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Rotavirus excretion by kids in a naturally infected goat herd

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Abstract

A cross-sectional study was carried out in a dairy goat herd, investigating the presence of rotavirus by means of ELISA, polyacrylamide gel electrophoresis (PAGE) and two latex agglutination tests in feces of 63 goat kids younger than 1 month, with and without diarrhea, and in feces of 19 adult goats during the first few days after parturition. All animals belonged to a herd located in the mountains of the León province (NW Spain). Rotaviruses were found in 18 out of 63 goat kid fecal samples but no significant association between shedding of rotavirus and presence of diarrhea could be established. Rotaviruses were found in kids aged 6 to 21 days, and more frequently between 6 and 10 days. No shedding of virus was detected in any of the adults. Considering ELISA as the reference test, PAGE and both latex agglutination tests were less sensitive. One of the latex tests was also highly non-specific. All PAGE-positive samples showed the typical electropherotype of group A rotavirus. Feces were also screened for other pathogens including *Escherichia coli, Clostridium perfringens* and *Cryptosporidium parvum. C. parvum* oocysts were detected in the feces of six out of 45 goat kids tested, all six suffering from diarrhea. This paper represents the first description of rotavirus infections in goats in Spain. The possible mechanisms of viral diffusion within the herd and its role as pathogen in goats are discussed.

Keywords: ELISA; Group A rotavirus; Goat kid; Latex agglutination; Polyacrylamide gel electrophoresis

1. Introduction

Rotaviruses have been described on several occasions as agents which cause diarrhoea in the newborn of several species, including man (Flewett and Woode, 1978; Holmes, 1979). Most animals experience infection at one stage or another throughout their lives, as evidenced by high percentages of seropositive animals found in several studies (Takahashi et al., 1979; Sato et al., 1981). This ease of infection is partly due to fecal excretion of virus at high concentrations, both by sick and asymptomatic carrier animals which are responsible of the perpetuation of the infection in the herd, making rotavirus infections enzootic. Furthermore, the virus is very resistant, which favours its diffusion. Although individuals of all ages can be infected, rotaviruses are a common cause of diarrhea mainly during the first 2 weeks of life in most animal species.

Rotaviruses were described for the first time in goat species by Scott et al. in 1978. Since then, both the presence of the virus in feces and specific antibodies in sera of goats has been reported on several occasions, although data on the diffusion of this infection in goat herds is still limited. They have been detected in goat feces in Chile, Hungary and France with variable prevalences (Berrios et al., 1988; Nagy et al., 1983; Ramisse et al., 1984; Yvore et al., 1984). The prevalence of seropositive animals has ranged from 60% reported by

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Takahashi et al. (1979) in Japan, to 25.7% detected by Iovane et al. (1988) in Italy. Nevertheless few details are known on aspects of epidemiology and pathogenicity of rotavirus infections in this species.

A wide variety of diagnostic tests have been used for the detection of rotaviruses in feces, such as electron microscopy, cell culture isolation, fluorescent antibody tests, ELISA, PAGE and latex agglutination tests. These last three are among the most frequently used for their simplicity and some of them are available as commercial kits.

The present study was carried out to determine the presence and role as a pathogen of rotavirus in a goat herd, as well as to obtain information about some epidemiological determinants, such as age distribution of infection and possible shedding of rotavirus by the does, which could contribute to better understanding of viral diffusion within a herd. With this purpose, fecal samples from kids of different ages as well as feces from their mothers were investigated for the presence of rotavirus. To evaluate the suitability of several diagnostic tests in the detection of rotavirus in goat feces, an ELISA test, PAGE and two commercial latex agglutination kits were used for testing the fecal samples.

2. Material and methods

Animals

The study was carried out in February and March 1989 on a commercial diary goat herd located in the mountains in the Province of León (Northwest Spain). Its hygienic conditions were good. The herd was representative of the average semiextensive dairy herd of the region. During the previous parturition season there was an outbreak of mild diarrhea, and although no analyses were done to determine the presence of rotavirus in feces, a high prevalence of rotavirus-seropositive animals was found among the adult goat population in this farm (data not shown).

