Research article

Risk Assessment of African Swine Fever Virus in Pork in Phnom Penh, Cambodia

VOUCHSIM KONG*
Department of Agro-Industry, Ministry of Agriculture, Forestry and Fisheries, Phnom Penh, Cambodia
Email: kongvouchsim@gmail.com

PHEA SUM
M's Pig ACMC (Cambodia) Co., Ltd, Phnom Penh, Cambodia

PHEARY MOL
M's Pig ACMC (Cambodia) Co., Ltd, Phnom Penh, Cambodia

KAROLIN CHAN
Faculty of Veterinary Medicine, Royal University of Agriculture, Phnom Penh, Cambodia

SOCHEATA RATHA
Faculty of Veterinary Medicine, Royal University of Agriculture, Phnom Penh, Cambodia

SEAVCHOU LAUT
Faculty of Veterinary Medicine, Royal University of Agriculture, Phnom Penh, Cambodia

VUTEY VENN
Department of Veterinary Paraclinical Science, Royal University of Agriculture, Phnom Penh, Cambodia

Received 25 January 2020 Accepted 30 April 2020 (*Corresponding Author)

Abstract African swine fever (ASF) is an acute infectious and deadly viral disease that affects domestic and wild pigs of all breeds and ages. ASF virus (ASFV) can spread vastly to non-infected pig population, but it cannot be transmitted from pigs to humans. In Cambodia, first ASF outbreak was transmitted from bordering countries and reported in five provinces. While estimated 70% of ASF widespread all over the country, local farmers experienced a greater economic loss. Among the four components of risk analysis, the study determines the risk assessment, and hazard identification plays a crucial step in risk assessment. The objectives of this study aimed to detect the presence of ASFV in pork on local markets through qualitative risk assessment approach and propose possible measurable recommendations to prevent ASF outbreak. The study was conducted during the period of ASF outbreak in August 2019, and the qualitative detection of ASFV was conducted on pork tissue samples selected from wet markets and supermarkets in Phnom Penh. Sample extractions were isolated from 30 pork tissue samples and detected virus by iiPCR. The qualitative result on detection of ASF virus is confirmed by PCR technique. The ASFV is found in pork tissue samples in wet markets and supermarkets. Of the 30 samples, 21 (70%) were found positive with ASFV, 6 in 9 (20%) tissue samples from supermarkets and 15 in 21 tissue samples from wet markets (50%) confirmed the presence of ASFV. With this result, it indicates that likelihood of the ASF virus transmission would be very likely to occur and the spread of ASF virus in pork tissue samples in wet markets and supermarkets is significantly prevalent, and the virus is likely to spread quickly. Scientifically, there is no vaccine to prevent ASF, and as recommended by FAO, the influenced policy-based implementation is required in place to minimize further production losses. The implementations must be strengthened through strict farm biosecurity guideline and slaughter of infected pigs, strict import regulation (border and movement control of live pigs) and heavy penalty to illegal import of live pigs. Based on this result, it may contribute to bring consumers' and relevant stakeholders' awareness to reduce high risk through early detection of ASFV at the slaughterhouses and markets by risk assessment approach. Further studies on risk management and risk communication to complete the risk analysis of ASFV in pork are highly recommended.

Keywords African swine fever virus, risk assessment, pork, markets, Cambodia

INTRODUCTION

African swine fever (ASF) is an acute infectious and deadly viral disease that affects domestic and wild pigs of all breeds and ages, and caused by a DNA virus belonging to *Asfarviridae* family (Guberti et al., 2018). ASF virus (ASFV) can spread vastly to non-infected pig population, but it cannot be transmitted from pigs to humans (FAO, 2008). Although humans are not susceptible to ASF, its outbreak causes noticeable socio-economic consequences to infected countries (Bellini, Rutili and Guberti, 2016). In Cambodia, first ASF outbreak transmitted from bordering countries was reported in Ratanakiri province by the Ministry of Agriculture, Forestry and Fisheries, MAFF (FAO, 2019). ASFV has continued its transmission and spread quickly to Tboung Khmum, Svay Rieng, Takeo and Kandal provinces. While estimated 70% of ASF widespread all over the country, local farmers in these five areas experienced a greater economic loss. Majority of small- and medium-scale pig farms stop to operate temporarily, and a small number of commercial farms are being operated (Siem Reap PDAFF, 2019).

