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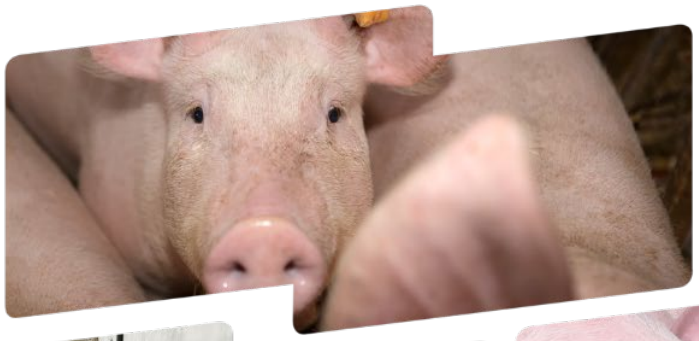
VEEPEILER VARKEN

Rapport d'activités 2022-2023



Inhoudsopgave

1 Introduction

Le programme 'Veepeiler Varken' a été créé dans le but de soutenir le secteur porcin en Belgique par des études pratiques et des conseils de seconde ligne. Veepeiler Varken a vu le jour à l'initiative de DGZ et des facultés de médecine vétérinaire de l'université de Gand et de l'université de Liège, et est soutenu financièrement par le Fonds sanitaire.
Veepeiler Varken repose sur deux piliers importants : la médecine vétérinaire de seconde ligne et des projets de recherche courts et axés sur la pratique.

Médecine vétérinaire de seconde ligne

Veepeiler Varken fournit des conseils de seconde ligne aux élevages qui rencontrent des problèmes dont la cause est toujours inconnue malgré les recherches. Les différentes parties prenantes (vétérinaire de Veepeiler, éleveur de porcs, vétérinaire de l'exploitation, conseiller en alimentation, conseiller d'exploitations d'élevage...) se réunissent pour étudier le problème de façon multidisciplinaire et de manière plus approfondie afin de trouver une solution. En accord avec le vétérinaire de l'exploitation, des études complémentaires peuvent être effectuées (par ex. études en laboratoire sur des échantillons biologiques, sur l'eau potable et les aliments, des autopsies, des inspections d'abattoirs, etc.). Après chaque visite d'exploitation, un rapport est rédigé. Il comporte des conseils et un plan d'approche. L'éleveur, le vétérinaire de l'exploitation et les éventuelles autres personnes concernées en reçoivent une copie. L'exploitation est visitée à plusieurs reprises en vue d'assurer un suivi de la problématique ainsi que d'aborder et d'évaluer les mesures prises.

Projets de recherche courts et axés sur la pratique

Outre l'apport de médecine vétérinaire de seconde ligne, Veepeiler Varken se consacre également à la réalisation de projets de recherche courts et axés sur la pratique concernant une problématique spécifique dans le cadre des soins de santé porcine.

2 Projets terminés

2.1 Effets du PVP sur les symptômes associés au CVP2 chez le porcelet et le porc d'engraissement flamands

2.1.1 Contexte

Les vétérinaires praticiens indiquent à Veepeiler qu'ils ont constaté que les charges virales sanguines sont plus souvent plus élevées que par le passé lors de tests PCR pour le circovirus porcin de type 2 (CVP2). Il ressort de données de recherche que la co-infection avec des parvovirus porcins (PVP) pourrait être à l'origine de ce constat (3,4,5,6,7). L'examen des résultats des analyses (PCR CVP2) de 2019 à 2021 effectuées à la DGZ met en effet en lumière une augmentation de la charge virale dans les échantillons de sang.

2.1.2 Protocole expérimental

Ce projet a pour objectif de déterminer s'il existe un lien entre les charges virales du CVP2 dans le sang (PCR) et l'apparition de certains types de PVP (Pathosense). À cet effet, 25 échantillons combinés (pooling) avec différentes charges de CVP2 (PCR), qui sont analysés à la DGZ, seront sélectionnés et examinés par Pathosense afin de détecter la présence de PVP.

2.1.3 Résultats

Les résultats ont montré une augmentation du nombre total de parvovirus en fonction de l'augmentation de la charge PCR du CVP2. L'augmentation était détectable pour les différents types de parvovirus, à l'exception du parvovirus de type 4 qui n'a pas été détecté du tout lorsque la charge PCR du CVP2 était élevée. Pour tout complément d'information, veuillez consulter le résumé à l'annexe 1.

2.2 Optimisation du nettoyage et de la désinfection des porcheries

2.2.1 Contexte

Le nettoyage et la désinfection constituent un élément important de la biosécurité dans les exploitations porcines, et l'une des mesures déterminantes de la lutte contre la propagation des infections au sein d'un élevage. Les surfaces et les matériaux souillés par le lisier peuvent perpétuer l'infection dans l'exploitation en réinfectant de manière répétée les animaux qui entrent en contact avec eux (De Wulf en Van Immerseel, 2018). En effet, il a été démontré qu'un très grand nombre de pathogènes (dont Brachyspira hyodysenteriae, Erysipelothrix rhusiopathiae, E. Coli, Lawsonia intracellularis, Pasteurella multocida, CVP2, le virus de la diarrhée épidémique porcine (vDEP), le virus du syndrome dysgénésique respiratoire porcin (VSDRP), Salmonella,

Streptococcus suis et le virus Influenza) peuvent être transmis indirectement par le lisier, entre autres (De Wulf en Van Immerseel, 2018). Un nettoyage adéquat garantit l'élimination de cette matière organique qui sert de substrat de reproduction aux micro-organismes. Il a également été démontré que le nettoyage entraîne une nette diminution du nombre de germes, qui diminue encore après la désinfection (Luyckx et al., 2015a).
Un projet Veepeiler sur le nettoyage et la désinfection des porcheries a déjà été mené en 2017. L'effet du nettoyage et de la désinfection peut être contrôlé à l'aide de plaques de contact Rodac ou d'hygiénogrammes. Ces plaques donnent une image de la contamination bactérienne. Sur les 111 exploitations qui ont participé, la moitié a dû effectuer un second contrôle, reconnaissons-le, après avoir reçu des conseils sur le protocole de nettoyage et de désinfection. Ces exploitations ont amélioré leur score d'hygiénogramme.

À la suite de l'apparition de la peste porcine africaine en Belgique, il a été décidé que chaque vétérinaire d'exploitation serait tenu de réaliser une enquête annuelle sur les risques dans les élevages de porcs. Cette mesure est en vigueur depuis l'année dernière. L'étude sur les risques porte, entre autres, sur le protocole de nettoyage et de désinfection. Les premiers résultats (n=3700) montrent que la plupart des exploitations (87 %) disposent d'un protocole de nettoyage et de désinfection qui est respecté. Malheureusement, seuls 8 % d'entre elles contrôlent le processus.

2.2.2 Protocole expérimental

Le projet a pour objectif de mettre en lumière les résultats obtenus avec le protocole de nettoyage et de désinfection dans les exploitations porcines flamandes et de les comparer avec ceux du projet de 2017. À cet effet, les exploitations pouvaient faire placer des plaques de contact dans des salles nettoyées et désinfectées et faire exécuter une analyse de l'eau de nettoyage. Si une exploitation adaptait son protocole sur la base des résultats, elle pouvait faire réaliser un nouveau contrôle.

2.2.3 Résultats

La batterie de porcelet obtient en moyenne de meilleurs résultats après le nettoyage et la désinfection que les salles de mise bas. Dans les deux salles, les endroits élevés tels que le plafond, le mur à hauteur des yeux et la ventilation, ainsi que les endroits difficiles à nettoyer tels que l'équipement et les mangeoires, obtiennent de moins bonnes notes que les endroits plus bas tels que le sol et les cloisons murales. Les exploitations qui ont adapté leur protocole ont réussi à obtenir un meilleur score en termes de plaques de contact. Les mesures les plus importantes sont l'utilisation d'une solution de trempage ou d'un désinfectant et, pour les exploitations qui utilisaient

déjà ces produits, leur utilisation correcte. Celle-ci comprend une quantité, une concentration et un temps de trempage suffisants, et ce conformément aux instructions du fabricant. Les résultats de ce projet sont tout à fait comparables à ceux du projet de 2017. Pour tout complément d'information, veuillez consulter l'annexe 2.

2.3 L'importance de la morsure d'oreille dans l'apparition de la nécrose d'oreille du porc (PEN).

2.3.1 Contexte

La nécrose d'oreille ou auriculaire du porc (en anglais porcine ear necrosis (PEN)) est une affection fréquente dans les élevages porcins. Des études antérieures ont montré que la prévalence de la PEN peut parfois dépasser 80 % et qu'elle peut présenter une variabilité considérable d'une exploitation à l'autre et d'une étude à l'autre. À ce jour, ni l'étiologie ni la pathogénie de cette maladie ne sont connues. Les facteurs de risque possibles peuvent être très divers, tels que les infections, les mauvaises conditions climatiques et d'hébergement, et les mycotoxines. Cependant, le rôle exact de ces facteurs et leur importance n'ont pas été élucidés. En outre, des porcelets avec et sans PEN peuvent cohabiter dans la même porcherie, ce qui suggère que des facteurs non présents au niveau de l'exploitation ou de la porcherie (par exemple, la composition des aliments ou le climat de la porcherie) peuvent également jouer un rôle. Malik et al. (2022 – en préparation) ont récemment montré que Mycoplasma hyopharyngis était commun dans les lésions caractéristiques de la PEN chez les porcs affectés, alors que cette bactérie est absente chez les animaux non affectés du même enclos (projet Veepeiler PVP-20-04.) Les observations réalisées au cours de l'étude ont également montré qu'il y avait des cas de morsure/succion des oreilles, ainsi que de morsure de la queue ou des flancs. Cependant, ces études n'ont pas quantifié l'ampleur de la succion ou de la morsure des oreilles, et n'ont pas non plus examiné dans quelle mesure les porcelets atteints de PEN présentaient des degrés de morsure différents des porcs non atteints.

