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A Comprehensive overview of Rabbit Hemorrhagic Disease Virus in Egypt

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ABSTRACT

A significant event occurred in Egypt in 1991 when Rabbit Hemorrhagic Disease (RHD) was introduced, causing a serious threat to the rabbit industry. Because of its high morbidity and mortality, RHDV is an industry-destructive agent, causing financial losses in Egypt's production of rabbits. RHDV is a *Lagovirus*, a member of the *Caliciviridae* family, which is the cause of RHD. Three RHD outbreaks have been identified in Egypt, the first, related to the classical RHD virus (RHDV), occurred in 1991; The second type, known as the variant virus (RHDVa), occurred in 2007; and the third, known as the variant virus (RHDVb/RHDV2), was identified in 2018, and the first part of 2019. RHD can affect rabbits of all ages, both domestic and wild. There are three ways that RHD manifests, peracute, acute, and subacute or chronic forms. Rates of mortality are often extremely high, particularly during per acute and acute phases, and are linked to necrotic hepatitis. and disseminated intravascular coagulopathy. The liver, lungs, and spleen have been found to have the most significant lesions. The diagnosis of RHDV relies on clinical picture and laboratory investigations such as molecular, histological and serological techniques can be used. Despite the availability of RHDV vaccinations, outbreaks of the virus continue to occur in several Upper and Lower governorates in Egypt. Therefore, the most significant factors of prevention and control methods are the use of preventative inactivated vaccines in conjunction with the carrying out of sanitary measures. So, in this overview study, the epidemiology, clinical and laboratory diagnosis, prevention, and control measures of RHD are highlighted, with reference to the Egyptian situation from 1991 to 2024.

Introduction

Viral hemorrhagic disease (VHD), sometimes referred to as rabbit hemorrhagic disease (RHD) caused by rabbit hemorrhagic disease virus (RHDV), is an extremely transmissible, acute, and incurable viral disease occurring in rabbits (*Oryctolagus cuniculus*). It is distinguished by sudden death, disseminated intravascular coagulation, liver necrosis, and 70 to 100 percentage mortality rates (Abrantes *et al*., 2012). Capucci *et al*. (2017) recognized that RHDV is an instance of viral hepatitis affecting cottontail rabbits, European rabbits, and hares. Previously, RHDV was referred to as Hemorrhagic septicemia syndrome, viral hemorrhagic pneumonia, rabbit plague, rabbit viral hemorrhagic disease, and rabbit viral sudden death (Mitro and Krauss, 1993; Vinjé *et al*., 2019).

Historical outlook

World outbreaks

In China in the 1980s, RHDV was first documented to have killed 14 million Angora rabbits in nine months in Europe (Liu *et al*., 1984; Xu, 1991). Within a few years, the disease became endemic after rapidly spreading over Asia and Europe (Le Gall-Recule *et al*., 2003; Alda *et al*., 2010; Abrantes *et al*., 2012). The first reports of a new RHDV variant (RHDVa) were published in Germany and Italy in 1998 and 1999 (Schirrmeier *et al*., 1999; Capucci *et al*., 1998). Outbreaks have been documented in Egypt, New Zealand, Australia, America, Asia, and North Africa (Liu *et al*., 1984; Gregg *et al*., 1991; McIntosh *et al*., 2007). In 2012, a novel variation was reported in Portugal (Abrantes *et al*., 2013). Curiously, prior to then, Portugal was known to have only one circulating genogroup, G1 (Müller *et al*., 2009; Abrantes *et al*., 2012). A novel strain, RHDV2, was discovered in France in 2010 and related to infection in rabbits of various ages and populations (Le Gall-Recule *et al*., 2011). In Europe, RHDV2 is currently regarded as endemic (Mahar *et al*., 2018). In France, Spain, Portugal, Australia, and other countries, GI.2 has replaced GI.1 as the current pandemic strain, according to molecular epidemiological investigations (Dalton *et al*., 2014; Lopes *et al*., 2015).

Outbreaks in Egypt

In the spring of 1991, the virus was discovered to be responsible for 90% of the deaths in the El-Sharkia governorate of Egypt (Ghanem and Ismail, 1992). The virus was then found in the governorate of Qalubia (Sharawi, 1992). In the winter of 1992, RHDV was endemic in the Upper Egypt (Assiut) governorate (Salem and El-Ballal, 1992). RHDV was identified from 14–16-week-old rabbits in the governorates of El-Minya, Assiut, and Sohag in 1993, with 26.7%–100% losses (El-Zanaty, 1994). Since then, more illness outbreaks have been discovered in various governorates around Egypt. Between 1994 and 1996, about 25 outbreaks of circulating RHDV were reported in the governorates of Marsa-Matroh, El-Kalubia,-Cairo, Kafr-El-Sheikh, Giza, El-Gharbia and El-Dakahlia (El-Mongy, 1998). In 2000, rabbit flocks in the Assuit governorate with RHDV showed clinical signs of epistaxis, gait incoordination, convulsions, and vaginal bloody exudate (Abd El-Ghafar *et al*., 2000). Numerous outbreaks of the disease were documented. Nonetheless, there is a presence of vaccination schedule for Egyptian rabbit flocks against RHDV (Mostafa, 2001; Abd El-Lateff, 2006; Ewees, 2007; El-Sissi and Gafer, 2008). El-Sissi and Gafer (2008) and Ewees (2007) have reported that RHDVa variant strains have been registered in Egypt since 2007. Up till now, various outbreaks have been documented in Upper Egypt's Assuit and New Valley due to these variant strains (Abodalal *et al*., 2021). In 2018 and early 2019, the novel *Lagovirus*

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RHDV2 was isolated in a few Egyptian governorates with notable death rates, particularly in suckling rabbits (Abodalal and Tahoon, 2020; Erfan and Shalaby, 2020; Hemida *et al*., 2020). According to current isolates' genotyping through sequencing and phylogenetic analysis of the VP60 gene, Egyptian isolates were shown to cluster with RHDV2 genotype I.2 (GI.2) strains (Awad and Kotb, 2018; Magouzi *et al*., 2019; Erfan and Shalaby, 2020). As shown in (Figure 1).

