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Specific prevention of duck virus hepatitis (review)

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ABSTRACT

Introduction. Duck virus hepatitis (DVH) is a highly contagious acute disease primarily affecting ducklings, causing significant economic losses in duck farming. It is included into the World Organization for Animal Health (WOAH) list of notifiable diseases. Three main virus types cause duck virus hepatitis, with type 1 being the most ubiquitous and the primary etiological agent in most regions worldwide.

Objective. Systematization and analysis of contemporary approaches to prevention of duck virus hepatitis, review of promising strategies for recombinant and multivalent vaccine development.

Materials and methods. Literature searches and analysis were performed using the Scopus, Web of Science, Google Scholar, PubMed, ScienceDirect, and RSCI (Russian Science Citation Index) databases.

Results. This article analyzes current DVH situation, and reviews key features of the disease pathogenesis, immune response and the disease diagnosis. Furthermore, it presents comparative data on various vaccine characteristics, including production methods, efficacy, immunization schedules, immune response profiles, advantages and disadvantages, and commercial availability. This article summarizes existing approaches to livestock protection and proposes directions for further research.

Conclusion. Although a large number of different foreign vaccines against DVH is available, embryo vaccine against DVH based on VGNKI-K strain is the only vaccine produced in the Russian Federation and it has a rather limited shelf life (9 months). In response to this need, the Federal Centre for Animal Health is carrying out research aimed at developing a live freeze-dried vaccine featuring extended shelf life.

Keywords: review, duck virus hepatitis, specific prevention

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Специфическая профилактика вирусного гепатита утят (обзор)

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РЕЗЮМЕ

Введение. Вирусный гепатит утят – высококонтагиозное острое заболевание молодняка уток, наносящее значительный экономический ущерб утководческим хозяйствам и входящее в перечень нотифицируемых болезней Всемирной организации здравоохранения животных. Наиболее распространены три типа вируса, из которых тип 1 встречается повсеместно и является основным этиологическим агентом в большинстве регионов мира.

Цель исследования. Систематизация и анализ современных подходов к профилактике вирусного гепатита утят, обзор перспективных стратегий разработки рекомбинантных и поливалентных вакцин.

Материалы и методы. Поиск и анализ литературных источников проводился с использованием баз данных Scopus, Web of Science, Google Scholar, PubMed, ScienceDirect и РИНЦ.

Результаты. В статье приведен анализ современной эпизоотической ситуации по вирусному гепатиту утят, рассмотрены особенности патогенеза, иммунного ответа и диагностики заболевания. Представлены сравнительные данные по характеристикам различных вакцин против вирусного гепатита утят: их способу производства, эффективности, схемам иммунизации, формированию иммунитета, преимуществам и недостаткам, наличию на коммерческом рынке. Обобщены существующие подходы к защите поголовья и предложены направления дальнейших исследований.

Заключение. Несмотря на наличие большого количества разнообразных зарубежных вакцин против вирусного гепатита утят, в Российской Федерации единственной производимой вакциной является вакцина против вирусного гепатита утят из штамма «ВГНКИ-К» эмбриональная (ФГБУ «ВНИИЗЖ», Россия), которая имеет достаточно ограниченный срок годности (9 мес.). В связи с этим в ФГБУ «ВНИИЗЖ» проводится научно-исследовательская работа по разработке живой лиофилизированной вакцины с увеличенным сроком годности.

Ключевые слова: обзор, вирусный гепатит утят, специфическая профилактика

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INTRODUCTION

Duck virus hepatitis (DVH) is an acute, highly contagious disease affecting susceptible ducklings under 3–4 weeks of age. It is characterized by liver damage, hemorrhagic diathesis, and such clinical signs as depression, jaundice, and high mortality in young birds [1, 2, 3]. There are three types of duck hepatitis virus (DHV). Type 1 virus (DHAV type I or DHV-1) belongs to the genus *Avihepatovirus*, family *Picornaviridae*, and includes 3 serotypes (DHAV-1 or DHAV-A; DHAV-2 or DHAV-B; DHAV-3 or DHAV-C). Type 2 and 3 viruses (DHAV type II or DHV-2; DHAV type III or DHV-3) represent the *Astroviridae* family: duck astrovirus type 1 (DAstV-1) and duck astrovirus type 2 (DAstV-2), respectively [4, 5]. Type 2 virus was isolated in England and type 3 virus was isolated in the USA [6].

It is of paramount importance to study DVH due to its severe clinical course in young birds, rapid transmissibility, and substantial economic impact arising from high mortality among ducklings. Besides, the recovered ducklings lag behind in growth and development, the adult flock demonstrates decreased productivity and the necessary restrictive measures impose an additional financial burden [2].

The purpose of this review is to systematize current data on DVH with an emphasis on specific prevention, in particular vaccination, as well as to analyze the disease situation in the Russian Federation, where only one registered domestically produced vaccine is used.

HISTORICAL REFERENCE

Duck virus hepatitis was first officially registered in 1949 in the USA. Subsequently, the disease spread rapidly across countries with well-established duck farming industries, including Canada, Brazil, Germany, France, Ukraine, England, Belgium, India, the Czech Republic, Japan, and Poland. The DVH-associated mortality rate ranged from 30 to 95% [7, 8]. In 1958, the first DVH outbreak was reported in the USSR in the Belgorod and Kharkov Oblasts [5].

Three different DHV types were described, and all of them were initially classified as picornaviruses, with type 1 considered to be the most similar to the viruses of the genus *Enterovirus*. Duck hepatitis virus types 2 and 3 were later assigned to the *Astroviridae* family and renamed as duck astroviruses (DAstV); DHV-1 is the most widespread and the most virulent among the three [9].