2.3.2 Protocole expérimental

L'étude a pour objectif principal d'examiner le rôle de la morsure/succion d'oreille dans l'apparition de la PEN chez le porc. À cette fin, les morsures d'oreille (nombre de morsures, durée des morsures) ont été étroitement surveillées et quantifiées. Une comparaison a été faite entre les porcs affectés et non affectés, et entre les bâtiments d'élevage avec une prévalence élevée et faible de PEN. À cette fin, 3 exploitations avec une forte prévalence de PEN (>30 %) chez les porcelets sevrés ont été sélectionnées.

2.3.3 Résultats

Les porcelets des enclos à forte prévalence de nécrose auriculaire ont montré deux fois plus de manipulations des oreilles que les porcelets évoluant dans des espaces à faible prévalence. La « manipulation » (sucer- mordiller-mâchouiller) des oreilles commence avant l'apparition des premières lésions et il y a toujours un décalage d'environ 2 à 3 semaines entre le pic des manipulations et le pic des lésions. Le nombre de manipulations a un effet plus important sur l'apparition des lésions que leur durée. Aucune corrélation n'a été trouvée entre le climat de la porcherie et les lésions ou entre la présence de différentes bactéries/virus et l'apparition de lésions. Les résultats montrent un effet significatif de la manipulation orale sur l'apparition de la nécrose de l'oreille. Pour tout complément d'information vous pouvez écouter podcast qui se trouve sur ce lien et lire l'article scientifique qui se trouve à l'annexe 3.

3 Projets en cours

3.1 Prise colostrale chez les porcelets

3.1.1 Contexte

La prise colostrale est essentielle pour les performances et la santé des porcelets, et Veepeiler Varken reçoit régulièrement des questions de vétérinaires d'élevage à ce sujet. Il existe plusieurs méthodes de test qui peuvent donner une indication sur l'ingestion colostrale chez le porcelet. Cependant, dans la pratique, il est rare que des analyses soient effectuées pour obtenir plus d'informations sur l'ingestion du colostrum. De plus, à notre connaissance, aucune donnée n'est disponible sur la gestion du colostrum et la prise colostrale chez le porcelet en Flandre.

3.1.2 Protocole expérimental

Le projet a pour objectif de mieux appréhender la gestion du colostrum et la prise colostrale chez le porcelet dans les élevages de porcs flamands. À cet effet, 75 élevages de porcs ont pu faire effectuer un échantillonnage après avoir répondu à une enquête. Il s'agissait d'échantillons de sang de 5 truies et, par truie, de 6 porcelets âgés de moins de 7 jours. En déterminant les titres d'anticorps pour le CVP2 et en comparant ces niveaux des porcelets avec ceux de la truie mère, il est possible de déterminer si les porcelets ont ingéré suffisamment de colostrum.

3.1.3 État d'avancement

Quatre-vingts exploitations ont répondu à l'enquête et ont envoyé un ensemble d'échantillons. Dix d'entre elles ont également effectué un deuxième échantillonnage par la suite. Les résultats du contrôle du colostrum et de l'enquête sont en cours de traitement.

3.2 Porcelets nés virémiques pour le VSDRP : prévention, approche et suivi

3.2.1 Contexte

Le virus du syndrome dysgénésique et respiratoire du porc (VSDRP) est présent de manière endémique dans les élevages porcins flamands. Il provoque des problèmes respiratoires chez le porcelet et le porc d'engraissement, et est également connu comme un virus immunosuppresseur. Si les porcelets sevrés les plus jeunes semblent être infectés par le VSDRP, nous supposons que le statut du VSDRP chez les truies n'est pas stable et que les porcelets sont déjà nés virémiques. Dans ce projet, nous visons à vérifier que c'est bien le cas.

3.2.2 Protocole expérimental

Le projet a pour objectif d'étudier si les porcelets naissent virémiques pour le VSDRP dans les exploitations où les porcelets sont testés positifs au VSDRP au sevrage. À cette fin, les exploitations où les porcelets sont testés positifs au VSDRP après le sevrage (PCR) ont pu faire tester 2 pools de fluides de traitement (FT) pour détecter l'activité du VSDRP à l'aide d'un test PCR. Les exploitations intéressées peuvent être suivies et/ou accompagnées par la suite pour vérifier les causes éventuelles et l'approche à adopter.

3.2.3 État d'avancement

Quatorze exploitations ont participé et ont été soumises à un test unique des fluides de traitement. Huit d'entre elles font l'objet d'un suivi ultérieur. Le projet est clôturé et les résultats, traités.

3.3 Causes de mortalité des truies

3.3.1 Contexte

Dans la pratique, quelque 45 % des truies sont remplacées en moyenne chaque année. En d'autres termes, une truie produit en moyenne 4 portées au cours de sa vie. La durée de vie utile d'une truie est donc d'environ 2 ans. D'un point de vue économique, la règle de base est de ne pas remplacer les truies trop tôt afin de pouvoir ventiler les coûts d'élevage sur une plus longue période. En effet, les meilleurs résultats de production sont obtenus entre les parités 3 et 6. Une élimination trop précoce peut être justifiée du point de vue de l'élevage, mais pas du point de vue financier. Après tout, l'achat d'une cochette représente un coût de production important. Par ailleurs, il convient de ne pas éliminer les truies trop tard. En effet, un trop grand nombre de truies relativement âgées (parité □7) dans une exploitation peut entraîner une augmentation de la mortalité embryonnaire, du nombre de porcelets mort-nés, de la variation du poids à la naissance

et de la taille de la cochonnée, du nombre de porcelets morts, du nombre de mamelles présentant des anomalies et des malformations des onglons et des pattes.

L'élimination de la plupart des truies est planifiée. Cela se produit dans la grande majorité des cas après le sevrage des porcelets. Les principales raisons sont des problèmes de fertilité, la vieillesse et des problèmes locomoteurs. Cependant, il arrive que cette élimination ne soit pas planifiée. De manière générale, le décès de la truie en est la cause. La perte de truies due à la mortalité était traditionnellement la quatrième cause d'élimination des truies. Dans une étude réalisée par De Letter (2002), la mortalité des truies dans 14 élevages flamands au cours de la période 1995-2001 était d'environ 3,6 %, passant de 3,1 % en 1995 à 4,8 % en 2001. Sur une base annuelle, la mortalité des truies ne devrait pas dépasser 5 %. Cependant, une très forte augmentation de la mortalité des truies a été observée ces dernières années. Dans un nombre croissant d'exploitations, la mortalité des truies dépasse la barre des 10 %. Dans une étude danoise portant sur 10 élevages de porcs, la mortalité annuelle des truies était de 14 % (min. 6,4 % – max. 18,5 %) (Kongsted et al., 2021). En d'autres termes, ces pertes représentent non seulement un coût économique très important pour l'éleveur, mais aussi un problème de bien-être animal. Qui plus est, il nuit à la motivation et au bien-être de l'éleveur.

Traditionnellement, le risque de mortalité des truies était plus élevé dans les grandes exploitations, pendant l'été, au cours de la période péripartale et chez les truies dont l'alimentation n'était pas optimale. Les principales causes signalées dans la littérature sont les troubles gastro-intestinaux (par exemple les torsions), l'insuffisance cardiaque, les infections urinaires, l'hyperthermie maligne, les problèmes péripartaux (par exemple, le prolapsus utérin) et les infections (érysipèle du porc, Clostridium) (rapport d'autopsie de la DGZ, 2020). Cependant, les causes de la mortalité des truies dans les élevages belges actuels ne sont pas connues. La situation ayant radicalement changé par rapport à celle qui prévalait il y a 20 ans par exemple (forte augmentation du nombre d'animaux produits, au moins un doublement de la mortalité des truies, taux de remplacement plus élevé, consommation d'aliments plus importante), il était urgent d'étudier les causes de la mortalité des truies.

3.3.2 Protocole expérimental

Quinze exploitations réparties sur l'ensemble du territoire belge sont incluses dans l'étude. Les critères de sélection sont la volonté de participer à l'étude et une mortalité des truies d'au moins 5 % au cours de l'année écoulée. Chaque exploitation sera visitée au début de l'étude et diverses données d'élevage lui seront demandées. Cette étude a pour objectif spécifique d'identifier les causes de la mortalité des truies dans les élevages de porcs belges. Elle peut conduire à la mise en œuvre de mesures de contrôle plus ciblées, à une réduction de la mortalité des truies et à une amélioration de la rentabilité des exploitations. Le suivi des élevages s'étendra sur un an. Dans

chacun d'entre eux, un maximum de 8 truies seront autopsiées. Le cas échéant, par exemple, si l'autopsie n'a pas permis d'expliquer clairement la mortalité, des tests de laboratoire supplémentaires seront effectués pour poser un diagnostic.

3.3.3 État d'avancement

Quinze exploitations présentant une mortalité des truies supérieure à 5 % ont participé au projet. Les dernières visites d'exploitations sont en cours et les résultats seront ensuite traités.