In such cases, risk analysis, the process composed of hazard identification, risk assessment, risk management and risk communication, is required. FAO/WHO (2006) defined risk assessment as the term that is generally used to describe the entire process of making a public health decision regarding a specific drug or agent and it is the scientific evaluation of known or potential adverse effects resulting from human exposure to food borne hazards. According to Venn et al. (2014) as cited in the Codex Alimentarius Commission (CAC, 1999), risk assessment is defined as integrated elements of the structure of risk analysis and to be based on the following steps as shown in Fig. 1 and Fig. 2.

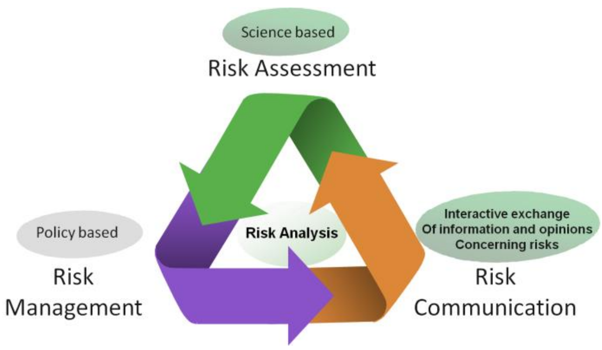

Fig. 1 Structure of risk analysis (Venn et al., 2014 cited in CAC, 1999)

Fig. 2 Risk assessment (Venn et al., 2014 cited in CAC, 1999)

ASFV can be transmitted and spread from infected pigs directly to uninfected populations. The cause of transmission is associated with virus-containing matter (blood, faeces, urine or saliva from infected pigs) that contact directly with brought-to-farm infected pork, farm inputs and in-farm used materials (bedding, pig cage, feed, equipment, clothing and footwear, means of transportation (vehicles), studied by Thomson (1985). In the research of Mellor et al. (1987), it has shown that ASFV can be airborne within a short distance, approximately less than 2 kilometers.

ASFV has a remarkable ability to survive for long periods in a protein environment, and therefore meat from pigs slaughtered in the infective stages of ASF or die naturally of the disease provides a good source of virus. Pork slaughtered from infected pigs with ASF provides a favorable protein environment for the virus to survive in the tissues for a long period of time. The virus is strongly resistant to high temperatures. The studies of McKercher et al. (1978) and Plowright et al. (1994) confirmed that fresh, frozen, processed, or preserved (salted and dried) pork may contain the infective virus. As ASFV is transmitted in a closer distance, it may be possible to prevent the occurrences by applying strict biosecurity rules and observation. While taking precautionary measures in use, it is necessary to limit accesses for people/ farm workers/ visitors and vehicles to enter the areas or farms where pigs are kept. This is also ensuring that the farm managers/ owners and veterinarians are disinfected before entering the critical areas/ farms with protective clothing and footwear, and free from inadvertent feeding of leftovers and pork (Penrith and Vosloo, 2009).

OBJECTIVE

This study aims to detect the presence of ASF virus in pork in Cambodian markets through risk assessment approach, and propose possible measurable recommendations to prevent ASF outbreak.

METHODOLOGY

The study was conducted during the period of ASF outbreak, and the qualitative detection of ASF virus was conducted on pork tissue samples using PCR technique certified by OIE approval number 20130108 for registration of diagnostic kits (GeneReach Biotechnology Corp., 2019). The samples were selected from 7 wet markets and 3 supermarkets in Phnom Penh in August 2019. Primary information was gathered by interviewing the key informants of the city slaughterhouses and sellers in both markets. The distribution of live pig movement and slaughtered pork was from smallholder famers, middlemen and traders who source for pigs from the small-scale farms and neighboring countries.

Sample extractions were isolated from 30 pork tissue samples and detected virus. The analysis samples were evaluated based on the insulated isothermal PCR technique that automates sample lysis, nucleic acid extraction and amplification and detection of the target sequences. Pork tissue samples were pretreated by weighting 40 mg of each pork tissue sample and placing them into a clean 1.5 ml microcentrifuge tube with 0.5 ml of Sample Storage Solution atc-lysis. The pork tissues were homogenized in the grinder and spined the tube for 1 minute in mini-centrifuge before transferring 200 µl of supernatant to an Extraction Cartridge. Then, Transfer Cartridge was prepared by placing one Premix and one Transfer Cartridge for each sample; labeling the sample ID and Premix ID on the side of Transfer Cartridge; removing Transfer Cartridge Cap; turning the notched side of the cartridge away, and snapping the Premix vial into well #3 of the Transfer Cartridge. The Extraction Cartridge was prepared by placing one Extraction Cartridge (B) for each sample; removing the Extraction Cartridge from the aluminum pack, labeling the sample ID on the side of Extraction Cartridge; slowly peeling off the aluminum film; turning notched side, and loading 200 µl of homogenized sample to the sample well of the Extraction Cartridge. After the preparations of Transfer Cartridge and Extraction Cartridge were completed, the Extraction lot number of the Extraction Cartridge was entered into the PCR; then the loaded Extraction Cartridge was place into the selected slot before the Reagent lot number of the Premix Reagent was entered. The Transfer Cartridge to the selected was loaded into the slot and the analysis were ready to run in the PCR.

