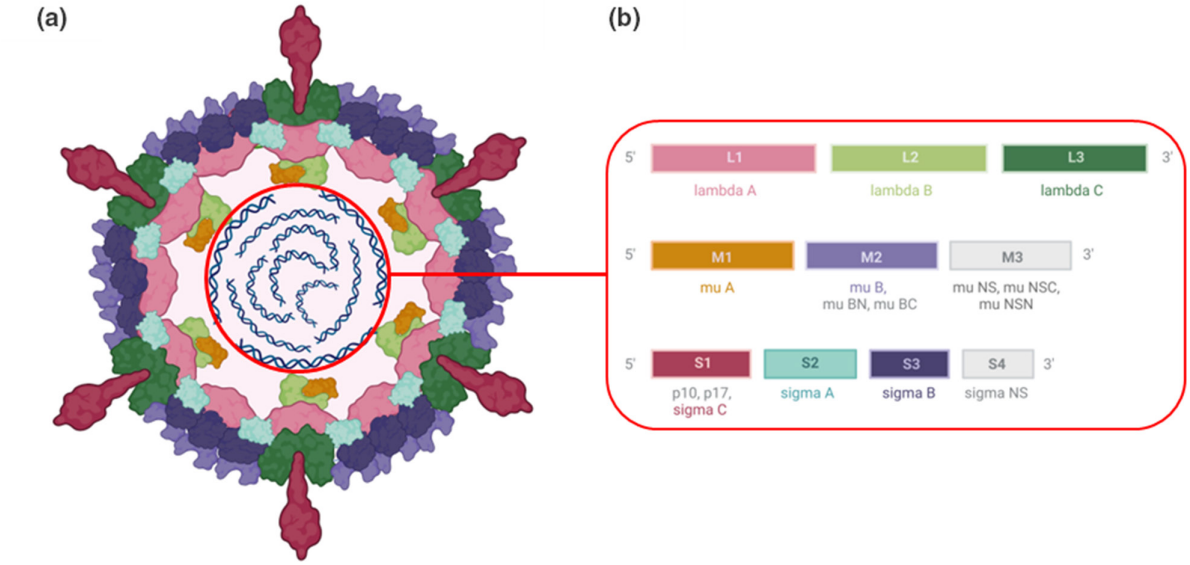


Fig. 1. (a) Avian reovirus virion structure. (b) Genome segments and their encoded proteins. Figure developed with BioRender.

HISTORICAL PERSPECTIVE

The *Reoviridae* family contains 15 genera and 75 species, isolated from many mammals, birds, reptiles, fish, arthropods, plants and fungi. The family is divided into two subfamilies, the *Spinareovirinae* and the *Sedoreovirinae*. Within the *Spinareovirinae*, the Orthoreovirus genus is further divided into ten species, of which avian orthoreovirus is one. Avian orthoreovirus, also known as avian reovirus (ARV), was first isolated from chickens' respiratory tract and tendons in the 1950s [1, 2], and was microscopically characterized in the 1970s [3]. In the 1980s, vaccines were first developed [4], which controlled disease in chickens initially. However, new variants emerged in the 2000s, complicating control efforts.

CLINICAL PRESENTATION

While many ARV infections are asymptomatic, pathogenic arthrotropic strains can cause arthritis and tenosynovitis, leading to lameness that may mean birds must be humanely culled. Flexor and gastrocnemius tendons can rupture in heavy-weight birds, associated with a green leg discolouration [5]. Pathogenic enterotropic ARV strains, in combination with other gut pathogens, can cause a malabsorption syndrome (MAS) and a running-stunting syndrome (RSS) [6], leading to growth retardation and abnormal feathering. ARV can also decrease egg production, and cause immunosuppression, respiratory and neurological signs [7].

