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Review Article
Diagnosis and Control of Foot and Mouth Disease (FMD) in Dairy Small Ruminants; Sheep and Goats

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Abstract

Foot and Mouth Disease (FMD) is one of the most contagious viral diseases of mammals that have an ability to cause high economic losses in susceptible cloven-hoofed animals. In addition to, losses in the milk productions occurred in the form of sudden and severe drop of milk yield. The aim of the present work was to throw light on the different methods for diagnosis and control of FMD that affect dairy small ruminants; sheep and goats. Sheep and goats play a role in the FMD epidemiology, as they become carriers and act as reservoirs of infection. Diagnosis of FMD achieved by many techniques such as virus isolation, Sandwich ELISA, Multiplex PCR, indirect ELISA (DIVA) and real time PCR. Virus isolation onto cell culture is considered as the "gold standard" technique for FMD diagnosis. Moreover, detecting of antibodies against the non-structural proteins (NSPs) of FMD using indirect-ELISA were successful for differentiation between infected and vaccinated animals (DIVA). Differentiation of the infected from the vaccinated animal is of great importance in the control program of FMD. The control program depends mainly on vaccination, treatment, effective quarantine measures, disinfection and hygiene and sanitation measures. Treatment protocols of small ruminants are showing typical clinical symptoms of FMD achieved by the use of antipyretic and analgesic medicine and a broad-spectrum long-acting antibiotic. The inactivated FMDV vaccine succeeded in reducing the outbreaks worldwide. It gives protection for all ruminants against FMDV for 1 year. Foot and mouth disease have the ability to cause milk production losses in small ruminants. Recent diagnostic tools urgently needed not only for the diagnosis, but also for following-up combating programs and control of FMD.

Key words: Foot and mouth disease, PCR, virus isolation, cloven-hoofed animals vaccines, analgesic medicine, long-acting antibiotic, non-structural proteins

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INTRODUCTION

Foot and Mouth Disease (FMD) is responsible for considerable economic losses through deaths in young animals or decrease in animal weight gain and milk production. The losses in milk production occurred due to severe and sudden drop in milk yield. Although, most adult livestock are able to recover clinically in 2-3 weeks, the re-establishment of the milk production level prior to FMD onset may require longer period resulting in severe economic losses[1]. Milk yield of different species and breeds of livestock studied before and after FMD infection. The FMD causes about 20 and 44% milk yield loss in cows and 19% losses in sheep. Moreover, FMD induced milk yield depression in Holstein, cross and local breed cows in rates of 37, 17 and 5%, respectively[2,3].

Viruses represent serious threats to animal health. Consequently, early and quick diagnosis and identification of viral pathogens are essential. The diagnosis of viral diseases are important in the determining of the control strategies. In addition to the identification of the prevalence of viruses in different forms such as; serotypes or isolates are important. Many viral diseases such as; foot and mouth disease virus, peste des petits virus, bluetongue virus and infectious bovine rhinotracheitis and herpes viruses were continually inducing serious economic complications in the field[4-8]. Several molecular-based techniques like Polymerase Chain Reaction (PCR), probe hybridization[9] and nucleic acid sequencing were widely used for this purpose[10,11]. Foot and mouth disease is one of the most contagious viral diseases of mammals that have the ability to cause high economic losses in susceptible cloven-hoofed animals[12].

In Egypt, the FMDV outbreaks have been reported[13] since 1950. Several foci detected during 1961-1970 and all infections were caused[14] by strain O1, but in 2011, serotype A was isolated from Egyptian cattle in addition to the serotype[15] O. In 2012, FMDV SAT2 reported in cattle and buffaloes in six outbreaks in 8 governorates in Egypt[16]. This FMDV serotype (SAT2) spread throughout Egypt, Libya and Palestine. Phylogenetic analysis of the isolated serotype (SAT2) showed that the circulating FMDV is genetically related to the isolated serotype from Saudi Arabia, Sudan, Eritrea and Cameroon[10,17] between 2000 and 2010. A more recent outbreak belongs to the A/AFRICA/G-IV lineage occurred in Egypt, during 2016, the genetic investigation revealed its relationship to the collected[16] viruses in Nigeria 2015 and Cameroon 2013.

Diagnosis of viral diseases depends on clinical signs, epidemiology, pathological lesions and specific detection of the viral antigen, viral genome or the specific antibodies in the tested samples by different serological tests and nucleic acid based assays[18]. The most common used diagnostic techniques are culture isolation, agar gel immunodiffusion (AGID), hemagglutination (HA) tests, immunocapture enzyme-linked immunosorbent assay (IC-ELISA) and competitive ELISA[19]. In addition to the previous techniques, Virus Neutralization Test (VNT) and reverse transcriptase polymerase chain reaction (RT-PCR) are used[20,21]. The control of FMD is not only necessary for reducing economic losses of the disease but also essential for increasing livestock production. The FMD control can also open up the new chance of export as it is a trans-boundary disease limit export of farm animal's products from the country [22]. The disease is endemic in Egypt as well as Africa, so the goal was to clarify the vision to reach a stage of control of the disease and declare those areas initially free of the disease with vaccination. Therefore, the aim of the current study was to throw light on the diagnosis and control of FMD that infect dairy small ruminants; sheep and goats.