The herd had 100 does of the Saanen and Alpine breeds, a part of which were not expected to kid in this parturition season since they were due the following autumn parturition season. Adults were kept indoors during the night and taken out to graze during the day. While indoors, all animals were housed in the same building, but separated by wooden fences into lots depending on age and breeding status. Kid goats received three colostrum doses directly from their mothers and then were split into small groups according to birthdates, i.e., all kids that were born in the same week were kept together. Groups were separated from one another by means of hay fences and from adults by a 3-m wide corridor and by wooden or hay fences. They were fed continually on a mixture of milk from the whole herd and artificial milk. They were kept indoors until their second or third month of life, when they were put out to graze. Parturition season started at the end of January and the number of live born kids, when this study reached its conclusion, was 65.

Collection and processing of fecal samples

The herd was sampled three times, starting at the end of February. The interval between samplings was 10 d. In each case, samples were only taken from the kids which were born after the previous sampling, in such a way that only one sample was obtained from each animal.

A total of 63 feces samples were taken from 1–30-d-old kids, 12 from diarrheic and 51 from non-diarrheic animals. Nineteen fecal samples were also taken from those 19 does that were between 0 and 3 d postparturition at the time of our samplings. Feces samples were taken with rectal swabs from the kids and immediately suspended in 1 ml of 0.1 M phosphate-buffered saline (PBS). Feces were collected from adults with plastic gloves directly from the rectum and diluted 1:10 in PBS.

Double antibody-sandwich ELISA

The method described by Ellens and De Leeuw (1977) was followed. Briefly, polystyrene microtiter plates were coated with pig anti-rotavirus IgG as capturing antibody and feces samples, diluted in PBS 0.05% Tween 80, were added. Rotavirus antigen was detected with rabbit anti-rotavirus IgG, and peroxidase-labelled goat anti-rabbit IgG (Nordic, Holland) was used as conjugate. The substrate-cromogen mixture was 0.005% hydrogen peroxide and 1 mg/ml 5-aminosalycilic acid diluted in phosphate-EDTA buffer. Between every step, plates were incubated for 1 h at 37°C in a water bath and were washed four to six times with PBS 0.05% Tween-80. The reading was done using a Multiskan (Titertek, Finland) ELISA reader at 450 nm of wavelength.

Latex agglutination

Two different commercial kits were used, following the manufacturers' instructions: Slidex-Rotakit II (Biomèrieux, France), a monoclonal antibody based test, and Rotalex (Orion Diagnostica, Finland), which uses rabbit anti-rotavirus polyclonal antibodies. In both of them one drop of fecal suspension was mixed with one drop of the latex reagent (latex beads coated with antirotavirus antibodies), while another drop of the same fecal suspension was mixed with a drop of control latex (non coated beads) on a glass or cardboard plate. After a thorough mixing of drops, plates were rotated for 2 to 5 min after which the reading was done. This reaction was considered to be positive when agglutination was observed with the latex reagent but not with the control latex, negative when no agglutination was observed in either drops and, finally, the reaction was considered to be non-specific when agglutination was observed with both reagents. In this case the test was considered non-valid for the sample.

Polyacrylamide gel electrophoresis of viral RNA

The method described by Herring et al. (1982) was followed. Feces samples were treated with sodium dodecyl sulphate and a 3:2 mixture (v/v) of phenol and chloroform to remove proteins and extract the viral RNA, and were subjected to electrophoresis. Laemmli's (1970) discontinuous buffer system was used, with 3% stacking acrylamide gels and 7.5% slab gels. Electrophoresis was carried out at 40 mA during 90 min at room temperature. Gels were stained with silver nitrate to visualize the RNA bands.

Detection of other enteric pathogens

Fecal smears were stained by the Ziehl-Neelsen method, modified by Henriksen and Polenz (1981) and examined microscopically to detect *Cryptosporidium parvum* oocysts.

To isolate *Clostridium perfringens*, feces samples were inoculated into cooked meat broth, as enriching medium, and afterwards plated onto tripticase sulfite neomicine agar (Biomèrieux, France), as a differential medium. Plates were incubated anaerobically and black colonies grown after 24 h of incubation were considered suspicious of *C. perfringens* and inoculated onto API 20A galleries (Biomerieux, France) for confirmation.

K99$^+$ *Escherichia coli* were detected after plating onto Minca agar (Guinee et al., 1976), by slide agglutination with anti-K99 rabbit antiserum (Rijksinstituut voor Volksgezondheid en Milieuhygiene, Bilthoven, Holland).

