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VEEPEILER VARKEN

# Activiteitenrapport 2022-2023





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## 1 Inleiding

Veepeiler Varken is in het leven geroepen om de varkenssector in België te ondersteunen met praktisch onderzoek en tweedelijnsadvies. Veepeiler Varken kwam tot stand op initiatief van DGZ en de faculteiten Diergeneeskunde van de Universiteit Gent en Université de Liège, en wordt financieel gesteund door het Sanitair Fonds.

Veepeiler Varken heeft twee belangrijke pijlers: tweedelijnsdiergeneeskunde en korte, praktijkgerichte onderzoeksprojecten.

### *Tweedelijnsdiergeneeskunde:*

Veepeiler Varken verleent tweedelijnsadvies op praktijkbedrijven die te kampen hebben met problemen waarvan de oorzaak na verschillende onderzoeken niet werd gevonden. De verschillende partijen (Veepeilerdierenarts, varkenshouder, bedrijfsdierenarts, voederadviseur, adviseur van de fokbedrijven, ...) zitten samen rond de tafel om het probleem multidisciplinair en met meer diepgang te benaderen en zo tot een oplossing te komen. In samenspraak met de bedrijfsdierenarts kunnen er aanvullende onderzoeken worden uitgevoerd (bv. laboratoriumonderzoeken van biologische monsters, drinkwater en voeder, autopsies, slachthuisonderzoek, enz.). Van elk bedrijfsbezoek wordt een verslag opgesteld, met adviezen en een plan van aanpak. De veehouder, bedrijfsdierenarts en de eventuele andere betrokken personen ontvangen een kopie van het verslag. Het bedrijf wordt meerdere keren bezocht om de problematiek verder op te volgen en de genomen maatregelen te bespreken en evalueren.

### *Korte, praktijkgerichte onderzoeksprojecten:*

Naast het leveren van tweedelijnsdiergeneeskunde richt Veepeiler Varken zich op het uitvoeren van korte, praktijkgerichte onderzoeksprojecten omtrent een specifieke problematiek binnen de varkensgezondheidszorg.



## 2 Afgelopen projecten

### 2.1 Rol van PPV bij Vlaamse biggen en vleesvarkens op PCV2 geassocieerde symptomen

#### 2.1.1 Situering

Praktijkdierenartsen melden aan veepeiler dat het hen opvalt dat wanneer ze PCR PCV2 laten uitvoeren de virale ladingen die in het bloed worden teruggevonden vaker hoger zijn dan in het verleden. Uit onderzoeksgegevens blijkt een co-infectie met porcien parvovirussen (PPV) aan de basis te kunnen liggen van deze vaststelling (3,4,5,6,7). Als we kijken naar de resultaten van de analyses (PCV2 PCR) van 2019 tot en met 2021 die werden uitgevoerd bij DGZ dan zien we inderdaad in stijging in de virale lading in de bloedmonsters.

#### 2.1.2 Proefopzet

Het doel van het project is nagaan of er een verband bestaat tussen de virale ladingen van PCV2 in het bloed (PCR) en het voorkomen van bepaalde types PPV (Pathosense). Hiervoor zullen 25 poolmonsters met verschillende lading PCV2 (PCR) die onderzocht worden bij DGZ geselecteerd worden en onderzocht worden via Pathosense op aanwezigheid van PPV.

#### 2.1.3 Resultaten

De resultaten toonden een stijging aan van de totale hoeveelheid parvovirus wanneer de PCV2 PCR load stijgt. De stijging is waarneembaar voor de verschillende types parvovirus met uitzondering van parvovirus type 4 die geen enkele keer gedetecteerd werd bij een hoge PCV2 PCT load op PCR. Meer details zijn terug te vinden in abstract in bijlage 1.

### 2.2 Optimalisatie van de reiniging en ontsmetting van de varkensstal

#### 2.2.1 Situering

Reinigen en ontsmetten is een belangrijk onderdeel van een goede bioveiligheid op varkensbedrijven en een van de belangrijkste maatregelen om de spreiding van infecties tegen te gaan binnen een bedrijf. Oppervlakken en materialen die bevuild zijn met mest kunnen een infectie op het bedrijf in stand houden door de dieren die hiermee in contact komen telkens opnieuw te besmetten (De Wulf en Van Immerseel, 2018). Voor heel wat pathogenen (Brachyspira hyodysenteriae, Erysipelothrix rhusiopathiae, E. Coli, Lawsonia intracellularis, Pasteurella multocida, PCV2, PEDV, PRRSV, Salmonella, Streptococcus suis, Influenza virus, ...) is namelijk aangetoond dat ze indirect kunnen worden overgedragen door onder ander mest (De Wulf en Van Immerseel, 2018). Een goede reiniging zorgt enerzijds voor verwijdering van dit organisch



materiaal dat dienst doet als voedingsbodem voor micro-organismen. Er werd ook aangetoond dat de reiniging zorgt voor een duidelijke daling van het kiemgetal dat vervolgens nog verder daalt na desinfectie (Luyckx et al., 2015a).

In 2017 werd reeds een Veepeilerproject uitgevoerd rond reiniging en ontsmetting van de varkensstallen. Het effect van reiniging en ontsmetting kan worden nagegaan aan de hand van afdrukplaatjes of hygiënogrammen. Deze plaatjes geven een beeld van de bacteriële verontreiniging. Van de 111 bedrijven die deelnamen voerde de helft een tweede controle uit na reiniging en ontsmetting, weliswaar nadat advies gegeven werd rond het reinigings- en ontsmettingsprotocol. Die bedrijven realiseerden een verbetering van hun hygiënogramscore.

Naar aanleiding van de uitbraak van Afrikaanse Varkenspest in België is beslist dat elke bedrijfsdierenarts verplicht is om jaarlijks een risico-enquête in te vullen op de varkensbedrijven. Dit is van kracht sedert vorig jaar. De risico-enquête gaat onder andere in op het reinigings- en ontsmettingsprotocol. Uit de eerste bevindingen (n=3700) blijkt dat de meeste bedrijven (87%) bezitten over een reinigings- en ontsmettingsprotocol dat wordt nageleefd. Helaas wordt het proces slechts in 8% gecontroleerd.

### 2.2.2 Proefopzet

Het doel van het project is het reinigings- en ontsmettingsprotocol op de Vlaamse varkensbedrijven in beeld te brengen en te vergelijken met de resultaten van het project uit 2017. Hiervoor konden bedrijven afdrukplaatjes laten nemen in een gereinigde en ontsmette afdelingen en een wateranalyse laten uitvoeren van het reinigingswater. Indien het bedrijf op basis van de resultaten aanpassingen doorvoert in zijn protocol kon het een hercontrole laten uitvoeren.

### 2.2.3 Resultaten

De biggenafdeling scoort gemiddelde beter na reinigen en ontsmetten in vergelijking met kraamstalafdelingen. In beide afdelingen scoren hoger gelegen plaatsen zoals plafond, muur op ooghoogte en ventilatie en moeilijk te reinigen plaatsen zoals materiaal en voederbak slechter dan lager gelegen plaatsen zoals vloer en wandtussenschot. Bedrijven die hun protocol aanpasten slaagden erin een betere score van de afdrukplaatjes te bekomen. De belangrijkste maatregelen zijn het gebruik van inwekmiddel of ontsmetting en voor bedrijven die deze producten al gebruikten, het correct gebruik ervan. Dit houdt in een voldoende hoeveelheid, concentratie en inwerkingstijd en dit volgens de voorschriften van de fabrikant. De resultaten van dit project zijn volledig vergelijkbaar met de resultaten van het project in 2017. Meer details zijn terug te vinden in bijlage 2.



## 2.3 Het belang van oorbijten bij het ontstaan van porcine ear necrosis (PEN).

### 2.3.1 Situering

Porcine oornecrose (PEN), ook wel oortopnecrose genoemd, komt veel voor bij varkens. Eerdere studies toonden aan dat de prevalentie van PEN soms meer dan 80% kan bedragen, en dat deze sterk kan variëren tussen verschillende bedrijven en studies. Tot dusver is de etiologie noch de pathogenese bekend. De mogelijke risicofactoren kunnen zeer divers zijn zoals infecties, slechte klimatologische en huisvestingsomstandigheden en mycotoxinen. De precieze rol van deze factoren noch hun belang zijn echter opgehelderd. Daarnaast zijn er binnen dezelfde stal biggen met en zonder PEN, wat suggereert dat ook factoren die niet op bedrijfs- of stalniveau aanwezig zijn (bijvoorbeeld voersamenstelling of stalklimaat), een rol kunnen spelen. Malik et al. (2022 – in voorbereiding) toonden onlangs aan dat *Mycoplasma hyopharyngis* vaak voorkomt in de PEN-lesies van aangetaste varkens, terwijl deze bacterie niet werd gevonden bij niet-aangetaste dieren uit hetzelfde hok (Veepeiler-project PVP-20-04). Ook bleek uit observaties tijdens het onderzoek dat bijten/zuigen aan oren, evenals bijten in staarten of flanken plaatsvond. De mate van oorzuigen of -bijten is in deze onderzoeken echter niet gekwantificeerd en er werd ook niet onderzocht in welke mate biggen met PEN in een andere mate gebeten werden dan niet-aangetaste varkens.

### 2.3.2 Proefopzet

Het belangrijkste doel van het onderzoek is om de rol van oorbijten/-zuigen bij het optreden van PEN bij varkens te onderzoeken. Hiertoe werd het oorbijten (aantal keer bijten, duur van bijten) nauwgezet opgevolgd en gekwantificeerd. Er werd een vergelijking gemaakt tussen aangetaste en niet-aangetaste varkens, en tussen hokken met hoge en lage prevalentie van PEN. Hiervoor werden 3 bedrijven worden geselecteerd met een hoge prevalentie van PEN (>30%) bij de gespeende biggen.

### 2.3.3 Resultaten

Biggen in hokken met een hoge prevalentie van oortopnecrose vertoonden dubbel zoveel manipulaties aan de oren in vergelijking met biggen in hokken met een lage prevalentie van oortopnecrose. De manipulatie (sabbelen) van de oren begint vooraleer de eerste oorletsels zichtbaar worden en er is steeds een tijdspanne van ongeveer 2 tot 3 weken tussen de piek in manipulaties en de piek in letsels. Het aantal manipulaties heeft een sterker effect op het voorkomen van letsels dan de duur van de manipulaties. Er werd geen correlatie gevonden tussen stalklimaat en letsels of tussen de aanwezigheid van verschillende bacteriën/virussen en het



voorkomen van de letsels. De resultaten tonen een significant effect aan van orale manipulatie op het voorkomen van oortopnecrose. Meer details over het project kom je te weten in de podcast die je terugvindt via deze [link](#) en in het wetenschappelijke artikel in bijlage 3.

## 3 Lopende projecten

### 3.1 Colostrumopname bij biggen

#### 3.1.1 Situering

Colostrumopname is essentieel voor de prestaties en de gezondheid van de biggen krijgt en Veepeiler Varken geregeld vragen van bedrijfsdierenartsen hierover. Er zijn verschillende testmethodes die een indicatie kunnen geven over de colostrumopname bij biggen. Toch worden in de praktijk maar zelden analyses uitgevoerd om meer informatie te bekomen over de colostrumopname. Bovendien zijn er voor zover wij weten geen gegevens beschikbaar over biestmanagement en colostrumopname bij biggen in Vlaanderen.

#### 3.1.2 Proefopzet

Het doel van het project was inzicht krijgen in het biestmanagement en de colostrumopname bij biggen op Vlaamse varkensbedrijven. Hiervoor kregen 75 varkensbedrijven de kans om, na het invullen van een enquête, een monsternamen te laten uitvoeren. Deze bestaat uit bloedmonsters van 5 zeugen en per zeug 6 biggen van minder dan 7 dagen leeftijd. Door de antistoftiters voor PCV2 te bepalen en deze gehalten van de biggen te vergelijken met deze van de moederzeug kan nagegaan worden of de biggen al dan niet voldoende colostrum hebben opgenomen.

#### 3.1.3 Stand van zaken

Tachtig bedrijven vulden de enquête in en kregen een staalnamepakket opgestuurd, 10 voerden nadien ook een tweede staalname uit. De resultaten van de biestcontrole en de enquête worden momenteel verwerkt.

### 3.2 PRRSV viremisch geboren biggen: voorkomen, aanpak en opvolging

#### 3.2.1 Situering

Porcien Reproductief en Respiratoir Syndroom virus (PRRSV) is endemisch aanwezig op de Vlaamse bedrijven. Het veroorzaakt respiratoire problemen bij biggen en vleesvarkens, en staat ook bekend als een immuniteitsonderdrukkend virus. Als de jongste gespeende biggen geïnfecteerd blijken te zijn met PRRSV, veronderstellen we dat de PRRSV-status bij de zeugen





niet stabiel is en dat de biggen reeds viremisch geboren worden. In dit project willen we nagaan of dit wel degelijk zo is.

### 3.2.2 Proefopzet

Het doel van het project is om na te gaan of biggen PRRSV viremisch geboren worden op bedrijven waar de biggen PRRSV positief testen bij spenen. Hiervoor kregen bedrijven waarbij de biggen na spenen PRRSV positief zijn (PCR) de kans om 2 pools processing fluids (PF) te laten onderzoeken op de aanwezigheid van PRRSV door middel van PCR. De bedrijven die hiervoor interesse hebben kunnen nadien verder opgevolgd/begeleid worden om eventuele oorzaken en aanpak na te gaan.

### 3.2.3 Stand van zaken

Veertien bedrijven namen deel en lieten eenmalig PF testen. Acht van de bedrijven worden verder opgevolgd. Het project wordt afgesloten en de resultaten worden verwerkt.

## 3.3 Oorzaken van zeugensterfte

### 3.3.1 Situering

In de praktijk wordt gemiddeld ongeveer 45 % van de zeugen jaarlijks vervangen. Dit houdt in dat een zeug in haar leven gemiddeld ongeveer 4 worpen voortbrengt. De gebruiksduur van een zeug is dus ongeveer 2 jaar. Vanuit economisch standpunt is de grondregel dat niet te snel tot vervanging moet worden overgegaan zodat de opfokkosten over een langere periode kunnen gespreid worden. De beste productieresultaten worden immers bekomen van pariteit 3 tot 6. Te vroeg opruimen is vanuit foktechnisch standpunt eventueel nog te verantwoorden, maar niet vanuit financieel standpunt. De aankoop van een gilt is immers een grote productiekost. Anderzijds mag er ook niet te laat opgeruimd worden. Teveel oudere zeugen (pariteit  $\geq 7$ ) op een bedrijf, kan immers leiden tot een toename van de embryonale sterfte, het aantal doodgeboren biggen, de variatie in geboortegewichten en toomgrootte, het aantal doodgelegene biggen, het aantal slechte uierpakketten en klauw- en beengebreeken.