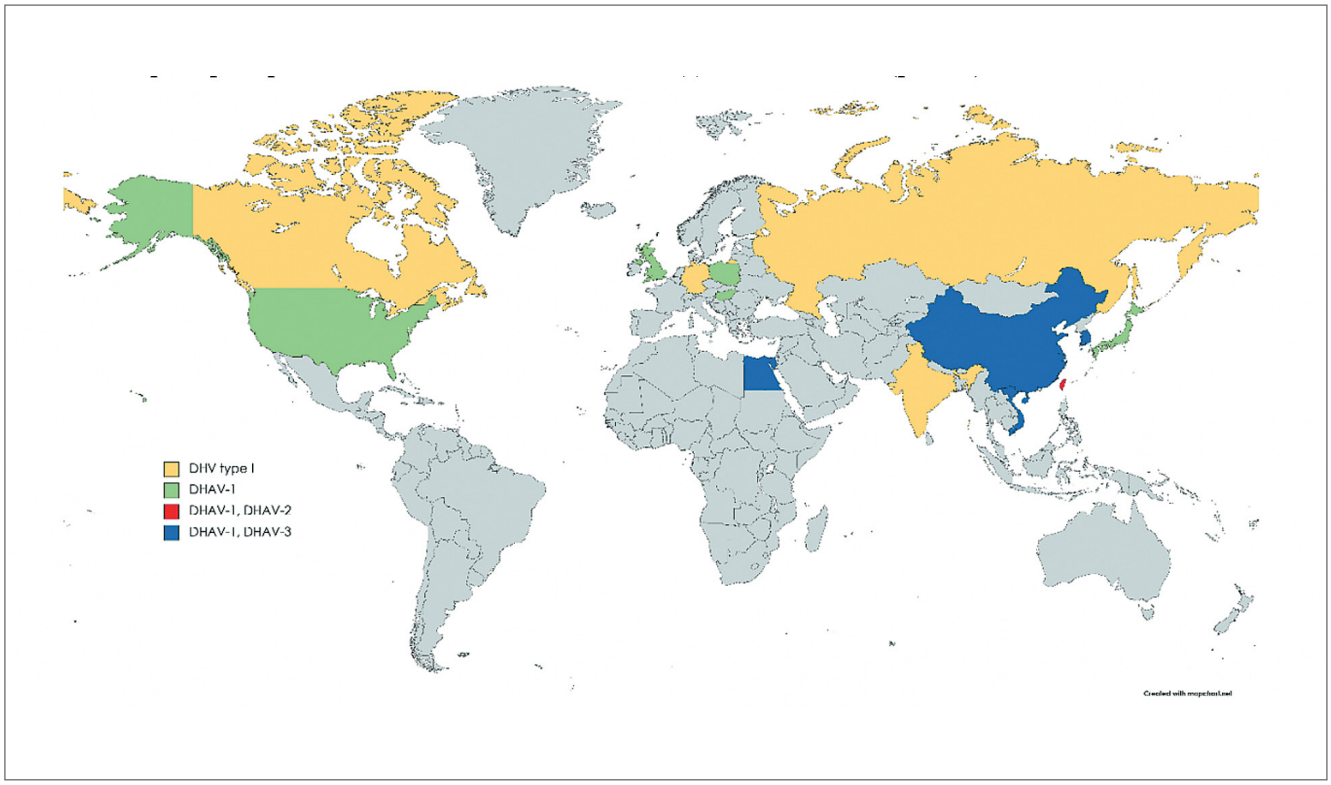

Fig. 1. Geographic distribution of DHV serotypes (for 2021) [10]

Serotype 1 (DHAV-1) is prevalent in many regions worldwide, such as England, Poland, China, and Egypt [10]. Serotype 2 (DHAV-2), on the contrary, is found only in Taiwan and China [9]. Serotype 3 (DHAV-3) is a new serotype that appeared in Korea in 2003, and then spread to China, Vietnam, and recently Egypt (Fig. 1) [10, 11, 12, 13].

Duck virus hepatitis outbreaks continue to be recorded now in various regions of the world, mainly in Asia, where the disease causes significant economic damage to farms. It is also of considerable importance for the Russian Federation. Since commercial duck farming is practiced in the country, the risk of introducing and spreading novel virus variants remains a significant concern.

CAUSATIVE AGENT

As a member of the family *Picornaviridae*, DHV is a small, non-enveloped, spherical virus measuring 20–40 nm in diameter, with a single-stranded positive-sense RNA, approximately 7,800 nucleotides in length. The complete open reading frame (ORF), which is surrounded by 5′ and 3′ non-coding regions, encodes three mature structural proteins: capsid proteins 0 (VP0), 1 (VP1), and 3 (VP3). The ORF also encodes nine non-structural proteins (2A1–2A2–2A3–2B–2C–3A–3B–3C–3D), while the major surface protein VP1 serves as an antigenic determinant that plays a significant role in virus pathogenicity, evolution, and virulence [6, 10].

Based on the genetic structure characteristics of the pathogen, it has been classified into three serotypes: serotype 1 (DHAV-A), which is found worldwide; serotype 2 (DHAV-B), isolated in Taiwan; and serotype 3 (DHAV-C), isolated in South Korea and China. DHAV-C mainly affects ducklings under 3 weeks of age. Its epidemiological profile, clinical manifestations, and post-mortem impact are very similar to those of DHAV-A, thereby hindering discrimination between these two serotypes. Comparison of the complete nucleotide and amino acid sequences of VP1, VP0, and VP3, as well as partial three-dimensional nucleotide sequences, revealed differences among the three DHV serotypes [5].

The pathogen lacks hemagglutinating activity yet replicates in avian embryos (duck, chicken, quail, and guinea fowl) and in primary trypsinized fibroblast cultures derived from these embryos. The virus induces a cytopathic effect in cell culture [7, 14, 15]. Goose embryo cell cultures were used for the virus replication, and there is also evidence of using primary and continuous cell lines, as well as various tumor tissues. It was found that tissue cultures derived from mammals, as well as other cell lines such as HEp-2, Detroit 6, HeLa, L-99, and GPk, display low susceptibility to DHV [14]. DHV type 2 replicates well in duck embryos and ducks themselves, as well as in duckling liver and kidney cell cultures. However, in contrast to DHV type 1, replication of DHV type 2 is limited in chicken and quail kidney cell cultures. In addition, DVH type 2 does not replicate in chicken embryos. Ducklings immune to DHV-1 become ill upon infection with DHV-3. The distinct replication patterns point to antigenic variations among the viruses causing DHV [15].

Duck hepatitis virus is highly stable in the environment: it persists in feeders for > 10 weeks, in litter for up to 37 days, in water for up to 74 days, and in soil for 105–157 days. DHV remained pathogenic on the wall surfaces of poultry houses for up to 20 to 40 days, while the pathogen survival depended on the air temperature. The virus remains viable for up to 25 days in spring and up to 105 days in winter. The maximum pathogen survival and persistence in different water environments was 74 days [15].

The virus exhibits resistance to ether, chloroform, and varying environmental pH levels. The pathogen remained viable for several years when stored at temperatures between minus 14 °C and minus 32 °C in a refrigerator [15].