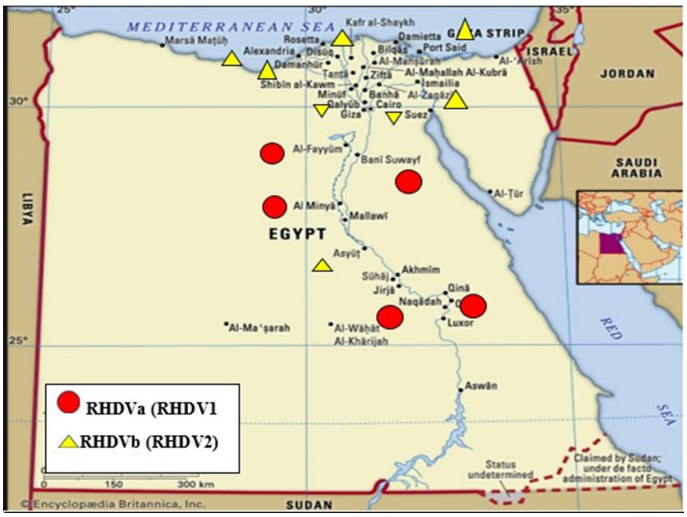

Fig.1 Geographical distribution of RHDV in Egypt.

Economic significance

Additionally, to their significance in nutrition and industry, rabbits are essential experimental animals used for research and diagnostics in biological and medical institutions (Abrantes *et al*., 2012). The rabbit business plays a significant role in the production of fur and meat. In order to prevent bacterial, viral, and parasite illnesses, it must concentrate on prevention strategies (Dalton *et al*., 2015; Carvalho *et al*., 2017; Isomursu *et al*., 2018). In the world, Egypt is the fourth-largest producer of rabbit meat. Because of its many health benefits and carcass qualities, Egyptians enjoy rabbit meat (Farnos *et al*., 2006; Alboghdady and Alashry, 2010). Like all other animals, rabbits are susceptible to a variety of infections (mycotic, viral, bacterial, parasitic) as well as non-infections (metabolic, genetic, toxins, and stress) that can lead to several disease problems and financial loss (Ghanem and Ismail, 1992; Eid and Ibraheem, 2006).

Etiology

Taxonomic classification and antigenic variations of RHDV

Prior to the initial RHDV outbreak in the early 1980s, the European brown hare syndrome virus (EBHSV), which infects hare species (*Lepus europaeus* and Lepus timidus), was discovered in Sweden. EBHSV is a member of the *Lagovirus* genus and family *Caliciviridae*, including the rabbit hemorrhagic disease virus (RHDV) (Gavier-Widen and Morner, 1993; Van Regenmortel *et al*., 2000; OIE, 2010; Abrantes, 2012). There are weak pathogenic and non-pathogenic virus strains that differ genetically from the pathogenic RHDV by around 20% (Capucci *et al*., 1996; Le Gall-Reculé *et al*., 2011). The VP60 gene's phylogenetic analysis indicates that all classical pathogenic strains of RHDV have a single serotype, which is further divided into three subtypes, the "classic RHDV," which includes the genogroups G1–G5 isolated from 1984 onward; the antigenic variant RHDVa/G6 discovered in 1996; nucleotide sequence similarities between these subtypes show a maximum difference of 14%; and the new variant "RHDVb" or "RHDV2," which was first documented in France in 2010 (Carvalho *et al*., 2017; Le Gall-Recule *et al*., 2013; OIE, 2018). RHDV1 and RHDV2 can have an average nucleotide identity of 82.4% and an amino acid similarity of roughly 89.2% (Kong *et al*., 2016). In recent times, they have been divided into two genotypes, GI.1a (G6/RHDVa), which includes other variants, GI.1b (G1), GI.1c (G2), and GI.1d (G3–G5), and GI.2 (Le Pendu *et al*., 2017). Variations in antigenicity, genetics, and pathogenicity influence the adaptability and dissemination of the virus (McIntosh *et al*., 2007; Wang *et al*., 2012). According to Droillard *et al*. (2021), the significant difference (>15%) between GI.1 and GI.2 may be the cause of the vaccines' insufficient or poor protection against GI.1 in GI.2 outbreaks. The RHDV strains identified in Qalubia governorate during 2019 are linked to the Egyptian RHDV2 strain based on phylogenetic analysis of the VP60 gene (Abido *et al*., 2020). El-Samadony *et al*. (2021) reported that four RHDV isolates out of eight RT-PCR positives were connected to the classic G3 (GI.1d/RHDV) phylogenetically based analysis and sequencing. Additionally, the sequences of the RHDV isolates and the vaccine strain (Giza-2006) differed by twelve amino acids. Abodalal *et al*. (2021) recorded that the selected 4 isolates were clustered as RHDV-1 variant strains (G3-G5) in Upper Egypt. Through the sequencing of an amplified fragment of the virus VP60 gene, Erfan and Shalaby (2020) and Desouky *et al*. (2023) distinguished two distinct genotypes, one that is primarily circulating in upper Egypt and is closely related to the classical G3-G5 virus strains, and another is circulating primarily in lower Egypt and is more closely related to the RHDV2 variant. Four isolates were identified as classical RHDV strains, and one isolate was classified as a RHDVa variant strain that shared the same HA pattern (Magouzi *et al*., 2019). Six isolates were chosen for phylogenetic and genetic sequence analysis, which showed that they clustered with RHDV2 strains that were firstly recorded in Upper Egypt. Nucleotide differences between the newly isolated strains and the commonly vaccinated strain were 23.1% (Giza 2006) (Ahmed *et al*., 2024a).

