Research internship

The trigger of Actinobacillus pleuropneumoniae…

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The trigger for *Actinobacillus pleuropneumoniae*…

Introduction

Actinobacillus pleuropneumoniae (App) is a gram-negative bacterium that is endemically present in many pig herds. At slaughter mostly all pigs are infected (Chiers et al, 2002, Maes et al., 2001). The effects of App have led to high economic losses. The economic losses result from mortality, depression in growth rate, decreased feed efficiency and increased marketing' of undersized pigs of culled pigs.

Isolates of App can be divided into serotypes. Eighteen different serotypes are currently recognized (Bosse et al., 2018). The bacterium produces different Apx toxins; Apx I, Apx II, Apx III and Apx IV. The presence of Apx I, Apx II and Apx III is specific to a serotype. In the Netherlands serotype 2, 5, 9 and 11 were most prevalent (Duinhof et al., 2013).

Respiratory signs due to App are the result of infection in the lower respiratory tract. The illness can be divided in an acute, subacute and chronic course of disease. In the acute cases the clinical signs are coughing, abdominal breathing, dyspnea and mortality (Zimmerman et al., 2012). Lung lesions consist of a fibrinous bronchopneumonia characterized by severe consolidations and a fibrinous exudate on the pleural surface. A large area of fibrinous pleuropneumonia involving the caudal lobe of a pig's lung is considered almost diagnostic for this disease (Zachary et al., 2012). By peracutely infected pigs, the most common sign is sudden death without clinical signs (Zimmerman et al., 2012). The pneumonic lesions are locally extensive in the dorsal aspects of the caudal lung lobes. Lobular congestion, consolidation and interlobular edema are visible (Zachary et al., 2012). Growth retardation is most seen in chronic infected pigs (Zimmerman et al., 2012). Pigs with the chronic form may have multiple pulmonary abscesses and large pieces of necrotic lung encapsulated by connective tissue. These changes are frequently seen at slaughterhouses (Zachary et al., 2012).

Characteristic outbreaks of disease start at a mean age of 12 weeks and have a duration of four days. The size of a characteristic outbreak is ~50% diseased pigs (Klinkenberg et al., 2014).Based on previous studies it is likely that clinical outbreaks of App are explained by simultaneous exposure of pigs to a so called "trigger". Firstly, the pigs are colonized with the bacterium without clinical signs. After exposure to a trigger, the pigs will develop pneumonia and show clinical signs.(Maes et al., 2001, Sanford et al., 1981, Rosendal et al., 1983, Klinkenberg et al., 2014). Identifying the trigger has still not been successful.

App bacteria are usually localized in tonsils, nasal cavities without causing clinical signs. These subclinically infected pigs are a source of infection for naïve subpopulations.
Pigs can be initially colonized with App at different moments of pig production. During the suckling period sows can transmit the bacterium to their piglets (Chiers et al., 2002, Tobias et al., 2014). At weaning ~30% of suckling pigs are colonized, which may increase to >50% of the piglets at ten weeks of age (Tobias et al., 2014).
Many risk factors can be the trigger for an outbreak of pleuropneumonia. To stop or to reduce the size of an outbreak on farms it is important to identify and remove or prevent the trigger. However, in practice it appears not straightforward to identify the trigger.

The risk factors of a trigger are the focus of this thesis. Identification of the risk factors on farms will help to control the outbreaks that are initiated by a trigger. The research question is: What standard must a protocol have in order to identify possible App triggers?

This thesis consists of two parts.
In part I, the aim is to set up and to test a protocol to identify the trigger of App in the field. In part II a data analysis of an existing dataset will be performed, made available by a pharmaceutical company, which contains data of serum profiles of pig herds. The aim of the data analysis is to evaluate to which pathogens pigs have or developed antibodies and therefore can be considered as potential infectious triggers for an App outbreak.

For reading this thesis it is good to know that the thesis consists of two separate parts, every part has its own discussion and conclusion.

References

Bossé, J.T., Li, Y., Sárközi, R., Fodor, L., Lacouture, S., Gottschalk, M., Amoribieta, M.C., Angen, Ø., Nedbalcova, K., Holden, M.T.G., Maskel. D.J., Tucker, A.W., Wren, B.W., Rycroft, A.N., Langford, P.R., 2018. Proposal of serovars 17 and 18 of *Actinobacillus pleuropneumoniae* based on serological and genotypic analysis. Veterinary Microbiology 217, 1-6.

Chiers, K., Donné, E., Van Overbeke, I., Ducatelle, R., Haesebrouck, F., 2002. *Actinobacillus pleuropneumoniae* infections in closed swine herd: infection patterns and serological profiles. Vet. Microbiology 85, 343-352.

Duinhof, T., Templmans-Plat, B., Niesink-Broekroelofs, J., Wellenberg, G.J., 2013. *Actinobacillus pleuropneumoniae* serotypes in The Netherlands: a survey based on serology and isolates from lung lesions. Tijdschrift Diergeneeskunde 138, 28-35.

Klinkenberg, D., Tobias, T.J., Bouma, A., Van Leengoed, L.A.M.G., Stegeman, J.A., 2014. Simulation study on the mechanism of outbreaks of clinical disease caused by *Actinobacillus pleuropneumoniae* in finishing pigs. Veterinary Journal 202, 99-105.

Maes, D., Chiers, K., Haesebrouck, F., Laevens, H., Verdonck, M., de Kruif, A., 2001. Herd factors associated with the seroprevalences of *Actinobacilus pleuroneumoniae* serovars 2, 3 and 9 in slaughter pigs from farrow-to-finish pig herds. Veterinary Research 32, 409-419.

Rosendal, S., Mitchell, W.R., 1983. Epidemiology of *Haemophilus pleuropneumoniae* infection in pigs: a survey of Ontario Pork Producers, 1981. Can. J. Comp. Med. 47, 1-5.

Sanford, S.E., Josephson, G.K., 1981. Porcine *Haemophilus pleuropneumonia* epizootic in southwestern Ontario: clinical, microbiological, pathological an some epidemiologic findings. Can. J. Comp. Med. 45, 2-7.

Tobias, T.J., Klinkenberg, D., Bouma, A., Van den Broek, J., Daemen, A.J.J.M., Wagenaar, J.A., Stegeman, J.A., 2014. A cohort study on *Actinobacillus pleuropneumoniae* colonisation in suckling piglets. Preventive Veterinary Medicine 114, 223-230.

Tobias, T.J., Bouma, A., Van den Broek, J., Van Nes, A., Daemen, A.J.J.M., Wagenaar, J.A., Stegeman, J.A., Klinkenberg, D., 2014. Transmission of *Actinobacillus pleuropneumoniae* among weaned piglets on endemically infected farms. Preventive Veterinary Medicine 117, 207-214.

Zachary, J.F., McGavin, M.D., 2012. Pathologic basis of veterinary disease, 5e edition. St. Louis, Missouris: Elsevier.

Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schawartz, K.J., Stevenson, G.W., 2012. Disease of swine, 10e edition. New Jersey, United States: Wiley-Blackwell.