3.4 Extension de la surveillance des souches du VSDRP à l'aide du séquençage du génome entier

3.4.1 Contexte

Le virus du syndrome dysgénésique respiratoire porcin (VSDRP) est endémique dans les élevages belges. Il provoque des problèmes respiratoires chez le porcelet et le porc d'engraissement, des troubles de la fertilité chez la truie et est également connu comme un virus immunosuppresseur. Présent dans le monde entier, le VSDRP peut être divisé en deux génotypes : le type nord-américain (NA) et le type européen (UE). Ils circulent tous deux en Belgique. Au sein de chaque type, différentes souches présentant éventuellement différents degrés de virulence sont détectées. L'introduction d'une nouvelle souche dans une exploitation ou dans une région particulière peut perturber l'équilibre endémique et donner lieu à de graves flambées épidémiques de VSDRP. Depuis 2020, l'Espagne a été aux prises avec plusieurs épisodes de ce type causés par une telle nouvelle souche. Ces foyers s'accompagnent de taux d'avortement élevés et d'une forte mortalité chez les porcelets sevrés. Cette nouvelle souche, de type européen et appelée Rosalia, est apparue après plusieurs recombinaisons avec d'autres souches européennes, dont la virulente souche PR40 provenant d'Italie. L'introduction de Rosalia ou d'autres souches virulentes en Belgique pourrait avoir des conséquences importantes pour notre secteur porcin. Une détection et une identification rapide de cette nouvelle souche se révèlent donc cruciales pour rester au fait de la situation. L'objectif est de surveiller les exploitations porcines touchées par un foyer grave de VSDRP afin de détecter rapidement la présence éventuelle de la souche Rosalia ou d'autres « nouvelles » souches en Belgique. En outre, il s'agit de surveiller toute importation de porcs (reproducteurs) vivants en provenance d'Espagne en utilisant le séquençage du génome entier via le séquençage rapide par nanopore (PathoSense BV).

3.4.2 Protocole expérimental

Afin de pouvoir identifier les souches de VSDRP, on utilisera le séquençage du génome entier par nanopore rapide de PathoSense (fournisseur de services certifié d'Oxford Nanopore Technologies). Seuls les échantillons dont la valeur du CT du test PCR VSDRP en temps réel est inférieure à 27 seront examinés. L'accent sera mis sur l'analyse d'échantillons de fœtus et de sérums provenant d'animaux malades (problèmes respiratoires) et d'animaux importés d'Espagne.

3.4.3 État d'avancement

Quarante-deux échantillons ont déjà été sélectionnés, ils seront examinés et la sélection des échantillons se poursuit.

4 Visites d'exploitations dans le cadre de la médecine vétérinaire de seconde ligne

4.1 Nombre de visites

En 2022, Veepeiler Varken a réalisé 64 visites d'exploitation dans 43 exploitations, 48 visites ont été effectuées par la DGZ et 16 par Ugent. Le nombre d'exploitations suivies par la DGZ était de 31 et de 12 par Ugent.

En 2023, Veepeiler Varken a réalisé 74 visites d'exploitation dans 44 exploitations, 47 visites ont été effectuées par la DGZ,16 par Ugent et 1 par ULG. Le nombre d'exploitations suivies par la DGZ était de 27 et de 16 par Ugent.

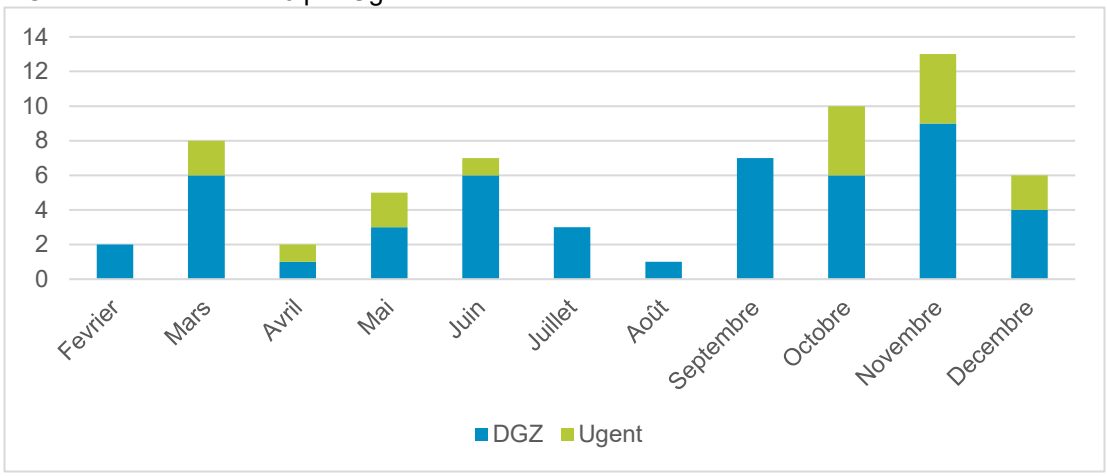


Figure 1: *Nombre mensuel de visites d'exploitations effectuées en 2022 dans le cadre de la médecine vétérinaire de seconde ligne du Veepeiler.*

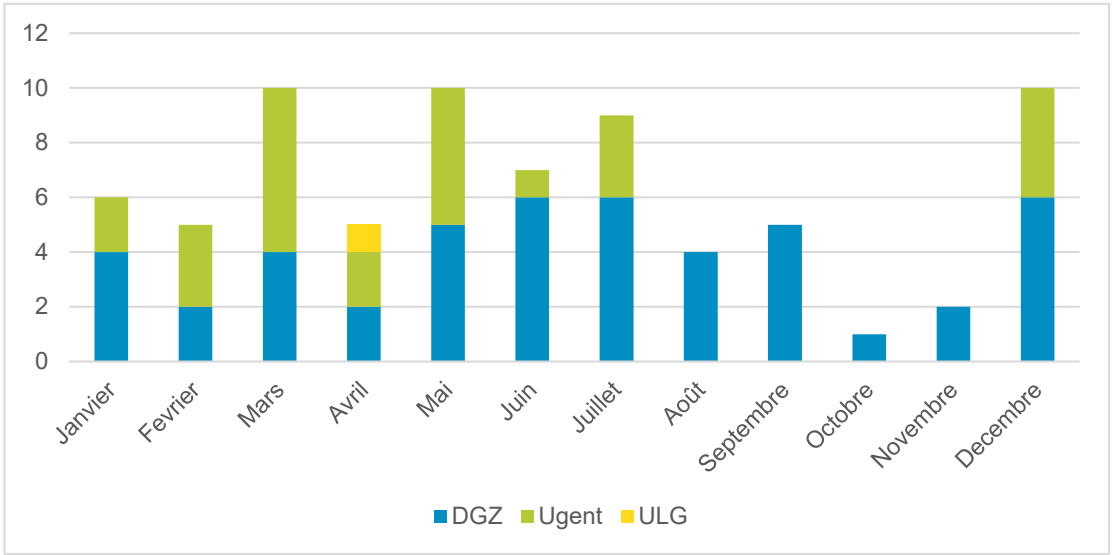


Figure 2: *Nombre mensuel de visites d'exploitations effectuées en 2023 dans le cadre de la médecine vétérinaire de seconde ligne du Veepeiler.*

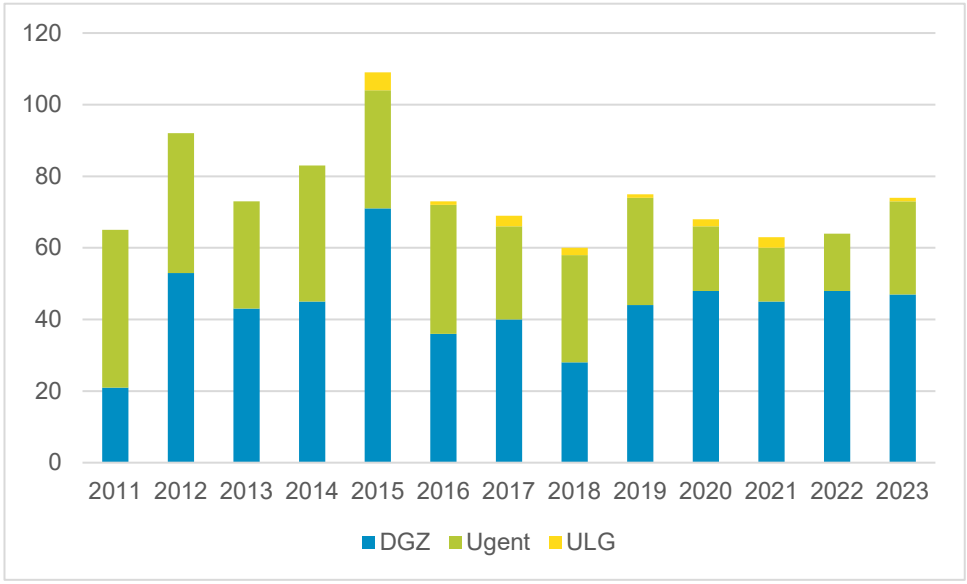


Figure 3: *Évolution du nombre de visites d'exploitations effectuées dans le cadre de la médecine vétérinaire de seconde ligne du Veepeiler au fil des années.*

