Indian Journal of Animal Sciences **91** (6): 443–448, June 2021/Article
https://doi.org/10.56093/ijans.v91i6.115441

Pathology and molecular characterization of classical swine fever virus from piggery units in Haryana

P MOUDGIL[1]✉, A PRAKASH[1], N JINDAL[1], D LATHER[1] and R GUPTA[1]

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana 125 004 India

Received: 26 June 2021; Accepted: 21 July 2021

ABSTRACT

Classical swine fever (CSF) is a highly contagious viral disease of pigs and is responsible for significant economic losses due to high morbidity and mortality. Pigs from nine different piggery units in Haryana were investigated for CSF suspected outbreaks during July 2017–June 2019. On the basis of clinical signs, pathology, reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing, the disease in all piggery units was confirmed as CSF. The overall morbidity rate, cumulative mortality and case fatality rate (CFR) due to CSF in these units were 14.3, 9.3% and 65.5%, respectively. Age-wise statistical analysis identified no significant difference in morbidities and mortalities among three age groups, i.e. adult, young and piglets. However, there was a significant difference in CFR with highest among piglets (79.3%) followed by young (68.9%) and adults (45.1%). Seasonal analysis revealed highest cumulative mortality and CFR in winter (29.9% and 91.1%, respectively) followed by rainy season (24.3% and 69.9%, respectively). The findings of the present study are of significant veterinary importance to check the dissemination of CSFV by prompt diagnosis which would help in imposing control measures for minimizing the losses suffered by the piggery units of Haryana, India.

Keywords: Classical swine fever, Histopathology, Molecular characterization, Outbreaks, Pigs

Classical swine fever (CSF) is one of the most significant notifiable diseases of swine. This is because of its rapid spread, enormous economic losses along with significant impact on international trade (Ganges *et al.* 2020). CSF has been considered as a transboundary animal disease by Food and Agriculture Organization (Chowdhuary *et al.* 2020). It is a highly contagious disease that affects domestic and wild swine of all age groups with high morbidity, mortality, stunted growth and poor reproductive performance (Leifer *et al.* 2010, Singh *et al.* 2018). The etiological agent of the disease is classical swine fever virus (CSFV), a member of the genus Pestivirus, family Flaviviridae (Smith *et al.* 2017).

CSF is endemic in swine population of Eastern Europe, South America, Africa and Asia (Brown and Bevins 2018) and it remains a major threat to pigs in South Asia, including India (Sarkar *et al.* 2018). The disease has been reported from most of the Indian states where pig rearing is practiced (Patil *et al.* 2010, Nandi *et al.* 2011, Singh *et al.* 2018). During the year 2000–2015, a total of 611 outbreaks of CSF were reported in India (Patil *et al.* 2018). The annual economic loss due to CSF outbreaks in India was reported to be INR 9.085 million (Singh *et al.* 2016a).

Present address: [1]College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana.
✉Corresponding author email: pallavi.moudgil@luvas.edu.in

The fast and accurate laboratory diagnosis of CSF is extremely essential due to the contagious nature of the disease. Early diagnosis also helps in reducing the spread of infection to the uninfected herds. Also, effective prophylactic measures are required to control the disease for proficient growth of the piggery industry. Thus, the present study was envisaged with an aim to perform clinico-pathological investigations, analysis of risk factors, i.e. age and season and molecular characterization of CSFV from suspected outbreaks in piggery units in Haryana.

MATERIALS AND METHODS

Risk factors, clinical findings and post-mortem examination: The disease outbreaks were investigated in nine different piggery units located in five districts (Jhajjar, Rewari, Hisar, Charkhi Dadri and Sirsa) of Haryana during the period July 2017–June 2019. The risk factors [age (piglets: from birth to 3 weeks, young: >3 weeks to ≤26 weeks, adults: >26 weeks) and season (summer, winter, spring and rainy)] of the outbreaks as well as association between risk factors and vital measures was analysed statistically. The clinical signs exhibited by affected pigs were recorded. Detailed necropsy of pigs was conducted and various changes in the organs like lungs, heart, liver, kidneys, lymph nodes, intestine, spleen and tonsils were recorded. Representative samples were collected for histopathology and molecular identification.