Part I Field Research

Design and evaluation of a protocol for the identification of the App trigger in the field

Introduction
The current explanation of outbreaks of pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (App) is the so-called "trigger mechanism" (Klinkenberg et al., 2014). For this mechanism, first pigs have to become colonized with the bacterium in the upper respiratory tract in mucous membrane, tonsils, nasopharynx or oropharynx. Colonized pigs are without clinical signs. After exposure to a trigger, clinical signs may develop (Klinkenberg et al., 2014).
Many risk factors can be related to function as a trigger for App. All circumstances causing acute stress are on the one hand likely to increase the probability to respiratory disease (Sassu et al., 2018). Examples of such stressors could be the policy of purchasing gilts, mixing piglets, poor biosecurity measures, season, or co-infections with other respiratory pathogens (Maes et al., 2001, Elbers et al., 1992). These risk factors have been associated also with a higher seroprevalence for App (Maes et al., 2001).

To stop or to reduce the size of an outbreaks on farms it is important to identify and remove or prevent the trigger (Tobias, 2014). However, in practice it appears not straightforward to identify the trigger. In case a simple and effective risk assessment protocol is available, veterinarians would be able to improve their advice to farmers. In addition, such a protocol enables future epidemiological studies on these triggers and potential interventions. Therefore, the aim of this research is to design and test a risk protocol for the identification of the App trigger in the field.

Material and Methods
This part of the study consisted of a protocol development phase (phase 1) and a phase of testing the protocol in the field (phase 2).

Phase 1
Based on literature and interviews a risk assessment protocol was composed for use on farms to identify a trigger that has resulted in a very recent App outbreak. A literature study was conducted to describe physiological, health and behavioral aspects of pigs. References/minimal conditions were formulated for these requirements, which were used to compare conditions on farms. Interviews were held with five pig farmers, two pig veterinarians from two veterinary practices (University Farm Animal Practice and Gelre Vets) and three ECPHM diplomates and experts of Utrecht University and MSD Animal Health. The conducted literature study and interviews resulted in a concept protocol for on farm identification of the trigger during App outbreaks.

Phase 2
Testing of the protocol in the field was performed by a retrospective cross sectional study. During an outbreak I have studied and verified whether risk factors are or have been present at the start of the outbreak, using the protocol designed in phase 1. At the moment of App outbreak (=outcome), the protocol was used to study the presence of risk factors (=retrospective). Several pig veterinarians have been contacted for calling the student or supervisor if they have visited a farm with App problems.

The protocol was tested on six farms with known and recurrent problems of App and outbreaks of App. For the inclusion of farms a few criteria were defined. Firstly, acute App outbreak with first clinical signs since equal or less than three days. Secondly, clinical signs of App are still visible: sudden death, dyspnea and depression. Thirdly, the herd veterinarian stated a strong suspicion for App as causative organism of the clinical signs.
During the visit to the farm information was get about the numbers of clinically affected pigs and the incidence of clinically affected pigs during the outbreak, relative to the applied interventions.

In addition, paired blood samples were taken from eight clinical representatives of affected pigs. Using blood samples we were able to test for antibodies for some specific pathogens during the

outbreak. Antibodies to the following pathogens were tested; porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, swine influenza, Porcine Circo Virus type 2 and to App as well. Ideally, paired serology will be conducted to relate the increase of antibody titer to the appearance of clinical signs.
Furthermore, long tissue samples were collected of clinical representative or affected pigs during an outbreak for confirming diagnosis of App by bacteriology. Also PCR was performed on lung tissue for detection DNA of App, *Mycoplasma hyopneumoniae*, swine influenza, Porcine Circo Virus type 2 and PRRSV.

Results

Phase 1 Design protocol

Literature study has resulted in an overview of physiological, health and behavioral aspects of pigs. If pigs are not able to meet basic needs, it can lead to stress which could be a trigger. Minimal conditions of the different parameters have been set. It can be divided in management-based measures and animal-based measures, as performed by WQ, 2009 and Weeghel et al. 2009. Management-based measures consisted of space allowance per pig, number of feeder and drinker places, climate parameters and soiling of pen area. Animal-based measures consisted of scoring tail and ear biting and clinical exam for health.
The management-based and animal-based measures were included in the protocol. Furthermore, the protocol consisted of general information about the farm and about recent changes at the farm. The protocol is enclosed in appendix A (in Dutch).

These minimal conditions were based on either legal requirements, information from the climate platform of Wageningen University Research (WUR), the Welfare Quality Assessment Protocol or clinical exam (Kuiper et al., 2016) (Table 1). Classification and explanation of the parameters is enclosed in appendix B (in Dutch).

Parameters	Pig 7-25 kg	Pig 25-100 kg	Reference
m2/pig	0,2-0,3 m²/pig	0,5-1,0 m²/pig	Law requirement
Number of feed places	1/5 pigs	1/8 pigs	Farmers advice WUR
Number of drink places	1/10 pigs	1/10 pigs	Farmers advice WUR
Temperature	22-26 °C	21-25 °C	Climate platform WUR
CO2	<2000-3000 ppm	<2000-3000 ppm	Climate platform WUR
NH3	<10 ppm	<10 ppm	Climate platform WUR
Humidity	50-80%	60-80%	Climate platform WUR
Air velocity	max 0,15 m/s	max 0,20 m/s	Climate platform WUR
Soiling pen area	1	1	WQ, 2009
Soiling of the body	0	0	WQ, 2009
Awareness	Alert	Alert	Clinical exam
Dog sitting posture	0	0	WQ, 2009
Tail biting	0	0	WQ, 2009
Ear biting	0	0	WQ, 2009
Skin lesions	1	1	WQ, 2009
Coughing	0	0	WQ, 2009
Sneezing	0	0	WQ, 2009
Dyspnea	0	0	WQ, 2009
Conjunctivitis	pink, clean	pink, clean	Clinical exam
Tear staining	absence	absence	Clinical exam
Nasal discharge	absence	absence	Clinical exam
Body temperature	39.5	40.0	Clinical exam

Table 1. Parameters with minimal conditions/references for pigs 7-25 kg and 25-100 kg. See appendix B for meaning of 0 and 1.

Phase 2 Testing of the protocol
Testing of the protocol was performed by a retrospective cross sectional study. Assessment using the protocol has taken 1 - 1.5 hours. In total six farms responded to the call. Two farms were excluded because there was not an acute outbreak. So they did not meet on criteria for participation.

By using the protocol and serology, in two of six farms a possible trigger was identified. But, the cause of an outbreak is still unknown in the other two farms.
In general, the complaint was the same in every company. In farm 2, possible trigger was found by the presence of a co-infection of swine influenza and *Mycoplasma hyopneumoniae.* This was supported by clinical signs and serology. In farm 4, the possible trigger may be related to findings of climatic parameters (e.g. high CO2). A summary of findings of the completed protocols were shown in table 2.