RESULTS AND DISCUSSION

The qualitative result on detection of ASF virus is confirmed by PCR technique. The ASFV is found in pork tissue samples in wet markets and supermarkets. Of the 30 samples, 21 (70%) were found positive with ASFV, 6 in 9 (20%) tissue samples from supermarkets and 15 in 21 tissue samples from wet markets (50%) confirmed the presence of ASFV (Table 1). With this result, it indicates that likelihood of the ASF virus transmission would be very likely to occur (OIE, 2010) and the spread of ASF virus in pork tissue samples in wet markets and supermarkets is significantly prevalent. Guberti et al. (2018 and 2019) confirmed that the virus is present in the meat of sick pigs and can survive for more than 3 months in the meat and offal, about one year in dry meat and fat, and more than one year in the frozen meat.

Table 1 Percentage of sample detection

Sample detection	Positive number (ratio%)	Negative number (ratio%)
Supermarkets (9 tissue samples)	20% (6/9)	10% (3/9)
Wet markets (21 tissue samples)	50% (15/21)	20% (6/21)

Source: Results from pork tissue samples detection of ASFV confirmed by PCR technique

Based on this early detection of ASFV, risk assessment as scientific-based approach identifies four components including hazard identification, hazard characterization, exposure assessment, and risk characterization are described in Table 2.

Table 2 Risk Assessment of ASF virus

Components of Risk Assessment	Identifications
Component 1. Hazard Identification	African Swine Fever Virus (ASFV), is a DNA virus in the *Asfarviridae* Family. The causative agent of the disease, the ASF virus (ASFV), is the only member of the *Asfaviridae* family, genus *Asfivirus.*
Component 2. Hazard Characterization	- Human consequence: ASF virus cannot be transmitted from pigs to humans. - Economic consequence: In Cambodia, first ASF outbreak was transmitted from bordering countries and reported in April 2019 in five provinces. While estimated 70% of ASF widespread all over the country, local farmers estimated in greater losses. The pig price is increasing. Infected pigs' weight 50kg loss approximately 90% of selling price, while pigs' weight 80kg loss about 45%. Majority of small- and medium-scale pig farms stop to operate temporarily, and a small number of commercial farms are being operated.
Component 3. Exposure Assessment	Spread of the disease related vehicles and staff movements at the farms and operation of kitchens inside the farms (bring-in infected pork/contaminated foodstuffs with ASF virus), and contamination of vehicles and staff movements and carcass at the slaughterhouse.
Component 4. Risk Characterization	Combining the results of the preceding three steps and advice for decision making (to continue conducting the study on risk management).

Source: Results from pork tissue samples detection of ASFV confirmed by PCR technique

As shown in Fig. 3, a timely detection and identification of an incursion of initial event (Event is NOT likely to occur) of infected pig in the farms can remove/ cull the sources of ASFV and break the routes of risk outcomes in slaughterhouses and markets (NO RISK). However, the result indicates that 70% of samples were found positive with ASFV and the contaminated pork are distributed in the markets that would be very likely to occur (RISK outcome of interest DOES Occur).

Economically, the sources of infected pigs may not be removed/ culled eventhough early detection is positive in farms which are still slaughtered and distributed in the markets. It seems the supply chain of pigs is less identical. There are a number of actors in the pig supply chain; in which, mostly buyers, middlemen and traders are those who source for pigs in the local villages. Sometimes farmers may contact buyers, middlemen and traders directly to sell their pigs. The purchases are operated daily, and pigs are transported by motorcycles or trucks from farms directly to the slaughterhouses. The research studies conducted by Thomson (1985) and Thomson and Tustin (1994) confirmed that ASFV can be transmitted and spread from infected pigs directly to uninfected populations. The transmission is associated with virus-containing matter (blood, faeces, urine or saliva from infected pigs). The infected pigs may be in contact directly with brought-to-farm infected meat, farm inputs and in-farm used materials such as bedding, pig cage, feed, equipment, clothing and footwear, and other means of transportation (motorcycles and vehicles). Mellor et al. (1987) noticed that ASFV can be airborne over in a short distance approximately less than 2 kilometers that may give a higher chance for the virus to spread in the affected populations/areas.