High temperatures adversely affect the virus, with a 60-minute treatment at 56 °C being sufficient to inactivate the pathogen. Ultraviolet rays destroy the pathogen within 10 minutes [7].

Disinfectants including xylonaphth, lysol, creolin, and soda ash fail to inactivate the pathogen when used at normal concentrations [15]. To decontaminate housing facilities in duck farms, several disinfectants are employed: a 1% formaldehyde solution; a 4% sodium hydroxide solution warmed to 40–45 °C and held for 12 hours; a sodium hypochlorite solution containing 1.5% available chlorine and 1.2% free alkali with an exposure time of 6 hours; and a 5% monochlorine iodide solution, also applied for 6 hours [7].

To inactivate the virus, aerosol disinfection of poultry houses may also be performed using a 40% formaldehyde solution (12-hour exposure) or a 20% aqueous formaldehyde solution (24-hour exposure). Compound feed contaminated with DHV requires one-hour heat treatment at 65–75 °C to achieve effective decontamination [7].

The evolutionary mechanisms of duck hepatitis virus resemble those of other picornaviruses. Key shared features include a rapid evolutionary rate and the ability to undergo viral RNA recombination, even among heterotypic strains. Both evolutionary history and epizootological evidence indicate that Asia could act as a center of diversification for duck virus hepatitis agent. DHAV-1 strains found in Europe are closely linked to those identified in China. This finding is consistent with earlier research on other closely related waterfowl-borne pathogens, which show overlapping geographic distributions between these remote regions. All these data may point to common factors or transmission routes for various waterfowl viruses between the Far East and European regions [10].

EPIZOOTIC PATTERNS

The disease affects ducklings and goslings, whereas birds of the order *Galliformes* and laboratory animals are not susceptible to it [7]. The reservoir of the causative agent includes birds that are either currently infected or have recovered from the disease. Convalescent ducklings continue to shed the virus via feces, nasal discharge and conjunctival fluids. Post-infection virus carriage can last between 60–75 days and 300–650 days, depending on the case. In nature, ducklings get infected via contaminated feed and water, with the airborne route also playing a significant role [2, 16]. A potential vertical transmission of the pathogen was noted by some researchers [17].

A characteristic feature of DVH epizooties, observed in nearly all cases, is the rapid increase in duckling mortality: the peak occurs on days 4–5, followed by

a decline on days 7–8, with a sharp drop in the number of dead birds by days 10–12 [15].

Disease progression is exacerbated by overcrowding, wet bedding, drafts, and low temperatures in the housing facility. Transmission can take place on water ranges when domestic birds come into contact with wild waterfowl [7].

The disease's extensive spread stems from the virus's high resistance to physicochemical agents, its prolonged persistence in recovered birds, its genetic variability, and persistently unfavorable DVH situation on affected farms [16].

Over the past 6 years, DHV was reported in China (2009–2021), Egypt (2019), India (2020–2023), and Guyana (2019–2024) [18, 19, 20, 21].

The emergence of new DHV genotypes and subgenotypes reduces effectiveness of the used vaccines. Due to the virus genetic variability, many existing vaccines provide protection only against certain strains, which makes these vaccines less effective [22].

PATHOGENESIS AND CLINICAL SIGNS

Upon infection, the virus enters the body through mucous membranes of the respiratory tract, the gastrointestinal tract, and damaged skin. DHV targets hepatocytes, replicating within their cytoplasm. The pathogen accumulates not only in the liver but also in the spleen, the bursa of Fabricius, and the brain. Infected birds develop hepatitis, characterized by hepatocyte necrosis, congestive hyperemia, and hemorrhages. Damage to the bile ducts impairs bile flow, resulting in delayed evacuation. This leads to systemic intoxication. Subsequently, myocardial dystrophy, pericarditis, and nephritis develop, resulting in the death of the bird [16]. Virus accumulation in the spleen and bursa of Fabricius suggests that the pathogen is capable of invading both central and peripheral immune organs. This, in turn, leads to a dysfunction of the immune system in infected ducklings. Consequently, even after recovering from infection caused by DHV, ducklings still remain susceptible to other pathogens due to weakened immunity [3].

Infected ducklings exhibit metabolic changes, including decreased levels of total protein, bilirubin, and albumin, reduced protective properties of serum colloids, lower activity of alkaline phosphatase, glutamate-pyruvate transaminase, bilirubin, and creatinine, alongside increased serum enzyme activity, which directly correlates with the presence of liver damage in infected birds. Necrosis of pancreatic acinar cells was observed, reflecting an impact on exocrine pancreatic function [3, 15].

The incubation period ranges from 1 to 5 days and is shorter following oral, intranasal, or aerogenic challenge compared with parenteral administration. Peracute and acute forms of the disease are mostly reported. Sick birds show depression, inappetance, incoordination, paresis, paralysis, opisthotonos (Fig. 2), narrowed palpebral fissures, and conjunctivitis. Full recovery is seldom observed. The ducklings that survive the infection remain the virus carriers. In the chronic form, birds exhibit impaired growth and development. The chronic

Fig. 2. DHV-caused opisthotonus (photo by: S. V. Glazer, V. Yu. Fomenko, V. N. Irza)

form is more often observed in 3–4-week-old ducklings. The disease lasts for 10–20 days, sometimes more, accompanied by diarrhea. Ducklings exhibit lethargy, with some developing swollen joints. Affected ducklings exhibit a penguin-like gait, moving with the body held in a vertical position [15].

Post-mortem findings resulting from the chronic form of the disease include a 1.5-fold liver enlargement. The liver is unevenly colored, leukemia-typical granulomas are found; the spleen is congested and contains foci of necrosis, and periarthritis develops [6, 15].

Recovered survivors show practically no differences from the healthy ducklings. However, the virus can be isolated from pathological samples (liver, brain) obtained from infected ducklings, and specific antibodies can be detected in blood serum samples [6].

Adult ducks are asymptomatic, with ovariosalpingitis noted occasionally [7].

The morbidity rate in ducklings under three weeks of age is 80–90%. In the peracute form of the disease, mortality in ducklings up to 10 days of age can reach 100%, whereas in the acute form it is 70–80%. On persistently affected farms, DVH is observed in ducklings aged 15–30 days and older. Mortality rate in certain batches can be as high as 5–10%. When non-immune ducklings are reintroduced into such a farm, mortality increases again from batch to batch, sometimes reaching 80–95% [8]. Embryos infected with DHV die at various stages of embryonic development in 75–90% of cases [16].

