

Erysipelas: Identifying risk factors for clinical cases in Dutch pig herds

A case control study



Source: Tineke van de Veerdonk (Dutch veterinarian specialized in pigs)

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Prefatory note

At Veterinary Medicine Utrecht University, every student has to fulfill a research project. This is the report of a research project carried out by M.G.H. van Heesbeen at GD Animal Health Deventer. This research was carried out as a pilot study in the framework of monitoring swine health. This study was funded by pig industry (Nederlandse vakbond varkenshouders (NVV) and Land- en Tuinbouw Organisatie Nederland (LTO)), the Ministry of Economic Affairs and contributions of farmers.

The aim of this project was to get more insight in the risk factors involved in the occurrence of swine erysipelas.

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Abstract

Factors associated with swine erysipelas were investigated in 47 Dutch pig herds (18 cases and 29 controls). Data related to the erysipelas outbreak, erysipelas vaccination, external biosecurity, internal biosecurity, diet and climate were collected by a survey for veterinarians specialized in pig health. Veterinarians from all over The Netherlands were approached to get information about the Dutch pig herd. For every case herd, two control herds were selected by the veterinarian (matched case-control study). Controls were the same herd type as the case and they had no history of erysipelas in the last five years. Factors related to erysipelas were identified by conditional logistic regression and Chi square analysis. Only location of the sow (farrowing barn) seemed to be significantly associated with erysipelas ($p=0.002263$). Further research is needed to identify risk factors present in farrowing barn. Hardly cleanable pens, non-application of all in all out and liquid feed consisting of wetfeed were marked as trends ($0.05 < p < 0.2$). However, none of the examined risk factors could be excluded definitely, because of the small dataset.

Keywords; swine erysipelas, risk factors, matched case-control, conditional logistic regression, Chi square analysis

Introduction

Swine erysipelas is a disease caused by *Erysipelothrix rhusiopathiae*. In 1882, this organism was first isolated from a pig by Louis Pasteur. When uncontrolled, swine erysipelas is an economically significant disease able to affect all stages of pork production. Sudden death or acute septicemia are responsible for most of these economic losses. However, lameness and arthritis in case of chronic erysipelas also lead to economic damage because of their effect on growth. The following introduction on swine erysipelas is based on the information given in 'Diseases of swine 10th ed.' by Jeffrey J Zimmerman.

Etiology

The genus *Erysipelothrix* is subdivided into two major species: *E. rhusiopathiae* and *Erysipelothrix tonsillarum* (1).

Erysipelothrix spp. strains can be subdivided into at least 28 serotypes (2, 3). Field cases of erysipelas are preponderantly caused by *E. rhusiopathiae* serotypes 1a, 1b or 2. Initially *E. tonsillarum* seemed to be avirulent for pigs. In a recent study *E. tonsillarum* was isolated from 3,4% of the carcasses with typical lesions for swine erysipelas, that were negative for *E. rhusiopathiae*. This indicates the potential significance of this *Erysipelothrix* species in erysipelas pathogenesis(4, 5).

E. rhusiopathiae is a nonmotile, nonsporulating, non-acid fast, slender gram-positive rods (6). Recent studies showed the presence of a capsule and suggested a role for it in virulence. The bacterium grows best in anaerobe conditions, however laboratory adapted cultures

grow also aerobically. The growth temperature ranges between 5 and 44 °C, with an optimum between 30 and 37 °C. Growth is best favoured by an pH between 7,2 and 7,6.

Epidemiology

E. rhusiopathiae is harbored by at least 50 species of mammals and 30 species of wild birds (7, 8). Among these potential reservoirs are: pigs, sheep, horses, cattle, dogs, fish, turkeys and chickens. The domestic pig is considered to be the most important reservoir. It is estimated that 30-50 per cent of healthy swine harbor the organism in their tonsils and other lymphoid tissues (9). *E. rhusiopathiae* can be shed in the feces and oronasal secretions of these pigs, creating an important source of infection. Acute infected swine shed *E. rhusiopathiae* in feces, urine, saliva and nasal secretions profusely, so they are a certain source of infection. Other pigs can be infected by ingestion of contaminated water or feed and by contamination of skin wounds.

The bacteria can also be transmitted indirectly via environmental contamination, for example contamination of soil and straw. However, no evidence of establishment of a stable population in the soil is found, survival in soil is less than 35 days (10).

Commonly available disinfectants can inactivate *E. rhusiopathiae* (11). Though structurally complex equipment which contains organic matter is harder to disinfect, especially without cleaning.

Pathogenesis

Very little is known about the pathogenesis of erysipelas. No toxin has been associated with *E. rhusiopathiae*. Virulence factors such as neuraminidase, polysaccharide capsules and a few surface proteins have been suggested to play a role in its pathogenicity.

Neuraminidase is probably one of the most important factors (12). A correlation between the virulence of strains and the average amount of neuraminidase produced in different media was noted (13). There was no neuraminidase activity detected in the non-pathogenic *Erysipelothrix* species (14). Moreover, in an experiment where parent strains of *E. rhusiopathiae* were compared with their lysogenic variants, a fall in neuraminidase in the lysogenic variants was accompanied by a decrease in virulence (15). Neuraminidase is responsible for cleavage of sialic acids from sialoglycoconjugates such as glycolipids, polysaccharides and glycoproteins. Sialic acids are widely spread across the surfaces of eukaryotic cells and on glycoproteins. The enzymatic reaction may serve the nutritional requirements of bacteria and disrupts many host functions as well. There are indications that neuraminidase causes vascular damage, thrombus formation and haemolysis (16). Bacterial adhesion and tissue invasion are provided by neuraminidase.

Surface proteins on the cell wall of *E. rhusiopathiae* may serve as virulence factors. These proteins include RspA, rspB, SpaA, 64-66 kDa, 43 kDa (12). RspA and RspB are adhesive surface proteins (17). They showed a high degree of binding to fibronectin, polystyrene and Type I and Type IV collagens. They have the ability to bind to both biotic and abiotic surfaces and therefore they participate in initiation of biofilm formation. In a biofilm, the bacteria will

survive better because a biofilm offers protection to antibiotics and the hosts' immune system.

Another important surface protein is surface protective antigen A (SpaA) (18, 19). The virulence of a spa deletion mutant of *E. rhusiopathiae* showed a more than 76-fold decrease in virulence compared to the wild-type strain in mice. The mutant strain was sensitive and the wild-type was resistant to bactericidal swine serum.

The mechanism of pathogenicity of the SpaA protein is not fully understood yet.

E. rhusiopathiae possesses a polysaccharide capsule to prevent itself from phagocytosis by polymorphonuclear leukocytes of its host (20). The mutant strains possessed no polysaccharide capsule and these strains were efficiently phagocytosed in normal serum. A recent study, however, questioned the polysaccharide capsule as a virulence factor (19). At electron microscopy, a virulent strain and an avirulent strain did not show significant morphological differences in capsule material.

The bacteria are transmitted by the feco-oral route or by contamination of skin abrasions. Once in the oral cavity the organism ends up in the tonsils or the gastrointestinal mucosa. The route from tonsils to blood stream is not known yet. Probably the tonsillar crypt epithelium is a location of persistent *E. rhusiopathiae* infection and the bacteria exploit cytokeratin 18 positive cells of the crypts as portals of entry into the body (21).

Sepsis distributes the organism through the body. In the early septicemic stage, damage occurs to venules and capillaries of most body organs and synovial tissue (22). Vascular damage leads to shock-like generalized coagulopathy, this includes subcutaneous inoculation, endothelial swelling, monocyte adherence to vascular walls and hyaline thrombosis (22). The shock-like generalized coagulopathy results in fibrinous thrombosis, diapedesis, invasion of vascular endothelium by bacteria and deposition of fibrin in perivascular tissues (22, 23). There could be connective tissue activation in predisposed sites of infection, like joints, skin and heart valves. Also, ischemic necrosis and hemolysis can occur in severe cases.

Clinical findings

Swine erysipelas is seen in three forms: acute, subacute and chronic (8). In the acute form of erysipelas the clinical signs could include: acute death, abortions, depression, lethargy, pyrexia (40-42 °C), withdrawal, lying down, painful joints, reluctance to move, vocalization during movement, partial or complete inappetence and characteristic pink, red, or purple raised firm rhomboid or squared 'diamond skin' lesions (10). These lesions are nearly pathognomonic for swine erysipelas. In dark skinned animals, the



Source: Herman Aa (Dutch veterinarian specialized in pig health)

lesions may hard to see, then the skin lesions are best appreciated by observing areas with raised hairs or by palpation. The skin lesions are predominantly located around the snout, ears, jowls, throat, abdomen, back and thighs.

The subacute form is clinically less clear than the acute form. Pigs do not appear as sick, temperatures are less high, appetite may remain unaffected, skin lesions may be absent or few in number, mortality will be lower and pigs will recover more rapidly (10).

The chronic form follows acute, subacute or subclinical erysipelas in a proportion of surviving animals (10, 12). Chronic erysipelas is characterized most by arthritis and endocarditis. In swine affected with chronic arthritis, the joints show various degrees of stiffness and enlargement. Affected pigs are mildly to markedly lame and show an associated reduction in feed intake, and thus a decrease in growth. Ischemic necrosis of the skin lesions and of the skin on the extremities is observed. The second expression of the chronic form is vegetative valvular endocarditis. This may lead to cardiac insufficiency, consequent pulmonary oedema, respiratory signs, lethargy and cyanosis. Sudden death may also occur.

Morbidity and mortality depends on several factors. An outbreak of acute erysipelas in a naïve herd could lead to a mortality rate of 20-40% (10). In chronically or subclinically infected herds the morbidity and mortality depends on herdsmanhip, environment and other concurrent infections.

Findings in pathology

Besides the skin lesions, typical lesions of septicemia are observed; enlarged and congested lymph nodes, enlarged spleen and edematous and congested lungs (10). Petechiae and ecchymoses could be found in the renal cortex, heart and occasionally elsewhere. Joint cavities may be filled with serofibrinous exudates.

Microscopic lesions

Acute erysipelas leads to microscopic lesions in blood vessels (10). Capillaries and venules are frequently dilated and congested. Bacterial emboli and microthrombi occlude vessels which leads to circulatory stasis and focal necrosis. Neutrophils infiltrate the body. These processes have an effect on brain, heart, lungs, kidney, liver, spleen and synovial membranes.

Diagnosis

A tentative diagnosis is often based on the typical skin lesions described earlier in this paper. However, skin lesions could also be observed with classical swine fever virus, porcine dermatitis and nephropathy syndrome or *Actinobaccillus suis* septicemia (10). In grow-finish pigs septicemia and sudden death due to *Salmonella choleraesuis*, *A. suis*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Streptococcus suis* and other bacteria are at the differential diagnosis list.

To make the right diagnosis, a variety of tests are available. Direct culture from noncontaminated specimens can be conducted using basic laboratory equipment (24). In most laboratories direct culture from fluids, blood or fresh tissues is used as the standard method for *E. rhusiopathiae* isolation.

Another method is the immunohistochemical (IHC) assay (25). This method is highly sensitive and specific in detecting *E. rhusiopathiae* antigens in formalin-fixed and paraffin-embedded tissues. IHC assay is particularly useful when pigs are treated with antibiotics and when direct cultures of organs were negative.

Also several polymerase chain reaction (PCR) methods have been developed. The most recent method is the multiplex real-time PCR assay (26). This method is capable of detecting and differentiating *E. rhusiopathiae*, *E. tonsillarum* and *Erysipelothrix* spp. strain 2. Moreover it is simple, rapid, reliable, specific and highly sensitive.

Serological tests may be useful in evaluation of vaccination, but it has limited practical application in the diagnosis of acute erysipelas (10).

Immunity

Both humoral and cell-mediated immunity play a role in host defense against *E. rhusiopathiae* infection (10). Humoral immunity is probably the most important of these two mechanisms. The SpaA proteins seem to be the major immunizing antigen of *E. rhusiopathiae* (27). Immunization with HisSpa 1.0 (N-terminal of SpaA and histidine hexamer) markedly enhanced the in vitro killing and phagocytic activity of pig neutrophils against *E. rhusiopathiae*. Moreover, passive immunization experiments in mice with SpaA.1 proteins (a protein nearly identical to SpaA) showed protective activity of rabbit serum (28). The role of cellular immunity in protection is less clear (10).

Cross protection between different serotypes of *Erysipelothrix* spp. does exist (29). Pigs immunized with serotype 2, did not show clinical signs of acute erysipelas after being challenged to serotype 1a, 1b, 5, 8, 11, 12, 18, 19 or 21 either, while nonvaccinated pigs developed acute erysipelas after the challenge these serotypes. An earlier study of Wood et al. did also confirm cross protection (2).

Treatment

First choice in treatment of erysipelas is penicillin. Most strains are also susceptible to ampicillin, cloxacillin, ceftiofur, tylosin, enrofloxacin and danofloxacin (30). Another option is a subcutaneous injection with an antiserum. Antiserum gives passive protection for up to 2 weeks.

Prevention

Due to the inability to fully remove the organism from the environment, a multifaceted approach comprised of sound husbandry, herd management, sanitation and immunization is recommended (10). The main way to prevent swine erysipelas is immunization. Vaccination is generally effective in preventing disease. The duration of immunity ranges from 6 and 12 months (31).

In western Europe vaccines usually consist of whole killed bacteria combined with a soluble immunogenic product produced by selected serotype 2 strains grown in liquid media (16). A protein fraction with a molecular weight of 64-66 kDa is the most active component of the soluble immunogenic product.

Aim of this project

Despite the use of vaccines, GD animal health still receives reports of clinical erysipelas in Dutch pig herds. To prevent pigs from getting erysipelas, it is necessary to identify the risk factors for this disease. So, the aim of this research project is to identify the risk factors for erysipelas in Dutch pig herds.

Materials and methods

Experimental design

To compare the presence of risk factors in Dutch pig farms with and without erysipelas a matched case-control study was performed. A questionnaire existing of two parts was set up. The first part contained questions about the situation at the case farm at the time of the erysipelas outbreak. In the second half of the survey nearly the same questions were asked about two control pig farms. Dutch veterinarians specialized in pig health responded to this questionnaire. After a five week period of data collection, the data was analyzed using the computer program R.