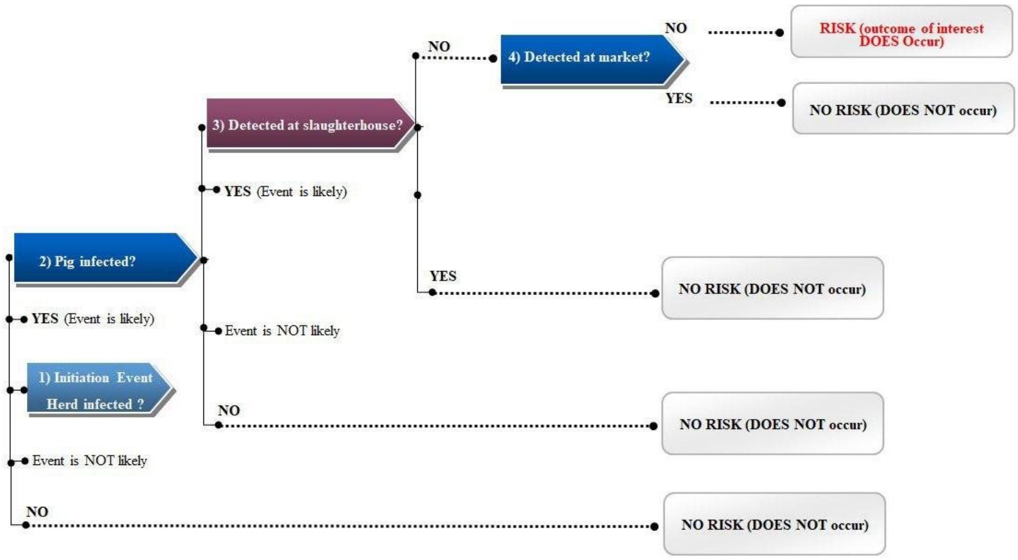

Fig. 3 A scenario tree outlining the biological pathways leading to swine selected from an infected herd, and being either accepted or rejected after it has been PCR tested

The pork is sold raw and distributed to local markets. Some semi-commercial farms have arranged the contracts with village famers and the farms will purchase the pigs when they reach a certain market weight (Mutua et al., 2011 and Levy et al, 2013). A main cause of concern linked to the spread of AFS is a typical practice of buyers, middlemen and traders visit villages and households regularly. Farmers may sell their infected pigs to the buyers and the spread of ASFV may get in contact with other pigs. After slaughter those infected pigs, ASFV can survive for long periods in a protein environment, and meat from pigs slaughtered in the infective stages of ASF or that die naturally of the disease provides a good source of virus and a favorable protein environment for the virus to survive in the tissues for a long period of time (FAO, 2010; Plowright et al. 1994 and McKercher et al. 1978). Bellini et al. (2016) emphasized on the improvement of efficiency of the disease control measures by early detection of ASF that the infected pigs may be identified at the farms/slaughterhouses/markets.

CONCLUSION

The results show that the spread of ASF virus in pork tissue samples in wet markets and supermarkets in Phnom Penh is significantly prevalent, and the virus is more likely to spread quickly in Cambodia. Scientifically, there is no vaccine to prevent ASF. Technically, influenced policy-based implementation is necessary to address to minimize further production losses. The implementations must be strengthened through strict farm biosecurity guideline and slaughter of infected pigs, strict import regulation (border and movement control of live pigs) and heavy penalty to illegal import of live pigs. Based on this result, it may contribute to bring consumers' and relevant stakeholders' awareness to reduce high risk through early detection of ASFV at the slaughterhouses and markets by risk assessment approach. Further studies on risk management and risk communication to complete the risk analysis of ASFV in pork are highly recommended.

ACKNOWLEDGEMENTS

The authors would like to express a sincere appreciation and thank to M's Pig ACMC (Cambodia) Co., Ltd for providing supports in conducting this study. The authors would like to extend the appreciation for the diligent, motivated and dedicated work done by research team, and the proofreader, Ms. Marianne Gudmundsdottir Ernst.