De meeste zeugen worden gepland afgevoerd. Dit gebeurt in veruit de meeste gevallen na het spenen van de biggen. De voornaamste redenen zijn vruchtbaarheidsproblemen, ouderdom en locomotieproblemen. Echter, soms vallen zeugen ook ongepland uit. Dit komt meestal door sterfte. Het verlies van zeugen door sterfte was traditioneel de 4de belangrijkste oorzaak van afvoer van zeugen. In een onderzoek van De Letter (2002) was de zeugensterfte op 14 Vlaamse zeugenbedrijven tijdens de periode 1995-2001 ongeveer 3,6%, en steeg deze van 3,1% in 1995 naar 4,8% in 2001. Op jaarbasis mag de zeugensterfte maximaal 5% bedragen. De laatste jaren is er echter een zeer forse toename in zeugensterfte waar te nemen. In meer en meer bedrijven



bedraagt de zeugensterfte meer dan 10%. De jaarlijkse zeugensterfte in een Deense studie op 10 varkensbedrijven bedroeg 14% (min. 6,4%-max. 18,5%) (Kongsted et al., 2021). Dit betekent niet alleen een zeer grote economische kost voor de varkenshouder, maar het is ook een probleem in het kader van dierenwelzijn. Verder is het ook nadelig voor de motivatie en het welbevinden van de varkenshouder.

Traditioneel was het risico voor zeugensterfte hoger op grote bedrijven, tijdens de zomer, in de peripartumperiode en bij niet-optimaal gevoederde zeugen. De voornaamste oorzaken vermeld in de literatuur zijn maagdarmsstoornissen (bv. torsies), hartfalen, urinaire infecties, maligne hyperthermie, problemen rond de partus (bv. uterusprolaps) en infecties (vlekziekte, Clostridium) (Autopsieverlag DGZ, 2020). Echter, het is echter niet gekend welke de oorzaken zijn van zeugensterfte in de huidige Belgische bedrijven. Gezien de situatie drastisch gewijzigd is in vergelijking met bv. 20 jaar geleden (sterke stijging productiegetal, minstens een verdubbeling van de zeugensterfte, groter vervangingspercentage, grotere voederopname), was dringend onderzoek nodig naar de oorzaken van zeugensterfte.

### 3.3.2 Proefopzet

Er zullen 15 bedrijven verspreid over België worden opgenomen in het onderzoek. De criteria om bedrijven te selecteren zijn bereidheid tot deelname aan het onderzoek en minstens 5% zeugensterfte in het afgelopen jaar. Elk bedrijf wordt bij de start van het onderzoek bezocht en verschillende bedrijfsgegevens worden opgevraagd. De specifieke doelstelling van dit onderzoek is om na te gaan wat de oorzaken zijn van zeugensterfte in Belgische varkensbedrijven. Dit kan leiden tot het implementeren van meer gerichte controlemaatregelen, een vermindering van de zeugensterfte en een betere rendabiliteit van de bedrijven. De bedrijven worden gedurende een jaar opgevolgd. Op elk van de bedrijven zal van maximaal 8 gestorven zeugen autopsie worden uitgevoerd. Indien nodig (bv. geen duidelijke autopsiebevinding die de sterfte kan verklaren), zal aanvullend laboratoriumonderzoek plaatsvinden om tot een diagnose te komen.

### 3.3.3 Stand van zaken

Vijftien bedrijven met meer dan 5% zeugensterfte namen deel aan het project. De laatste bedrijfsbezoeken worden uitgevoerd en de resultaten zullen worden verwerkt.



## 3.4 Uitbreiding van de PRRSV stam surveillance met behulp van whole genome sequencing

### 3.4.1 Situering

Porcien Reproductief en Respiratoir Syndroom virus (PRRSV) is endemisch aanwezig op de Belgische bedrijven. Het veroorzaakt respiratoire problemen bij biggen en vleesvarkens, vruchtbaarheidsstoornissen bij zeugen en staat ook bekend als een immuniteitsonderdrukkend virus. PRRSV komt wereldwijd voor en kan onderverdeeld worden in twee genotypes: het Noord-Amerikaans (NA) type en het Europees (EU) type. Beide types circuleren in België. Binnen elk type worden verschillende stammen gedetecteerd. Deze stammen kunnen verschillen in virulentie. De introductie van een nieuwe stam op een bedrijf of in een bepaalde regio kan het endemisch evenwicht verstoren en zo aanleiding geven tot ernstige ziekte-uitbraken. Spanje wordt sinds 2020 geconfronteerd met meerdere zware PRRSV-uitbraken veroorzaakt door een dergelijke nieuwe stam. Deze uitbraken gaan gepaard met hoge abortusaantallen en uitgesproken sterfte bij de gespeende biggen. Deze nieuwe stam, van het Europese type en Rosalia genaamd, is ontstaan na meerdere recombinaties met andere Europese stammen waaronder de virulente PR40-stam uit Italië. De introductie van Rosalia of andere virulente stammen in België zou grote gevolgen kunnen hebben voor onze varkenshouderij. De vinger aan de pols houden met een snelle detectie en identificatie van deze nieuwe stam is dan ook cruciaal. Het doel is om varkensbedrijven te monitoren die te kampen hebben met een ernstige PRRSV-uitbraak om de eventuele aanwezigheid van de Rosalia-stam, of andere 'nieuwe' stam(men), in België snel op te pikken. En daarnaast ook het monitoren van de eventuele invoer van levende (fok)varkens uit Spanje. Dit met behulp van whole genome sequencing via snelle nanopore sequencing (PathoSense BV).

### 3.4.2 Proefopzet

Om de PRRS stammen te kunnen identificeren zal gebruik gemaakt worden van kost-efficiënte whole genome sequencing met behulp van nanopore sequencing bij PathoSense (Certified Service Provider van Oxford Nanopore Technologies). Enkel monsters waarvan de CT-waarde van de real-time PCR-test voor PRRSV minder dan 27 bedraagt, zullen onderzocht worden. Er zal gefocust worden op het analyseren van monsters van foeti en sera van zieke dieren (respiratoire problematiek) en dieren ingevoerd vanuit Spanje.

### 3.4.3 Stand van zaken

Tweeëntwintig monster werden reeds geselecteerd, deze zullen onderzocht worden en de selectie van de monsters loopt verder.

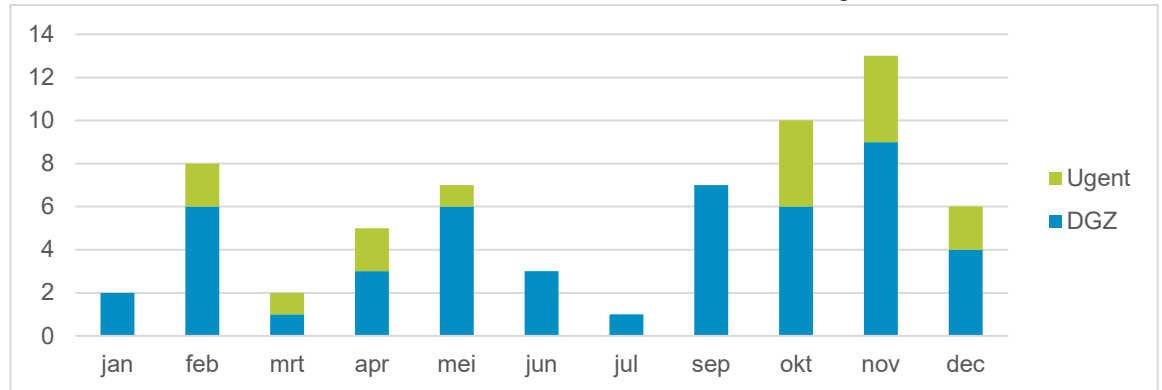


## 4 Bedrijfsbezoeken tweedelijnsdiergeneeskunde

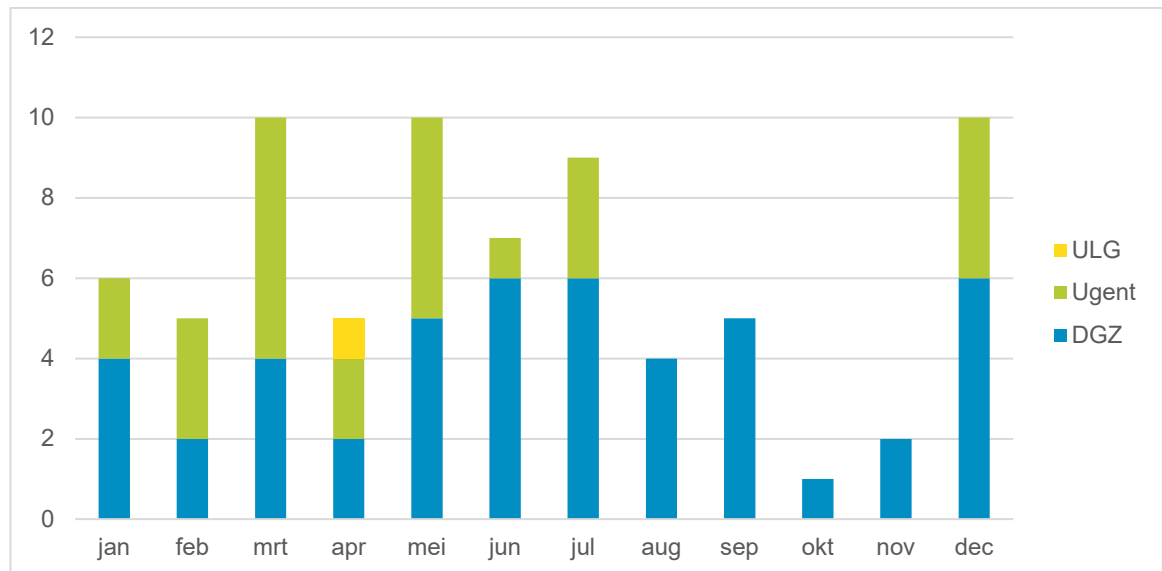
### 4.1 Aantal bezoeken

In 2022 werden 64 bedrijfsbezoeken uitgevoerd op 43 unieke beslagen, 48 bezoeken werden uitgevoerd door DGZ en 16 bezoeken door Ugent. Het aantal individuele bedrijven begeleid door DGZ was 31 en 12 door Ugent.

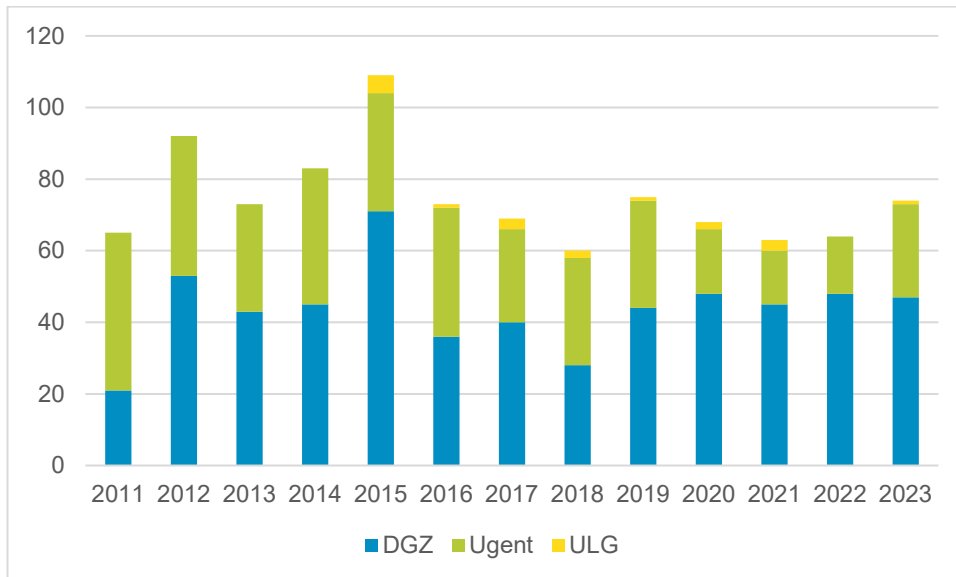
In 2023 werden 74 bezoeken uitgevoerd op 44 unieke beslagen, 47 bezoeken werden uitgevoerd door DGZ, 26 door Ugent en 1 door ULG. Het aantal individuele bedrijven begeleid door DGZ was 27 en door Ugent 16.



**Figuur 1:** Maandelijks aantal bedrijfsbezoeken uitgevoerd in 2022 in het kader van tweedelijnsdiergeneeskunde van Veepeiler Varken.

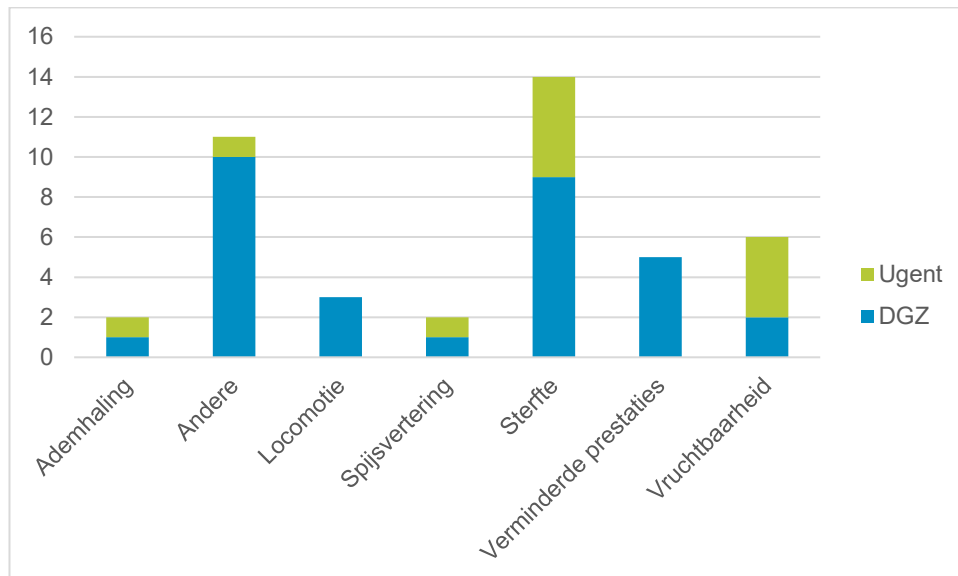


**Figuur 2:** Maandelijks aantal bedrijfsbezoeken uitgevoerd in 2023 in het kader van tweedelijnsdiergeneeskunde van Veepeiler Varken.

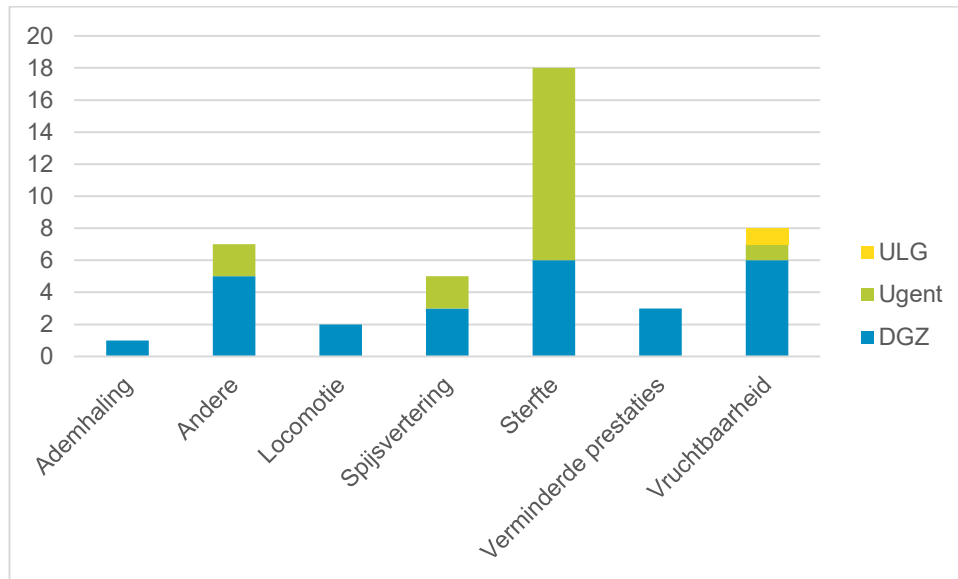


**Figuur 3:** Evolutie aantal bedrijfsbezoeken in het kader van tweedelijnsdiergeneeskunde van Veepeiler Varken over de jaren heen.

