Review Article

Submitted: 01/09/2011

Accepted: 04/12/2011

Published: 22/12/2011

Ovine rotaviruses

S. Gazal¹, I.A. Mir¹, A. Iqbal^{2,*}, A.K. Taku¹, B. Kumar¹ and M.A. Bhat¹ ¹Division of Veterinary Microbiology and Immunology, Faculty of Veterinary Sciences and Animal Husbandry, R.S.Pura, SKUAST-Jammu-181102, India ²Division of Veterinary Epidemiology and Preventive Medicine, Faculty of Veterinary Sciences and Animal Husbandry, R.S.Pura, SKUAST-Jammu-181102, India

Abstract

Rotavirus has been recognized as a predominant cause of acute diarrhea in young animals and humans. Rotavirus has segmented genome composed of 11 segments of double stranded RNA. The virus has a triple layered protein shell consisting of a core, an inner capsid and an outer capsid. The inner capsid protein is responsible for group specificity and based on it rotaviruses are classified into seven groups. Ovine rotavirus strains have only been identified into two serogroups (A and B). The two outer capsid proteins (VP7 and VP4) are responsible for G and P typing of rotavirus, respectively. Although rotavirus has been frequently reported in many animal species, data regarding ovine rotavirus strains is very scanty and limited. Only a few ovine rotaviruses have been isolated and characterized so far. Recently, the G and P types circulating in ovines have been identified. The ovine rotavirus strain NT isolated from a diarrheic lamb in China is being considered as a promising vaccine candidate for human infants.

Keywords: Ovine, Rotavirus, Electropherotypes, G and P types, Vaccine

Introduction

Rotavirus is responsible for causing economically significant malady in neonates of many domestic animals (Kapikian and Chanock, 1996). It has been observed that young animals succumb to infectious agents during neonatal period, thereby adversely affecting the economic stability of many animal farming ventures. In ovines, rotaviruses are known to cause enteritis and diarrhea, especially in neonatal lambs (Wani *et al.*, 2004).

In fact, a study at the U.S. Sheep Experiment Station showed that diarrhea accounted for 46% of lamb mortality (Schoenian, 2007). Increasing evidence suggests direct transmission of rotavirus strains between animals and humans.

Rotavirus is also a cause of major concern in human gastroenteritis cases. It has been estimated that about 39% of childhood diarrhea hospitalizations are caused by rotaviruses and nearly half a million children die from rotavirus infections each year worldwide (Parashar *et al.*, 2003).

Furthermore, rotavirus mortality is concentrated in the developing countries on the Asian subcontinent, in Africa, and in Latin America, where access to health care facilities is limited (Phua *et al.*, 2006). The virus has also been implicated as a cause of encephalitis in children in Australia and Germany (Goldwater *et al.*, 2001; Kehle *et al.*, 2003).

Rotaviruses, members of the family Reoviridae, are characterized by segmented genomes comprising of 11 segments of double stranded RNA contained within a triple layered protein shell composed of a core, inner capsid and outer capsid. Sixty spikes, 4.5 to 6.0 nm in length and each with a knob at its distal end, extend from the smooth surface of the outer shell.

The name rotavirus was suggested on the basis of the characteristic wheel like appearance with a sharply defined circular outline of the outer capsid, short spokes and a well-defined rim, when examined by negative-stain electron microscopy (Flewett *et al.*, 1974).

Intact rotavirus particles are about 70 nm in diameter and have an icosahedral symmetry (Estes, 2001). The virus is composed of 6 structural (VP1, VP2, VP3, VP4, VP6 and VP7) and 6 nonstructural (NSP1-NSP6) proteins.

Three structural proteins, VP1, VP2 and VP3, form the core of the rotavirus particle. VP6 proteins form the inner capsid while the outer capsid of the virus is made up of the VP7 and VP4 proteins. The group specificity of the virus is determined by epitopes on the VP6 protein and based on it; rotaviruses can be divided into seven distinct groups (A - G).

