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Molecular Detection and Phylogenetic Analysis of Chicken Astrovirus Associated with Poultry Enteritis

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ABSTRACT

Background: Poultry enteritis is an important multifactorial disease. Chicken Astrovirus (CAstV) usually associated with enteritis. The aim of this study was to investigate the occurrence of CAstV in poultry enteritis cases, its molecular characterization, phylogenetic analysis and gross and microscopic examination of intestine and liver specimen affected with CAstV.

Methods: Total 604 dead poultry birds from commercial poultry farms affected with enteritis were examined for presence of CAstV. Intestinal samples of four birds were pooled to make one biological sample. CAstV was detected by reverse transcriptase PCR (RT-PCR) using ORF-1b gene specific primers. Molecular characterization was carried out by partial gene sequencing.

Result: CAstV was detected in 20.52% (31/151) of samples. Highest prevalence (49.29%) was observed in 0-1 week old chicks. The partial molecular characterization revealed high similarity of the nucleotide sequence from India (97% to 93%) and from USA, Brazil, Poland and Korea (94 to 92%). Further similarity of amino acid sequences of CAstV from India (100% to 98%) and from USA, Brazil, Poland and Korea (98 to 97%) was observed. Histopathological examination revealed villous atrophy, congestion and atrophic cystic glands in sub-mucosa of intestine. Further severe congestion and hemorrhages along with infiltration of inflammatory cells in liver parenchyma was observed.

Key words: Chicken astrovirus, Histopathology, Phylogenetic analysis, Poultry enteritis, RT-PCR.

INTRODUCTION

Absorption and assimilation of nutrients depends on the health of gastrointestinal tract (GIT). Any abnormality in the functioning of GIT results in decreased growth, feed conversion ratio (FCR) and production losses (Devaney *et al.,* 2016 and Lobani *et al.,* 2016). Enteric viruses such as CAstV, Avian coronavirus (ACV), Avian rotavirus (ARV), Avian reovirus (AReoV), Fowl adenovirus-I (FAdV-I) and Chicken parvovirus (ChPV), have been detected in chickens with diarrhoea and other signs of Runting and Stunting Syndrome (RSS) (Kaithal *et al.,* 2016; Nunez *et al.,* 2016 and Mettifogo *et al.,* 2014). CAstV is recognized as the causal agent of enteritis in chicken primarily in young birds (Mettifogo *et al.,* 2014 and Pantin-Jackwood *et al.,* 2011). Involvement of CAstV with an unusual condition in chicks known as “white chicks” has been reported from Brazil (Nuñez *et al.,* 2016b).

CAstV is a small, round, non-enveloped, positive-sense single-stranded RNA virus having 5 or 6-pointed star-like surface projections. It belongs to the genus *Avastrovirus* of *the Astroviridae* family (Nuñez and Ferreira, 2013). Its genome is approximately 7 kb in length having three ORFs, it begins with a 5’ untranslated region (UTR) followed by three Open Reading Frames (ORF); ORF1a, ORF1b and ORF2 (Nuñez and Ferreira, 2013). CAstV has been detected from poultry enteritis and RSS from UK, USA, India, Netherland, Korea and Brazil (Day *et al.,* 2007, Pantin-Jackwood *et al.,* 2007, Kaithal *et al.,* 2016; Saraswat *et al.,* 2021; Todd *et al*., 2009; Smyth *et al*., 2009, Kang *et al.,* 2012, Koo *et al.,* 2013 and Nuñez *et al.,* 2016b).

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Present study was aimed to find out the occurrence of CAstV in poultry enteritis cases from unexplored areas of Rajasthan state of India, describe the partial molecular characterization of CAstV and observe gross and microscopic changes in the intestine and liver of the affected birds.

MATERIALS AND METHODS

The present study was conducted at Department of Veterinary Microbiology, College of Veterinary and Animal Science, Navania, Udaipur, Rajasthan. To investigate the

problem of poultry enteritis associated with CAstV, in commercial poultry farms in four districts *viz*. Ajmer, Dungarpur, Sirohi and Pratapgarh of Southern Rajasthan during the period of January 2019 to February 2020. A total of 604 dead birds had signs of enteritis, rough feather and poor weight gain, were included in this study. The birds were subjected to post-mortem and molecular screening for CAstV. Intestine and liver were subjected to histopathological examination.