MICROBIAL CHARACTERISTICS: PHENOTYPIC AND GENOTYPIC FEATURES

ARV belongs to the family *Reoviridae,* genus *Orthoreovirus*. The viral particle has a diameter of 80 nm, is non-enveloped, and has two icosahedral capsids. The genome comprises double-stranded RNA, divided into ten segments, the sum of which is 23.5 kb in length. The segments are divided into three large (L1, L2, L3), three medium (M1, M2, M3) and four small (S1, S2, S3, S4), according to their migration pattern by gel electrophoresis [8]. The ARV genome encodes eight structural and four non-structural proteins (Fig. 1). The sigma (σ)C protein, encoded by S1, mediates cell attachment, and elicits type-specific neutralizing antibodies.

Received 05 June 2023; Accepted 19 September 2023; Published 06 October 2023
Author affiliations: [1]Department of Animal and Avian Sciences, University of Maryland, College Park, MD, 20742, USA.
***Correspondence:** Andrew J. Broadbent, ajbroad@umd.edu;broadbent.jmm@gmail.com
Keywords: ARV; avian orthoreovirus; immunosuppression; malabsorption; poultry; viral arthritis-tenosynovitis.
Abbreviations: ARV, Avian reovirus; BSL, Biosafety level; CARV, chicken avian reovirus; DRV, Duck reovirus; dsRDRP, dsRNA-dependent RNA polymerase; ELISA, Enzyme-linked immunosorbent assay; FAST, Fusion-associated small transmembrane; GRV, geese reovirus; MAS, Malabsorption syndrome; ML, maximum-likelihood; NS, non-structural; ORF, open reading frame; RSS, Runting-stunting syndrome; RTPCR, reverse transcription polymerase chain reaction; TARV, Turkey avian reovirus; WOAH, World Organization for Animal Health.

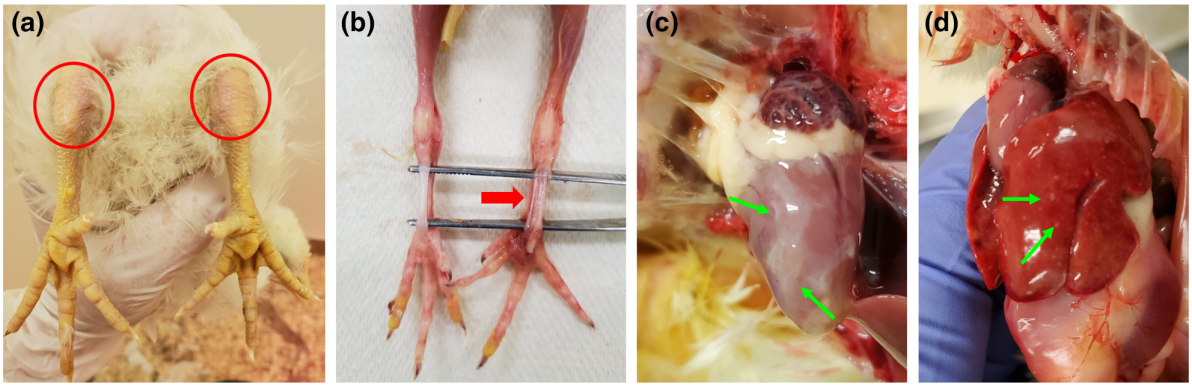


Fig. 2. Macroscopic lesions of avian reovirus. (a) Enlarged hock joints represented by the red circles. (b) Enlarged unilateral digital-flexor tendon represented by the arrow. (c) The arrows represent necrotic foci in the pericardium. (d) Necrotic foci in the liver are represented by the arrows.

CLINICAL DIAGNOSIS, LABORATORY CONFIRMATION AND SAFETY

Clinical diagnosis

ARV can be suspected by observing the tarsometatarsal (hock) joint, which is typically enlarged (Fig. 2a), and birds may be reluctant to move. Moreover, growth retardation and uneven uniformity in the flock might be attributable to RSS and MAS. In some cases, macroscopic lesions may include enlarged digital-flexor tendons (Fig. 2b), and necrotic foci in the pericardium (Fig. 2c), myocardium, spleen and liver (Fig. 2d). Since ARV infections can be asymptomatic, definitive diagnosis and differentiation from other leg, joint and malabsorption problems require laboratory confirmation.