4.2 Motifs des demandes de visites d'exploitation

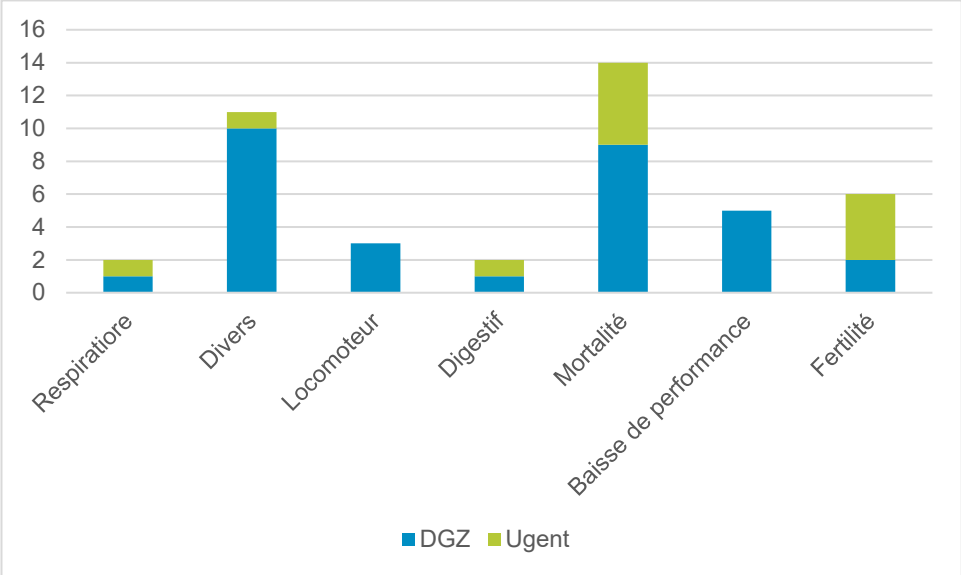


Figure 4: *Motifs des demandes de visites des exploitations dans le cadre de la médecine vétérinaire de seconde ligne du Veepeiler Varken en 2022.*

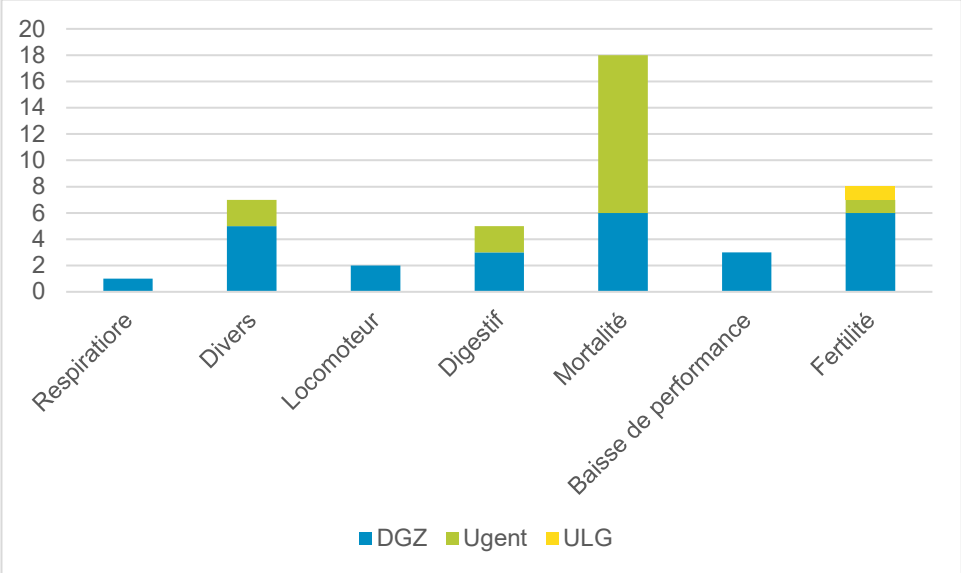


Figure 5: *Motifs des demandes de visites des exploitations dans le cadre de la médecine vétérinaire de seconde ligne du Veepeiler Varken en 2023.*

La raison principale des demandes des conseils de deuxième ligne en 2022 et 2023 étant la mortalité. La plupart des exploitations ayant des problèmes de mortalité ont participé au projet sur la mortalité des truies. Les problèmes respiratoires chez les porcs d'engraissement et les cochettes comprennent la rhinite atrophique et Mycoplasma hyopneumoniae. La catégorie « Autres »

comprend les problèmes liés au SDRP, aux troubles nerveux, à la nécrose des mamelles, à l'agressivité, à la colostrum et au nettoyage et à la désinfection. La locomotion concerne les élevages ayant des porcelets boiteux et raides. Les problèmes digestifs concernent la diarrhée chez les porcelets. Enfin, la fertilité comprend les problèmes péripartaux, l'absence de gestation et des porcelets mort-nés.

4.3 Causes probables des problèmes observés dans les exploitations

Dans de nombreuses exploitations, les causes des problèmes sont multifactorielles. Veepeiler Varken encourage à les examiner de plus près et se pose en intervenant indépendant entre les différentes parties prenantes (laboratoires, spécialistes en alimentation, etc.). On peut ainsi arriver à un diagnostic étiologique dans le but de trouver des solutions ou des moyens d'améliorer la problématique.

Il n'est toutefois pas toujours possible d'établir un diagnostic étiologique et les problèmes découlent souvent d'une gestion déficiente sur laquelle vient se greffer une cause infectieuse.

4.4 Tendances observées : – comparaison des motifs de demandes et des causes probables

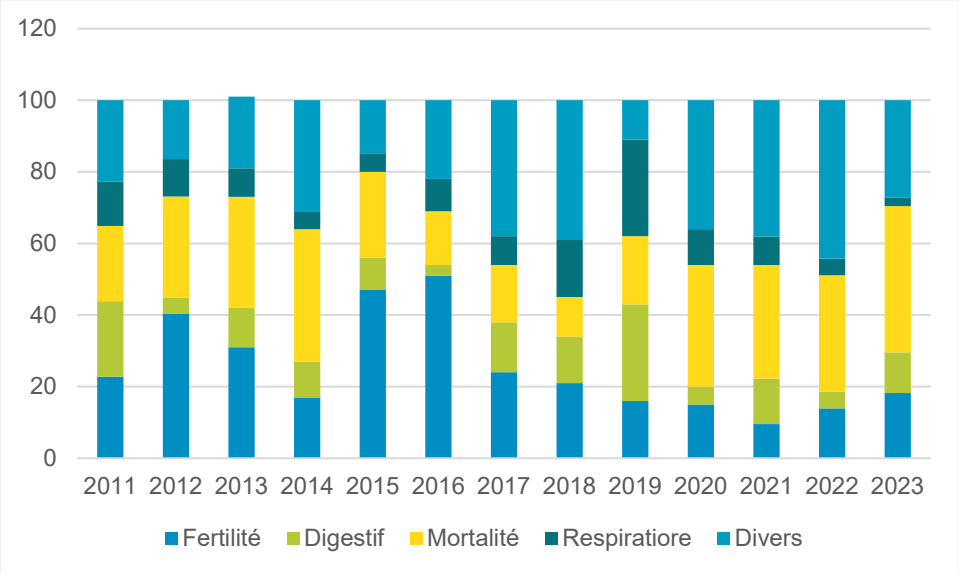


Figure 6: *Pourcentage des motifs de demandes de visites d'une exploitation dans le cadre de la médecine vétérinaire de seconde ligne de Veepeiler Varken au cours des dernières années.*

Lors de l'interprétation des chiffres dans le graphique ci-dessus, il convient de tenir compte du fait que les quantités sont relativement réduites et que quelques visites en plus ou en moins peuvent déjà engendrer une grande différence de pourcentage.

5 Autopsies

Les carcasses présentées chez DGZ en vue d'une autopsie dans le cadre de la médecine vétérinaire de seconde ligne sont toujours en rapport avec une visite réalisée dans l'exploitation concernée. En 2021, DGZ a réalisé 52 dossier d'autopsies pour Veepeiler.

