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Norovirus en las unidades de producción cunícola tras la pandemia por COVID-19

Norovirus in rabbit production units after the COVID-19 pandemic

Norovírus em unidades de produção de coelhos após a pandemia de COVID-19

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Resumen

Norovirus (NoV) es uno de los principales agentes etiológicos de gastroenteritis aguda en humanos y animales. Es un virus altamente infeccioso y de distribución mundial. La infección se realiza a través de la interacción con receptores HBGA, presentes en diferentes células epiteliales superficiales y en fluidos corporales de humanos, murinos, bovinos y murciélagos. Estos receptores también están presentes en las células epiteliales intestinales (enterocitos) de los conejos, razón por la cual se favorece la infección por NoV. La cunicultura en México surge como alternativa a la escasez de alimentos y la falta de empleo en las zonas rurales, por ello es importante apoyar en el fortalecimiento de la actividad. El objetivo de esta investigación consistió en detectar infecciones producidas por NoV en los conejos en unidades de producción cunícola del Estado de México, mediante la técnica de RT-PCR. Como resultado obtuvimos una frecuencia de 22.34% de NoV presente en conejos con signología diarreica. Estos resultados nos dan la pauta para continuar con el estudio de NoV en conejos y poder evaluar su posible potencial zoonótico que contribuya al conocimiento del estado zoosanitario y además permita la mejora en la salud pública de la población.

Palabras clave: Norovirus, infección, conejos, traspatio, México.

Abstract

Norovirus (NoV) is one of the main etiological agents of acute gastroenteritis in humans and animals. It is a highly infectious virus and worldwide distribution. The infection is carried out through the interaction with HBGA receptors, present in different superficial epithelial cells and in body fluids of humans, murine animals, cattle and bats. These receptors are also present in the intestinal epithelial cells (enterocytes) of rabbits, which is why NoV infection is favored. Rabbit farming in Mexico emerges as an alternative to food shortages and lack of employment in rural areas, so it is important to support the strengthening of the activity. The objective of this research was to detect infections caused by NoV in rabbits in rabbit production units of the State of Mexico, using the RT-PCR technique. As a result, we obtained a frequency of 22.34% of NoV present in rabbits with diarrheal signology, these results give us the guideline to continue with the study of NoV in rabbits and to evaluate its possible zoonotic potential that contributes to the knowledge of the animal health status and allows the improvement in the public health of the population.

Keywords: Norovirus, infection, rabbits, backyard, Mexico.

Resumo

O norovírus (NoV) é um dos principais agentes etiológicos de gastroenterites agudas em humanos e animais. É um vírus altamente infeccioso com distribuição mundial. A infecção é realizada através da interação com receptores HBGA, presentes em diferentes células epiteliais superficiais e em fluidos corporais de humanos, murinos, bovinos e morcegos. Esses receptores também estão presentes nas células epiteliais intestinais (enterócitos) de coelhos, razão pela qual a infecção por NoV é favorecida. A criação de coelhos no México surge como uma alternativa à escassez de alimentos e à falta de emprego nas zonas rurais, por isso é importante apoiar o fortalecimento da atividade. O objetivo desta pesquisa foi detectar infecções causadas por NoV em coelhos em unidades de produção de coelhos no Estado do México, utilizando a técnica de RT-PCR. Como resultado obtivemos uma frequência de 22,34% de NoV presente em coelhos com sinais diarreicos. Estes resultados dão-nos orientação para continuar com o estudo do NoV em coelhos e poder avaliar o seu possível potencial zoonótico que contribui para o conhecimento do estado zoossanitário e também permite a melhoria na saúde pública da população.

Palavras-chave: Norovírus, infecção, coelhos, quintal, México.

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Introduction

Norovirus (NoV) is one of the most common causes of viral gastroenteritis in humans and animals (Chhabra *et al*., 2019). They are the most common etiologic agent of acute gastroenteritis (Ahmed *et al*., 2014) and are responsible for approximately 20% of all cases of acute gastroenteritis worldwide, with a similar prevalence in high- and low-income countries (Liao *et al.*, 2021).