Company	**1**	**2**	**3**	**4**
Complaint	Mortality, coughing	Mortality, coughing, decreased feed intake	Mortality, coughing, decreased feed intake	Mortality, coughing, decreased feed intake
General information	Farrow-to-finish farm	Farrow-to-finish farm	Farrow-to-finish farm	Feeder-to-market farm
Living conditions	Within references	Within references	Within references	Within references
Climate	Ground channel ventilation, measures within references	Ceiling ventilation	Ceiling ventilation	Central corridor ventilation CO2 >3000 ppm T 25 (reference 22,5)
Recent alternations	Composition piglets changed, no coughing	-	-	-
Clinical exam	Coughing, depressive, abdominal breathing fattening pigs, dog sitting posture	Comp 1 Coughing, abdominal breathing Comp 2 No abnormalities	Some coughing	Abdominal breathing, dyspnea, coughing
Serology	APP positive, no increase co-infections	APP positive, no increase co-infections	APP positive, MH, FLU possible positive	APP positive, no increase co-infections
BO/PCR lung tissue	Bact: APP PCR: APP	Bact: APP PCR: APP, PCV-2, MH	Bact: *S. suis, P. multocida* PCR: APP, MH	Bact: APP PCR: APP

Table 2.Summary of findings of the performed protocols. Comp = compartment, bact = bacteriology, T = temperature.

Discussion

The aim of this part of study was to design and evaluation of a protocol for the identification of the App trigger under field conditions. The protocol was used to studying and verifying whether risk factors are or have been present at the start of the outbreak. Results of implementation of the protocol with results of serology have given information of the current situation of the farm. It was possible to identify a trigger.

In two of six participation farms a possible trigger was identified. In general, the complaint was the same in every company. In farm 2, possible trigger was found by the presence of a co-infection of swine influenza and *Mycoplasma hyopneumoniae.* This was supported by clinical signs and serology. In farm 4, the possible trigger can be related to findings of climatic parameters (e.g. high CO2).

Also, some limitations were evidently. Criteria for participating were very important. If the criteria were not present, the protocol could not be related to an outbreak of App. The observations did not give information about the presence or absence of risk factors.

It appeared very difficult to comprehend what happens on farms during or before an outbreak of App. The criteria for acute App outbreak with first clinical signs since three days were not accurate to set presence or abundance of risk factors. The course of an outbreak develops rapidly and within three days a lot may have happened on the farm. At the moment of clinical signs, the trigger may have disappeared. The time between trigger and outcome of clinical signs is unknown. An important part of the protocol showed to be the anamnesis. Results of the protocol were easier to interpret with the anamnesis. Additional questions have to be attached, to get more information.

Also the course of the outbreak did not become clear. The course of an outbreak could be different. For example it can affect a few departments, but it can also start affecting finishing pigs and develop to weaned pigs.

The explanation of the course of an outbreak could be related to the transmission of App. The transmission rate within a pen is higher than across pens (Tobias et al., 2014). Also, it is dependent of the presence of a colonized piglet. Result of the simulation of transmission showed that one colonized pig is able to colonize other pigs by direct contact (Tobias et al., 2014).

As reported before, outbreaks are impossible to predict en develop rapidly, so that is hardly to monitor the course of colonization and infection (Klinkenberg et al., 2014).

The aim of this study was to set up a protocol and to identify risk factors in the field. Recommended improvements are to get information to determine the course of an outbreak. Such as, detailed information about the number of affected pigs, development of an outbreak within department or with multiple departments. Also, during the outbreaks in this study a control group (non affected pigs) was not included. In the field, this is hardly to realize if you think of simultaneously weaning/ the same climate conditions, the composition of food etc. However, from a scientific point of view data on a control group on farms may benefit the validation of an observed risk factor for App outbreaks.

In conclusion, performing the protocol together with the results of serology has given information about the current situation on the farm. Identifying the trigger was possible. But, the cause of an outbreak still can be unknown. Further research needs to identify causes of a trigger in the field.

References

Boerenadvies VIC Sterksel. Aantal vreet- en drinkplaatsen: biggen en vleesvarkens bij droogvoerverstrekking. Geraadpleegd op 16 oktober 2017, van https://www.wur.nl/upload_mm/2/9/e/3d490b6b-b0fe-4691-9971-ff2f747d098f_aantal%20vreet%20en%20drinkplaatsen%2C%20biggen%20en%20vleesvarkens%20bij%20droogvoerverstrekking.pdf.

Elbers, A.R.W., Tielen, M.J.M., Cromwijk, W.A.J., Hunneman, W.A., 1992. Variation in seropositivity for some respiratory disease agent in finishing pigs: epidemiological studies on some health parameters and farm and management conditions in the herds. Veterinary quarterly 14: 8-13.

Klinkenberg, D., Tobias, T. J., Bouma, A., van Leengoed, L. A. M. G., & Stegeman, J. A., 2014.Simulation study of the mechanisms underlying outbreaks of clinical disease caused by glycoprotein analysis of porcine bronchoalveolar lavage fluid reveals potential biomarkers corresponding to resistance to *Actinobacillus pleuropneumoniae* infection in finishing pigs. *Veterinary Journal*, **202**, 99– 105.

Kuiper, R., van Nieuwstadt, R.A., 2016. Het klinisch onderzoek van paard en landbouwhuisdieren, 5th edition. Houten, The Netherlands: Springer media.

Maes, D., CHiers, K., Haesebrouck, F., Laevens H., Verdonck, M., de Kruif A., 2001. Herd factors associated with the seroprevalences of *Actinobacillus pleuropneumoniae* serovars 2, 3 and 9 in slaughter pigs from farrow-to-finish pig herds. Vet. Res. 32, 409-419.

Richtlijnen klimaatinstellingen. (2014, augustus). Geraadpleegd op 16 oktober 2017, van https://www.wur.nl/upload_mm/d/e/9/2156ebbe-3618-457a-ae50-48736949e888_RICHTLIJNEN%20klimaatinstellingen%20augustus%202014.pdf

Sassu, EL, Bossé, JT, Tobias, TJ, Gottschalk, M, Langford, PR, Hennig-Pauka, I., 2018. Update on *Actinobacillus pleuropneumoniae*—knowledge, gaps and challenges. *Transbound Emerg Dis*. 65(Suppl. 1): 72– 90.

Tobias, T., 2014. *Actinobacillus pleuropneumoniae* transmission and clinical outbreaks. General Discussion. PhD thesis Faculty of Veterinary Medicine, Utrecht University.

Tobias, T.J., Bouma, A., van den Broek, J., van Nes, A., Daemen, A.J.J.M., Wagenaar, J.A., Stegeman, J.A., Klinkenberg, D., 2014. Transmission of *Actinobacillus pleuropneumoniae* in weaned piglets on endemically infected farms. Preventive Veterinary Medicine 117 (1): 207-214.

Weeghel, H.J.E. van; Cornelissen, J.M.R.; Greef, K.H. de; Lansbergen, L.M.T.E.; Lauwere, C.C. de; Ursinus, W.W.; Vermeer, H.M.; Zonderland, J.J., 2009. Projectteam 'Diergericht Ontwerpen voor varkens', 2009. Wat wil het varken? WUR Livestock research.