Laboratory confirmation

Most diagnostic laboratories use reverse-transcription PCR (RT-PCR), targeting conserved ARV segments to diagnose infection [9–11]. Molecular characterization is encouraged for surveillance and can be done by sequencing the σC encoding segment [11, 12]. For research and vaccine development, detection of ARV can be achieved based on virus isolation from clinically affected flocks. The specimen type depends on the clinical signs and pathological findings and can include tendons, synovial fluid, pericardium, liver or intestinal content. ARV isolation can occur in primary cell cultures such as chicken embryonic fibroblasts (CEFs), chicken embryonic kidney or liver cells, or continuous cell lines such as chicken liver male hepatoma (LMH), Vero, or BHK cells. Syncytia formation is a critical cytopathic effect that can be visualized through light microscopy that signifies ARV infection. ARV isolation can also be done after inoculating the yolk sac of specific pathogen-free (SPF) embryonated eggs. Since ARV is difficult to isolate, it could require up to three or four blind passages [11]. Virus isolation can be confirmed by RT-PCR or by immunostaining. In addition, several commercial ELISA kits are available for monitoring flock vaccination schedules.

Laboratory safety

ARV is not a zoonotic agent; however, because it is an agricultural threat, diagnostic and/or research laboratories should comply with local biosafety regulations as advised by the World Organization for Animal Health (WOAH) [13].

TREATMENT AND RESISTANCE

Currently, there is no antiviral treatment for ARV infection. Treatment aimed at reducing the symptoms in pet birds could be attempted depending on local rules and prescribing practices, but this is challenging to manage in production settings. Given no treatment, there is no reported antiviral resistance in ARV. However, escape from immunity conferred by vaccines has occurred (see 'Prevention' section).

PATHOGENIC STRATEGIES: HOST RANGE, TRANSMISSION, INFECTION AND VIRULENCE FACTORS

Host range

ARVs have been subcategorized into chicken avian reovirus (CARV), turkey avian reovirus (TARV), duck reovirus (DRV) and goose reovirus (GRV), although ARVs have also been isolated from other species such as quail, pheasants, parrots and