NoV, is a member of the Caliciviridae family, its genome is composed of a positive-sense linear RNA, which is approximately 7.6 kb in length (Jiang *et al*., 1993). It has three open reading frames (ORFs) 1, 2 and 3, which encode six viral proteins (VPs). ORF-2 and ORF-3 encode the virion structural components VP1 and VP2; ORF-1 encodes non-structural proteins, including the NoV protease and RNA-dependent RNA polymerase (Thorne and Goodfellow, 2014).

It is currently organized into ten genogroups (G) (GI to GX), which are divided into 50 genotypes. The genogroups that have been identified in humans are GI, GII, GIV.1, GVIII and GIX, in animals a wide variety of genogroups are identified: porcine (GII.11, GII.18 and GII.19), bovine (GIII.1 and GIII.2), sheep (GIII.3), rodents (GV.1 and GV.2), felines (GIV.2, GVI.1 and GVI.2), lions (GIV.2), canines (GVI.1, GVI.2 and GVII), harbour porpoises (GNA1), sea lions (GNA2) and bats (GX) (Villabruna et *al* ., 2021).

Histoblood group antigens (HBGA), and in particular the Lewis, Secretor and ABO phenotype families, serve as NoV binding receptors (Dabelsteen, 2002; Marionneau *et al*.,2001). It has been shown that some animals express receptors of the HBGA *family*, which favor NoV infection, the reported species are murine (Taube *et al*., 2009), cattle (Zakhour *et al*., 2009), canines (Caddy *et al*., 2014), and bats (Kocher *et al*., 2018). Rabbits also express HBGA binding receptors (A, B, and H type 2 [Lewis Y]) in the duodenum region, in addition to other anatomical regions, such as the trachea and bile ducts where several types of HBGA are weakly expressed (Nyström *et al*., 2011). Pigs do not express HBGA receptors, however, they do develop NoV infections although the receptors are unknown (Silva *et al*., 2015).

The rabbit farming sector in Mexico carries out the breeding, fattening and reproduction of rabbits with the aim of obtaining high-quality white meat for human consumption, since this meat has a high percentage of proteins, vitamins and minerals, and is also low in calories, fat and cholesterol (SADER, 2016). The characteristics of rabbit meat are used to solve malnutrition problems that affect vulnerable populations and/or those with limited economic resources in several states of the Mexican Republic (Jaramillo *et al*., 2015).

Mexico has an inventory of 11,560 production units (UP) that report an inventory of 1,108,350 domestic rabbits. Since 2012, the State of Mexico has remained the main producer and consumer of rabbit meat at the national level and has an inventory of 66,806 registered sows in 3,885 PU and 293,332 rabbits (SENASICA, 2020). This production is mainly related to the tourist corridor "Los Volcanes" and "La Ruta de Sor Juana", which includes the municipalities of Tepetlixpa, Ozumba and Amecameca (EdoMex, 2017). In the country, rabbit farming is classified into three types: high-tech, semi-high-tech and backyard, the latter being commonly developed with few sanitary measures (SENASICA, 2020b). The breeds that are commonly used for meat production are the following: New Zealand White, California, Chinchilla, Mariposa, Red Satin, Black Aztec and hybrids (Rabbit Product System Committee, 2012).

Materials and methods

This research is qualitative, in which a descriptive, comparative, observational, cross-sectional, non-experimental and intentional study was carried out. A non-probabilistic and convenience sampling was used in order to show that there is a certain trait in a study population.

The study population consisted of rabbits with enteric disease, 35-65 days old, of all breeds and both sexes from rabbit UPs within region I of the State of Mexico, which includes the municipalities of Valle de Chalco, Chalco, Temamatla, Cocotitlán, Tlalmanalco, Juchitepec, Tenango del Aire, Ayapango, Amecameca, Atlautla, Ozumba, Tepetlixpa and Ecatzingo.

The field study period was carried out from December 2021 to December 2022, where information was also collected through a questionnaire with data of interest on the generalities of rabbit production units.