REFERENCES

Bellini, S., Rutili, D. and Guberti, V. 2016. Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. Acta veterinaria Scandinavica, 58 (1), 82. Doi: 10.1186/s13028-016-0264-x

Codex Alimentarius Commission. 1999. Procedural manual, tenth edition. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, 1997. Definitions for "risk management" and "risk communication" were adopted as revised texts at the Twenty-third Session of the Codex Alimentarius Commission.

Food and Agriculture Organization of the United Nations. 2008. African swine fever in the Caucasus. EMPRES Watch, 1-4. Retrived from http://www.fao.org/3/a-aj214e.pdf

Food and Agriculture Organization of the United Nations. 2010. Good practices for biosecurity in the pig sector. FAO Animal Production and Health Paper, No. 169, Rome, FAO. Retrived from http://www.fao.org/3/a-i1435e.pdf

Food and Agriculture Organization of the United Nations. 2019. ASF situation in Asia update. Retrieved from http://www.fao.org/ag/againfo/programmes/en/empres/ASF/situation_update.html

FAO/WHO. 2006. Food safety risk analysis. A guide for national food safety authorities. FAO Food and Nutrition, Paper 87, Food and Agriculture Organization, Rome, Italy.

GeneReach Biotechnology Corp. 2019. POCKIT™ central nucleic acid analyzer. Retrived from http://www.genereach.com/index.php?func=product&action=view&product_no=13

Guberti, V., Khomenko, S., Masiulis, M. and Kerba, S. 2018. Handbook on African swine fever in wild boar and biosecurity during hunting. FAO Animal Production and Health Manual. Rome, FAO and OIE.

Guberti, V., Khomenko, S., Masiulis, M. and Kerba, S. 2019. African swine fever in wild boar ecology and biosecurity. FAO Animal Production and Health Manual, No. 22, Rome, FAO, OIE and EC.

Levy, M., Dewey, C., Weersink, A., Mutua, F. and Poljak, Z. 2013. Pig marketing and factors associated with prices and margins in Western Kenya. Journal of Agricultural Economics and Development, 2, 371-383.

McKercher, P.D., Hess, W.R. and Hamdy, F. 1978. Residual viruses in pork products. Applied Environmental Microbiology, 35, 142-145.

Mellor, P.S., Kitching, R.P. and Wilkinson, P.J. 1987 Mechanical transmission of capripox virus and African swine fever virus by *Stomoxys calcitrans.* Research in Veterinary Science, 43, 109-112.

Mutua, F., Dewey, C., Arimi, S., Ogara, W., Githigia, S., Levy, M. and Schelling, E. 2011. Indigenous pig management practices in rural villages of Western Kenya, Livestock Research for Rural Development. Retrived from http://www.lrrd.org/lrrd23/7/mutu23144.htm

Plowright, W., Thomson, G.R. and Neser, J.A. 1994 African swine fever. In Coetzer J.A.W., Thomson, G.R. and Tustin, R.C. (Eds.), Infectious Diseases of Livestock, with Special Reference to Southern Africa, 1. Oxford University Press, Cape Town, 568-599.

Penrith, M.L. and Vosloo, W. 2009. Review of African swine fever: Transmission, spread and control. Journal of the South African Veterinary Association, 80 (2), 58-62. Retrieved from http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S1019-91282009000200001&lng=en&tlng=en.

Siem Reap Provincial Department of Agriculture, Forestry and Fisheries. August 2019. Strengthening the prevention of infectious diseases, especially the African swine flu. Retrieved from https://www.siemreap.maff.gov.kh/post/5d638a2e4834b

Thomson, G.R. 1985. The epidemiology of African swine fever: The role of free-living hosts in Africa. Onderstepoort Journal of Veterinary Research, 52, 201-209.

Thomson, G.R. and Tustin, R.C. (Eds.). 1994. Infectious diseases of livestock, with special reference to Southern Africa. Vol. 1, Oxford University Press, Cape Town, 568-599.

Venn, V., Vouchsim, K., Borarin, B., Thong, K., Loinda, R.B. and Sopheareth, M. 2014. Risk assessment of veterinary drug residues: The dietary exposure assessment of benzylpenicillin and tetracycline residues in pork consumed on Filipino market. Journal of Life Sciences, 8 (11), 872-879.

World Organisation for Animal Health. OIE. 2010. Introduction and qualitative risk analysis. Handbook on Handbook on Import Risk Analysis for Animals and Animal Products, Vol. 1, 2nd Ed.