The questionnaire

The questionnaire consists of 101 questions that assess the presence of potential risk factors in Dutch pig farms. These possible risk factors are partly based on literature and partly derived from epidemiology or pathogenesis.

In literature, the following risk factors were mentioned: farrowing (32, 33), poor hygienic conditions (34), extreme temperatures, parasitic infection, low humidity (35), inadequate vaccination (16), sudden diet changes and outdoor pig keeping with unpaved soil (6, 34, 36). This questionnaire is used to see if these risk factors are also applicable in the Netherlands. Using the epidemiology and pathogenesis of erysipelas a few potential risk factors were derived and added to the risk factors found in literature.

The fact that infected animals shed the bacteria in their feces and urine may result in the pens being a risk factor. *E. rhusiopathiae* could also be transmitted by contamination of skin abrasions, so the presence of skin abrasions could be a risk factor. Straw and soil may be contaminated with *E. rhusiopathiae*, so housing on straw probably increases the risk of swine erysipelas. Vermin could also transmit the bacteria, so in the survey is asked about the level of pest control. In addition the general principles of infectious diseases should be taken into account, such as factors involved in transmission of infectious agents. From this point of view high stocking density and regrouping pigs may increase the risk of swine erysipelas.

The risk factors were transformed into both open and closed questions. To make the questionnaire logically and clear, the questions were divided into seven categories; general information, information about the case (erysipelas outbreak), vaccination, external biosecurity, internal biosecurity, diet and climate. Information about the case and climate was only asked for in the first part of the survey. The other five categories were the same in the questions for the case herd and for the control herds.

The questionnaire was available in hard copy and digital to make it more user-friendly. There were no differences between these two versions.

The full questionnaire (in Dutch) is provided in Appendix A.

The veterinarians

Only Dutch veterinarians specialized in pig health took part in the questionnaire. Our target population was all Dutch pig herds. So, it was required to use data from veterinarians from all over the country.

The only selection criterium for the case herds was at least one clinical case of swine erysipelas in the last two years. The veterinarians were selected using three methods. First a call was made on members of a nationwide study group of veterinarians specialized in pig health. The second method consisted of approaching veterinarians which had called 'Veekijker' to report a clinical case of erysipelas or to ask a question about this disease. 'Veekijker' is a telephonic helpdesk for farmers and veterinarians on pig health related questions. The first two methods did not lead to a sufficient number of cases. So a third option was used, calling veterinary practices spread across The Netherlands with at least one veterinarian specialized in pig health.

Herds

One case herd and two control herds had to be selected by the veterinarian. Both herds had to meet several criteria. Case herds had experienced an erysipelas outbreak in the last two years. The period of two years was determined in order to make sure the veterinarian remembered the situation at the moment of the erysipelas outbreak. The inclusion criteria for the control herds were: erysipelas free for the last five years, the control herd described had to be visited by the veterinarian themselves to avoid the same herd being included twice and the possibility that incorrect information was given in the questionnaire and the control herd had to be the same type of herd as the case (i.e. breeding or rearing). Preferably the control herd had to be the same size as the case herd.

Data analysis

All data were collected in an Excel file. This file was used to make graphs and tables for a better understanding of the data. To be comparable, the graphs and tables showed percentages of both the cases and controls.

In this study cases and controls were matched. As the graphs and tables did not take account of this matching, all risk factors were further analysed using the statistical program R. Univariate conditional logistic regression was executed in order to identify the risk factors for swine erysipelas. This model was used because it takes into account the fact that cases and controls were matched.

Three variables applied only to the cases; location of the sow, changes in vaccination scheme and sudden diet change. These variables were examined using Chi-square analysis.

A significance level of $p < 0.05$ was used in both statistical methods. Factors with a p-value between 0.05 and 0.2 were marked as trends. A small dataset made it hard to find low p-values, so a p-value of 0.2 was chosen as the upper limit for a trend.

Only factors associated with erysipelas ($p < 0.2$ at univariate conditional logistic regression) were then selected in the second step to complete a multivariate analysis. The Akaike

information criterion (AIC) was used to find the factors best suited for this multivariate model. A lower AIC indicated the factor(s) were stronger linked to erysipelas. The coefficients served to identify confounding factors. After omitting one of the associated factor from the multivariate model, coefficients were compared with the coefficients in the complete multivariate model. When coefficients were changed more than 15%, the omitted factor and the factor where the coefficient was changed were marked as confounding factors.

Results

Eighteen veterinarians took part in the survey. These veterinarians worked in 17 different veterinary practices spread across The Netherlands. Information was obtained about 47 herds (18 cases and 29 controls). Veterinarians were not able to answer all questions. There were several questions where data was missing. Therefore, size of the dataset is given each time figures are reported.

The report of the results consists of four parts. First a summarizing overview of the herds described by the veterinarians in the survey. The second part gives some information about the cases. Then the presence of potential risk factors at both the case and control farms is described. It does not take into account the fact that cases and controls were matched. Per factor, all herds are presented in one table or graphic. Only graphics and tables that showed potential significant differences are included in this report. The rest is added in Appendix B. In the last part, results of the statistical analysis in R are given.

A summarizing overview of the herds described by the veterinarians

The three graphics below show the distribution of herd types, number of sows at the farms and the number of fattening pigs at the farms respectively.

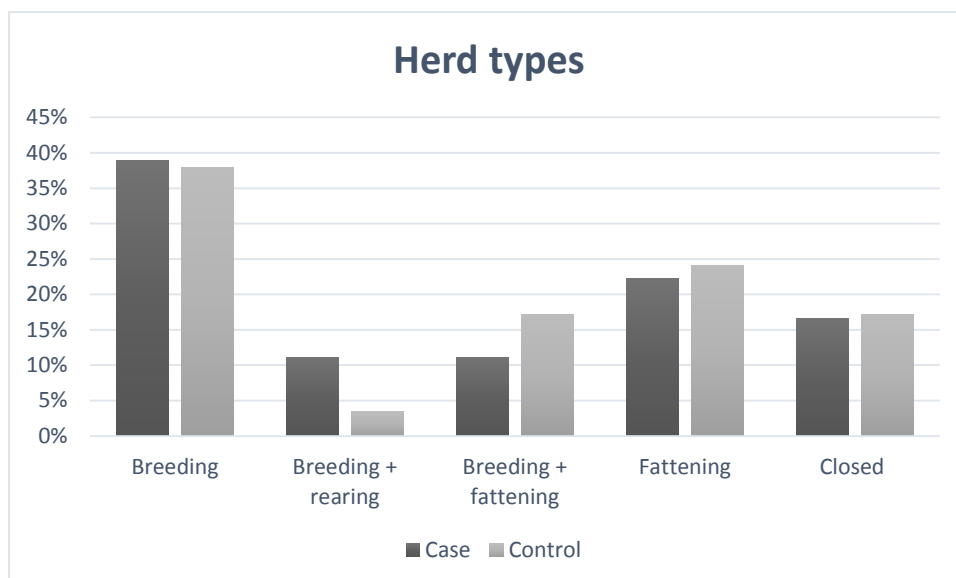


Figure 1

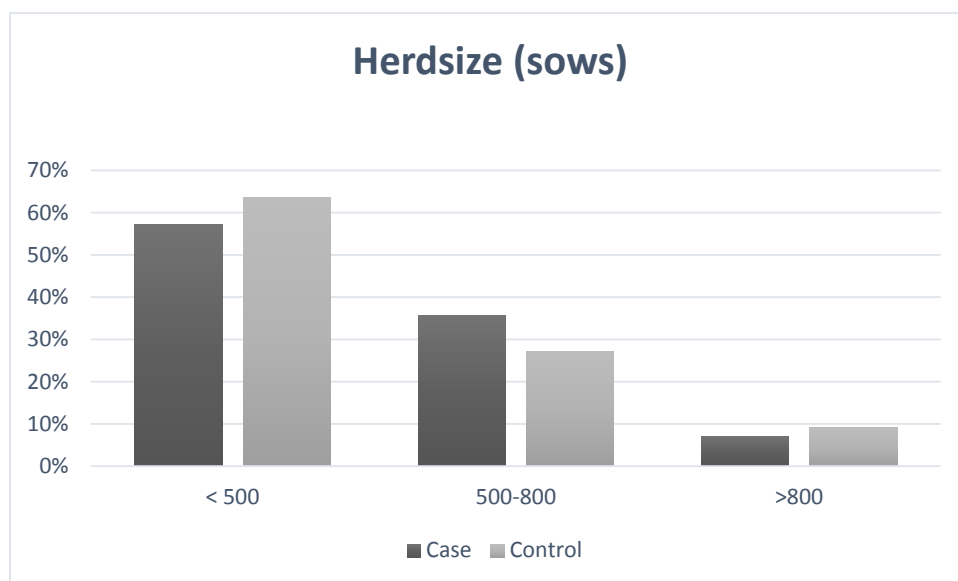


Figure 2

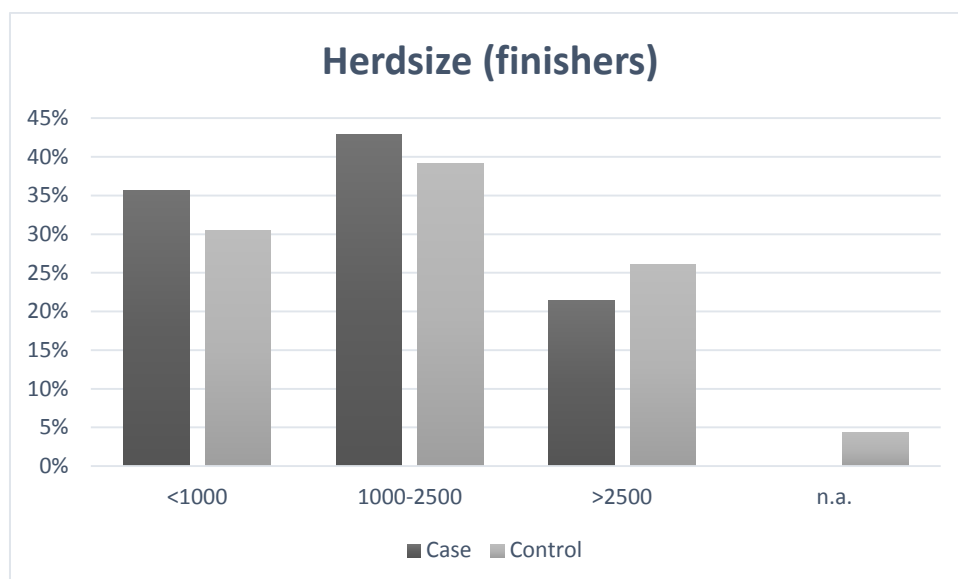


Figure 3

Information about the cases

All herd types were included in this research project to obtain as much information as possible. Table 1 shows which production stages encountered problems with swine erysipelas. At some farms, more animal categories were diagnosed with erysipelas.

| Animals diagnosed with swine erysipelas | Number of herds |
|---|-----------------|
| Sows | 5 |
| Sows and sucking piglets | 2 |
| Sows and fattening pigs | 1 |
| Sows and gilts | 2 |
| Sows, gilts and boars | 1 |
| Fattening pigs | 7 |

Table 1

In the survey a wide range in age of the diseased case pigs was found. Sows (n=9) of different parities were diagnosed with erysipelas. Suckling piglets were involved at two cases. In both cases the piglets showed signs at the age of 14 days. Fattening and rearing pigs (n=10) had signs of erysipelas at 70-210 days. In this large interval, no age group was found to be typical for the occurrence of erysipelas.

In the majority of the outbreaks, more than five animals were involved (67%). Erysipelas seemed also a recurring problem at the major part of the farms (61%). The diagnosis of erysipelas was often only based on the appearance of the typical skin lesions (Figure 4).

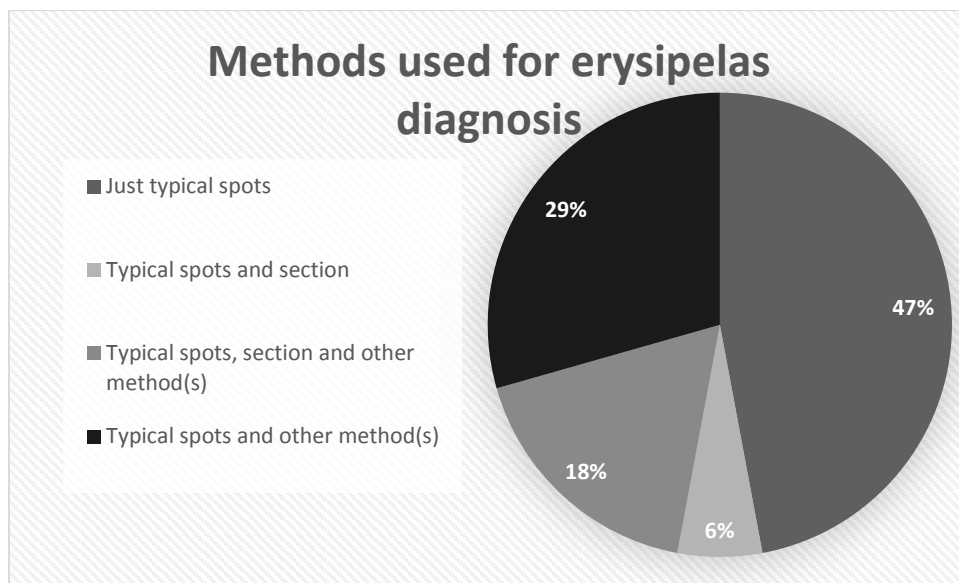


Figure 4

Potential risk factors

Location of the sow

In the survey was asked where the sow was located (if the case was a sow) at the moment of getting erysipelas. The veterinarians had three options: farrowing barn, insemination barn and gestation barn. Sows (n=11) were diagnosed in farrowing barn (82%) and in gestating sows barn (18%). The survey did not report any case in the insemination barn.