DIAGNOSIS OF FOOT AND MOUTH DISEASE

Clinical picture: The diseased animals showed a prompt and severe lameness. So, the animals tend to set down frequently and have unwillingness to rise or move. Blisters found on the hoof, dental pad and sometimes tongue[23,24]. Small ruminants considered as the maintenance host for the FMD virus, not the propagative host like pigs. The clinical signs of FMD in sheep and goats are not severe like that of cattle and buffaloes and include high fever (41°C), salivation, decrease in food consumption and milk production, inability to move, oral vesicles, interdigital spaces ulcers, lesions on the dental pad and death of young animals (Fig. 1). In sheep and goats, the most frequent clinical sign is lameness. Affected animals develop fever, show reluctance to walk and may separate itself from the rest of the flock[25]. The incubation period of FMD in small ruminants is about 3-8 days. About 25% of the affected sheep do not develop vesicles and 20% have lesions only at one site or develop visible vesicles that last for few days. Vesicles may also observe on the teats especially of milking sheep and goats affecting milk yield and rarely on the vulva and prepuce[26]. Ewes may abort. unweaned lambs and kids usually die due to heart failure[18,27].

Post-mortem examinations: The postmortem findings associated with FMD include vesicles and erosions of the mucous membranes of the mouth, rumen, teats and interdigital spaces, ulcers and lesions on the dental pad (Fig. 2)[28].

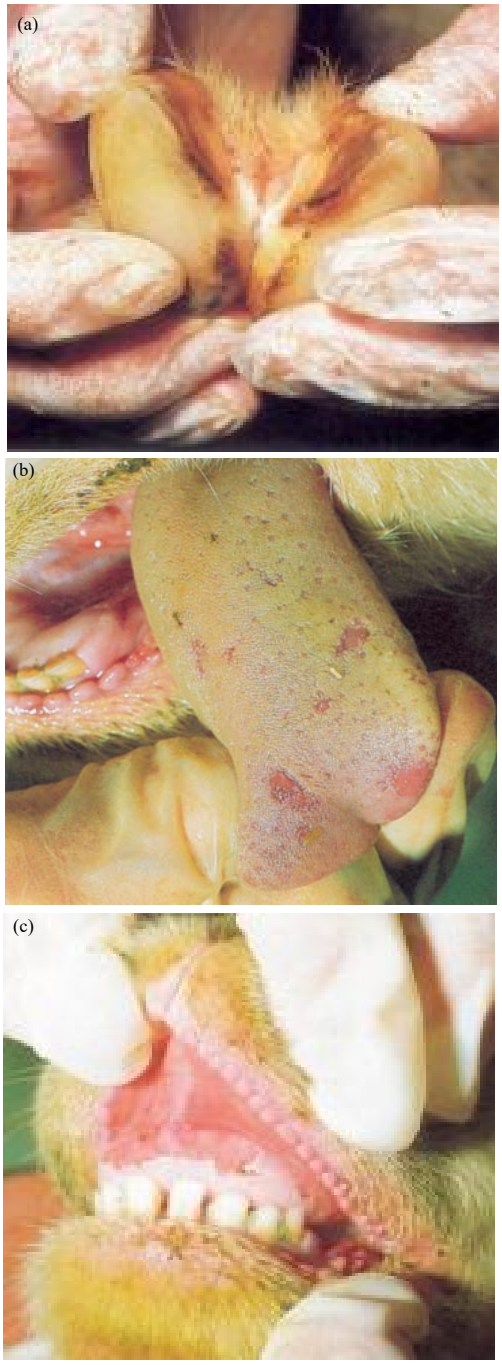

Fig. 1(a-c): Different PM clinical signs of FMD
Source: DEFRA[28]

Carrier status: Sheep and goats may also become virus carriers after exposure. Around 50% of recovering sheep insistently infected for up to 9 weeks and a small number of animals may carry the virus for up to 9 months. Some breeds of sheep can persist as carriers for up to 5 months after exposure. A number of mechanisms suggested for persistent

