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Original Research Article

Seroprevalence Rate and Factors Causing African swine fever Outbreak in Kupang City and Regency of East Nusa Tenggara Province, Indonesia

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Abstract: African Swine Faver (ASF) disease was reported in Indonesia in 2019. ASF outbreaks occurred in East Nusa Tenggara Province in 2020 and 2021, with hundreds of thousands of pig deaths. An effective vaccine to prevent the transmission of ASF is currently undiscovered. The diagnosis of ASF is based on the observation of clinical symptoms, observation of the epidemiology of the disease, and laboratory examinations of both serological, virological, and post- mortem tests. Rapid and accurate serology tests are indispensable to prevent the transmission of ASF infection. A risk- based approach to infectious sources needs to be taken to prevent the emergence of ASF outbreaks. This study aims to determine ASF seroprevalence and identify factors that play a role in the transmission of the ASF virus. The results of the study obtained a seroprevalence rate of 52.9%. Risk factors that act as a source of ASF transmission are the origin of pig livestock from the animal market (6.7%), feed from swill feeding (61.7%), non-routine health checks carried out (90%), pig carcasses thrown into landfills (20%), manure waste left alone (33.3%) and sick pigs immediately sold (60%).
Keywords: ASF, seroprevalence, Risk factors, kupang.

INTRODUCTION

African Swine Fever (ASF) is a disease of viral origin that is hemorrhagic in pigs with a very high mortality rate that can even reach 100% (Oura, 2020; Netherton *et al.*, 2019). ASF is caused by the African Swine Fever Virus (ASFV) of the Asfarviridae family and is a double-stranded DNA virus (Galindo and Alonso, 2017; Njau *et al.*, 2021; Revilla *et al.*, 2017).

ASF has caused huge losses as well as economic consequences for the pig farming industry in recent years. An effective vaccine to prevent ASF disease has not yet been found and has implications for not only hindering the health and well-being of animals but also adversely affecting the biodiversity and livelihoods of farmers (WOAH, 2019).

The Central Statistics Agency recorded the number of pig populations in East Nusa Tenggara Province (NTT Province) in 2018 as many as 2.141.246 heads of the total pig population in Indonesia of 8.542.488 heads and is the largest in Indonesia, with the number of active farmers reaching 900 thousand households (BPS, 2019). According to the NTT Provincial Livestock Service in 2022, as many as 122 thousand pig livestock owned by residents died due to the ASF virus (de Rosari, 2022). Looking at the data on cases of ASF disease that caused such a large number of deaths, this is very detrimental economically to most pig farmers in NTT Province.

The ASF virus can survive for a long period in blood, feces, and tissues, raw or undercooked pork products. In addition, the appropriate natural environment is the best place for the long-term survival of the ASF virus such as in the bone marrow of the femoral bone and the tibia bone of the buried pig carcass (Hranush *et al.*, 2021). After the pig contracts the ASF virus, infected pigs are the most important source of the virus for susceptible pigs. Pigs infected with less virulent (avirulent) viral germs can transmit the virus to susceptible pigs for 1 month after being infected through the blood (Oura, 2020). In addition, pigs infected with ASF and recovered can be career and chronic in nature (Abworo *et al.*, 2017). The rate of spread and transmission of ASF on a pig farm is strongly influenced by several factors that are at risk of increasing the number of ASF cases. Several analytical studies have been conducted to identify the risk factors involved in the transmission and spread of ASF on a pig farm. Risk factors that can play a role in the spread of

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the ASF virus include poor farm management and low levels of biosecurity applied (OI Sevskis *et al*., 2016; Belini *et al.*, 2016; Lamberga *et al.*, 2020), swill feeding and pig slaughter in livestock areas (Arias *et al.*, 2018), ASF transmission through the purchase of pigs and the purchase of pork and processed products (Boklund *et al*., 2020), human behavior and activities, environmental factors, and culture or customs of the community. In addition, other factors considered risk factors in the transmission of ASF include the density of the pen and the number of pig populations (Gulenkin *et al.,* 2011), the extensive and open farming system (Mur *et al.,* 2016), the location of farms adjacent to farms that are contracting ASF disease (Boklund *et al*., 2020), the use of cement for artificial insemination (IB) of lusted sows from outside farms (cement out of farm), contact with unsalted external pigs or wild boars (Jurado *et al*., 2018) as well as improper disposal of dead pig carcasses, including waste disposal that is not done well (Lamberga *et al.,* 2020).