Histopathology: Representative tissue specimens (liver, heart, lungs, kidneys, intestine, spleen and lymph nodes) were collected in 10% buffered formalin. The formalin fixed tissues were processed by paraffin embedding technique and were cut serially at a thickness of 3–4 µm and stained with haematoxylin and eosin stain (Luna 1968).

Reverse transcription-polymerase chain reaction (*RT-PCR) amplification:* Total RNA was extracted from tissue (liver, heart, lungs, kidneys, intestine, spleen and lymph nodes) and blood samples using QIAmp Viral RNA Mini Kit (Qiagen, Germany) as per the manufacturer's instructions. The purity and quantification of extracted RNA was determined by spectrophotometer (Biophotometer Plus, Eppendorf, Germany). Reverse transcription was carried out with RevertAid^{TM} first strand cDNA synthesis kit (Thermo Scientific). The obtained cDNA was subjected to PCR amplification using protocol and primers (specific to *p120* gene of CSFV) of Harding *et al*. (1996). PCR amplification of target genome fragments was carried out in 25 µL reaction volume: 5 µL cDNA, 12.5 µL of 10 × Top Taq master mix (Qiagen), and 0.5 µL of 50 picomole of forward and reverse primer each adjusted to final volume with nuclease free water.

Positive control (CSF vaccine) and non-template control was included in each run. Amplified products were gel electrophoresed in 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml) along with 100 bp molecular weight marker.

Sequencing and phylogenetic analysis: Virus-positive PCR products were purified using Gel extraction kit (Qiagen) and then got custom sequenced in both directions (Thermo Fisher Scientific, India). The CSFV sequences of this study were subjected to BLAST analysis to confirm the virus identity. The sequences were aligned and compared with CSFV sequences retrieved from GenBank database. The sequences were submitted to GenBank using web-based sequence submission tool 'BankIt'. The sequence alignment was carried out using Clustal W algorithm available in MEGA X programme (Kumar *et al.* 2018). The evolutionary relationship was determined by constructing a phylogenetic tree with bootstrap value of 1000 replicate using Neighbor-Joining method in MEGA X programme.

Statistical analysis: Vital measures of disease (morbidity, cumulative mortality and case fatality rate) and their 95% confidence intervals (CIs) were calculated using STATA^{TM}/IC 15.1 (Stata Corp, College Station, TX) with clustered standard error, outbreak being the cluster variable. Modified Chi square test for clustered data as described by Rao and Scott (1992) was conducted using Microsoft Office 10 Excel software with some modifications (in case of single herd in one cluster one dummy herd was added without altering the proportion) to ascertain the association between risk factors (age and season) and vital measures of the disease. A P value <0.05 was considered as significant.

RESULTS AND DISCUSSION

Risk factors analysis: Nine piggery units in five districts of Haryana were investigated during the study period. Out of 1628 pigs in these nine units, 232 (adults: 71, young: 74, piglets: 87) were clinically affected with 152 (adults: 32, young: 51, piglets: 69) deaths. The overall morbidity rate, cumulative mortality and CFR due to CSF in these units were 14.3%, 9.3% and 65.5, respectively (Table 1). Risk factor analysis identified no significant difference in morbidities and mortalities among three age groups (P>0.05). However, significant difference was observed in CFR (P<0.05) with this parameter being the highest among piglets (79.3%) followed by young (68.9%) and adults (45.1%) (Table 1). Season-wise analysis of the data revealed significant differences in morbidity, mortality, and CFR (P<0.05) with highest mortality and CFR in winter season (29.9% and 91.1%, respectively) followed by rainy season (24.3 and 69.9%, respectively) (Table 2). However, summer and spring seasons evinced significantly lower mortality and CFR of 0.4% and 40% and 4.2% and 43.5%, respectively (Table 2).