Ovine rotavirus strains belong to serogroup A (Kaminjolo and Adesiyun, 1994) and B (Theil *et al.*, 1995). VP7, a glycoprotein, is the major component of the outer capsid while VP4, a protease-sensitive protein, is a minor component which forms spikes on the outer capsid (Prasad *et al.*, 1988). VP4 and VP7 proteins are able to elicit independent neutralizing antibody responses. The two outer capsid proteins form the basis of binary system of classification of

^{*}Corresponding Author: Dr. Asif Iqbal, Faculty of Veterinary Sciences and Animal Husbandry, R.S.Pura, SKUAST-Jammu-181102, India. *Tel.*: +919906490854. Email: <u>asifent2008@gmail.com</u>

rotaviruses in which rotavirus strains are classified into VP4 or P serotypes and VP7 or G serotypes (Estes and Kapikian, 2007). While G serotype designations largely coincide with G genotype designations, this is not the case with P serotypes and genotypes.

Therefore, a dual nomenclature has been adopted for VP4 antigenic and genetic classification (Estes and Kapikian, 2007). The P serotype (when known) is denoted by an Arabic number (sometimes followed by a capital letter) and the P genotype is denoted immediately after the P serotype number by a number within squared brackets (Estes, 2001). So far 19 G and 27 P genotypes have been identified (Matthijnssens *et al.*, 2008). Strains that have more than 89% amino acid identity are considered to be belonging to the same genotype (Gentsch *et al.*, 1996; Estes, 2001).

Various physical and chemical properties of rotaviruses have also been analyzed. They are known to survive in fecal material for long periods and remain a source of infection to susceptible populations (Steele *et al.*, 2004).

They are stable at low and high relative humidity, at pH range 3-9; and exhibit a reduction in infectivity at higher temperatures (Steele *et al.*, 2004). Further, the rotaviruses are not inactivated in presence of ether, chloroform, quaternary ammonium disinfectants and sodium hypochlorite (Steele *et al.*, 2004). However, ethanol, phenol, formalin and lysol are suitable disinfectants; and 37% formaldehyde (1:10), 0.75% hexachlorophene (1:3) and 67% chloramine-T (1:5) can effectively destroy rotaviruses (Tan and Schnagl, 1981; Steele *et al.*, 2004).

Epidemiology

Despite the huge contribution of sheep to the livestock sector, the data regarding rotaviruses in the ovine species are very scanty. The epidemiology of lamb rotavirus strains is still largely unknown, possibly due to a lack of surveillance within animal populations (Ciarlet *et al.*, 2008). Only few reports of rotavirus in sheep have been noted.

Ovine rotavirus has been identified as causing neonatal lamb diarrhea in United Kingdom, Japan, United States (Makabe *et al.*, 1985; Chasey and Banks, 1986; Theil *et al.*, 1995; Schoenian, 2007), Egypt (Khafagi *et al.*, 2010) and India (Wani *et al.*, 2004; Gazal *et al.*, 2011). Khafagi *et al.* (2010) determined the prevalence of rotavirus associated with diarrhea in lambs and kids in Egypt by latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA). Rotavirus was detected in 12.3% samples from diarrheic lambs.

A typical group B rotaviruses have been associated with diarrhea in lambs (Holland, 1990; Theil *et al.*, 1995). However, Munoz *et al.* (1996) detected group A rotavirus from lambs in Spain. Similarly, Wani *et*

al. (2004) associated group A rotavirus with lamb diarrhea in an outbreak in Kashmir, India. Wani *et al.* (2004) detected group A rotavirus in 25% diarrheic lambs using sandwich ELISA and RNA-polyacrylamide gel electrophoresis (RNA-PAGE). The association of group A rotavirus with diarrheic lambs was contrary to the findings of other investigators who mostly reported group B associated with lamb diarrhea (Holland, 1990; Theil *et al.*, 1995; Theil *et al.*, 1996).

Only recently Gazal *et al.* (2011) determined the prevalence of group A rotavirus in diarrheic lambs in Jammu and Kashmir, India. Five hundred diarrheic fecal samples collected over a period of three years were screened for presence of group A rotavirus by LAT, RNA-PAGE and reverse transcription polymerase chain reaction (RT-PCR). The prevalence of group A rotavirus in lambs was found to be 13.2% by LAT, 9.8% by RNA-PAGE and 10.4% by RT-PCR.