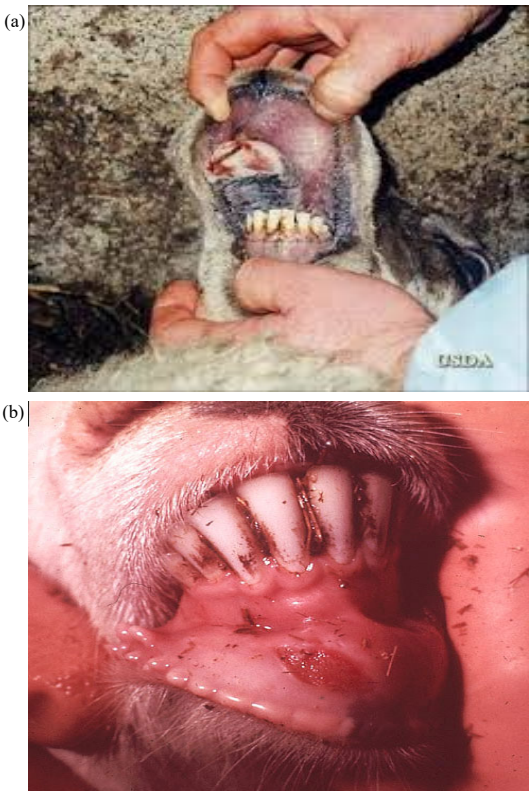

Fig. 2(a-b): Different PM lesion of FMD
Source: DEFRA[28]

infection with FMD virus. The virus persists in the pharynx. Sheep and goats are less susceptible for FMD than cattle and pigs and the disease often has unapparent nature in these animals. Sheep and goats played a vital role in the FMD epidemiology as they become carriers and act as reservoirs of infection[29]. Animals could be carriers in the following cases; after recovery, sub-clinical form of FMD and when the vaccinated animals are subjected to the infection. All the three previous cases lead to the carrier's state. The duration of the carrier state differs according to the species. Carrier state lasted for 5 years, 3 years and 9 months in African buffaloes, cattle and sheep and goats, respectively. Deer and antelope can convey the virus for long periods. Pigs do not become carriers[30].

Differential diagnosis: Differential diagnosis of FMD applied to differentiate the diseases that misdiagnosed with it. These included the following; Peste des petit ruminants (PPR) can be excluded by signs of pneumonia and diarrhea, Bluetongue disease excluded by signs of facial oedema and nasal ulceration, Pock lesions exclude Capripox, Contagious ecthyma excluded by lack of vesicular stomatitis and

lameness, Pneumonic Pasteurellosis and Caprine Pleuropneumonia are characterized by respiratory illness alone[31].

Laboratory diagnosis

Virus isolation: According to the World Organization for Animal Health (OIE)[32] , Terrestrial Manual, virus isolation onto cell culture is considered as the "gold standard" technique for FMD diagnosis[33]. This method is highly sensitive, but it is time-consuming, lasting between 1 and 4 days and requires extraordinary laboratory facilities. The most sensitive cell culture to most of FMDV serotypes is the primary bovine thyroid[34], but they are difficult and exclusive and usually lose its susceptibility to FMDV after numerous passages[35]. Primary lamb kidney (LK) cells are very sensitive to FMDV and vary from primary bovine thyroid (BTY) cells in preserving of their sensitivity to FMDV infection after cryopreservation. Cell lines like baby hamster kidney (BHK-21) is much easier to preserve, but are less susceptible to specific animal-derived FMDV[36]. The summary of different assays for the diagnosis of FMD[29] are given in Table 1.

Virus neutralization test: The virus neutralization test (VNT) is the "gold standard" technique for detection of antibodies to structural proteins of FMDV and is an approved test for the certification trade of animals and animal products[37]. The test sensitivity varies due to its dependence on various types of primary cells and cell lines cultures which have variable degrees of sensitivities to the FMD. In addition, VNT is time- consuming, liable for contamination and requires special facilities in comparison to other serological tests that can use inactivated viruses as antigens.

Nonstructural protein (NSP) antibody tests: Detecting of the antibodies against the non-structural proteins (NSPs) of FMDV successfully used for differentiation between infected and vaccinated animals (DIVA)[38]. Differentiation of the infected from the vaccinated animal is of great importance in the control program of FMD. This differentiation depends on the FMD vaccine quality that must be free from any live virus particles. A series of competitive and indirect ELISAs using 3AB3, 3ABC and truncated 2C (2 Ct) NSPs of FMDV was developed to achieve DIVA[22,39]. The 3ABC indirect ELISA routinely applied for general screening of bovines[39]. The sensitivity and specificity of the 3ABC indirect ELISA are 96 and 96.4%, respectively[19]. This assay has the ability to detect antibodies-3 ABC from 10-900 days post-infection in experimentally infected cattle. Recently, 3NSP based assays depending on 3B, 2B and 3D were developed and validated in India[40,41].

Sandwich ELISA: Sandwich ELISA is rapid and simple to perform. It is the primary test for FMD diagnosis. The assay depends on serotype-specific polyclonal antibodies prepared in guinea pig and rabbits for the detection of FMDV structural proteins. The test gave 100% specificity and 80% sensitivity in FMDV detection[29].