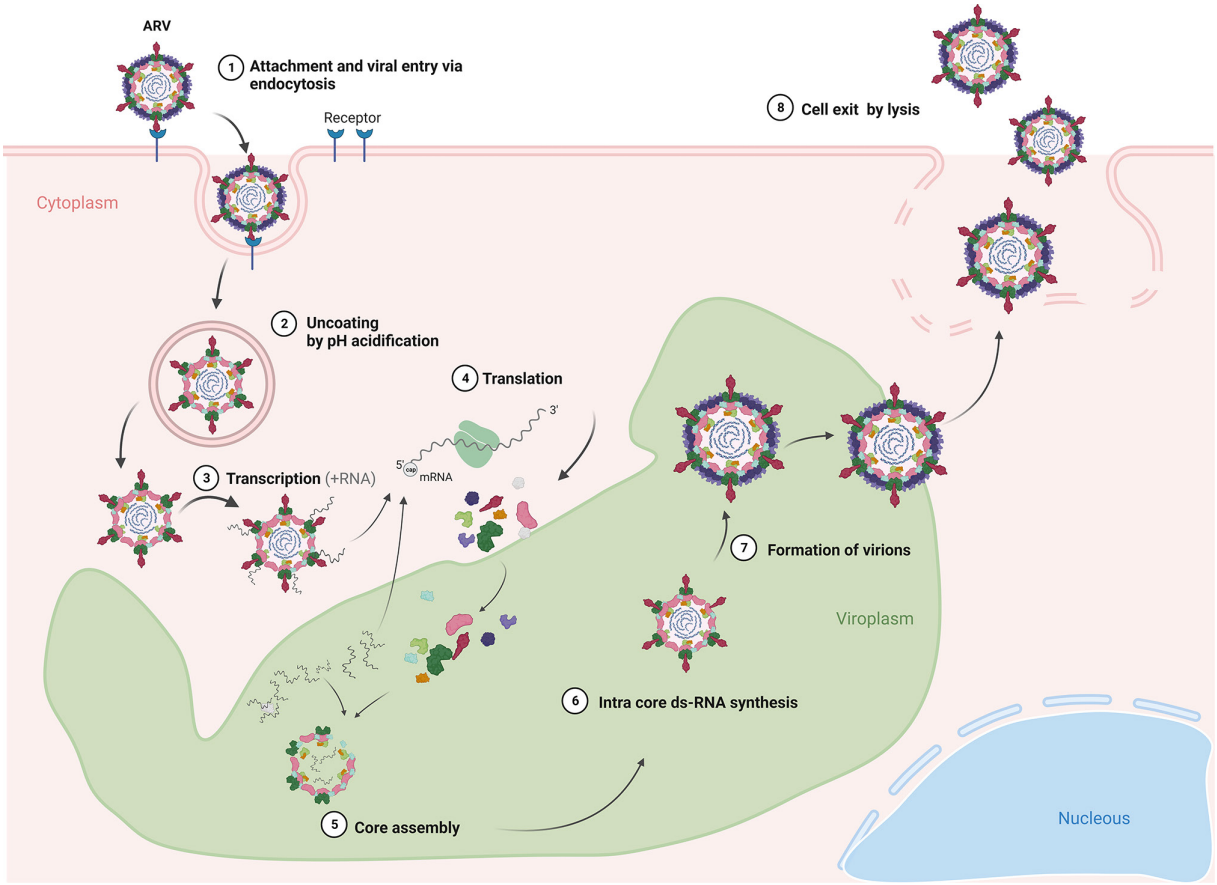


Fig. 3. The ARV reproductive cycle. Figure developed with BioRender.

pigeons [6]. While some members of the Orthoreovirus genus are zoonotic, for example, Nelson Bay orthoreoviruses, which are of bat origin and can cause respiratory infections in humans, ARVs are not known to be zoonotic.

Transmission

ARV can be horizontally and vertically transmitted. Horizontal transmission occurs when virus particles in intestinal and respiratory excretions enter through the oral route. Infection through the respiratory system and broken skin has also been demonstrated experimentally [14, 15]. ARV vertical transmission can occur between breeders and their progeny, although the transmission rate is low [16].

Infection

ARV replication starts with the attachment of the σC protein to the cell receptor, which remains unknown. The virion enters an endosome, where acidification leads to viral uncoating and the release of the virion core into the cytoplasm. Transcription occurs inside the core by the viral polymerase, and positive-sense RNA produced from each gene segment is released into the cytoplasm, which is translated into viral proteins by the host-cell ribosomes. A viroplasm forms, where newly synthesized virus cores assemble, into which positive-sense RNA is packaged and then used as a template to make the negative strand of the dsRNA genome by the polymerase within each core particle. The original core particle may also be recruited to the viroplasm as it expands. Mature virions are then released predominantly through cell lysis (Fig. 3) [17]. This reproductive cycle initially occurs in the intestinal epithelium, and the virus disseminates to different organs through the blood, leading to the above-described diseases. ARV infection can also cause immunosuppression, by depleting B lymphocytes in the bursa of Fabricius, which can exacerbate secondary infections. The incubation period varies between 1 to 14 days, depending on the route of infection and host factors [6].