Vaccination

Fattening pigs are rarely vaccinated. Therefore, in this report vaccination status is only evaluated for cases with sows. Vaccination status is displayed in two figures. Figure 5 shows whether the rearing stock (n=25) was vaccinated or not. Vaccinated means vaccinated three times during rearing (12, 16 weeks and just before mating or Topigs scheme: 11, 15 weeks and just before mating(37)). In figure 6 vaccination status of the sows (n=27) themselves is displayed. Sows were considered to be vaccinated when vaccination was carried out every cycle.

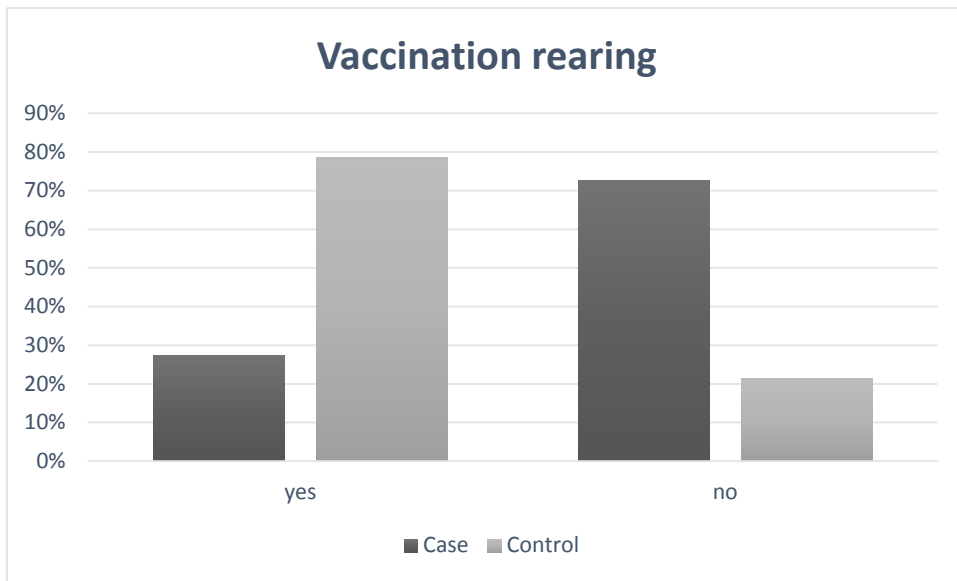


Figure 5

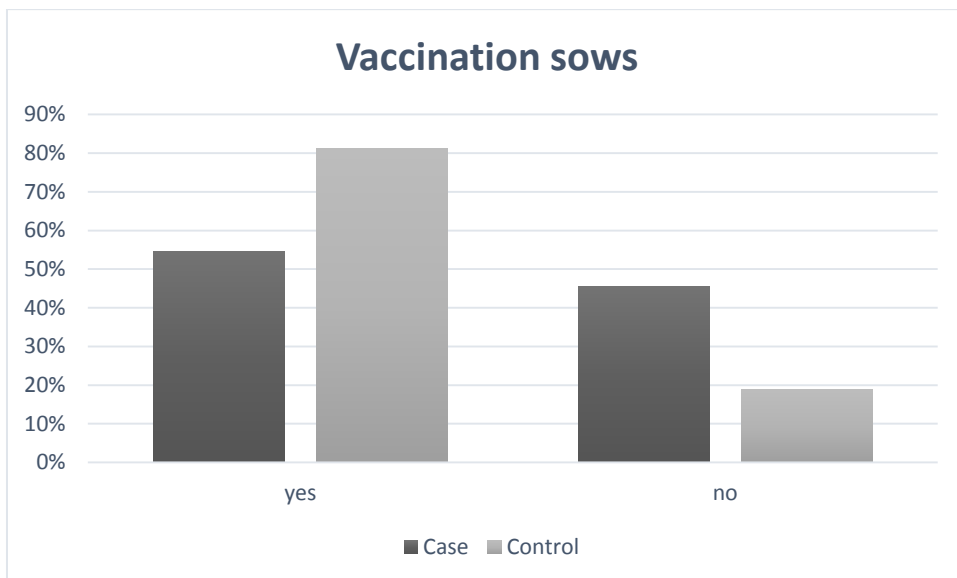


Figure 6

Pest control

Veterinarians were asked to rate the level of pest control on a scale of 1 (very poor) to 5 (very good). All veterinarians completed these questions, so there was data of all 47 herds. Figure 7 shows two different waves, where the top of the cases is situated at average pest control and the top of the controls at good pest control.

Erysipelas: Identifying risk factors for clinical cases at Dutch pig herds

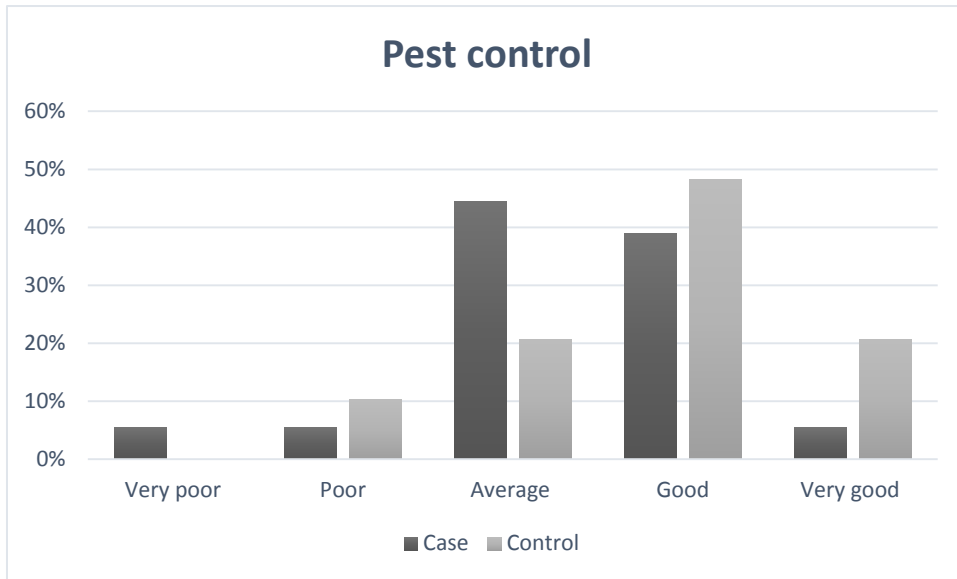


Figure 7

Dirty pens and foaming manure pits

The degree of dirt was also evaluated by a corresponding unit scale. Data was obtained from 46 herds (17 cases and 29 controls). Similar to the graphic of pest control, figure 8 also shows two waves. The cases have their optimum at clean pens, while controls have their optimum at the average.

The question about the presence of foaming manure pits was also answered for 46 herds. At 2 of 17 (12%) case farms, the veterinarian had seen foaming manure pits at least once. For the controls this ratio amounted 7 of 29 (24%). Just like the rate of dirty pens, this indicates that cases were housed in cleaner pens than the controls.

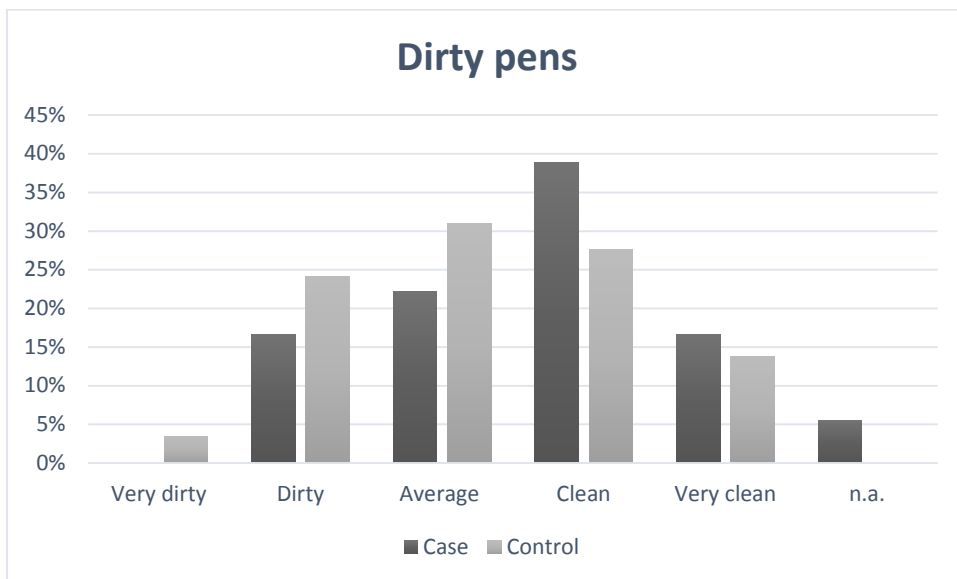


Figure 8

Cleaning of the pens

Case farms (n=17) had hardly cleanable pens in 35% of the cases. This compared with rates of 11% at the control farms (n=27).

In the survey was asked which measures were taken before new pigs arrived at the pens. A lot of different combinations of measures were mentioned by the veterinarians. Therefore, only remarkable differences were included in the analysis. In the first place, the level of cleaning was assessed by looking if any cleaning took place or not. Of the 29 control farms, just 1 farm (3%) did not take any measures before new pigs were put in the pens. At the case farms (n=18) the percentage of no cleaning measures was much higher,

In addition, it was examined if there were differences between cold and hot water cleaning. Cases (n=10) did use cold water cleaning relatively more often, 90% used cold water against 80% of the control farms (n=20). The influence of disinfectants was also analysed. Exactly half of the case farms (n=17) used disinfectants between two groups of pigs. More than half of control farms (n=27) disinfected their pens (56%).

All-in all out

Figure 9 shows the percentages of farms (n=45, 17 cases and 28 controls) that apply an all-in all out system. This figure is a general overview, differences between piglets and fattening pigs are not taken into account. Herds are categorised as partly when the all-in all out system is applied just to one part of the herd (i.e. piglets).

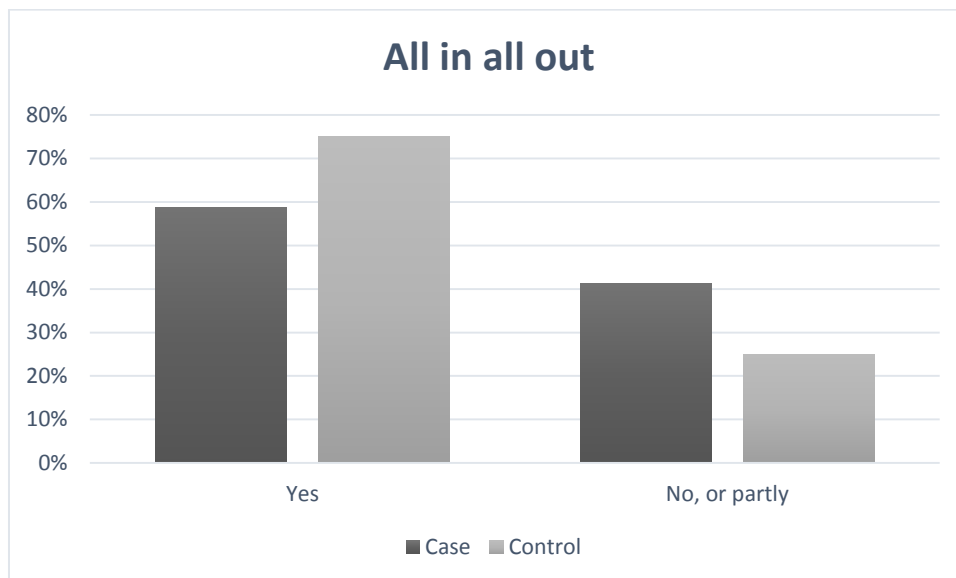


Figure 9

Nutrition

An analysis of the type of feed fed at case and control farms has been carried out. In the survey, the veterinarians had three options: dry feed, liquid feed consisting of wetfeed or liquid feed only consisting of dry feed. Figure 10 reflects the differences between cases (n=18) and controls (n=29). At 39% of the case farms, animals received feed consisting of wetfeed. At control farms, this was 17%.

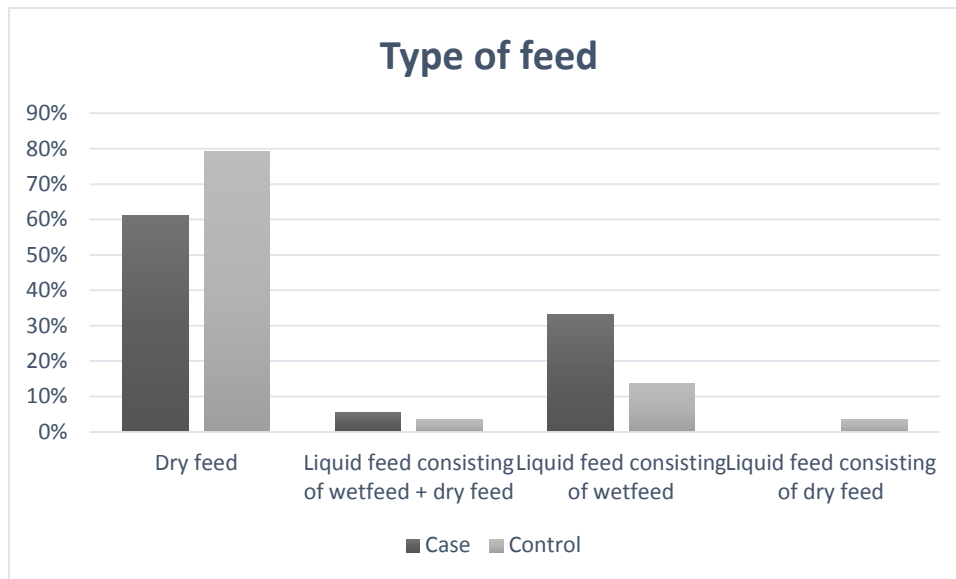


Figure 10

Often pigs had to cope with sudden diet changes in the month before erysipelas was diagnosed. In 35% of the cases, the pig(s) underwent a sudden diet change. It is unknown what kind of diet change took place.