Post-mortem examination

All visceral organs were examined for gross lesions. Intestine of four birds of same age group were pooled to make one biological sample for molecular testing, while a piece of intestine and liver were collected in 10% buffered formalin for histopathology.

Molecular detection

Pooled Intestinal samples were subjected to molecular detection of the CAstV using RT-PCR. Tissue samples were processed as described (Saraswat, 2019). The supernatants were treated with TRIzol™ reagent (Sigma life science technologies) for the extraction of RNA according to the manufacturer's instruction. For RT-PCR nuclease-free water was used as a negative control. While the CAstV positive samples stored in our laboratory was used as a positive control.

The RNA obtained was subjected to a reverse transcription reaction to obtain complementary DNA (cDNA) as described (Saraswat, 2019), cDNA obtained was submitted to PCR.

Polymerase chain reaction (PCR)

ORF -1b gene specific primers has been used in this study as described (Day *et al*., 2007). With forward and reverse sequences 5′-GAYCARCGAATGCGRAGRTTG-3′ and 5′-TCAGTGGAAGTGGGKARTCTAC-3′ respectively. The PCR reaction mix of 20 µl contained the following components: 0.5 µl of each forward and reverse primers (10 pmol), 10 µl of 2x PCR master mix (Thermo Scientific), 2 µl of cDNA as template and 7 µ nuclease free water. PCR amplification was performed under the following conditions a cycle of 95°C for 5 min as initial denaturation, 35 cycles at 95°C for 30 sec, 51°C (annealing temperature) for 1min and 72°C for 1 min and a cycle of 72°C for 10 min as final extension. The amplified product (362 bp) was submitted to electrophoresis in 1.5% agarose gel.

DNA sequencing and phylogenetic analysis

The amplified products of the ORF1b gene of CAstV from 2 positive samples were purified using Sure Trap Gel Extraction Kit as described by the manufacturer (Genetix biotech Asia Pvt. Ltd.). Each purified product was sequenced in forward and reverse direction using Applied Biosystem by life science technology 3130 XL sequencer (at the Department of Biochemistry, University of Delhi, South Campus, New Delhi).

Nucleotide sequences were edited in Bio-Edit software. The aligned sequences were analysed on the NCBI website http://www.ncbi.nlm.gov using BLAST to confirm their identity. The nucleotide sequences were then aligned using MEGAX version 5 software by CLUSTAL W method, using partial sequences of 16 astroviruses downloaded from the NCBI. The nucleotide phylogenetic tree was inferred using the neighbour-joining method with 1,000 bootstrap replicates that were integrated into MEGA X version 5 software (Saitou and Nei, 1987 and Kumar *et al.,* 2018).

GenBank accession numbers

The sequence data were submitted to the GenBank

Table 1: Sequence comparison of chicken astrovirus sequences CAstV/RAJ NAV 20 CAV1/India/Chicken and CAstV/RAJ NAV 20 CAV2/India/Chicken.

Gene/virus	Avian astrovirus sequences	Country	Gen bank accession no.	Host species	Similarity with nucleotide sequence	Similarity with RDRP protein
Polymerase gene of CAstV	CAstV/GUT475/DPR_IND/2016	India	MF491634	Chicken	97%	100%
	CAstV/PDRC/264/South Zone/2011	India	JX945871	Chicken	95%	100%
	CAstV/PDRC/573/West Zone/2011	India	JX945876	Chicken	95%	99%
	CAstV/INDIA/ANAND/2016	India	KY038163	Chicken	94%	98%
	CAstV/5/16/HR-Ind/2016	India	MF416951	Chicken	95%	98%
	CAstV/1125/2014/HR(India)/2014	India	KT386330	Chicken	96%	100%
	CAstV/1125/2014/HR(India)/2016	India	MF416959	Chicken	94%	98%
	CAstV/1194/2012/HR(India)/2012	India	KT386329	Chicken	93%	95%
	CAstV /USP336-1/Brazil/2009	Brazil	GU585493	Chicken	94%	97%
	CAstV/Brazil/2009/USP358-4	Brazil	GU014472	Chicken	94%	97%
	CAstV/USP401-4B/2010	Brazil	JF309112	Chicken	92%	95%
	CAstV/GA-SEP-A364-05	USA	DQ324839	Chicken	93%	99%
	CAstV/GA-SEP-A368-05	USA	DQ324842	Chicken	93%	97%
	CAstV/GA-SEP-A369-05	USA	DQ324843	Chicken	93%	97%
	CAstV/PL/CK/G038/2013	Poland	KX779113	Chicken	92%	98%
	CAstV/Kr/ADL120203/2012	South Korea	KC593408	Chicken	92%	98%

database and were assigned accession numbers MT263725 and MT263726 (Table 3). Table 1 shows the Gene Bank accession numbers of sequences used for molecular analysis in the present work.