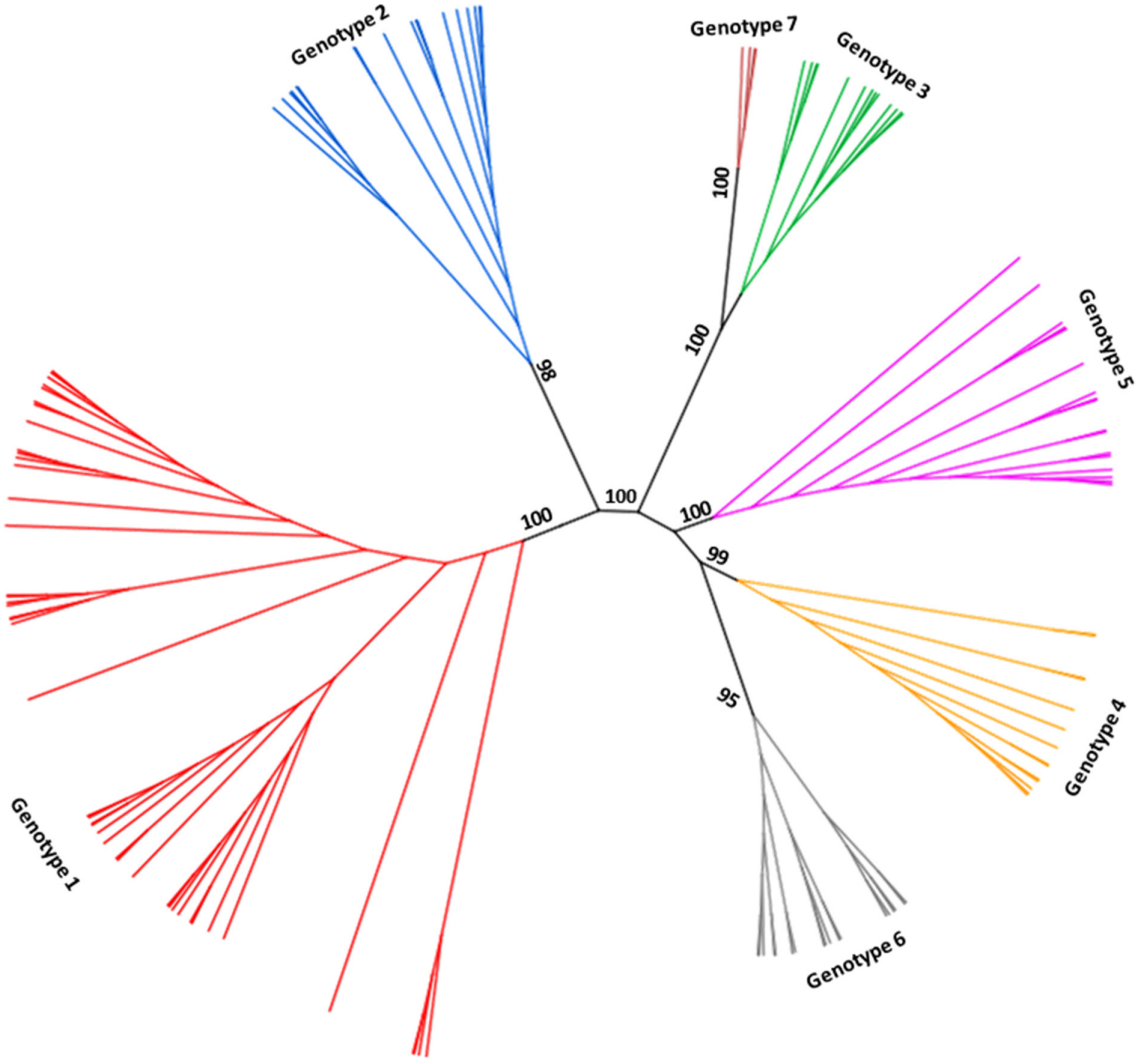


Fig. 4. Maximum-likelihood (ML) phylogenetic tree for ARV genetic classification using a partial σC encoding segment (875 bp) with published sequences in GenBank [11, 12, 23, 24, 26]. The phylogenetic tree was produced using Geneious Prime Software 2023.1.2 (https://www.geneious.com). The internal node numbers indicate bootstrap support values expressed as percentages for 1000 replicates.

Virulence factors

ARV virulence is multifactorial. The primary receptor binding protein (σC) likely plays a role in tissue tropism and disease. However, p10, encoded by the ORF1 of segment S1, is also a virulence factor, a fusion-associated small transmembrane (FAST) protein that induces cell syncytia formation and plays a role in apoptosis [18, 19]. In addition, σA, encoded by segment S2, inhibits type I interferon responses by binding to ds-RNA and inhibiting the dsRNA-activated protein kinase [20–22], and strain-dependent differences may influence virulence.