In a study conducted by Gupta *et al.* (1986), morbidity and mortality rates due to CSF in Haryana ranged from 8.9–100% and 4.2–57.1%, respectively; whereas in another study, the overall morbidity rate, mortality rate and CFR during the outbreaks in piggery units in Ambala and Hisar districts of Haryana were 54.9, 36.6 and 66.6%, respectively (Jindal *et al.* 2008). The overall morbidity and mortality in these two studies are higher as compared to the present study but CFR observed in the present study is comparable to that reported by Jindal *et al.* (2008). Also, the variability in these indices in the current study could be explained by the fact that no follow up was done and values of epidemiological indices in the present study represented one time data. Actual morbidity and mortality due to the disease may vary. Higher mortality and CFR in young pigs and piglets as compared to adults could be attributed to the different pathogenicity and virulence of virus in different age groups as the dose causing nearly 70% mortality in young animals may cause nearly asymptomatic infection in adults (Brown and Bevins 2018). Significant difference in % mortality and CFR in different seasons could be due to fact that in winter season animals have more close contact with each other particularly during night time and the low environmental temperature favours long survival of the virus (up to one month) in the contaminated pens (Blome *et al.* 2017).

Clinical and post-mortem findings: The disease in the affected pigs in all nine piggery units was characterized by high rise of body temperature (103–106ºF), anorexia, and bluish discolouration of skin around nostrils, abdomen, legs, tail, snout and eyes, swaying movement of the hind quarters, respiratory distress and death. However, nervous signs were observed in only two piggery units. The course of the disease varied between 2–7 days. Gross post-mortem examination revealed purple discoloration of the skin of the ears, neck, hock, abdominal and inguinal region, pneumonic lungs, congestion of spleen and mesenteric lymph nodes and petechial haemorrhages on heart. The

Table 1. Epidemiological data of classical swine fever outbreaks investigated

Outbreak No.	Age of population	Total population	Affected	Died	Morbidity (%) (95% CI)	Mortality (%) (95% CI)	CFR (%) (95% CI)
1	Total	450	5	2	1.11 (0.36–2.57)	0.44 (0.05–1.6)	40 (5.27–85.34)
	Adults	300	5	2	1.67 (0.54–3.85)	0.67 (0.08–2.39)	40 (5.27–85.34)
	Young	150	0	0	0 (0–2.43)	0 (0–2.43)	–
2	Total	195	31	19	15.9 (11.06–21.8)	9.74 (5.97–14.8)	61.29 (42.19–78.15)
	Adults	45	7	4	15.56 (6.49–29.46)	8.89 (2.48–21.22)	57.14 (18.41–90.1)
	Young	150	24	15	16.00 (10.13–21.87)	10.00 (5.20–14.80)	62.5 (40.59–81.2)
3	Total	135	13	6	9.63 (5.23–15.9)	4.44 (1.65–9.42)	46.15 (19.22–74.87)
	Adults	40	0	0	0 (0–8.81)	0 (0–8.81)	–
	Young	60	3	1	5 (1.04–13.92)	1.67 (0.04–8.94)	33.33 (0.84–90.57)
	Piglets	35	10	5	28.57 (14.64–46.3)	14.29 (4.81–30.26)	50 (18.71–81.29)
4	Total	15	8	7	53.33 (26.59–78.73)	46.67 (21.27–73.41)	87.5 (47.35–99.68)
	Adults	5	3	2	60 (14.66–94.73)	40 (5.27–85.34)	66.67 (9.43–99.16)
	Young	10	5	5	50 (18.71–81.29)	50 (18.71–81.29)	100 (47.82–100)
5	Total	130	82	60	63.08 (54.17–71.37)	46.15 (37.38–55.11)	73.17 (62.24–82.36)
	Adults	50	17	10	34 (21.21–48.77)	20 (10.03–33.72)	58.82 (32.92–81.56)
	Young	30	25	20	83.33 (65.28–94.36)	66.67 (47.19–82.71)	80 (59.3–93.17)
	Piglets	50	40	30	80 (66.28–89.97)	60 (45.18–73.59)	75 (58.8–87.31)
6	Total	25	6	3	24 (9.36–45.13)	12 (2.55–31.22)	50 (11.81–88.19)
	Adults	15	6	3	40 (16.34–67.71)	20 (4.33–48.09)	50 (11.81–88.19)
	Young	10	0	0	0 (0–30.85)	0 (0–30.85)	–
7	Total	106	20	11	18.87 (11.92–27.62)	10.38 (5.3–17.81)	55 (31.53–76.94)
	Adults	36	3	1	8.33 (1.75–22.47)	2.78 (0.07–14.53)	33.33 (0.84–90.57)
	Young	70	17	10	24.29 (14.83–36.01)	14.29 (7.07–24.71)	58.82 (32.92–81.56)
8	Total	122	37	34	30.33 (22.33–39.3)	27.87 (20.13–36.71)	91.89 (78.09–98.3)
	Adults	42	0	0	0 (0–8.41)	0 (0–8.41)	–
	Piglets	80	37	34	46.25 (35.03–57.76)	42.5 (31.51–54.06)	91.89 (78.09–98.3)
9	Total	450	30	10	6.67 (4.54–9.38)	2.22 (1.07–4.05)	33.33 (17.29–52.81)
	Adults	300	30	10	10 (6.85–13.97)	3.33 (1.61–6.04)	33.33 (17.29–52.81)
	Piglets	150	0	0	0 (0–2.43)	0 (0–2.43)	–
	Total	1628	232	152	14.25 (4.87–35.07)	9.34 (2.69–27.76)	65.52 (47.75–79.80)
	Adults	833	71	32	8.52 (3.40–19.79)	3.84 (1.37–10.34)	45.07 (30.82–60.18)
	Young	480	74	51	15.42 (4.91–39.16)	10.63 (3.13–30.44)	68.92 (54.21–80.60)
	Piglets	315	87	69	27.62 (4.96–73.62)	21.90 (4.09–64.83)	79.31 (58.81–91.14)
P value					0.2431	0.076	0.037