Complement fixation test (CFT): It is an old method in the history of clinical virology. The complement attacks antigen-antibody compound. Presence of the Ag-Ab complex triggers the complement to bind. Sensitized sheep red blood cells (RBCs) used as an indicative agent. Positive results are associated with no hemolysis. Although the CFT is convenient and requires low-cost materials, it is labor intensive and lacks sensitivity[42].

Table 1: Different assays for the diagnosis of FMD

FMD diagnostic assay	Sensitivity	Specificity	Advantages	Disadvantages
Sandwich ELISA	80%	100%	Easy to perform, Suitable for handling large no. of samples	Less sensitive, Not suitable for certain type of clinical samples
Multiplex PCR	Minimum detection limit of 1×10^{-1} TCID 50 mL^{-1}	100% specific for cross serotype detection	Rapid and sensitive, Suitable for samples like semen and milk	High risk of generating false positives
TaqMan real-time PCR	Minimum detection limit of $10^{1.0}$ TCID 50 mL^{-1}	100% specific for cross serotype detection	More sensitive and specific than gel based assay	High risk of generating false positives
Virus isolation and neutralization	-	-	Gold standard assay for FMD diagnosis	Slow takes 1-4 days for confirmatory results
LAMP RNA	Minimum detection limit up to 1.1×10^{-4} TCID 50 mL^{-1}	-	Require no specialized instruments, can be used as point-of-care diagnosis	High risk of generating false positives
3AB3 I-ELISA	-	99.1-96.4%	Sensitive and specific	Only for bovine species
3ABC C-ELISA	-	96%	Specific assay Universal for all species	Less sensitive than I-ELISA

Source: Sharma *et al.*[29]

Nucleic acid recognition methods

RT-PCR assay: Five FMDV serotypes were distinguished by the formerly published conventional RT-PCR techniques depending on the magnification of the VP1 gene[43]. Conventional RT-PCR techniques are serotyping specific. Consequently, conventional RT-PCR recommended just for amplification of the VP1 region due to its incompatibility to serotyping followed by further nucleotide sequence analysis[10,37].

RT-LAMP: It is an isothermal nucleic acid amplification technique, which carried out at a constant temperature and does not require a cycler like PCR[44]. RT-LAMP is extremely sensitive molecular analyses for the simple and rapid detection of FMDV. A total of 38/50 samples were positive by RT-LAMP and the identified serotypes were A (15/50), O (15/50) and Asia-1 (8/50)[45]. The RT-LAMP succeeded in the amplification of 199, 209 and 187 bp of the target sequence of the 3D polymerase gene of serotypes A, O and Asia-1 at 60°C during 15-60 min, respectively. A reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) developed using general and serotype-specific genes in a single tube. This test easily performed and can detect FMDV at serotype level in about 60 min. In addition, it has a comparable sensitivity and specificity to reverse transcriptase PCR and real-time PCR[46].

Multiplex PCR: The technique is more rapid and sensitive than conventional virus isolation[47]. Assays were established and directed against the conserved 3D region and 5' UTR region of the FMD virus. Afterward, the multiplex PCR applied for detect FMDV (mPCR) directed to the VP1 region was developed and differentiated the serotypes[36]. In this assay, 2 primers sets were used, the first targeting the 1D region and the second direct to 2 B region. The technique succeeded in the identification of FMD serotype. Products of different sizes (249, 376 and 537 bp) were obtained which are specific for serotypes O, A and Asia1, respectively. The minimum detection limit of the mPCR has been valued[10,48] as 1×10^{-1} $TCID_{50}$/mL for serotypes O, A and Asia 1.

Real-time RT-PCR: These assays settled for the identification of FMDV all over the world. A PCR assay directed to the 1D region established in a multiplex design for concurrent detection and identification of FMDV serotypes in the samples[49]. The RT-PCR assay was very sensitive, because of the great sensitivity and specificity of the RT-PCR assay, it was suggested as the main test for the FMDV detection in persistently carriers. This technique is of extreme significance in disease control as it can detect the carrier animals[50].

CONTROL OF FOOT AND MOUTH DISEASE

Quarantine measures and disinfectants: It is necessary to apply thorough cleaning and proper disinfection to all premises and infected materials, such as implements, cars and clothes. Hygienic removal of carcasses, bedding and contaminated animal products are important[32]. In free areas like Britain, USA and Sweden applied extensive program for FMDV control that depends on stamping-out; killing and destruction of all infected animals and their immediate susceptible contacts followed by thorough cleaning and disinfection of the affected premises[24,51]. The FMDV is liable for wide variety of disinfectants such as; sodium hydroxide, carbonate, citric acid and Virkon-S[26]. The FMD virus is defenseless to excesses of pH. Therefore, both acids (e.g., citric acid) and bases (e.g., caustic soda or sodium hydroxide) have the ability to destroy the virus. There are many marketable types of disinfectants that be used in the elimination of the FMD virus. Strict precautions and proper usage of the exact concentration of the disinfectant according to the manufacturer instructions recommended[52].