## 4.2 Problematiek voor de aanvraag van de bedrijfsbezoeken



**Figuur 4:** Problematieken waarvoor Veepeiler Varken bedrijfsbezoeken uitvoerde in 2022.



**Figuur 5:** Problematieken waarvoor Veepeiler Varken bedrijfsbezoeken uitvoerde in 2023.

Voornaamste reden om tweedelijnsbegeleiding aan te vragen in 2022 en 2023 is sterfte, de meeste van de bedrijven met sterfteproblematiek waren dan ook deelnemers aan het project zeugensterfte. Ademhaling hield problemen bij vleesvarkens en gelten in met onder ander Atofische Rinitis en Mycoplasma hyopneumoniae. Onder 'andere' komen problemen met PRRS, trilbiggen, uier necrose, agressiviteit, biestopname en reinigen en ontsmetten voor. Locomotie gaat over bedrijven met manken en stijve biggen en slecht beenwerk. Spijsverteringsproblemen waren diarree bij gespeende en kraamstal biggen. Vruchtbaarheid tot slot omvatte peripartale problemen, teveel terugkomers, niet bronstig of niet drachtig worden en te veel doodgeboren biggen.

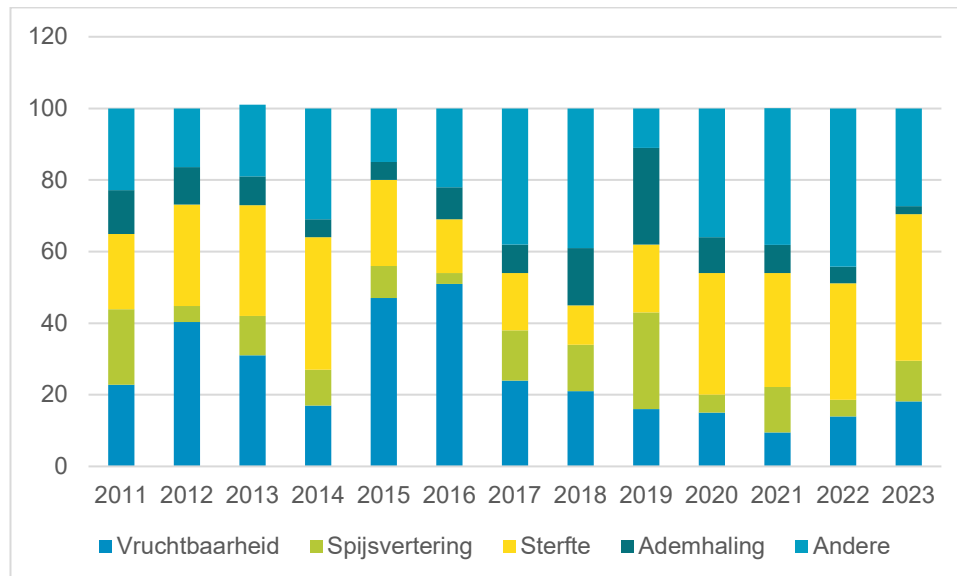
### 4.3 Vermoedelijke oorzaken van de problematiek op bedrijven

Bij veel bedrijfsproblemen is de oorzaak multifactorieel. Veepeiler Varken zet aan tot verder onderzoek en treedt op als onafhankelijke partij tussen de verschillende partners (laboratoria, voederspecialisten, ...). Zo kan tot een etiologische diagnose gekomen worden gericht op het oplossen of verbeteren van de problematiek.

Het is echter niet steeds mogelijk om met zekerheid een etiologische diagnose te stellen en vaak zijn de problemen het gevolg van een combinatie van tekortkomingen in het management met daarbovenop een infectieuze oorzaak.



#### 4.4 Trendobservatie: vergelijking van redenen tot aanvraag



**Figuur 6:** Percentage redenen tot aanvraag van een bedrijfsbezoek in het kader van tweedelijnsdiergeneeskunde van Veepeiler Varken in de voorbije jaren.

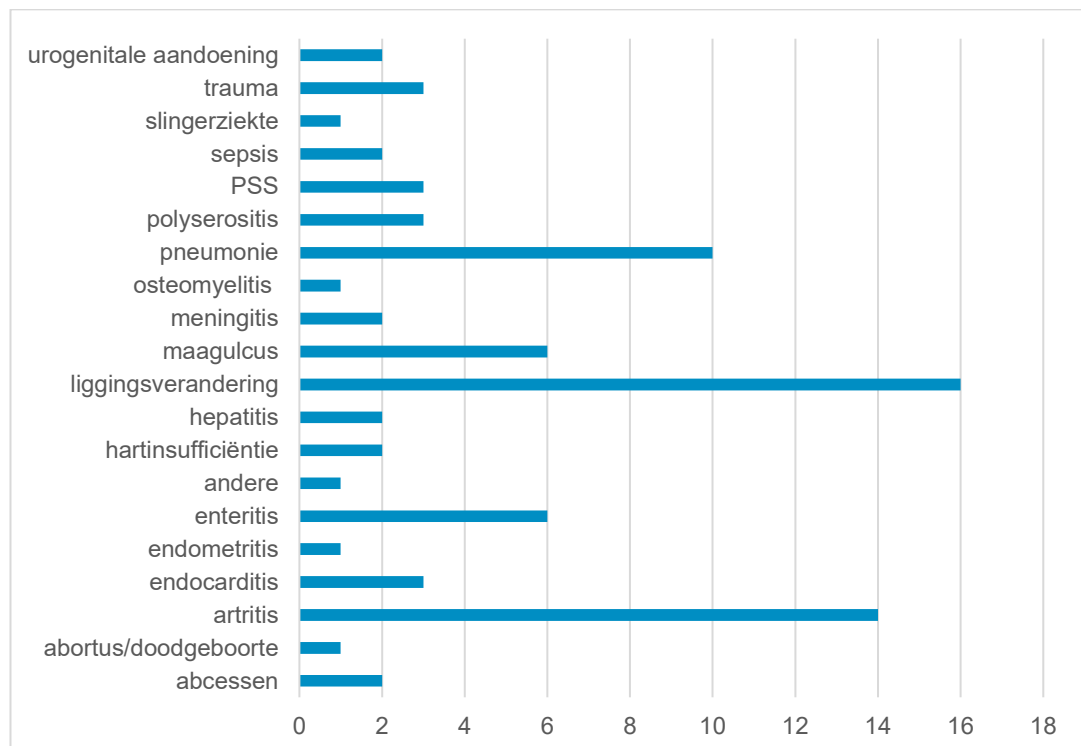
Bij de interpretatie van de cijfers in bovenstaande grafiek moet rekening worden gehouden met het feit dat de aantallen vrij klein zijn en dat enkele bezoeken meer of minder procentueel al een groot verschil kunnen teweegbrengen.



## 5 Autopsies uitgevoerd voor Veepeiler Varken

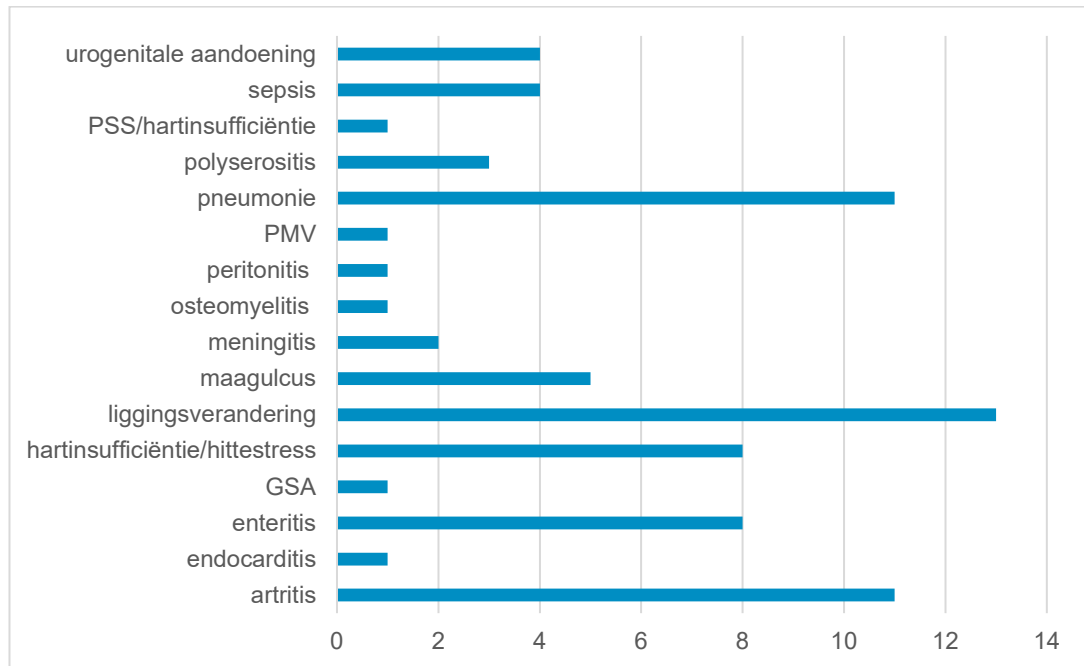
De kadavers aangeboden bij DGZ voor autopsie in het kader van tweedelijnsdiergeneeskunde staan steeds in verband met een bedrijfsbezoek dat op het betrokken bedrijf werd uitgevoerd. In 2021 waren er voor Veepeiler 52 autopsiedossiers bij DGZ.

### 5.1 Vastgestelde doodsoorzaken bij autopsie



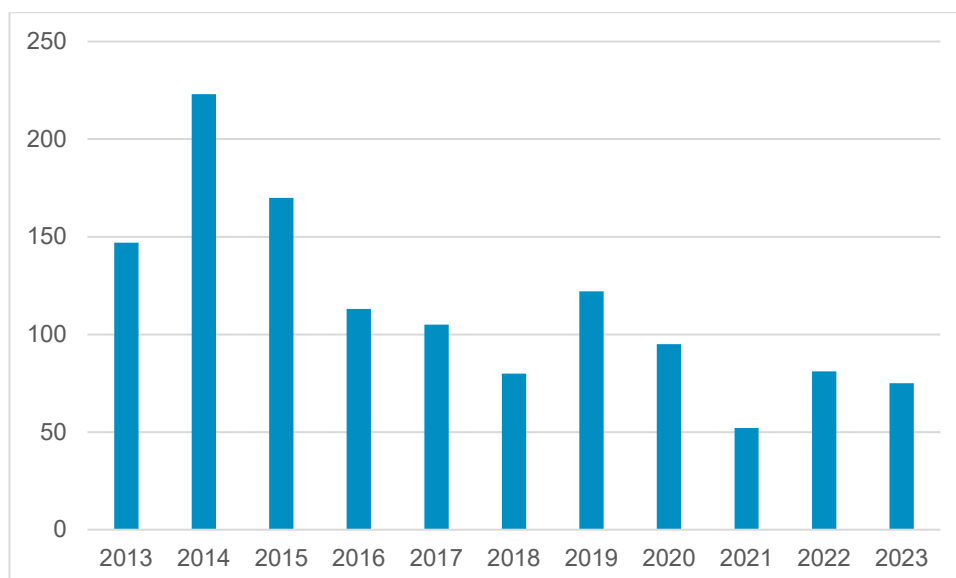
**Figuur 7:** Vastgestelde afwijkingen van kadavers aangeboden voor autopsie in het kader van tweedelijnsdiergeneeskunde van Veepeiler Varken in 2022.





**Figuur 8:** Vastgestelde afwijkingen van kadavers aangeboden voor autopsie in het kader van tweedelijnsdiergezondheidszorg van Veepeiler Varken in 2023.

## 5.2 Trendobservatie – vergelijking met voorgaande jaren



**Figuur 9:** Evolutie van het aantal autopsies uitgevoerd in het kader van Veepeiler Varken per jaar.



## 6 Publicaties Veepeiler Varken 2022-2023

Datum	Type	Magazine/gelegenheid	Titel
1/01/2022	Persbericht	Varkensbedrijf	Minder zeugensterfte dankzij betere drinkwateropname
21/01/2022	Presentatie	IPVS refresher course	Infection levels and immunity for <i>M. hyopneumoniae</i> in breeding animals
1/04/2022	Presentatie	Large animals residents symposium	Porcine ear necrosis: infectious or behavioral problem?
29/04/2022	Persbericht	Drietand	Veepeilerprojecten leveren inzichten op voor de varkenssector
12/05/2022	Poster	ESPHM 2022	Porcine ear necrosis in piglets: development of lesions and germs.
12/05/2022	Presentatie	ESPHM 2022	A cross-sectional study on the prevalence of <i>Mycoplasma hyopneumoniae</i> in breeding animals
19/05/2022	Persbericht	Boer en Tuinder	Kreupelheid vermijden
9/06/2022	Wet. Pub	Porcine Health Management <a href="https://doi.org/10.1186/s40813-022-00267-w">https://doi.org/10.1186/s40813-022-00267-w</a>	Influence of parity and reproductive stage on the prevalence of <i>Mycoplasma hyopneumoniae</i> in breeding animals in Belgian farrow-to-finish herds
16/06/2022	Nieuwsbrief	NB DGZ	Schrijf je in voor een nieuw Veepeilerproject en pak PRRSV bij je biggen aan
23/06/2022	Poster	IPVS 2022	Pathogenesis of porcine ear necrosis in nursery piglets.
23/06/2022	Presentatie	IPVS 2022	Influence of parity and reproductive stage on the prevalence of <i>Mycoplasma hyopneumoniae</i> in breeding animals
17/10/2022	Nieuwsbrief	NB DGZ	Krijg inzichten in je biestmanagement met nieuwste Veepeilerproject
3/11/2022	Persbericht	Landbouwleven	Reinigingsprotocol verbeteren, hoe proper is jouw stal
7/12/2022	Nieuwsbrief	NB DGZ	Hoe proper is jouw stal echt?
14/02/2023	Wet. Pub	Microbiol Spectrum 11(1) <a href="https://doi.org/10.1128/spectrum.04123-22">https://doi.org/10.1128/spectrum.04123-22</a>	Predictive Power of Long-Read Whole-Genome Sequencing for Rapid Diagnostics of Multidrug-Resistant <i>Brachyspira hyodysenteriae</i> Strains.
5/04/2023	Nieuwsbrief	NB DGZ	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België