The profile picture of pig farming in NTT Province, especially in Kupang City and Regency, is illustrated by risk factors that have the potential to be a source of transmission and spread of ASF disease such as most farmers providing swill feeding, extensive or semi-intensive livestock systems with open-air cage construction that allows free access, improper disposal of pig carcasses, management of waste treatment that is not done properly, the use of IB cement from outside the farm (Angi *et al*., 2014). In addition, another factor that also causes the easy transmission of ASF in the NTT Province area is the geographical condition of the NTT Province area, where it is difficult to carry out strict supervision of the traffic in and out of pigs from areas at risk, especially areas bordering other countries (Timor Leste) as well as supporting factors for low knowledge of pig farmers about preventing ASF transmission. According to (Gallardo *et al*., 2022), the diagnosis of ASF is based on the observation of clinical symptoms, observation of disease epidemiology, and laboratory examination of serological, virological, and post-mortem tests. Rapid and accurate virological tests are indispensable to prevent the transmission of ASF infection. Programs that are carried out in a controlled manner to eradicate ASF in areas with endemic tendencies such as in NTT Province must be reviewed and carried out properly and supported by laboratory examinations and followed by regular inspections or clinical inspections examinations. The best use of diagnostic tools is to combine the detection of viruses and ASF antibodies to increase the effectiveness of disease control measures, regardless of the nature of the circulating ASF virus strains (Matsumoto *et al*., 2020). An accurate evaluation of the results of serological and virological assays should be carried out, taking into account all clinical and epidemiological findings, within the framework of the investigation that will be carried out if there is a suspicion or confirmation of ASF (Gallardo *et al.,* 2022). This study aims to determine the seroprevalence of ASF after the outbreak in 2020 - 2021 by looking at the results of the ASF-Ag rapid test kit testing on serum samples taken from pig farms in the Kupang City and Regency areas and identify factors that play an important role in the spread of the ASF virus.

MATERIALS AND METHODS

Ethical approval dan Informed consent

The study was approved by Ethics Commission for the Use of Animals in Research and Education, Faculty of Veterinary Medicine, Nusa Cendana University, Indonesia (approval no.042/KEH/SKet/IX/2022). Verbal consent was obtained from each participant before the study.

Study period and location

Blood was collected to collect serum samples at several pig farms in Kupang City and Regency, East Nusa Tenggara Province - Indonesia. ASF-Ag rapid test kit testing location was carried out at the Animal Health Laboratory of the State Agricultural Polytechnic of Kupang. The duration of the study starts from May to December 2022.

Design and sample population

The study was conducted with cross-sectional studies. Pig blood sampling is carried out using simple random sampling. The sample size was determined using Win Episcope 2.0 software, with a confidence level of 90%, while the expected prevalence was 85% and an error rate of 5%. The number of pig blood samples taken as research samples was 138 samples. The first stage of the study was to take blood samples from several locations in Kupang city and the regency of NTT Province Indonesia using blood samples taken from pigs each ± 10 ml (from the jugular vein or auricular vein) using a sterile syringe without anticoagulant (EDTA) directly, then put into a centrifugation tube. Placed in a tube rack with an inclined position in a cool box so that agglutination does not occur. The tube containing the blood was centrifuged at a speed of 3000 rpm for 15 minutes. After centrifugation will form 2 layers of liquid. The transparent yellow clear layer that sits on the top is serum. Next, the serum is taken using a sterile drip pipette/micropipette and transferred to another clean, sterile tube for further testing. The second stage is by testing serum samples with the ASF-Ag Rapid Test Kit (Ring Biotechnology Co Ltd Production, China, ISO9001 &ISO 13485 Certified Manufacturer). Test principle, this kit applies the principle of Colloidal Gold Immunochromatography assay. The sample will move together with the colloidal gold marker along the chromatography membrane. If African Swine Fever Virus (ASFV) antigen exists in the samples, it will combine with the colloidal gold marker and the antibody in the detection line, then it will show a purple color. Otherwise, it will not show the color reaction. Assay procedure, Allow all kit components and samples to reach room temperature before testing. Take the sample with the pipette, add 2 drops (about 60 μL) of serum/plasma supernatant to the sample diluent vials, and mix fully. Tear the aluminum foil bag of the detection card and take out the detection card, and put it on a smooth, clean table, Add 4~5 drops (about 120 μL) of the mixture to the sample well(S) for detection vertically and slowly (avoid foaming). Incubate for 10 to 20 minutes and then judge the results immediately. Judgment of result, negative only the control line region (C) shows a line in the observation well. Positive, both the test line region (T) and the control line region (C) show a line in the observation well. Invalid, No line shows in the observation well of the control line region. Interpretation of the result, the negative result reveals that there is no ASFV antigen in the sample. If there is a corresponding acute symptom, then ASFV infection cannot be excluded. The positive result reveals that there is ASFV an antigen in the sample. It might be infected with ASFV, and the result should be combined with other methods to analyze (Ringbio, 2020).