Morphology and genomic structure of RHDV

RHDV is a naked, icosahedral, spherical, single-stranded, positive-sense RNA virus with a diameter of 32–44 nm. The organism's genome is 7,442 bp and is arranged into two closely overlapping open reading frames (ORFs) (OIE, 2012; Lopes *et al*., 2015; Ismail *et al*., 2017). ORF1 contains the primary RHDV antigenic determinants, which are found on the VP60's C-terminal end (Fitzner, 1994; Gall and Schirrmeier, 2006). Both RHDV and RHDV2 have two open reading frames (ORFs) and similar genomic sequences. ORF1 is a large polyprotein with 10–7044 nucleotide residues that is cleaved by a virus-encoded protease to produce the major capsid protein (VP60) at its Cterminus and several non-structural (NS) proteins, such as a helicase, a protease, a polymerase, and the RNA-dependent RNA polymerase. (Meyers *et al*., 2000; Le Gall-Reculé *et al*., 2013; Dalton *et al*., 2015). ORF2 generates VP10, a small structural protein, and is made up of nucleotide residues 7025–7378 (Du, 1991; Wirblich *et al*., 1996). The host immune response target against RHDV is demonstrated by the antigenic epitope found in VP60 (Capucci *et al*., 1998). As a result, molecular analyses of both partial and whole VP60 gene sequences were conducted in order to identify genetic differences among RHDV strains (El Bagoury *et al*., 2014; Wang *et al*., 2012) and include the hypervariable region E, which is responsible for antigenic variations (Wang *et al*., 2013). According to certain research, VP10 may enhance viral multiplication and encourage cell apoptosis (Liu *et al*., 1984). The RHDV capsid is made up of an exposed shell (S) domain that is buried and includes the N-terminal, which is hinged to the protruding (P) domain that encloses the C-terminal region. There are two sub-domains inside the P domain, P1 (the stem of the arch) and P2 (the top of the arch) (Neill, 1992). VP60 is the primary immunogenic protein and viral structure of RHDV (Mikschofsky *et al*., 2009). VP60 has six different sections (A to F); the most genetically varied regions are C and E, which are found in the exposed P2 subdomain in the most exposed region of the capsid (Neill, 1992; Capucci *et al*., 1998; Schirrmeier *et al*., 1999). According to phylogenetic studies of the VP60 nucleotide sequence and amino acid alignment, RHDVa has a stronger antigenetic relationship with classic RHDV, but RHDVb is more strongly associated with rabbit calicivirus (RCV) than with classic RHDV isolates

(Qi *et al*., 2019). The three subtypes of RHDV (classic RHDV, RHDVa, and RHDVb) differ in VP60, which can lead to changes in immunology, epidemiology, and antigenicity. Three domains make up VP60 (nt 1-1740 coded for 579 aa), the protrusion (P, aa 238–579), which is further subdivided into two subdomains (P1 (aa 238–286) and P2 (aa 287–449 and 467–483)), the shell (S, aa 66–229), a brief hinge (aa 230–237), and the Nterminal arm (NTA, aa 1–65) (Li *et al*., 2017). According to phosphorylation site analysis. Classic RHDV, RHDVa, and RHDVb all contained 19, 21, and 13 phosphorylation sites in their VP60, some phosphorylation sites in RHDVa and RHDVb were different from those in classic RHDV, which could potentially explain why classic RHDV, RHDVa, and RHDVb differ in virulence (Qi *et al*., 2019).

RHDV resistance to chemical and physical agents

RHDV is resistant to ether and chloroform Because it lacks the fatty envelope (Abd El-Ghany, 2020). According to Liu *et al*. (1984), the agglutination titer of RHDV could be significantly decreased by repeatedly freezing and thawing, and 0.4% formaldehyde eradicated the virus's infectivity while conserving its immunogenicity. In contrast, Smid *et al*. (1991) found that RHDV lost its ability to infect when treatment was extended to three days at room temperature. RHDV was completely inactivated by 0.2–0.4%. formalin, 4% biethyleneamine, and 0.05% beta-propiolactone, which were effective after 48, 24, and 5 hours of incubation, respectively (Fitzner *et al*., 1993). Formalin (1%–2%) or sodium hydroxide (1%) or 0.2–0.5% beta-propiolactone was used to inactivate RHDV but does not reduce its immunogenicity; therefore, these inactivators were recommended for the development of RHDV vaccines. The OIE, Terrestrial Animal Health Code recommends using 3 % formalin for disinfection (OIE, 2018). Smid *et al*. (1991) reported that RHDV could survive for a minimum of 225 days in an organ suspension kept at 4 °C, 105 days in its dried state on fabric at room temperature (about 20°C), and at least two days at 60 °C. RHDV can withstand 50 °C of heat for an hour. According to OIE (2018), it lives at pH 3.0 and is stable at pH 4.5–10.5, however it becomes inactive at pH >12 The most effective disinfectants for RHDV are 0.5% sodium hypochlorite and substituted phenolics (e.g., 2% Onestroke Environ®). Trypsin, ether, and chloroform do not lessen viral infectivity (Eleraky *et al*., 2002). RHDV was inactivated by UV light using an electronic UV crosslinker. that had a UV intensity of 0.0078 W. When rabbits were injected with this inactivated virus, neither antibodies nor clinical symptoms appeared (Henning *et al*., 2005a). RHDV in animal tissues, such as rabbit carcasses, can survive for at least three months in the field and may establish a persistent virus reservoir that could lead to new disease outbreaks. In addition, viruses that have been exposed directly to environmental conditions, such as dried excreted viruses, are alive for less than a month (Henning *et al*., 2005b).