Duck virus hepatitis is often complicated by the concurrent development of other diseases of viral, bacterial, and fungal origin, including influenza, salmonellosis, mycoplasmosis, colibacillosis, and aspergillosis. Importantly, DVH plays a pivotal role in development and progression of such coinfections [15].

Postmortem examination reveals liver lesions (Fig. 3). The liver is enlarged and flabby, unevenly colored ranging from ochre-yellow to grayish-clay, and contains numerous hemorrhages beneath the capsule and throughout the parenchyma. The gallbladder is markedly distended with bile. The spleen occasionally appears enlarged and mottled. In the majority of cases, the kidneys are enlarged and congested, and the renal blood vessels are hyperemic. The cerebral blood vessels are congested. The heart looks like cooked meat, exhibits granular degeneration, and the coronary vessels are congested. The lungs are edematous, and fibrinous-diphtheritic deposits are observed on the air sac wall [7, 16]. Young birds often develop catarrhal enteritis, which is a consequence of hepatitis complicated by bacterial infection, particularly salmonellosis [15].

Histological examination of the affected liver reveals hepatocellular necrosis with complete karyolysis, granular degeneration, fatty metamorphosis, and lymphocytic-plasmacytic proliferation, and less commonly, hyperemia of the interlobular capillary blood vessels. Using the Noble tetrazine-floxin staining

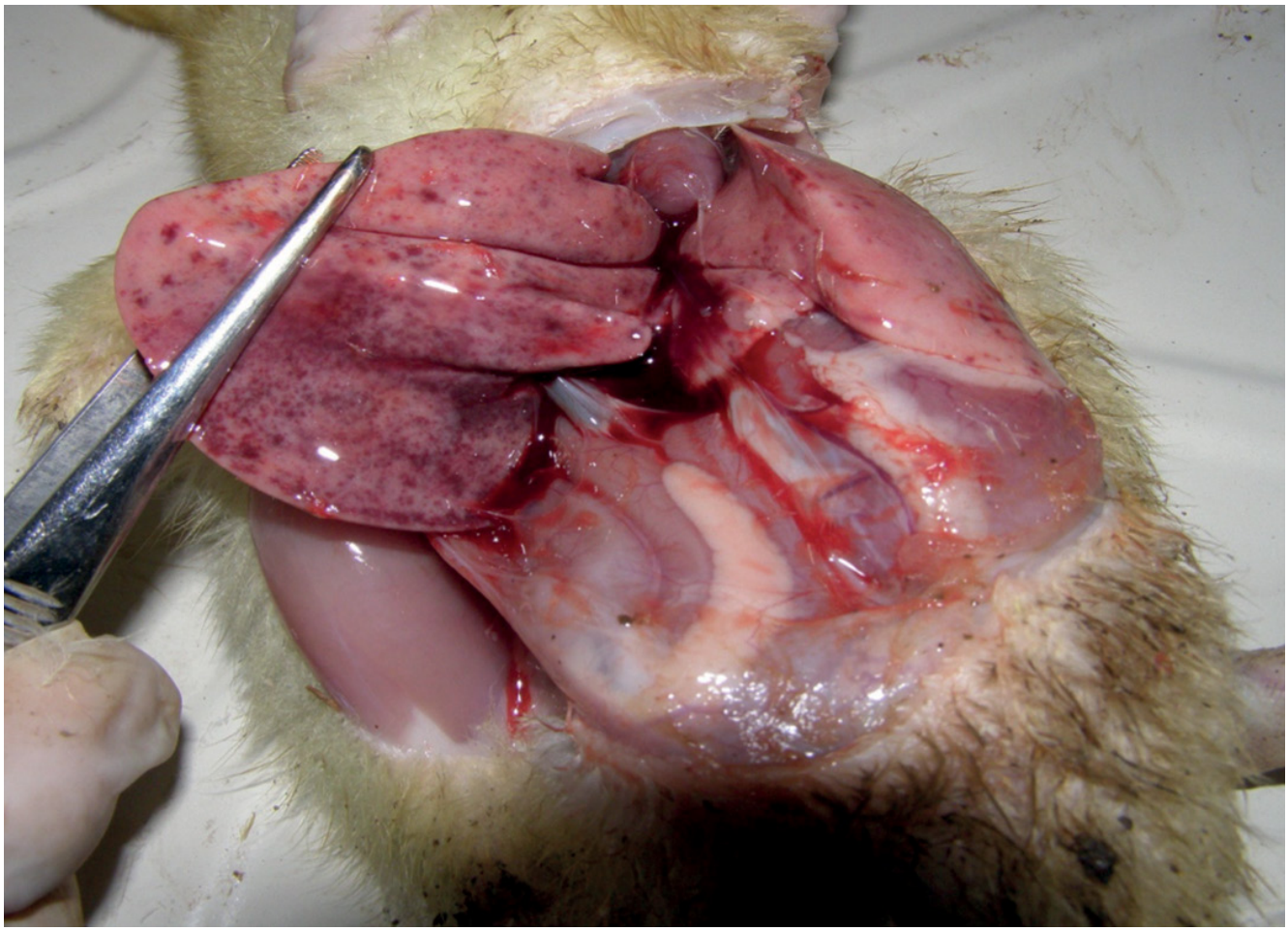

Fig. 3. DHV-caused liver lesions (photo by: S. V. Glazer, V. Yu. Fomenko, V. N. Irza)

method, oval or spherical inclusion bodies ranging from 1 to 8 μm in size can be detected in histological sections of liver tissue [7, 16]. Virus-like particles can be detected in hepatocytes already in the first 1–18 hours after infection. Within 24 hours, the first signs of liver changes appear, including necrobiosis and apoptosis of hepatocytes; in some cases, extensive necrotic foci and hemorrhages are observed. Subsequently, small focal proliferates and disintegration of affected cells are observed within the hepatic lobules and adjacent to the sinusoidal capillaries. Perivascular infiltration is observed around blood vessels and bile ducts, consisting of granulocytes, lymphocytes, and plasma cells. In convalescent birds, the onset of regenerative activity may be observed within the hepatic parenchyma [6].

Active regressive changes are observed in the spleen, progressing to necrosis [7, 16]. Following infection, specific pathomorphological changes are observed in the bursa of Fabricius, including hyperemia, marked serous exudate infiltration, and proliferation of plasma cells and hemocytoblasts. Proliferation becomes pronounced 72 hours after infection, which is associated with an immune response to the viral antigen. If the infection persists for 2–3 weeks, the number of plasma cells in the bursa of Fabricius and spleen increases 3- to 4-fold. There are no significant post-mortem lesions in the thymus. The nature of the cerebral lesions is indicative of serous encephalitis [6].