Histopathological examinations

From CAstV positive case, intestine and liver samples preserved in buffered formalin were subjected to histopathological examination by paraffin embedding using acetone and benzene technique (Lillie, 1965 and Luna *et al.,* 1968). Further, the histopathological lesions were analysed using a research microscope at 10x and 40x magnifications.

RESULTS AND DISCUSSION

Samples

A total of 151 pooled Samples from 604 birds were collected from affected flocks of four districts of southern Rajasthan (Table 2).

Molecular detection

All the collected intestinal samples were subjected to molecular detection, by PCR which amplified the ORF1b gene of CAstV and obtained an amplicon of 362 bp in agarose gel electrophoreses as shown in Fig 1. CAstV was detected in 20.52% (31/151) of pooled samples. The highest CAstV occurrence in poultry enteritis was recorded from Ajmer district 37.83% followed by Sirohi 20.83%, Pratapgarh 17.64% and minimum occurrence was recorded as 7.69% in Dungarpur district (Table 2). This variation in prevalence among these districts of Rajasthan may be due to size of chicken flocks reared and number of samples collected. Most of the samples from Ajmer district were collected from commercial poultry farms, containing large number of chickens reared in a close confined area. While from other three districts most of the samples were from small flocks. So the close contact is relatively less and probably low chance of horizontal transmission. In our study, high prevalence of enteric CAstV infection was observed in 0-1 week of age group birds as 19/71 (26.76%) whereas the prevalence among 2-12 weeks of age birds was 12/80 (15%).

Saraswat (2019) reported 3.33% a lower prevalence of CAstV in and around Udaipur district of Rajasthan during 2018-19. High occurrence of CAstV (87.69%) in Haryana state has been reported by Kaithel *et al*. (2016). The variation among various state of same country has also been reported by De la Torre *et al.* (2018) 12 states of Brazil and found CAstV in only two state this shows that occurrence of the virus can vary between regions (De la Torre *et al.,* 2018). Globally various researchers also reported the prevalence of CAstV *i.e.* De la Torre *et al.* (2018) from Brazil 8.1%, Lobani *et al.* (2016) from Jordan 29.7%, Mettifogo *et al.,* (2014) from Brazil 21.1%, Koo *et al.* (2013) from Korea 38.2%. Roussan *et al.* (2012) 38.6% and Pantin-Jackwood *et al.* (2008) reported 86% prevalence of CAstV. Low prevalence in our study may also be due to low titres of

Table 2: District-wise prevalence of enteric CAstV.

Poultry enteric virus	Ajmer (37 pooled samples)		Pratapgarh (51 pooled samples)		Dungarpur (39 pooled samples)		Sirohi (24 pooled samples)		Overall prevalence (151 pooled samples)	
	Positive	Prevalence %	Positive	Prevalence %	Positive	Prevalence %	Positive	Prevalence %	Positive	Prevalence %
CAstV	14	37.83	9	17.64	3	7.69	5	20.83	31	20.52

virus in samples, although to increase the titre of virus in one biological sample we have pooled four birds from same age group and same flocks.

The relation of CAstV and poultry enteritis has been shown by Baxendale and Mebatsion, who conducted an experimental study in which they inoculated orally in day-old specific pathogen free (SPF) white leghorn chicks with the CAstV. The inoculated chicks were developed clinical signs within 4 days such as mild diarrhoea with moderately digested food in faeces and distension of small intestine (Baxendale and Mebatsion, 2004).