Biochemical properties of RHDV

RHDV2 effectively agglutinates human-type (O) red blood cells, according to Le Gall-Recule *et al*. (2013), who also confirmed the application of HA as a common diagnostic method for identifying RHDV2 in contaminated samples. Burmakina *et al*. (2016) identified that all classic and variant RHDV field isolates are hemagglutinating viruses; the RHDV variant was able to hemagglutinate the human-type (O) RBCs, indicating that the genetic factor influencing the HA activity among RHDV strains is still unknown. On the other hand, El-Sissi and Gafer (2008) found that variant strains of RHDVa with negative HA activity were responsible for several RHD outbreaks in numerous Egyptian regions, indicating that the non-Hemagglutinating RHDV strains represent antigenic variations of the virus. Ruvoën-Clouet *et al*. (1995) clarified that the inclusion of histo-blood group antigens (ABH antigens) is necessary for the RHD virus's unique ability to agglutinate human O-type erythrocytes. The circulation of non-hemagglutinating RHDV was documented by Abd El-Moaty *et al*. (2014) and Bazid *et al*. (2015) in various parts of Egypt, including Munofia. Numerous previous investigations have documented the existence of RHDV isolates that do not hemagglutinate (Salman, 1999; El-Sissi and Gafer, 2008; Salman, *et al*., 2010).

Epidemiology

Host and age susceptibility

In addition to rabbits, caliciviruses are found in humans, dogs, pigs, chimps, and sea mammals (whales, seals, sea lions, dolphins, and walruses) mustelids (skunks and minks), cattle, reptiles, and felids (cheetahs and cats) (Van Regenmortel *et al*., 2000). In fact, the only hosts for the EBHSV and RHDV *Lagoviruses* are rabbits and hares, respectively. It has been demonstrated that RHDV does not affect other leporid species (Gregg *et al*., 1991). Both wild and domestic members of the *Oryctolagus cuniculus* (European rabbit) species are susceptible to rabbit hemorrhagic disease (Müller *et al*., 2009; Miao *et al*., 2019). According to Mahar *et al*. (2018), RHDV2 is the strain that is most prevalent in both wild and domestic rabbits. Hare species like the European brown hare (*Lepus europaeus*) and other hare species (*L. corsicanus, L. capensis,* and *L. timidus*) are not affected by RHDV/RHDV classical strains. But in other hare species, such as the mountain hare (*L. timidus*), the European brown hare (*L. europaeus*), and the Italian hare (*L. corsicanus*), It was found that the currently identified RHDV2 can infect and cause a condition similar to RHDV (OIE, 2018). First, the age at which rabbits are susceptible to RHDV is contradicted. Adult rabbits may be more susceptible to the virus than young ones because deaths in rabbits younger than four weeks are uncommon. This might have to do with the fact that certain receptors are present in adults but absent in juvenile animals. Additionally, only adults are killed by GI.1 RHDV. while sub-adult rabbits and kits as young as 11 days are also killed by GI.2 (Dalton *et al*., 2012). Seasons and breeds are equally susceptible; female rabbits, especially those who are pregnant or nursing, lactating does are more susceptible to disease (El-Sissi and Gafer, 2008).

Transmission and source of infection

Because certain virus receptors are mostly located in the upper digestive and respiratory tracts of susceptible animals, transmission can happen orally, nasally, or conjunctivally (Ruvoën-Clouet *et al*., 2000; Lavazza and Capucci, 2018). Because the RHDV is highly stable and resistant to environmental factors, it can spread through contaminated food, water, fomites, cages, equipment and clothing (Chasey, 1997). The virus can survive in flies for nine days, making them extremely efficient mechanical vectors for transmission (OIE, 2008). Animals in the wild can mechanically spread the virus. Predators and scavengers (such dogs and foxes) may not seem to replicate the virus, but they can still expel RHDV in their feces after eating infected rabbits (CFSPH, 2006). Direct oral, nasal, or conjunctival contact with infected animals can result in RHDV infection (Campagnolo *et al*., 2003). In addition, exposure to an infected animal's fur or carcass, as well as through contaminated food, bedding, and water are important factors in the disease's spread. Importing contaminated rabbit flesh may be one of the primary ways of spreading RHD to a new area. Meat has a lot of virus-infected blood that keeps well at freezing temperatures. RHDV may also be transmitted through contaminated bedding containing urine and feces from infected animals (Cooke and Fenner, 2002). Additionally, under extreme environmental conditions, RHDV can survive and spread for 12 weeks on dead or decomposed carcasses (McColl *et al*., 2002).

Pathogenesis

However, the pathogenesis of naturally occurring RHDV infections is poorly understood. A better knowledge of the pathogenesis of RHDV would result from identifying the cellular receptor or receptors that the

virus uses to induce infection (Ruvoën-Clouet*et al*., 2000). Following viral entrance, RHDV binds to HBGA receptors in the liver, the primary target organ, the digestive tract, and the upper respiratory tract (Nyström *et al*., 2011). Virus antigen was detected in the liver of animals older than nine weeks, primarily in periportal regions, between 12 and 24 hours post-infection. Apoptotic symptoms start throughout the next 24 hours when antigen levels skyrocket (Prieto *et al*., 2000). Several systemic organs have petechial hemorrhages as part of the pathogenesis of RHD due to virus-induced hypercoagulopathy; the liver, trachea, and lungs exhibit extremely severe type of these lesions (OIE, 2010). Moreover, RHDV causes fatal hepatitis, particularly in adult rabbits (Le Gall-Recule *et al*., 2011). The targets of virus's early life cycle have been identified in adult rabbits. In fact, the liver contains viral antigens within the first few hours after RHDV infection, and the cytoplasm of hepatocytes, which are primarily found in centriacinar regions, is where viral replication takes place (Moussa *et al*., 1992; Jung *et al*., 2000; Kimura *et al*., 2001). Throughout the course of the disease, the number of infected hepatocytes increases visibly, peaking between 36 and 48 hours later (Gelmetti *et al*., 1998; Jung *et al*., 2000). The initial spread of the virus may be aided by the presence of replicating viruses in alveolar macrophages, which come into contact with the bloodstream. Once the virus has infected the liver, Kupfer cells might play a crucial role in the infection's spread to other organs (Kimura *et al*., 2001). The spleen contains viral antigens, specifically in the macrophages found in the red pulp (Prieto *et al*., 2000; Kimura *et al*.,2001). The lungs' alveolar macrophages and kidney (Prieto *et al*., 2000).