Treatment of FMD in small ruminants: Treatment protocols of small ruminants are showing typical clinical symptoms of FMD achieved by the use of antipyretic and analgesic medicine (Vetalgin-Intervet) and a broad-spectrum long-acting antibiotic (Terramycine/LA Pfizer) and both protective dressing or immunomodulatory was applied[53]. Localized treatment by rinse the ulcerative vesicles found on the mouth, tongue, legs, claws and teat with one of the following solutions; normal saline, citric acid 1% potassium permanganate 1% or alum 2% were helpful[54].

FMD VACCINES

Attenuated vaccines: Attenuated strains produced by the passage in the laboratory animals like mice and rabbits or in the embryonated eggs until their virulence for infection were weak or lost[55]. This vaccine has some disadvantages, it does not allow discrimination of naturally infected and vaccinated animals, attenuated vaccine virus spreading to non-vaccinated livestock and may cause the development of virus carriers[56]. There many types of FMD attenuated vaccines such as; Novel Attenuated Vaccines, Nucleic Acid Vaccines, Synthetic Peptide

Table 2: The FMD vaccines in Egypt

Producer	Product name	Type	Strain	Adjuvant	Licensed countries
Veterinary Serum and Vaccine Research Institute	Bivalent inactivated foot and mouth disease vaccine	Killed	O1 93, AEGY/06	Aluminum hydroxide	Egypt
	Polyvalent inactivated foot and mouth disease oil vaccine	Killed	O, A, SAT2	Oil	Egypt
Middle East for Veterinary Vaccine (ME VAC)	Tri-APHTHOVAC	Killed	A, O, SAT 2	Montonide ISA-50	Egypt
	APHTHOVAC	Killed	O-PanAsia 2, O-Sudan, O-Manisa, SAT-2, A-African, A-Iran 05	Oil	Egypt
	Bi-APHTHOVAC	Killed	Not Available	Oil	Egypt

Source: CFSPH[26]

Vaccines, Viral Capsids Vaccines, Virus-Like Particles Vaccines (VLP) and Nanoparticle Vaccines, but all of them are under research or not authorized.

Inactivated killed vaccine: The vaccine produced from live FMDV amplified in BHK-21, then inactivated by special chemicals like formalin, purified and mixed with the adjuvant. It is of great importance to insure the freedom of the vaccine from any live virus particle to avoid post vaccinal infection and allow the DIVA[51]. The inactivated FMDV vaccine succeeded in reducing the outbreaks worldwide. There are a number of limitations with its use in emergency control programs; one of these limits is that the incomplete inactivation may lead to infective vaccines[57]. The inactivated vaccine gives protection for all ruminants against FMDV for one year. The first dose for cattle and buffaloes is 3 mL and for sheep and goats is 1.5 mL. A booster dose injected subcutaneously 3-4 weeks after the first dose. The dairy cattle, breeding calves, sheep and goats vaccinated at 6-8 months age[58]. The details of some FMD vaccines is given in Table 2[26].

CONCLUSION

Foot and mouth disease (FMD) is one of the most contagious viral diseases of mammals that have the ability to cause high economic losses in susceptible cloven-hoofed animals. The most frequent clinical signs associated with FMD in sheep and goats are lameness, high fever (41°C), salivation, oral vesicles, interdigital spaces ulcers, lesions on the dental pad, severe drop in milk yield in dairy animals and death of young animals. Diagnosis of FMD achieved by many techniques such as; virus isolation, Sandwich ELISA, Multiplex PCR, indirect ELISA (DIVA) and real time PCR. The control program of FMD depends mainly on vaccination, effective quarantine measures and hygiene and sanitation measures. The inactivated FMDV vaccine succeeded in reducing the outbreaks worldwide. It gives protection for all ruminants against FMDV for one year.

SIGNIFICANCE STATEMENT

Foot and mouth disease is one of the most contagious viral diseases that have the ability to cause high economic losses in susceptible cloven-hoofed animals. It causes severe drop in milk yield in the dairy small ruminants. Early and accurate diagnosis is necessary and the recent diagnostic tools urgently needed not only for the diagnosis but also for following-up combating programs and control of FMD. This study found that the inactivated FMDV vaccine succeeded in reducing the outbreaks and recommended in endemic areas. Moreover, this study discussed the different methods used in diagnosis and control of FMD that will help the researchers in exploring and discovering the methods suitable in their countries.