5.1 Anomalies les plus fréquemment rencontrées à l'autopsie

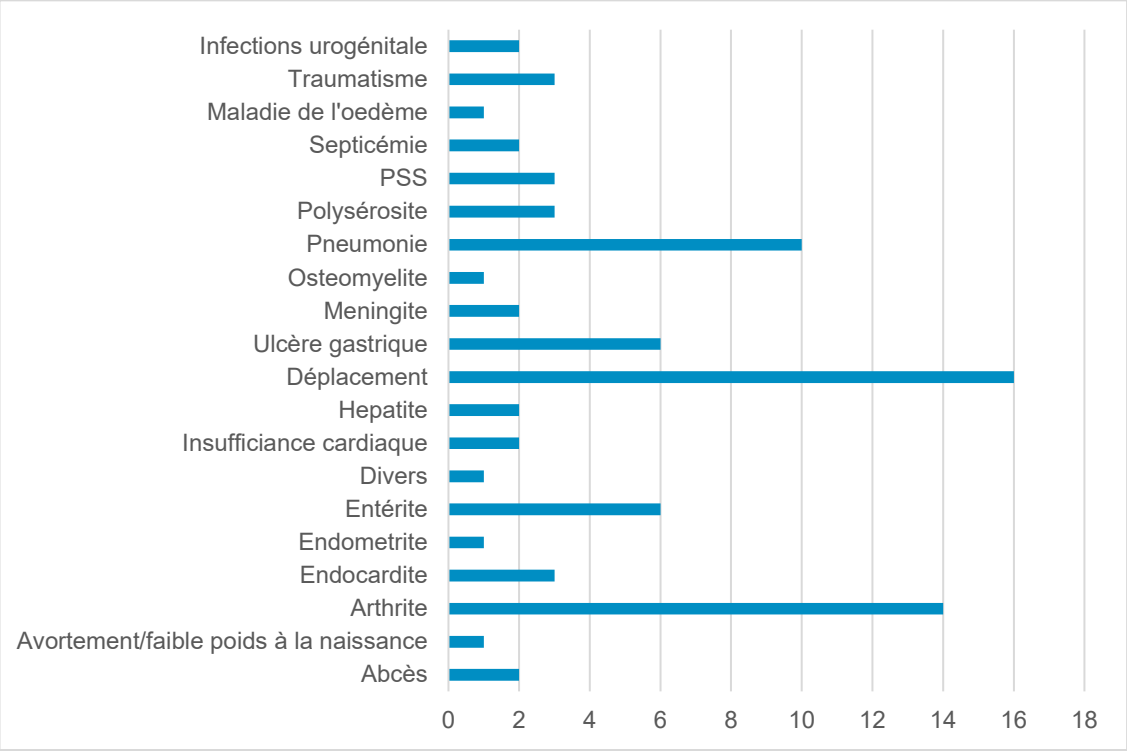


Figure 7: *Anomalies constatées Sur des cadavres autopsiés dans le cadre de la médecine vétérinaire de seconde ligne du Veepeiler Varken en 2022.*

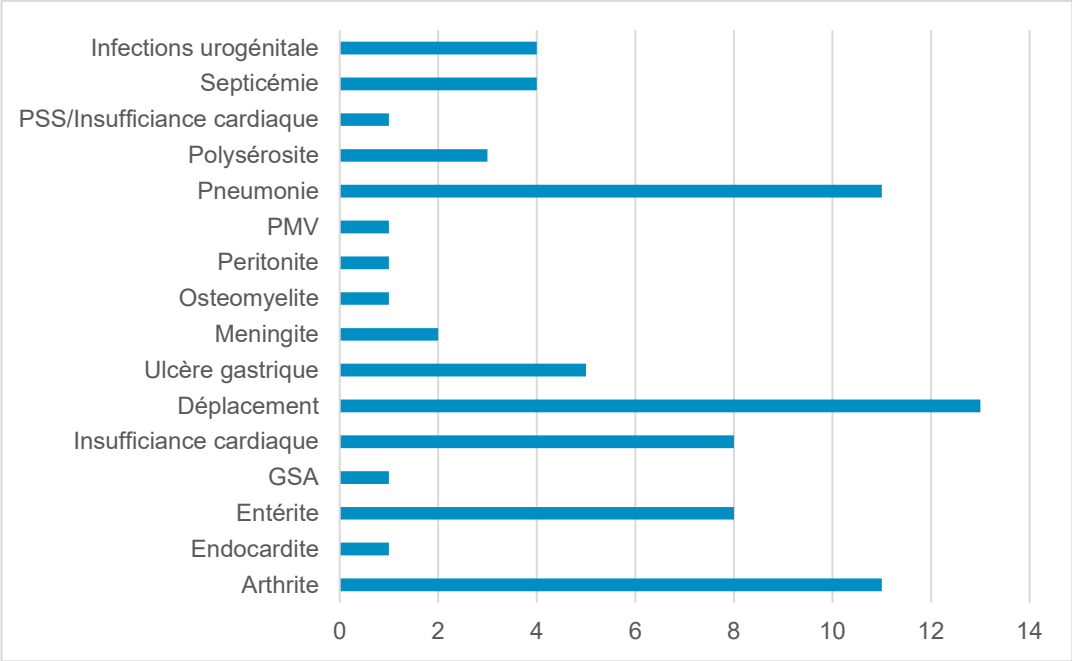


Figure 8: *Anomalies constatées Sur des cadavres autopsiés dans le cadre de la médecine vétérinaire de seconde ligne du Veepeiler Varken en 2023.*

5.2 Tendances observées – comparaison avec les années précédentes

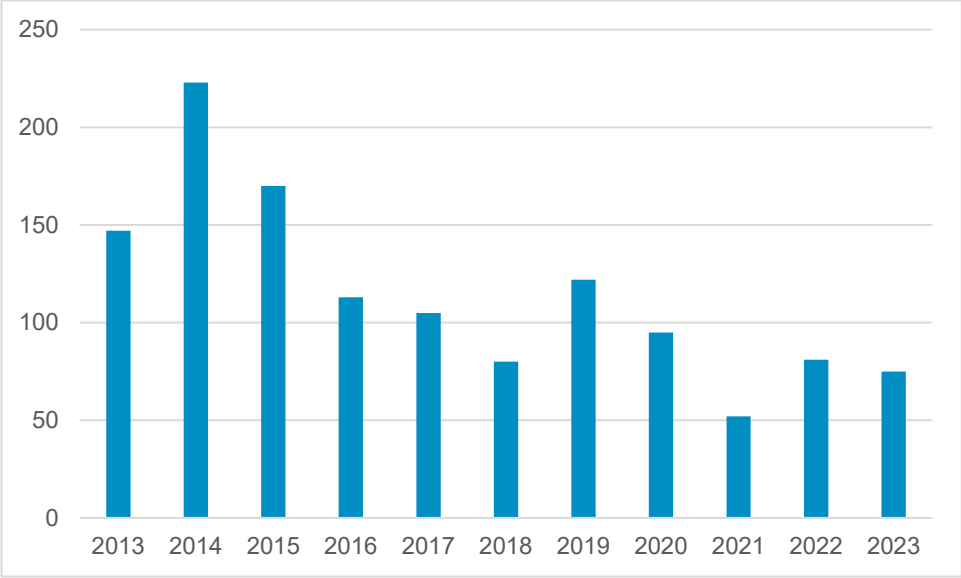


Figure 9: *Évolution du nombre d'autopsies effectuées dans le cadre du Veepeiler Varken par année.*

6 Publications Veepeiler Varken 2022-2023

Date	Type	Magazine/occasion	Titre
1/01/2022	communiqué de presse	Varkensbedrijf	Minder zeugensterfte dankzij betere drinkwateropname
21/01/2022	présentation	IPVS refresher course	Infection levels and immunity for M. hyopneumoniae in breeding animals
1/04/2022	présentation	Large animals residents symposium	Porcine ear necorsis: infectious or behavioral problem?
29/04/2022	communiqué de presse	Drietand	Veepeilerprojecten leveren inzichten op voor de varkenssector
12/05/2022	présentation	ESPHM	Porcine ear necrosis in piglets: development of lesions and germs.
12/05/2022	présentation	ESPHM	A cross-sectional study on the prevalence of Mycoplasma hyopneumoniae in breeding animals
19/05/2022	communiqué de presse	Boer en Tuinder	Kreupelheid vermijden
9/06/2022	Sc. Pub	Porcine Health Management https://doi.org/10.1186/s40813-022-00267-w	Influence of parity and reproductive stage on the prevalence of Mycoplasma hyopneumoniae in breeding animals in Belgian farrow-to-finish herds
16/06/2022	newsletter	NB DGZ	Schrijf je in voor een nieuw Veepeilerproject en pak PRRSV bij je biggen aan
23/06/2022	poster	IPVS	Pathogenesis of porcine ear necrosis in nursery piglets.
23/06/2022	présentation	IPVS	Influence of parity and reproductive stage on the prevalence of Mycoplasma hyopneumoniae in breeding animals
17/10/2022	newsletter	NB DGZ	Krijg inizchten in je biestmanagement met nieuwste Veepeilerproject
3/11/2022	communiqué de presse	Landbouwleven	Reinigingsprotocol verbeteren, hoe proper is jouw stal
7/12/2022	newsletter	NB DGZ	Hoe proper is jouw stal echt?

14/02/2023	Sc. Pub	Microbiol Spectrum 11(1) https://doi.org/10.1128/spectrum.04123-22	Predictive Power of Long-Read Whole-Genome Sequencing for Rapid Diagnostics of Multidrug-Resistant *Brachyspira hyodysenteriae* Strains.
5/04/2023	newsletter	NB DGZ	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
1/05/2023	communiqué de presse	Dierenartsenwereld	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
31/05-2/06/2023	poster	ESPHM 2023	The role of behaviour and ear biting on the occurrence of porcine ear necrosis.
4-7/06/2023	poster	ICPR 2023	Farrowing traits associated with prolonged farrowing duration and high birth intervals in hyperprolific sows.
29/06/2023	newsletter	NB DGZ	Veepeiler start nieuw project om PRRSV-stammen in beeld te krijgen
29/06/2023	communiqué de presse	Landbouwleven	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
1/07/2023	communiqué de presse	VedaScoop	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
6/07/2023	communiqué de presse	Boer en Tuinder	Nieuwe Spaanse PRRS-stam Rosalia nog niet gedetecteerd in België
28/08/2023	Sc. Pub.	Porc Health Manag 9(1) http://hdl.handle.net/1854/LU-01HCYWZB9RRC2XP696C4GY4KTP	Relationship between piglets' survivability and farrowing kinetics in hyper-prolific sows.
5/09/2023	newsletter	NB DGZ	Optimaliseer de gezondheid van je dieren en je bedrijf met behulp van Veepeiler Varken
29/09/2023	Sc. Pub.	Vet Res, 54:85 https://doi.org/10.1186/s13567-023-01218-1	Porcine ear necrosis: lesions, associated pathogens and factors.
2/10/2023	newsletter	NB DGZ	Krijg PRRSV-stam in beeld met de hulp van Veepeiler