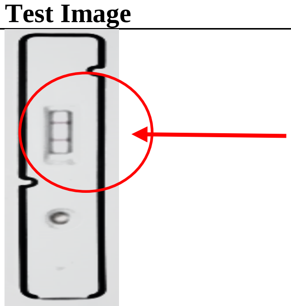
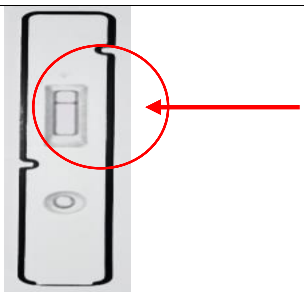
Identification of risk factors that play a role in ASF transmission

The questionnaire survey was conducted to see the profile of pig farms in the Kupang City and Regency areas and their maintenance management related to risk factors that play an important role in the transmission of ASF disease. Data collection was carried out by interviewing 60 pig farmers through a survey using a structured questionnaire. The selection of respondents was based on several criteria such as the length of breeding, the population of pigs raised, as well as the maintenance management carried out such as housing, and feed availability. Questions in the fill-in-the-blank list include aspects of maintenance management and prevention of ASF post-outbreak diseases, aspects of hygiene and sanitation of cages and sewage treatment, aspects of ticks (*Ornithodoros sp.*) as an infectious vector of ASF, aspects of counseling and sources of information, and aspects of farmers' knowledge about ASF. Before data collection is carried out, questionnaire data validation is carried out to see the extent of the validity and reliability of the questions asked.

RESULTS AND DISCUSSION

The test results of pig serum samples with tests found 73 positive samples or contained ASF virus in serum, 10 invalid samples, and 55 negative samples (no viral antigens in serum). The seroprevalence rate of ASF in Kupang City and Regency after the 2020-2021 outbreak was 52.9% (Table 1).

Table 1: Test Results of 138 serum from pig blood samples

Number	Test Image	Test Results	Total Number	Seroprevalence (%)
1		(+) Positive	73	52.9 %
2		(-) Negative	55	39.9 %
3		Invalid	10	7.3%

The ASF seroprevalence rate in the Republic of East Timor which is still on one island (Timor Island) with Kupang City and Regency is 34.4% (23). The high number of ASF cases tested with ASF-Ag rapid test kits is caused by several factors, including the ability of the ASF virus to survive in its natural conditions (Hranush, *et al*., 2021), where most of the ASF cases that occur in most countries in Africa tropical climate conditions and regional geography are almost the same as climatic and geographical conditions in the Kupang City and Regency area of NTT Province. The complete eradication after the ASF outbreak in the Kupang City and Regency areas was also not carried out optimally, causing the ASF virus to survive for a long time. ASF virus particles can resist and transmit in maintaining their stability after contamination with the carcass of a dead pig, soil, water, or other biological products. In addition, the presence of the virus in the body of pig livestock that survives after being infected with ASF can act as a carrier of the ASF virus which is likely to cause the persistence of the disease and spread the infection to other pig livestock so that ASF cases remain high (Chenais *et al*., 2019). In addition to the suboptimal end-to-end eradication factor and the ASF virus' ability to survive over a long period, the factors of pig trafficking that returned to normal conditions after the ASF outbreak included the pig trade in Kupang City and Regency. The ASF virus showed a high degree of evolution and spread over time, with no signs of decline to date. This is in line with the increasing pig population and the high traffic of pig trade between countries and between regions which causes the virus to continue to circulate and be maintained (Alkhamis, *et al.*, 2018). ASF is a cross-border animal disease (WOAH, 2020; Beltran-Alcrudo and Arias, 2020; Penrith, 2009) so the focus on prevention and control includes formal and informal interventions in national and international trade (Simulundu *et al.*, 2017). Although quarantine measures are known, the pathway of disease recognition is difficult to understand and is used for risk estimation (Beltrand-Alcrudo *et al*., 2019). The high number of ASF cases in Kupang City and Regency is also caused by t farmers' frequent neglect of implementing biosecurity measures. According to (Chenais *et al*., 2017) although the application of biosecurity is often applied, sometimes breeders still commit violations or omissions in the application of biosecurity, resulting in an increase in cases or the emergence of ASF outbreaks.