REFERENCES

1. Shankar, B., S. Morzaria, A. Fiorucci and M. Hak, 2012. Animal disease and livestock-keeper livelihoods in Southern Cambodia. Int. Dev. Plan. Rev., 34: 39-63.
2. Tufan, M., 1993. An evaluation of the monitoring and control of foot and mouth disease in Turkey. University of Reading, United Kingdom.
3. Adibes, M., M. Gundogan, N. Evgin, N. Bacak and C. Erginoz *et al*., 1998. Detailed Investigations, Using Farmer Interview, to Assess the Losses Caused by Foot and Mouth Disease Outbreaks in Turkey. FMD Research Institute, Ankara.
4. Mahmoud, M.A. and S.A. Galbat, 2017. Outbreak of foot and mouth disease and peste des petits ruminants in sheep flock imported for immediate slaughter in Riyadh. Vet. World, 10: 238-243.
5. Elshemey, T.M. and M.A. Mahmoud, 2011. Seroprevalence of antibodies against peste des petits ruminants (PPR) virus in sheep and goat in Kingdom Saudia Arabia. Alex. J. Vet. Sci., 32: 175-182.
6. Ghazy, A.A., W.M. Ahmed, M.A. Mahmoud and L.A. Ahmed, 2007. Prevalence of infectious bovine rhinotracheitis and bovine viral diarrhoea viruses in female buffaloes with reproductive disorders and parasitic infections. Int. J. Dairy Sci., 2: 339-347.

7. Mahmoud, M.A. and S.A. Ahmed, 2009. Prevalence of bovine herpesvirus-1 in sheep and goats in Egypt. Global Vet., 3: 472-479.
8. Ata, E.B., A. Zaghawa, A.A. Ghazy, A. Elsify and R.M. Shaapan, 2018. Equine herpes virus type-1 infection: Etiology, epidemiology, pathogenesis, identification and recent diagnosis. Asian J. Epidemiol., 11: 34-45.
9. Minakshi, P., R. Singh, K. Ranjan, P. Kumar, C.G. Joshi, Y.K.M. Reddy and G. Prasad, 2012. Complete genome sequence of bluetongue virus serotype 16 of goat origin from India. J. Virol., 86: 8337-8338.
10. Salam, H.M., A.A. Mahmoud, A.M. Khadr, S.A. Salem, T.M. Elshemey, A.H. Abdelrahman and M.A. Mahmoud, 2015. Isolation, sequencing and phylogenetic analysis of FMD strains during 2011-2012 outbreaks in Egypt. Int. J. Agri. Innov. Res., 3: 1023-1038.
11. Stewart, M.E. and P. Roy, 2015. Structure based identification of functional residues in the nucleoside 2'O methylase domain of Bluetongue virus VP4 capping enzyme. FEBS Open Bio., 56: 138-146.
12. OIE., 2017. Foot-and-mouth disease situation: Monthly report. OIE and Food and Agriculture Organization of the United Nations, USA.
13. Aidaros, H.A., 2002. Regional status and approaches to control and eradication of foot and mouth disease in the Middle East and North Africa. Rev. Sci. Technol., 21: 451-458.
14. El-Shehawy, L.I., H.I. Abu-Elnaga, S.A. Rizk, A.S. Abd El-Kreem, A.A. Mohamed and H.G. Fawzy, 2014. Molecular differentiation and phylogenetic analysis of the Egyptian foot-and-mouth disease virus SAT2. Arch. Virol., 159: 437-443.
15. El-Shehawy, L., H. Abu-Elnaga, A. Talat, E. El-Garf, A. Zakria and A. Azab, 2011. A nucleotide sequencing of foot-and-mouth disease virus Egyptian strains. J. Anim. Sci., 7: 430-435.
16. Abd El-Moety, M.S., M.M. Abd El-Aty, H.M. Fakry, H.M. Daoud and E.E.S. Ibrahim *et al*., 2013. Isolation and molecular characterization of foot and mouth disease SAT2 virus during Outbreak 2012 in Egypt. J. Vet. Adv., 3: 60-68.
17. Kandeil, A., R. El-Shesheny, G. Kayali, Y. Moatasim and O. Bagato *et al*., 2013. Characterization of the recent outbreak of foot-and-mouth disease virus serotype SAT2 in Egypt. Arch. Virol., 158: 619-627.