7 Annexe 1

VVD – Virology and Viral Diseases

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

C. Bonckaert 1 , C. Rigauts 1 , C. Brossé 1 , T. Vandersmissen 1 , S. Theuns 2 , H. Nauwynck 3

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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 108 . 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 (105-7 PCV2 genome copies) and 3 (108-10 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[1], Charlotte RIGAUTS[1], Charlotte Brossé[1], Sebastiaan THEUNS[2], Hans NAUWYNCK[3]
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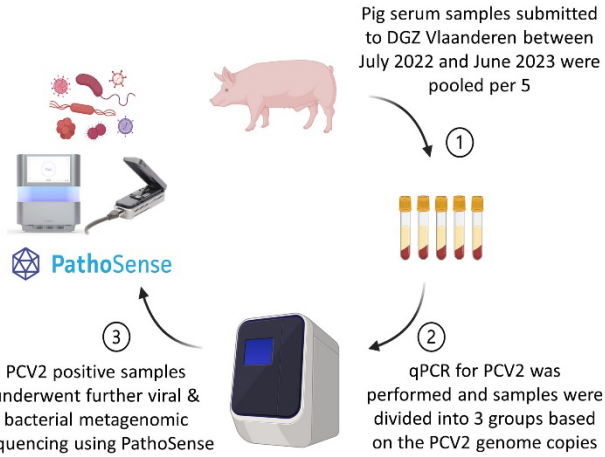
INTRODUCTION

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[1]. 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[2-6]. 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

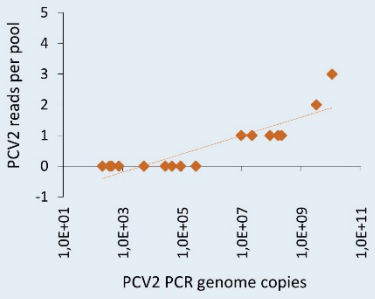


Figure 1. Comparison of semi-quantitative PCV2 reads to PCV2 PCR genome copies. A positive correlation (R^2=0.7749) is observed. However, in samples containing <10^4 PCV2 PCR genome copies, no PCV2 reads were detected. PCV2 reads are semi-quantitative with very low=1, low=2, medium=3, high=4 and very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

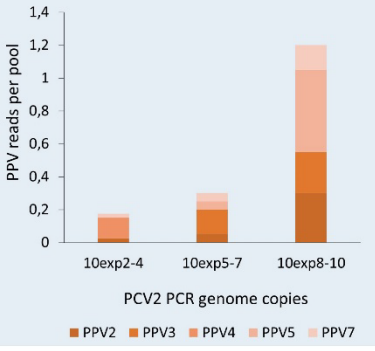


Figure 2. Mean number of PPV reads per group of PCV2 PCR genome copies. Mean number of PPV reads per pool is calculated as: (n reads PPV2+n reads PPV3+n reads PPV4+n reads PPV5+n reads PPV7 with very low=1, low=2, medium=3, high=4 and very high=5 as semi-quantitative values/5xnumber of pigs)

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

REFERENCES

[1]Ruifang W, *Changes on the viral capsid surface*, Virus Evolution, Volume 5, Issue 2, July 2019
[2]Ouyang, Co-Infection of Swine with Porcine Circovirus Type 2, Viruses 2019 Feb 21
[3]Miłek D., *Do PPV1-PPV7 impact PCV2*?, Vet Microbiol. 2020 Mar;242:108613.
[4]Bokma J., *Evaluation of nanopore sequencing*. J Of Clinical Microbiology. 2021, 59(12).
[5]Theuns S., *Nanopore sequencing as a revolutionary diagnostic tool*. Scientific Reports, 2018, 8:9830
[6]Ellis JA, Coinfection by porcine circoviruses. J Vet Diagn Invest. 2000 Jan;12(1):21-7.

8 Annexe 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[1]. 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.

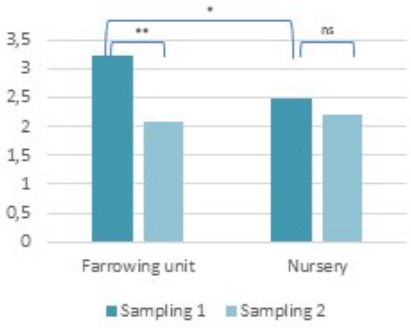

Figure 1: The mean score of the RODAC plates in the farrowing unit and the nursery before (sampling 1) and after (sampling 2) receiving advice. * p-value < 0.01; ** p-value < 0.001; ns = not significant

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 Annexe 3

Veterinary Research

RESEARCH ARTICLE

Open Access

Porcine ear necrosis: characterization of lesions and associated pathogens

Mateusz Malik[1*], Koen Chiers[2], Sebastiaan Theuns[4], Nick Vereecke[3,4], Ilias Chantziaras[1], Siska Croubels[2] and Dominiek Maes[1]

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

Handling editor: Marcelo Gottschalk.

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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 β-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 timepoints. 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 × *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 µm 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, MacConckey agar, and Columbia horse agar were used; which were incubated for 48 h at 37 °C in CO_2 (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 × *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), α-zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, α-zearalenol-glucuronide, β-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 (°C), relative humidity (%), and dew point (°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® SPSS® 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

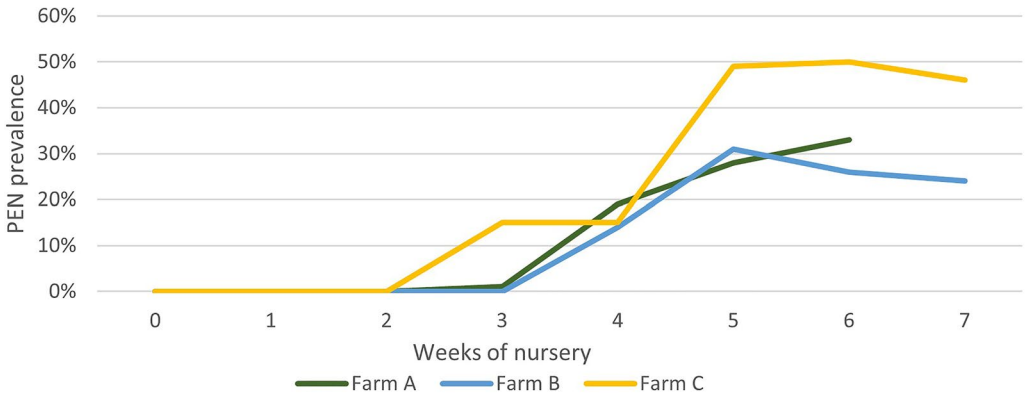

Figure 1 Weekly prevalence of porcine ear necrosis (PEN) lesions in nursery pigs from three farms. The number of piglets at weaning on farms **A**, **B**, and **C** was 485, 527, and 268, respectively.

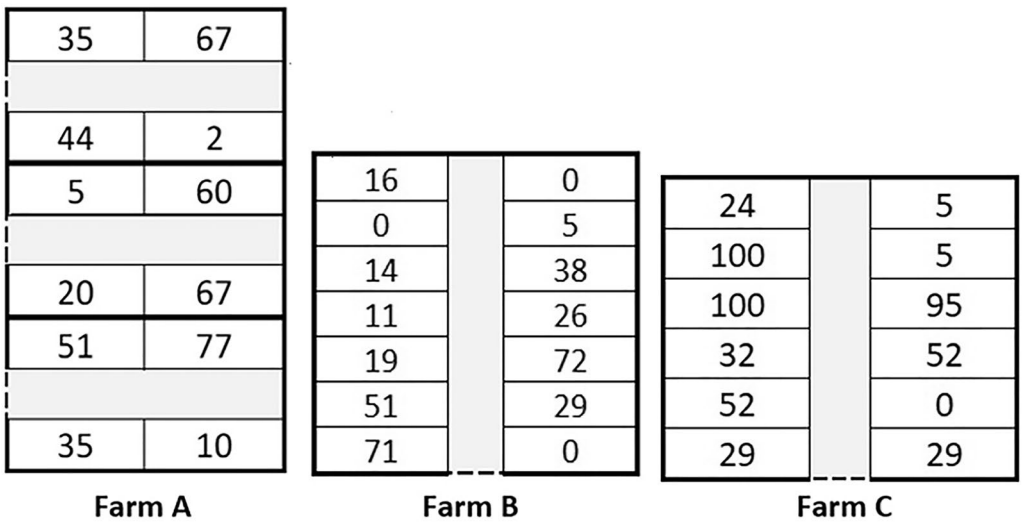

Figure 2 Percentage of animals affected with porcine ear necrosis per pen, at the end of nursery period in farms A, B, and C. The mean number of pigs per pen was 40, 38, and 25 in farms **A**, **B**, and **C**, respectively.