Embryos infected with the hepatitis virus that succumb between days 20 and 25 of incubation exhibit yolk sac vascular congestion, passive congestion, and subcutaneous edema affecting the head, neck, and back. Occasionally, edema affects the limbs, and multiple hemorrhages are commonly seen. Embryos succumbing 15 days post-incubation exhibit hyperemia of the yolk sac and allantoic vessels. The allantoic cavity contains a transparent, viscous, sometimes opalescent fluid with a greenish tint. Affected embryos often show evidence of dwarfism, and limb muscle atrophy may occur in some instances. The liver of affected embryos that succumb to infection is enlarged and exhibits uneven coloration, varying from light brown to dark green. Due to the alternation of affected and normal of parenchyma areas, the liver takes on a marbled appearance. Foci of necrosis can be found on the liver surface [6].

DIAGNOSTIC TESTS

A diagnosis of duck virus hepatitis is made using epizootological, clinical, and gross pathological data, in conjunction with laboratory findings.

During postmortem examination of dead ducklings with acute disease, key findings include hemorrhagic and necrotic hepatitis, glomerulonephritis, serous encephalitis, and myocardiodystrophy. Subacute duck hepatitis is typically characterized by catarrhal enteritis. In ducklings under 3 weeks of age, a pathognomonic sign is the presence of hemorrhages of varying shapes and intensity across the entire liver surface [6].

In laboratory testing, the virus is first isolated using sensitive biological systems. For this purpose, a 10–20% suspension is prepared from liver, spleen, and brain samples collected from 10–15 duckling carcasses. The resulting supernatant is then injected into 2- to 14-day-old ducklings, inoculated into 9- to 12-day-old duck embryos or 8- to 10-day-old chicken embryos, or added to primary cell cultures of duck or chicken embryonic kidney, liver, and fibroblasts. In dead embryos, the allantoic fluid and yolk sac contents take on a greenish tint. Hemorrhages are observed on the embryo surface, along with edema of the thoracic and abdominal cavities accompanied by hemorrhages. The liver is loose, friable and gray-yellow, with necrosis foci observed. Nephrosis-nephritis is also observed in dead embryos [15]. Duck embryos show greater susceptibility than chicken embryos, earlier embryonic mortality and more severe lesions. Upon serial passaging, severity of the lesions described above becomes more pronounced. Embryos that die on days 5–8 post-infection are often reduced in size, a sign of dwarfism [6].

For rapid diagnosis of DHV-1, indirect fluorescent antibody test (IFAT) can be used. For identification of the isolated virus, virus neutralization test (VNT) is used on ducklings, embryos, and cell cultures. For retrospective diagnosis, several authors have used indirect hemagglutination test (IHAT), indirect enzyme-linked immunosorbent assay (ELISA), agar gel diffusion precipitation test (AGDP), and counter immuno electrophoresis (CIE) [16].

In 1982, plaque reduction neutralization test (PRNT) was described for the first time. This method demonstrated far greater sensitivity than VNT in embryos.

In 1988, a microneutralization assay in duck embryo kidney cell culture was also described for serological diagnosis of DVH-1. At the time, this method was more practical, faster, and more cost-effective compared to other alternative methods, but the plaque reduction assay remained more sensitive.

These methods remain popular in laboratory diagnostics for their simplicity and accessibility. However, it should be borne in mind that they have a number of disadvantages. When evaluated based on parameters such as sensitivity, specificity, and speed of obtaining a final result, the above diagnostic methods are not optimal.

Currently, ELISA is the method of choice for detecting antibodies against DHV-1. When evaluating different methods for detecting antibodies to DHV-1 in blood serum, it was found that solid-phase ELISA is close in sensitivity to VNT, while AGDP test was far less sensitive [6].

The ELISA method developed in 1998 for detecting specific antibodies to DHV-1 in blood serum proved to be highly sensitive and specific. Due to the key role of humoral immunity in DHAV-1 infection, S. Mao et al. indirect ELISA methods were developed for detection of serum immunoglobulins: IgG, IgM and IgA. The results obtained by the authors showed that the correlation between indirect ELISA and the VNT was 95.2% for IgG and IgA, and 75% for IgM. According to the authors, the new indirect ELISA is a promising and practical method for assessing the humoral response to DHAV-1. This method can be useful in tests aimed at studying the immune response to hepatitis virus infection [23].

Currently, indirect ELISA variants have been proposed in which the VP1 and VP3 DHV-1 proteins are used as sorbing antigens to detect specific antibodies to the virus. This ELISA version is faster, simpler and more practical than the conventional one. An ELISA test system (VP1-ELISA) was developed for detecting antibodies to DHV-1. Recombinant VP1 protein, cloned and expressed in *Escherichia coli*, served as the coating antigen. This method demonstrated high specificity (92.5%) and sensitivity (96.7%) compared to VNT. Due to the specificity of the VP1 protein for recognizing antibodies to DHV-1, VP1-ELISA does not react with antiserum to other viral infections of ducks. The developers believe that the proposed VP1-ELISA is a highly sensitive and specific test suitable for screening for infection caused by DHAV-1, as well as for monitoring the level of antibodies to DHV-1 [24].

The first indirect ELISA using the recombinant VP3 protein of DHAV-1 was developed by Y. Shen et al., allowing for simultaneous detection of antibodies to both DHAV-1 and DHAV-3. This VP3-based method is characterized by high sensitivity, specificity, and reproducibility, and is as effective as the indirect ELISA based on the DHAV-1 for serological tests [25].

Advances in immunology and molecular biology resulted in emergence of innovative laboratory diagnostic methods. Polymerase chain reaction (PCR) is one of such methods, which has now become widely used for DHAV-1 detection and identification [6].

In China, DHV is associated with three types: DHAV-1, DHAV-3 and DAstV-1 [26, 27, 28]. Regardless of virus type that infected the ducklings, they show similar signs: a short incubation period, rapid disease development, high mortality, opisthotonus and enlarged liver with profuse hemorrhages. A tentative diagnosis of "duck virus hepatitis type 1" is made on the basis of clinical manifestations and post-mortem findings; nevertheless, ascertaining which virus type or combination thereof has caused the infection remains difficult. In 2008, a multiplex PCR was developed to differentiate between DHAV-1 and DHAV-3 strains [29]. In a more recent advancement, scientists developed a duplex RT-PCR that enables rapid, cost-effective laboratory detection of mixed DHAV-1 and DHAV-3 infections in ducklings [30]. However, none of the above-mentioned PCR assays allowed for the detection of DAstV-1.