18. Balamurugan, V., D. Hemadri, M.R. Gajendragad, R.K. Singh and H. Rahman, 2014. Diagnosis and control of peste des petits ruminants: a comprehensive review. Virus Disease, 25: 39-56.
19. Mahmoud, M.A.E.F., M.K. Elbayoumy, D. Sedky and S. Ahmed, 2017. Serological investigation of some important RNA viruses affecting sheep and goats in Giza and Beni-Suef governorates in Egypt. Vet. World, 10: 1161-1166.
20. Anderson, J., J.A. McKay and R.N. Butcher, 1991. The use of monoclonal antibodies in competitive ELISA for the detection of antibodies to rinderpest and Peste des Petitis Ruminants viruses. Panel Proc. IAEA-SM-318. International Sumposium Nuclear and Related Tech. Anim. Prod. Hlth., Vienna, Austria.
21. Forsyth, M.A. and T. Barrett, 1995. Evaluation of polymerase chain reaction for the detection and characterisation of rinderpest and peste des petits ruminants viruses for epidemiological studies. Virus Res., 39: 151-163.
22. PARC., 2017. Research council control of foot and mouth diseases. Report. Pakistan Agricultural Research Council, November 22, 2017, Pakistan.
23. Alexandersen, S., Z. Zhang, A.I. Donaldson and A.J.M. Garland, 2003. The pathogenesis and diagnosis of foot-and-mouth disease. J. Comp. Pathol., 129: 1-36.
24. Dorea, F.C., M. Noremark, S. Widgren, J. Frossling, A. Boklund, T. Halasa and K. Stahl, 2017. Evaluation of strategies to control a potential outbreak of foot-and-mouth disease in Sweden. Front. Vet. Sci., Vol. 4. 10.3389/fvets.2017.00118.
25. Queensland Government, 2017. Foot-and-mouth disease guide for Queensland veterinarians, including diagnosis. Business Queensland, The State of Queensland.
26. CFSPH., 2015. Foot and mouth disease, Collage of Veterinary Medicine Lowa State University, Fiebre Aftosa. J. Sci. Technol., 23: 1-9.
27. Abdul-Hamid, N.F., N.M. Hussein, J. Wadsworth, A.D. Radford, N.J. Knowles and D.P. King, 2011. Phylogeography of foot-and-mouth disease virus types O and A in Malaysia and surrounding countries. Infect. Genet. Evol., 11: 320-328.
28. DEFRA., 2017. Annual report and accounts 2017-18 Annex 2-commentary on sustainable performance. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/725030/defra-annual-report-2017-18-print. pdf
29. Sharma, G.K., S. Mahajan, R. Matura, S. Subramaniam and R. Ranjan *et al*., 2015. Diagnostic assays developed for the control of foot-and-mouth disease in India. World J. Virol., 4: 295-302.
30. Ganter, M., W.D. Graunke, G. Steng and H. Worbes, 2001. [Foot and mouth disease in sheep and goats]. Deutsche Tierarztliche Wochenschrift, 108: 499-503, (In German).
31. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2007. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th Edn., W.B. Saunders Company, Philadelphia, USA., ISBN-13: 978-0702027772, Pages: 2065.
32. OIE., 2012. Foot and Mouth Disease. In: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees), OIE (Eds.). 7th Edn., Vol. 1, Chapter 2.1.5, World Organization for Animal Health, Paris, France, ISBN: 978-92-9044-879-2, pp: 145-173.
33. Segundo, F.D.S., G.N. Medina, C. Stenfeldt, J. Arzt and T. de los Santos, 2017. Foot-and-mouth disease vaccines. Vet. Microbiol., 206: 102-112.
34. Snowdon, W.A., 1966. Growth of foot-and-mouth disease virus in monolayer cultures of calf thyroid cells. Nature, 210: 1079-1080.