Welfare Quality ®, 2009. Welfare quality® assessment protocol for pigs (sows and piglets, growing and finishing pigs). Welfare quality® consortium, Lelystad, Netherlands.

Appendix A Protocol

Protocol

Datum: Tijdstip:

Bedrijfsniveau		
Type bedrijf	Gesloten / Vermeerdering / Vleesvarkens	
	Vleesvarkens?	
	Afkomst	Subfokker
		Aantal:
Genetica		

Opmerkingen:

Afdelingsniveau			
Aantal hokken (n)			
	Hok 1	Hok 2	Hok 3
Aantal dieren (n)			
Hok breedte (m)			
Hok diepte (m)			
Voerbak type			
Voerbak aantal (n)			
Voerbak lengte (m)			
Drinknippels aantal (n) Functioneel			
Buitentemperatuur (°C)			
Binnentemperatuur (°C)			
CO_2 (ppm) dierniveau			
NH_3 (ppm) dierniveau			
Type ventilatie			
Ventilatie %			
Diameter ventilatie (m)			
P-waarde (°C)			
Luchtvochtigheid (%)			
Luchtsnelheid (m/s)			

Opmerkingen:

Schets hok

Dierniveau			
	Hok 1	Hok 2	Hok 3
Speendatum/Oplegdatum			
Leeftijd			
Samenstelling veranderd?	Wel / Niet	Wel / Niet	Wel / Niet
Hoesten voor uitbraak?			
Voerverandering?			
Hokbevuiling			
Bewustzijnsniveau			
Rillen (n)			
Huddling (n)			
Hondenzit(n)			
Bevuiling varkens			
Staartbijten(n + mate)			
Oorbijten (n + mate)			
Schrammen			
Hoesten(n/5 min)			
Niezen (n/5min)			
Dyspneu (n)			
Conjunctiva			
Traanstreep			
Neusuitvloeiing			
Temperatuur			
Klinisch App verschijnselen			
Niet-klinische App verschijnselen			

Opmerkingen:

Appendix B Classificatie en toelichting bij het protocol

Algemeen – opschrijven wat de bevindingen zijn, classificeren kan achteraf.

Afdelingsniveau
Afmetingen hok in meters, één decimaal.
Binnentemperatuur meten op dierniveau.
CO2 en NH3 meten op dierniveau.

Dierniveau
Hokbevuiling – mate waarin een hok vies is door mest wat niet op de juiste plaats (=roosters) ligt.
Classificatie:

0 = 0-20% van het hok is bevuild
1 = 20-50% van het hok is bevuild
2 = meer dan 50% van het hok is bevuild

Bewustzijnsniveau – mate van alertheid en interesse in omgeving
Classificatie:

0 = alert, komt overeind, komt naar je toe
1= soporeus, blijft liggen ondanks aansporingen

Rillen - langzaam en onregelmatig trillen van een lichaamsdeel of het hele lichaam.
Classificatie:

0 = geen rillen
1 = 20% rillen
2 = meer dan 20% rillen

Huddling - een varken ligt voor meer dan de helft met zijn lichaam in contact met een ander varken. Het normale zij aan zij liggen wordt hiermee niet bedoeld, meer het half op elkaar liggen.
Classificatie:

0 = geen huddling
1 = 20% huddling
2 = meer dan 20% huddling

Hondenzit – varken zit op de kont met de voorpoten uit elkaar.
Classificatie

0 = geen enkel dier heeft een hondenzit
1 = 20% heeft een hondenzit
2 = meer dan 20% heeft een hondenzit

Vieze varkens - een varken dat bevuild is met mest
Classificatie:

0 = 0-20% van de dieren is bevuild
1 = 20-50% van de dieren is bevuild
2 = meer dan 50% van de dieren is bevuild

Staartbijten – wondjes aan de staart, die ontstaan door bijten van andere varkens. Tellen hoeveel dieren zijn aangetast en in welke mate.
Classificatie:

0 = gezond
1 = oude/kleine wondjes
2 = verse wond, recent

Oorbijten – wondjes aan de rand van de oorschep die ontstaan door bijten van andere varkens. Tellen hoeveel dieren zijn aangetast en in welke mate.
Classificatie

0 = gave oorrand
1 = zwart/oude aangetaste oorrand
2 = rood/verse aangetaste oorrand

Schrammen–Kijken in welke mate er schrammen en laesies zich bevinden op de huid van het varken. Ook benoemen qua ernst, grootte van de laesies.
Classificatie

0 = geen schrammen of laesies zichtbaar op de huid
1 = 20% van de varkens heeft schrammen of laesies op de huid
2 = meer dan 20% van de varkens heeft schrammen of laesies op de huid

Hoesten–Nadat de dieren overeind zijn gekomen, luisteren hoe vaak er gehoest wordt ongeveer 5 minuten. Scoren op basis van gemiddelde frequentie van hoesten per afdeling.
Classificatie

0 = variërend van geen hoest tot 20 keer hoesten binnen 5 minuten
1 = vaker dan 20 keer hoesten binnen 5 minuten

Niezen – Nadat de dieren overeind zijn gekomen, luisteren hoe vaak er geniest wordt. Scoren op basis van gemiddelde frequentie van niezen per afdeling.
Classificatie

0 = variërend van geen hoest tot 20 keer hoesten binnen 5 minuten
1 = vaker dan 20 keer hoesten binnen 5 minuten

Dyspneu - zichtbaar benauwd zijn
Classificatie:

0 = normaal ademen, bek dicht
1 = buikslag zichtbaar, bek dicht
2 = ernstig benauwd; open bek

Conjunctivitis – Kijken hoe de conjunctiva eruit ziet.
Classificatie

0 = schoon, roze
1 = roze, vuil
2 = rood, vuil

Traanstreep - Kijken of er een traanstreep zichtbaar is.
Classificatie:

0 = niet aanwezig
1 = wel aanwezig

Neusuitvloeiing – vocht dat uit de neus komt. Bekijken of het aanwezig is en met welk exsudaat; waterig, helder, purulent enz.
Classificatie

0 = niet aanwezig
1 = wel aanwezig

Temperatuur – opnemen bij dieren met klinische App klachten en bij dieren zonder klinische App klachten. Ongeveer 5 dieren per hok.

Part II Dataset

Identifying associations between App and co-infections

Introduction

Many risk factors can be related to function as a trigger for *Actinobacillus pleuropneumoniae* (App). All circumstances causing acute stress are on the one hand likely to increase probability to respiratory disease (Sassu et al., 2018). As mentioned before, several stressors can be the trigger such as mixing piglets, poor biosecurity measures and season. Also, co-infection with other respiratory pathogens could be a trigger (Maes et al., 2001, Elbers et al., 1992).
It is already known that other respiratory pathogens can worsen the severity of the course of an App outbreak. Especially *Mycoplasma hyopneumoniae* is mentioned in the literature (Marois et al., 2009, Sakano et al., 1993). The role of respiratory pathogens as infectious triggers for an App outbreak is less mentioned.