Statistics

Two group comparisons were made with the χ^2 test at $\alpha = 0.05$.

3. Results

3.1. Animals

The first diarrheic animal appeared at the end of February, a month after parturitions began. From this moment until the first week of March, five new cases appeared, three of which occurred in 20-d-old animals. The feces were soft and bright yellow. Later, the problem became more serious and all the animals born in the last days of the parturition season underwent this process. In this 'second stage', diarrhea became more severe, the feces were yellowish-green and of a more liquid nature. Diarrhea appeared towards the sixth day of life and lasted approximately a week. The animals showed anorexia and growth rate slowed down. Diarrheic kids were separated from the rest and were orally administered a glucose-saline solution. There were no deaths.

3.2. Rotavirus

All fecal samples were examined by all four tests. Results obtained are shown in Table 1. ELISA detected rotavirus antigen in 17 out of 63 kid samples tested, while ten, eight and seven samples were scored as positive by PAGE, Slidex-Rotakit II and Rotalex, respectively. All ten PAGE-positive samples showed identical migration patterns resembling the character-

Table 1
Rotavirus detection in kid feces by ELISA, PAGE, Slidex-Rotakit II and Rotalex

	ELISA	PAGE	Slidex-Rotakit II	Rotalex
Positive	17	10	8	7
Non-specific	–	–	1	36
Negative	46	53	54	20

istic electropherotype of group A rotaviruses. A high percentage of non-specific reactions was observed with the Rotalex kit (57.1%), while only 1.4% of the samples reacted non-specifically with the Slidex-Rotakit II. Rotaviruses were not detected in feces samples from does either by ELISA, latex agglutination or PAGE. For further evaluation and considerations on epidemiological factors, any sample scored as positive by any of the four tests was considered as containing rotavirus.

Rotavirus excretion and diarrhea

There were 18 positive results out of 63 (25.4%) kid samples tested, 15 were from healthy and three from diarrheic animals (Table 2). There were no statistically significant differences ($P > 0.05$) in rotavirus excretion between healthy and diarrheic animals.

Table 2
Shedding of rotavirus by diarrhoeic and non-diarrhoeic kids

Shedding of rotavirus	Diarrhea		Total
	yes	no	
Yes	3	15	18
No	9	36	46
Total	12	51	63

Table 3
Influence of age on rotavirus shedding

Age[a]	Sampled	Positive	%
<6	12	0	0
6–10	23	13	56.2
>10	28	5	17.8
Total	63	18	

[a]Age in days.

Table 4
Shedding of *Cryptosporidium parvum* oocysts by diarrhoeic and non-diarrhoeic kids

Shedding of *C. parvum*	Diarrhea		Total
	yes	no	
Yes	6	0	6
No	3	36	39
Total	9	36	45

Rotavirus excretion and age

To evaluate the influence of age on virus shedding, three groups were made depending on the age of the kids, as shown in Table 3. Rotavirus shedding began at 6 d of age, reached a peak between 6 and 10 d, and decreased from d 10 onwards. The virus was not detected in animals older than 21 d of age.

Two of the three diarrheic animals that excreted rotavirus were 20 d and were ill during the 'first stage', when diarrhea was not very serious. The other animal was a 6-d-old kid goat which suffered from a mixed rotavirus and *C. parvum* infection.

3.3. Other agents

C. parvum oocysts were detected in the feces of six out of 45 animals tested, all six suffering from diarrhea (Table 4). The association between diarrhea and excretion of oocysts was highly significant ($P < 0.001$). Kids shedding oocysts were between 5 and 15 d old and were diarrheic during the 'second stage', towards the end of the parturition season, when the problem became more serious. Neither *C. perfringens* or K99$^+$ *E. coli* were found in any sample.

4. Discussion

An association between shedding of rotavirus and diarrhea in goat kids could not be demonstrated in the present study, thus, these results do not allow us to consider rotavirus as the main cause of this diarrheal outbreak. It is known that several factors exist which decisively influence the outburst of diarrhea in rotavirus-infected animals. Among them are the age of the animal, immune status, hygiene and management conditions, concurrence of other enteric pathogens (Radostits and Acres, 1980; Snodgrass et al., 1982) and the pathogenicity of the virus itself. Thus, the presence of rotavirus in a herd may be of variable significance.