35. Minakshi, P., K. Ranjan, B. Brar, S. Ambawat and M. Shafiq *et al*., 2014. New approaches for diagnosis of viral diseases in animals. Adv. Anim. Vet. Sci., 2: 55-63.
36. House, C. and J.A. House, 1989. Evaluation of techniques to demonstrate foot-and-mouth disease virus in bovine tongue epithelium: Comparison of the sensitivity of cattle, mice, primary cell cultures, cryopreserved cell cultures and established cell lines. Vet. Microbiol., 20: 99-109.
37. Jamal, S.M. and G.J. Belsham, 2013. Foot-and-mouth disease: Past, present and future. Vet. Res., Vol. 44. 10.1186/1297-9716-44-116.
38. Paton, D.J., K. de Clercq, M. Greiner, A. Dekker and E. Brocchi *et al*., 2006. Application of non-structural protein antibody tests in substantiating freedom from foot-and-mouth disease virus infection after emergency vaccination of cattle. Vaccine, 24: 6503-6512.
39. Mahajan, S., J.K. Mohapatra, L.K. Pandey, G.K. Sharma and B. Pattnaik, 2013. Truncated recombinant non-structural protein 2C-based indirect ELISA for FMD sero-surveillance. J. Virol. Methods, 193: 405-414.
40. Mohapatra, A.K. , J.K. Mohapatra, L.K. Pandey, A. Sanyal and B. Pattnaik, 2014. Diagnostic potential of recombinant nonstructural protein 3B to detect antibodies induced by foot-and-mouth disease virus infection in bovines. Arch. Virol., 159: 2359-2369.
41. Mahajan, S., J.K. Mohapatra, L.K. Pandey, G.K. Sharma and B. Pattnaik, 2015. Indirect ELISA using recombinant nonstructural protein 3D to detect foot and mouth disease virus infection associated antibodies. Biologicals, 43: 47-54.
42. Souf, S., 2016. Recent advances in diagnostic testing for viral infections. Biosci. Horizons: Int. J. Student Res., Vol. 9. 10.1093/biohorizons/hzw010.
43. Ahmed, H.A., S.A.H. Salem, A.R. Habashi, A.A. Arafa and M.G.A. Aggour *et al*., 2012. Emergence of foot-and-mouth disease virus SAT 2 in Egypt during 2012. Transboundary Emerg. Dis., 59: 476-481.
44. Parida, S., 2009. Vaccination against Foot-and-mouth disease virus: Strategies and effectiveness. Expert Rev. Vaccine, 8: 347-365.
45. Maryam, S., T. Rasheed, A. Latif, R. Zahra and A.B. Zahur *et al*., 2017. One-step Real-Time Loop-Mediated Isothermal Amplification (RT-LAMP): evaluation and its application for the detection of foot-and-mouth-disease virus and its serotypes. Turk. J. Vet. Anim. Sci., 41: 435-443.
46. Farooq, U., A. Latif, H. Irshad, A. Ullah and A.B. Zahur *et al*., 2015. Loop-mediated isothermal amplification (RT-LAMP): A new approach for the detection of foot-and-mouth disease virus and its sero-types in Pakistan. Iran. J. Vet. Res., 16: 331-334.
47. Sharma, G.K., S. Mahajan, B. Das, R. Ranjan, A. Kanani, A. Sanyal and B. Pattnaik, 2014. Comparison of stabilisers for development of a lyophilised multiplex reverse-transcription PCR mixture for rapid detection of foot and mouth disease virus serotypes. Rev. Sci. Tech. Off. Int. Epiz., 33: 859-867.
48. Giridharan, P., D. Hemadri, C. Tosh, A. Sanyal and S.K. Bandyopadhyay, 2005. Development and evaluation of a multiplex PCR for differentiation of foot-and-mouth disease virus strains native to India. J. Virol. Methods, 126: 1-11.
49. Selvan, R.P.T., 2010. Analysis of replication dynamics of mixed foot-and-mouth disease virus populations using serotype differentiating multiplex QPCR. Ph.D. Thesis, Deemed University, Indian Veterinary Research Institute, Izat Nagar, U.P., India.
50. Zhang, Z. and S. Alexandersen, 2003. Detection of carrier cattle and sheep persistently infected with foot-and-mouth disease virus by a rapid real-time RT-PCR assay. J. Virol. Methods, 111: 95-100.
51. Knight-Jones, T.J.D. and J. Rushton, 2013. The economic impacts of foot and mouth disease-What are they, how big are they and where do they occur? Prev. Vet. Med., 112: 161-173.
52. USDA., 2012. Potential pesticides to use against the causative agents of selected foreign animal diseases in farm settings. https://www.aphis.usda.gov/animal_health/emergency_management/downloads/fad_epa_disinfectants.pdf
53. El-Bayoumy, M.K., K.A. Abdelrahman, A.M. Allam, T.K. Farag, H.A.A. Abou-Zeina and M.A. Kutkat, 2014. Molecular characterization of foot-and-mouth disease virus collected from Al-Fayoum and Beni-Suef governorates in Egypt. Global Vet., 13: 828-835.
54. Tho, L.V., 2018. Prevention and treatment of Foot and Mouth Disease (FMD). http://www.biopharmachemie.com/technical/health-management-disease-prevention-and-treatment-in-cattle-goat-sheep-horse/prevention-and-treatment-of-foot-and-mouth-disease-fmd.html
55. Grubman, M.J. and B. Baxt, 2004. Foot-and-mouth disease. Clin. Microbiol. Rev., 17: 465-493.
56. Jamal, S.M., G. Ferrari, S. Ahmed, P. Normann and G.J. Belsham, 2011. Molecular characterization of serotype Asia-1 foot-and-mouth disease viruses in Pakistan and Afghanistan; emergence of a new genetic group and evidence for a novel recombinant virus. Infect. Genet. Evol., 11: 2049-2062.
57. Guo, H.C., S.Q. Sun, Y. Jin, S.L. Yang and Y.Q. Wei *et al*., 2013. Foot-and-mouth disease virus-like particles produced by a SUMO fusion protein system in *Escherichia coli* induce potent protective immune responses in guinea pigs, swine and cattle. Vet. Res., Vol. 44. 10.1186/1297-9716-44-48.
58. Shawky, S.M., N.S. Thabet, S.H. Orabi and M.A. Nayel, 2016. A comparative study on the hemato-biochemical and immunological effects of the hexavalent FMD vaccine alone or in combination with trivalent FMD vaccine in cattle. J. Biosci. Med., 4: 16-26.