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EPIDEMIOLOGICAL STUDIES ON ROTAVIRUS ASSOCIATED WITH DIARRHOEA AMONG CALVES AND CHILDREN IN KADUNA STATE, NIGERIA

Aliyu A.M[1]., Aminu, M[2]., Ado, S.A[2]. and Jatau, E.D[2].
[1]Department of Applied Science, C.S.T. Kaduna Polytechnic, Kaduna, Nigeria
[2]Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria
Correspondence author: aisha2zra@yahoo.com +2348035890640

ABSTRACT
Rotaviruses are enteric pathogens causing acute, watery, dehydrating diarrhoea in various host species, including birds and mammals. A total of 716 diarrhoeic stool samples were collected comprising of 249 from calves and 467 from children within Kaduna State Nigeria. Rotavirus antigen was detected in the stools of calves by NSP3 Real-Time Reverse Transcription-Polymerase Chain Reaction, while RV antigens were detected in children using Enzyme Linked Immunosobent Assay (ELISA). Rotavirus was detected in 34 of the 249 stool sample from calves (13.7%). The infection was higher in males (15.7%:16/102) compared to females calves (12.3%: 11/147). The highest number of cases was detected among calves 2-3 months of age (22.9%:8/35). Further analysis of the result showed no significant difference between rotavirus infection in calves with mode of feeding and presence of other animals in the farm (p>0.05). There was significant difference between rotavirus infection and sanitation on farm and source of water for calves (p<0.05). An overall RV prevalence of 31.0% (143/467) was obtained in children. The infection was higher in females (32.1%:79/246) compared to males (29.9%:66/221). The highest burden was detected in children 25-36 months of age (36.4%:22/77). There was no significant association between source of drinking water, boiling of drinking water, attendance of day care and playing with toys and the prevalence of rotavirus (P> 0.05). The study has revealed that rotavirus remains an important cause of acute diarrhoea among calves and children in Kaduna State, Nigeria. Hence the need for improvement in sanitation and the implemention of the vaccines into the childhood immunization programme.
Key words: Rotaviruses, Stools, Calves, Children, Risk factors, Diarrhoea.

INTRODUCTION

Rotaviruses are enteric pathogens causing acute, watery, dehydrating diarrhoea in various host species, including birds and mammals. Rotavirus is the cause for approximately 500,000 child deaths yearly, mainly in developing countries (Rajendran and Kang, 2014). The virus is the single most important cause of infectious, severe, dehydrating diarrhoea and death worldwide in children less than 5 years (Pennap and Umoh, 2010).

Rotavirus gastroenteritis is a mild to severe disease, with incubation period of about 1-2 days. The symptoms often starts with fever, nausea, and vomiting, followed by abdominal cramps and frequent watery diarrhea, which may last for 3-8 days. Infected children may also have a cough and runny nose. (Junaid *et al.*, 2011)

Bovine rotaviruses are important causative agents of neonatal calf diarrhoea throughout the world, and rotavirus infection is a significant cause of economic loss in the cattle industry (Hoshino, 2009). Rotavirus infections constitute the basic causes of economic loss owing to growth delay, birth of weak calves and high mortality levels in herds (Silver *et al.*, 2012). The disease occurs predominantly in intensively reared animals and is characterized by a short incubation period, anorexia and diarrhoea. The onset of the disease is rapid and the clinical signs include depression, anorexia, diarrhoea and dehydration (Mulherin *et al.*, 2008).

Rotaviruses are members of the family *Reoviridae*, nonenveloped and are characterized by the presence of 11 segment of double stranded RNA surrounded by 3 separate shells, the core, inner capsid and outer capsid (Junaid *et al.*, 2011).

The study therefore aimed at determining the demographic and possible risk factors that might be associated with rotavirus diarrhoea among calves and children in Kaduna state Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in Kaduna state, Nigeria. The state has a total number of 23 Local Government Areas (LGAs), and three senatorial districts, that include south, north and central senatorial zones. Six of the LGAs were selected by simple random sampling for this research. These LGAs include Kachia, and Kagarko (south), Chikun, and Giwa (central), Soba, and Sabon gari (north). Farms were selected in each LGA for calve collection centers, and a hospital for children collection centers.

Determination of Sample Size

A prevalence rate of 20.0% as reported in a bovine study by Amupitan (2011) was used to calculate the sample size using the equation by Sarmukaddam and Garad (2006). The sample size calculated was 246 which was the least number of samples to be used for the study. Two hundred and fourty nine (249) samples were collected from calves. A prevalence of 36.6.0% as reported in a human study by Wada-Kura (2011) was used to calculate a sample size using the equation by (Sarmukaddam and Garad, 2006). The sample size calculated was 357 as the minimum number of sample to be collected. But to have a good representation of the target population and to increase the chances of having positive samples, a total of 467 stool samples were collected from children.