Analysis in R

Univariate

In several conditional logistic regression models, values were hard to estimate due to the small dataset. When the model was able to estimate values, the p-value is given in table 2. Conditional logistic regression did not show any significant association ($p < 0.05$) between a risk factor and swine erysipelas. There were three factors marked as a trend ($0.05 < p < 0.2$): hardly cleanable pens, non-application of all-in all out and liquid feed consisting of wetfeed.

| Variables | p-value |
|-------------------------------------|-------------|
| Use of already opened vaccines | 0.86 ; 0.77 |
| Other animals at the farm | 0.89 |
| High stocking density | 0.76 ; 0.58 |
| Foaming manure pits | 0.51 |
| Hardly cleanable pens | 0.11 |
| Use of disinfectants | 0.48 |
| Dynamic groups of sows | 0.76 |
| Feed station in gestating sows barn | 0.64 |
| Housing of sows on straw | 0.34 |
| Non-application of all-In all out | 0.13 |
| Liquid feed consisting of wetfeed | 0.054 |

Table 2: Results of conditional logistic regression

Factors only examined at case herds were analysed using Chi-square analysis. Only the location of the sow was found significantly related to erysipelas ($p=0.002263$). Erysipelas in sows occurred more often in farrowing barn. The p-value might be even lower when the length of stay in the different barns would be taken into account. Changes in vaccination

schemes and sudden diet changes turned out not to be linked with erysipelas ($p=0.4386$ and $p=0.2253$ respectively).

Multivariate

Four variables were retained in the univariate analysis ($p < 0.2$). The factors found associated with swine erysipelas were: location of the sow, hardly cleanable pens, non-application of all-in all out and liquid feed consisting of wetfeed. It was not possible to put 'location of the sow' in the multivariate conditional logistic regression model, because there is just data from case herds.

Variables most strongly linked to erysipelas

| Variables | AIC value |
|--|------------------|
| 1. Hardly cleanable pens, non-application of all-in all out and liquid feed consisting of by products | 24.72629 |
| 2. Hardly cleanable pens and non-application of all-in all out | 27.52462 |
| 3. Hardly cleanable pens and liquid feed consisting of by products | 26.41256 |
| 4. Non-application of all-in all out and liquid feed consisting of wetfeed | 22.72817 |
| 5. Hardly cleanable pens | 29.14418 |
| 6. Non-application of all-in all out | 25.52778 |
| 7. Liquid feed consisting of wetfeed | 25.52778 |

Table 3: AIC values of different conditional logistic regression models

The AIC value indicates the adequacy of the fit of the model. The AIC is a relative value, a lower AIC means the model fits better. If the model fits better, this suggests a stronger link between the variable and outcome (erysipelas). Therefore, non-application of all-in all out and liquid feed consisting of wetfeed are stronger linked to erysipelas than hardly cleanable pens.

Confounding

In model 3 (Table 3) the coefficient for hardly cleanable pens changed with more than 15%. This suggests hardly cleanable pens and non-application of all-in all out might be confounders.

Discussion

In the current study, Dutch veterinarians specialized in pig health were contacted by phone and mail. They were asked if they had seen any erysipelas cases in the period of November 2014 to October 2016. When they did, they were asked to fill in the survey. The survey revealed that 47% of the cases were diagnosed only based on clinical findings (mainly typical spots). Although it is assumed veterinarians specialized in pig health are able to make a correct diagnosis (erysipelas is associated with typical clinical findings), there is still a chance they did not establish the right diagnosis.

The same veterinarian did also select one or two control herds. Controls had to be of the same herd type as the case. Figure 1 shows all herd types have almost the same proportion. This assumes matching went well. However, in one case, the control herd was not the same herd type as the case farm. The case was a breeding establishment with rearing and the control was a breeding establishment with fattening pigs. In the context of getting enough data, it was still decided to use this case. Moreover, these two herds are not that much different from each other.

Other small differences were caused by the fact that some cases were accompanied by one control and others by two controls. Some veterinarians did not have enough suitable herds to select two control herds. The conditional logistic regression takes account of the different amounts of controls per case (38). Preferably, veterinarians had to select a control herd in the same size category. This is seen in figure 2 and 3, comparing amounts of sows and fattening pigs at the farms. Once again some minor differences are caused by the different amount of controls per case.

It is possible that veterinarians subconsciously selected a 'better' control herd. This is further examined and given the results of the survey this may not be probable. Since, for several risk factors cases and controls scored the same. At two risk factors (dirty pens and foaming manure pits) cases scored even better than controls.

While creating the survey, an appropriate balance had to be found between a brief survey for enough responses and enough questions to find the risk factors. A briefer survey may also result in missing some important risk factors. However, extensive research is done to select the most likely risk factors for erysipelas in Dutch pig herds. Nevertheless, some risk factors deserved more detailed examination. One of these factors is vaccination, also vaccination of fattening pigs had to be asked.

Some questions were not clear enough and could have been interpreted in several ways. These were; herd type, changes in the vaccination schedule and sudden diet changes. The veterinarian also categorised herds with sows and fattening pigs as closed. Fortunately, this has no effect on the results, because herd types were just used for matching.

When asked about changes in vaccination schemes, there should have been referred to vaccination schemes for erysipelas. Some respondents might have thought they had to mention every change of the vaccination scheme. Since changes in vaccination schemes did not show any association with erysipelas, it is not very likely that change in erysipelas vaccination is associated with erysipelas. When looking at the question about sudden diet changes, there might have been a miscommunication. When sows go to farrowing barn, they are always confronted with diet changes. However, the question was drawn up to get some information about changes in feed suppliers.

In this study, it was hard to find veterinarians suitable to participate in this survey. Despite the fact that (nearly) all veterinarians who could participate did fill in the survey, the amount of data is still relatively small (18 cases and 29 controls).

Unfortunately, two questions had to be deleted, because most of the veterinarians did not have data on department temperature and humidity, both mentioned in literature (34, 35).

The small size of the dataset also lead to problems in the statistical program R. Some models were not able to predict values. Therefore, some risk factors may be missed, while a larger dataset might have resulted in a statistical relevant relation between a factor and erysipelas. To clarify the consequences of a small dataset, Appendix C displays vaccination status of sows for pairs cases and controls (this means per veterinarian ID (n=10)). So there are 10 pairs of data (case + control = pair). As this is a matched case control study, the conditional logistic regression model was used. This model only works with pairs that show differences in vaccination between case and control. As Appendix C shows, there were 3 pairs with a different vaccination status of case and controls. This means that there were 3 pairs left for analysis, which is not enough to find associations.

In this study a significant relation was found between 'location of the sow' and swine erysipelas. Sows were diagnosed with erysipelas in farrowing barn more often. At first this question was included in the survey because literature mentioned farrowing as a risk factor (32, 33). According to this article this is related to the stress during farrowing. It is suggested that stress supresses the immune system, making it easier for *E. rhusiopathiae* to migrate from the tonsils into blood stream. However, there is no evidence for this reasoning. There might be other factors in farrowing barn having an effect on occurrence of erysipelas. For example, often vaccination is repeated in farrowing barn. Erysipelas in farrowing barn should be further examined to find the factors associated with erysipelas.

Three variables were marked as trends ($0.05 < p < 0.2$) after completing univariate conditional logistic regression for all factors; hardly cleanable pens, non-application of all-in all out and liquid feeding consisting of wetfeed. The first two seemed to be confounding factors. There was no relationship found between these two factors. They might have to do with an inadequate management both.

Remarkably, the three factors found to be associated with erysipelas were not mentioned in literature. They were derived from epidemiology. None of them declare why 30-50% of the pigs harbour *E. rhusiopathiae* in their tonsils and most of them will not get sick. There is got to be some factors involved with bacterial migration from tonsils to bloodstream. However, the fact that none of the risk factors mentioned in literature were found significantly related, does not mean they are excluded as risk factors for erysipelas in Dutch pig herds. Like mentioned before, because of the small dataset R was not able predict several values. If there was more data, there might have been significant findings.

Contrary to the expectations, this study did not find a relation between inadequate vaccination to erysipelas and swine erysipelas. Relationships have previously been mentioned between inadequate vaccination and erysipelas (16). Moreover, vaccination was the factor most often mentioned by veterinarians when they were told about the aim of this research. Further research specifically dedicated to erysipelas vaccinations is needed to better understand the influence of inadequate vaccination on the chance of getting erysipelas. It is not only important that pigs are vaccinated against erysipelas, but also how the vaccination process takes place. Despite the lack of association found in this study, it is still recommended to vaccinate herds against erysipelas. Namely, the dataset is too small to

find associations. Furthermore, erysipelas is one of the cheapest vaccinations and an outbreak of erysipelas is more expensive.

Conclusion

Sows are significantly more often diagnosed with erysipelas in farrowing barn. Whether this has to do with farrowing or other factors present in farrowing barn is still unclear in this study. Further research is needed to identify these factors.

In addition, hardly cleanable pens, non-application of all-in all out and liquid feeding consisting of wetfeed showed an association with erysipelas. The last two seemed particularly strongly related to one another.

However, none of the examined risk factors could be excluded definitely, because of the small dataset.

Research specifically dedicated to the vaccination might be useful, because literature and veterinarians both suggest a relationship between inadequate vaccination and erysipelas.

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References

1. Takahashi T, Fujisawa T, Tamura Y, Suzuki S, Muramatsu M, Sawada T, et al. DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum*. *Int J Syst Evol Microbiol*. 1992;42(3):469-73.
2. Wood RL. Specificity in response of vaccinated swine and mice to challenge exposure with strains of *Erysipelothrix rhusiopathiae* of various serotypes. *Am J Vet Res*. 1979 Jun;40(6):795-801.
3. KUCSERA G. Proposal for standardization of the designations used for serotypes of *Erysipelothrix rhusiopathiae* (Migula) Buchanan. *Int J Syst Evol Microbiol*. 1973;23(2):184-8.
4. Takahashi T, Fujisawa T, Umeno A, Kozasa T, Yamamoto K, Sawada T. A taxonomic study on *Erysipelothrix* by DNA-DNA hybridization experiments with numerous strains isolated from extensive origins. *Microbiol Immunol*. 2008;52(10):469-78.
5. Bender JS, Irwin CK, Shen HG, Schwartz KJ, Opriessnig T. *Erysipelothrix* spp. genotypes, serotypes, and surface protective antigen types associated with abattoir condemnations. *J Vet Diagn Invest*. 2011 Jan;23(1):139-42.
6. Brooke CJ, Riley TV. *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *J Med Microbiol*. 1999;48(9):789-99.
7. Reboli AC, Farrar WE. *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clin Microbiol Rev*. 1989 Oct;2(4):354-9.
8. Grieco MH, Sheldon C. *Erysipelothrix rhusiopathiae*. *Ann N Y Acad Sci*. 1970;174(2):523-32.
9. Stephenson EH, Berman DT. Isolation of *Erysipelothrix rhusiopathiae* from tonsils of apparently normal swine by two methods. *Am J Vet Res*. 1978 Jan;39(1):187-8.
10. Zimmerman, Jeffrey J., John Wiley & Sons.,. *Diseases of swine* 750-757. Chichester: Wiley-Blackwell; 2013.
11. Fidalgo SG, Longbottom CJ, Riley TV. Susceptibility of *Erysipelothrix rhusiopathiae* to antimicrobial agents and home disinfectants. *Pathology*. 2002;34(5):462-5.
12. Wang Q, Chang BJ, Riley TV. *Erysipelothrix rhusiopathiae*. *Vet Microbiol*. 2010 1/27;140(3-4):405-17.
13. Krasemann C, Müller HE. The virulence of *Erysipelothrix rhusiopathiae* strains and their neuraminidase production (author's transl). *Zentralbl Bakteriol [Orig A]*. 1975;231(1-3):206-13.
14. Wang Q, Chang BJ, Mee BJ, Riley TV. Neuraminidase production by *Erysipelothrix rhusiopathiae*. *Vet Microbiol*. 2005 5/20;107(3-4):265-72.
15. Nikolov P, Abrashev I, Ilieva K, Avramova T. Virulence and neuraminidase activity in *Erysipelothrix rhusiopathiae*. *Acta Microbiol Bulg*. 1978;VOL 2:62-5.

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16. Haesebrouck F, Pasmans F, Chiers K, Maes D, Ducatelle R, Decostere A. Efficacy of vaccines against bacterial diseases in swine: what can we expect? *Vet Microbiol.* 2004 6/3;100(3–4):255-68.
17. Shimoji Y, Ogawa Y, Osaki M, Kabeya H, Maruyama S, Mikami T, et al. Adhesive surface proteins of *Erysipelothrix rhusiopathiae* bind to polystyrene, fibronectin, and type I and IV collagens. *J Bacteriol.* 2003 May;185(9):2739-48.
18. Borrathybay E, Gong F, Zhang L, Nazierbieke W. Role of surface protective antigen A in the pathogenesis of *Erysipelothrix rhusiopathiae* strain C43065. *Journal of microbiology and biotechnology.* 2015;25(2):206-16.
19. Li Y, Zou Y, Xia Y, Bai J, Wang X, Jiang P. Proteomic and Transcriptomic Analyses of Swine Pathogen *Erysipelothrix rhusiopathiae* Reveal Virulence Repertoire. *PloS one.* 2016;11(8):e0159462.
20. Shimoji Y, Yokomizo Y, Sekizaki T, Mori Y, Kubo M. Presence of a capsule in *Erysipelothrix rhusiopathiae* and its relationship to virulence for mice. *Infect Immun.* 1994 Jul;62(7):2806-10.
21. Harada T, Ogawa Y, Eguchi M, Shi F, Sato M, Uchida K, et al. *Erysipelothrix rhusiopathiae* exploits cytokeratin 18-positive epithelial cells of porcine tonsillar crypts as an invasion gateway. *Vet Immunol Immunopathol.* 2013 6/15;153(3–4):260-6.
22. Schulz L-, Drommer W, Seidler D, Ehard H, Leimbeck R, Weiss R. Experimenteller Rotlauf bei verschiedenen Spezies als Modell einer systemischen Bindegewebskrankheit II. Chronische Phase mit besonderer Berücksichtigung der Polyarthritits. *Beiträge zur Pathologie.* 1975 January 1975;154(1):27-51.
23. SCHULZ L, HERTRAMPF B, EHARD H, GIESE W, DROMMER W. SIGNIFICANCE OF COAGULATION TROUBLES AND OF INFLAMMATORY DEFENCE IN AN EXPERIMENTAL-MODEL OF RHEUMATOID-ARTHRITIS CAUSED BY MICROBIAL INFECTION. 1. SYSTEMIC, SHOCK-LIKE COAGULOPATHY AND FIBRIN INCORPORATION AS INDICATORS OF RHEUMATOID VISCERAL MANIFESTATION, AS SEEN IN ERYSIPELAS MODEL. *Z Rheumatol.* 1976b;35(9-10):315-23.
24. Bender JS, Kinyon JM, Kariyawasam S, Halbur PG, Opriessnig T. Comparison of conventional direct and enrichment culture methods for *Erysipelothrix* spp. from experimentally and naturally infected swine. *J Vet Diagn Invest.* 2009 Nov;21(6):863-8.
25. Opriessnig T, Bender JS, Halbur PG. Development and validation of an immunohistochemical method for rapid diagnosis of swine erysipelas in formalin-fixed, paraffin-embedded tissue samples. *J Vet Diagn Invest.* 2010 Jan;22(1):86-90.
26. Pal N, Bender J, Opriessnig T. Rapid detection and differentiation of *Erysipelothrix* spp. by a novel multiplex real-time PCR assay. *J Appl Microbiol.* 2010;108(3):1083-93.
27. Imada Y, Mori Y, Daizoh M, Kudoh K, Sakano T. Enzyme-linked immunosorbent assay employing a recombinant antigen for detection of protective antibody against swine erysipelas. *J Clin Microbiol.* 2003 Nov;41(11):5015-21.
28. Shimoji Y, Mori Y, Fischetti VA. Immunological characterization of a protective antigen of *Erysipelothrix rhusiopathiae*: identification of the region responsible for protective immunity. *Infect Immun.* 1999 Apr;67(4):1646-51.