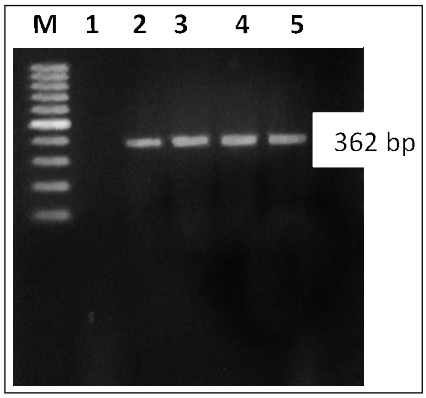

Fig 1: Detection of CAstV by PCR; PCR was carried out to amplify ORF1b gene specific primers of CAstV from enteric samples. Agrpse gel electrophoresis of PCR product: Well no-1: Negative control; 2 -positive control and 3, 4 and 5 are field samples positive for CAstV.

DNA sequencing and phylogenetic analysis of enteric CAstV

The use of a particle-associated nucleic acid sequencing technique led to the identification and sequencing of CAstV, showing the ORF 1b gene to be the most conserved region. The sequences of CAstV from enteritis-affected birds were edited and compared with other sequences from genBank using the BLAST tool. The results showed high similarity with other CAstV. A fragment of 362 bp from sequences of CAstV from the intestine of the affected bird in the present work was compared with other sequences. The Indian sequences of CAstV obtained here revealed a high similarity to the nucleotide (nt) (97%-92%) and amino acid (aa) of (100%-98%) between the sequences. Comparison with sequences from other countries also showed a high similarity of nucleotide (Table 1 and Fig 2). Various authors (Kaithel *et al.,* 2016; De la Torre *et al.,* 2018; Nunez *et al*., 2016b; Koo *et al*., 2013 and Pantin-Jackwood *et al.,* 2006) had also reported similar findings. In the present study, a part of polymerase gene was amplified; determination of the complete genome therefore needs to be considered for genotyping of CAstVs. Though the present study was conducted in a specific region, screening of suspected enteritis cases in large geographical region may help to uncover CAstVs infection circulating in poultry. Further, phylogenetic tree was constructed using neighbour-joining method algorithm, which is a distance based method. The evolutionary relationship among 18 sequences were

Fig 2: Phylogenetic tree based on ORF 1b gene sequences of CAstV. Sequences in bold with GenBank accession numbers are from the present study and the sequences (unbold) with GenBank accession numbers are previously published sequences. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood Method Evolutionary analyses were conducted in MEGA-X.

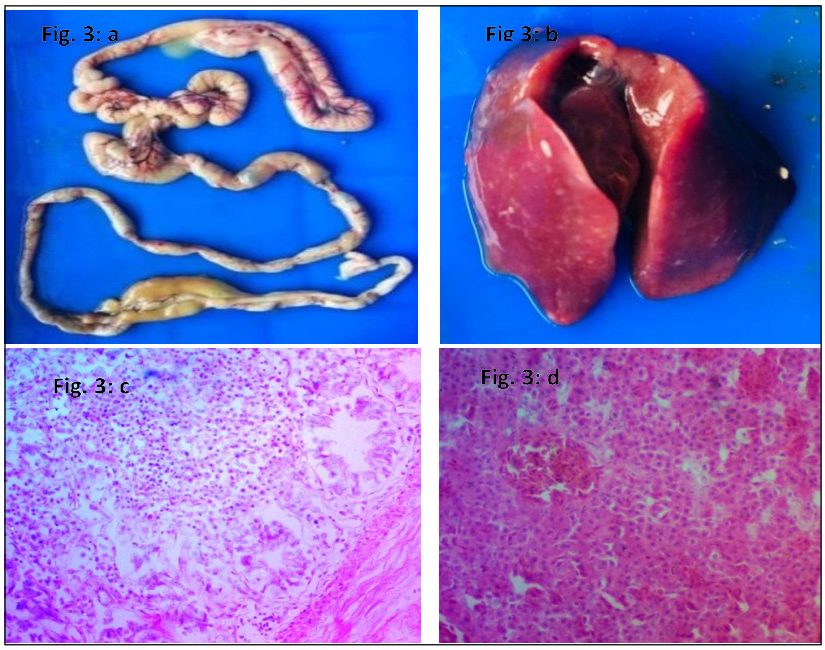

Fig 3: (a) Intestine of 18 days aged enteritis affected bird showing severe haemorrhage. (b) Liver of 10 days aged enteritis affected poultry showing severe haemorrhages and necrotic patches. (c) (H & E 40x) CAstV affected poultry intestine of 18 days aged bird showing severe degeneration of villi, crypts and epithelial cells, severe villous atrophy Infiltration of mononuclear cells in lamina propria, lumen is obliterated with the necrotic debris, atrophy of sub mucosal gland, degenerative layer and cystic submucosal glands. (d) (H & E 40X) Liver of 10 days aged enteritis affected poultry showing mild degenerative changes, severe congestion and haemorrhages noticed; infiltration of inflammatory cells notices near central vein.

