POTENTIAL HIGH-RISK PATHOGENS FOR FREE-RANGING PIGS

Highlighting the potential high-risk pathogens that are transmissible between humans, wildlife and free-ranging pigs at Den Elshorst

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Research report

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Den Elshorst Tref 't landelijk leven...

Abstract

Nature inclusive agriculture with outdoor husbandry could pose certain risks of pathogens transmitted via wildlife and via humans. Outdoor husbandry is less controllable when it comes to infection chances with pathogens rather than conventional indoor settings. During this study possible risk pathogens that could be transmitted by humans and wildlife to free-ranging pigs (*Sus scrofa domesticus*) and back to humans have been investigated. The wildlife that has been seen on wildlife cameras has been selected. In this literature study was focused on pigeons (*Columba spp.*), kestrels (*Falco spp*.), rats (*Rattus spp.)*, foxes *(Vulpes spp.)*, martens (*Martes spp.)* and humans. A total of 20 pathogens that can be transmitted through these animals, were enlisted based on relevance for transmission in the area of Den Elshorst and relevant in terms of risks. From these 20 pathogens, 17 are zoonotic pathogens and three are non-zoonotic pathogens. Some pathogens can be transmitted by multiple animal species, which makes them a possible higher treat. Beside the amount of pathogens transmitted by the studied animals, other risk factors has been studied. Those risk factors include survival in the environment, occurrence in the Netherlands, and availability of vaccinations. Regarding the amount of risk factors, *Leptospira spp*. (Leptospirosis) scored highest, whereas *Varicellovirus spp*. (Aujeszky's disease) and *Pestivirus spp*. (Classical Swine Fever) scored lowest. However, pathogens with a lower number of risk factors can still be a risk when there is an outbreak. Concluding, there are definitely risks with keeping pigs outside, because of the higher risks of other animals getting close to the free-ranging pigs and thus being able to transmit pathogens. To prevent this transmission of pathogens, it is important to maintain good animal control at and around the farm. Additionally, it is important to install good fencing at the different pig pens to avoid contact with wild animals. To prevent transmission through meat consumption it is necessary to have continuous abattoir control and to cook the meat properly.

Preface

This research project was commissioned by the Dutch Ministry of Agriculture, Nature and Food Safety ("Ministerie van Landbouw, Natuur en Voedselveiligheid") and is a follow up study about the possible presence of pathogens in the area of pig farm Den Elshorst in Diessen. Den Elshorst is a pig farm in the Netherlands on which pigs are housed outside, making it an interesting farm for research. Just like this research project, previous project was also conducted by students from the HAS University of Applied Sciences supervised by Dr. Liesbeth Dingboom. The aim of this study was initially the identification of the presence of Trichinella spp. and Toxoplasma gondii in wildlife with ELISA, but was adjusted to a literature study about the presence of pathogens at and around Den Elshorst later. Reason for this was the low number of cadavers found. Moreover, the results of this study will contribute to a bigger program which discusses the possibilities to sustain nature inclusive agriculture in the Dutch pig industry. This could improve the welfare and housing of pigs, but poses risks regarding the transmission of pathogens. In this report, we give an overview of pathogens (focused on Den Elshorst), discuss the risks of free-ranging pigs and give advice on how to manage the risks of these pathogens.

We would like to express our gratefulness to Drs. Martijn Stijntjes from the Dutch Ministry of Agriculture, Nature and Food Safety, Ben Bruurs and Michiel Bruurs from Den Elshorst and the HAS University of Applied Sciences for giving us the chance to be part of this project. In addition, we would like to thank Dr. Liesbeth Dingboom for her expert assistance and guidance. We also gratefully acknowledge Dr. Frits Franssen and Dr. Rolf Nijsse for their support and expertise on *Trichinella spp.* and *Toxoplasma gondii*.

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1. Introduction

Den Elshorst is a nature inclusive farm where free-ranging domesticated pigs (*Sus scrofa domesticus*) are used for management of nature and agricultural land. The pigs walk outside in their natural habitat (Rijksoverheid, 2017). Nature inclusive agriculture could pose certain risks due to the keeping conditions of the animals. Outdoor husbandry is less controllable when it comes to infection chances with pathogens rather than conventional indoor settings. Wildlife has a relevant role in the transmission of diseases, because it can carry pathogens over large distances, even between countries (Gortázar et al., 2007). Those pathogens can impair animal health and welfare and can be transmitted to humans (Pietrosemoli & Tang, 2020). However, nature inclusive agriculture helps to optimize ecosystem services and increases biodiversity (Arcena, 2019).