Sample Collection and Processing

A total of 249 diarrhoeic stools were collected from calves' 0-8months across the six selected LGAs with the assistance of fields' men of the National Commission for Nomadic Education (NCNE). About 5gm or 5ml of each stool sample was scooped with a wooden spatula or decanted respectively into clean, labeled screw capped tubes. A total of 467 stool samples were collected from children 0-5 years of age across the six selected LGAs. All samples were transported in ice box to the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria and stored frozen at -20^{0}C.

Extraction of RNA from Calves Stool Samples

The extraction of RNA from the calves stool samples was done at the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria. The RNA was extracted using QIAGEN RNA extraction kit (QIAGEN GmbH Germany) according to manufacturer's instructions.

Analysis of Extracted RNA

The extracted RNA were transported within iceboxes to the Centers for Disease Control and Prevention (CDC) Atlanta, Georgia, USA for rotavirus detection using NSP3 Real-Time Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) as previously described by Mijatovic-Rustempasic *et al.* (2013).

NSP3 Real-Time Reverse Transcription-Polymerase Chain Reaction

The extracted RNA were amplified to detect rotaviruses by qRT-PCR using rT*th* NSP3 kit (Applied Biosystems, Inc., Foster City, CA) according to manufactures instructions. Each 25µl reaction mixture contained 7.5µl of nuclease-free water, 1µl EZ buffer, 300 nM (each) deoxynucleoside triphosphates (dNTP), 2.5 mM Mn(OAc)2, 0.1 U r*Tth* polymerase, 500 nM (each) forward and reverse primer, 150 nM probe, and 5µl of undenatured RNA extract. The forward primer NVP3-FDeg (5′-ACC ATC TWC ACR TRA CCC TC-3′), reverse primer NVP3-R1 (5′-GGT CAC ATA ACG CCC CTA TA-3′), and probe NVP3-Probe (5′-A 6-carboxyfluorescein [FAM] TG AGC ACA ATA GTT black hole quencher 1 [BHQ1] AAA AGC TAA CAC TGT CAA-3′) previously described were used (Freeman *et al.,* 2008). After adding the reaction mixture and undenatured RNA to MicroAmp Fast Optical 96-well reaction plates (Applied Biosystems, Inc., Foster City, CA), template denaturation, RT, and PCR amplification were carried out on an ABI 7500 Fast real-time PCR system (Applied Biosystems, Inc., Foster City, CA) in standard mode.

The qRT-PCR was run using the following thermocycling condition: 1 cycle 95^{0}C for 5 minutes (dsRNA denaturation), 1 cycle 50^{0}C for 30 minutes (reverse transcription), 1 cycle 95^{0}C for 1 minute (RNA-cDNA denaturation), 45 cycles of: 95^{0}C for 15 seconds and 60^{0}C for 60 seconds (2-step amplification; quantification). A test result was considered positive if a sigmoidal amplification curve crossed the threshold before 45 cycles and all positive and negative control reactions gave expected results. Samples with cycling threshold (CT) values ≤30 were considered to be positive.

ELISA for Children Stool Samples

Each 10% fecal suspension was screened for the presence of rotavirus antigens using commercially available enzyme immunoassay (EIA) kit (Premier Rotaclone Meridian Bioscience, Inc. USA).

Analysis of Results

Data obtained from the questionnaires and the results of the laboratory analysis were analyzed using Statistical Package for Social Sciences Version 16.0 (SPSS Incorporation, 2007 Chicago, USA). Chi-Square test was employed to test for association between the variables and seroprevalence obtained at 95% confidence interval and a *P* value ≤0.05 was considered significant.

RESULTS AND DISCUSSION

Out of the total 249 bovine feacal samples screened for NSP3 of rotavirus by Real-Time RT-PCR, 13.7% (34/249) were positive for rotavirus. Out of the total 467 faecal samples screened for the presence of Human rotavirus in children, 145 (31.0%) were positive for rotavirus antigens.

Table 1: Age and Sex Distribution of Rotavirus Infection among Calves in Parts of Kaduna State.

Parameter	No examined	No positive (%)	p-value
Age group (months)			
0-1	36	2(5.6)	
2-3	35	8(22.9)	0.169
4-5	78	14(17.9)	
>5	100	10(10.0)	
Sex			
Male	102	16(15.7)	0.437
Female	147	18(12.3)	

In this study, rotavirus was detected in calves with a prevalence of 13.7%. This contrasts studies conducted in Zaria among calves which reported 20.0% (Amupitan, 2006). The result however agrees with the 16.83% reported in India (Dash, *et al.*, 2011).