<b>1/05/2023</b>	Persbericht	Dierenartsenwereld	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
<b>31/05-2/06/2023</b>	Poster	ESPHM 2023	The role of behaviour and ear biting on the occurrence of porcine ear necrosis.
<b>4-7/06/2023</b>	Poster	ICPR 2023	Farrowing traits associated with prolonged farrowing duration and high birth intervals in hyperprolific sows.
<b>29/06/2023</b>	Nieuwsbrief	NB DGZ	Veepeiler start nieuw project om PRRSV-stammen in beeld te krijgen
<b>29/06/2023</b>	Persbericht	Landbouwleven	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
<b>1/07/2023</b>	Perbericht	VedaScoop	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
<b>6/07/2023</b>	Persbericht	Boer en Tuinder	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
<b>28/08/2023</b>	Wet. Pub.	Porc Health Manag 9(1) <a href="http://hdl.handle.net/1854/LU-01HCYWZB9RRC2XP696C4GY4KTP">http://hdl.handle.net/1854/LU-01HCYWZB9RRC2XP696C4GY4KTP</a>	Relationship between piglets' survivability and farrowing kinetics in hyperprolific sows.
<b>5/09/2023</b>	Nieuwsbrief	NB DGZ	Optimaliseer de gezondheid van je dieren en je bedrijf met behulp van Veepeiler Varken
<b>29/09/2023</b>	Wet. Pub.	Vet Res, 54:85 <a href="https://doi.org/10.1186/s13567-023-01218-1">https://doi.org/10.1186/s13567-023-01218-1</a>	Porcine ear necrosis: lesions, associated pathogens and factors.
<b>2/10/2023</b>	Nieuwsbrief	NB DGZ	Krijg PRRSV-stam in beeld met de hulp van Veepeiler



## 7 Bijlage 1

VVD – Virology and Viral Diseases

### **PCV2 VIRAL LOADS IN PIGS ARE CORRELATED WITH CONCURRENT PORCINE PARVOVIRUS INFECTION(S).**

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#### **Background and Objectives**

PCV2 causes several syndromes, commonly known as porcine circovirus-associated disease (PCAD). Despite this vaccination, veterinarians have reported an increase in PCV2 viral loads over the years. The clinically most important and best described porcine parvovirus (PPV) is PPV1. However, little is known about various other PPV types (PPV2-7) circulating on farms in Belgium. Furthermore, research data show that co-infection(s) with porcine parvovirus(es) (PPV) may underlie the rise in PCV2 load. Therefore, this study aims to investigate if increasing PCV2 viral loads in pigs on Flemish farms can be correlated with systemic PPV infections.

#### **Material and Methods**

Pig serum samples submitted to DGZ Vlaanderen between July 2022 and June 2023 were pooled up to five samples and analyzed by qPCR for PCV2. PCV2 positive samples were selected and categorized based on the viral PCV2 load into different groups for further viral & bacterial metagenomic sequencing at PathoSense: Group 1 consisted of PCV2 samples with a viral load of  $10^8$ . In this way, the PCV2 loads were linked to the detection of PPV co-infections in a semi-quantitative manner. Results Samples with a viral PCV2 load of  $<10^3$  genome copies/ml with PCR (group 1) were tested negative for PPV. Sequencing by PathoSense confirmed the presence of PCV2 in the samples in group 2 ( $10^5$ - $7$  PCV2 genome copies) and 3 ( $10^8$ - $10$  PCV2 genome copies) and also at least one PPV type in those groups. In total, five different types of parvoviruses (not PPV1) were detected by PathoSense: PPV2, 3, 4, 5 and 7. Importantly, an increase of total PPV in samples with a high PCV2 load was observed.

#### **Discussion and Conclusion**

The suspicion of field veterinarians that PCV2 is more present has been confirmed in this study. In addition, we found evidence that different PPVs can be found in association with high PCV2 loads. This is logical from a pathogenetic point of view, as both viruses rely on proliferating lymphoblasts, which is the result of an activated immunity during co-infections.



## Higher PCV2 viral loads in pigs can be explained by underlying porcine parvovirus infection(s).

Caroline BONCKAERT<sup>1</sup>, Charlotte RIGAUTS<sup>1</sup>, Charlotte Brossé<sup>1</sup>, Sebastiaan THEUNIS<sup>2</sup>, Hans NAUWYNCK<sup>3</sup>

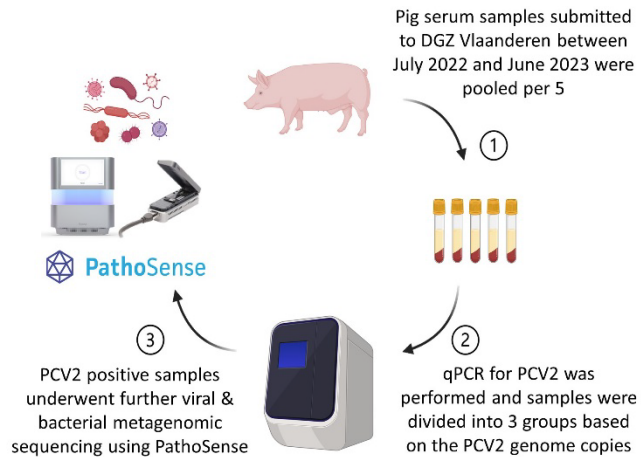
<sup>1</sup>Diergezondheidszorg Vlaanderen, Hagenbroeksesteenweg 167, 2500 Lier; <sup>2</sup>PathoSense, Pastoriestraat 10, 2500 Lier; <sup>3</sup>Laboratory of Virology, Faculty of Veterinary Sciences, Ghent University, Salisburylaan 133, 9820 Merelbeke

### INTRODUCTION

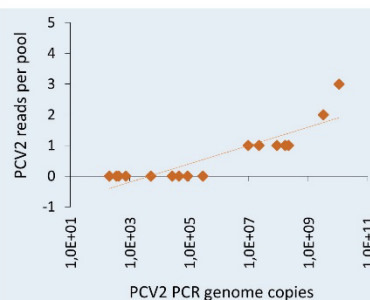


Despite extensive vaccination against porcine circovirus type 2 (PCV2), veterinarians have reported an increase in PCV2 viral loads over the years. Research has shown a PCV2 genotype shift from PCV2a to a better replicating PCV2d, which can at least partly explain the increase in viral load<sup>1</sup>. While most farms vaccinate sows against the most common type of porcine parvovirus (PPV1), it is not known whether the various other PPV types circulate on farms in Belgium and whether there is cross-protection by the PPV1 vaccine. Furthermore, research data show that co-infection with PPV may underlie the rise in PCV2 load<sup>2-6</sup>. Therefore, this study aims to confirm the increasing PCV2 viral loads in pigs on Flemish farms and to investigate whether this can be linked to underlying PPV infections.

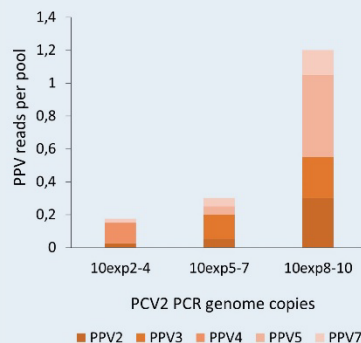
### METHODS



### RESULTS



**Figure 1. Comparison of semi-quantitative PCV2 reads to PCV2 PCR genome copies.** A positive correlation ( $R^2=0.7749$ ) is observed. However, in samples containing  $<10^4$  PCV2 PCR genome copies, no PCV2 reads were detected. PCV2 reads are semi-quantitative with very low=1, low=2, medium=3, high=4 and very high=5.



**Figure 2. Mean number of PPV reads per group of PCV2 PCR genome copies.** Mean number of PPV reads per pool is calculated as: (n reads PPV2+n reads PPV3+n reads PPV4+n reads PPV5+n reads PPV7 with very low=1, low=2, medium=3, high=4 and very high=5 as semi-quantitative values/5xnumber of pigs)

### CONCLUSION

The suspicion of field veterinarians that PCV2 is more present has been confirmed in this study. In addition, we found evidence that different PPVs can be found in association with high PCV2 loads. This is logical from a pathogenetic point of view, as both viruses rely on proliferating lymphoblasts, which is the result of an activated immunity during co-infections.

### ACKNOWLEDGEMENTS

This research was funded by Veepeiler Varken. Figures created with BioRender.com

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## 8 Bijlage 2

### **Title**

Effectiveness of cleaning and disinfection protocols on pig farms in Northern-Belgium.

### **Autors**

Tamara Vandersmissen, Caroline Bonckaert, Charlotte Brossé, , Charlotte Rigauts

### **Background & Objectives**

Manure-contaminated surfaces are the perfect source to perpetuate infection on a farm, given that this indirect transmission route has been demonstrated for many pathogens<sup>1</sup>. To prevent these pathogens from surviving and infecting the next group, it is essential to clean and disinfect properly between batches. This project aimed to assess the effect of the farmer's cleaning and disinfection protocol using RODAC plates.

### **Material & Methods**

44 farms were included, all applying all-in/all-out with cleaning and/or disinfecting per department. After cleaning and/or disinfection, 13 surfaces in the farrowing unit (n=10), the nursery (n=20), or both (n=14) were sampled using RODAC plates. A hygiene score (0-5) was defined based on the number of bacteria grown on the plates, with a high number indicating poorer hygiene (0: zero CFU/plate; 1: 1-40 CFU/plate; 2: 41-120 CFU/plate; 3: 121-400 CFU/plate; 4: >400 CFU/plate; 5: uncountable). After receiving advice on improving their cleaning and disinfection protocol, 5 farrowing units and 7 nurseries were sampled a second time.

### **Results**

The mean hygiene score of the farrowing unit was significantly higher compared to the nursery (2.6 versus 2.1, respectively). Surfaces at animal height had better hygiene scores than higher surfaces (2.19 versus 2.87, respectively). In the farrowing units a significantly lower hygiene score was achieved at the second sampling after receiving advice and improving their cleaning and disinfection protocol (2.07 versus 3.22 at first sampling).

### **Discussion & Conclusion.**

The results show that farrowing units are not cleaned as effectively as nurseries. This may be due to insufficient time for cleaning and disinfecting between two batches. Additionally, the results show that more attention should be paid to higher places as they score worse than surfaces at animal height. As an improved hygiene score was obtained after receiving advice, the project proves the importance of informing the farmer of a proper cleaning and disinfection protocol.



## Effectiveness of cleaning and disinfection protocols on pig farms in Northern-Belgium.

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### OBJECTIVES

Manure-contaminated surfaces are the perfect source to perpetuate infection on a farm. So proper cleaning and disinfection between batches, are vital to prevent pathogens from survival and transmission to the next group. This project aimed to assess the effect of the farmer's cleaning and disinfection protocol using RODAC plates.

### MATERIALS & METHODS

44 farms were included, all applying all-in/all-out with cleaning and/or disinfecting per department. After cleaning and/or disinfection, 13 surface samples from farrowing unit (n=12), nursery (n=25), or both (n=14) were taken (sampling 1), using RODAC plates (picture 1). A mean score was defined based on the number of bacteria (CFU) grown on the 13 plates (table 1). Following protocol improvement advice, 5 farrowing and 7 nursery units were resampled (sampling 2).



Picture 1: Sampling by using RODAC plates

RODAC plate score	CFU/plate	Interpretation
0	0	excellent
1	0-40	very good
2	41-120	good
3	121-400	median
4	>400	poor
5	uncountable	very poor

Table 1: Interpretation of the hygiene score with RODAC plates

### RESULTS

The mean score of the RODAC plates of the first sampling was significantly higher ( $p < 0.01$ ) in the farrowing unit compared to the nursery (3.22 versus 2.48, respectively). Surfaces at animal height had significantly better scores ( $p < 0.001$ ) than higher surfaces (2.19 versus 2.87, respectively). Improvements in cleaning and disinfection protocols after receiving advice, significantly reduced mean scores ( $p < 0.001$ ) in farrowing units (2.07 at second sampling versus 3.22 at first sampling) but not significantly in nurseries (2.46 versus 2.22, respectively). Proper use of soaking solution (4/12 farms) and disinfection products (7/12 farms) were the most effective measures to improve the scores.

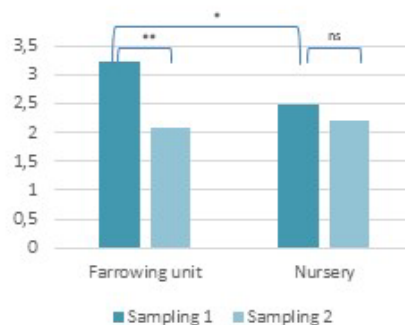


Figure 1: The mean score of the RODAC plates in the farrowing unit and the nursery before (sampling 1) and after (sampling 2) receiving advice. \*  $p$ -value < 0.01; \*\*  $p$ -value < 0.001; ns = not significant

### CONCLUSION and DISCUSSION

The results show that farrowing units are not cleaned as effectively as nurseries. This may be due to insufficient time for cleaning and disinfecting between batches. Additionally, the results show that more attention should be paid to higher places as they score worse than surfaces at animal height. As an improved score was obtained after receiving advice, the project proves the importance of control measurements, followed by informing the farmer of a proper protocol.





## 9 Bijlage 3



RESEARCH ARTICLE

Open Access



# Porcine ear necrosis: characterization of lesions and associated pathogens

Mateusz Malik<sup>1\*</sup>, Koen Chiers<sup>2</sup>, Sebastiaan Theuns<sup>4</sup>, Nick Vereecke<sup>3,4</sup>, Ilias Chantziaras<sup>1</sup>, Siska Croubels<sup>2</sup> and Dominiek Maes<sup>1</sup>

## Abstract

Porcine ear necrosis (PEN) is characterized by ulcerative lesions of the ear auricle. To investigate that problem, three farms with PEN in nursery pigs were included, and the study aim was to characterize PEN and the potential role of pathogens and mycotoxins. Within each farm, one batch of weaned piglets was included and the prevalence and severity of PEN were monitored for 6–7 weeks. Within each batch, 30 PEN-affected/non-affected animals were randomly selected. Blood samples were taken from these animals, to assess the systemic presence of pathogens and mycotoxins, as well as punch biopsies from the ear auricle for histopathological examination. From 10 animals, scrapings and swabs from the lesions were subjected to nanopore metagenomic sequencing and bacteriological cultivation, respectively. In all three farms, lesions appeared within 3–4 weeks post-weaning. The prevalence at the end of the nursery was 33%, 24%, and 46% for farms A, B, and C, respectively. Most affected pigs had mild to moderate lesions. Blood samples revealed low to very low levels of pathogens and mycotoxins. Different bacteria such as *Staphylococcus*, *Streptococcus*, *Fusobacterium*, *Mycoplasma*, and *Clostridium* species were identified by sequencing in the scrapings. The first two pathogens were also most often identified in bacterial cultures. *Mycoplasma hyopharyngis* was only found in PEN-affected pigs. Histopathological changes were primarily observed in the outer layer of the epidermis. The results suggest that PEN lesions develop by damage to the outer part of the skin e.g. by ear suckling or biting, followed by multiplication of opportunistic pathogens.