Nowadays in the Netherlands, the main focus has been on a high production per hectare or per animal, yet this affects ecosystems and results in loss of biodiversity (Erisman et al., 2016; Tsiafouli et al., 2015). Nature inclusive agriculture is a policy concept to ensure food security and economic development without negatively affecting the environment (Van Doorn et al., 2016). This will eventually lead to a more sustainable form of agriculture where different kinds of animals will contribute to a stable ecosystem (Runhaar, 2017). This concept improves animal welfare since pigs are able to explore the environment and express natural behaviour (Brace & Hopster, 2006). Free-ranging pig farm Den Elshorst is a nature inclusive farm with a governmental Green Deal. Green Deals provide the opportunity to enable sustainable and innovative initiatives that would not be possible under the current legal regulations (Rijksoverheid, 2021a).

Den Elshorst uses its pigs for management of agricultural land and for meat consumption. The pigs walk outside in fenced pens, which are adjacent to a nature conservation area. Wildlife and humans have been seen in, above and around the fenced pens and cause a potential threat for pathogen transmission to the pigs (van Blitterswijk et al., 2020). There are three important transmission routes for pathogens (Taylor et al., 2001). Pathogens can be transmitted via direct physical contact, such as sexual contact, through wounds or ingestion of tissue of infected intermediated hosts. Another transmission route is indirect contact, via food, water, feces, inhalation or environmental reservoirs such as soil (Taylor et al., 2001; Stelzer et al., 2019**;** WUR, 2020a). Additionally, vectors can play an important role in the transmission of pathogens (Taylor et al., 2001). Transmission from domestic pigs to humans is also possible by ingestion of raw or undercooked meat (Dorny et al., 2009).

Zoonotic pathogens can infect multiple species, which can cause infectious disease outbreaks in wildlife, livestock and humans (Woolhouse, 2002). For this reason, zoonotic pathogens are regarded to emerge almost twice as likely than non-zoonotic pathogens (Taylor et al., 2001). All types of pathogens, including fungi, parasites, bacteria and viruses, can cause a zoonotic disease or infection (Bueno-Marí et al., 2015). Zoonotic diseases with significant public health, agricultural, and ecological impacts are known to impose financial burdens on farmers and governments around the world. These economic consequences can include mortality and an overall production loss in pigs and there can be sanctions on the trade of exporting the animal products from the infected region (Chua, 2003; Miller et al., 2013; Uddin Khan et al., 2013). An example of a zoonotic diseases is rabies (RIVM, 2019; Singh et al., 2017; WUR, 2021a). Rabies is a zoonotic disease with high-risk transmission (Hemachudha et al., 2013). If there is rabies outbreak, for example in the wildlife, that will have economic and social consequences, such as starting a vaccination program for humans or vaccinating all domestic pigs and cats in a specific area (De Rosa et al., 2018). De Rosa (2018) noted that the rabies outbreak in The Netherlands in 1988, immediately initiating a vaccination programs.