Table 2. Seasonal pattern of classical swine fever outbreaks in piggery units of Haryana, India

Season	No. of outbreaks	Total population	Affected	Died	Morbidity % (95% CI)	Mortality % (95% CI)	CFR % (95% CI)
Spring	4	716	69	30	9.64 (5.20–17.17)	4.19 (1.66–18.18)	43.48 (30.62–57.28)
Summer	1	450	5	2	1.11 (0.36–2.57)	0.44 (0.05–1.60)	40.00 (5.27–85.33)
Rainy	2	325	113	79	34.77 (8.66–74.98)	24.31 (5.85–62.41)	69.91 (61.16–77.42)
Winter	2	137	45	41	32.85 (25.60–41.01)	29.93 (23.99–36.62)	91.11 (88.63–93.10)
Total	9	1628	232	152	14.25 (12.59–16.04)	9.34 (7.97–10.85)	65.52 (59.02–71.62)
P value					<0.0001	<0.0001	<0.0001

petechial haemorrhages were also observed in subcapsular region of kidney in some of the succumbed pigs. Kidney upon cross-section showed congestion at the cortico-medullary junction (Fig. 1 a, b, c).

The typical clinical signs of CSF exhibited by the affected pigs were also reported by various workers in India as well as abroad (Saini *et al.* 2000, Freitas *et al.* 2012, Malswamkima *et al.* 2015, Sarkar *et al.* 2018, Borca *et al*. 2019). The clinical signs generally depend upon the virulence of the strain, host responses and secondary bacterial infections (Blome *et al.* 2017). The clinical findings and the course of the disease as observed in this study was suggestive of acute nature of disease.

Histopathological examination of tissues: Histopathologically, the disease was characterized by vascular (congestion and haemorrhages) and necrotic changes in different organs (spleen, kidney, lymph nodes, liver, and heart) and severe bronchopneumonia of lungs.

