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Research Article

Detection of Group a Rotaviruses in Dairy Cows in Greece

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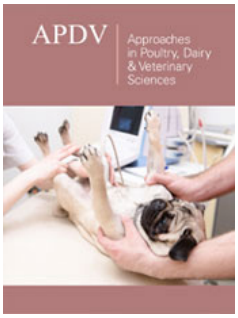
ISSN: 2576-9162



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Submission: January 22, 2022

Published: January 31, 2022

Volume 9 - Issue 1

How to cite this article: Chatzopoulos DC, Athanasakopoulou Z, Papalexiou I, Sofia M, Spyrou V, Antonia Touloudi, Billinis C. Detection of Group a Rotaviruses in Dairy Cows in Greece. Appro Poult Dairy & Vet Sci 9(1). APDV. 000701. 2022.
DOI: 10.31031/APDV.2022.09.000701

Abstract

Group A Rotaviruses (GARVs) are a major causative agent of neonatal diarrhoea. Heifers and adult cows may also be infected by the virus resulting in mild or no clinical signs. In this study, the detection of GARVs in faecal samples of two adult cattle is reported. Both infected animals were suffering from intermittent diarrhoea and were clinically healthy on the day of samples collection. GARVs carriage in heifers and dairy cattle may play a crucial role in virus dispersion within the farm, further contributing to the virus exposure of young calves early in their life.

Keywords: Group a rotaviruses; Dairy cattle; Diarrhoea; Zoonoses

Abbreviations: GARVs: Group A Rotaviruses; BVDV: Bovine Viral Diarrhea Virus; IBRV: Infectious Bovine Rhinotracheitis Virus

Introduction

Group A Rotaviruses (GARVs) infections are considered to be among the predominant causes of neonatal diarrhoea in calves resulting in significant economic losses due to the suboptimal raising rates of the calves and the costs required for virus control [1]. The severity of GARV infections varies greatly depending mainly on the viral strain, the animals' age and the immune response [2]. Considering that newborn calves (younger than ten days) have an immature immune system, they are more vulnerable to virus infections [3,4]. GARVs may lead to the establishment of enteritis in cattle farms, posing a constant threat to all newborns. Occasionally morbidity rates in calves may reach 90%, while mortality varies from 1 to 50% [5-7]. Infrequently, GARV is also detected in adult dairy cattle, where its role has been rather underestimated and poorly understood. Although it is well documented that GARV infections in adult animals remain asymptomatic, few studies have reported the occurrence of mild to severe diarrhoea [8-11]. Nevertheless, GARVs carriage in heifers and dairy cattle may play a crucial role in virus dispersion within the farm, further contributing to the virus exposure of young calves early in their life. In addition, the zoonotic potential of the virus has also been demonstrated, given that dairy cattle can serve as a constant source of virus transmission to humans [12]. We herein report the detection of GARVs in faecal samples of two dairy cows.

Case Presentation

In order to evaluate whether the reduction of productivity and of the reproductive performance of cattle is caused by heat stress or by infections, samples were collected from 2018 to 2021 in Greece. All the tested animals were adults, with at least one partition and clinically healthy on the sampling day. They were regularly vaccinated against the most common pathogens causing reproductive disorders, including Bovine Viral Diarrhea Virus (BVDV) and Infectious Bovine Rhinotracheitis Virus (IBRV), and they had received no therapeutic medication for at least two weeks before sampling. Owners of the eight farms included in the study reported the previous occurrence of sporadic, mild, self-limited and intermittent diarrhoea. According to the data kept on these farms, the presence of diarrhoea

was not induced by stressful conditions and the typical diarrhoeic episodes duration did not exceed three days. In order to ensure that the animals were free of diseases that could potentially affect their productivity and/or reproductive performance, faecal samples were collected from those individuals (n=215) and were processed for bacteriological, virological, and parasitic investigation (Table 1). Faecal samples were collected directly from the cows' rectum by harvesting at least 20g of faeces into the gloved hand of the investigator. The samples were maintained in cold storage until their transport to the laboratory within 12h.

Table 1: Characteristics of the farms included in the study. *Animals positive in both the rapid immunochromatography (IC) tests as well as in the RT-PCR assay.

Farm	Total Number of Animals in the Farm	Number of Sampled Animals with Sporadic, Self-Limited Diarrhoea	Animals Positive for Rotavirus IC/PCR*
F1	189	20	-
F2	202	22	-
F3	201	20	-
F4	432	35	1
F5	250	26	-
F6	180	20	-
F7	220	30	-
F8	850	42	2
Total	2524	215	3

For the detection of Rotavirus antigen (Ag) in faeces, rapid immunochromatography tests (IC) was applied, using two commercially available rapid test kits (VIKIA Rota-Adeno; Biomerieux-BoviD-4, BioNote, Gyeonggi-do, Republic of Korea). Interestingly, the presence of Rotavirus Ag was identified in samples of three cows derived from two different farms, while all the other tests performed were negative. In the GARVs Ag positively tested samples, the virus RNA was also detected by an RT-PCR assay amplifying the VP6 core gene [13,14]. RNA extraction was performed using a commercially available RNA kit (Ambion RNA kit; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturers' instructions. Following electrophoresis on 2% agarose gel, amplicons were visualized under ultra-violet light. The RT-PCR was considered affirmative for the virus RNA presence when amplicons size was equal to 379bp.

Discussion

The current study demonstrated for the first time in Greece the presence of GARVs in dairy cows which did not present any clinical signs. Our findings are aligned with previous studies that reported a limited detection rate of GARVs in asymptomatic cattle [15,16]. Two out of the 215 (0.9%) adult animals suffering from intermittent diarrhoea were found to be carriers of the virus. The presence of Rotaviruses in two of the eight tested farms underlines the extended spread of the virus in dairy cattle farms. At the herd level, diarrhoea outbreaks were sporadically reported on both farms that presented GARVs cases and were addressed using broad-spectrum antibiotics. Morbidity and mortality rates of approximately 30% and 10% had been recorded in the first herd, while in the second one, morbidity and mortality had been estimated to be 15% and 5%, respectively. Tests for identifying bovine Rotavirus had never been performed previously on both farms, and thus the causative agent of these outbreaks remained unclear.

To date, there is extended research regarding the contribution of Rotaviruses in neonatal diarrhoea of calves, classifying the virus among the most critical intestinal pathogens. In contrast, the available data regarding the prevalence of GARV infections in adult cattle are scanty, and therefore virus' role remains unclear. Viral intestinal infection could also be established in dairy cows without causing clinical disease, while healthy carriers could play a significant role in spreading Rotaviruses within the farm. At the late stage of the pregnancy or during lactation, infected adults may excrete the virus through their faeces, further constituting a source of infection for their offspring. Moreover, via the intermittent excretion of the virus, infected animals could serve as a constant threat and mechanical carriers, further affecting the efficiency of GARV control measures and strategies.

Acknowledgment

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (Acronym: the summer cow, project code: T1EDK-01078).

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