Important infectious diseases in the domestic pig sector are African Swine Fever (*Asfivirus spp*.), Classical Swine Fever (*Pestivirus spp*.) and Aujeszky's disease (*Varicellovirus spp*.) (Sánchez-Vizcaíno et al., 2015; Guinat et al., 2016; Dixon et al., 2019). These three infectious diseases can be transmitted through multiple transmission routes (Dixon et al., 2019; GD, 2021a; Guinat et al., 2016; Ribbens et al., 2004; Sánchez-Vizcaíno et al., 2015; WUR, 2021c). In the recent years, African Swine Fever received much attention in The Netherlands. African Swine Fever is a disease caused by the genus *Asfivirus spp*., which is very infectious for domestic pigs and other species (WUR, 2020b). Transmission from this virus to domestic pigs is possible through multiple ways (Dixon et al., 2019; Guinat et al., 2016; Sánchez-Vizcaíno et al., 2015; WUR, 2020b) African Swine Fever virus can be transmitted to domestic pigs through direct contact with wild boars (*Sus scrofa*) or via indirect contact such as meat products from infected pigs or wild boars (WUR, 2020b). Transmission of the African Swine Fever virus to wild boars is possible through direct contact with an infected wild boar or through indirect contact of kitchen waste, food scraps and meat products from infected pigs or wild boars left in the field by humans (f.e hunters) (WUR, 2020b). Another way of transmission to domestic pigs is with the use of infected materials and transport vehicles (WUR, 2020b). Transmission of this virus is also possible from an infected domestic pig to another domestic pig (WUR, 2020b). An infection with this virus does not always lead to clinical symptoms in domestic pigs (WUR, 2020c). If domestic pigs develop clinical symptoms, these symptoms become more severe in a short time with high mortality rates up to 100% (WUR, 2020c). The clinical symptoms for the African Swine Fever can look like the clinical symptoms for Classic Swine Fever (Sánchez-Vizcaíno et al., 2015; WUR, 2020b). If African Swine Fever is localized in the Netherlands, the consequences will be enormous (WUR, 2018). Since the pigs will be rendered on the farm where African Swine Fever is localized, there will be a negative financial impact for the farmer and a negative economic impact for the pig sector, (WUR, 2018).

Classical Swine Fever is caused by the genus *Pestivirus spp*. A part of the infected domestic pigs can survive this disease (WUR, 2021b). Classical Swine Fever can be transmitted through direct and indirect transmission (GD, 2021b). Direct transmission consists of contact between domestic pigs, while indirect transmission consists of transmission through infected materials and transport vehicles, kitchen waste, food scraps and meat products from infected pigs or infected manure (GD, 2021b; Ribbens et al., 2004; Weesendorp et al., 2014; WUR, 2021b). Humans are also an important transmission route to domestic pigs through indirect contact of infected clothes, shoes or hands (GD, 2021b). In case of an outbreak of Classical Swine Fever, there could be a negative economic impact for the pig sector. Besides this negative economic impact, pig farm businesses will also be affected, because their pigs will be rendered to prevent transmission of the disease. Not only the pigs from the infected farm will be rendered, but also all pigs within a radius of 1 km or more of the infected farm. An outbreak in the Netherlands in 1997 and 1998 resulted in the rendering of more than 8 million pigs, of which 6.5 million pigs were rendered based on animal welfare (Terpstra & De Smit, 2000).

Like African Swine Fever and Classical Swine Fever, Aujeszky's disease can be transmitted through direct and indirect transmission (GD, 2021c; WUR, 2021c). Aujeszky's disease can be transmitted directly between domestic pigs however most transmission occurs viaindirect transmission through the air and through kitchen waste, food scraps and meat products from infected pigs (GD, 2021c; WUR, 2021c). Humans can also transmit the virus indirectly through hands, clothes and materials (GD, 2021c). Infection with Aujeszky's disease can lead to sudden death (WUR, 2021c). Aujeszky's disease can also cause great economic damage (WUR, 2021c). An outbreak of Aujeszky's disease can result in, among other things, export bans and vaccinations of infected pigs (GD, 2021c; WUR, 2021c).

Preventive actions concerning transmission, such as fencing, feeding and water management, are primary options to control diseases caused by transmission from wildlife to cattle (Mantovani, 1992;

Chomel, 2008; Maurice 2008; Rodolakis & Mohammed, 2010; WHO, 2020). A preventive action of pathogen transmission is culling of the wildlife population of vaccination of wildlife (Gortázar et al., 2007). Another preventive action is vaccination of the domestic pigs themselves (Romagosa et al., 2011). This can result in virus shedding and reduction of clinical signs in domestic pig but there is limited information on how vaccination may prevent transmission to wildlife and/or humans (Romagosa et al., 2011). Surveillance and monitoring of wildlife can support the early detection of disease outbreaks, which can be crucial for public health (Artois et al., 2009). Most transmission from domestic pigs to humans is through the eating of meat products (Dorny et al., 2009). This can be prevented by cooking the meat products if the appropriate temperature is reached in the core of meat product (Dorny et al., 2009).