Erysipelas: Identifying risk factors for clinical cases at Dutch pig herds

29. Takahashi T, Takagi M, Sawada T, Seto K. Cross protection in mice and swine immunized with live erysipelas vaccine to challenge exposure with strains of *Erysipelothrix rhusiopathiae* of various serotypes. *Am J Vet Res.* 1984 Oct;45(10):2115-8.
30. Yamamoto K, Kijima M, Yoshimura H, Takahashi T. Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae* isolated from pigs with swine erysipelas in Japan, 1988–1998. *Journal of Veterinary Medicine, Series B.* 2001;48(2):115-26.
31. Swan R, Lindsey M. Treatment and control by vaccination of erysipelas in farmed emus (*Dromaius novo-hollandiae*). *Aust Vet J.* 1998;76(5):325-7.
32. Eriksson H, Jansson DS, Johansson K, Båverud V, Chirico J, Aspán A. Characterization of *Erysipelothrix rhusiopathiae* isolates from poultry, pigs, emus, the poultry red mite and other animals. *Vet Microbiol.* 2009 5/28;137(1–2):98-104.
33. Garg SR. Zoonoses : bacterial diseases. New Delhi: Daya Pub. House; 2014.
34. Risco D, Llario P, Velarde R, García W, Benítez J, García A, et al. Outbreak of Swine Erysipelas in a Semi-Intensive Wild Boar Farm in Spain. *Transboundary and emerging diseases.* 2011;58(5):445-50.
35. Tuovinen VK, Gröhn YT, Straw BE. Farrowing unit housing and management factors associated with diseases and disease signs of importance for feeder pig quality. *Acta Agriculturae Scandinavica A—Animal Sciences.* 1997;47(2):117-25.
36. Vlekziekte [Internet].; 26-2-2009 []. Available from: <http://www.pigbusiness.nl/diergezondheid/aandoeningen/vlekziekte/nieuws/541/vlekziekte>.
37. Topigs Norsvin entschema [Internet]. []. Available from: <http://topigs.ipg.nl/pls/ppig/f?p=903:8:::NO::>.
38. Dohoo, Ian., Martin, Wayne., Stryhn, Henrik.,. *Veterinary epidemiologic research*, p 369. In: Charlottetown (PEI, Canada): Atlantic Veterinary College, University of Prince Edward Island; 2003.

Appendix A: Survey (in Dutch)

Onderzoek naar de risicofactoren voor vlekziekte bij Nederlandse varkenspopulaties

Beste varkensdierenarts,

Met behulp van deze enquête probeer ik de risicofactoren voor vlekziekte bij Nederlandse varkens te bepalen. Er dienen drie keer (bijna) dezelfde vragen beantwoord te worden, namelijk één keer voor de case en twee keer voor de controlebedrijven. Als case neemt u een bedrijf waar u in de afgelopen twee jaar vlekziekte als waarschijnlijkheidsdiagnose heeft gesteld. Alle vragen kunt u beantwoorden naar de situatie op het bedrijf op het moment van het klinische geval van vlekziekte. Als controlebedrijf neemt u twee bedrijven uit uw klantenbestand die qua productiesysteem en bedrijfsgrootte matchen met het case-bedrijf. Op het controlebedrijf mag de afgelopen vijf jaar geen vlekziekte als waarschijnlijkheidsdiagnose zijn gesteld. Hoe u een controlebedrijf precies selecteert staat in het tweede deel van de enquête uitgelegd.

De enquête dient uiterlijk 21 november 2016 ingevuld te zijn. Ik kom de enquête die dag persoonlijk ophalen. De enquêtes kunnen ook opgestuurd worden middels de bijgeleverde envelop.

Het kan zijn dat u het antwoord op een vraag echt niet weet, geef dit dan a.u.b. ook aan. Mocht u vragen hebben, dan kunt u mailen naar: m.g.h.vanheesbeen@students.uu.nl of bellen naar: 06-81604536.

Bij voorbaat dank,

Marly van Heesbeen

Oproep Gezondheidsdienst Deventer

De GD is op zoek naar bedrijven waar de afgelopen drie maanden vlekziekte is gediagnosticeerd. Het is belangrijk dat de varkens op dit moment nog in de afdeling aanwezig zijn. Als u een bedrijf kent dat aan deze voorwaarden voldoet dan kunt u uw gegevens mailen naar: m.houben@gddiergezondheid.nl. Er zal dan contact met u opgenomen worden.

DEEL 1: CASE-BEDRIJF

Als case-bedrijf neemt u een bedrijf waar u in de periode van november 2014 t/m oktober 2016 de waarschijnlijkheidsdiagnose vlekziekte heeft gesteld.

Algemene informatie case-bedrijf

1. Wat was het bedrijfstype? U kunt meerdere opties selecteren.

- vermeerderingsbedrijf
- vleesvarkensbedrijf
- opfok zeugen

2. Hoeveel zeugen telde het bedrijf?

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- <500
- 500 – 800
- > 800
- niet van toepassing

3. Hoeveel vleesvarkens telde het bedrijf?

- < 1000
- 1000-2500
- > 2500
- niet van toepassing

4. Betrof het een biologisch bedrijf?

- ja
- nee

5. Hadden de varkens de mogelijkheid tot vrije uitloop op onverharde bodem?

- ja
- nee

6. Wat zijn de kengetallen van de zeugen van het jaar waarin vlekziekte gediagnosticeerd werd op het bedrijf? (Niet invullen indien niet van toepassing voor dit bedrijf)

| | |
|--------------------------------|------------|
| Leeftijd eerste dekking | dagen |
| Worpindex | |
| Vervangingspercentage zeugen | % |
| Uitvalspercentage onder zeugen | % |
| Dier dag dosering | dagen/jaar |

7. Wat zijn de kengetallen van de vleesvarkens van het jaar waarin vlekziekte gediagnosticeerd werd op het bedrijf? (Niet invullen indien niet van toepassing voor dit bedrijf)

| | |
|-------------------|------------|
| Uitvalspercentage | % |
| Dagelijkse groei | g/dag |
| Voederconversie | |
| Dier dag dosering | dagen/jaar |

Vragen over de case

8. Bij welke dieren werd de waarschijnlijkheidsdiagnose vlekziekte gesteld? U kunt meerdere opties selecteren.

- zeugen
 zuigende biggen
 gespeende biggen
 vleesvarkens
 gelten
 beren

9. Wat was de leeftijd van het dier/de dieren?

..... dagen

10. Hoeveel dieren zijn er met vlekziekte gediagnosticeerd?

- één
 twee – vijf dieren
 > vijf dieren

11. Was vlekziekte een incidenteel of een terugkerend probleem op dit bedrijf?

- incidenteel
 terugkerend

12. Hoe is de (waarschijnlijkheids)diagnose vlekziekte gesteld?

- vlekken
- sectie
- overig,

Vraag 13 hoeft alleen ingevuld te worden indien de casus een zeug betrof.

13. Waar bevond de zeug zich op het moment dat zij met vlekziekte gediagnosticeerd werd?

- kraamstal
- dekstal
- dragende zeugen stal

14. Wat waren de meest voorkomende ziekteproblemen op dit bedrijf op het moment van de vlekziekte uitbraak? Geef een top 3.

.....

.....

.....

Vaccinatie op het case-bedrijf

Vraag 15 en 16 hoeven alleen beantwoord te worden als er zich zeugen op het bedrijf bevonden.

15. Wat was het vaccinatieschema voor vlekziekte voor zeugen op dit bedrijf? Geef hierbij s.v.p. aan met **welk vaccin** de dieren gevaccineerd werden, **welke dieren** gevaccineerd werden en op **welke leeftijd**.

.....

.....

.....

16. Op welk moment werd de vaccinatie herhaald? (bv. in de kraamstal)

.....

Vraag 17 hoeft alleen beantwoord te worden indien er opfok op het bedrijf aanwezig was.

17. Wat was het vaccinatieschema voor vlekziekte voor de opfok op dit bedrijf? Geef hierbij s.v.p. aan met **welk vaccin** de dieren gevaccineerd werden, **welke dieren** gevaccineerd werden en op **welke leeftijd**.

.....
.....
.....

18. Werden de vaccins op dit bedrijf gekoeld en apart bewaard?

- ja
 nee
 weet ik niet

19. Werden er op dit bedrijf al aangeprikte vaccins op een later moment nog eens gebruikt?

- ja
 nee
 weet ik niet

20. Is er ten tijde van de vlekziekte uitbraak een verandering in het vaccinatieschema opgetreden?

- ja
 nee
 weet ik niet

Externe biosecurity op het case-bedrijf

21. Was er een hygiënesluis aanwezig op het bedrijf?

- ja
 nee

22. Wat was uw indruk van de ongediertebestrijding op het bedrijf? Geef aan op een schaal van 1 tot 5, waarbij 1 = erg slecht en 5 = erg goed.

- 1 2 3 4 5

23. Waren er ook andere dieren aanwezig op het bedrijf? Zo ja, geef aan welke dieren.

- ja,
- nee
- weet ik niet

Vraag 24 is alleen van toepassing indien het bedrijf een vleesvarkensbedrijf betreft.

24. Worden alle varkens van hetzelfde vermeerderingsbedrijf aangevoerd?

- ja
- nee, het bedrijf heeft 2 of 3 vaste leveranciers
- nee, de varkens komen van 4 of meer verschillende vermeerderingsbedrijven
- weet ik niet

Interne biosecurity op het case-bedrijf

25. Wat was de bezettingsgraad op dit bedrijf?

- laag (<90%)
- gemiddeld (90-100%)
- hoog (>100%)
- weet ik niet

26. Waren er op dit bedrijf veel dieren met krassen en huidbeschadigingen? Geef aan op een schaal van 1 tot 5, waarbij 1= erg veel dieren met krassen en huidbeschadigingen en 5 = erg weinig dieren met krassen en huidbeschadigingen.

- 1 2 3 4 5

27. Hoe zou u de hokbevuiling op de afdeling waar het dier/de dieren met vlekziekte zich bevond(en) omschrijven? Geef aan op een schaal van 1 tot 5, waarbij 1 = erg vuil en 5 = erg schoon

- 1 2 3 4 5

28. Zag u op de afdeling waar u vlekziekte heeft gediagnosticeerd wel eens schuimende putten?

- ja
- nee

29. Waren de hokken gemaakt van goed te reinigen en te ontsmetten materiaal?

- ja
- nee
- weet ik niet

30. Welke maatregelen werden er genomen voordat er nieuwe dieren in een hok kwamen? Er kunnen meerdere antwoorden geselecteerd worden.

- koud water reiniging
- warm water reiniging
- reiniging met zeep
- gebruik ontsmettingsmiddelen
- geen maatregelen
- weet ik niet

Vraag 31, 32, 33 en 34 zijn alleen van toepassing wanneer er zich zeugen op het bedrijf bevonden.

31. Werden de dragende zeugen in stabiele of dynamische groepen gehuisvest?

- stabiele groepen
- dynamische groepen

32. Hoe groot waren de groepen waarin dragende zeugen gehuisvest werden?

.....zeugen

33. Werden de dragende zeugen met voerstations gevoerd?

- ja
- nee

34. Werden de dragende zeugen op stro gehuisvest?

- ja
- nee

35. Werden er biggen op dit bedrijf dubbel opgelegd?

- ja, regelmatig
- ja, af en toe
- nee
- niet van toepassing
- weet ik niet

36. Hanteerde het bedrijf een all-in all-out principe bij de biggen en/of vleesvarkens? U kunt meerdere opties selecteren.

- ja, bij de biggen
- ja, bij de vleesvarkens
- nee
- niet van toepassing
- weet ik niet

Voeding op het case-bedrijf

37. Wat voor type voer werd er op dit bedrijf gevoerd?

- droog voer
- brijvoer bestaande uit bijproducten
- brijvoer bestaande uit brok

38. Had het dier waarbij de waarschijnlijkheidsdiagnose vlekziekte is gesteld in de maand voorafgaand aan de diagnose een voerverandering ondergaan?

- ja
- nee

Klimaat op het case-bedrijf

39. In welke maand deed de case zich voor?

- december, januari, februari of maart
- april, mei, oktober of november
- juni, juli, augustus of september

40. Is de temperatuur bekend van de afdeling waar de case zich bevond? Zo ja, wat was deze temperatuur?

ja, die was: , °C (afroonden op één cijfer achter de komma) niet bekend

41. Is de luchtvochtigheid bekend van de afdeling waar de case zich bevond? Zo ja, wat was het luchtvochtigheidspercentage?