Electropherotypes

The 11 segments of rotavirus genome vary in their molecular weights and can be resolved to produce a characteristic migration pattern in polyacrylamide gels referred to as electropherotypes.

Electropherotypes showing a 4-2-3-2 migration pattern is characteristic of group A rotavirus, whereas electropherotypes showing a 4-2-2-3 pattern is characteristic of group B rotavirus (Snodgrass *et al.*, 1984; Steele *et al.*, 2004). This technique has become a useful method for virus detection, molecular epidemiology studies (Chanock *et al.*, 1983), studying the genomic variation and tracing mixed rotavirus infection (Bhat *et al.*, 2005). Wani *et al.* (2004) found electropherotypes of group A rotavirus in ovine diarrheic samples. Similarly, Gazal *et al.* (2011) detected 4-2-3-2 migration pattern characteristic of group A rotavirus in diarrheic fecal samples of ovine origin in India.

G and P Genotypes

Little information is available on the genotypes of ovine rotavirus strains globally. However, Fitzgerald *et al.* (1995) conducted serological and genomic characterization of group A rotavirus from lambs. Four lamb rotaviruses were characterized serologically by reactions with monoclonal antibodies and genomically by hybridization assays and sequencing. The viruses were found to belong to serotypes G3, G6, G10 and G9. The corresponding P types were P1, P11, P14 and P9. This was the first report of G9 from a species other than humans.

Munoz *et al.* (1995) studied the prevalence of neutralizing antibodies to 9 rotavirus strains representing 7 G-serotypes in sheep sera. Neutralizing antibodies to 9 rotavirus strains representing serotypes G1, G3, G5, G6, G8, G9, and G10 were investigated

in 212 sheep serum samples from three different age groups, comprising of 1 week old lambs, 2-3 months old lambs and adult sheep. All the sera samples from one week old lambs had neutralizing antibodies to all 9 rotavirus strains.

Both neutralizing antibody titers and prevalence to all 9 strains markedly decreased in 2-3 months old lamb group and increased again in the adult sheep group. In addition, adult sheep sera neutralized a larger number of rotavirus strains than 2-3 months old lamb sera. The highest neutralizing antibody titers and prevalence were found to strains B223 and K923 (representing serotype G10), to strain RRV (representing serotype G3) and to strain NCDV (representing serotype G6), indicating that these could be the predominant 3 rotavirus serotypes in Spanish sheep.

Ciarlet *et al.* (2008) isolated an ovine rotavirus strain 762 in the feces of a 30-week-old lamb affected with severe gastroenteritis in the province of Zaragoza, Spain. Subsequent characterization of the VP4, VP7, VP6, NSP4, and NSP5/NSP6 genes were performed.

Ovine rotavirus strain 762 was classified as a P[14] rotavirus, as the VP4 and VP8* (trypsin-cleavage product of the VP4 protein) revealed the highest amino acid (aa) identity (94% and 97%, respectively) with that of the P11[14] human rotavirus strain PA169, isolated in Italy.

Analysis of the VP7 gene product revealed that ovine rotavirus strain 762 possessed G8 serotype specificity, a type common in ruminants. Moreover, ovine rotavirus strain 762 displayed a bovine-like NSP4 (genotype E2), NSP5/NSP6 (genotype H3) and a VP6 genotype I2, as well as a long electropherotype pattern. This was the first report of a lamb rotavirus with P[14] and G8 specificities, providing additional evidence for the wide genetic and antigenic diversity of group A rotaviruses.

Recently Chen *et al.* (2009) characterized a lamb rotavirus strain NT isolated from a diarrheic lamb in China. It was found to possess genotype G10P[15]. The lamb rotavirus strain NT shared a high degree of similarity with ovine rotavirus strain 762 (84.2%). The lamb rotavirus strain for future development. Comparative genomic analysis of the lamb-NT strain with 17 reference strains revealed that gene reassortments between rotaviruses circulating in different species had occurred.

Gazal *et al.* (2011) recently determined the genotypes of ovine rotavirus strains circulating in Jammu and Kashmir, India. Genotype G6P[11] was found to be the most predominant rotavirus strain circulating in ovines (Gazal *et al.*, 2011). This is in contrast with the work by Galindo-Cardiel *et al.* (2011) who reported G8P[1] as the primary cause of ovine diarrheic syndrome in weaned lambs.