Wildlife have been observed with wildlife cameras around, in and above the fenced pens of farm Den Elshorst (Blisterwijk et al., 2020). To evaluate the risks of transmission of pathogens from the wildlife and humans to free-ranging pigs, a literature study was been done. The aim of this literature study was to enlist which pathogens can be transmitted by six species selected from animals caught on camera and how these pathogens can be transmitted to free-ranging pigs and humans. The various risk factors have been studied to make an estimation of the risk of transmission. An advice about the preventive and control measures is included in the discussion.

2. Methods

2.1 Literature study

To answer the research questions, relevant articles and reports were searched. The databases GreenI, Google scholar, PubMed, ScienceDirect and ResearchGate were used. Additionally, the websites of Wageningen University, NVWA, RIVM, and GD Dierengezondheid were consulted.

2.2 Selection criteria

Farm Den Elshorst practices nature inclusive agriculture and is a Dutch farm that keeps its pigs free ranging outside instead of in complete indoor facilities. In a previous study conducted at Den Elshorst, multiple wildlife species were observed in and around the pig pens by wildlife cameras. In total, 12 species have been seen. From these 12 species, six were selected for their role in transmission based on different aspects. Birds were observed the most, therefore pigeons (*Columba spp.*) and kestrels (Falco spp.) are included in this study. Other species that are included are rats (*Rattus spp.)* because of their abundancy and their bad reputation in transmission of pathogens*,* foxes *(Vulpes spp.)* and martens (*Martes spp.)* because they are scavengers and humans because they visit the pigs, possible feeding and petting them. From the diseases that could be transmitted by these animals, 18 pathogens where selected based on their presence in the Netherlands and on the number of selected animals able to transmit the disease.

2.3 Risk analysis

For the results, 18 pathogens with 20 causal pathogens were enlisted, which are able to infect domestic pigs, humans and one or more of the wildlife species. Some diseases can be caused by multiple pathogens. Therefore, we have focused on the two pathogens *Trichophyton spp*. and *Microsporum spp*. for Dermatophytosis and *Echinococcus multilocularis* and *Echinococcus granulosus* for the disease Echinnococosis. For the 18 enlisted pathogens a risk analysis has been made. The parameters that have been included as risk weighting factors are pathogen being zoonotic or not, whether the pathogen is present in Europe and/or in The Netherlands, the pathogens chance of survival in the environment or being food-borne, whether there are vaccinations available for pigs in The Netherlands and whether the pathogen is lethal for pigs and/or humans. Furthermore, it was assessed whether these diseases were included in the following Dutch laws: “Gezondheids- en welzijnswet van Dieren” and/or “Wet Publieke Gezondheid”. Risk factors were assessed per pathogen with a binary choice, “yes” being in agreement with the risk factor, “no” being in disagreement. The amount of risk factors answered with a “yes” was then counted.

3. Results

A total of 20 pathogens were enlisted based on relevance for transmission in the area of Den Elshorst and relevant in terms of risks (table 1). From these 20 pathogens, 17 are zoonotic pathogens and three are non-zoonotic pathogens. Moreover, seven of these pathogens are not only infectious for humans but can also be lethal. Six pathogens can be lethal for pigs, three of these six pathogens are not zoonoses. These pathogens are *Varicellovirus spp.* (Aujeszky's disease), *Asfivirus spp.* (African Swine Fever) and *Pestivirus spp.* (Classical Swine Fever). Pathogens which can be lethal for both humans and pigs are *Lyssavirus spp.* (Rabies) and *Leptospira spp.* (Leptospirosis). Regarding the amount of risk factors, *Leptospira spp.* (Leptospirosis) scored highest, whereas *Varicellovirus spp.* (Aujeszky's disease) and *Pestivirus spp.* (Classical Swine Fever) scored lowest (*table 1*).