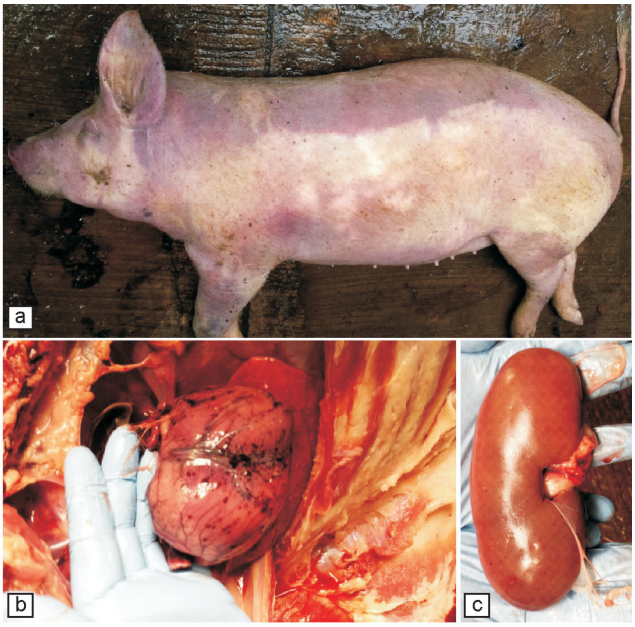

Fig. 1. Gross examination of pig affected with classical swine fever. (a) Purple discolouration of skin; (b) Epicardial haemorrhages; (c) Petechial haemorrhages on kidney.

On microscopic examination, spleen revealed severe haemorrhages, infarcts, hemosiderosis in red pulp, depletion of lymphocytes in white pulp zone and area of coagulative necrosis of lymphocytes in the marginal centres (Fig. 2a and b). Kidneys revealed areas of focal congestion and haemorrhages in the cortical and medullary blood vessels with necrosis of tubular epithelial cells and mild infiltration of leucocytes at places. Lung sections revealed marked emphysema along with severe fibrinous broncho-pneumonia, presence of serofibrinous exudates with infiltration of neutrophils, lymphocytes and macrophages in alveolar lumen and interstitium along with congested pulmonary blood vessels and haemorrhages with marked hemosiderosis. Proliferation of peribronchiolar lymphocytes was also evident in some sections (Fig. 2c). Mediastinal and mesenteric lymph nodes showed acute lymphadenitis characterized by congestion and haemorrhages, and depletion of lymphocytes in the cortical areas with cystic spaces and reticular cell hyperplasia (Fig. 2d). Liver sections revealed centrilobular necrosis of hepatocytes and severe telangiectasis, haemorrhages and infiltration of leucocytes particularly lymphocytes in portal areas. Heart revealed congestion, haemorrhages, thrombosis and coagulative necrosis and fragmentation of myocardial muscle fibres.

The observations of the present study pertaining to the pathological changes are in corroboration with the findings of previous studies (Rout and Saikumar 2012, Malaswamkima *et al.* 2015, Singh *et al.* 2016b, Sangeetha *et al.* 2018, Izzati *et al.* 2021). The pathological findings in CSF directly depend upon the course of the viral infection. In acute course, the lymph nodes, spleen and vital organs are involved with splenic infarction being the pathognomonic lesion (Van 1999), as observed in the present study. The infected piglets exhibit immuno-suppression, leukopenia and thrombocytopenia, further substantiating to secondary infections (Brown and Bevins 2018).

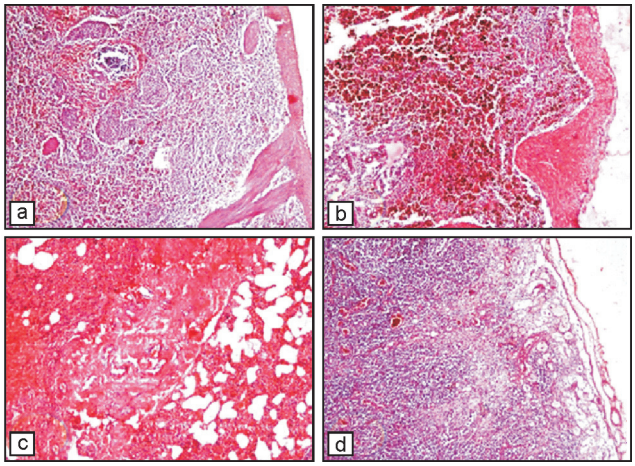

Fig. 2. Histopathological examination of tissues of CSFV affected pigs. (a) Spleen section showing areas of coagulative necrosis of lymphocytes particularly at the marginal regions were evident along with reticuloendothelial proliferation; (b) Spleen section revealed severe hemorrhages, hemosiderosis in red pulp areas and depletion of lymphocytes in white pulp zone; (c) Lung section revealed marked emphysema along with severe fibrinous bronchopneumonia, presence of serofibrinous exudates and infiltration of neutrophils, lymphocytes and macrophages in alveolar lumen and interstitium; (d) Lymph nodes section showing acute lymphadenitis characterized by congestion and haemorrhages and depletion of lymphocytes in the cortical areas with cystic spaces and reticular cell hyperplasia.