Rotavirus was detected in calves with a higher prevalence in age group 2-3 months and a lower prevalence in age group 0-1months. There was no statistically significant association between age and the prevalence of rotavirus infection in calves. This high prevalence in age group 2-3 months could be as a result of loss of protection from maternally acquired antibodies, calves pick up infection as the maternal antibodies wane. Low prevalence in 0-1 months could be as a result of maternally acquired antibodies that protects young calves.

Rotavirus was detected slightly at higher rates in males than in females this agrees with studies conducted in Zaria (Aminu *et al.,* 2014). The higher prevalence in males could be assumed that the size of the male at birth induces dystocia, and consequently decrease colostrum absorption, or it could be due to their behavioral activity. The males are more active than the females hence the more likelihood to pick up infection, or it could be by chance.

Table 2: Demographic Distribution of Human Rotavirus Infection among Children in Parts of Kaduna State

Parameter	No examined	No positive (%)	p-value
Age group (months)			
0-12	113	37(32.7)	
13-24	208	63(30.3)	
25-36	77	28(36.4)	0.517
37-48	35	11(31.4)	
40-60	34	6(17.6)	
Sex			
Male	221	66(29.9)	
Female	246	79(32.1)	0.536

Rotavirus antigen was detected in children 0-5years in parts of Kaduna state with a prevalence of 31.0% in this study. The result agrees with 36.5% recorded in Kano Northern Nigeria (Wada-Kura, 2011). The finding also agrees with results in other African Countries and other parts of the world. However, the prevalence is higher than those reported in Zaria 15.6%, 23.8% (Pennap and Umoh, 2010; Gambo, 2014).

Rotavirus was recorded in all age groups 0-60 months. Although highest prevalence was recorded in age group 25-36 months, there was no statistically significant difference between age and the prevalence of rotavirus. This agrees with the findings of Wada-Kura (2011) who reported higher prevalence in 41-50 months age group among children in Kano. But contradict the report of Junaid *et al,* (2011) and Aminu *et al*, (2014) in studies conducted in Jos and Zaria. The higher prevalence recorded among this age group (25-36 months) in this study could be due to behavioral activities of children at this age, who tend to play outside with possibly feacally contaminated materials. Least prevalence was recorded in older children. This could be due to the fact that older children tend to become protected from severe form of rotavirus infection as a result of protection acquired from multiple reinfection (Pennap and Umoh, 2010).

There was no statistically significant association between rotavirus infection and gender. This agrees with the findings of Pennap and Umoh (2010). Though in this study, females had a slightly higher prevalence than males.

Table 3: Prevalence of Bovine Rotavirus Infection among Calves in Relation to Some Possible Risk Factors.

Risk factor	No examined	No positive (%)	No negative (%)	*P*-value
Mode of feeding				
Suckling	248	34(13.7)	214(86.3)	
Bucket feeding	1	0(0.0)	1(100.0)	0.690
Sanitation on farm				
Poor	93	19(20.4)	74(79.6)	
Good	156	15(9.6)	15(90.4)	0.016
Other animal on farm				
Dog	97	12(12.4)	85(87.6)	
Sheep	123	19(15.4)	104(84.6)	
Goat	10	2(20.0)	8(80.0)	0.905
Others	4	0(0.0)	4(100)	
None	15	1(6.7)	14(93.3)	
Source of water on farm				
Borehole	5	2(40.0)	3(60.0)	
Well	83	13(15.7)	70(84.3)	0.044
Stream	144	14(9.7)	130(90.3)	
Pond	17	5(29.4)	12(70.6)	

There was no statistically significant association between mode of feeding, presence of other animals on the farm, and the prevalence of rotavirus infection in calves. There was statistically significant association between sanitation on farm, source of water, and the prevalence of rotavirus infection in calves. This agrees with the findings of (Aminu *et al.*, 2014) who identified sanitation on farms and source of water as risk factors associated with rotavirus infection in calves. Close association between herdsmen with their livestock can serve as a potential risk in the transmission of the virus.

Table 4: Risk Factor Distribution of Rotavirus Infection among Children in Parts of Kaduna State.

Risk factors	**No tested**	**No positive (%)**	**OR**	**CI on OR**	**p-value**
Source of water					
Well	195	66(33.8)			
Tap	152	52(34.2)			
Pound	22	4(18.2)			0.216
Stream	47	13(27.7)			
Borehole	51	10(19.6)			
Boiling water					
Yes	75	19(25.3)			
No	392	126(32.1)	0.783	0.456-1.231	0.786
Attendance of day care					
Yes	117	41(35.0)			
No	350	104(29.7)	0.981	0.789-1.123	0.369
Play with toys					
Yes	200	56(29.5)			
No	267	86(32.2)	0.881	0.234-0.734	0.531

There was no statistically significant association between source of water, boiling of drinking water, attendance of day care and playing with toys and the presence of rotavirus infection. This agrees with the finding of Pennap and Umoh (2010) who reported that attendance of day care facility, type of drinking water had no statistically significant association with rotavirus prevalence and contradicts the finding of Junaid *et al*. (2011) who identified playing with toys and attendance of day care facility as risk factors associated with rotavirus infection in children.