**Keywords** Porcine ear necrosis, lesions, ear biting, mycotoxins, ear tag, behavior, weaned pigs

## Introduction

Porcine ear necrosis (PEN) is characterized by ulcerative, bloody, and wet lesions of the ear auricle, localized mostly on the ear tips [1]. Different names have been used for this condition such as ear-tip necrosis (ETN), ear-biting, porcine ear necrosis syndrome (PENS), ulcerative spirochetosis, or streptococcal auricular dermatitis. The condition should be considered a welfare problem, especially in case of severe lesions. A recent study showed that mild PEN lesions did not affect pig growth [2] but likely severe PEN lesions do decrease performance. In addition, the skin wounds at the ears may serve as an entry point for opportunistic bacteria, as has been shown for tail-biting lesions [3]. Such bacteria may subsequently spread throughout the body and cause abscesses in the lung and

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other internal organs, leading to condemnations of the carcasses at slaughter [4].

The cause and pathophysiology of PEN are largely unknown. Three major pathogenesis hypotheses have been described in scientific literature thus far: (1) obstruction of small blood vessels due to cold agglutinins [5]; (2) damage of the epidermis caused by staphylococcal exfoliative toxins; and (3) ear-biting leading to skin injury followed by  $\beta$ -hemolytic streptococcal infection [6, 7]. Spirochetes of the genus *Treponema* have also been associated with PEN [8]. However, experimental intradermal inoculation of *T. pedis* did not result in PEN lesion formation [9]. Porcine circovirus type 2 (PCV2) infections were mentioned by Pejsak and colleagues [10] as a possible risk factor, though no direct influence was shown so far. Since PCV2 frequently infects nursery pigs and might exert immunosuppressive effects, its significance in herds affected by PEN is questionable. Recently, Costa et al. [11] could reproduce PEN via intradermal inoculation of suspensions made from tissue collected from fresh PEN lesions, but only mild lesions were found.

Several non-infectious risk factors have been suggested including high stocking density, low availability of feeders and drinkers, mycotoxin contamination of feed, fully slatted floors, high humidity, and high environmental temperature [6, 12]. A recent study including different successive batches of nursery pigs [2] reported that pens with a high and low prevalence of affected pigs can exist next to each other, and that lesion prevalence in specific pens is not consistent over time. This suggests that pigs affected with PEN are not always present in specific “high-risk” pens over successive batches.

So far, the ear lesions of pigs affected with PEN have not been fully characterized neither were the pathogens involved assessed using more recent and advanced diagnostic procedures. To further elucidate the pathogenesis of the condition, the aims of the present study were to investigate PEN lesions in nursery pigs of different farms, to characterize the lesions and to assess the pathogens involved using nanopore metagenomic sequencing and bacterial culture.

## Materials and methods

The study protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine and the Faculty of Bioscience Engineering, Ghent University (EC2020-095), as well as by the Flemish governmental agency for animal welfare (DWZ/KF/21/1.15/40).

### Study design and farm characteristics

Three commercial single-site farrow-to-finish pig farms were included. The farms had been suffering from PEN in nursery pigs for more than 1 year, as confirmed by the

farmer and the farm veterinarian. The research was conducted between May and November 2021. On each farm, one entire batch of piglets was observed from weaning to the end of the nursery, spanning a period of 6–7 weeks. The weaning age was 21 days in farm A, and 28 days in farms B and C. The farms were visited by the first author to collect appropriate farm data using a questionnaire and to evaluate the severity of the problem. The general characteristics obtained by the questionnaire are summarized in Additional file 1. The number of nursery pens that were monitored during the study differed between farms. In Farm A, 12 pens (on average 40 pigs/pen) were divided over three compartments, in Farm B, 14 pens (38 pigs/pen) were in one compartment, and in Farm C, 12 pens (25 pigs/pen) were present in the same compartment. The farms were visited weekly, and the prevalence and severity of PEN of the entire weaning group were assessed. During the last two visits, various samples namely scrapings (10/farm), swabs (10/farm), and biopsies of the ears (30/farm), plasma (30/farm), and serum (18–30/farm and pooled by 6, depending on present severity score) were collected for further analysis, including metagenomic sequencing, bacterial culture, histopathology, and blood for mycotoxins analysis. The samples were taken towards the end of the nursery, as at that time, lesions of different severity were present and could be sampled. There were no other animal health problems on the farms during the study.

### Assessment of PEN prevalence and severity

In all three farms, every pen of the weaning group was monitored weekly, and the animals were restrained individually in the pen, and visually evaluated one by one for the presence and severity of PEN lesions. This also allowed us to assess the distribution of the total number of affected animals over the different pens in the different compartments. A five-point scoring system to assess the severity of the lesions was used as described previously [7]. In short, the scores (0 to 4) corresponded to the following conditions: 0=no deviations, 1=incipient red discoloration or a crust at the tip of the ear, 2=more black-like discoloration and a rounded ear tip, 3=severe necrosis with a part less than one-third of the ear missing at the tip, and 4=piglets lost more than one-third of the ear. After performing the evaluations on farm A, it was decided to record other lesions (scratches/small wounds) different than PEN on both ears from each individual animal on farms B and C. This was done at all scoring time-points. Piglets were ear tagged on all farms (official ear tag with farm number and country code) but in farm C, ear tags were consistently placed at the same side of the animal. This allowed us to evaluate the effect of the ear tag on PEN lesions in that farm.

### Sampling

In total, 1280 piglets were evaluated in this study. The number of pigs in the entire weaning batches of the three farms were as follows: farm A 485, farm B 527, and farm C 268 piglets. Within each farm, pigs with lesions of different severity were sampled.

Blood samples were collected via jugular vein puncture from 18 to 30 randomly chosen animals with different lesion severity scores 2 weeks before the end of the nursery period. Clotted blood samples were centrifuged for 20 min at  $2000 \times g$  and sera were collected. Within each farm, individual samples of six animals were pooled based on the PEN severity score present on the farm. To this end, on farms A and B, three pooled samples were prepared (from pigs with score 0, 1, or 2), and on farm C, five pooled samples were obtained (from pigs with score 0, 0, 1, 2, or 3). Blood of 30 animals per farm were taken to obtain plasma for further mycotoxins analysis.

To investigate possible differences in the microbiota present in the lesions from the animals that were blood sampled in each farm, 10 of them were selected at random to obtain scrapings namely 6 from affected (all severity scores) and 4 from non-affected animals. Scrapings of the lesions and underlying tissue from PEN-affected animals, as well as ear tip skin scrapings of unaffected animals, were collected with a scalpel blade. The lesions or the area around the lesions was not cleaned before sampling. On farms B and C, 5 scraping samples were taken 1 week later. The later sampling likely had only minor or no effect on the results.

### Metagenomic analysis

Serum and scrapings were analyzed using nanopore metagenomic sequencing as described previously [13, 14]. In short, (pooled) serum samples were pre-purified using a patented sampler (EP 19183233.6). Scrapings were crushed in dPBS using a 1.5 mL Eppendorf tube squisher (Zymo H1001) and filtered through a 1.5 mL Eppendorf tube 0.8  $\mu\text{m}$  centrifuge filter (Vivaclear, Sartorius) at 2000 rpm for 5 min. The resulting filtrates were subjected to enzymatic host depletion and *ad random* amplification procedure. The resulting DNA was subjected to rapid library preparation using the RBK096 library prep (ONT), multiplexing up to 24 samples per run. Sequencing was performed on R9.4.1 flow cells (ONT) using the GridION device, which allows real-time data acquisition, super accurate base calling, and demultiplexing via Guppy (v.6.1.5). The reads were taxonomically classified using in-house validated bioinformatics pipelines. A spike-in virus was used to perform normalization between samples and to give a semi-quantitative report as described before [15]. This allowed us to report

both viruses and bacteria in five categories, including very low, low, medium, high, and very high. The bacterial classification was limited to the genus level as previously discussed [14]. If two or fewer absolute reads were classified, they were not reported [13, 15].

### Bacteriology culturing

The animals that were selected for scrapings sampling for nanopore metagenomic sequencing were also selected for bacteriological culture. The swabs were taken just after, and from the same area of the ears as was done for the scrapings. They were collected with cotton sterile swabs with Amies transport medium, and transported within 4 h to the Animal Health Care Flanders laboratory (DGZ Vlaanderen, Torhout, Belgium), where standard aerobic and anaerobic agar culturing was performed. Namely for the aerobic pathogens Columbia sheep agar, MacConkey agar, and Columbia horse agar were used; which were incubated for 48 h at 37 °C in CO<sub>2</sub> (7.5%). For the anaerobic pathogens, fastidious anaerobic agar and Colombia agar were used, which were incubated for 24 h in anaerobic conditions (AnaeroGen, Thermo Scientific, USA). The samples were inoculated on the media until a pure culture was obtained. These pure cultures were identified using MALDI-TOF. The bacterial number or load in the sample was expressed semi-quantitatively namely: low (yellow) 0–10 colonies on plate, intermediate (orange) 11–50 colonies, and high (red) > 50 colonies on plate.

### Histopathology

Six millimeters punch biopsies were collected from the same 30 animals per farm that were blood sampled. From the affected animals, a biopsy was taken from the margin of the lesion. From non-affected animals, a biopsy was taken from the ear tip, 5–10 mm from the ear edge. The collected samples were first fixed in 4% neutral buffered formalin and embedded in paraffin before being stained with hematoxylin and eosin followed by microscopic evaluation.

### Mycotoxin analyses and feed composition

Blood samples from 90 animals were also collected in EDTA tubes (uncoagulated blood). The samples were centrifuged for 10 min at 4 °C at  $3725 \times g$  to obtain plasma, which was then stored at –20 °C until further analysis. The plasma samples were analyzed using liquid chromatography combined with triple quadrupole tandem mass spectrometry. The analysis has been validated for pig plasma as a multi-mycotoxin LC–MS/MS method [16] to assess the presence of mycotoxins, with phase I and II metabolites simultaneously. The following toxins were included: 3-acetyl deoxynivalenol, 15-acetyl

deoxynivalenol, alternariol, alternariol monomethyl ether (AME), aflatoxins: B1, B2, M1, M2, G1, G2, beauvericin, de-epoxy-deoxynivalenol, deoxynivalenol (DON), deoxynivalenol-glucuronide, deoxynivalenol-sulfate, enniatins: A, A1(ENNA1), B (ENNAB), B1 (ENNAB1), fumonisins (FBs): fumonisin B1 (FB1), B2, B3, HT-2 toxin, ochratoxin A (OTA), tenuazonic acid (TEA), T2 toxin, zearalenone (ZEN),  $\alpha$ -zearalanol,  $\beta$ -zearalanol,  $\alpha$ -zearalenol,  $\beta$ -zearalenol,  $\alpha$ -zearalenol-glucuronide,  $\beta$ -zearalenol-glucuronide, zearalanone, zearalenone-glucuronide and zearalenone-sulfate.

Additionally, two feed samples were collected in each farm, one from the feed that was provided during the first week post-weaning, and a second from the feed that was provided during the fourth week post-weaning. Each sample was obtained by pooling feed samples from different (four to six) feeding troughs throughout the compartment. The samples were stored at room temperature, and the presence of DON, FBs, and ZEN was determined by a multi-mycotoxin method—LC—MS/MS.

#### Drinking water quality

On each farm, water samples were taken from the drinking nipple in the stable and transported within 24 h to the DGZ Laboratory. After 30 s of continuous flow from the nipples, the water sample was collected into specific bottles provided by the laboratory. Bacteriological (the number of coliforms, intestinal enterococci, sulfite-reducing clostridia, overall aerobic bacteria) and chemical (ammonia, nitrates, sulfates, hardness, and salinity concentration) analyses were performed.

#### Stable climate

While carrying out the study on farm A, it was decided to assess as additional descriptive information different stable climate parameters in farms B and C. To this end, loggers were placed in the middle of the compartment, at a height of one meter above the ground. The following parameters were measured every 30 min using a Tinytag Plus2 logger (Gemini Data Loggers, UK): ambient temperature ( $^{\circ}\text{C}$ ), relative humidity (%), and dew point ( $^{\circ}\text{C}$ ).

#### Statistical analysis

A generalized linear mixed model (logistic regression with a binomial probability distribution) was run to determine the effect of wearing an ear tag and the effect of the week, on having a scratch or not in the first 3 weeks after weaning on farm C. The effects of pen and pig were included in the model as random effects.

Additionally, a cumulative odds ordinal logistic regression with proportional odds (generalized linear mixed model) was run to determine the effect of wearing an ear tag (present on the left or right ear) and the effect of the

week, on having a higher PEN score. The effects of pen and pig were included in the model as random effects.

Also, we performed a non-parametric test (Mann–Whitney test) to check whether the presence of PEN lesions was associated with the levels of mycotoxins (as tested in plasma samples).

A cumulative odds ordinal logistic regression with proportional odds was run to determine the effect of a higher number of mycotoxins on having a higher severity score for PEN. The farm was also considered in the model (fixed effect).

Finally, we made use of a non-parametric test (Spearman's rho) to check the correlations between PEN severity and the load for all viruses or bacteria revealed in the metagenomics analysis.

Statistical analysis was performed using IBM<sup>®</sup> SPSS<sup>®</sup> Statistics for Windows Version 29 (IBM Corp., Armonk, N.Y., USA). *P*-values below 0.05 were considered statistically significant.

## Results

### PEN prevalence

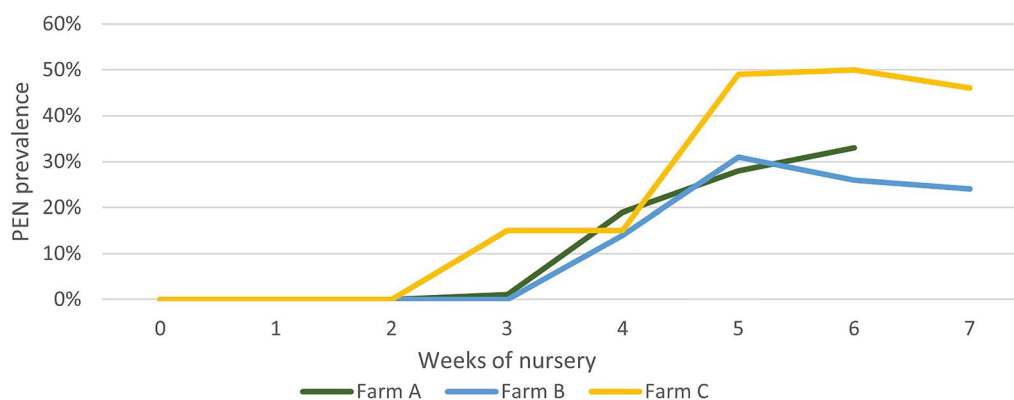
The entire weaning batch in farms A, B, and C included 485, 527, and 268 piglets, respectively. Figure 1 depicts the weekly prevalence of PEN. No animals showed signs of PEN at weaning. The prevalence of PEN in each batch increased with the number of weeks post-weaning. The maximum prevalence was 33%, 31%, and 50% in farms A, B, and C, respectively, what was reached at 5–6 weeks post-weaning. In the final 2 weeks of the nursery, the prevalence in farms B and C decreased slightly. The mortality on farms A, B and C was 2.7%, 0.8%, and 1.5%, respectively.