The results of the ASF-Ag rapid test kit conducted with high seroprevalence are illustrated from the portrait of pig farms in Kupang City and Regency, but the ability to test ASF at the disease point allows a quick response to the outbreak and control of the spread of the disease in an endemic situation. A fast and reliable diagnosis is very important as an early warning when there is a case of ASF to intervene promptly and monitor ASF (Arias *et al.*, 2017). Optimal testing should be possible to detect all genotypes and variants of the ASF virus. Identification of diagnostic results of ASF-infected pigs with high sensitivity and specificity should be validated by OIE guidelines (Blome *et al*., 2020). In addition, the diagnosis must be easy to handle, allow for quick interpretation, and be available at a reasonable cost at all times. Testing with the ASF-Ag Rapid Test Kit will also be required to co-partner with the vaccine candidate if an effective vaccine has been found for the prevention of ASF. Important things that must be considered from the ASF-Ag Rapid Test Kit are that the test results are qualitative tests. For confirmation of results, it is recommended also to look at clinical symptoms as well as compared them with other detection methods (Ringbio, 2020).

Relationship between risk factors and aspects of maintenance management and prevention of post-outbreak disease ASF

The maintenance management aspects seen and observed in this study include the type of pig, the origin of the pigs raised, and the source of feed given to pigs. The breed of pigs raised in the highest crossed breed with a figure of 46.7%, followed by purebreds (Duroc, Landrace, or Yorkshire) at 31.7%, while the rest are local pigs (native pigs) at 21.7%. The origin of pig livestock or saplings comes from or is purchased from home farmers or traditional breeders 71.7%, commercial farmers (intensive farms) in East Nusa Tenggara Province 18.3%, from the animal market 6.7%, while 3.3% of the origin of pigs raised is a gift of relatives. The survey results also obtained data where the feed given to pigs from factory-processed commercial feed was 28.3%, the self-produced feed was 10.0%, and the most were home or restaurant waste or market waste or slaughterhouses at 61.7%. Home or restaurant leftover feed (swill feeding) is usually directly given to the pig or mixed with other feed such as bran or commercial feed, where the largest percentage of the survey of waste farmer respondents (swill feeding) is directly given to the pig by 38.3%. From the information of farmers, information was also obtained that most of the garbage feed was food obtained from stall owners or tent stalls for pork sei or pork rice which usually sells from the afternoon to the evening. Swill feeding is one of the main risk factors under which the ASF virus enters a farm (Nantima, *et al*., 2015; Cheng and Ward, 2020), so farmers or pig owners themselves unconsciously carry the ASF virus to their pigs.

The relationship between risk factors and aspects of pig health management

The aspect of health management that can be seen from the farmer respondents is that most pig farmers in the Kupang City area keep their pigs in pens (98.3%) with semi-woven cage buildings made of wood (71.7%). The system of its maintenance, for the most part, keeps the livestock of pigs with separation by age and sex (83.3%). The important thing that can be seen from the aspect of health management is that there are no regular health checks on pigs raised (90.0%), including never being given antiparasitic drugs (85%). The service factor in handling pig health also reduces the risk of being infected with the ASF virus (Minoungou *et al*., 2019; Bellini *et al*., 2016). Another thing that is also related to the transmission of ASF is that if the pig dies during maintenance the respondents, there are 8.3% of pig carcasses are thrown into landfills. Although the percentage is small, the dead pigs can likely be caused by ASF cases. Disposal of dead pigs or parts of dead pigs at the appropriate location to avoid the spread of material infected with ASF disease (Bellini *et al*., 2016). Improving the condition or maintaining the condition of pigs during the winter season or hostile weather and giving vitamins as a complement to the nutritional content of the feed given, data was obtained that most farmers only give their pigs vitamins when the pig condition is sick (60 %). Vaccinations carried out to prevent diseases that have become endemic diseases in the East Nusa Tenggara region such as Hog Cholera also obtained data that almost most farmers do not give the Hog Cholera vaccine to their pigs (80%). According to (Sanchez-Vizcaino *et al*., 2014), The clinical symptoms of ASF disease are almost similar to Hog Cholera, so laboratory examinations need to be carried out to distinguish the two diseases that occur.