The pathogens *Yersinia pestis* (Plague), *Lyssavirus spp.* (Rabies), *Trichinella spp.* (Trichinellosis), *Varicellovirus spp.* (Aujeszky's disease), *Asfivirus spp.* (African Swine Fever) and *Pestivirus spp.* (Classical Swine Fever) are currently not present within the Netherlands. However, all of these pathogens are still present within Europe, with exception of *Yersinia pestis* (*table 1*). From the 20 enlisted pathogens, a number of 19 pathogens can reside and survive in the environment, of which most pathogens can reside in surface and drinking water (table 1 & 2). Additionally, from the 20 pathogens, only four were not transmittable by ingesting of contaminated food but by direct contact. These pathogens are *Trichophyton spp.* (Dermatophytosis), *Microsporum spp.* (Dermatophytosis), *Sarcoptes scabiei* (Scabies) and *Lyssavirus spp.* (Rabies). Despite the presence of many of the pathogens in the Netherlands and lethality of some of them, currently for only four of the 20 pathogens vaccines for pigs are available within the Netherlands. Pathogens with available vaccines for pigs are *Varicellovirus spp.* (Aujeszky's disease) (but only as an emergency vaccine), *Pestivirus spp.* (Classical Swine Fever), *Escherichia coli* (Shigatoxigenic *Escherichia coli,* also known as STEC) and *Salmonella spp.* (Salmonellosis) (this vaccine is only recently developed and tested). Finally, all pathogens are stated in either the "Gezondheids- en Welzijnswet voor Dieren" or in the "Wet Publieke Gezondheid", seven of the pathogens are even stated in both (*table 1*).

Table 1. Risk analysis. The 20 pathogens were enlisted which were found to be relevant for possible transmission in the area of Den Elshorst. Further columns are defined to be risk weighting factors which were added per pathogen (column Amount of risk factors) and laws in which the diseases are described.

Name disease	Pathogen	Zoonosis	Present in Europe	Present in the Netherlands	Survival in environment	Food-borne disease	No vaccine for pigs available in the Netherlands	Lethal for pigs	Lethal for humans	Amount of risk factors	Described in "Gezondheids- en Welzijnswet voor Dieren"	Described in "Wet Publieke Gezondheid"	References
African Swine Fever	*Asfivirus spp.*		X		X	X	X	X		5	X		NVWA, 2021; WUR, 2021; Sánchez-Vizcaíno *et al.* , 2012
Aujeszky's disease	*Varicellovirus spp.*		X		X	X	*	X		4	X		WUR, 2021; GD, 2021; Groot *et al.,* 2012
Botulism	*Clostridium botulinum*	X	X	X	X	X	X		X	7		X	RIVM, 2021; Vlaanderen *et al.* , 2020; Peck, 2006; WUR, 2021
Campylobacteriosis	*Campylobacter spp.*	X	X	X	X	X	X			6	X	X	RIVM, 2021; Vlaanderen *et al.* , 2020; Havelaar *et al.,* 2012; WUR, 2021
Classical Swine Fever	*Pestivirus spp.*		X		X	X		X		4	X		GD, 2021; NVWA, 2021; WUR, 2021
Cryptosporidiosis	*Cryptosporidium parvum*	X	X	X	X	X	X			6		X	RIVM, 2021; Vlaanderen *et al.* , 2020; Havelaar *et al.* , 2012; Groot *et al.,* 2012; WUR, 2021
Dermatophytosis (tinea capitis)	*Trichophyton spp. or Microsporum spp.*	X	X	X	X		X			5		X	RIVM, 2021; Vlaanderen *et al.* , 2020
Echinococosis	*Echinococcus miltilocularis, Echinococcus granulosus*	X	X	X	X	X	X		X	7	X		RIVM, 2021; Vlaanderen *et al.* , 2020; WUR, 2021
Leptospirosis	*Leptospira spp.*	X	X	X	X	X	X	X	X	8	X	X	RIVM, 2021; Vlaanderen *et al.* , 2020; GD, 2021
Listeriosis	*Listeria monocytogenes*	X	X	X	X	X	X			6	X	X	RIVM, 2021; Vlaanderen *et al.* , 2020; Havelaar *et al.* , 2012; WUR, 2021
Methicillin-resistant *Staphylococcus aureus* (MRSA)	*Staphylococcus aureus*	X	X	X	X	X	X		X	7		X	RIVM, 2021; Vlaanderen *et al.* , 2020; Havelaar *et al.* , 2012; WUR, 2021; Sergelidis & Angelidis, 2017
Plague	*Yersinia pestis*	X			X	X	X		X	5	X	X	RIVM, 2021; Vlaanderen *et al.* , 2020; WUR, 2021; Marshall *et al.* , 1972
Rabies	*Lyssavirus spp.*	X	X		X		X	X	X	6	X	X	RIVM, 2021; Vlaanderen *et al.* , 2020; WUR, 2021; Iowa State University, 2021
Salmonellosis	*Salmonella spp.*	X	X	X	X	X	**			5	X	X	RIVM, 2021; Vlaanderen *et al.* , 2020; Groot *et al.,* 2012; Havelaar *et al.* , 2012; WUR, 2021; GD, 2021; Peeters, 2019
Scabies	*Sarcoptes scabiei*	X	X	X	X		X			5		X	RIVM, 2021; Vlaanderen *et al.* , 2020; WUR, 2021
Shigatoxigenic *Escherichia coli* (STEC)	*Escherichia coli*	X	X	X	X	X		X		6		X	RIVM, 2021; Vlaanderen *et al.* , 2020; Havelaar *et al.,* 2012; WUR, 2021; NVWA, 2021
Toxoplasmosis	*Toxoplasma gondii*	X	X	X	X	X	X			6	X		RIVM, 2021; Vlaanderen *et al.* , 2020; Groot *et al.,* 2012; Havelaar *et al.,* 2012; WUR, 2021; GD, 2021
Trichinellosis	*Trichinella spp.*	X	X			X	X			4	X	X	RIVM, 2021; Vlaanderen *et al.* , 2020; Gottstein *et al.,* 2009; Groot *et al* ., 2012; WUR, 2021