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 ± 8%) and 60% (SD ± 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

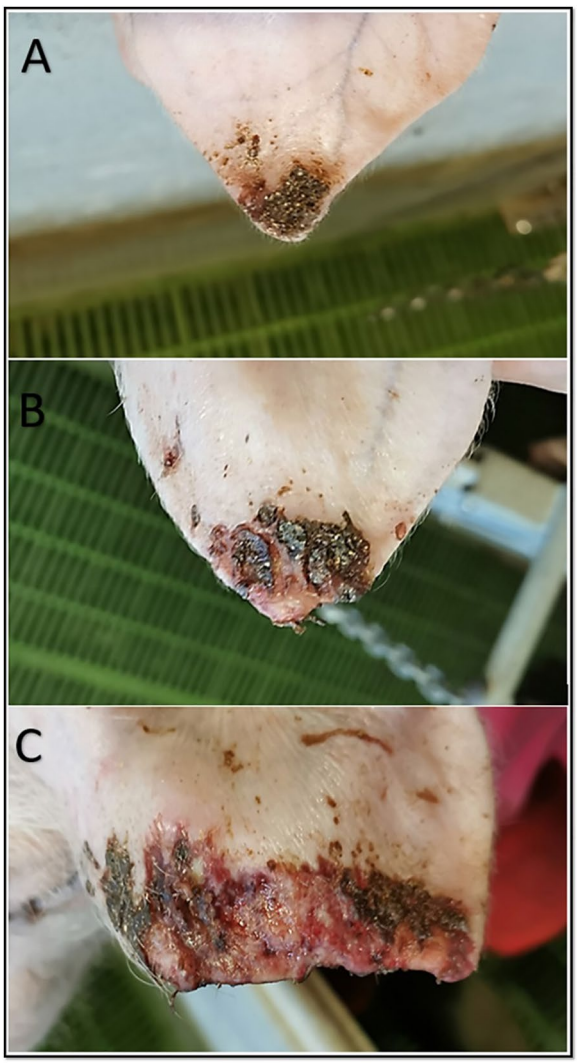

Figure 3 Example of ear lesions evaluated during the study. **A** Piglet with mild lesions (score 1)—light reddening and small crusts, **B** ear of a moderately affected piglet (score 2)—rounded ear tip with crusts and wet tissue beneath, **C** ear of severely affected piglet (score 3)—partially missing 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 16 S 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,

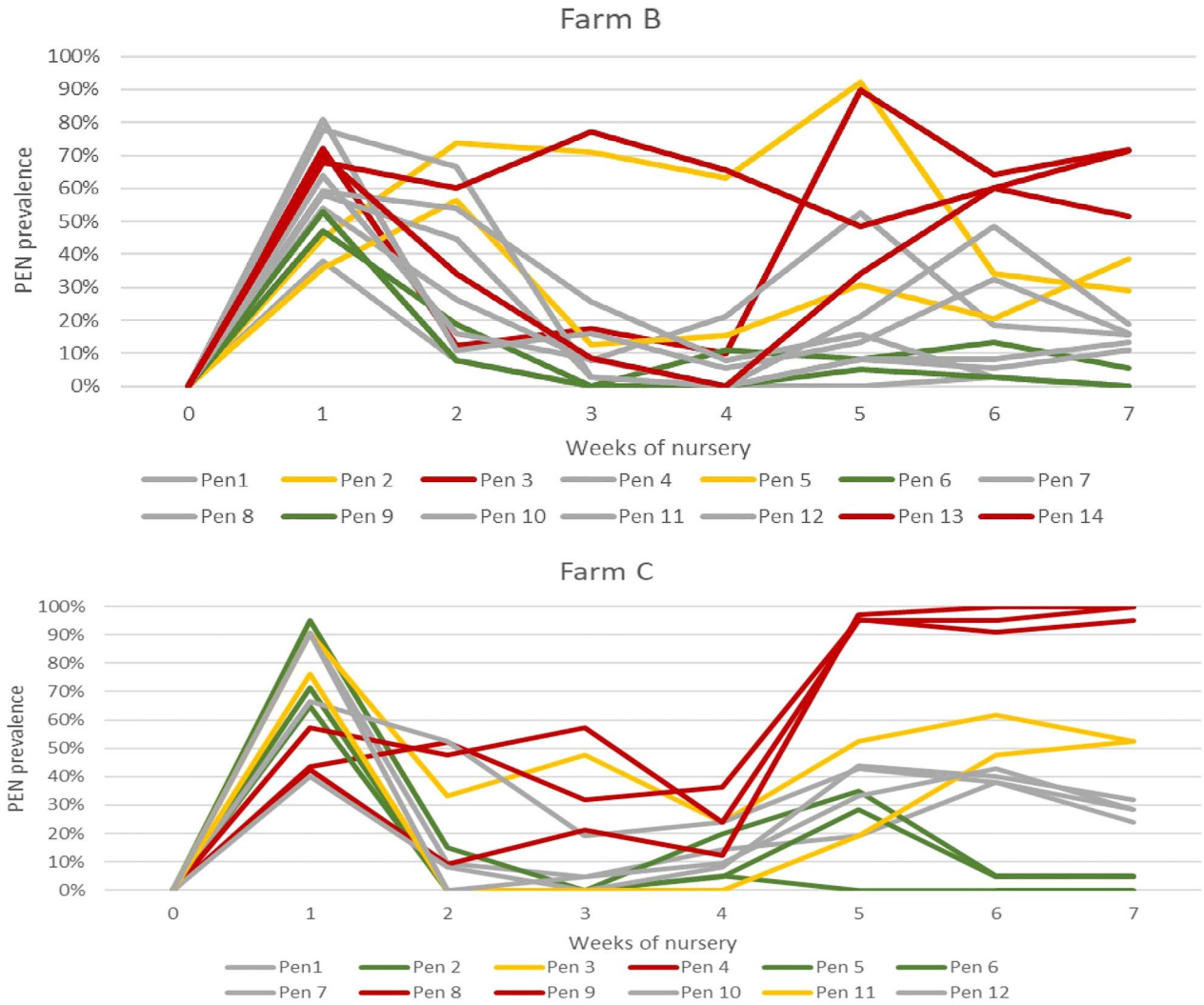

Figure 4 Weekly prevalence of all recorded ear lesions/alternations recorded at the pen level on farms B and farm C. Colors of the lines related to the prevalence: red—very high, yellow—high, grey—moderate, green—low.

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

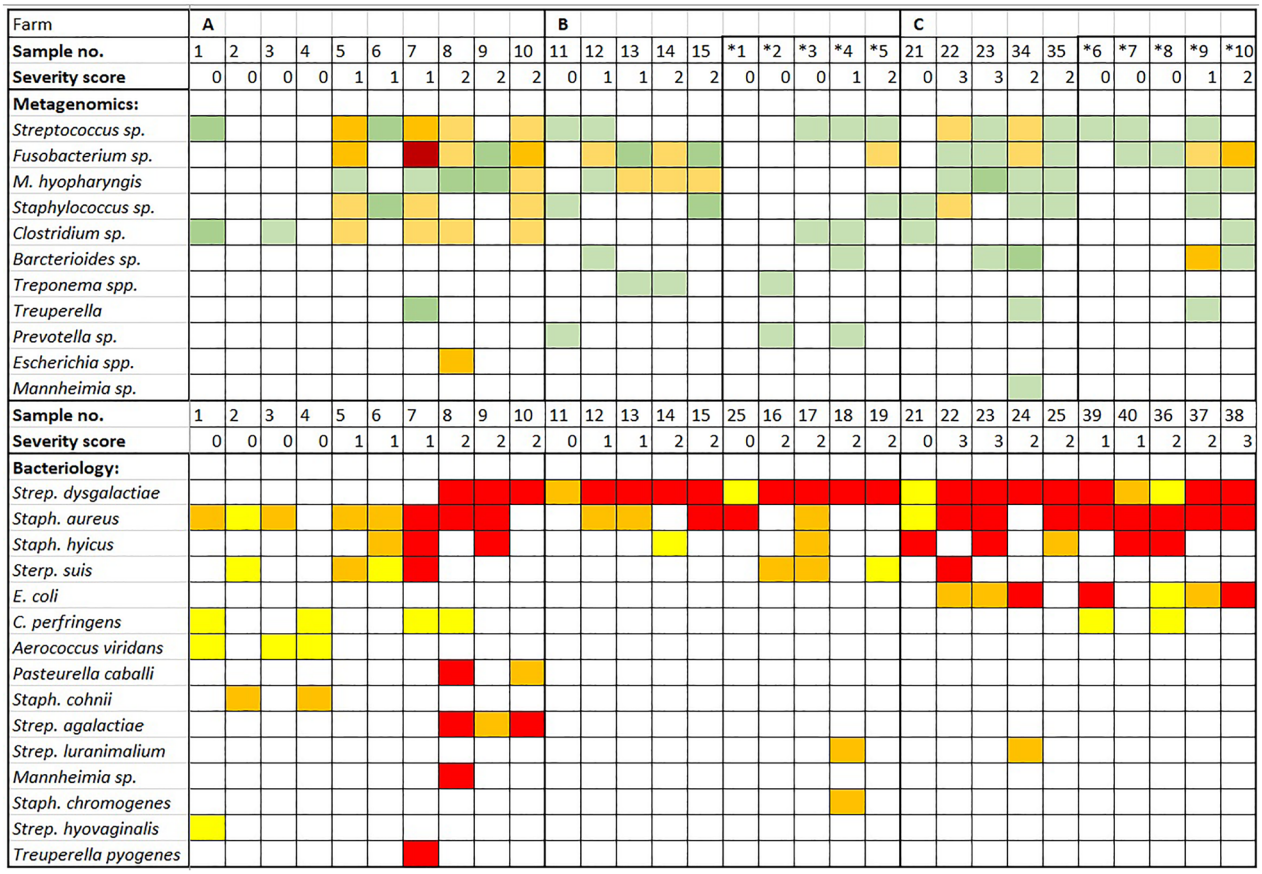

Figure 5 Heatmap of bacteria found in scraping samples, with their loads, analyzed by nanopore sequencing (white—negative, light green—very low, green—low, light orange—moderate, orange high, red—high loads) together with the bacteriology results load (white—negative, yellow—low, orange—moderate, red—high).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