Relationship between risk factors and aspects of sanitation and disinfection and waste management

Aspects of sanitation and disinfection of cages as well as the processing of waste from domesticated livestock waste such as pigs play an important role in the transmission of a disease, where the condition of the cage and the cleanliness of the cage greatly affect the transmission of disease. Strict sanitary and disinfection controls will guarantee the prevention of ASF transmission. Data and direct observations in the field generally pig farmers in Kupang City and Regency clean the pens and places to eat and drink quite well, where farmers routinely clean their pens before feeding and drinking their pigs. The respondent farmers from the aspect of sanitation and disinfection of the pen can be said to be good where most of the respondent farmers regularly clean the pens and feeding places for pigs in their maintenance. The average farmer respondent cleaned their pens and feeding places in the morning and evening when they wanted to feed and drink their pigs (98.3%) and were routinely carried out (93.3%). Since there is no effective vaccine against the virus, farm biosecurity and good farm practices are the only effective tools to prevent the spread of the ASF virus in a pigsty. Therefore, an important component of biosecurity in the field of animal husbandry is the procedure for cleaning, sanitizing, and disinfection of the coop (de Lorenzi *et al*., 2020).

Most pig farmers in Kupang City and Regency raise pigs in the area adjacent to their houses; even some farmers build pens directly adjacent to the walls of their homes. Data from interviews and direct observations in the field show that the average farmer has a special channel of waste or is discharged into a simple-made sewer whose main use is devoted mainly to avoiding odors from the results of pig manure disposal. In the case of an outbreak of ASF disease on one farm, the right waste treatment needs to be applied to minimize the spread of ASF (Dharmayanti, *et al*., 2021). Most farmers let pig waste either liquid (25%) or solid (33.3%) waste into the sewers around the pen (33.3%). Poor waste management in pig farms also has the potential to be an infectious agent of the ASF virus. Pigs become infected with the ASF virus mainly through the oro-nasal route after contact with an infected pig or after eating pork containing the virus or contaminated products. The results of excretion and secretion of pigs infected with ASF such as feces, urine or saliva, and blood still contain the ASF virus and can persist for a long time (Gellardo *et al*., 2015).

The relationship between risk factors and aspects of ticks (*Ornithodoros sp.*) that have the potential to be an infectious disease

Pigs can contract ASF disease as a result of the bite of an infected tick (*Ornithodoros sp*), so the disease is categorized as an arthropod-borne disease (Boinas *et al*., 2011). ASF began to enter East Nusa Tenggara by road from the country of East Timor at the end of 2019 and began attacking pig livestock on the island of Timor until it spread throughout the archipelago in East Nusa Tenggara. While the State of East Timor most of its processed food products including pork directly related story of Portugal. ASF disease is a hemorrhagic disease that often affects local pigs and wild boars, where the tick *Ornithodoros erraticus sensu stricto argasid* is the only biological vector of the ASFV virus known to occur in Europe, especially in Portugal (Ribeiro *et al*., 2015). So that the possibility of ASF transmission in pigs occurs in East Nusa Tenggara Province including Kupang City and Regency according to data reported due to tick-infected vectors whose strains are the same as those in Timor Leste and Portugal. Most breeders are unaware of the tick *Ornithodoros sp*. as an infectious disease ASF (91.7%). At the time when breeders noticed the presence of ticks *Ornithodoros sp*. in their pig cages, most farmers shut down directly mechanically (53.3%).

Relationship between risk factors and counseling aspects, sources of information, and knowledge of farmers related to ASF disease

Farmer knowledge also plays an important role in preventing or reducing risk factors for the onset of ASF disease in pigs. The low knowledge and lack of attention of breeders to ASF disease make it difficult to prevent ASF outbreaks (Guinat *et al.*, 2016; Chenais *et al*., 2017). The results of a questionnaire survey on pig farmers in Kupang City and Regency, where farmers knew ASF through print and electronic media by 48.3%, through seminars or counseling by 33.3%., received ASF information from neighbors or farmers by 11.7 %. The survey results were only 6.7% of farmers never heard of ASF. The survey results also showed that most breeders knew the cause of ASF, namely the virus, and 96.7% and 3.3% of farmers did not know the cause of ASF. However, there is a major problem or also a risk factor for the spread of ASF disease, where farmers immediately sell their pigs if they show symptoms of illness with clinical symptoms leading to ASF (60%). The habit of selling pigs after an outbreak, where the condition of pigs is sick is a risk factor for the transmission of ASF (Woowong *et al*., 2020).

CONCLUSION

Based on the results of the study, the seroprevalence rate of ASF in Kupang City and Regency is 52.9%. The high number of ASF cases is caused by several contributing factors, including most of the pigs raised by farmers come from home farmers (traditional farms) including from animal markets that are poorly controlled health status, feed sources mostly come from swill feeding, health checks are not carried out regularly, pigs that die carcasses are thrown into landfills and if the pigs are sick, farmers immediately sell their pigs. Some suggestions that can be given from this study include the need to implement better livestock management in terms of feeding and feed sources given to pigs not contaminated with the ASF virus, health checks of pigs must be routine and scheduled, and the application of biosecurity must be tightened.

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