Table 3: GenBank accession numbers of the sequences of this study.

Gene/virus strain name	Country	GenBank accession number	Host species
CAstV/RAJ NAV 20 CAV1/India/Chicken	India	MT263725	Chicken
CAstV/RAJ NAV 20 CAV2/India/Chicken	India	MT263726	Chicken

depicted and analysed. It divided majorly in two branches I and II which further subdivided in four clusters namely A, B, C and D. Branch I, which having cluster A and B contains all Indian variants of CAstV including recently submitted sequences. Where, branch II which comprises with cluster C and D having variants from South Korea, Poland, USA and Brazil. These finding suggest the closest phylogenetic relationship of CAstV sequences (MT263726 and MT263725) with sequence (MF491634) submitted from Hyderabad, Telangana, India and other Indian sequences found in cluster A. However, cluster B comprises with single sequence found distantly related with recently submitted sequences, in spite of country-wise origin. They share a common ancestry with other Indian sequences as well sequences found in different countries.

Pathological finding of intestine and liver affected from poultry enteritis

Gross examination of intestine and liver

On gross examination of intestine showed severe congestion with watery fluid and undigested material in the lumen. Some part of intestine showed distension and ballooning with thin transparent in appearance and in liver hepatomegaly, severe congestion with pinpoint necrotic patches. asess andend severe congestion with pinpoint necrotic pathesin transparent appearence (Fig 3a and b).

Histopathological examination of intestine and liver

Histopathological examination of affected intestine reveled mild degenerative changes and necrosis of the mucosal villi with severe infiltration of mononuclear cells into mucosa and submucosa. Submucosal glandes were atrophied and some become cystic. Mild congestion was observed in the muscularis layer (Fig 3c). Liver showed mild to moderate degenerative changes, severe congestion and hemorrhages along with infiltration of inflammatory cells especially mononuclear cells infiltration (Fig 3d). These observations are well in accordance with those described by Rosenberger and Direction (2012); Qamar *et al.* (2013) and Hauck *et al.,* (2016).

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REFERENCES

Baxendale, W. and Mebatsion, T. (2004). The isolation and characterisation of astroviruses from chickens. Avian Pathology. 33(3): 364-370.

Day, J.M., Spackman, E. and Pantin-Jackwood, M. (2007). A multiplex RT-PCR test for the differential identification of Turkey astrovirus type 1, Turkeyastrovirus type 2, CAstV, Avian nephritis virus and Avian rotavirus. Avian Diseases. 51(3): 681-684.

De la Torre, D.I., Nuñez, L.F., Astolfi-Ferreira, C.S. and Piantino Ferreira, A.J. (2018a). Enteric virus diversity examined by molecular methods in Brazilian poultry flocks. Veterinary Sciences. 5(2): 38. doi: 10.3390/vetsci5020038.

Devaney, R., Trudgett, J., Trudgett, A., Meharg, C. and Smyth, V. (2016). A metagenomic comparison of endemic viruses from broiler chickens with runting-stunting syndrome and from normal birds. Avian Pathology. 45(6): 616-629.

Hauck, R., Gallardo, R.A., Woolcock, P.R. and Shivaprasad, H.L. (2016). A coronavirus associated with runting stunting syndrome in broiler chickens. Avian Diseases. 60(2): 528-534.

Kaithal, B., Jindal, N., Kumar, P. and Mor, S.K. (2016). Detection and molecular characterization of enteric viruses in enteritis-affected commercial broiler chickens in India. Acta Virologica. 60(4): 361-371.

Kang, K.I., El-Gazzar, M., Sellers, H.S., Dorea, F., Williams, S.M., Kim, T. *et al.* (2012). Investigation into the aetiology of runting and stunting syndrome in chickens. Avian Pathology. 41(1): 41-50.

Koo, B.S., Lee, H.R., Jeon, E.O., Han, M.S., Min, K.C., Lee, S.B. and Mo, I.P. (2013). Molecular survey of enteric viruses in commercial chicken farms in Korea with a history of enteritis. Poultry Science. 92(11): 2876-2885.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution. 35(6): 1547-1549.