### PEN prevalence by location in the stable

Figure 2 shows an outline of the pens in the compartments as well as the percentage of affected animals in each pen at the end of the nursery period. The prevalence of affected animals per pen ranged from 2 to 77%, 0–72%, and from 0 to 100% in farms A, B, and C, respectively.

The lesions in animals from all three farms developed first as a dry small crust on the ear tip or as reddening with edema and a small dry wound at the tip (Figure 3A). Some of these lesions progressed from moderate (Figure 3B) to severe wet wounds with partial ear pinna loss. The tissue beneath was often fresh and moist (Figure 3C).

In the first week post-weaning in farms B and C, small scratches/wounds were present in 80–95% of the pigs (Figures 4A, B). The prevalence decreased to less than 20% in most of the pens (20/26) 2–3 weeks post-weaning. In the six remaining pens where the prevalence of scratches/wounds remained 20% or higher, four pens



**Figure 1** Weekly prevalence of porcine ear necrosis (PEN) lesions in nursery pigs from three farms. The number of piglets at weaning on farms A, B, and C was 485, 527, and 268, respectively.

Farm A		Farm B		Farm C	
35	67	16	0	24	5
44	2	0	5	100	5
5	60	14	38	100	95
20	67	11	26	32	52
51	77	19	72	52	0
35	10	51	29	29	29
		71	0		

**Figure 2** Percentage of animals affected with porcine ear necrosis per pen, at the end of nursery period in farms A, B, and C. The mean number of pigs per pen was 40, 38, and 25 in farms A, B, and C, respectively.

reached the highest PEN prevalence (95–100%) at the end of the nursery.

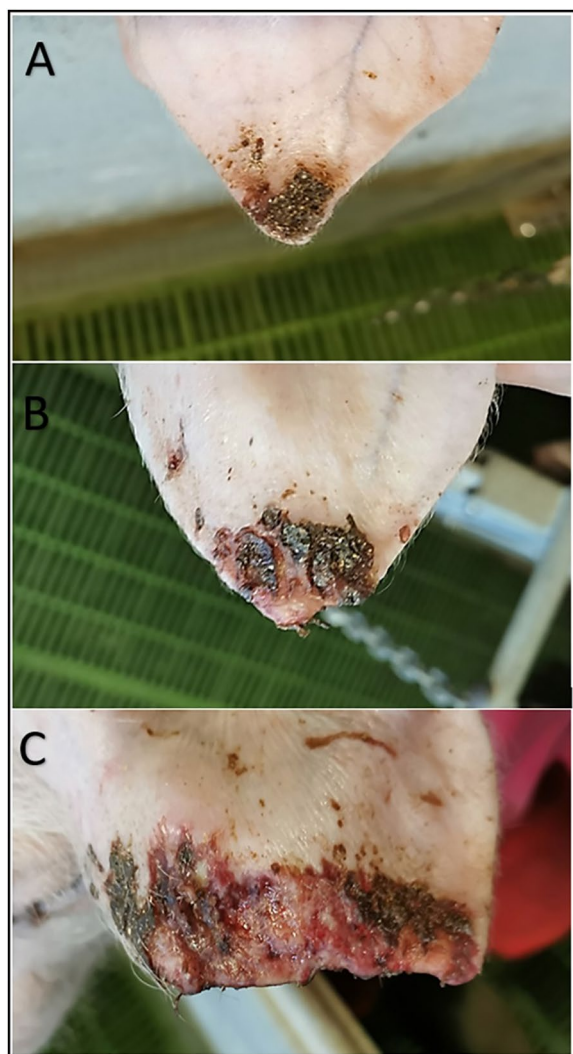
In farm C, the ear tags were placed in the right ear only, 2–3 cm from the edge and tip of the ear. The prevalence of all small wounds/scratches after the first week post-weaning ( $n=269$  pigs), was 26% and 62% in the right and left ear, respectively. In pen numbers 3, 4, 7, and 8, the prevalence of wounds/scratches did not decrease below 20% in week 2–3 post-weaning ( $n=66$  pigs), and the average prevalence for the first 3 weeks was 11% ( $SD \pm 8\%$ ) and 60% ( $SD \pm 17\%$ ) in the right (with the ear tag) and left ear (without ear tag), respectively.

The odds of having a scratch on an ear without an ear tag was 7.42 (95% CI 5.20 to 10.62) times that for ears with an ear tag,  $t=10.998$ ,  $p<0.001$ . Week 1 was

associated with an increase in the odds of having a scratch when compared with Week 3, with an odds ratio of 10.01 (95% CI 6.95 to 14.42),  $t=12.384$ ,  $p<0.001$ . Week 1 was associated with an increase in the odds of having an increase in scratches when compared with Week 2, with an odds ratio of 10.42 (95% CI 6.95 to 14.42),  $t=12.49$ ,  $p<0.001$ . Week 2 was not associated ( $t = -0.206$ ,  $p=0.837$ ) with a change in the odds of having a scratch when compared with week 3, with an odds ratio of 0.96 (95% CI 0.63 to 1.46).

**Severity of the PEN lesions**

The results of the severity scoring of the piglets in the three farms are shown in Table 1. On farms A and B, only 1–3% of the pigs had a lesion score of 2, whereas, on farm



**Figure 3** Example of ear lesions evaluated during the study.

**A** Piglet with mild lesions (score 1)—light reddening and small crusts, **B** ear of a moderately affected piglet (score 2)—rounded ear tip with crusts and wet tissue beneath, **C** ear of severely affected piglet (score 3)—partially missing ear pinna, with remaining crusts and fresh wet tissue.

C, 34% of animals developed lesions with a score of 2 or 3. Very severe lesions with a score of 4 were not found in any of the farms.

In farm C where the ear tags were always placed on the right ear, for the last 3 weeks of the nursery, the average PEN severity score was assessed ear separately for the left and right in three pens with the highest PEN prevalence. The average severity scores were 1.06 (SD ± 0.31) and 1.75 (SD ± 0.20) for the ears on the right and the left side, respectively ( $n=135$ ). In those pigs, the average PEN prevalence severity score of 3 at the end of the nursery was 0% and 29% on the right and left ears, respectively.

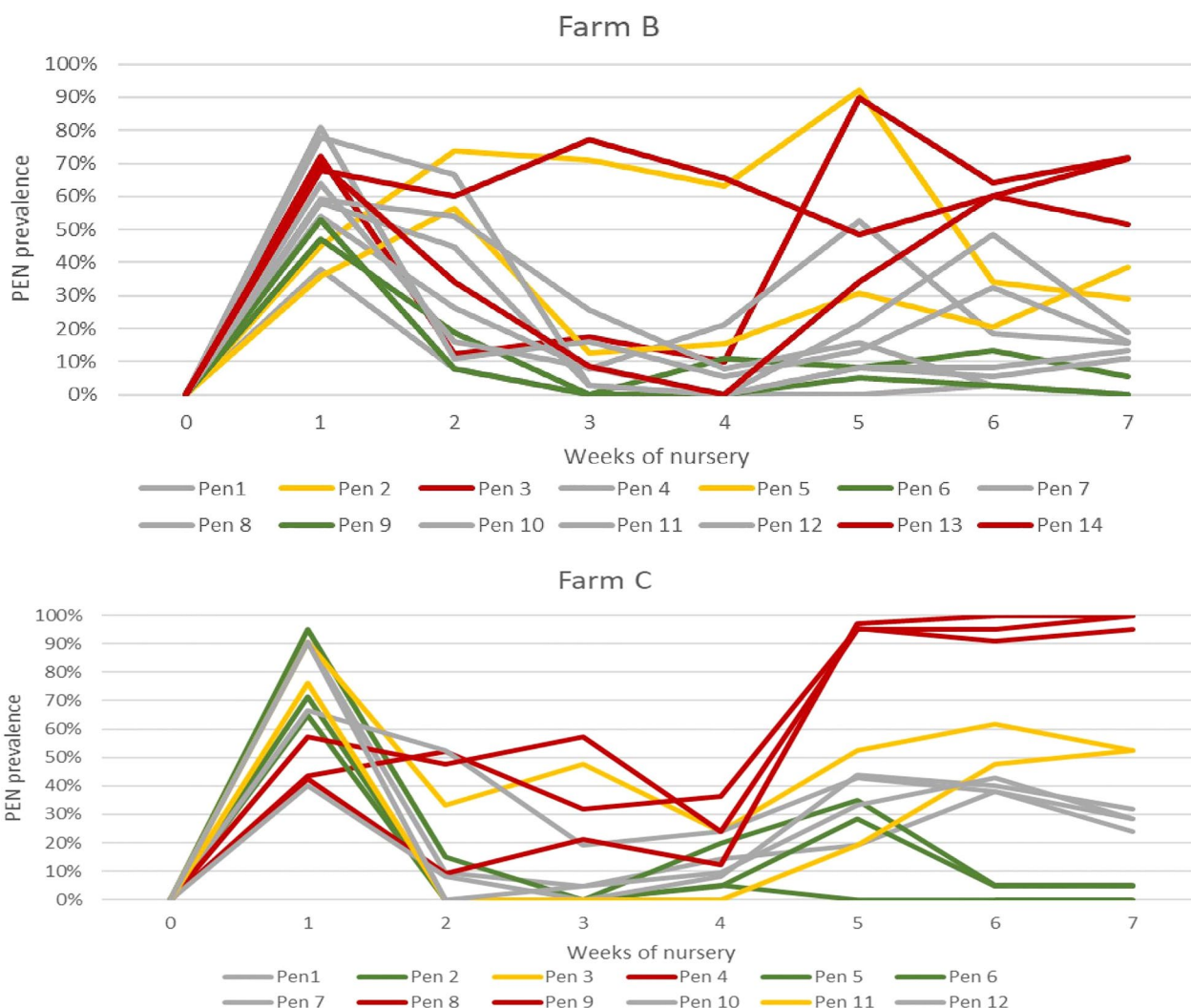
The odds of having a higher PEN score on an ear without an ear tag was 2.42 (95% CI 1.82 to 3.22) times that for ears wearing an ear tag,  $t=6.05$ ,  $p<0.001$ . Week 7 was associated with an increase in the odds of having an increase in PEN score when compared with Week 5, with an odds ratio of 1.77 (95% CI 1.31 to 2.38),  $t=3.764$ ,  $p<0.001$ . Week 6 was associated with an increase in the odds of having an increase in PEN score when compared with Week 5, with an odds ratio of 1.75 (95% CI 1.30 to 2.36),  $t=3.694$ ,  $p<0.001$ . Week 7 was not associated ( $t=-0.77$ ,  $p=0.939$ ) with a change in the PEN score when compared with Week 6, with an odds ratio of 0.99 (95% CI 0.74 to 1.32).

#### Metagenomic profiling of viruses and bacteria

Overall, many different pathogens were detected in the serum by nanopore metagenomic sequencing, mostly at low and/or very low levels. Porcine Parvovirus (PPV) type 5 (3/3 pooled samples in farm B) and PPV type 2 (4/5 pooled samples in farm C) were found to be present at high levels. Also, torque teno sus virus (TTSuV) was found on all three farms (farm A: 3/3; farm B: 3/3; farm C: 3/5), but at low levels. The porcine reproductive and respiratory syndrome virus (PRRSV), and porcine bocaparvovirus, were present at low levels. For the bacteria, *Streptococcus* sp. was found in one pooled sample from farm B and one from farm C.

For the ear scrapings, the most prevalent viruses were Bocaparvovirus (27/30, ranging from very low to medium levels), porcine parvoviruses, including type 2 (2/30, very low to low), 5 (5/30, very low to high), 6 (1/30 very low), and 7 (1/30 very low), TTSuV (4/30, very low to low), swine pneumovirus (4/30 very low), porcine cytomegalovirus (1/30 very low), PRRSV (1/30 low), porcine polyomavirus (1/30 very low), and porcine adenovirus 5 (1/30 very low). Also, other viruses (kobuvirus, rotavirus, picobirnavirus, enterovirus) were detected in various samples (Additional file 2).

The most prevalent bacterial genera in scrapings were *Fusobacterium* sp. (18/30, very low to very high; likely *F. necrophorum*), *Streptococcus* sp. (17/30, very low to high), *Mycoplasma* sp. (15/30, very low to medium; likely *M. hyopharyngis* when considering full-length 16S rRNA gene sequences), *Staphylococcus* sp. (12/30, very low to medium), and *Clostridium* sp. (10/30, very low to medium). Some less abundant bacterial genera were *Campylobacter* sp. (5/30, very low to medium; likely *C. mucosalis* when considering full-length 16S rRNA gene sequences), *Trueperella* sp. (3/30, very low to low), *Porphyromonas* sp. (3/30 very low), *Treponema* sp. (3/30 very low), *Actinobacillus* sp. (3/30, very low to medium), *Mannheimia* sp. (1/30 very low), and *Escherichia* sp. (1/30 medium). Also, other bacteria were identified,



**Figure 4** Weekly prevalence of all recorded ear lesions/alterations recorded at the pen level on farms B and farm C. Colors of the lines related to the prevalence: red—very high, yellow—high, grey—moderate, green—low.

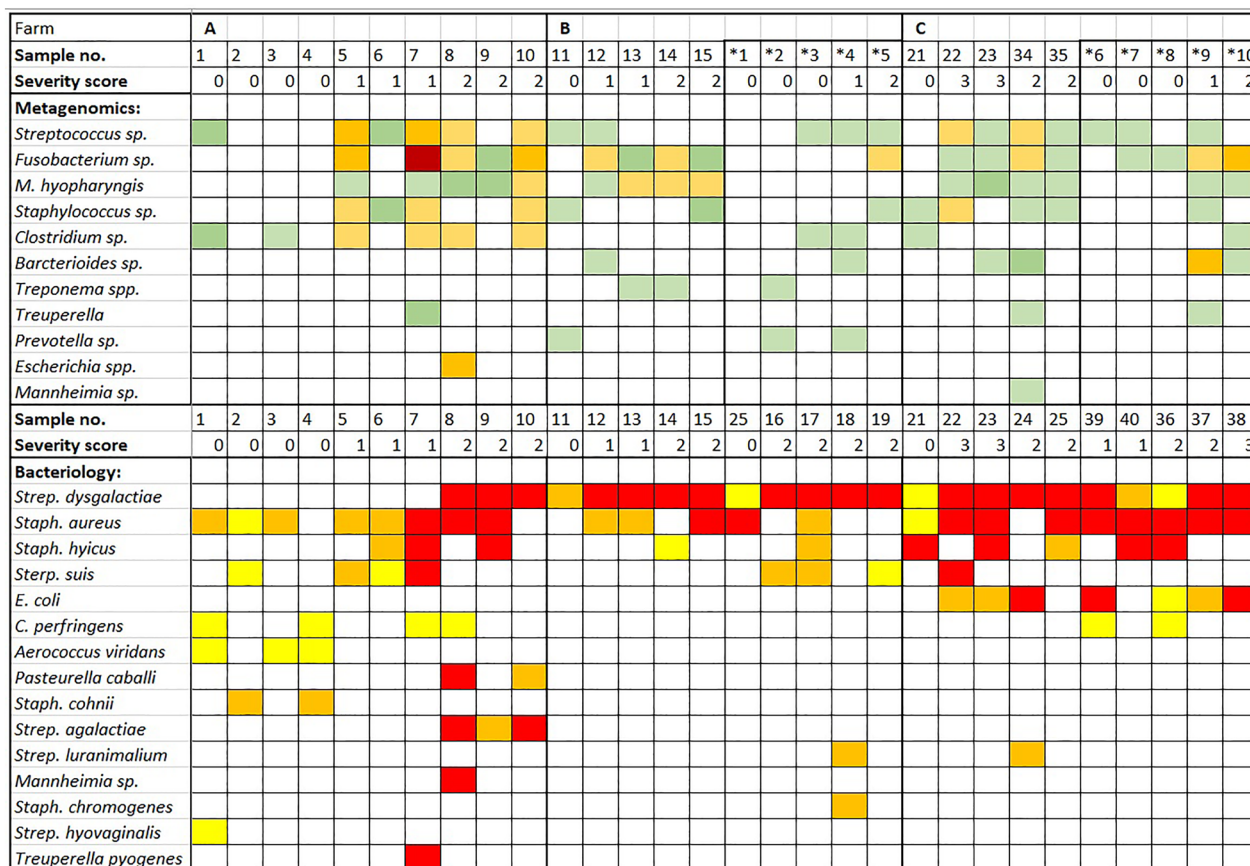
**Table 1** Distribution of different lesion scores in nursery pigs of the three farms at the end of the nursery.