Lamb rotavirus as vaccines candidate for infants

A notable characteristic of rotavirus strains is that the gene segments are prone to genetic reorganization (reassortments) in nature or under experimental conditions. As a result of this, a great deal of genetic diversity is observed in rotaviruses circulating in humans and animals (Estes and Cohen, 1989).

Animal rotaviruses tend to be attenuated naturally in human hosts and easier to be cultivated in cell culture (Chen *et al.*, 2009). The fact that natural infection with one rotavirus genotype induces protection against different genotypes indicates that protection is heterotypic (Velazquez *et al.*, 1996).

Based on the characteristics of rotaviruses and using gene recombination technology, third generation rotavirus vaccine strains are being developed. Currently, two live-attenuated rotavirus vaccines which were licensed in 2006 are available for use in human infants.

These are the monovalent human rotavirus vaccine (Rotarix) (Ruiz-Palacios *et al.*, 2006) and the pentavalent bovine-human reassortant vaccine (RotaTeq) (Vesikari *et al.*, 2006). Both vaccines have demonstrated very good safety and efficacy profiles and are currently in widespread use.

A rotavirus vaccine, referred to as LLR, is a monovalent (G10P[12]) live-attenuated oral vaccine derived from a lamb strain of rotavirus (developed and produced by the Lanzhou Institute of Biological Products, China). The vaccine was developed by passing a wild-type group A serotype G10P[12] lamb rotavirus through primary calf kidney cells. Although the efficacy of this vaccine is unknown, it is currently used to vaccinate children (aged 2 to 36 months) against rotavirus disease in China and may be an additional vaccine candidate for use in human infants. However, the vaccine is relatively expensive in China, costing \$18.4 per dose; as a result, few children received more than one dose (Fu et al., 2007). At present there is no rotavirus vaccine licensed for use in ovines.

Summary

Rotavirus is the leading etiological agent of acute viral gastroenteritis in young ones of many species of animals (including ovines) and human. The importance of rotavirus with reference to lamb diarrhea, its physico-chemical properties, electropherotypes, G and P types and the potential of lamb rotaviruses as alternative vaccine candidate for human infants have been discussed.

References

Bhat, M.A., Wani, S.A., Taku, A. and Dutta, T.K. 2005. Molecular epidemiology of rotavirus infection in animals in India: An update. Indian J. Anim. Sci. 75, 1276-1282.

- Chanock, S.J., Wenske, E.A. and Fields, B.N. 1983. Human rotaviruses and genomic RNA. J. Infect. Dis. 148, 49-50.
- Chen, Y., Zhu, W., Sui, S., Yin, Y., Hu, S. and Zhang, X. 2009. Whole genome sequencing of lamb rotavirus and comparative analysis with other mammalian rotaviruses. Virus Genes 38(2), 302-310.
- Chasey, D. and Banks, J. 1986. Replication of atypical ovine rotavirus in small intestine and cell culture. J. Gen. Virol. 67, 567-576.
- Ciarlet, M., Hoffmann, C., Lorusso, E., Baselga, R., Cafiero, M.A., Bányai, K., Matthijnssens, J., Parreño, V., de Grazia, S., Buonavoglia, C. and Martella, V. 2008. Genomic characterization of a novel group A lamb rotavirus isolated in Zaragoza, Spain. Virus Genes 37(2), 250-265.
- Estes, M.K. 2001. Rotaviruses and their replication. In Fields Virology, Eds., Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B. and Straus, S.E. 4th ed., vol. 2: Lippincott Williams and Wilkins, Philadelphia, pp: 1745-1785.
- Estes, M.K. and Cohen, J. 1989. Rotavirus gene structure and function. Microbiol. Rev. 53, 410-449.
- Estes, M.K. and Kapikian, A. 2007. Rotaviruses. In Fields Virology, Eds., Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E., 5th ed. Kluwer Health/Lippincott, Williams and Wilkins, Philadelphia, pp: 1917-1974.
- Fitzgerald, T.A., Munoz, M., Wood, A.R. and Snodgrass, D.R. 1995. Serological and genomic characterization of group A rotaviruses from lambs. Arch. Virol. 140(9), 1541-1548.
- Flewett, T.H., Bryden, A.S., Davies, H., Woode, G.N., Bridger, J.C. and Derrick, J.M. 1974. Relation between viruses from acute gastroenteritis of children and newborn calves. Lancet 2(7872), 61-63.
- Fu, C., Wang, M., Liang, J., He, T., Wang, D. and Xu, J. 2007. Effectiveness of Lanzhou lamb rotavirus vaccine against rotavirus gastroenteritis requiring hospitalization: a matched case-control study. Vaccine 25, 8756-8761.
- Galindo-Cardiel, I., Fernández-Jiménezc, M., Lujánd, L., Buesac, J., Espadae, J., Fantovae, E., Blancof, J., Segalésa, J. and Badiolad, J.J. 2011. Novel group A rotavirus G8 P[1] as primary cause of an ovine diarrheic syndrome outbreak in weaned lambs. Vet. Microbiol. 149 (3-4), 467-471.
- Gazal, S., Taku, A.K. and Kumar, B. 2011. Predominance of rotavirus genotype G6P[11] in diarrhoeic lambs. The Vet. J. *in press*.