Legend			
X	Yes	4	4 risk factors
	No	5	5 risk factors
*	Vaccine is available, but is an emergency vaccine	6	6 risk factors
**	Vaccine is only recently developed and tested.	7	7 risk factors
		8	8 risk factors

Table 2. Transmission. The 20 pathogens with their pathogen type, the animals able to transmit them and places on which they can survive in the environment. Pathogen types include viruses, bacteria, protozoa, fungi, a mite, a cestode and a nematode. From these pathogens, Toxoplasma gondii can be transmitted by the highest number of animals focused on. Moreover, 19 pathogens can reside and survive in the environment, of which most pathogens can reside in surface and drinking water.

Manner of transmission	Diseases	References
Animals		NVWA, 2021; WUR, 2021; GD, 2021; RIVM, 2021; Vlaanderen *et al.*, 2020; Havelaar *et al.*, 2012; Groot *et al.*, 2012; Sergelidis & Angelidis, 2017; Gottstein *et al.*, 2009
Humans	African swine fever, botulism, dermatophytosis, echinnococosis, methicillin-resistant *Staphylococcus aureus* (MRSA), plague, salmonellosis, scabies, shigatoxigenic *Escherichia coli* (STEC), toxoplasmosis	
Pigs	African swine fever, Aujeszky's disease, botulism, campylobacteriosis, classical swine fever, cryptosporidiosis, dermatophytosis, echinnococosis, leptospirosis, listeriosis, methicillin-resistant *Staphylococcus aureus* (MRSA), plague, rabies, salmonellosis, scabies, shigatoxigenic *Escherichia coli* (STEC), toxoplasmosis, trichinellosis	
Birds	Botulism, campylobacteriosis, listeriosis, plague, salmonellosis, shigatoxigenic *Escherichia coli* (STEC), toxoplasmosis	
Foxes	Cryptosporidiosis, echinnococosis, leptospirosis, scabies, toxoplasmosis	
Martens	Rabies, scabies, toxoplasmosis, trichinellosis	
Rats	African swine fever, Aujeszky's disease, botulism, campylobacteriosis, classical swine fever, cryptosporidiosis, dermatophytosis, echinnococosis, leptospirosis, listeriosis, methicillin-resistant *Staphylococcus aureus* (MRSA), plague, rabies, salmonellosis, scabies, shigatoxigenic *Escherichia coli* (STEC), toxoplasmosis, trichinellosis	
Environment		
Aerosol	Aujeszky's disease, classical swine fever, leptospirosis, methicillin-resistant *Staphylococcus aureus* (MRSA), plague, rabies	
Bedding	Dermatophytosis	
Plants	Salmonellosis, shigatoxigenic *Escherichia coli* (STEC), toxoplasmosis	
Soil	Botulism, listeriosis, toxoplasmosis	
Textile surfaces	Dermatophytosis, scabies	
Water	Campylobacteriosis, cryptosporidiosis, echinnococosis, leptospirosis, listeriosis, salmonellosis, shigatoxigenic *Escherichia coli* (STEC), toxoplasmosis	
Various other surfaces	African swine fever, Aujeszky's disease, classical swine fever, methicillin-resistant *Staphylococcus aureus* (MRSA)	