Score	Farm A	%	Farm B	%	Farm C	%
0–4	n = 472	100	n = 523	100	n = 262	100
0	314	66	393	75	140	53
1	154	33	115	22	35	14
2	4	1	15	3	62	24
3	0	0	0	0	25	10
4	0	0	0	0	0	0

In animals with different scores on both ears, the highest score was counted.

including *Prevotella* sp., *Bacteroides* sp., *Neisseria* sp., and some Lactobacillaceae. *Mycoplasma hyopharyngis* was also the only bacterium present in PEN affected pigs (75%) and absent in PEN-negative pigs (100%). The

number of different viruses and bacteria detected on farms A, B, and C were 13, 11, and 17, respectively. Figure 5 shows a heatmap of selected bacteria found in the ear-scraping samples of the nursery pigs from the three



**Figure 5** Heatmap of bacteria found in scraping samples, with their loads, analyzed by nanopore sequencing (white—negative, light green—very low, green—low, light orange—moderate, orange high, red—high loads) together with the bacteriology results load (white—negative, yellow—low, orange—moderate, red—high). \*Samples with the asterisk were collected 1 week later.

farms, along with their semiquantitative abundance, and ranked by prevalence. Complete output files (viruses and bacteria) are shown in Additional file 2.

The only pathogens showing a statistically significant correlation to PEN lesions were: *M. hyopharyngis* (0.590,  $p < 0.001$ ), Porcine Parvovirus 2 (0.438,  $p = 0.015$ ), *Fusobacterium* spp. (0.404,  $p = 0.027$ ), Rotavirus C (0.385,  $p = 0.035$ ).

**Bacteriology**

Bacteriological culture of skin and lesion swabs revealed the growth of *Staphylococcus aureus* (*S. aureus*) and *hyicus* (*S. hyicus*), *Streptococcus suis* (*S. suis*), and *Streptococcus dysgalactiae* ssp. *equisimilis* (*S. dysgalactiae* ssp. *equisimilis*) as well as *Escherichia coli* (*E. coli*) in both PEN-affected and non-affected pigs. *Clostridium perfringens*, *Streptococcus hyovaginalis*, *Streptococcus agalactiae*, *Staphylococcus cohnii*, *Staphylococcus sciuri*, *Aerococcus viridans*, *Pasteurella caballi*, *Trueperella pyogenes*, and *Mannheimia* sp. were less common. More bacterial species were cultivated from samples from affected

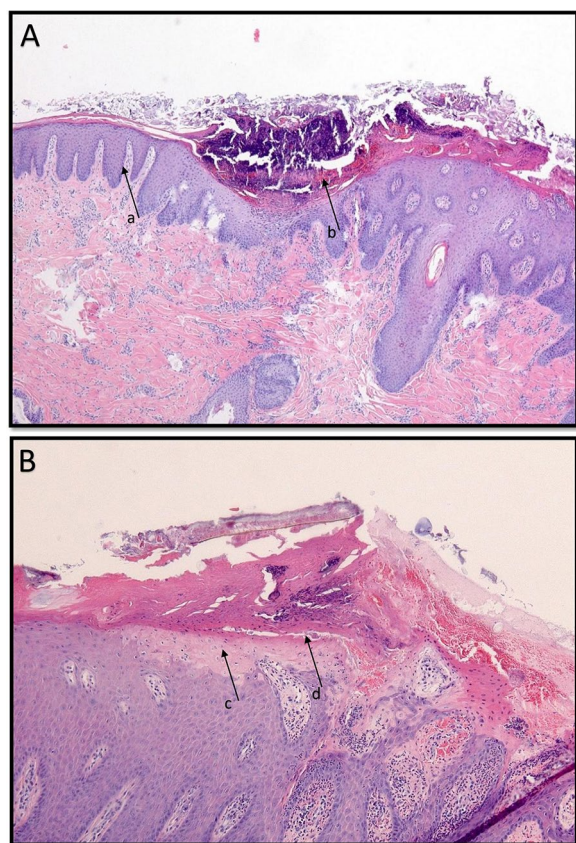
ears (13), compared to samples from non-affected ears (9). Farm A had the most diverse bacterial species namely 12. Figure 5 shows a heatmap of all bacteria cultured on agar, with their load, sorted by prevalence.

**Histopathology**

In total, 89 biopsy samples (30 pigs in each farm; one sample from farm C was lost) from ears were analyzed histologically. Epidermal hyperplasia and hyperkeratosis were identified in all afflicted tissue samples. As illustrated in Figure 6, the epidermis was eroded and covered with a crust composed of degenerated leukocytes, nuclear debris, hemorrhage, and coagulated serum proteins (serocellular crust).

In tissues with gross lesion scores 2 and 3, epidermal pallor (hydropic degeneration keratinocytes and/or spongiosis) was often marked adjacent to the hyperkeratosis and was associated with the separation of the hyperkeratotic layer (Figure 6). Neutrophilic epidermal (pustular) and dermal inflammation as well as the formation of granulation tissue was most severe in gross lesion scores





**Figure 6** Microscopic picture of biopsy of affected ear. **A** Sample with gross lesion score 3. Severe epidermal hyperplasia (a) with focal erosion covered by a serocellular crust (b). **B** Sample with gross lesion score 1. The epidermal pallor of the superficial stratum spinosum (c) and separation of the hyperkeratotic layer (d).

2 and 3. Bacterial coccoid microcolonies were found in numerous samples of afflicted tissue. In three samples (PEN lesion score 2 and 3) and one sample (PEN lesion

score 2), vasculitis and thrombosis were present. The histopathological findings of all samples are summarized in Table 2.

Modest epidermal hyperplasia and hyperkeratosis were identified in a few samples of animals with no visible ear abnormalities.

**Mycotoxins analysis**

At least one mycotoxin was present in plasma samples of 89% of affected and 100% of non-affected piglets. The most frequently found mycotoxins were OTA, ENNB1, and DON (Table 3).

On farm A, the most prevalent mycotoxins were DON, OTA, and ENNB, followed by ENNB1 and TEA. On farm B, OTA and ENNB mycotoxins were found almost exclusively, and on farm C, the main mycotoxin was OTA. In six out of nine cases, the average concentration of the mycotoxins was lower in PEN-affected animals compared to non-affected pigs.

We failed to reject the null hypothesis for all tested mycotoxins, namely DON ( $p=0.227$ ), OTA ( $p=0.266$ ), ENNB1 ( $p=0.654$ ), ENNB ( $p=0.084$ ), TEA ( $p=0.243$ ) and AME ( $p=0.331$ ). Also, an increase in the number of mycotoxins (isolated from plasma samples) was not associated (Wald  $\chi^2 = 1.91$ ,  $p=0.167$ ) with a change in the odds of having an increase in the severity of PEN, with an odds ratio of 0.761 (95% CI 0.517 to 1.121).

Analysis of the feed revealed the presence of four mycotoxins namely DON, FB1 and FB2, and ZEN (Table 4). The feed on farm A was the most contaminated in terms of concentration and number of different mycotoxins.

**Drinking water analysis and stable climate**

The results of the drinking water analyses showed a high concentration of ammonia in farm A, while the water was contaminated with *enterococci* on farms B and C

**Table 2** Prevalence of histopathological lesions in ear biopsies from nursery pigs with different severity of lesions.

Farm	A (n = 30)				B (n = 30)				C (n = 29)			
	0 (n = 8)	1 (n = 11)	2 (n = 11)	3 (n = 0)	0 (n = 10)	1 (n = 12)	2 (n = 8)	3 (n = 0)	0 (n = 10)	1 (n = 6)	2 (n = 6)	3 (n = 7)
Epidermal hyperplasia	2	11	11	–	5	12	8	–	1	6	6	7
Epidermal hyperkeratosis	2	11	11	–	2	12	8	–	5	6	6	7
Epidermal pallor	0	0	1	–	0	5	2	–	0	1	5	2
Epidermal separation	0	8	5	–	0	8	4	–	0	4	6	7
Epidermal erosion	0	11	10	–	0	11	8	–	0	4	6	7
Serocellular crust	1	11	10	–	0	9	7	–	0	5	4	5
Bacterial microcolonies	0	8	5	–	0	4	3	–	0	4	3	6
Inflammation neutrophils	1	11	11	–	0	11	8	–	1	4	6	7
Dermal granulation tissue	0	8	11	–	0	7	8	–	0	3	4	4
Vasculitis	0	0	0	–	0	0	1	–	0	0	1	1
Thrombosis	0	0	0	–	0	0	2	–	0	0	1	1

**Table 3** Percentage of mycotoxin positive plasma samples (x), and their average concentration (y) [ng/mL] obtained from pigs with and without porcine ear necrosis (PEN).

Mycotoxins	Farm	A		B		C		
		PEN	PEN+ (n = 21)	PEN- (n = 8 <sup>a</sup> )	PEN+ (n = 20)	PEN- (n = 10)	PEN+ (n = 19 <sup>a</sup> )	PEN- (n = 10)
DON	x		71	50	0	0	0	0
	y		0.68	0.64				
OTA	x		52	50	60	80	84	90
	y		0.22	0.20	0.32	0.35	0.48	0.62
ENNB1	x		24	38	0	0	0	0
	y		0.14	0.17				
ENNB	x		90	100	70	100	16	30
	y		0.28	0.37	0.26	0.38	0.17	0.12
TEA	x		38	50	0	0	0	0
	y		1.57	1.88				
AME	x		5	0	5	0	0	0
	y							

DON: deoxynivalenol, OTA: ochratoxin a, ENNB1: enniatin B1, ENNB: enniatin B, TEA: tenuazonic acid, AME: alternariol monomethyl ether.

<sup>a</sup> One sample analysis failed, and one sample got lost.

**Table 4** Mycotoxin concentration of feed samples from the nursery unit in each of the three farms (A, B and C).

Sample	Farm A		Farm B		Farm C	
	1	2	1	2	1	2
Mycotoxin (µg/kg)						
DON	97.1	132.0	21.8	24.6	27.4	32.3
FUM B1 + B2	70.9	35.5			32.4	51.4
ZEA	20.4	23.4				

One feed sample (1) was taken 1 week post-weaning, the other feed sample (2) was taken 4 weeks post-weaning.

EU reference value in feed for pigs and piglets for deoxynivalenol (DON) is 900 µg/kg, fumonisin (FUM B1 + B2) 5000 µg/kg, and for zearalenone (ZEA) 100 µg/kg, respectively.

(Table 5). The pH of the drinking water was 3.7, 7.5, and 7.3 in farms A, B, and C, respectively.

The results of the stable climate measurements on farms B and C were as follows: average temperature (± SD): farm B 27.5 °C (± 1.1), farm C 27.3 °C (± 1.0), relative humidity (± SD): farm B 59% (± 8), farm C 65% (± 6) (Figure 7).

### Discussion

The present study applied a wide variety of diagnostic approaches to assess the pathogens involved in PEN and characterize PEN lesions in nursery pigs from three farms. The ear lesions appeared at 3 to 4 weeks after weaning, but the prevalence and severity varied between farms and pens within a single weaning batch. Many different bacteria were identified by nanopore sequencing as well as by bacterial cultures, including *Staphylococcus*, *Fusobacterium*, and *Mycoplasma*. The main histopathological changes were epidermal splitting of the corneum and spinosum, hyperplasia, hyperkeratosis, and

ulceration of the epidermis with serocellular crusts. They were observed primarily in the outer layer of the epidermis, suggesting that damage is initiated from the outer part of the skin.

### Prevalence of lesions

The PEN lesions appeared in pigs of 7 to 9 weeks of age, or 3 to 4 weeks post-weaning. This is similar to previous studies [2, 17]. None of the animals showed PEN lesions at weaning. The highest prevalence of 50% was reached on farm C. This is higher than in our previous study but lower than the 80–100% prevalence reported [18, 19]. The number of affected animals steadily increased towards the end of the nursery on farm A, while there was a slight decrease in the last 2 weeks in the other two farms. Farm C, with the highest floor space availability (0.33 m<sup>2</sup>/piglet—see Additional file 1), had the highest prevalence and severity of ear lesions, the prevalence in the other two farms with less floor space for the pigs was 27% (farm A) and 12% (farm B). There was a large variation in the

**Table 5** Results of bacteriological, biochemical, and macroscopic analysis of the drinking water in the three farms.

Parameter	Farm A	Farm B	Farm C	Laboratory reference values
Macroscopic evaluation				
Physical appearance	Bright	Bright	Brown sediment	Bright
Smell	Light smell	No	Light smell	No
Color	Colorless	Colorless	Colorless	Colorless
Bacteriological analysis				
Number of coliforms (cfu/mL)	0	0	10	< 100
Intestinal enterococci (cfu/100 mL)	2	> 100	> 100	< 1
Sulfite-reducing clostridia (cfu/20 mL)	0	0	21	< 1
Aerobic bacteria (22 °C) in total (cfu/mL)	1010	1300	7000	< 100000
Aerobic bacteria (37 °C) in total (cfu/mL)	650	13	800	< 100000
Chemical analysis				
pH	3.7	7.5	7.3	4–9
Ammonia (mg/L)	145.7	0.2	0.9	≤ 2
Nitrates (mg/L)	69.0	< 10.0	12.0	≤ 200
Nitrites (mg/L)	< 0.1	< 0.1	< 0.1	≤ 0.5
Sulfates (mg/L)	115.0	81.0	20.0	≤ 250
Total hardness (°D)	29.9	13.0	4.4	≤ 20
NaCl (mg/L)	93.0	69.0	27.0	≤ 3000

prevalence of PEN between the pens of a compartment. Pens with a high and low prevalence of PEN lesions were often found next to each other. This was also observed in previous studies [2, 20], and indicates that it is important to include different pens to assess the overall prevalence in a compartment.