- Gentsch, J.R., Woods, P.A., Ramachandran, M., Das, B.K., Leite, J.P., Alfeiri, A., Kumar, R., Bhan, M.K. and Glass, R.I. 1996. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J. Infect. Dis. 174, S30-S36.
- Goldwater, P.N., Rowland, K., Power, R., Thesinger,
 G., Abbott, K., Grieve, A., Palombo, E.A.,
 Masendycz, P.J., Wilkinson, I. and Bear, J. 2001.
 Rotavirus encephalopathy: pathogenesis reviewed.
 J. Paediatr. Child Health 37, 206-209.
- Holland, R.E. 1990. Some infectious causes of diarrhea in young farm animals. Clin. Microbiol. Rev. 3, 345-375.
- Kaminjolo, J.S. and Adesiyun, A.A. 1994. Rotavirus infection in calves, piglets, lambs and goat kids in Trinidad. Br. Vet. J. 150(3), 293-299.
- Kapikian, A.Z. and Chanock, R.M. 1996. Rotaviruses. In Fields Virology, Eds., Fields, B.N., Knipe, D.M., Howley, P.M., Chanock, R.M., Melinick, J.L., Monath, T.P., Roizman, B. and Straus, S.E. 3rd ed., vol 2. Lippincott-Raven, Philadelphia, pp. 1657-1707.
- Kehle, J., Metzger-Boddien, C., Tewald, F., Wald, M., Schuurman, J. and Enders, G. 2003. First case of confirmed rotavirus meningoencephalitis in Germany. Pediatr. Infect. Dis. J. 22, 468-470.
- Khafagi, M.H., Mahmoud, M.A. and Habashi, A.R. 2010. Prevalence of rotavirus infections in small ruminants. Global Veterinaria 4(5), 539-543.
- Makabe, T., Sato, K., Ouneda, S. and Inaba, Y. 1985. Haemagglutination with ovine rotavirus. Arch. Virol. 90, 153-158.
- Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M. K., Gentsch, J. R., Iturriza-Gómara, M., Kirkwood, C., Martella, V., Mertens, P.P.C., Nakagomi, O., Patton, J.T., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Desselberger, U. and Ranst, M.V. 2008. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch. Virol. 153(8), 1621-1629.
- Munoz, M., Lanza, I., Alveraz, M. and Carmenes, P. 1995. Prevalence of neutralizing antibodies to 9 rotavirus strains representing 7 G-serotypes in sheep sera. Vet. Microbiol. 45(4), 351-361.
- Munoz, M., Alvarez, M., Lanza, I. and Carmenes, P. 1996. Role of enteric pathogens in the aetiology of neonatal diarrhoea in lambs and goat kids in Spain. Epidemiol. Infect. 117, 203-211.
- Phua, K.B., Emmanuel, S.C., Goh, P., Quak, S.H., Lee, B.W., Han, H.H., Ward, R.L., Bernstein, D.I., De Vos, B. and Bock, H.L. 2006. A rotavirus vaccine for infants: The Asian experience. Ann. Acad. Med. Singapore 35, 38-44.