The samples obtained for this study were feces from rabbits from UP cunicolas, which were collected and identified in sterile bags for easy handling and transported in refrigerated containers at 4-8 °C for processing, analysis and storage at the Biotechnology, Molecular Biology and Genetics Laboratory of the CU UAEM Amecameca.

Total RNA was extracted individually using the described phenol-chloroform technique with Trizol from Invitrogen®. The extracted RNA was stored frozen at -75 °C until analysis. Subsequently, cDNA synthesis was performed using the ImProm-II™ commercial kit, a reverse transcription system following the manufacturer's instructions.

For molecular identification and amplification of NoV, the Polymerase Chain Reaction (PCR) was used, using the oligoinitiators reported by Kojima *et al*. (2002), directed to the capsid region, which are very efficient for amplification and rapid diagnosis of NoV infection, where a fragment of 300 base pairs (bp) is obtained. The PCR was performed with the following conditions: initial denaturation 94°C for 5 minutes 40 cycles; denaturation 94°C for 30 seconds, annealing 50° C for 30 seconds and extension of 72°C for 40 seconds, with a final extension of 72°C for 5 minutes. PCR products were separated on 3% agarose gels using 100 volt electrophoresis for 30 minutes, then the gels were stained with ethidium bromide to be visualized under a UV transilluminator.

Results

The total population was 5,327 rabbits belonging to 15 meat producing farms in the municipalities of region I, State of Mexico. Within this population, 94 rabbits with enteric signs were identified and samples obtained, in which, through molecular analysis using the oligoinitiators designed by Kojima *et al*. (2002), NoV was identified through RT-PCR in a total of 21 samples representing a frequency of 22.34% of NoV in rabbits from the rabbit breeding facilities.

NoV-positive rabbits were collected from farms in the municipalities of Tlalmanalco, Amecameca, Atlautla, Ozumba and Tepetlixpa, as shown in Table 1.

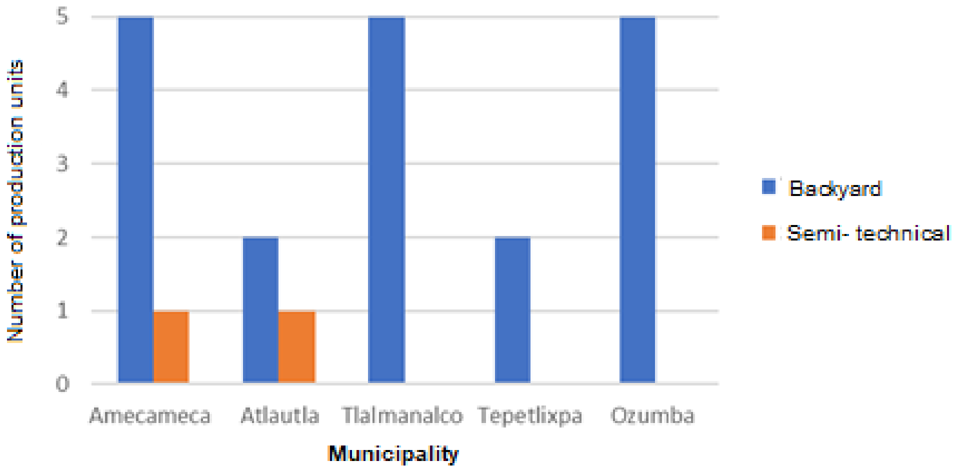
Table 1. Origin of NoV-positive rabbits and type of production.

Municipality of origin	Type of production	Rabbit Identification
Amecameca	Backyard	C20
Atlautla	Backyard	C22
Tlalmanalco	Semi-technical	C24
Tlalmanalco	Semi-technical	C25
Tepetlixpa	Backyard	C27
Tlalmanalco	Semi-technical	C28
Tlalmanalco	Semi-technical	C29
Tlalmanalco	Semi-technical	C30
Ozumba	Backyard	C32
Ozumba	Backyard	C33
Ozumba	Backyard	C35
Atlautla	Semi-technical	C38
Amecameca	Backyard	C40
Amecameca	Backyard	C41
Amecameca	Backyard	C42
Ozumba	Backyard	C45
Ozumba	Backyard	C46
Amecameca	Semi-technical	C49
Atlautla	Backyard	C58
Tepetlixpa	Backyard	C62
Amecameca	Backyard	C69

Source: Own elaboration.