< 60%

tussen 60% en 75%

> 75%

niet bekend

DEEL 2: DE CONTROLEBEDRIJVEN

De twee controlebedrijven waar u nu (bijna) dezelfde vragen voor in gaat vullen dient aan de volgende vijf eisen te voldoen:

- 1 Op dit bedrijf mag de afgelopen 5 jaar geen waarschijnlijkheidsdiagnose vlekziekte zijn gesteld
- 2 Het bedrijfstype komt overeen met het bedrijf waarop de casus zich afspeelde (oftewel vraag 1, 42 en 72 dienen hetzelfde beantwoord te worden)
- 3 Het betreft een bedrijf waar u zelf de bedrijfsbegeleiding uitvoert (of u bent er zeker van dat uw collega's die ook deze enquête invullen niet hetzelfde bedrijf als controlebedrijf nemen)
- 4 Het aantal zeugen dient in dezelfde categorie te vallen (oftewel vraag 2, 43 en 73 dienen hetzelfde beantwoord te worden)
- 5 Het aantal vleesvarkens dient in dezelfde categorie te vallen (oftewel vraag 3, 44 en 74 dienen hetzelfde beantwoord te worden)

Wanneer u geen bedrijf kunt vinden dat qua bedrijfsgrootte vergelijkbaar is met het case-bedrijf (eisen 4 en 5) vermeld dit dan hieronder. U kunt dan alsnog deze enquête invullen voor de controlebedrijven. Als u geen bedrijf kunt beschrijven dat aan eisen 1, 2 en 3 voldoet, dan kunt u de enquête niet invullen voor het controlebedrijf.

Algemene informatie controlebedrijf 1

42. Wat is het bedrijfstype? U kunt meerdere opties selecteren.

vermeerderingsbedrijf

vleesvarkensbedrijf

opfok zeugen

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43. Hoeveel zeugen telt het bedrijf?

- <500
 500 – 800
 > 800
 niet van toepassing

44. Hoeveel vleesvarkens telt het bedrijf?

- < 1000
 1000-2500
 > 2500
 niet van toepassing

45. Betreft het een biologisch bedrijf?

- ja
 nee

46. Hebben de varkens de mogelijkheid tot vrije uitloop op onverharde bodem?

- ja
 nee

47. Wat zijn de huidige kengetallen van de zeugen op het bedrijf? (Niet invullen indien niet van toepassing voor dit bedrijf)

| | |
|--------------------------------|------------|
| Leeftijd eerste dekking | dagen |
| Worptest | |
| Vervangingspercentage zeugen | % |
| Uitvalspercentage onder zeugen | % |
| Dier dag dosering | dagen/jaar |

48. Wat zijn de huidige kengetallen van de vleesvarkens op het bedrijf? (Niet invullen indien niet van toepassing voor dit bedrijf)

| | |
|-------------------|------------|
| Uitvalspercentage | % |
| Dagelijkse groei | g/dag |
| Voederconversie | |
| Dier dag dosering | dagen/jaar |

49. Wat zijn de meest voorkomende ziekteproblemen op dit bedrijf? Geef een top 3.

.....

.....

.....

Vaccinatie op controlebedrijf 1

Vraag 50 en 51 hoeven alleen beantwoord te worden als er zich zeugen op het bedrijf bevinden.

50. Wat is het vaccinatieschema voor vlekziekte voor zeugen op dit bedrijf? Geef hierbij s.v.p. aan met **welk vaccin** de dieren gevaccineerd worden, **welke dieren** gevaccineerd worden en op **welke leeftijd**.

.....

.....

.....

51. Op welk moment wordt de vaccinatie herhaald? (bv. in de kraamstal)

.....

De volgende vraag hoeft alleen beantwoord te worden indien er opfok op het bedrijf aanwezig is.

52. Wat is het vaccinatieschema voor vlekziekte voor de opfok op dit bedrijf? Geef hierbij s.v.p. aan met **welk vaccin** de dieren gevaccineerd worden, **welke dieren** gevaccineerd worden en op **welke leeftijd**.

.....

.....

.....

53. Worden de vaccins op dit bedrijf gekoeld en apart bewaard?

- ja
- nee
- weet ik niet

54. Worden er op dit bedrijf al aangeprikte vaccins op een later moment nog eens gebruikt?

- ja
- nee
- weet ik niet

Externe biosecurity op controlebedrijf 1

55. Is er een hygiënesluis aanwezig op het bedrijf?

- ja
- nee

56. Wat is uw indruk van de ongediertebestrijding op het bedrijf? Geef aan op een schaal van 1 tot 5, waarbij 1 = erg slecht en 5 = erg goed.

- 1
- 2
- 3
- 4
- 5

57. Zijn er ook andere dieren aanwezig op het bedrijf? Zo ja, geef aan welke dieren.

- ja,
- nee
- weet ik niet

Vraag 58 is alleen van toepassing indien het bedrijf een vleesvarkensbedrijf betreft.

58. Worden alle varkens van hetzelfde vermeerderingsbedrijf aangevoerd?

- ja
- nee, het bedrijf heeft 2 of 3 vaste leveranciers
- nee, de varkens komen van 4 of meer verschillende vermeerderingsbedrijven
- weet ik niet

Interne biosecurity op controlebedrijf 1

59. Wat is de bezettingsgraad op dit bedrijf?

- laag (<90%)
- gemiddeld (90-100%)
- hoog (>100%)
- weet ik niet

60. Zijn er op dit bedrijf veel dieren met krassen en huidbeschadigingen? Geef aan op een schaal van 1 tot 5, waarbij 1= erg veel dieren met krassen en huidbeschadigingen en 5 = erg weinig dieren met krassen en huidbeschadigingen.

- 1
- 2
- 3
- 4
- 5

61. Hoe zou u de hokbevuiling op dit bedrijf over het algemeen omschrijven? Geef aan op een schaal van 1 tot 5, waarbij 1 = erg vuil en 5 = erg schoon

- 1
- 2
- 3
- 4
- 5

62. Ziet u wel eens schuimende putten op dit bedrijf?

- ja
- nee

63. Zijn de hokken gemaakt van goed te reinigen en te ontsmetten materiaal?

- ja
- nee
- weet ik niet

64. Welke maatregelen worden er genomen voordat er nieuwe dieren in een hok komen? Er kunnen meerdere antwoorden geselecteerd worden.

- koud water reiniging
- warm water reiniging
- reiniging met zeep
- gebruik ontsmettingsmiddelen
- geen maatregelen

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weet ik niet

Vraag 65, 66, 67 en 68 zijn alleen van toepassing wanneer er zich zeugen op het bedrijf bevinden.

65. Worden de dragende zeugen in stabiele of dynamische groepen gehuisvest?

- stabiele groepen
- dynamische groepen

66. Hoe groot zijn de groepen waarin dragende zeugen gehuisvest worden?

.....zeugen

67. Worden de dragende zeugen met voerstations gevoerd?

- ja
- nee

68. Worden de dragende zeugen op stro gehuisvest?

- ja
- nee

69. Worden biggen op dit bedrijf dubbel opgelegd?

- ja, regelmatig
- ja, af en toe
- nee
- niet van toepassing
- weet ik niet

70. Hanteert het bedrijf een all-in all-out principe bij de biggen en/of vleesvarkens? U kunt meerdere antwoorden selecteren.

- ja, bij de biggen
- ja, bij de vleesvarkens
- nee
- niet van toepassing

weet ik niet

Voeding op controlebedrijf 1

71. Wat voor type voer wordt er op dit bedrijf gevoerd?

- droog voer
- brijvoer bestaande uit bijproducten
- brijvoer bestaande uit brok

Neem nu een tweede controlebedrijf in gedachten.

Algemene informatie controlebedrijf 2

72. Wat is het bedrijfstype? U kunt meerdere opties selecteren.

- vermeerderingsbedrijf
- vleesvarkensbedrijf
- opfok zeugen

73. Hoeveel zeugen telt het bedrijf?

- <500
- 500 – 800
- > 800
- niet van toepassing

74. Hoeveel vleesvarkens telt het bedrijf?

- < 1000
- 1000-2500
- > 2500
- niet van toepassing

75. Betreft het een biologisch bedrijf?

- ja
- nee

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76. Hebben de varkens de mogelijkheid tot vrije uitloop op onverharde bodem?

- ja
- nee

77. Wat zijn de huidige kengetallen van de zeugen op het bedrijf? (Niet invullen indien niet van toepassing voor dit bedrijf)

| | |
|--------------------------------|------------|
| Leeftijd eerste dekking | dagen |
| Worpindeks | |
| Vervangingspercentage zeugen | % |
| Uitvalspercentage onder zeugen | % |
| Dier dag dosering | dagen/jaar |

78. Wat zijn de huidige kengetallen van de vleesvarkens op het bedrijf? (Niet invullen indien niet van toepassing voor dit bedrijf)

| | |
|-------------------|------------|
| Uitvalspercentage | % |
| Dagelijkse groei | g/dag |
| Voederconversie | |
| Dier dag dosering | dagen/jaar |

79. Wat zijn de meest voorkomende ziekteproblemen op dit bedrijf? Geef een top 3.

.....

.....

.....

Vaccinatie op controlebedrijf 2

De volgende twee vragen hoeven alleen beantwoord te worden als er zich zeugen op het bedrijf bevinden.

80. Wat is het vaccinatieschema voor vlekziekte voor zeugen op dit bedrijf? Geef hierbij s.v.p. aan met **welk vaccin** de dieren gevaccineerd worden, **welke dieren** gevaccineerd worden en op **welke leeftijd**.

.....

.....
.....

81. Op welk moment wordt de vaccinatie herhaald? (bv. in de kraamstal)

.....

Vraag 82 hoeft alleen beantwoord te worden indien er opfok op het bedrijf aanwezig is.

82. Wat is het vaccinatieschema voor vlekziekte voor de opfok op dit bedrijf? Geef hierbij s.v.p. aan met **welk vaccin** de dieren gevaccineerd worden, **welke dieren** gevaccineerd worden en op **welke leeftijd**.

.....
.....
.....

83. Worden de vaccins op dit bedrijf gekoeld en apart bewaard?

- ja
 nee
 weet ik niet

84. Worden er op dit bedrijf al aangeprikte vaccins op een later moment nog eens gebruikt?

- ja
 nee
 weet ik niet

Externe biosecurity op controlebedrijf 2

85. Is er een hygiënesluis aanwezig op het bedrijf?

- ja
 nee

86. Wat is uw indruk van de ongediertebestrijding op het bedrijf? Geef aan op een schaal van 1 tot 5, waarbij 1 = erg slecht en 5 = erg goed.

- 1 2 3 4 5

87. Zijn er ook andere dieren aanwezig op het bedrijf? Zo ja, geef aan welke dieren.

- ja,
- nee
- weet ik niet

Vraag 88 is alleen van toepassing indien het bedrijf een vleesvarkensbedrijf betreft.

88. Worden alle varkens van hetzelfde vermeerderingsbedrijf aangevoerd?

- ja
- nee, het bedrijf heeft 2 of 3 vaste leveranciers
- nee, de varkens komen van 4 of meer verschillende vermeerderingsbedrijven
- weet ik niet

Interne biosecurity op controlebedrijf 2

89. Wat is de bezettingsgraad op dit bedrijf?

- laag (<90%)
- gemiddeld (90-100%)
- hoog (>100%)
- weet ik niet

90. Zijn er op dit bedrijf veel dieren met krassen en huidbeschadigingen? Geef aan op een schaal van 1 tot 5, waarbij 1= erg veel dieren met krassen en huidbeschadigingen en 5 = erg weinig dieren met krassen en huidbeschadigingen.

- 1 2 3 4 5

91. Hoe zou u de hokbevuiling op dit bedrijf over het algemeen omschrijven? Geef aan op een schaal van 1 tot 5, waarbij 1 = erg vuil en 5 = erg schoon

- 1 2 3 4 5

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92. Ziet u wel eens schuimende putten op dit bedrijf?

- ja
- nee

93. Zijn de hokken gemaakt van goed te reinigen en te ontsmetten materiaal?

- ja
- nee
- weet ik niet

94. Welke maatregelen worden er genomen voordat er nieuwe dieren in een hok komen? Er kunnen meerdere antwoorden geselecteerd worden.

- koud water reiniging
- warm water reiniging
- reiniging met zeep
- gebruik ontsmettingsmiddelen
- geen maatregelen
- weet ik niet

Vraag 95, 96, 97 en 98 zijn alleen van toepassing wanneer er zich zeugen op het bedrijf bevinden.

95. Worden de dragende zeugen in stabiele of dynamische groepen gehuisvest?

- stabiele groepen
- dynamische groepen

96. Hoe groot zijn de groepen waarin dragende zeugen gehuisvest worden?

.....zeugen

97. Worden de dragende zeugen met voerstations gevoerd?

- ja
- nee

98. Worden de dragende zeugen op stro gehuisvest?

- ja
- nee

99. Worden biggen op dit bedrijf dubbel opgelegd?

- ja, regelmatig
- ja, af en toe
- nee
- niet van toepassing
- weet ik niet

100. Hanteert het bedrijf een all-in all-out principe bij de biggen en/of vleesvarkens? U kunt meerdere antwoorden selecteren.

- ja, bij de biggen
- ja, bij de vleesvarkens
- nee
- niet van toepassing
- weet ik niet

Voeding op controlebedrijf 2

101. Wat voor type voer wordt er op dit bedrijf gevoerd?

- droog voer
- brijvoer bestaande uit bijproducten
- brijvoer bestaande uit brok

Dit is het einde van de enquête. Heeft u vragen gemist? Dan kunt u hieronder uw suggesties noteren.

