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Molecular and Antigen Detection of Norovirus in Human and Dogs in Diyala Governorate

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Introduction

Noroviruses were classified within the family *Caliciviridae* as a separate genus. Their genome is ribonucleic acid of single strand and positive sense, the size of the genome was estimated to be 7500 bases. There are three open reading frames (ORF) in NoV genome, included ORF-1, ORF-2 and ORF-3. RNA-dependent RNA polymerase enzyme that regarded as non-structural protein was under the control of OFR-1, whereas, ORF-2 and ORF-3 are encoded for capsid protein VP1 and VP2 respectively (Shaheen ,2019). According to the differences in VP1 noroviruses are classified into different genogroups , these genogroups are GI to GVII (La Rosa *et al*.,2007; Shaheen, 2019).

Genogroups GI,GII and GIV were associated with human infections, and GVI and GVII are associated with canine infections, but humans generally infected with GI and GII (Ando et al., 2000; Kageyama *et al*., 2003; Jan , 2015). These genogroups are subdivided into genotypes or genetic clusters that included many strains (Zheng *et al*., 2006; Al-Mashhadani *et al.*,2008).

Noroviruses are worldwide in distribution. They are the most common cause of gastroenteritis in USA (Hall *et al*., 2018), the disease occurred yearly and reaches its peak during winter as seasonal outbreaks in healthcare institutions and long-term care facilities (Osuka *et al*.,2018). The disease is causing economic impact and significant burdens in many other countries including United Kingdome (Harris *et al*., 2017) , Taiwan (Burke *et al*., 2018), Latina America (O'Ryan *et al* . , 2017), Guatemala (Bierhoff *et al*., 2018), and China (Zhau *et al*., 2018) and Iraq (Al-Tabtabai *et al*., 2020; Diraa *et al*., 2020).

There are nine genotypes in NoV GI and 23 genotypes in GII (Kroneman, *et al*., 2011 ; Shaheen , 2019). Furthermore GII of NoV have wider distribution than GI and plays an important roles in acute gastroenteritis (Vinje ,2015). During the year 1968 the first outbreak due to NoV was reported. Then the virus was reported in all age groups worldwide (Maunula *et al*., 2005; Goodgame *et al.*,2007 ; Robilotti *et al.*, 2015). The mode of transmission was reported to be through environmental contamination, person-to-person, contaminated food and water, vomits, and fecal oral route (Goodgame,2007 ; Robilotti *et al*., 2015).

Noroviruses infect all ages but it was reported to occur mainly in children below five years of age (Petrignani *et al*., 2018). It is well known that HuNoV infections are of self-limiting characters and can be treated with rehydration and supportive therapy (Glass, 2009).

Patients with immunodeficiency or immunocompromised due to transplantation suffered from chronic infection of human norovirus (HNoV) (Glass ,2009; Gairard-Dory , 2014; Angarone , 2016, , Echenique , 2016; vanBeek *et al*, 2017).

Presence of emerging strains of NoVs was contributed to infections occurred in immunocompromised patients (Brown *et al*., 2017) but the original strain of NoVs may get mutations during the course of disease in ordinary patients (Eden *et al.*, 2017). Most morbidity and mortality among children was associated with acute gastroenteritis that was attributed to annually 1.45 million death worldwide (Lozano , 2012).

Infection of (dogs with NoV was reported by many workers worldwide. The first identification of canine norovirus CaNoV) was occurred in Italy in 2007, when a dog showed clinical signs of enteritis (Martella *et al*., 2008).

Then CaNoVs were described in dogs in many other locations of Asia and Europe (Mesquita *et al*., 2010; Mesquita and Nascimento 2012; Caddy *et al*., 2013; Soma *et al.,* 2015). A seroprevalance study conducted on serum samples collected from dogs in 14 countries from Europe showed that 39% of these samples were positive to CaNoV, and gave asuggestion that CaNoVs may infect human especially those in close contact with pet dogs and veterinarians (Mesquita *et al*., 2013;Mesquita *et al*., 2014). In industerilized countries pet dogs are part of individual of family life, this close relationship makes CaNoVs as a public health concern of possibility of zoonotic transmission of CaNoVs (Day, 2011;Summa *et al*., 2012).

Recently human norovirus GII.4 was detected in outbreak affecting dogs in Thailand, those dogs showed fever, acute watery diarrhea and mild dehydration (Charoenkul *et al*., 2020). The same workers mentioned that noroviruses from dogs are closely related to human NoVs and those NoVs in GII genogroup. Dogs must carry Histo-blood group antigens (HBGAs) to be susceptible to human norovirus, these antigens must be carried on epithelial cells of gastrointestinal tracts. No resemblance was detected between human system and dogs in blood system. Furthermore, erythrocytes of dogs cannot be agglutinated (Hutson *et al*.,2003). Some other workers were refer to that dogs expressed HBGAs on surfaces of intestinal epithelium and their saliva (Caddy *et al*.,2014). Accordingly they express the factors for attachment of Norovirus and susceptible to HuNoV (Caddy *et al*., 2015).

In Iraq many studies were conducted on detection of norovirus in human in different Provinces of Iraq including Kurdistan (Al-Mashhadani *et al*., 2008), Al-Najaf province (Al-Ameedi and AL-Amar, 2015), Babylon (Al-Tabtabai *et al*., 2020), Diyala (Diraa *et al.,* 2020), Baghdad (Khalid *et al.*,

2020). It was also reported in American troops (David, 2005) and British troops (Mark *et al*., 2005).

No report was documented or published about NoV detection or infections among dogs in Iraq, Accordingly this study was designed and aimed to:

1- Molecular detection of HuNoVs .
2- Molecular detection of CaNoVs .
3- Phylogenetic analysis of detected virus in comparison to those of NCBI data to point out variations.
4- Study the possibility of cross reactivity among detected viruses in ELISA and qRT-PCR.