RT-PCR and sequence analysis: The tissue and blood samples from all suspected outbreaks were positive for CSFV by RT-PCR assay, confirming the presence of disease in all nine piggery units. All positive samples yielded a 508 bp amplicon specific to *p120* gene (Fig. 3). The sequences of three PCR products were aligned using Clustal W programme and phylogenetic tree was created using Neighbor-joining method (Fig. 4). The phylogenetic analysis revealed that three viral isolates of this study (GenBank accession numbers: MT514515, MT514516 and

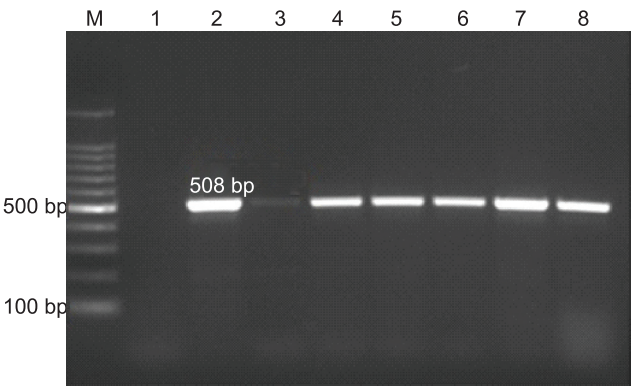

Fig. 3. RT PCR amplification targeting *p120* gene of CSFV. M, 100 bp ladder; Lane 1, Negative control; Lane 2, CSF vaccine control; Lane 3–8, Suspected outbreak samples.

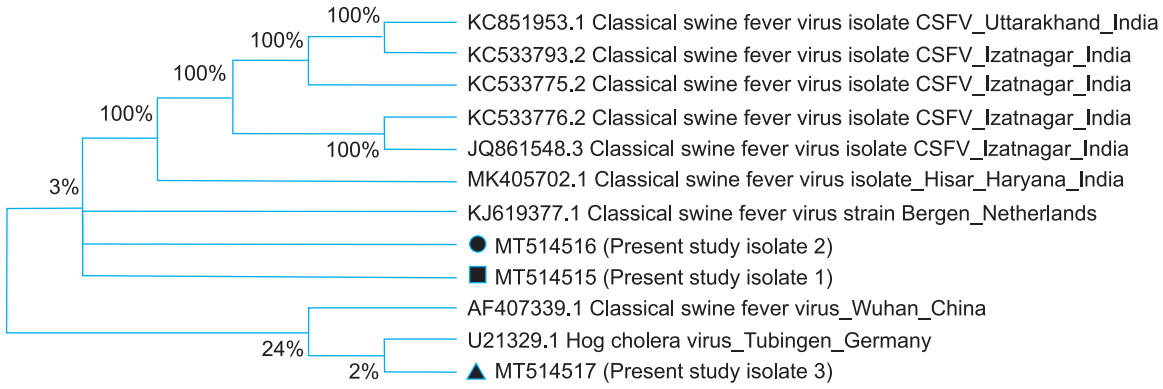

Fig. 4. Phylogenetic tree based on partial nucleotide sequences of *p120* gene (corresponding to nucleotide positions 674–1040 of reference strain Alfort and Brescia strains, Accession no. JX964755) of Hog Cholera virus (CSFV). Phylogenetic tree was constructed by the neighbour-joining method using 1000 bootstrap replicates value in Mega X software.

MT514517) clustered in a separate group and two (MT514515 and MT514516) of the three viral isolates had 100% similitude to each other. Hence, the isolates with accession number MT514515 and MT514516 showed similitude with the sequences having accession numbers KC851953.1, KC533793.2, KC533775.2, KC533776.2, JQ861548.3, MK405702.1 and KJ619377.1.

The nucleotide sequence homology exhibited by the CSFV isolates of this study with the sequences retrieved from GenBank was more than 98% and exhibited higher similitude with the isolates (published in NCBI database) belonging to subgenotype 2.2 (Fig. 4). All the aforementioned CSFVs were retrieved from pigs of North India, i.e. from Uttarakhand, Izatnagar (Uttar Pradesh) and Hisar (Haryana) except one isolate from Netherlands (KJ619377.1). The third isolate with accession number MT514517 exhibited similitude with the isolates from Wuhan, China (Accession number: AF407339.1) and Tubingen, Germany (Accession number: U21329.1).