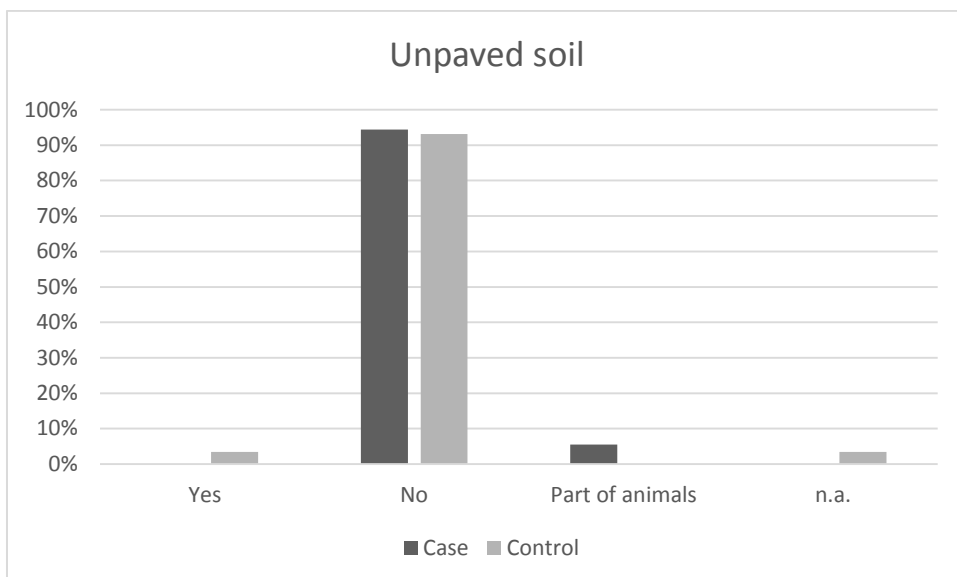
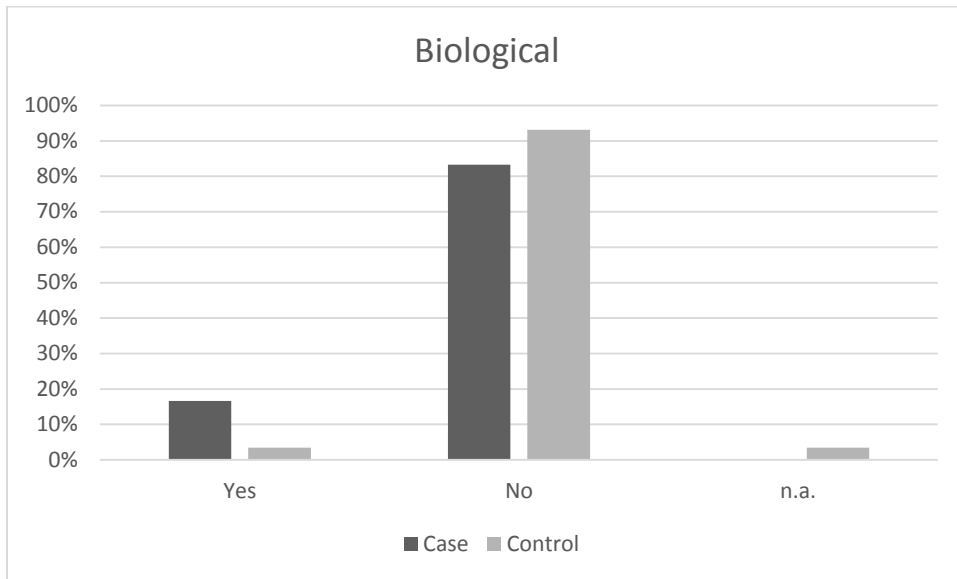
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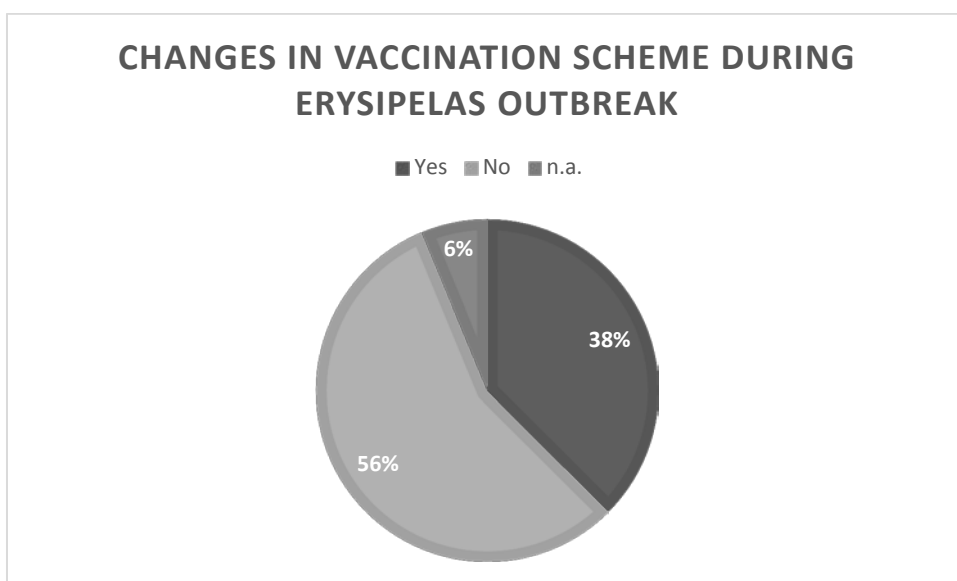
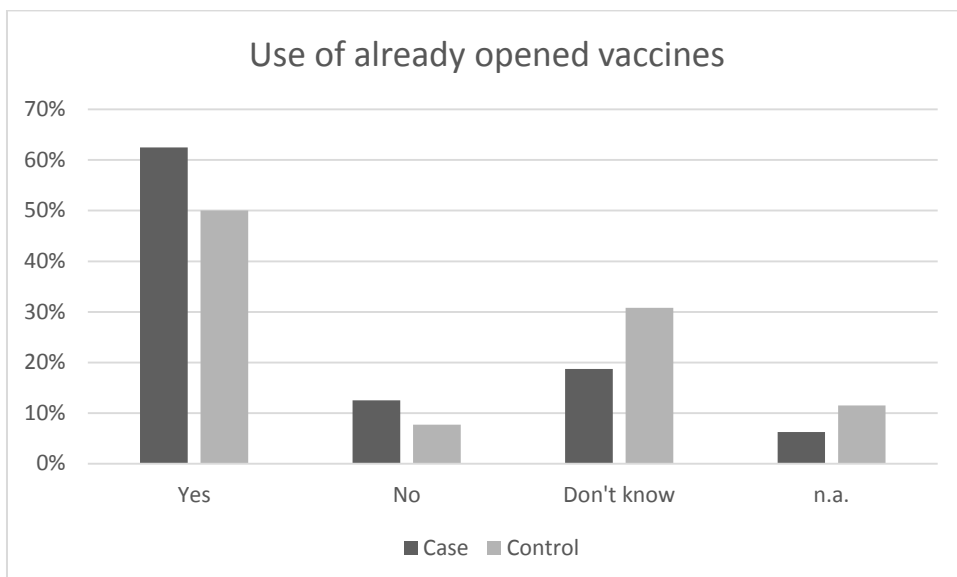
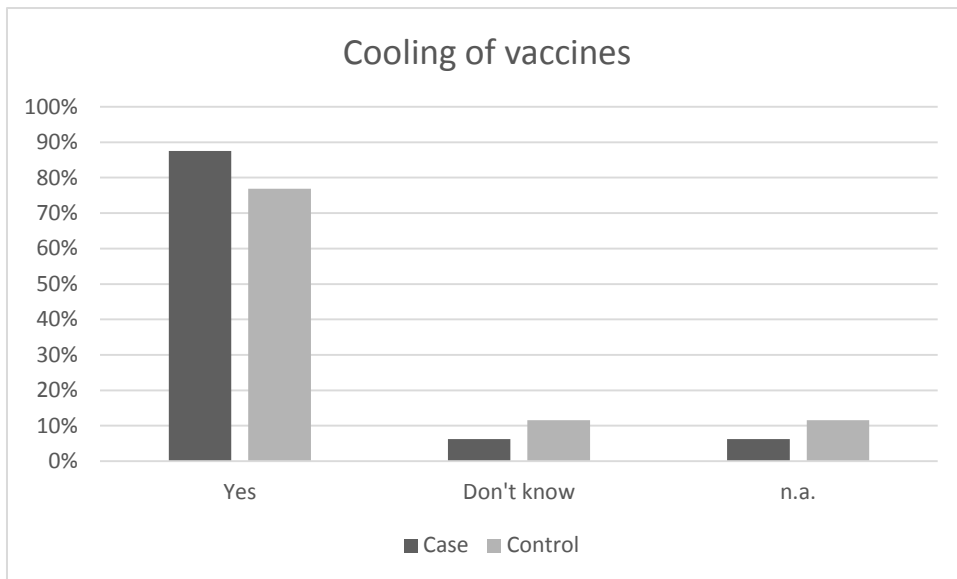
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Ik wil u hartelijk danken voor het deelnemen aan deze enquête!

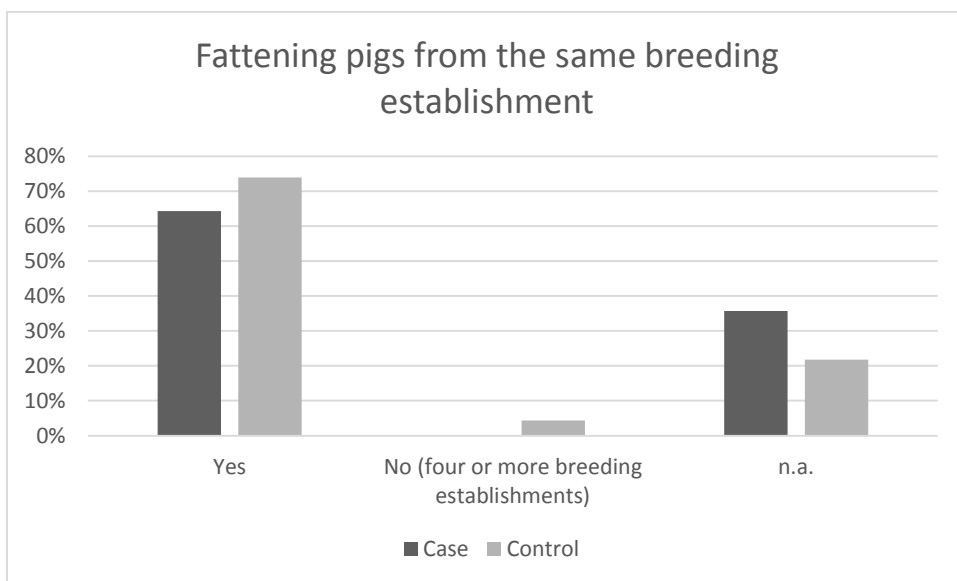
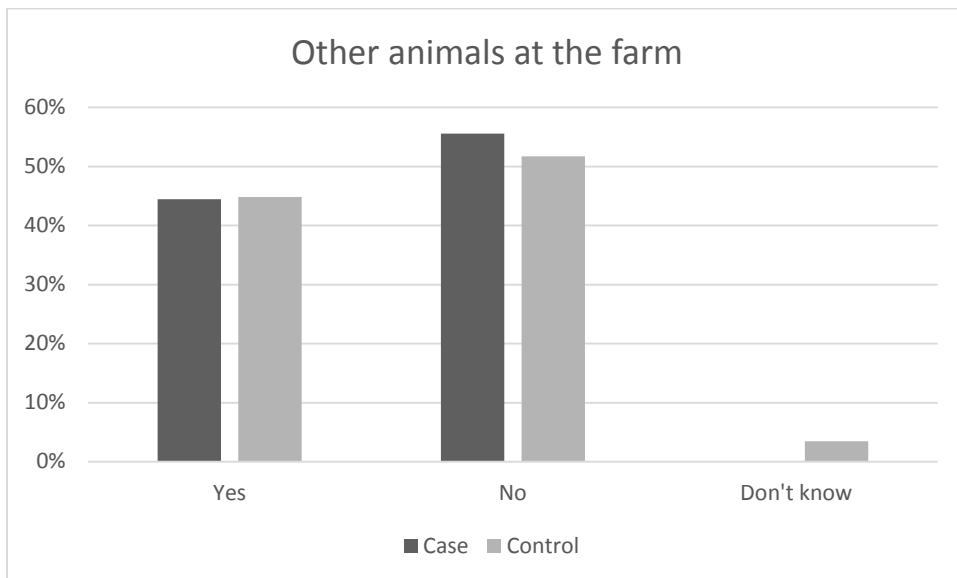
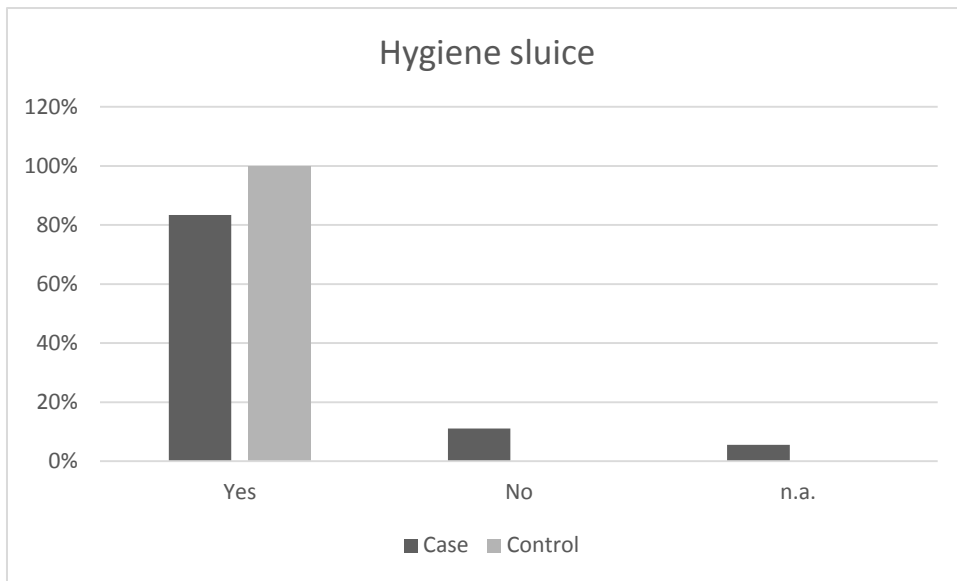
Appendix B: Graphics and tables with little differences between cases and controls