No studies about the pathogenicity of rotavirus in goats under experimental conditions have been published so far and, under natural conditions, most authors have failed to demonstrate the association between rotavirus shedding and the presence of diarrhea (Nagy et al., 1983; Yvore et al., 1984, Berrios et al., 1988), which agrees with our observation. Only Nagy et al.

(1987), in another study, found a positive association between the presence of rotavirus and diarrhea in one out of three herds. Good hygiene and management conditions, such as lack of overcrowding and separation of kids into small groups, may have contributed to the mildness of the symptoms in the case reported here.

The peak of animals shedding rotavirus found by us was between 6 and 10 d of life, below that previously described. Scott et al. (1978) detected rotavirus in the feces of two 4-mo-old diarrheic goats. Berrios et al. (1988) observed that 16 out of 25 goats shedding rotavirus on the same farm were older than 1 month, whereas Nagy et al. (1987) found rotavirus excretion between 7 and 30 d of age (mean 16). This divergency in the data has also been observed for other species and suggests that many factors, such as the immune status of the herd, may influence age of rotavirus infection. Rotavirus was detected in feces from 6 d of age and onwards. Incubation period for the rotavirus infection is usually between 24 and 72 h, and although it is generally admitted that in a herd where rotavirus infection is enzootic most animals get infected on their d 1, excretion is not usually detected in feces until d 4–5 or even much later due to the presence of colostral antibodies in the gut. These passively acquired antibodies may have contributed to prevent the development of more severe symptoms in this herd and could also explain the fact that rotavirus was not detected in animals below 6 d of age, as kids were continually fed on maternal colostrum and milk, and does were known to possess specific antibodies (data not shown) that might pass to the gut of the newborn resulting in an increase of the incubation period or even in the prevention of infection.

Another reason for this delay could be that animals may not get in contact with the virus or receive low doses during the very first few days of life, while they remain with their mothers. No rotavirus shedding was detected in the does. There has been a lot of controversy on the role the mother plays in the transmission of the virus to the newborn. Studies that have been carried out in other species, such as bovine and porcine, have led to heterogeneous results, with some authors demonstrating excretion of virus in mother's feces (Rubio et al., 1989; Kodituwakku and Harbour, 1990) and others not (Fu and Hampson, 1989; García-Sanchez et al., 1993). It is believed that cows may shed virus intermittently or in such low amounts that can not be detected by the commonly used diagnostic tests but that are enough to perpetuate the infection in a herd by infecting their offspring. Nevertheless, they would not be the main source of infection, but the other calves that excrete virus in high concentrations contributing to progressive contamination of soil and hay. For this reason, lack of overcrowding on a farm makes facilities less contaminated and lowers the infective dose that young animals receive.

Regarding the participation of other agents, a high statistically significant association was established between diarrhea and shedding of *C. parvum* oocysts in feces, which allows consideration of this parasite as the main cause of diarrhea on the farm, at least in its more serious 'second stage'. However, two 20-d-old kids that suffered a mild diarrhea during the 'first stage' were shedding only rotavirus.

Interaction between rotavirus and *C. parvum* is difficult to evaluate and unlikely to have happened as rotavirus infections occurred mainly in the so-called 'first stage', while *C. parvum* was clearly associated with the more severe 'second stage'. Only in one animal, a mixed rotavirus-*C. parvum* infection was detected. These concurrent infections have already been described in animals from the same herd and less frequently in the same individual (Nagy et al., 1983, 1987; Ramisse et al., 1984). However, so far no studies have been performed of the possible enhancing synergistic effect of coinfection by both enteropathogens in goats.

In order to compare the diagnostic tests used in this study, ELISA was considered as the reference test, as the method used by us has been proven by others to be highly sensitive and specific in the detection of rotavirus in feces (Ellens, 1978). The sensitivity of both latex test kits was very low, having detected only eight out of 13 positive samples by Slidex-Rota-Kit II and seven out of 13 by Rotalex. Previous studies have shown the lower sensitivity of latex agglutination as compared with ELISA and PAGE for detection of rotavirus in stool specimens (Dennehy et al., 1988). Latex agglutination tests are even less sensitive late in the course of diarrhea (Miotti et al., 1985), when low quantities of virus are shed. The same occurs for detection of asymptomatic animals excreting rotavirus, which was the case for most of the goat kids tested. Rotalex has its beads coated with polyclonal antibodies against a bovine G6 serotype strain (NCDV) (Sukura