Rotavirus is resilient and highly contagious, therefore, improvement in water and sanitation are unlikely to be effective preventive measures of rotavirus disease.

CONCLUSION

The study has revealed that rotavirus is an important cause of diarrhoea among calves and children in Kaduna state with a prevalence of 13.7% and 31.0% respectively. Factors associated with rotavirus diarrhoea in calves were; sanitation on farm and herd's source of water. None of the factors tested was found to be associated with rotavirus diarrhoea in children.

REFERENCES

Aminu, M., Amupitan, E., Umoh, J.U., Dzikwi, A. and Esona, M.D. (2014). Detection of Rotavirus Antigens From Calves in Zaria, Nigeria. A paper presented at the Eleventh International Rotavirus Symposium 3-5 September New Delhi India 165-166.

Amupitan, E. (2011). Prevalence of Group A Bovine and Human Rotavirus Antigens from Bovine Calves in Zaria and Environs. An unpublished M.Sc research thesis submitted to the school of postgraduate studies, Ahmadu Bello University, Zaria.

Dash, S. K., Tewari, A., Kumar, K., Goel, A., and Bhattia, A. K. (2011). Detection of Rotavirus from Diarrhoeic Cow Calves in Mathura, India. *Veterinary World,* 4**(12):** 554-556.

Freeman, M.M., Kerin, T., Hull, J., McCaustland, K. and Gentsch, J. (2008). Enhancement of Detection and Quantification of Rotavirus in Stool Using a Modified Real-time RT-PCR Assay. *Journal of Medical Virology,* 80: 1489-1496.

Gambo, A. (2014). Prevalence of Rotavirus and *Cryptosporidium Pavum* Infections and Their Co-infection Among Children with Acute Gastroenteritis in Zaria, Nigeria. An unpublished M.Sc research thesis submitted to the school of postgraduate studies, Ahmadu Bello University, Zaria.

Hoshino, Y.A. (2009). Longitudinal cohort study in calves evaluated for rotavirus infections from 1-12 months of age by sequential serological assays. Archives Virology, 154 **(5):** 755-763.

Junaid, S.A., Umeh, C., Olabode, A.O., and Banda, J.M. (2011). Incidence of Rotavirus in Children with Gastroenteritis Attending Jos University Teaching Hospital, Nigeria. *Virology Journal,* 8**(1)**: 233-238.

Mijatovic-Rustempasic, S., Tam, K.I., Kerin, T.K., Lewis, J.M., Gautam, R., Quaye, O., Gentsch, J.R. and Bowen, M.D. (2013). Sensitive and Specific Quantitative Detection of Rotavirus a by One-step Real –time Reverse Transcription PCR Assay without Antecedent Double-Stranded-RNA Denaturation. *Journal of Clinical Microbiology,* 51**(9)**: 3047-3054.

Mulherin, E., Bryan, J., Beltman, M., O'Grady, L., Pidgeon, E., Garon, L., Lloyd, A., Bainbridge, J., O'Shea, H., Whyte, P., Fanning, S. (2008). Molecular Characterization of a Bovine-like Rotavirus Detected from a Giraffe. *BMC Veterinary Research,* 4: 46-54.

Pennap, G. and Umoh, J. (2010). The Prevalence of Group A Rotavirus Infection and Some Risk Factors in Pediatric Diarrhea in Zaria, North Central Nigeria. *African Journal of Microbiology Research,* 4**(14):** 1532-1536.

Rajendran, P. and Kang, G. (2014). Molecular Epidemiology of Rotavirus in Children and Animals and Characterization of an unusual G10P[15] Strains Associated with Bovine Diarrhoea in South India. *Vaccine*, 32S: A89-A94.

Sarmukaddam, S. B. and Garad S. G. (2006). On Validity of Assumptions While Determining Sample Size. *Indian journal of Community Medicine*, 29**(2):** 2004 – 2006.

Silver, F. D. F., Gregori, F., Goncalves, A. C. S., Samara, S. I. and Buzinaro, M. G. (2012). Molecular Characterization of GroupA Bovine Rotavirus in South-eastern and Central- western Brazil, 2009-2010. *Pesquisa Veterinari Brasileira* 32**(3):** 237-242.

Wada-Kura, A. (2011). Molecular Characterization of Rotaviruses Detected in Children Under the Age of Five Years with Diarrhoea in Kano State-Nigeria. An unpublished M.Sc research thesis submitted to the school of postgraduate studies, Ahmadu Bello University, Zaria.