EPIDEMIOLOGY, PREVENTION AND RISK GROUPS

Epidemiology

ARVs are ubiquitous in poultry production facilities and are distributed worldwide. Pathogenic variant strains have emerged in the past two decades and continue to evolve, causing outbreaks in different geographic regions [11, 12, 23–26]. Genotypic variation can occur due to antigenic drift and reassortment, and recently, seven genotypes have been described, based on the sequence of the gene encoding σC [11] (Fig. 4). However, the variability within genotypes is still high, and similar genotypes can also cause different pathology [27], so the molecular classification still needs to be improved. Moreover, other variable outer capsid proteins might play a role in ARV antigenicity, for example, lambda (λ)B and mu (μ)B [28, 29], and should be considered

when typing strains. Finally, ARV is resistant to a wide range of heat, pH, and disinfectants, and virus particles can remain viable on wood shavings, feathers, shells, soil, water, feed, and other fomites for prolonged times [6], further hampering control efforts.

Prevention

ARV prevention involves the application of strict biosecurity measures such as cleaning and disinfection of barns and equipment, and pest control. In commercial production settings, all-in, all-out systems are also employed, which avoid mixing different ages and species in the same environment, and birds are vaccinated. Breeder flocks typically receive a vaccination schedule that includes commercial live and inactivated vaccines, so maternal antibodies protect their progeny. However, because of antigenic drift, current commercial vaccine strains are poorly matched to variant field strains, and many producers also use inactivated autogenous vaccines to improve control.

Risk groups

The risk groups include non-vaccinated birds, chicks that lack maternal antibodies and immunosuppressed birds.

OPEN QUESTIONS

(1) How can the current molecular genotypic classification be improved, and should genes encoding proteins other than σC be included in genotyping?
(2) What is the relationship between sequence diversity and antigenic diversity?
(3) What virus genes influence virulence?
(4) How can the efficacy of ARV vaccines be enhanced?
(5) Does co-infection with different viruses influence ARV pathogenicity?