Diagnosis

The clinical picture, molecular characterization of the virus, and antibody measurement utilizing the Enzyme-Linked Immuno-Sorbent Assay (ELISA) or the hemagglutination (HI) inhibition test are the main methods used to diagnose RHD (Lavazza and Capucci, 1996).

Field diagnosis

Incubation period

Deaths occur two to three days after infection, as the incubation period of RHDV ranges from 16 to 48 hours. The disease could last for as long as 30 days. The severity of clinical symptoms varies depending on the animal's breed, age, immunity, location, the amount of the infectious virus, and the route of infection (Abd El-Ghany, 2020).

Morbidity and mortality

El-Zanaty (1994) reported that RHDV was discovered in rabbits in Sharkia Province in the spring of 1991 and was linked to 90% losses, with mortality rates among rabbits aged 14–16 weeks ranged from 26.7% to 100%. Adult rabbits with the disease have significant mortality rates (70–100%), but young kits are sub clinically affected. Although the cause of this variation in illness susceptibility is unknown, it might be brought on by certain receptors that develop when young rabbits mature into adults (Dalton *et al*., 2012). Within two to three days of infection, RHDV usually kills over 90% of susceptible adult animals (Abrantes *et al*., 2012). The mortality rate of RHDV2 infection varies from 5 to 70%, with experimentally infected rabbits having an average mortality of 20%; adult and nursing rabbits can die as early as fifteen days of age (Le Gall-Recule *et al*., 2011). On the other hand, new studies have shown that RHDV2 pathogenicity has significantly increased in recent years (Capucci *et al*., 2017).

Clinical signs

The disease's clinical progression might be classified as acute, subacute, chronic, or per acute (Marcato *et al*., 1991). RHVD-affected animals exhibit a fever for 12 to 36 hours followed by a sudden death in the peracute stage. Acute RHD animals live longer but exhibit general illnesses including sadness, a lack of appetite, ocular mucous membrane redness, and in some cases, pulmonary malfunction (such as cyanosis, blood-stained foamy rhinal discharge, and dyspnea) as well as ocular or rhinal hemorrhages. Other neurological conditions that some rabbits suffer include restlessness, paddling, and backward head and neck arching (CF-SPH, 2021). The subacute form has little symptoms, and nearly all rabbits survive and develop antibodies that guard against RHDV reinfection. The severe and widespread jaundice, lethargy, and off-food, abdominal distension, diarrhea or constipation, and Death follows in a few of weeks are the hallmarks of the chronic form. Rabbits who survive the sickness exhibit a strong seroconversion. but those that die after 1-2 weeks (Abrantes *et al*., 2012). Young kittens under 4–8 weeks old may have a subclinical RHD infection (Belz, 2004). Trzeciak-Ryczek *et al*. (2015) and Ahmed *et al*. (2024a) observed that neurological symptoms, including ataxia, convulsions, opisthotonos, and paralysis, as well as vulvar hemorrhages in pregnant does.

Postmortem lesions

The cause of death is extensive malfunction of the circulation linked to necrotizing hepatitis lesions and disseminated intravascular coagulation (Marcato *et al*., 1991; Plassiart *et al*., 1992). Necrotizing hepatitis, splenomegaly, pneumonia, frothy exudates in the trachea, liver congestion, hemothorax, subcutaneous abscesses, nasal mucosal congestion and ulceration, hemorrhages, multiple lung abscesses, and a congested brain are among the pathological alterations that occur after death (Embury-Hyatt *et al*., 2012). Dead infected rabbits exhibit congestion in the trachea and nasal cavities, as well as hemorrhages and swelling of the spleen, kidney, liver, and other organs (Abrantes *et al*., 2012; McIntosh *et al*., 2007; Ahmed *et al*., 2024a). GI.2 infection causes significant hemorrhages by causing diffuse intravascular coagulation and liver necrosis (Neimanis *et al*., 2018). According to Hamed *et al*. (2013), animals who died during the subacute phase had icterus and catarrhal enteritis. Additionally, the primary reasons of increased mortality are seriously infected organs such the kidneys, liver, lungs, and spleen during RHD outbreaks. As shown in Figure 2.

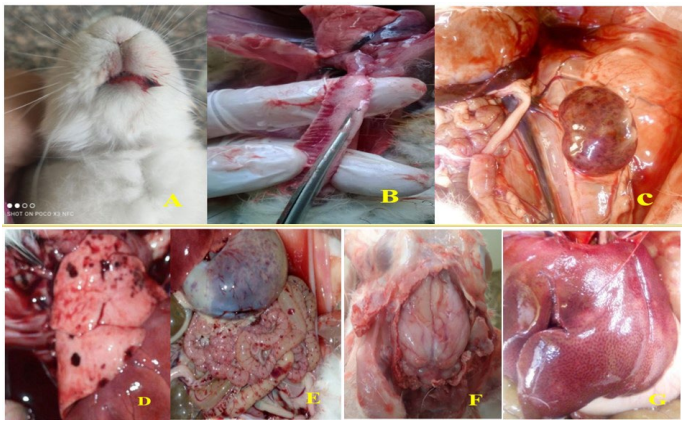

Fig. 2. Rabbits infected with RHDV showing: A. Blood expectoration from mouth B. Frothy blood present in trachea. C. Enlarged Kidney with severe hemorrhage. D. Congestion of lung with petechial and ecchymotic hemorrhages. E. Hemorrhages, and hyperemic intestine and stomach with petechiae. F. Congestion, and hemorrhages of brain. G. enlarged liver with pale reticular pattern of necrosis.