Apart from PEN lesions, the study also showed that many pigs were affected by abrasion-like lesions or scratches during the first week after weaning. These lesions healed very fast and were different from the PEN lesions which occurred later. The former lesions are likely due to aggressive biting, targeting mainly the head, and front body part of animals. Such biting behavior may occur during the hierarchy formation after regrouping animals, and/or when competing for feed [21]. The present study also clearly shows that the presence of an ear tag (close to the ear tip/edge) decreased almost 5 times the risk of ear mild lesions/scratches occurrence shortly after weaning. Further research is needed to confirm this result and elucidate whether biting or other mechanisms may be involved in PEN.

#### Severity of lesions

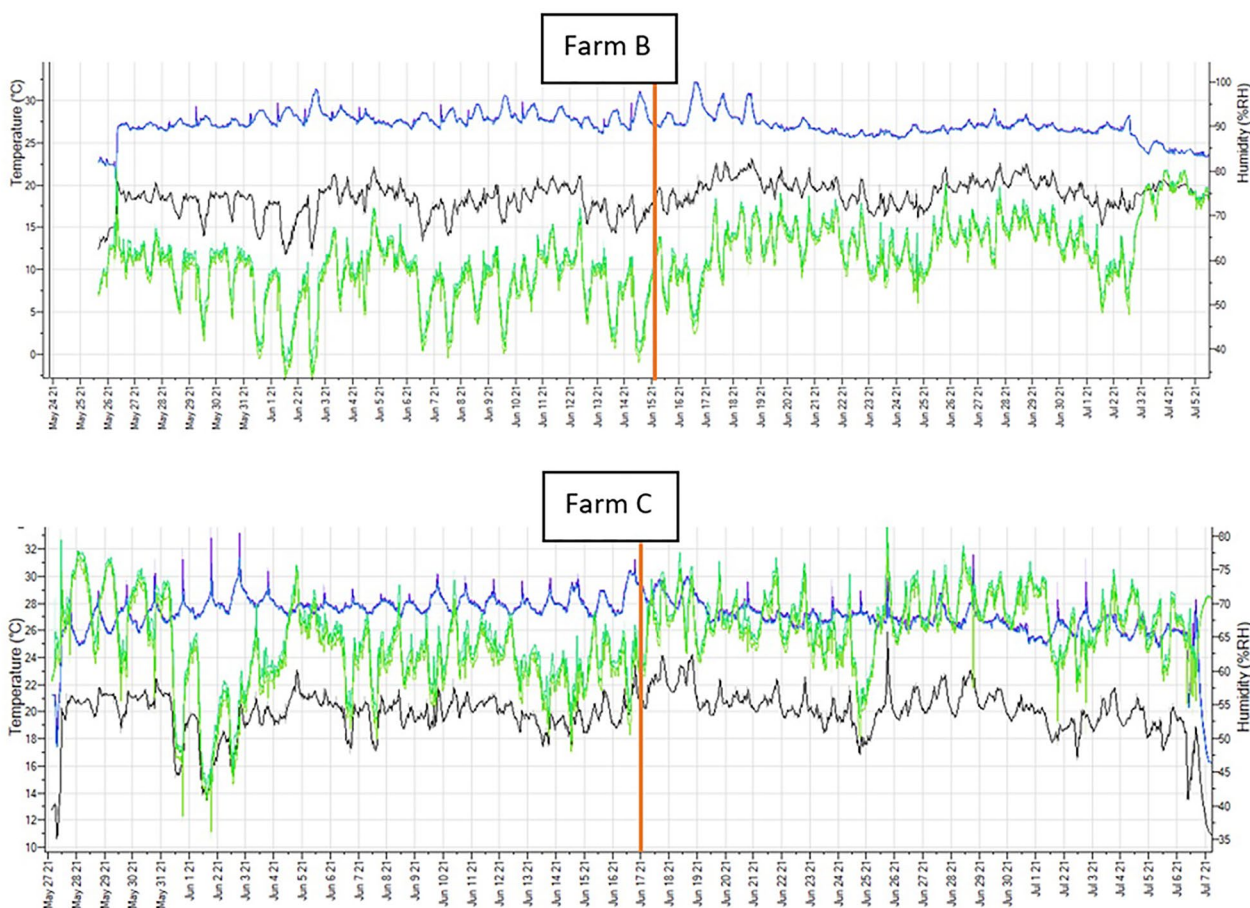
The results showed a large variation in PEN lesion severity between pigs and between farms. Farms A and B were affected mainly with lesion score 1 (88–97%), whereas Farm C was most severely affected. Half of the animals had lesions with a score of 2, and one-fifth had lesions

with a score of 3. Several affected pigs had lost parts of the ear tissue and suffered from large moist wounds.

This is more severe compared to the previous study on a different farm in which 85% of affected animals developed lesions scored as 1, and 14% with score 2. The study of Pejsak [10] noticed an overall PEN prevalence of 11–13%, with 7–8% for lesions corresponding to score 2 presented in the present study. In terms of the severity, the current study clearly showed the protective effect of an ear tag.

#### Results of nanopore metagenomic sequencing in serum and scrapings

The nanopore metagenomic sequencing of serum samples revealed the presence of several pathogens, mainly viruses. The high PPV2 and PPV5 loads found on farms B and C in both PEN-affected and non-affected animals point to an ongoing viremia. There are currently seven (1–7) PPVs described, with PPV1 being the cause of still-birth, mummification, embryonic death, and infertility [13], but the role of PPV2–7 is not well investigated [22, 23]. Some old studies link the presence of PPV (NADL-8 and Kresse strains) to skin lesions [24, 25], as in this case, there are actively growing cells with a high mitotic index, creating favorable conditions for these viruses. However, only 11% of ear scrapings in the current study (Farm B and C) contained PPV. Torque teno sus virus was another virus found in serum, but also in this case, no association



**Figure 7** Two graphs illustrating temperature (blue line), humidity (green line), and dew point (black line) measured in the compartment during the nursery period on farms B and C. The red vertical line is a timepoint when the first PEN lesions started to appear.

with the severity could be established. TTSuV is found all over the world [26, 27] there is no clear link between the virus and any clinical signs [28].

The nanopore sequencing results of the ear scrapings revealed a wide range of viruses and bacteria in the three farms, present in the lesions as well as on the healthy skin. The predominant viruses were bocaparvovirus, various parvovirus types, and TTSuV. Other viruses such as astrovirus, picobirnavirus, and rotaviruses were abundantly detected, but these are thought to be rather environmental contaminants [13]. Bocaparvovirus was first detected by Blomström et al. [29], but its pathogenicity remains unclear till now [30, 31].

Bacterial genera belonging to *Streptococcus*, *Staphylococcus*, *Fusobacterium*, *Mycoplasma*, and *Clostridium* were identified in many samples. In samples of affected animals, the bacterial loads were higher than in samples from pigs without PEN lesions. Moist and bloody wounds are a good environment for bacterial growth, so it is not clear whether the bacteria have an etiological role or whether the high load is a result of the favorable

environment to multiply. Environmental contaminating bacteria such as *Prevotella* sp. and *Bacteroides* sp. were also identified [32].

*Clostridium* and *Fusobacterium* are anaerobes that can infect open wounds and are common in the environment. The relatively high prevalence in the studied samples (53–56%) may be indicative of their potentially important role in PEN. *Fusobacterium necrophorum* is known for its ability to cause skin lesions or necrosis in various animal species [33], whereas *Clostridium perfringens* is able to induce myonecrosis and gas gangrene [34]. At histology, bacteria with a morphology of *Clostridia* sp. (rod-shaped) or *Fusobacterium* sp. (filamentous) invading the dermis were not observed. The *Staphylococcus* and *Streptococcus* species found in many samples might be involved in the pathogenesis. However, microcolonies of cocci were mainly observed associated with a superficial serocellular crust. Likely they might act as secondary invaders of the wounds. *Mycoplasma* sp. (most likely *M. hyopharyngis*) was found in 83% of affected ears and was absent in non-affected ears. This bacterium is poorly

described in the scientific literature. Kobisch and Friis reported its presence in the pharynx of a pig in 1996 [35], but no information about its possible pathogenicity has been released since then. The presence of this bacterium on affected ears might originate from ear-biting, or at least contact between the mouth of pigs and the ears of pen mates. Moreover, this suggests ear-biting might be involved in or contribute to the development of PEN lesions. Park et al. [6] discussed ear-biting as a potential cause of PEN. These findings, combined with ear-biting and chewing observed during the farm visits in the present study, may indicate the importance of the behavioral component in the genesis of severe PEN lesions, although further research is needed to confirm this. Weissenbacher-Lang [19] investigated the presence of *M. suis* by PCR in 9 PEN-positive farms, but only 2 out of 72 samples were positive. In the present study, *Mycoplasma suis* was not detected, nor were the associated clinical signs (hemolytic anemia and icterus with fever) or the lesions (vascular thrombosis and coagulopathy) [36, 37]. Therefore, the importance of *M. suis* in the pathogenesis of PEN is rather limited in the current study.

### Bacteriology

Bacteriological culturing on agar revealed that more diverse bacteria were detected in samples from affected than in non-affected ears. The most common bacteria were *Streptococcus dysgalactiae* ssp. *equisimilis* (77%), *S. suis* (30%), *Staphylococcus aureus* (73%), and *S. hyicus* (33%).

*Streptococcus dysgalactiae* ssp. *equisimilis* belongs to  $\beta$ -hemolytic streptococci and has been related to endocarditis, arthritis, and meningitis. The main route of infection is vaginal secretion where the transmission occurs mainly via injuries to the feet and skin lesions [38]. *S. suis* colonizes pigs during parturition. Clinical signs include arthritis, meningitis, and septicemia. Most pigs carry the pathogen without showing any clinical signs [39]. *S. aureus* and *S. hyicus* can be commonly found on the skin surface of healthy pigs [40]. Both pathogens can produce exfoliative toxins that can damage the epidermis. However, in many lesions, epidermal pallor (hydropic degeneration keratinocytes and/or spongiosis) with subsequent separation of the epidermis was present, which differs from the splitting of the epidermis at the level of the stratum granulosum mediated by exfoliative toxins.

A difference has been also found by Weissenbacher-Lang [19] in bacterial loads of streptococci and staphylococci between affected and non-affected animals. Those cocci can have a negative effect on the injured epidermis. Costa et al. [11] aimed to reproduce PEN lesions experimentally by using material obtained from affected animals. The inoculum contained a high concentration of

*Staphylococcus* spp. and *Streptococcus* spp. However, only mild lesions and inflammation were induced, which disappeared within 21 days after inoculation.

Metagenomic sequencing has been shown to allow accurate and semi-quantitative detection of bacteria in different types of samples [13, 15]. For most of the bacteria, there was an overall moderate to good correlation with the results of the bacterial culture. However, there were also some differences. Metagenomic sequencing allowed us to show the presence of difficult-to-grow bacteria such as *Mycoplasma*, *Fusobacterium*, and *Campylobacter* species. Differences with bacteriological culture were also observed for *Streptococcus* and *Staphylococcus*, which might be related to the growth characteristics of these bacteria. It could be because of their difficult-to-lyse Gram-positive and easy-to-(over)grow features, respectively. Finally, the sampling procedure for both diagnostic assays was different, using scrapings for nanopore metagenomics and swabs for bacterial cultures. Hence this might also impact the final output.

### Gross lesions and histology

The gross lesions were similar on the three farms. The principal histological findings included epidermal splitting of the corneum and spinosum as well as hyperplasia, hyperkeratosis, and ulceration of the epidermis with serocellular crusts together with the presence of bacteria microcolonies and neutrophils and neutrophils in the dermis. Vasculitis, thrombosis, and the involvement of cartilage occurred in less than 5% of affected tissue samples. These findings are not suggestive of an acute trauma affecting the ear auricle, but merely the influence of more repetitive external damage in combination with bacterial infection and toxin production.

The mild lesions differ slightly from those defined by others [1, 41]. They found intra-epidermal abscesses, intracellular keratinocyte edema, para-keratotic hyperkeratosis of the stratum corneum, neutrophil infiltration, vacuolar degeneration and necrosis of basal cells, and the subsequent formation of intra-epidermal vesicles. A focal epidermal necrosis in the early stages, with subsequent extension to broader areas, degeneration of the blood vessels, and vesicular dermatitis was reported. Vesicular dermatitis was not found in the present study [19].

### Mycotoxins

The mycotoxins OTA and ENNB were found in plasma samples on all farms. Other detected mycotoxins in plasma such as DON, ENNB1, and TEA were only found in farm A. The feed analysis data also showed that farm A had the highest level of mycotoxins contamination (DON, FUM B1 + B2, and ZEA). On farm A, the DON concentrations in the feed samples were 97 and 130  $\mu\text{g/l}$

kg, and DON was detected in 65% of the plasma samples. In a previous study [2], DON concentrations in the feed were 60 to 175 g/kg, whereas it was not detected in plasma.

There were no major differences in the mycotoxin levels in the plasma of PEN-affected and non-affected animals. This corroborates with previous studies [2, 19]. Actually, in six out of nine samples, the average concentration was higher in the animals without PEN lesions. No associations between mycotoxins in the plasma and PEN severity score were seen.

### Drinking water, and stable climate

The results of the drinking water analyses showed a high concentration of ammonia in farm A and contamination with *enterococci* on farms B and C. The origin of the high ammonia concentration is not clear, but it might be related to the acidification procedure or the use of fertilizers in the close area to the farm, as deep drainage water was used. Contamination with sewage or animal wastes is less likely in this case, as much higher bacterial contamination would be expected [42]. The high levels of enterococci can point to contamination of the drinking water with manure [43]. The drinking water quality should be improved, but the role of deviations in these parameters in the PEN problems in these farms is not clear. Deviations are also often seen in other farms without PEN problems.

No major abnormalities were present in ambient temperature and relative humidity in the nursery units of farms B and C. The relative humidity slightly increased in the week when PEN lesions appeared. The increase was minor and likely not relevant to the present study.

The evolution of PEN lesions was similar in the three farms. The lesions appeared 3 to 4 weeks after weaning and peaked towards the end of the nursery. The prevalence and severity of the lesions varied substantially between pens. The most abundant bacteria detected by nanopore sequencing were *Staphylococci*, *Streptococci*, *Clostridium*, *Fusobacterium*, and *Mycoplasma*. Interestingly, bacteria, namely *Mycoplasma hyopharyngis*, was only detected in PEN-affected pigs. Histopathological lesions were mainly found in the outer layer of the epidermis. Therefore, the results suggest that the suckling, chewing, or biting of pigs on the ear of pen mates followed by bacterial infection and skin damage may be important in the pathogenesis. Further research focusing on monitoring the behavior of pigs throughout the nursery phase is therefore warranted.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13567-023-01218-1>.

**Additional file 1. Questionnaire results.** Characteristics of the farms.

**Additional file 2. Metagenomic analysis results.** Full results of the metagenomic analysis performed on ear scrapings of affected and non-affected pigs.

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### Authors' contributions

MM designed the study, performed the field trial, together with retrieving results and drafting and finalizing the manuscript. KC supervised histopathological examination. ST and NV were responsible for metagenomic investigation. IC performed the statistical analysis, helped to design the study. SK supervised the blood mycotoxins analyses. DM supervised the study, major contributor in designing the study and writing the manuscript. All authors read and approved the final manuscript.

### Declarations

#### Competing interests

The authors declare that they have no competing interests.

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