and Neuvonen, 1990). Sanekata et al. (1991) observed a higher sensitivity of a latex test using antibodies anti-OSU strain in detecting the homologous virus than in detecting viruses of other serotypes such a G1, G3 and G6. In agreement, Goyal et al. (1987) found that Rotalex was more sensitive in the detection of bovine rotavirus than in the detection of rotaviruses from pigs and turkeys. Molyneaux et al. (1989) proved that a latex test intended for human diagnosis was less sensitive for detecting animal rotaviruses.

A great number ($n=36$) of the samples tested by Rotalex, agglutinated the control latex, which led to inconclusive results. This kit has been used by others in the detection of rotaviruses in porcine, bovine, and equine species (Imagawa et al., 1987; Rubio et al., 1989; Sukura and Neuvonen, 1990) and in no case was the percentage of non-specific reactions over 23%. Composition of goat feces could have been responsible of the self-agglutinating effect, and makes the use of this test kit unadvisable for diagnosis of rotavirus infections in goats.

PAGE has been regarded as a very sensitive and specific technique for detection of rotavirus RNA in stool samples (Herring et al., 1982; Arens and Swierkosz, 1989). However, it only detected ten out of 18 positive samples, probably because the test was performed from the suspension liquid of rectal swabs instead of feces, and thus, the sample quantity was lower. Furthermore, PAGE has been compared with other techniques for detection of rotavirus in diarrheic rather than in asymptomatic animals, in which the sensitivity could be lower because of the smaller amount of virus excreted, as shown by the faint RNA bands observed in the positive samples. In fact, PAGE detected all three ELISA-positive samples from diarrheic kids.

The final choice of a method for detection of rotavirus depends on facilities and requirements of each laboratory. Latex tests are easy and quick to perform and do not need to be automated, but it must be taken into account that some commercial kits can not be suitable to use in goat species as shown in this study, and may have a lower sensitivity when detecting rotavirus from asymptomatic animals. PAGE has a higher sensitivity and the advantage that detects non-group A rotaviruses, but both special equipment and trained personnel are needed. ELISA seems to be a sensitive technique when detection of rotavirus has to be done in asymptomatic animals, but also needs special equipment. This paper represents the first description of the presence of rotavirus infections in goats in Spain.

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Resumen

Muñoz, M., Lanza, I., Alvarez, M. and Cármenes, P., 1994. Eliminación de rotavirus en una explotación de ganado caprino infectada naturalmente. *Small Rumin. Res.*, 14: 83–89.

Se realizó un estudio de corte en una explotación de ganado caprino lechero investigando la presencia de rotavirus mediante ELISA, electroforesis en gel de poliacrilamida (PAGE) y 2 pruebas de aglutinación en látex en las heces de 63 cabritos menores de un mes, con y sin diarrea, y en las heces de 19 cabras durante los primeros días tras el parto. Todos los animales pertenecían a una explotación situada en la montaña de la provincia de León (NO de España). Se encontraron rotavirus en 18 de las 63 muestras fecales de cabritos analizadas, aunque no pudo demostrarse la existencia de asociación estadísticamente significativa entre la eliminación del virus y la presencia de diarrea. Las edades de los cabritos en que se detectó eliminación de rotavirus estaban comprendidas entre los 6 y los 21 días y más frecuentemente entre los 6 y los 10. Considerando ELISA como la prueba de referencia tanto PAGE como ambas pruebas de aglutinación en látex fueron menos sensibles. Una de las pruebas de aglutinación en látex fue también muy inespecífica. En todas las muestras positivas mediante electroforesis se observó el electroferotipo característico de los rotavirus del grupo A. También se estudió la presencia en las heces de otros patógenos como *Escherichia coli, Clostridium perfringens* y *Cryptosporidium parvum*. Se detectaron ooquistes de *C. parvum* en las heces de 6 de 45 cabritos investigados, los 6 padecían diarrea. Este trabajo representa la primera descripción de la infección por rotavirus en la especie caprina en España. Se discuten los posibles mecanismos de difusión del virus dentro de la explotación así como su papel como patógeno en cabras.