Funding information
This work was supported by University of Maryland startup funds to A.J.B.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Fahey JE, Crawley JF. Studies on chronic respiratory disease of chickens II. Isolation of a virus. *Can J Comp Med Vet Sci* 1954;18:13–21.
2. NO O, DA M. Infectious synovitis control by medication; effect of strain differences and pleuropneumonia-like organisms. *Am J Vet Res* 1957;18:735–739.
3. Walker ER, Friedman MH, Olson NO. Electron microscopic study of an avian reovirus that causes arthritis. *J Ultrastruct Res* 1972;41:67–79.
4. van der Heide L, Kalbac M, Brustolon M. Development of an attenuated apathogenic reovirus vaccine against viral arthritis/tenosynovitis. *Avian Dis* 1983;27:698–706.
5. Jones RC, Kibenge FSB. Reovirus-induced tenosynovitis in chickens: the effect of breed. *Avian Pathol* 1984;13:511–528.
6. Pitcovski J, Goyal SM. Avian reovirus infections. In: *Diseases of Poultry*. 2020.
7. Dandár E, Bálint A, Kecskeméti S, Szentpáli-Gavallér K, Kisfali P, *et al*. Detection and characterization of a divergent avian reovirus strain from a broiler chicken with central nervous system disease. *Arch Virol* 2013;158:2583–2588.
8. Benavente J, Martínez-Costas J. Avian reovirus: structure and biology. *Virus Res* 2007;123:105–119.
9. Bruhn S, Bruckner L, Ottiger H-P. Application of RT-PCR for the detection of avian reovirus contamination in avian viral vaccines. *J Virol Methods* 2005;123:179–186.
10. Tang Y, Lu H. Whole genome alignment based one-step real-time RT-PCR for universal detection of avian orthoreoviruses of chicken, pheasant and turkey origins. *Infect Genet Evol* 2016;39:120–126.
11. Sellers HS. Avian reoviruses from clinical cases of tenosynovitis: an overview of diagnostic approaches and 10-year review of isolations and genetic characterization. *Avian Dis* 2022;66:420–426.
12. Kant A, Balk F, Born L, van Roozelaar D, Heijmans J, *et al*. Classification of dutch and German avian reoviruses by sequencing the sigma C protein. *Vet Res* 2003;34:203–212.
13. (OIE) WO for AH. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual),
14. Roessler DE, Rosenberger JK. In vitro and in vivo characterization of avian reoviruses. III. Host factors affecting virulence and persistence. *Avian Dis* 1989;33:555–565.
15. al Afaleq AI, Jones RC. Localisation of avian reovirus in the hock joints of chicks after entry through broken skin. *Res Vet Sci* 1990;48:381–382.
16. Menendez NA, Calnek BW, Cowen BS. Experimental egg-transmission of avian reovirus. *Avian Dis* 1975;19:104–111.
17. Benavente J, Martinez-Costas J. Early steps in avian Reovirus Morphogenesis. *Reoviruses Entry, Assem Morphog* 2006:67–85.
18. Salsman J, Top D, Boutilier J, Duncan R. Extensive syncytium formation mediated by the reovirus FAST proteins triggers apoptosis-induced membrane instability. *J Virol* 2005;79:8090–8100.
19. Chen X, He Z, Fu M, Wang Y, Wu H, *et al*. The E3 ubiquitin ligase Siah-1 suppresses avian reovirus infection by targeting p10 for degradation. *J Virol* 2018;92:e02101-17.
20. Martínez-Costas J, González-López C, Vakharia VN, Benavente J. Possible involvement of the double-stranded RNA-binding core protein sigmaA in the resistance of avian reovirus to interferon. *J Virol* 2000;74:1124–1131.
21. González-López C, Martínez-Costas J, Esteban M, Benavente J. Evidence that avian reovirus sigmaA protein is an inhibitor of the double-stranded RNA-dependent protein kinase. *J Gen Virol* 2003;84:1629–1639.
22. Gao L, Liu R, Luo D, Li K, Qi X, *et al*. Avian reovirus σA protein inhibits type I interferon production by abrogating interferon regulatory factor 7 activation. *J Virol* 2023;97:e0178522.

23. Palomino-Tapia V, Mitevski D, Inglis T, van der Meer F, Abdul-Careem MF. Molecular characterization of emerging avian reovirus variants isolated from viral arthritis cases in Western Canada 2012-2017 based on partial sigma (σ)C gene. *Virology* 2018;522:138–146.
24. Ayalew LE, Gupta A, Fricke J, Ahmed KA, Popowich S, *et al*. Phenotypic, genotypic and antigenic characterization of emerging avian reoviruses isolated from clinical cases of arthritis in broilers in Saskatchewan, Canada. *Sci Rep* 2017;7:3565.
25. Egaña-Labrin S, Hauck R, Figueroa A, Stoute S, Shivaprasad HL, *et al*. Genotypic characterization of emerging avian reovirus genetic variants in California. *Sci Rep* 2019;9:9351.
26. Lu H, Tang Y, Dunn PA, Wallner-Pendleton EA, Lin L, *et al*. Isolation and molecular characterization of newly emerging avian reovirus variants and novel strains in Pennsylvania, USA, 2011–2014. *Sci Rep* 2015;5.
27. Egaña-Labrin S, Jerry C, Roh HJ, da Silva AP, Corsiglia C, *et al*. Avian reoviruses of the same genotype induce different pathology in chickens. *Avian Dis* 2021;65:529–539.
28. Wickramasinghe R, Meanger J, Enriquez CE, Wilcox GE. Avian reovirus proteins associated with neutralization of virus infectivity. *Virology* 1993;194:688–696.
29. Yin C, Qin L, Sun M, Gao Y, Qi X, *et al*. Antigenic analysis of monoclonal antibodies against different epitopes of σB protein of avian reovirus. *PLoS One* 2013;8:e81533.