Chen L. et al. were the first to develop a multiplex RT-PCR capable of simultaneously identifying DHAV-1, DHAV-3, and DAstV-1 in clinical samples. The results showed that this method is highly specific for the DHAV and does not detect genetic material from other duck pathogens. Importantly, differential diagnosis of DHAV-1, DHAV-3, and DAstV-1 can be completed within hours in a single reaction, unlike conventional methods such as cross-neutralization tests, which require several days. Thus, this multiplex PCR represents a rapid, effective, and practical method for differential diagnosis of mixed infections caused by three DHV types, as well as for epizootological monitoring of DHAV-1 [31].

The DHAV-1 molecular biological diagnosis is constantly evolving and being enriched with new techniques. Given the variability of the epizootic situation in Southeast Asia, where two serotypes of duck hepatitis virus (DHAV-1 and DHAV-3) are circulating, a duplex real-time PCR (qRT-PCR) assay was developed. This method allows for simultaneous and quantitative detection of DHAV-1 and DHAV-3 in samples [32].

A multiplex PCR assay was also developed to rapidly detect multiple duck pathogens, including DHAV-1, duck virus enteritis (DVE) virus, Muscovy duck parvovirus and reovirus, and avian influenza virus subtype H9N2. The results showed that the multiplex PCR system is effective for detecting viral nucleic acids in duck embryos infected with six common viruses, as well as in clinical samples. According to the researchers, this multiplex PCR assay offers specific, sensitive, and high-throughput detection of all six duck viruses, making it suitable for clinical identification and diagnosis of viral infections in ducks [33].

Importantly, existing molecular methods cannot distinguish the DHAV-1 vaccine strain from the field strain. Only K. P. Li et al. have developed a qRT-PCR method combined with high-resolution melting (HRM) curve analysis for rapid detection and differentiation of the vaccine strain from field strains of DHAV-1. The developed method demonstrates high specificity to DHAV-1 and is capable of detecting about 100 copies of viral RNA. Researchers have demonstrated the possibility to DHAV-1 in ducklings' faecal samples as early as 6 hours after infection. Based on this, the authors conclude that the developed qRT-PCR and HRM assay may be of potential use for DHAV-1 diagnosis and monitoring of its spread [34].

IMMUNITY

The immune response to DVH involves innate, humoral, cellular, and maternal protection mechanisms.

The innate immune response serves as the first line of defense and is initiated by the rapid recognition of viral components by pattern recognition receptors (PRRs), including toll-like receptors 7 and 3 (TLR7, TLR3), retinoic acid-inducible gene 1 (RIG-1), and melanoma differentiation-associated gene 5 (MDA5), which are typically involved in combating RNA virus infections. DHAV-infected ducklings exhibit substantial induction of type I and type II interferons, along with pro-inflammatory cytokines (IL-2, IL-6), whereas expression of selected cytokines correlates with the birds' age. Thus, innate immunity determines the outcome of early stages of infection and influences the subsequent development of the immune response [35].

The cellular response develops after antigen presentation on the surface of macrophages and dendritic cells, which subsequently activate T lymphocytes. The cellular immune response involves activation of multiple components, including specific lymphocyte receptors, major histocompatibility complex, dendritic lymphoid follicles, T and B cells transitioning from the G0 to G1 phase, as well as cytokines and lymphokines that mediate signal transduction. This coordinated process ultimately leads to antigen elimination [8]. T lymphocytes play a critical role in both eliminating infected liver cells and establishing long-term immunological memory. A robust IFN-γ response from CD8+ T cells promotes viral clearance, whereas ducklings with impaired T cell function show greater susceptibility to infection – highlighting the critical role of cellular immunity [35].

A critical component of protective immunity is production of virus-neutralizing antibodies, mainly targeting the capsid protein VP1 – the primary immunogenic element of DHAV [36]. Neutralizing antibodies appear in the serum of ducklings as early as on day 4 post-immunization, reaching peak levels on days 7–9 [8]. Nevertheless, research into the dynamics of virus-specific antibodies against hepatitis remains scarce, with most studies concentrating on development of detection techniques and on assessment of post-vaccination protection [35].

Passive protection provided to offspring via egg yolk from vaccinated or recovered ducks plays an important role. The immune system of day-old ducklings is not yet sufficiently mature and is therefore unable to fully resist infectious diseases. Acquired immunity develops gradually over the first few weeks after hatching, making the transfer of maternal antibodies crucial for protecting the offspring. Antibodies from the yolk sac enter the bloodstream of the developing embryo and the newly hatched duckling. This process underpins vaccination strategies in commercial poultry farming to control infectious diseases. Vaccinating young birds before the onset of egg laying enables the development of sufficient protective antibody levels that persist throughout their productive lifespan, thereby ensuring the transfer of maternal antibodies to their offspring [6]. Passively transferred antibodies remain detectable in ducklings for 2–3 weeks, establishing an "immunological window" that requires early vaccination. Experiments showed that 5 weeks after immunization of the parent duck flock, antibody titers reached (8.4 ± 1.3) $\log_2$ and remained at (9.0 ± 1.1) $\log_2$ through 36 weeks. The rate of antibody transfer to offspring was (12.8 ± 3.0)%, with a half-life in ducklings of approximately (3.4 ± 1.1) days [37]. Ducklings hatched from immune ducks, as well as birds older than 6 weeks of age, are typically resistant to the virus in 70–100% of cases [38].

TREATMENT AND PREVENTION

For treatment, serum and blood plasma from convalescent birds can be used, which are prepared after slaughtering adult poultry. Hyperimmune serum from horses and adult ducks was used in the past, but due to the low effectiveness of this approach, the use of such sera was discontinued [7, 39].

Nonspecific prevention requires veterinary and sanitary measures for poultry management, including isolated housing of young stock, regular disinfection of eggs, incubators, facilities, and water bodies, and limiting exposure to wild birds [7]. However, the primary preventive measure is vaccination of poultry to induce robust immunity.

The main characteristics of existing DVH vaccines are given in section *Supplementary files* at https://doi.org/10.29326/2304-196X-2026-15-2-110-122.