Lillie, R.D. (1965). Histopathological Technique and Practical Histochemistry, 3rd edn., McGraw Hill Book Co., New York and London. 493-495.

Lobani, A.M., Gharaibeh, S.M. and Al-Majali, A.M. (2016). Relationship between different enteric viral infections and the occurrence of diarrhea in broiler flocks in Jordan. Poultry Science. 95(6): 1257-1261.

Luna, L.G. (1968). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd edn., McGraw Hill Book Company. New York, USA, 34-157.

Mettifogo, E., Nuñez, L.F., Chacón, J.L., Santander Parra, S.H., Astolfi-Ferreira, C.S., Jerez, J.A. and Piantino Ferreira, A.J. (2014). Emergence of enteric viruses in production chickens is a concern for avian health. The Scientific World Journal. https://doi.org/10.1155/2014/450423.

Nuñez, L.F.N. and Ferreira, A.P. (2013). Viral agents related to enteric disease in commercial chicken flocks, with special reference to Latin America. World's Poultry Science Journal. 69(4): 853-864.

Nuñez, L.F.N., Parra, S.H.S., Astolfi-Ferreira, C.S., Carranza, C., De La Torre, D.I., *et al.* (2016). Detection of enteric viruses in pancreas and spleen of broilers with runting-stunting syndrome (RSS). Pesquisa Veterinária Brasileira. 36(7): 595-599.

Nuñez, L.F.N., Santander Parra, S.H., Carranza *science*, C., Astolfi-Ferreira, C.S., Buim, M. R. and Piantino Ferreira, A.J. (2016b). Detection and molecular characterization of CAstV associated with chicks that have an unusual condition known as "white chicks" in Brazil. Poultry. 95(6): 1262-1270.

Pantin-Jackwood, M.J., Day, J.M., Jackwood, M.W. and Spackman, E. (2008). Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Diseases. 52(2): 235-244.

Pantin-Jackwood, M.J., Spackman, E. and Woolcock, P.R. (2006). Molecular characterization and typing of chicken and *Turkey astroviruses* circulating in the United States: Implications for diagnostics. Avian Diseases. 50(3): 397-404.

Pantin-Jackwood, M.J., Spackman, E., Michael Day, J. and Rives, D. (2007). Periodic monitoring of commercial turkeys for enteric viruses indicates continuous presence of astrovirus and rotavirus on the farms. Avian Diseases. 51(3): 674-680.

Pantin-Jackwood, M.J., Strother, K.O., Mundt, E., Zsak, L., Day, J.M. and Spackman, E. (2011). Molecular characterization of avian astroviruses. Archives of Virology. 156(2): 235-244.

Qamar, M.F., Aslam, H. and Jahan, N. (2013). Histopathological Studies on Stunting Syndrome in Broilers, Lahore, Pakistan. Veterinary Medicine International. 1-6.

Rosenberger, J. and Direction, C.S. (2012). Update on the Runting-Stunting Syndrome. Ceva Eggs Program Online.

Roussan, D.A., Shaheen, I.A., Khawaldeh, G.Y., Totanji, W.S. and Al-Rifai, R.H. (2012). Simultaneous detection of astrovirus, rotavirus, reovirus and adenovirus type I in broiler chicken flocks. Polish Journal of Veterinary Sciences. 15(2): 337-344.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4(4): 406-425.

Saraswat, A. (2019). Prevalence of enteric viruses of poultry in and around Udaipur city of Rajasthan. M.V.Sc. Thesis submitted to RAJUVAS, Bikaner.

Saraswat, A., Sharma, D.K., Singathia, R., Joseph, B., Patidar, C., Suthar, P. and Singh, G. (2021). Molecular survey of enteric viruses associated with poultry Enteritis in Southern Rajasthan, India. International Journal of Livestock Research. 11(1): 75-81.

Smyth, V.J., Jewhurst, H.L., Adair, B.M. and Todd, D. (2009). Detection of chicken astrovirus by reverse transcriptase-polymerase chain reaction. Avian Pathology. 38(4): 293-299.

Todd, D., Wilkinson, D.S., Jewhurst, H.L., Wylie, M., Gordon, A.W. and Adair, B.M. (2009). A seroprevalence investigation of chicken astrovirus infections. Avian Pathology. 38(4): 301-309.