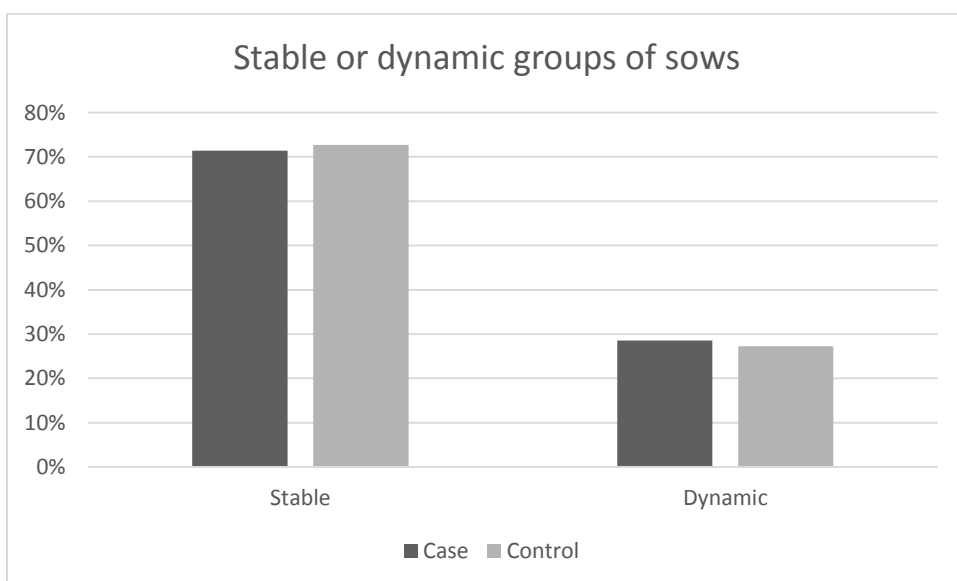
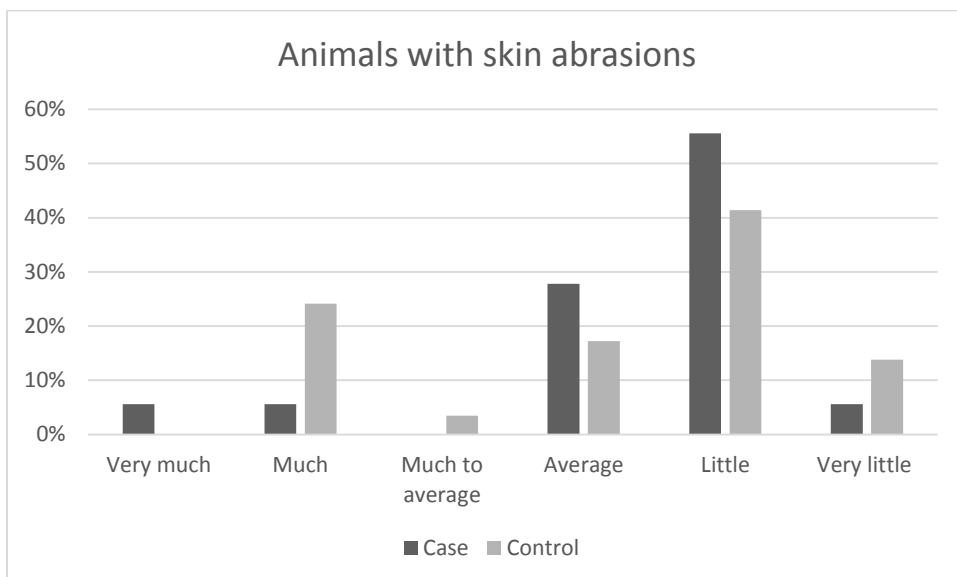
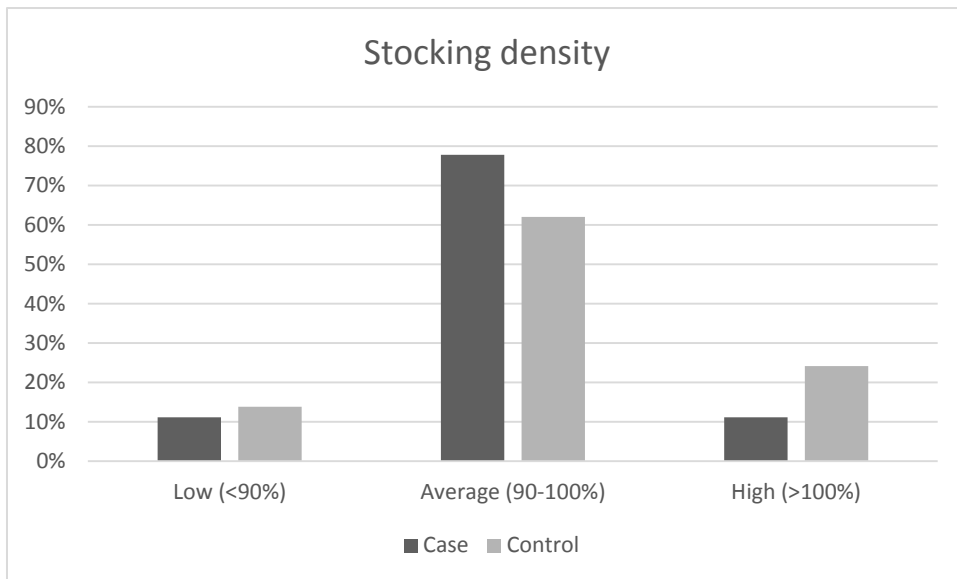
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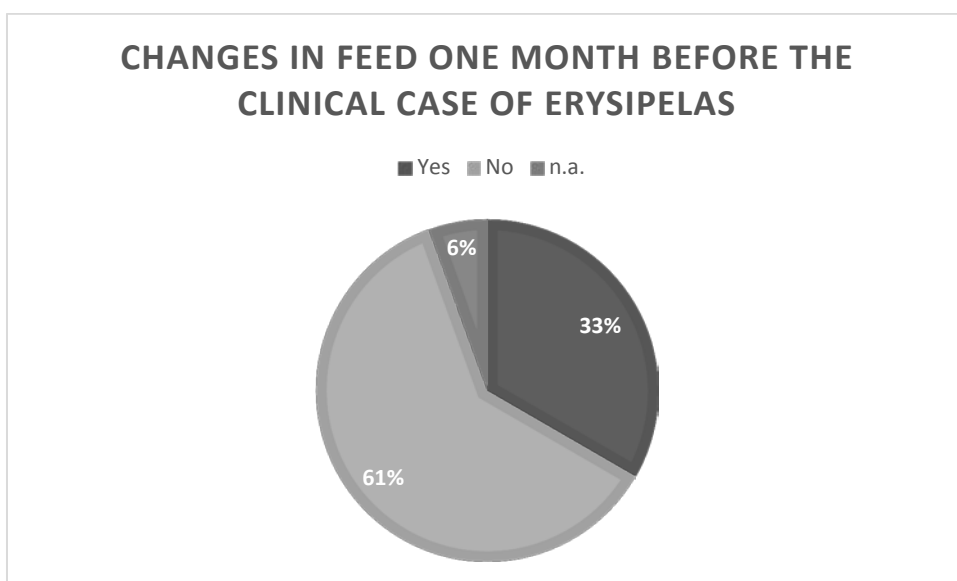
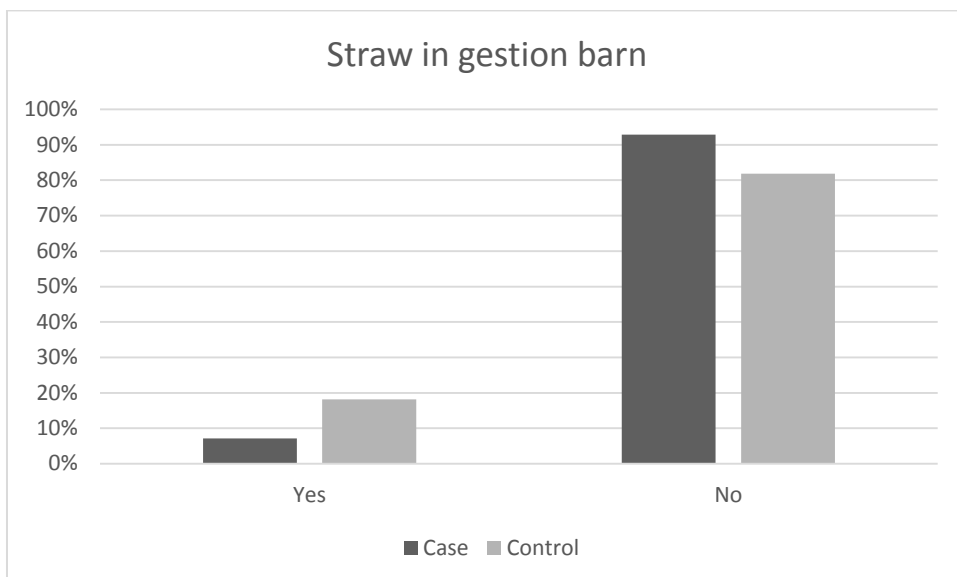
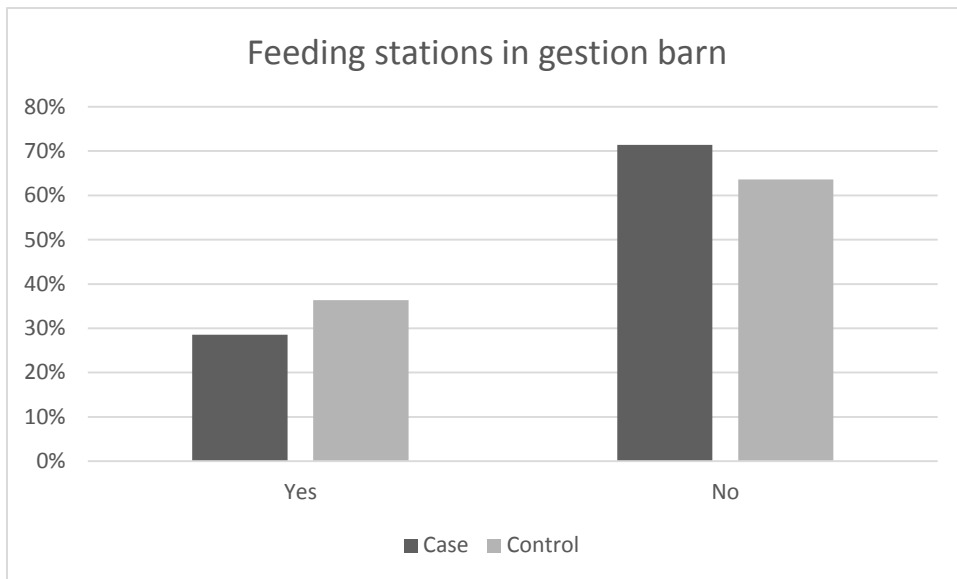
Erysipelas: Identifying risk factors for clinical cases at Dutch pig herds



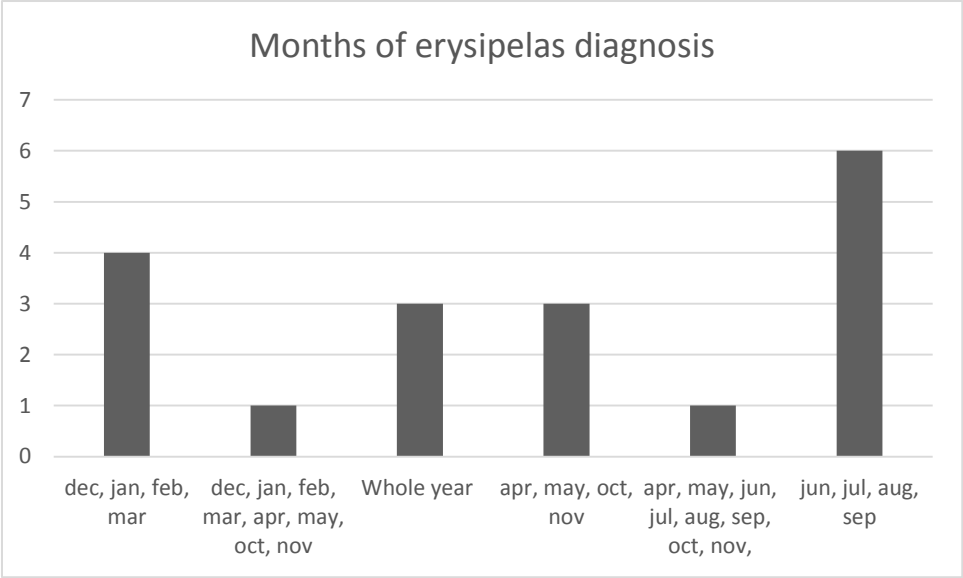
Erysipelas: Identifying risk factors for clinical cases at Dutch pig herds



Erysipelas: Identifying risk factors for clinical cases at Dutch pig herds



Erysipelas: Identifying risk factors for clinical cases at Dutch pig herds



Appendix C: Table vaccination sows, case, veterinarian ID

, , da = 3

Vaccination sows
Case 0 1
0 0 2
1 1 0

, , da = 4

Vaccination sows
Case 0 1
0 0 2
1 1 0

, , da = 7

Vaccination sows
Case 0 1
0 1 0
1 1 0

, , da = 10

Vaccination sows
Case 0 1
0 0 2
1 0 1

, , da = 11

Vaccination sows
Case 0 1
0 1 1
1 1 0

, , da = 12

Vaccination sows
Case 0 1
0 0 1
1 0 1

, , da = 14

Vaccination sows
Case 0 1
0 0 2
1 0 1

, , da = 15

Vaccination sows
Case 0 1
0 1 0
1 1 0

Erysipelas: Identifying risk factors for clinical cases at Dutch pig herds

, , da = 17

Vaccination sows
Case 0 1
0 0 1
1 0 1

, , da = 18

Vaccination sows
Case 0 1
0 0 2
1 0 1