Live attenuated vaccines are the most commonly employed for the immunization of ducklings at the age of 1–3 days. They provide long-lasting immunity and protection from the first days of life, which is especially important given the age-related susceptibility to DHV. Virus isolates are attenuated by multiple passages in various biological systems (chicken and duck embryos, primary chicken and duck embryo fibroblast cell cultures, and others) [39].

In the Russian Federation, a live embryo vaccine against DVH from the VGNKI-K strain is produced by the Federal Center for Animal Health. Immunity develops within 48 hours after one inoculation and it persists in ducklings for at least 30 days, while in ducks it lasts for at least 6 months after two doses [40]. Vietnam produces AVAC DVH Live vaccine, which is based on attenuated DHAV-1 strain grown in chicken embryos and has an activity of no less than $10^{3.3}$ ELD_{50} per dose. It induces immunity on day 3–5 and full protection by day 7 post vaccination [41]. Internationally, several vaccines are produced against DHAV-1: a modified live vaccine (USA), Hepatovax (Merial, France), and a live vaccine based on CH60 strain (Huapai Biotechnology Group, China). In the vaccine produced in the United States, the virus was attenuated via serial passage through chicken embryos. This vaccine is administered once subcutaneously in the neck area at a dose of 0.5 mL to one-day-old ducklings that lack maternal antibodies against the hepatitis virus. However, the duration of immunity in ducklings remains undetermined. Additionally, the vaccine can be administered to laying ducks at a dose of 0.5 mL subcutaneously in the neck region at 16, 20, and 24 weeks of age, and then every 12 weeks throughout the egg-laying period. The first three immunizations are necessary to provide passive protection for ducklings, which can persist for 2–4 weeks after hatching. The live vaccine based on CH60 strain was produced following 60 serial passages in chicken embryos. A single dose (0.25 mL) of this vaccine is administered intramuscularly into the thigh muscle of ducklings aged 1 to 7 days, providing immunity lasting more than 1 month. Laying ducks can be vaccinated 1 week before the onset of egg laying with a single dose (0.25 mL), providing passive immunity to their offspring for 6 months [42].

Additionally, live vaccines against DHAV genotype 3 have been developed using AP-04203-P100 strain (Republic of Korea), which underwent 100 passages in chicken embryos, and SD70 strain (China), which underwent 70 passages in chicken embryos with the viral titer of $10^{7.5}$ EID_{50}/mL. The vaccine based on SD70 strain did not cause clinical signs in day-old ducklings and protected against DHAV-3 challenge following a single injection [43, 44].

In several countries where both DHAV genotype 1 and 3 circulate simultaneously, a bivalent live attenuated vaccine (DHAV-1 + DHAV-3) has been developed, providing protection for ducklings against both genotypes. At 1 day of age, the birds received the bivalent vaccine via intramuscular injection. Immunized ducklings were resistant to challenge with virulent DHAV-1 and DHAV-3 strains as early as 2–3 days post-vaccination. Furthermore, the ducklings exhibited a strong humoral immune response, which peaked at 3 weeks and persisted for 6 weeks post-vaccination [45].

We investigated efficacy of oral booster immunization with a live vaccine following primary intramuscular administration of an inactivated vaccine. Breeding ducks developed virus-neutralizing antibodies in high titers that persisted for 36 weeks, and none of the offspring died following virus challenge at 1, 7, or 14 days of age. These results indicate that live vaccines against DVH can be used

to generate high and sustained antibody levels in breeders and to provide reliable passive immunity in offspring [46]. The advantage of this method is that the vaccine can be mass-administered via drinking water, making immunization more practical and effective in farm settings. However, live DHAV vaccines are typically given by intramuscular or subcutaneous injection, whereas studies on the oral route of administration are still scarce.

The molecular mechanisms underlying virus attenuation have been studied through serial passaging. Genomic sequence analysis of the DHAV-1 strain after 40 passages in chicken embryos revealed a gradual accumulation of nucleotide and amino acid mutations. Most of these changes were observed in the 2C, 3D, and VP1 proteins. Furthermore, 100 serial passages of DHAV-3 strain in chicken embryos resulted in a steady accumulation of mutations across various regions, including 3D, VP2, 2C, and the 3′-untranslated region. In the attenuated DHAV-3 strain resulting from 70 passages in chicken embryos, a total of 12 amino acid substitutions and three nucleotide mutations were identified. Notably, Y180C mutation in VP1 protein and S286N mutation in 2C protein resulted in the loss of potential glycosylation sites. Comparison of VP1 genes of the field and attenuated DHAV-1 strains revealed a number of consistent amino acid substitutions that may be associated with virus attenuation. It should be noted that all of these mutations were identified by sequencing attenuated DHV strains following a series of passages, and their direct role in virus attenuation has not been confirmed. A detailed understanding of the molecular basis of DHV virulence could be a foundation for developing novel live attenuated vaccines [42, 44, 47].

Safety represents another potential issue associated with live vaccines. Given that the manufacturing of live DHAV vaccines requires serial passaging of virulent strains in chicken or duck embryos, thorough safety assessment of these vaccines is essential. Despite evidence that the live DHAV-3 vaccine did not revert to virulence following multiple back-passaging cycles in ducks, other findings suggest that the DHAV-3 strain remained virulent even after 80 serial passages in chicken embryos, and loss of pathogenicity was not observed until after 90 passages. Importantly, there are documented cases of reversion to virulence of attenuated hepatitis virus following passaging in ducks. A similar situation was observed with two DHV strains, which, despite having undergone 90 passages in chicken embryos, regained their virulence upon serial passaging in ducklings. Therefore, prolonged DHV passaging in chicken embryos may result in significant attenuation of the virus, but the possibility of reversion to virulence – particularly under field conditions – cannot be ruled out. Therefore, continuous safety monitoring of live vaccines against DHV is essential [42, 44].

The production of live vaccines against DVH, like that of many other live poultry vaccines, largely depends on the availability of specific pathogen-free (SPF) embryos. Given certain drawbacks of this production method, researchers have pursued the development of stable cell lines suitable for DHV propagation. Specifically, DHAV-1 has been shown to replicate in and induce cytopathic changes in a modified cell line derived from duck embryo fibroblasts, indicating its suitability for production of live vaccines against DVH. Additionally, a goose embryo epithelial (GEE) cell line has been established that supports high-titer replication of DHAV-1, suggesting that GEE holds potential for use in live vaccine manufacturing [48]. However, it is worth noting that virus replication in these cell lines was assessed on a small scale, under laboratory conditions. There is currently no data on large-scale vaccine production against DVH using cell cultures. It is important to note that fetal bovine serum, used in cell culture, can inhibit the replication of DHAV-1 and DHAV-3 by suppressing viral attachment to cells and virus release. Therefore, the development of a serum-free cell culture platform appears to be a promising approach for the future manufacturing of live DHV vaccines based on cell culture systems [42, 49, 50].