The aim of the data analysis is to evaluate to which pathogens pigs have or developed antibodies and therefore can be considered as potential infectious triggers for an App outbreak.

Material and method

The data analysis was performed on an existing dataset 'Respig 2015-2016', made available by a pharmaceutical company. This dataset contains data of serum profiles of 503 pig herds.
Firstly, the dataset was explored by frequency tables (Appendix A). Secondly, biological hypothesis was set between clinical signs and infections. Another biological hypothesis was set between App and co-infections.
Clinical signs consisted of coughing/respiratory tract infections in weaned piglets or finishing piglets. The prevalence of several infections with different diagnostics were included in biological hypotheses; Porcine reproductive and respiratory syndrome virus (PRRSV) ELISA piglets 10 and 22 weeks, PRRSV PCR saliva samples finishing pigs, *Mycoplasma hyopneumoniae* PCR saliva samples piglets 10 weeks and finishing pigs, App OMP titer piglets 10 and 22 weeks, swine influenza PCR saliva samples piglets 5, 10 weeks and finishing pigs, *Haemophilus parasuis* (Glasser's disease) piglets 10 and 16 weeks, Porcine Circovirus type 2 (PCV2)ELISA titer piglets 10 and 22 weeks, PCV2 PCR saliva samples piglets 5, 10 weeks and finishing pigs.
Thirdly, contingency tables (2x2) were made and odds ratio were determined of the biological hypotheses. P-value was set on<0.2. Before contingency tables were made for biological hypotheses, the dataset was recoded in dummy variable (0-1). In appendix B, explanation of dummy variables was represented for the biological hypotheses.
The different steps of statistical analysis were performed on different subsets of the dataset. First subset all farms were included (503 data), second subset farrow-to-finish farm and farrow-to feeder farm (362 data), third subset farrow-to-finish farm and feeder-to-market farms (272 data).
All statistical analyses were performed using statistical software R version 3.4.4. (R Development Core Team, 2010). The script of the analysis is in appendix C.

Results

Exploring the dataset

For several infections diagnostic methods were not performed. These data were excluded. By excluding these data, the dataset was more limited than have been estimated in advance.

Biological hypotheses

For the first biological hypothesis contingency tables were made between clinical signs (coughing/respiratory tract infections) and several infections. Odds ratio and p-values were determined. A significant association was found between clinical signs and swine influenza PCR saliva samples of weaned piglets (P=0.004). No other significant associations or trends were visible. Results were shown in table 1 and 2, respectively weaned piglets and finishing pigs.

Coughing/Respiratory tract infections weaned piglets	P-value	OR	95% CI
PRRS Elisa piglets	0,45	1,88	0,76-1,87
PRRS PCR saliva piglets	0,15	1,49	0,87-2,58
MH PCR saliva piglets	0,95	0,94	0,12-5,83
APP OMP titer piglets	0,65	0,89	0,57-1,43
FLU PCR saliva piglets	0,0004	2,98	1,64-5,51
HPS Elisa piglets	0,18	1,78	0,77-4,21
PCV Elisa piglets	0,27	0,79	0,52-1,20
PCV PCR saliva piglets	0,27	0,79	0,52-1,20

Table 1. Association between clinical signs of weaned piglets and several infections with different diagnostic methods. Results are based on subset farrow-to finish farms and farrow-to-feeder farms (362 data). OR = odds ratio, 95%CI = 95% confidence interval.

Coughing/Respiratory tract infections finishing pigs	P-value	OR	95% CI
PRRS Elisa finishing pigs	0,6	1,15	0,61-2,21
PRRS PCR saliva finishing pigs	0,15	1,61	0,83-3,16
MH PCR saliva finishing pigs	1	1	0,38-2,60
App OMP titer finishing pigs	0,29	0,65	0,29-1,44
FLU Elisa finishing pigs	0,6	1,11	0,68-1,83
FLU PCR saliva finishing pigs	0,28	1,57	0,69-3,66
HPS Elisa finishing pigs	0,36	0,73	0,36-1,42
PCV Elisa finishing pigs	0,44	1,21	0,74-1,96
PCV PCR saliva finishing pigs	0,21	1,49	0,80-2,81

Table 2. Association between clinical signs of finishing pigs and several infections with different diagnostic methods. Results are based on subset farrow-to-finish farms and feeder-to-market farms (272) data. OR = odds ratio, 95%CI = 95% confidence interval.

For the second biological hypothesis, contingency tables were made between App and co-infections. Odds ratio and p-values were determined. Results were shown in table 3 and 4, respectively weaned piglets and finishing pigs. No significant associations were found. Also no trends were visible.

APP OMP titer weaned piglets	P-value	OR	95% CI
PRRS Elisa weaned piglets	0,17	1,39	0,86-2,25
PRRS PCR saliva weaned piglets	0,71	0,89	0,48-1,65
MH PCR saliva weaned piglets	0,77	1,4	0,21-27,73
FLU PCR saliva weaned piglets	0,65	1,17	0,60-2,37
HPS Elisa weaned piglets	0,85	0,97	0,41-2,51
PCV Elisa weaned piglets	0,74	1,08	0,68-1,71

PCV PCR saliva weaned piglets	0,22	0,68	0,37-1,27

Table 3. Association between App (OMP titer) infections in weaned piglets and co-infections. Results are based on subset farrow-to-finish farms and farrow-to-feeder farms (362 data).OR = odds ratio, 95%CI = 95% confidence interval.

APP OMP titer finishing pigs	P-value	OR	95% CI
PRRS Elisa finishing pigs	0,69	0,80	0,23-2,21
PRRS PCR saliva finishing pigs	0,49	0,67	0,21-2,12
MH PCR saliva finishing pigs	0,49	2,07	0,38-38,84
FLU Elisa finishing pigs	0,69	0,84	0,36-1,88
FLU PCR saliva finishing pigs	0,79	0,83	0,23-3,91
HPS Elisa finishing pigs	0,13	2,67	0,84-11,85
PCV Elisa finishing pigs	0,72	1,15	0,53-2,64
PCV PCR saliva finishing pigs	0,57	1,37	0,48-4,38

Table 4. Association between App (OMP titer) in finishing pigs and co-infections. Results are based on subset farrow-to-finish farms and feeder-to-market farms (272) data. OR = odds ratio, 95%CI = 95% confidence interval.

Discussion

The aim of this part of study was to evaluate to which pathogens pigs have or develop antibodies and therefore can be considered as potential triggers for an App outbreak. The biological hypothesis of clinical signs and infection have resulted in a significant association between coughing/respiratory tract infections and positive swine influenza PCR saliva samples of weaned piglets (P=0.004). A probable association between App and swine influenza was mentioned before. Pmorska-Mól et al. have shown that a clinical course of a co-infection of App and swine influenza is more severe than a single infection from App or influenza.

In finishing pigs, no association was found for the biological hypothesis of clinical sign and respiratory infection. Also, in this dataset no significant associations or trends were found between App and co-infections.