The results obtained indicate that the samples positive for NoV were obtained mostly from backyard UP (90.48%) and to a lesser extent from semi-technical UP (9.52%), as shown in Figure 1.

Figure 1. Type of production associated with NoV-positive rabbits belonging to UP of the State of Mexico.



Source: Own elaboration.

NoV infection was predominant in hybrid rabbits obtained mainly from the New Zealand-California cross 13/21 (61.91%) and from the California breed in 8/21 (38.09%).

Discussion

NoVs are genetically diverse, infect a wide range of mammalian hosts including humans, and are worldwide in distribution (Kapikian *et al*., 1972). NoV infections represent a global economic burden of up to US$60 billion (Bartsch *et al*., 2016).

Although a definitive cellular receptor for NoVs has not yet been identified, HBGA receptors have been shown to bind to the VP1 protein and facilitate binding and/or entry into the cell (Rockx *et al*., 2005). As Nyström refers to *et al*. (2011), rabbits express HBGA receptors in intestinal epithelial cells making them susceptible to NoV infection and demonstrated in this study by implementing the oligoinitiators reported by Kojima *et al*., (2002), which allow a rapid, specific and highly sensitive NoV diagnosis through RT-PCR.

In this study, it was also observed that rabbits that tested positive for NoV infection presented acute diarrheal episodes, as has been associated in reported outbreaks in other animal species, such as cattle (Di Felice *et al*., 2016), pigs (Shen *et al*., 2012), cats (Pinto *et al*., 2012) and dogs (Mesquita and Nascimento, 2012). However, it is not ruled out that animals that did not present enteric signs are not infected with NoV, since an epidemiological study carried out in asymptomatic sheep reports the detection of NoV (Wolf *et al*., 2009).

Diseases that occur in rabbit UP are a factor of great importance for rabbit farming, since they reduce profitability and, therefore, the economy of producers (Lebas *et al*., 2002).

The development of rabbit farming in Mexico depends on agroecological, social, economic and technological factors (Vélez *et al*., 2021); however, it is also necessary to include studies that strengthen the epidemiological zoosanitary factor in which the identification of all pathogens that influence production processes is carried out and thus contribute to the development of this activity.

More than 90% of the stool samples from rabbits with positive enteric signs for NoV come from backyard farms, where, as reported by Reynoso *et al*. (2019), production is deficient in infrastructure and management, and there is also a close relationship with other animal species, such as canines, felines, pigs, horses, sheep and fattening birds, which can favor the interspecies transmission of different diseases, as well as the presentation of possible zoonotic events.

In Mexico and around the world, the livestock sector is facing crises associated with the COVID-19 pandemic (ECLAC, 2020). Rabbit farming was no exception but also faced a resurgence of Rabbit Hemorrhagic Fever that affected around 2,113 families who make a living from rabbit farming, mainly in the State of Mexico, Aguascalientes, Hidalgo, Jalisco, Puebla and Tlaxcala (SENASICA, 2021), generating significant economic losses estimated at millions of pesos (Infobae, 2020).

Conclusions

It has been proven that NoV also infects the species (*Oryctulagus cuniculus*); infections are generally associated with the presence of diarrhea in backyard rabbits.

The rabbit meat industry in Mexico is still in the transition stage. However, it has great potential for development as it meets consumer demand in terms of nutrition and also supports the national economy.

Contributions to future lines of research

It is necessary to continue monitoring and/or surveillance of infections caused by NoVs in rabbit PUs, in order to achieve the sequencing of the complete genome of NoV and, in this way, contribute to the identification of strains that infect rabbits and their relationship with NoVs that infect humans, in order to provide new information that helps to elucidate the possible zoonotic potential of NoV.

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