Live DHV vaccines offer several advantages: they are well tolerated by ducklings, induce rapid immunity, can be mass-administered via drinking water, and stimulate both humoral and cellular immune responses. Live vaccines also present a number of disadvantages, such as the prolonged process of screening attenuated vaccine strains, the risk (however low) of virulence reversion, the requirement for temperature-controlled storage, and the high cost of SPF embryos. It should be noted that, due to their rapid induction of immunity, live vaccines remain the primary choice for prevention of DVH in ducklings. Live vaccines can also be used as a booster following primary immunization of young breeding stock with inactivated vaccines [42].

Inactivated vaccines are primarily used to immunize parent flocks for the purpose of transferring maternal antibodies to progeny. Domestic producers inactivate the DHV using aminoethylethylenimine or the biocide INAK, with oil emulsions or aluminum hydroxide serving as adjuvants [51, 52, 53, 54, 55].

A number of tests indicate that live vaccines against DVH are more effective than the inactivated ones. It was shown that none of the inactivated vaccines, when used for primary immunization of 16-week-old ducks, induced antibody production at a sufficient level. Antibody titers increased only when ducks received three vaccine doses at 8, 16, and 22 weeks of age; this regimen provided protection to their offspring up to 3 weeks of age. In contrast, ducks vaccinated with a live vaccine at 2–3 days of age developed strong and persistent antibodies that lasted up to 30 weeks, and the ducklings hatched from these birds were resistant to virus challenge through 3 weeks of age. It has also been reported that the immunogenicity of an inactivated DVH vaccine in breeding ducks was low but improved when live vaccines were used for primary immunization [42]. However, another study showed that a bivalent inactivated vaccine against DHAV-1 and DHAV-3 is highly immunogenic and effective, providing immunity in ducklings that lasts for more than 5 weeks [56]. The reasons for the discrepancies between the findings of these studies are unknown. The global market currently offers at least two commercial inactivated vaccines for duck virus hepatitis: Orniduck (Bioveta, the Czech Republic)

and a bivalent vaccine targeting DHAV-1 and DHAV-3 (Yebio, China) [42].

To develop vector vaccines, various research groups have worked on expressing genes of DHV protective antigens – such as VP0 gene of DHAV-1, VP1 gene of DHAV-1 or DHAV-3, and P1 and 3C genes of DHAV-3 – in different regions of DVE virus genome. The recombinant viruses exhibited high immunogenicity against both DVH and DVE, indicating their potential for use as bivalent vaccines for ducks [57, 58, 59]. Several studies have reported that recombinant avian adeno-associated viruses expressing VP1 or VP3 genes of DHAV-1 induced protective immunity in ducks [60, 61]. Furthermore, the VP1 genes of DHAV-1 and DHAV-3 were co-expressed in a Newcastle disease virus vector, and the recombinant virus induced the production of virus-neutralizing antibodies in ducks [62]. Vector vaccines offer several advantages, including stable expression of foreign antigens, no need for antigen concentration, induction of humoral, cellular, or mucosal immunity, and high genetic stability and safety. However, producing such vaccines requires complex molecular engineering of the viral genome and specialized technologies [42].

Recombinant subunit protein vaccines represent a class of vaccines comprising protective elements derived from pathogens that are capable of inducing a host immune response. Given the drawbacks of egg-based vaccine manufacturing platforms, subunit protein vaccines offer a promising alternative to conventional vaccines against DVH.

The VP1 protein of the hepatitis virus is the primary target of neutralizing antibodies and is involved in receptor binding; therefore, this protein is commonly expressed as a protective antigen in various systems [63]. The VP1 protein of DHAV-1 was expressed in Pichia pastoris, which induced a humoral and cellular immune response in ducklings [64]. Additionally, VP1 of DHAV-3 was expressed in *E. coli* as a vaccine antigen, but it provided only 25% protection against virus challenge. The combination of VP1 with flagellin significantly enhanced the humoral, cellular, and cytokine responses in ducklings and provided 75% protection [65]. The advantages of recombinant protein subunit vaccines include the ability to produce them on a large scale in cell-based systems, low cost, and immunogenicity comparable to that of whole-virus vaccines. However, antigen purification and systematic optimization may be required to increase antigen yield across different expression systems.

Another type of vaccine against DVH is based on virus-like particles (VLPs). They resemble natural viral particles in morphology and size, but do not contain nucleic acids. Such vaccines are safe for animals, since VLPs is unable to replicate in the host body, and induce cellular and humoral immunity. Research on VLP-based vaccines against DVH is limited, but a similar strategy has been used to develop VLP vaccines for various picornaviruses. P1 gene encodes structural proteins of picornavirus, and P1 polyprotein is cleaved by the viral 3CD protease into capsid proteins that assemble to form empty viral capsids [66]. Thus, the general strategy for producing VLPs of picornaviruses such as poliovirus, enterovirus type 71, and Coxsackievirus A16 involves the co-expression of P1 and 3CD genes in insect cells or plants [67, 68, 69]. Similarly, A. Wang et al. demonstrated that DHAV-1 VLPs could be formed in insect cells infected with a recombinant baculovirus co-expressing P1 and 3CD genes, and immunization with the VLP-based vaccine elicited a strong humoral response and provided robust protection [70]. Although such vaccines represent a promising platform for development of novel DHV vaccines, experimental parameters must be optimized to maximize VLP yield, and antigen purification may lead to increased costs.

CONCLUSION

Duck virus hepatitis is a significant infection that causes substantial economic losses to duck farming operations. To prevent the occurrence of DVH on farms, vaccination of the flock is necessary.

Despite the availability of a wide range of foreign vaccines, to date the only officially registered product for DVH prevention in the Russian Federation remains the live vaccine derived from the VGNKI-K strain, manufactured by the Federal Centre for Animal Health [71]. The vaccine reduces morbidity and mortality in young birds, but it has a limited shelf life (9 months). At present, work is underway at the institution to prolong the shelf-life of the vaccine. The vaccine is expected to be manufactured as a freeze-dried product, thereby providing enhanced product stability.

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