These results were not in accordance with other studies. According to Marois et al., *Mycoplasma hyopneumoniae* can worsen App infection if pigs were already colonized with App. Another association is probable between App and PRRSV according to veterinarians in the Netherlands because of the resulted pleuritis for both pathogens (Fables et al 2012). Although, experimental studies have not shown these results. Previous infection of PRSSV will not always enhance the severity of App infection (Pol et al., 1997). Still, the exact mechanism between these pathogens is not clear.

Probable explanation of the results of this study was the aim of Respig. Respig is a monitoring service of a pharmaceutical company. Twice a year farms were able to send blood samples. The samples were taken cross sectional on a farm of different ages of piglets. Results of blood samples gave information of different respiratory pathogens on a farm. For this study, to examine the association of App and other infections it was necessary to have blood samples available of an acute App outbreak. It is doubtful if there were enough samples available by Respig. For testing an immune response against App a serologic test was used on detection of antibodies against outer membrane protein (OMP). OMP titers were expressed in values between 6 and 15 log2. Values below 6-6.6 were negative. Piglets of 10 weeks had average OMP titers of 8. OMP titers increased on endemically infected farms without clinical signs of App. Values of ≥14 indicated an acute outbreak. These titers

will develop 3-4 weeks after clinical signs (Personal communication MSD veterinarian, interpretation serology results Respig). Exploring the dataset by use of frequency tables have shown a total samples of 76 farms with OMP titer >14.Problably, this number of samples was too little for statistical modelling.
As described, the dataset was recoded. The values in the dataset were resulted of five blood samples, which have been taken for Respig. In this study the values were recoded to dummy variables (0-1). So, results of diagnostics method to get information of respiratory pathogens were often combined. These several steps have influenced results. Also, the results were influenced by some reporting bias. The presence of clinical signs was based on the reported anamnesis of the veterinarian. Only data of clinical signs (coughing/respiratory tract infections) were used for analysis of the first biological hypothesis.

In conclusion, in this dataset a significant association was found between coughing/respiratory tract infections and positive influenza PCR saliva samples of weaned piglets (P=0.004). In finishing pigs, no association was found for the biological hypothesis of clinical sign and respiratory infection. Also, in this dataset no significant associations or trends were found between App and co-infections. Further research needs to identify association between App and co-infections. The biological hypothesis of this study can be repeat. More data of serological results of App with OMP titer ≥14 will be necessary to search for an association.

References

Elbers, A.R.W., Tielen, M.J.M., Cromwijk, W.A.J., Hunneman, W.A., 1992. Variation in seropositivity for some respiratory disease agent in finishing pigs: epidemiological studies on some health parameters and farm and management conditions in the herds. Veterinary quarterly 14: 8-13.

Fablet, C., Marois-Créhan, C., Simon, G., Grasland, B., Jestin, A., Kobisch, M., … Rose, N. (2012).Infectious agents associated with respiratory diseases in 125 farrow-to-finish pig herds: A cross-sectional study. *Veterinary Microbiology*, **157**, 152– 163.

Maes, D., CHiers, K., Haesebrouck, F., Laevens H., Verdonck, M., de Kruif A., 2001. Herd factors associated with the seroprevalences of *Actinobacillus pleuropneumoniae* serovars 2, 3 and 9 in slaughter pigs from farrow-to-finish pig herds. Vet. Res. 32, 409-419.

Marois, C., Gottschalk,M., Morvan, H., Fablet, C., Madec, F., Kobisch, M., 2009. Experimental infection of SPF pigs with *Actinobacillus pleuropneumoniae* serotype 9 alone or in association with *Mycoplasma hyopneumoniae*. Veterinary Microbiology,Volume 135, Issues 3–4, Pages 283-291.

Pol, J. M., van Leengoed, L. A., Stockhofe, N., Kok, G., & Wensvoort, G., 1997. Dual infections of PRRSV/influenza or PRRSV/*Actinobacillus pleuropneumoniae* in the respiratory tract. *Veterinary Microbiology*, **55**, 259– 264.

Pomorska-Mól, M., Dors, A., Kwit, K., Kowalcyk, A., Stasiak, E., Pejsak, Z., 2017. Kinetics of single and dual infection of pigs with swine influenza virus and *Actinobacillus pleuropneumoniae*. Veterinary Microbiology, Volume 201, Pages 113-120.

Sakano, T., Shibata, I., Samegai, Y., Taneda, A., Okada, M., Irisawa, T., & Sato, S., 1993. Experimental pneumonia of pigs infected with Aujeszky's disease virus and *Actinobacillus pleuropneumoniae*. *Journal of Veterinary Medical Science*, **55**, 575– 579.

Sassu, EL, Bossé, JT, Tobias, TJ, Gottschalk, M, Langford, PR, Hennig-Pauka, I., 2018. Update on *Actinobacillus pleuropneumoniae*—knowledge, gaps and challenges. *TransboundEmerg Dis*. 65(Suppl. 1): 72– 90.

Appendix A Frequency tables

Explore dataset Respig 2015+2016 by frequency tables.

Type Bedrijf	Aantal
1 Gesloten	171
2 Vermeerdering	191
3 Vleesvarkens	101
4 Opfok	40

Kliniek	Gesloten bedrijf	Vermeerdering	Vleesvarkens
5 biggen: geen bijzonderheden	16	37	-
6 biggen: hoest (app)/luchtweginfecties	70	89	1
7 biggen: niezen en of oog- neusuitvloeiing	23	16	-
12 biggen: te hoge uitval	12	11	2
15 vleesvarkens: geen bijzonderheden	15	-	18
16 vleesvarkens: hoest (app)/luchtweginfecties	71	1	54
17 vleesvarkens: niezen en of oog-neusuitvloeiing	3	-	6
22 vleesvarkens: te hoge uitval	7	-	8

Slachthuisgegevens	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 Geen afwijkingen	41	-	27
2 Pleuritis te hoog (>18%)	41	-	8
3 Longaandoeningen te hoog (>10%)	4	-	1
4 Pleuritis + longaandoeningen te hoog	4	-	6
99 Onbekend	100	-	58

Prevalenties

PRRSV profiel zeugen	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	26	35	-
2 indifferent (EU=US)	21	28	-
3 US	17	17	-
4 EU	106	111	-

PRRS biggen 5 wkn PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	59	63	4
2 pos EU	15	18	-
3 pos US	-	-	-
4 niet gedaan	59	64	-

PRRS biggen 10 wkn ELISA	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1< 0,4 (alle uitslagen negatief)	58	53	36
2 ≥ 0,4 (1 of meer positieve uitslagen)	113	136	65

PRRS biggen 10 wkn PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	66	58	23
2 pos EU	38	58	17
3 pos US	1	-	2
4 niet gedaan	28	30	39

PRRS vleesvarkens 22 wkn ELISA	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1< 0,4 (alle uitslagen negatief)	31	-	15
2≥ 0,4 (1 of meer positieve uitslagen)	140	-	86

PRRS vleesvarkens PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	49	-	30
2 pos EU	35	-	28
3 pos US	-	-	1
4 niet gedaan	47	-	22