4. Discussion

For this study multiple pathogens that are a potential high risk for both humans and free-ranging pigs are investigated. Twenty pathogens have been identified as high-risk pathogens since they are transmissible between multiple host species to both humans and pigs. Three out of the 20 found pathogens are not zoonotic, but are still dangerous for pigs. Because, humans can also transmit pathogens after visiting for example, other farms. *Leptospira spp.* has the highest number of risk factors with a total of eight. However, pathogens with a lower number of risk factors can still be a risk when there is an outbreak. The zoonotic pathogens *Yersinia pestis*, *Lyssavirus spp.* and *Trichinella spp.* are currently not present within the Netherlands, but can be a risk if they emerge again. From the 17 found zoonotic pathogens, nine are transmittable via humans and seven via avian species such as pigeons and kestrel. Carnivoran species such as foxes and martens are found to transmit four to five zoonotic pathogens. Pathogens that are currently not present within the Netherlands can still be dangerous if they emerge again. The risk of emergence in the Netherlands is higher for *Lyssavirus spp.* and *Trichinella spp.,* because those pathogens are still present in other European countries. The period of time these pathogens can survive in the environment varies for each pathogen.

To prevent transmission of pathogens between wildlife, pigs and humans, it is important to apply appropriate disease management. Gortázar et al. (2007) reviewed multiple disease management possibilities. When reviewing wildlife disease control there are three basic methods: prevention of introduction of pathogens, control of the existing pathogens and the complete eradication of the pathogens via extensive changes in habitat (Wobeser, 2002. IN: Gortázar et al., 2007).

First foremost, the most important way of prevention of introduction of pathogens is by surveillance via testing. Serologic tests are a possible way of surveillance by screening for the presence of pathogens in the animals. However, mammals have a latent period of about one week before antibodies can be detected, and mammalian antibody production peaks around two weeks post-exposure (Zimmerman, 2018). Greiner et al. (2000) showed that the duration of the infection affects the sensitivity of ELISA serodiagnosis in porcine trichinellosis because of this latency. A realistic assumption for serologic tests is that the sensitivity varies with the stage of infection and with the immune status of the host species. Therefore, the sample size of the serum panels for screening should be great enough to include a range of disease stages, titers, and other relevant conditions, in order to provide an appropriate conclusion (Zimmerman, 2018). Prevention of foodborne pathogens derived from pigs can be seen as a disease management strategy as well. High biodiversity and outdoor pig husbandry may increase the risk of pathogen transmission to fattening pigs, which eventually can manifest as foodborne pathogen after slaughter (Wacheck et al., 2010). Therefore it would be advisable to indicate the consumer on the foodborne pathogen risks and the correct preparation methods prior to consumption (Lahou et al., 2015). Another way of prevention is the vaccination of domestic pigs. This can result in virus shedding and reduction of clinical signs in domestic pigs (Romoagosa et al., 2011). A result of this study is that for most of the high-risk pathogens no vaccine is available in the Netherlands. Perhaps in other countries vaccinations are available for those pathogens, which gives opportunities for keeping pigs outside. Vaccinations can be expensive and there is a risk that even after several years a vaccination program is non profitable for the public health sector (Rijksoverheid, 2021b; Dehove et al.,2012). For now, there is limited information on how vaccination may prevent transmission to other animals (Romoagosa et al., 2011).