- Prasad, B.V.V., Wang, G.J., Clerx, J.P. and Chiu, W. 1988. Three dimensional structure of rotavirus. J. Mol. Biol. 199, 269-275.
- Parashar, U.D., Hummelma, E.G., Bresee, J.S., Miller, M.A. and Glass, R.I. 2003. Global illness and deaths caused by rotavirus diseases in children. Emerg. Infect. Dis. 9, 565-572.
- Ruiz-Palacios, G.M., Perez-Schael, I., Velazquez, F.R., Abate, H., Breuer, T., Clemens, S.C., Cheuvart, B., Espinoza, F., Gillard, P., Innis, B.L., Cervantes, Y., Linhares, A.C., Lopez, P., Macias-Parra, M., Ortega-Barria, E., Richardson, V., Rivera-Medina, D.M., Rivera, L., Salinas, B., Pavia-Ruz, N., Salmeron, J., Ruttimann, R., Tinoco, J.C., Rubio, P., Nunez, E., Guerrero, M.L., Yarzabal, J.P., Damaso, S., Tornieporth, N., Saez-Llorens, X., Vergara, R.F., Vesikari, T., Bouckenooghe, A., Clemens, R., De Vos, B. and O'Ryan, M. 2006. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N. Engl. J. Med. 354(1), 11-22.
- Schoenian, S. 2007. Diarrhea (scours) in small ruminants. Comp. Ext. J. Maryland University. <u>http://www.sheepandgoat.com/articles/scours.html</u>
- Snodgrass, D.R., Herring, J.A., Campbell, I., Inglis, J.M. and Hargreaves, F.D. 1984. Comparison of atypical rotavirus from calves, piglets, lambs and man. J. Gen. Virol. 65, 909-914.
- Steele, A.D., Geyer, A. and Gerdes, G.H. 2004. Rotavirus infections. In Infectious diseases of Livestock, Eds. Coetzer, J.A.W. and Tustin, R.C. pp. 1256-1264. Oxford University Press, Southern Africa

- Tan, J.A. and Schnagl, R. 1981. Inactivation of a rotavirus by disinfectants. Med. J. Aust. 1, 19-32.
- Theil, K.W., Grooms, D.L., McCloskey, C.M. and Redman, D.R. 1995. Group B rotavirus associated with an outbreak of neonatal lamb diarrhea. J. Vet. Diagn. Invest. 7, 148-150.
- Theil, K.W., Lance, S.E. and McCloskey, C.M. 1996. Rotaviruses associated with neonatal lamb diarrhea in two Wyoming shed-lambing operations. J. Vet. Diagn. Invest. 8, 245-248.
- Velazquez, F.R., Matson, D.O., Calva, J.J., Guerrer, L., Morrow, A.L., Carter-Campbell, S., Glass, R.I., Estes, M.K., Pickering, L.K. and Ruiz-Palacios, G.M. 1996. Rotavirus infection in infants as protection against subsequent infections. N. Engl. J. Med. 335, 1022-1028.
- Vesikari, T., Matson, D.O., Dennehy, P., Van Damme, P., Santosham, M., Rodriguez, Z., Dallas, M.J., Heyse, J.F., Goveia, M.G., Black, S.B., Shinefield, H.R., Christie, C.D., Ylitalo, S., Itzler, R.F., Coia, M.L., Onorato, M.T., Adeyi, B.A., Marshall, G.S., Gothefors, L., Campens, D., Karvonen, A., Watt, J.P., O'Brien, K.L., DiNubile, M.J., Clark, H.F., Boslego, J.W., Offit, P.A. and Heaton, P.M. 2006. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N. Engl. J. Med. 354(1), 23-33.
- Wani, S.A., Bhat, M.A., Nawchoo, R., Munshi, Z.H. and Bach, A.S. 2004. Evidence Of rotavirus associated with neonatal lamb diarrhea In India. Trop. Anim. Health Prod. 36(1), 27-32.