M. hyopneumoniae biggen 10 wkn PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1neg	101	114	40
2 pos	3	2	2
3 niet gedaan	29	30	39

M. hyopneumoniae vleesvarkens PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	69	-	53
2 pos	14	-	6
3 niet gedaan	47	-	22

App biggen 10 wkn OMP titer	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 OMP <6,6	2	5	1
2 OMP 7 (6,6-7,4)	41	52	22
3 OMP 8-13 (7,5-13,4)	128	133	77
4 OMP ≥14 (13,5-…)	-	-	1

App vleesvarkens 22wkn OMP titer	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 OMP<6,6	1	-	-
2OMP 7	18	-	9
3OMP 8-13	100	-	69
4OMP ≥14	52	-	23

Influenza zeugen	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 10% positieve testen opfok	-	-	-
2 20% positieve testen opfok	-	1	-
3 40% positieve testen opfok	4	1	-
4 60% positieve testen opfok	5	3	-
5 80% positieve testen opfok	22	34	-
6 100% positieve testen opfok	139	152	-

Influenza vleesvarkens	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 0% positieve testen	20	-	8
2 20% positieve testen	11	-	12
3 40% positieve testen	16	-	17
4 60% positieve testen	13	-	9
5 80% positieve testen	22	-	14
6 100% positieve testen	89	-	39

Influenza biggen 5 wkn PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	49	58	3
2 pos	25	23	1
3 niet gedaan	59	64	-

Influenza biggen 10wkn PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	73	81	31
2 pos	32	35	11
3 niet gedaan	28	30	39

Influenza vleesvarkens PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	64	-	50
2 pos	20	-	9
3 niet gedaan	47	-	22

Glasser'sdisease biggen 10 weken	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 alles negatief	2	163	76
2 20% positieve testen	-	17	11
3 40% positieve testen	-	5	8
4 60% positieve testen	-	5	4
5 80% positieve testen	-	-	2
6 100% positieve testen	-	-	-

Glasser'sdisease vleesvarkens 16 wkn	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 alles negatief	116	-	-
2 20% positieve testen	25	-	-
3 40% positieve testen	4	1	-
4 60% positieve testen	5	-	-
5 80% positieve testen	11	-	-
6 100% positieve testen	8		-

PCV zeugen	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 alles negatief	39	45	-
2 20% positieve testen	37	39	-
3 40% positieve testen	27	38	-
4 60% positieve testen	21	25	-
5 80% positieve testen	21	26	-
6 100% positieve testen	25	18	-

PCV gelt	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 alles negatief	34	38	-
2 20% positieve testen	19	24	-
3 40% positieve testen	15	17	-
4 60% positieve testen	16	25	-
5 80% positieve testen	22	23	-
6 100% positieve testen	63	63	-

PCV biggen 10 wkn titer	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 alles negatief	35	18	14
2 20% positieve testen	15	16	15
3 40% positieve testen	23	24	17
4 60% positieve testen	26	30	15
5 80% positieve testen	28	29	21
6 100% positieve testen	44	73	19

PCV vleesvarkens 22 wkn titer	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 alles negatief	67	-	67
2 20% positieve testen	38	-	38
3 40% positieve testen	20	-	20
4 60% positieve testen	14	-	14
5 80% positieve testen	12	-	12
6 100% positieve testen	20	-	20

PCV biggen 5 wkn PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	54	54	4
2 pos (weakly)	10	15	-
3 pos	10	12	1
4 niet gedaan	59	64	-

PCV biggen 10 wkn PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	58	82	19
2 pos (weakly)	21	15	7
3 pos	26	19	17
4 niet gedaan	28	30	38

PCV vleesvarkens PCR speekselmonsters	Gesloten bedrijf	Vermeerdering	Vleesvarkens
1 neg	30	-	24
2 pos (weakly)	6	-	8
3 pos	48	-	26
4 niet gedaan	47	-	22

Appendix B Dummy variables

Recoding dataset

- Clinical signs (coughing/respiratory tract infections) divided in true or false.
- Disease - all not performed diagnostics are exclude.
- App is subdivided into OMP <6.6, OMP 7, OMP 8-13 and OMP ≥14. The OMP is recoded into OMP <6.6 - 7 (= 0) and OMP 8 - ≥14 (=1).
- Swine influenza saliva samples piglets 10 weeks and finishing pigs: negative (=0), positive (positive EU + US)
- Swine influenza finishing pigs: negative (0%, 20%,40%,60% positive samples), positive (80+100% positive samples)
- Glasser's disease piglets 10 weeks and finishing pigs: negative (=0), positive (20%+40%+60%+80+100% positive samples)
- PCV2 piglets 10 weeks and 22 weeks: negative (negative + 20%positive samples), positive (40%+60%+80+100% positive samples)
- PCV2 piglets 5, 10 weeks PCR saliva samples: negative (=negative), positive (positive weakly+positive)
- PCV2 finshing pigs PCR saliva samples: negative (=negative+positive weakly), positive (=positive)