Laboratory diagnosis

Sampling

The liver is thought to be the best organ for RHDV detection since it has the highest viral content, particularly in cases of acute or chronic disease (Abd El-Motelib, 1993; Ahmad *et al*., 2011; Ahmed *et al*., 2024a). Additionally, the spleen may be a preferred sample, particularly in cases

of chronic or subacute disease (Lavazza and Capucci, 1996). However, RHDV can also be found in biological fluids like urine, feces and serum (Neimanis *et al*., 2018), as well as other organs such the bone marrow lung, and kidney (Moss *et al*., 2002; Abrantes *et al*., 2012).

Biological and serological tests

The HA test, which uses human type "O" erythrocytes (OIE, 2008) and may agglutinate sheep and Guinea pig erythrocytes, is the first step in the laboratory diagnosis of RHDV (Sahar *et al*., 2011). RHDV's capacity to effectively agglutinate type 0 human red blood cells (RBCs) was first documented in 1984 and the HA depends on the binding to glycolipid ligands on the surface of the red blood cells (Liu *et al*., 1984). According to Abd El-Moaty *et al*. (2014), relying just on hemagglutination for RHDV diagnosis is not a reliable test because some isolates may not be hemagglutinating, while others may exhibit hemagglutination following passaging in susceptible rabbits. Additionally, it was shown that the RHDV agglutination activity of human red blood cells of blood types O, A, B, and AB is similar. As a result, ABH blood group antigens were discovered to be attachment factors that RHDV uses to start infections (Ruvoën-Clouet *et al*., 2000). The reduction or lack of RHDV hemagglutination activity in RBCs from other species can be explained by the presence of ABH antigens in adult human RBCs but their absence in RBCs from other mammalian species (Xu, 1991). Since 2007, RHDV variant strains have been circulating in Egyptian rabbit flocks. These strains are non-Hemagglutinating but exhibit the same symptoms, lesions, and deaths rates similar to classical RHDV strains, suggesting that the HA test's sensitivity and specificity are insufficient (Ewees, 2007; El-Sissi and Gafer, 2008). RHD diagnosis may not be reliant on RHDV HA features Since certain variant strains displayed shifting HA, meaning that when they were passed via susceptible rabbits, negative HA strains turned into positive ones (Abd El-Moaty *et al*., 2014). In contrast, Magouzi *et al*. (2019) found that both the classical strains of RHDV and its variant strains (RHDVa) could agglutinate human red blood cells of type O. The analysis based on the hemagglutination assay (HA) test against various mammalian (sheep and human O-type) and avian (pigeon and chicken) erythrocytes, 33% of the samples under examination had viral titers of 5 log2 to 8 log2 hemagglutination of human O-type erythrocytes, whereas sheep, chicken, and pigeon erythrocytes had virus titers of 28%, 11%, and 28%, respectively (El-Samadony *et al*., 2021). C-ELISA, Indirect ELISA (I-ELISA), and hemagglutination inhibition (HI) are the three basic methods used for the serological diagnosis of RHDV (Liu *et al*., 1984; Capucci *et al*., 1991). Every one of these techniques has advantages and disadvantages. The most practical method is HI, which is followed by I-ELISA and C-ELISA, respectively. However, Both ELISAs, are quicker and simpler to use than HI, particularly when assessing an enormous number of samples. Compared to the other two methods, C-ELISA's specificity is noticeably higher (Capucci *et al*., 1991). For better serological interpretation and accurate diagnosis of rabbit immunological state, a combination of ELISA techniques that detect IgG, IgM, and IgA antibody responses is also available (Cooke and Fenner, 2000).

Molecular assay

Reverse transcriptase (RT-PCR) is regarded as an accurate and quickly diagnostic test for RHD and is more sensitive than other serological assays (Soliman *et al*., 2016). RT-PCR is 104 times more accurate than HA and ELISA and can identify RHDV strains that are HA negative (Yang *et al*., 2008). The reverse-transcription PCR (RT-PCR) assay employed as a common screening method for hare caliciviruses, and rabbit has chosen the viral VP60 gene as its target (Le Gall-Reculé *et al*., 2017). Thus, the identification of genetic variations across RHDV strains is thus dependent upon molecular analyses, which were performed on both partial and entire VP60 gene sequences (Tian *et al*., 2007; Wang *et al*., 2012; El Bagoury *et al*., 2014). Lavazza and Capucci (2018) suggested the use of a single-step RT-PCR using a set of primers built to amplify every RHDV GI.1 variations as well as GI.2. For partial amplification (538 bp) of the VP60 region c-terminal region, specific oligonucleotide primers (Metabion, Germany) were developed, P33, CCACCACCAACACTTCAGGT and P34, CAGGTTGAACACGAGTGTGC (Fahmy *et al*., 2010). Members of the calicivirus family were studied by Neill (1992). An amino acid sequence alignment investigation of the RHDV capsid protein revealed six unique areas (designated as regions A–F) inside the capsid precursors. The N-terminal section of the capsid protein of three isolates from geographically and chronologically distinct RHD outbreaks was sequenced by Guittre *et al*. (1996) using the RT-PCR assay. 96.6% to 98.7% homology was found in the results. showed that this section of the RHDV capsid protein was extremely conserved. By sequencing a section of VP60 that corresponded to the amino acid sequence from 236 to 509. Capucci *et al*. (1998) discovered the first consistent genetic and antigenic variant of RHDV. Over ninety percent of amino acid substitutions are in the E region (75%) and C region (15%). The local isolate used to prepare the RHDV inactivated vaccine (Giza 2006) was described by Magouzi *et al*. (2019). After cloning and sequencing the entire length of VP60 (1750 bp), multiple sequence alignment showed that the strains from the USA and the Egyptian isolate Giza (2006) shared more than 95% of their nucleotide homology.