The *p120* is highly conserved non-structural gene and valuable in characterization of CSFV and discriminating between porcine and ruminant pestiviruses (Harding *et al.* 1996) and hence was also targeted in the present study. CSFV has one serotype with three major genotypes (1, 2 and 3) and 10 subgenotypes (1.1, 1.2, 1.3; 2.1, 2.2, 2.3; 3.1, 3.2, 3.3, and 3.4) (Ganges *et al.* 2020). Based on phylogenetic analysis, the Indian isolates of domestic pigs are grouped into two subgenotypes, 1.1 and 2.2; whereas the wild boars exhibited the presence of genotypes of strain 2.1 (Rajkhowa *et al.* 2014). The sequences retrieved in the present study exhibited homology with the sequences of the GenBank, which are grouped under the subgenotype 2.2, which is in accordance with the findings of Rajkhowa *et al.* (2014).

Detection and characterization of CSFV is of paramount importance as this is a leading disease in swine affecting the livelihood of small and marginal farmers. The impact of the disease is not only measured in term of mortality but also in terms of high morbidity and loss of production potential of pigs.

The present study analyzed age and season as risk factors for CSF outbreaks, as well as pathology and molecular characterization of CSFV strains circulating in Haryana. The findings of the present study are of significant veterinary importance to check the dissemination of CSFV by prompt diagnosis which would help in imposing control measures for minimizing the losses suffered by the piggery units of Haryana, India.

ACKNOWLEDGEMENTS

Authors are thankful to the Director of Research, Lala Lajpat Rai University of Veterinary and Animal Sciences to provide the necessary facilities to carry out the study.

REFERENCES

Blome S, Staubach C, Henke J, Carlson J and Beer M. 2017. Classical swine fever—an updated review. *Viruses* **9**(86): 1–25.

Borca M V, Vuono E A, Ramirez Medina E, Azzinaro P, Berggren K A, Singer M, Rai A, Pruitt S, Silva E B, Velazquez Salinas L, Carrillo C and Gladue D P. 2019. Structural glycoprotein E2 of classical swine fever virus interacts with host protein dynactin subunit 6 (DCTN6) during the virus infectious cycle. *Journal of Virology* **94** (1): e01642–19.

Brown B R and Bevins S N. 2018. A review of classical swine fever virus and routes of introduction into the United States and the potential for virus establishment. *Frontiers in Veterinary Science* **5**: 31.

Chowdhary M G A, Islam S K S, Khair A, Hossain M M, Ahmed G, Brum E, Debnath N C, Badhy S C, Habib M A, Rumi T B, Rahman A A and Ward M P.2020. A further outbreak of classical swine fever in indigenous pigs in Kurigram district, Bangladesh. *Transboundary Emerging Diseases* **67**: 1922–29.

Freitas T R P, da Souza A C, Esteves E G and da Paula Lyra T M. 2012. Classical swine fever in Brazil: An update. *Journal of Agricultural Science and Technology* **2**(8A): 997.

Ganges L, Crooke H R, Bohorquez J A, Postel A, Sakoda Y, Becher P and Ruggli N. 2020. Classical swine fever virus: the past, present and future. *Virus Research* **289**:198151.

Gupta S C, Singh J, Kulshreshtha R C, Kaushik R K and Arora A K. 1986. Prevalence of swine fever in pigs in Haryana. *Indian Journal of Virology* **2**: 102–03.

Harding M J, Prud'homme I, Gradil C M, Heckert R A, Riva J, McLaurin R, Dulac G C and Vydelingum S. 1996. Evaluation

of nucleic acid amplification methods for the detection of hog cholera virus. *Journal of Veterinary Diagnostic Investigation* **8**: 414–19.

Izzati U Z, Hoa N T, Lan N T, Diep N V, Fuke N, Hirai T and Yamaguchi R. 2021. Pathology of the outbreak of subgenotype 2.5 classical swine fever virus in northern Vietnam. *Veterinary Medicine and Science* **7**(1): 164–74.