Appendix C R-Studio script

```
library(readxl)
Respig<- read.table("DGK/Onderzoek/Respig data ingevoerd.csv", header=T,dec=",",sep=";")
attach(Respig)
detach(Respig)
names(Respig)
summary(Respig)
############################

Biological hypothesis - clinical signs and infection
table(Klin.6,PRRSspPCR)
table(Klin.6,PRRSspPCR, useNA="ifany")
table(Klin.6,PRRSb10W, useNA="ifany")
table(Klin.6,PRRS10PCR, useNA="ifany")
table(Klin.6,MHsp10W)
table(Klin.6,APP10W)
table(Klin.6,FLUsp5W, useNA="ifany")
table(Klin.6,FLUsp10W)
table(Klin.6,HPSb10W)
table(Klin.6,PCV10W)
table(Klin.6,PCVsp5W)
table(Klin.6,PCVsp10W)
table(Klin.16,APPv22W)
table(Klin.16,PRRSspVL.V)
table(Klin.16,PRRSv22W)
table(Klin.16,MHspVLV)
table(Klin.16,APPv22W)
table(Klin.16,FLUv, useNA="ifany")
table(Klin.16,FLUspvlv)
table(Klin.16,HPSv16W)
table(Klin.16,PCV22W)
table(Klin.16,PCVvlv)
##################

P-value + Odds ratio + 95% CI of biological hypothesis - clinical signs and infection
library(readxl)
Respig2 <- read.table("DGK/Onderzoek/Respig data ingevoerdaangepas 0-1.csv", header=T, dec=",", sep=";")
attach(Respig2)
detach(Respig)
library(readxl)
Bedrijfselectie2 <- read.table("DGK/Onderzoek/Selectie bedrijf gesloten+vermeerdering.csv", header=T, dec=",", sep=";")
attach(Bedrijfselectie2)
detach(Bedrijfselectie2)
fit<-glm(Klin.6~PRRSspPCR, family="binomial")
summary(fit)
exp(0.4560)
exp(confint(fit))
fit<-glm(Klin.6~PRRSb10W, family="binomial")
summary(fit)
exp(0.1727)
exp(confint(fit))
fit<-glm(Klin.6~PRRS10PCR, family="binomial")
summary(fit)
exp(0.4002)
exp(confint(fit))
fit<-glm(Klin.6~MHsp10W, family="binomial")
summary(fit)
exp(-0.05449)
exp(confint(fit))
fit<-glm(Klin.6~APP10W, family= "binomial")
summary(fit)
```

```
exp(-0.1060)
exp(confint(fit))
fit<-glm(Klin.6~FLUsp5W, family="binomial")
summary(fit)
exp(1.1213)
exp(confint(fit))
fit<-glm(Klin.6~FLUsp10W, family="binomial")
summary(fit)
exp(1.0924)
exp(confint(fit))
fit<-glm(Klin.6~HPSb10W, family="binomial")
summary(fit)
exp(0.5759)
exp(confint(fit))
fit<-glm(Klin.6~PCV10W, family="binomial")
summary(fit)
exp(-0.2353)
exp(confint(fit))
fit<-glm(Klin.6~PCVsp5W, family="binomial")
summary(fit)
exp(0.2134)
exp(confint(fit))
fit<-glm(Klin.6~PCVsp10W, family="binomial")
summary(fit)
exp(-0.2352)
exp(confint(fit))
fit<-glm(Klin.16~PRRSv22W, family="binomial")
summary(fit)
fit<-glm(Klin.16~PRRSspVL.V, family="binomial")
summary(fit)
fit<-glm(Klin.16~MHspVLV,family="binomial")
summary(fit)
fit<-glm(Klin.16~APPv22W, family="binomial")
summary(fit)
fit<-glm(Klin.16~FLUv, family="binomial")
summary(fit)
fit<-glm(Klin.16~FLUspvlv, family="binomial")
summary(fit)
fit<-glm(Klin.16~HPSv16W, family="binomial")
summary(fit)
fit<-glm(Klin.16~PCV22W, family="binomial")
summary(fit)
fit<-glm(Klin.16~PCVvlv, family="binomial")
summary(fit)
#########

Biological hypothesis - App and co-infection
table(APP10W,PRRSspPCR)
table(APP10W,PRRS10PCR)
table(APP10W,PRRSb10W)
table(APP10W,MHsp10W)
table(APP10W,FLUsp5W)
table(APP10W,FLUsp10W)
table(APP10W,HPSb10W)
table(APP10W,PCV10W)
table(APP10W,PCVsp5W)
table(APP10W,PCVsp10W)
table(APPv22W,PRRSv22W)
table(APPv22W,PRRSspVL.V)
table(APPv22W,MHspVLV)
table(APPv22W,FLUv)
table(APPv22W,FLUspvlv)
table(APPv22W,HPSv16W)
```

```
table(APPv22W,PCV22W)
table(APPv22W,PCVvlv)

P-value + Odds ratio + 95% CI of biological hypothesis - App and co-infection
fit<-glm(APP10W~PRRSspPCR,family="binomial")
summary(fit)
exp(-0.6253)
exp(confint(fit))
fit<-glm(APP10W~PRRS10PCR, family="binomial")
summary(fit)
exp(-0.1162)
exp(confint(fit))
fit<-glm(APP10W~PRRSb10W, family="binomial")
summary(fit)
exp(0.3328)
exp(confint(fit))
fit<-glm(APP10W~MHsp10W, family="binomial")
summary(fit)
exp(0.3374)
exp(confint(fit))
fit<-glm(APP10W~FLUsp5W,family="binomial")
summary(fit)
exp(0.1123)
exp(confint(fit))
fit<-glm(APP10W~FLUsp10W, family="binomial")
summary(fit)
exp(0.1582)
exp(confint(fit))
fit<-glm(APP10W~HPSb10W, family="binomial")
summary(fit)
exp(-0.0274)
exp(confint(fit))
fit<-glm(APP10W~PCV10W, family="binomial")
summary(fit)
exp(0.0766)
exp(confint(fit))
fit<-glm(APP10W~PCVsp5W, family="binomial")
summary(fit)
exp(-0.2570)
exp(confint(fit))
fit<-glm(APP10W~PCVsp10W, family="binomial")
summary(fit)
exp(-0.3862)
exp(confint(fit))
fit<-glm(APPv22W~PRRSv22W, family="binomial")
summary(fit)
fit<-glm(APPv22W~PRRSspVL.V, family="binomial")
summary(fit)
fit<-glm(APPv22W~MHspVLV, family="binomial")
summary(fit)
fit<-glm(APPv22W~FLUv, family="binomial")
summary(fit)
fit<-glm(APPv22W~FLUspvlv, family="binomial")
summary(fit)
fit<-glm(APPv22W~HPSv16W, family="binomial")
summary(fit)
fit<-glm(APPv22W~PCV22W, family="binomial")
summary(fit)
fit<-glm(APPv22W~PCVvlv, family="binomial")
summary(fit)
##############

#Dataset geselecteerd op bedrijf gesloten+vlv
```

```
library(readxl)
Bedrijfselectie <- read.table("DGK/Onderzoek/Selectie bedrijf gesloten+vlv.csv", header=T,dec=",",sep=";")
attach(Bedrijfselectie)
fit<-glm(Klin.16~PRRSv22W, family="binomial")
summary(fit)
exp(0.1382)
exp(confint(fit))
fit<-glm(Klin.16~PRRSspVL.V, family="binomial")
summary(fit)
exp(0.4802)
exp(confint(fit))
fit<-glm(Klin.16~MHspVLV,family="binomial")
summary(fit)
exp(1.119e-16)
exp(confint(fit))
fit<-glm(Klin.16~APPv22W, family="binomial")
summary(fit)
exp(-0.4236)
exp(confint(fit))
fit<-glm(Klin.16~FLUv, family="binomial")
summary(fit)
exp(0.1085)
exp(confint(fit))
fit<-glm(Klin.16~FLUspvlv, family="binomial")
summary(fit)
exp(0.4537)
exp(confint(fit))
fit<-glm(Klin.16~HPSv16W, family="binomial")
summary(fit)
exp(-0.3167)
exp(confint(fit))
fit<-glm(Klin.16~PCV22W, family="binomial")
summary(fit)
exp(0.1883)
exp(confint(fit))
fit<-glm(Klin.16~PCVvlv, family="binomial")
summary(fit)
exp(0.3972)
exp(confint(fit))
fit<-glm(APPv22W~PRRSv22W, family="binomial")
summary(fit)
fit<-glm(APPv22W~PRRSspVL.V, family="binomial")
summary(fit)
fit<-glm(APPv22W~MHspVLV, family="binomial")
summary(fit)
fit<-glm(APPv22W~FLUv, family="binomial")
summary(fit)
fit<-glm(APPv22W~FLUspvlv, family="binomial")
summary(fit)
fit<-glm(APPv22W~HPSv16W, family="binomial")
summary(fit)
fit<-glm(APPv22W~PCV22W, family="binomial")
summary(fit)
fit<-glm(APPv22W~PCVvlv, family="binomial")
summary(fit)
```