Virus propagation

Since the virus cannot be grown in cell cultures, susceptible rabbits were inoculated in order to identify and characterize the virus (OIE, 2012; Ismail *et al*., 2017). Since 1984, there have been several attempts to cultivate RHDV in different cell culture systems. RHDV has been cultivated in primary rabbit cells (lung, liver, and testis kidney) and cell lines (VERO, BHK-21, HeLa, MA-104, IBRS-2, and PK-15) (Du, 1991; Chen, 1988; XIA, 1988). However, none of these attempts have been successful in adapting RHDV to cell cultures. The pathogenicity of the isolated strains of RHDV can be experimentally detected by inoculating susceptible animals with the virus (Abd El-Ghany, 2020). Rabbit inoculation is the only method of isolating, propagating, and titrating the infectivity of the virus because, there is no effective in-vitro replication system for the RHDV, and cell culture isolation cannot be included in the diagnostic techniques (Metwally and Madboully, 2005; Embury-Hyatt *et al*., 2012). The pathogenicity of isolated strains can be tested by inoculating susceptible animals with RHDV (Guittre *et al*., 1996; Soliman *et al*., 2016). Rabbit must be older than two months and free of RHDV antibodies. A liver suspension that has been filtered and treated with antibiotics and administered intramuscularly, intravenously, or via the nose can be used to replicate RHD (OIE, 2014). RHDV pathogenicity demonstrated that the infected rabbits exhibited the same clinical signs of a natural RHD infection, with a 90% mortality rate and death occurring 3–6 days post infection (Ismail *et al*., 2017; Abodalal *et al*., 2021; Ahmed *et al*., 2024a).

Histopathological examination

Pathological examination is crucial to the detection of RHDV (Hamed *et al*., 2013). microscopic lesions are Pathognomic RHDV. If tissues are sufficiently fresh for analysis, histopathological examination alone can diagnose RHD; nevertheless, further testing is essential to confirm the presence of the virus (Fuchs and Weissenbock, 1992). It is believed that viremia with widespread circulatory dysfunction is the cause of the pathological alterations (Xu *et al*., 1985). Poor blood coagulation causes petechial hemorrhage and widespread congestion in nearly every organ (Xu and Chen, 1988). Disseminated intravascular coagulopathy or liver failure are the causes of death (Marcato *et al*., 1991). McIntosh *et al*. (2007) and Abrantes *et al*. (2012) indicated that dead rabbits reveal hemorrhages and swelling of the kidney, spleen, liver, and other organs, in addition to nasal and tracheal congestion. Necrosis of the liver and the GI.2 infection is the cause of extensive intravascular coagulation, which results in

substantial hemorrhage (Neimanis *et al*., 2018). According to Wanting *et al*. (2022) and Ahmed *et al*. (2024a), the liver had the most notable histological abnormalities, which included vacuolar alterations, disassociation of the hepatic cords, acute periportal to midzonal hepatic degeneration and necrosis, and hypereosinophilia. The white pulp had necrotic splenic lymphocytes, while the red pulp was bleeding and congested. There were some alveolar lesions. While some alveolar gaps were fully filled, others were constricted and showed slight hemorrhages. No noticeable anomalies were found in the kidney or heart. Lung abnormalities ranged from interstitial pneumonia to simple congestion and alveolar edema, which suggested an acute evolution. According to several studies (Marcato *et al*., 1991; Maddison and Mesquite, 2009; Duarte *et al*., 2015; Lopes *et al*., 2015; Ahmed *et al*., 2024a), rabbits infected with RHDV and RDHV2 frequently have severe necrotic liver lesions. The most significant microscopic lesions were found in the brain, including lymphoplasmacytic meningoencephalitis in the brain and interstitial lymphoplasmacytic nephritis in the kidney (Carissa *et al*., 2012; Ahmed *et al*., 2024a).

Prevention

Biosecurity

RHDV is highly contagious and can transmit to birds, fomites, insects, and scavenger animals. Therefore, eradication can be achieved through disinfection, surveillance, depopulation, and quarantines. However, eradication is not possible in areas where RHDV is present in wild rabbits, and biosecurity measures such as vaccination, closed colony maintenance, and sanitation and disinfection are used for controlling the disease in domestic rabbits (OIE, 2019). Importing live rabbits or rabbit products from regions where RHDV is endemic is still a possible way for the virus to spread due to its abrupt onset and quick progression. However, there are options for managing this disease, including vaccines, eradication, and prevention, or a combination of these. It appears that both domestic and free-ranging rabbits still need to undergo virological or serologic testing to confirm the presence of disease (Ahmad *et al*., 2011). According to OIE (2018), RHD is notifiable, meaning that when an outbreak occurs, authorities must notify it immediately. In order to control RHD outbreaks, the field viral monitoring procedure and the epidemiological status of the disease in the area to identify any novel genetic and antigenic variations are of critical importance (Abrantes *et al*., 2012).

Cleaning and disinfection of premises

Strategies for RHDV prevention and control must include thorough cleaning and disinfection. It is necessary to use disinfectants such 0.2-0.5 % beta-propiolactone, 1% sodium hypochlorite, 1-2% formalin, 1.0-1.4 % formaldehyde, 10% sodium hydroxide or 10 ppm chlorine dioxide for inactivation viruses (Eleraky *et al*., 2002). Consideration should also be given to all mechanical causes of RHDV infection and for the control of RHD, fly and field rats' eradication should be applied (Abd El-Ghany, 2020).