Jindal N, Sharma P C, Mittal D, Tiwari A K, Narang G and Shukla C L. 2008. Occurrence of swine fever in vaccinated piggery units in Haryana: Detection by RT-PCR. *Indian Journal of Virology* **19**: 44–46.

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**: 1547–49.

Leifer I, Hoffmann B, Hoper D, Rasmussen T B, Blome S, Strebelow G, Horeth-Bontgen D, Staubach C and Beer M. 2010. Molecular epidemiology of current classical swine fever virus isolates of wild boar in Germany. *Journal of General Virology* **91**(11): 2687–97.

Luna L G. 1968. *Manual of Histologic Staining Method of Armed Forces Institute of Pathology*. 3rd edn. Mc Graw Hill Book Company, New York.

Malswamkima D, Rajkhowa T K, Chandra R and Dutta TK. 2015. Pathology and molecular diagnosis of classical swine fever in Mizoram. *Veterinary World* **8**(1): 76.

Nandi S, Muthuchelvan D, Ahuja A, Bisht S, Chander V, Pandey A B and Singh R K. 2011. Prevalence of classical swine fever virus in India: A 6 year study (2004–2010). *Transboundary and Emerging Diseases* **58**(5): 461–63.

Patil S S, Hemadri D, Shankar B P, Raghavendra A G, Veeresh H, Sindhoora B, Chandan S, Sreekala K, Gajendragad M R and Prabhudas K. 2010. Genetic typing of recent classical swine fever isolates from India. *Veterinary Microbiology* **141**: 367–73.

Patil S S, Suresh K P, Saha S, Prajapati A, Hemadri D and Roy P. 2018. Meta-analysis of classical swine fever prevalence in pigs in India: A 5-year study. *Veterinary World* **11**(3): 297–303.

Rajkhowa T K, Hauhnar L, Lalrohlua I and Mohanarao G J. 2014. Emergence of 2.1. subgenotype of classical swine fever virus in pig population of India in 2011. *Veterinary Quarterly* **34**: 224–28.

Rao J N and Scott A J. 1992. A simple method for the analysis of clustered binary data. *Biometrics* **48**: 577–85.

Rout M and Saikumar G. 2012. Pathology of classical swine fever in slaughtered pigs. *Indian Journal Veterinary Pathology* **36**(2): 136–42.

Saini S S, Dhand N K, Sharma D R and Sood N K. 2000. An outbreak of swine fever in Punjab. *Indian Journal Veterinary Pathology* **24**: 135–36.

Sangeetha R, Hemalatha S, Ramesh A, Vijayarani K and Kumanan K. 2018. A classical swine fever outbreak-A recent postulation of laboratory pathogenicity. *Indian Veterinary Journal* **95**: 9–13.

Sarkar S, Hossain M E, Gurley E S, Hasan R and Rahman M Z. 2018. An outbreak of classical swine fever in pigs in Bangladesh, 2015. *Veterinary Medicine and Science* **4**(1): 45–52.

Singh B, Bardhan D, Verma M R, Prasad S, Sinha D K and Sharma V B. 2016a. Incidence of classical swine fever in pig in India and its economic valuation with a simple mathematical model. *Animal Science Reporter* **10**: 3–9.

Singh N, Mahajan V B, Kaur A, Bhat P, Leishangthem G, Banga H S and Brar R S. 2016b. Localization of classical swine fever virus antigen in young piglets by immunohistochemistry. *Indian Journal Veterinary Pathology* **40**: 359–61.

Singh V K, Rajak K K, Kumar A and Yadav S K. 2018. Classical swine fever in India: current status and future perspective. *Tropical Animal Health and Production* **50**: 1181–91.

Smith D B, Meyers G, Bukh J, Gould E A, Monath T, Muerhoff A S, Pletnev A, Rico-Hesse R, Stapleton J T, Simmonds P and Becher P. 2017. Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae. *Journal of General Virology* **98**: 2106–12.

Van Oirschot J T. 1999. *Classical Swine Fever (Hog Cholera), 8th edn*, pp. 159–72. (Eds) Straw B E, D'Allaire S, Mengeling W L and Taylor D J. Iowa State University Press, Ames, IA, USA.