Ways of controlling pathogens in pig pens is by limiting the number of animals kept in the pen and by placing fences on the borders of the pens to keep wildlife outside (Jankowska-Makosa & Knecht, 2015). Placing fences is particular a good method to avoid the transmission between a wild animal, that is infected with a pathogen, and the free-ranging pigs. For example, animals like wild boar and deer

(*Cervidae spp.*) are able to maintain the transmission cycles over long periods of time (Parra et al., 2005). Fencing might be a good solution because wildlife such as deer and cattle (*Bos taurus*) are unable to travel freely between private properties or pens due to fences. However, it should be taken into account that different types of fencing are necessary to prevent the entering of different types of animals. Thus, this might also be a good solution for the wildlife species included in this study. However, wild boars may still be a factor in the spread of the disease, as they are capable of invading fences (Parra et al., 2005). Wild boar might currently not be present at Den Elshort, however, they occur in the surrounding area and are a big risk factor for the transmission of the pathogens for Aujeszky's disease, African Swine Fever and Classical Swine Fever (van Tulden et al., 2020). In the Netherlands, wild boar are being controlled by culling and then screened for the prevalence of these pathogens with serological tests (Stegeman et al., 2017). Since seven pathogens are transmittable via avian species, it is important to separately look at pathogen prevention for these species. Avian species are able to transmit pathogens via their faeces (Boon et al., 2007). However, culling or for example pigeons is ineffective since the removed birds are quickly replaced by new juveniles (Magnino et al., 2009). Management strategies against infection via avian faeces would work best by adding plastic or metal spikes to buildings or fences in order to prevent them to sit down in the area (Magnino et al., 2009).

From the animals living in the area of Den Elshorst, rats are found to be a host in all of the 20 found pathogens. Therefore, a good way of pathogen control will be the prevention of rat-pig contact. Proper ways of rat control can be through either poising baiting or trapping and baiting. However, rat habitat modification is most likely the most effective method of rat control (Himsworth et al., 2013). Rodents in general are also a risk factor because they can cause major problems due to destruction and contamination of food, and also by the spread of various diseases (Backhans & Fellström, 2012). Considering that climate change can be suspected of supporting temperate zone rodent populations, the issue is likely to become more difficult to overcome in the future. Therefore, the use of rodent-proof buildings would be critical when developing new pig farms (Backhans & Fellström, 2012). However, this might be difficult to realize in free- ranging pigs farms because the animals need to be able to shelter at will, which gives the opportunity to rodents to enter the shelters.

Other ways of pathogen control and eradication is via habitat management. When a wildlife host population density reduces, with for example hunting or a higher distribution, the spreading chance of pathogens might also be reduced. Additionally, feeding restrictions for example, can reduce the habitat carrying capacity of ungulates and eventually reduce population density and aggregation around the free-ranging pig farm (Acevedo et al., 2007). These two factors are a key in the transmission of infectious diseases (Acevedo et al., 2007). Some pathogens are more persistent of living in moist soil- or surface water habitats, which is also the case in for example *Salmonella spp*. (Guan & Holley, 2003). In colder soils (4 to 6 °C), most pathogens can survive for at least 30 days (Guan & Holley, 2003). Temperature gradients can influence overall infection risk, mediated by latitude and terrain elevation on a regional scale and mediated by canopy cover on a local scale (Becker & Zamudio, 2011; Liu et al., 2013; Nowakowski et al., 2016; Raffel et al., 2010). A complete avoidance of infection risks could be achieved by extensive habitat modification to create a thermally unsuitable habitat for the pathogens (Catenazzi et al., 2014). Viral pathogens in moist soil, or also called slurry, also have the ability to survive under a variety of environmental conditions because they have different physical adjustments due to being from different RNA virus families. As is the case in the classical swine virus and the swine influenza virus (Bøtner & Belsham, 2012). A way of reducing the number of pathogens in the slurry is by adjusting the pH to 6,5 and then by air-drying it (Guan & Holley, 2003).

4.1 Conclusions & recommendations

There are multiple pathogens that can be transmitted via contact with wildlife, human-to-pig contact and via pigs-to-human contact. An increased biodiversity from the nature inclusive agriculture concept leads to an increase in pathogen risks, since there are several different host species involved in the transmission of the pathogens. Because of the negative economic effects of disease outbreaks for the farmer and involved governments, it is important to provide good transmission prevention and controlling methods. Such as fencing, serological testing of wildlife and free-ranging pigs and culling of wildlife. Additionally, habitat management is a good way to control and eradicate pathogens. Further literature research is necessary to examine transmission routes between wildlife and outdoor pig husbandry. Environmental samples can confirm the presence of possible (zoonotic) pathogens that can be a risk for the free-ranging pigs.

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