Vaccination

Around the world, RHDV vaccines have been manufactured from the tissues of diseased animals, following being chemically inactivated (Smid *et al*., 1991). It was demonstrated that the RHDV vaccination obtained from cell culture (Vero cells) is more effective than the vaccine derived from liver tissue suspension (Khodeir and Daoud, 2002). It has been investigated for treatment of RHDV by using genetically modified virus-like particles (VLP) as a therapeutic strategy (Win *et al*., 2011). The capsid protein accumulates in VLP, which is distinct from the original virions and devoid of viral RNA (Nagesha *et al*., 1995). VLP is thought to be an immunogenic antigen that triggers cell-mediated and humoral immunity in diseased animals (Crisci *et al*., 2009). Vaccination is a key control method for the rabbit hemorrhagic disease virus. Salem and El-Ballal (1992) conducted the first production trial for an inactivated formalized tissue vaccine of the RHDV in Egypt. Other research was conducted to produce a formalin-inactivated, aluminum hydroxide-adjuvanted RHDV vaccination depending on local strain in Egypt ((Egypt 96) Classic, RHDV strain) (Daoud *et al*., 1998). Beginning in 2008, the conventional RHDV strain was replaced in vaccine production by the RHDVa variant strains, which were discovered in 2006 (Salman, 2007). In Egyptian rabbitries, the virus continues to spread despite the existence of several effective vaccination programs for RHDV prevention (Metwally and Madbouly, 2005; Abd El-Lateff, 2006; Ewees, 2007; El-Sissi and Gafer, 2008; El-Bagoury *et al*., 2014). Local commercial Egyptian vaccines (IZOVAC-MEVAX) and SVRS-Vac provide 100% protection against RHDV when administered to 1.5-month-old rabbits, followed by a booster dose fifteen days later, and then the immunization is repeated every four to six months. (Eid and Ibraheem, 2006). Outbreaks of RHD with extreme mortality were documented in rabbit flocks vaccinated with variant strains or classic of RHDV (RHDVa / RHDV) (Ewees, 2007). According to Erfan and Shalaby (2020), the monovalent RHDV vaccine may not be effective against all strains of the virus that are circulating in Egypt, including RHDVa and RHDV2. OIE (2018) recommended that rabbits should be vaccinated with a vaccine that contains both RHDVa and RHDV2 antigenic types. According to Abodalal and Tahoon (2020), a novel bivalent RHDV vaccine was developed containing both variant strains (RHDVa,RHDVb). El Bagoury*et al*. (2014) reported that a bivalent P. multocida lipopolysaccharide and RHDV vaccine was manufactured. The period of immunity and protection depends on the type of adjuvant following vaccination; this period is longer for oil-emulsified vaccines (Pages 1989). The widespread use of inactivated RHDV vaccines, either imported from Spain (with oil adjuvant CUNIPRAVAC-RHD) or locally (with Al (OH)3 gel adjuvant) is essential for controlling of RVHD in Egypt (Salman 2007; Taha *et al*., 2009). In Egypt, VSVRI continues to produce inactivated RHDV vaccine using aluminum hydroxide gel adjuvant in accordance with the previously published techniques by Daoud *et al*. (1998); Salman (1999) and Salman (2007). Oil-emulsified (OE) vaccines were made locally with paraffin oil (Salman *et al*., 2009). Additionally, Montanide ISAs were used to make combined bivalent oil-emulsified vaccinations against rabbit pasteurellosis and RVHD (Taha *et al*., 2009). Montanide ISAs—a group of adjuvants made up of various oils, emulsion properties, emulsifiers, and immunomodulators are used in the manufacturing of various oil emulsion veterinary vaccines due to their ability for enhancement of the immune response (Mark *et al*., 2012). Alumnium hydroxide gel vaccines provide immunity to RHDV for at least six months, whereas oil-emulsified tissue vaccines have potencies that continue for at least a year (Huang, 1991; Abido *et al*., 2020; Ahmed *et al*., 2024b). According to Desouky *et al*. (2023), there is nothing noticeable difference in the immunological responses to imported and locally developed vaccines. Desouky *et al*. (2023) and Ahmed *et al*. (2024b) indicated that The RHDV2 strains exhibit indications of cross-protection. but there is no cross-protection between RHDV2 and RHDVa. So, It is advised that rabbits should be vaccinated with a vaccine containing the same strains that were found during the epidemic or with a bivalent vaccine containing both antigenic types (RHDVa and RHDV2) in order to control both RHD virus outbreaks in Egypt and reduce financial losses (Abodalal and Tahoon, 2020; Desouky *et al*., 2023; Ahmed *et al*., 2024b).

Control

Rosell *et al*. (1989) suggested that inactivated RHDV vaccination might be utilized as an emergency during outbreaks. According to Salman (2007), a formalinized tissue vaccination might prevent the RHDV's spread within three to four days. Hyper-immune antiserum is used exclusively to prevent RHD and provides temporary protection, this treatment produced a 100% protection rate against RHD, although it might only work if there were no clinical symptoms of infection In Egypt in 1993 (Abd

El-Motelib, 1993).

Zoonotic potential

There is no proof that humans can be infected by RHDV. There have been no reported incidences of infection among those who regularly work in the field. In a study of 259 individuals exposed to RHDV at work, neither serological nor clinical signs of RHDV infection were found (Carman *et al*., 1998).

Conclusion

Despite RHDV's existence for more than 40 years RHDV is still one of the causes of the worldwide economic losses in the rabbit business due to its significant mortality rates in both young and adult rabbits The whole genome sequences of RHDV strains' VP60 are necessary to identify any modifications to the virus's sequences and update the vaccination strain. The RHDV strains that are circulating in Egypt should be continuously monitored and investigated RHDVa and RHDVb were the strains of RHDV that were now in circulation in Egypt In order to eradicate RHD infection in any area it is necessary that the epidemiological condition of this disease be continuously monitored. The most effective strategy to reduce the morbidity and mortality linked to outbreaks is vaccination In order to prevent infection with both types we advised using the bivalent RHDV vaccination which contains both the RHDV-1 and RHDV-2 variant strains as there is no cross-protection immunity between them.

Conflict of interest

The authors declare no competing interests.

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