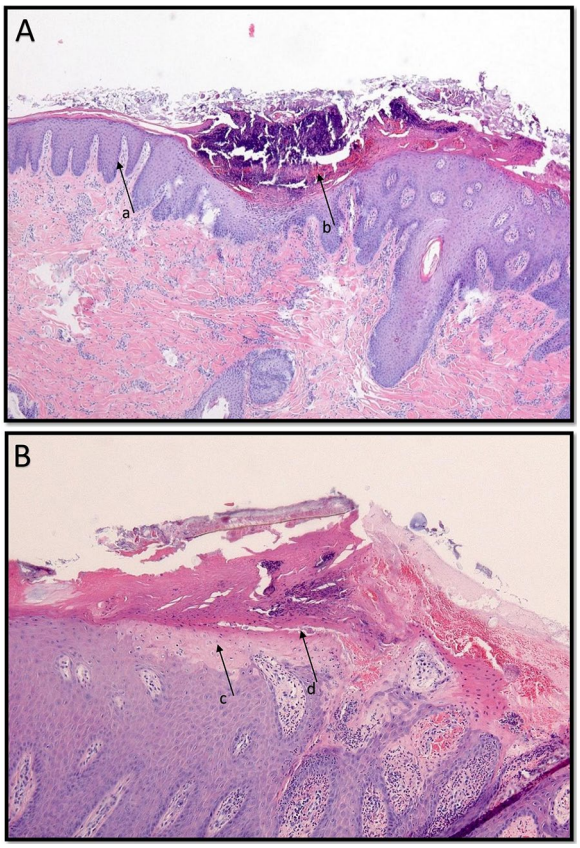

Figure 6 Microscopic picture of biopsy of affected ear. **A** Sample with gross lesion score 3. Severe epidermal hyperplasia (a) with focal erosion covered by a serocellular crust (b). **B** Sample with gross lesion score 1. The epidermal pallor of the superficial stratum spinosum (c) and separation 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 ($n=29$)			
Lesion score	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− ($n = 8$[a])	PEN+ ($n = 20$)	PEN− ($n = 10$)	PEN+ ($n = 19$[a])	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.

[a] 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^2/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	< 100 000
Aerobic bacteria (37 °C) in total (cfu/mL)	650	13	800	< 100 000
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 stillbirth, 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

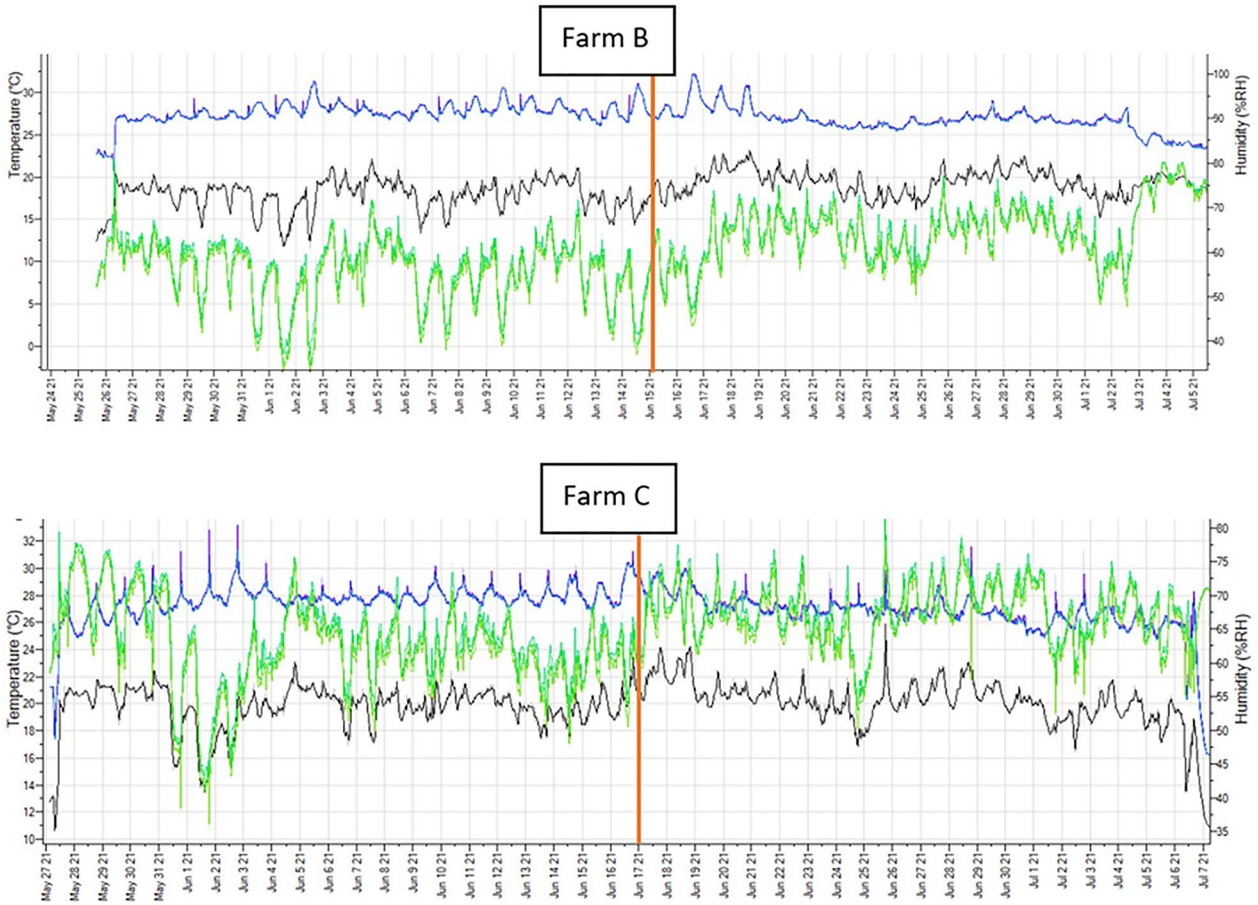

Figure 7 Two graphs illustrating temperature (blue line), humidity (green line), and dew point (black line) measured in the compartment during the nursery period on farms B and C. The red vertical line is a timepoint when the first PEN lesions started to appear.

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 β-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 µg/

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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References

1. Richardson JA, Morter RL, Rebar AH, Olander HJ (1984) Lesions of porcine necrotic ear syndrome. Vet Pathol 21:152–157
2. Malik M, Schoos A, Chantziaras I, Donkers D, Croubels S, Doupovec B, Maes D (2021) Porcine ear necrosis in weaned piglets: prevalence and impact on daily weight gain. Porcine Health Manag 13:61
3. Gomes A, Romeo C, Ghidini S, Vieira-Pinto M (2022) The relationship between carcass condemnations and tail lesion in swine considering different production systems and tail lengths. Animals 12:949
4. Kritas SK, Morrison RB (2007) Relationships between tail biting in pigs and disease lesions and condemnations at slaughter. Vet Rec 160:149–152
5. Pejsak Z, Truszczyński M (2009) Zespół martwicy uszu świń (porcine ear necrotic syndrome). Życie Weterynaryjne 84:716–718 (in Polish)
6. Park J, Friendship RM, Poijak Z, DeLay J, Slavic D, Dewey CE (2013) An investigation of ear necrosis in pigs. Can Vet J 54:491
7. Malik M, Chiers K, Boyen F, Croubels S, Maes D (2021) Porcine ear necrosis. Vet J 271:105655
8. Pringle M, Backhans A, Otman F, Sjölund M, Fellström C (2009) Isolation of spirochetes of genus *Treponema* from pigs with ear necrosis. Vet Microbiol 139:279–283
9. Karlsson F, Rosander A, Fellström C, Backhans A (2017) Experimental inoculation of Treponema pedis T A4 failed to induce ear necrosis in pigs. Porcine Health Manag 3:26
10. Pejsak Z, Markowska-Daniel I, Pomorska-Mól M, Porowski M, Kołacz R (2011) Ear necrosis reduction in pigs after vaccination against PCV2. Res Vet Sci 91:125–128

11. de Costa MO, Nosach R, Harding JCS, Huang Y (2021) Partial reproduction of ear-tip necrosis suggests an infectious, initially bacterial aetiology. Vet Microbiol 262:109246
12. Smulders D, Hautekiet V, Verbeke G, Geers R (2008) Tail and ear biting lesions in pigs: an epidemiological study. Anim Welf 17:61–69
13. Vereecke N, Zwickl S, Gumbert S, Graaf A, Harder T, Ritzmann M, Lillie-Jaschniski K, Theuns S, Stadler J (2023) Viral and bacterial profiles in endemic influenza a virus infected swine herds using nanopore metagenomic sequencing on tracheobronchial swabs. Microbiol Spectr 11:e0009823
14. Theuns S, Vanmechelen B, Bernaert Q, Deboutte W, Vandenhole M, Beller L, Matthijnssens J, Maes P, Nauwynck HJ (2018) Nanopore sequencing as a revolutionary diagnostic tool for porcine viral enteric disease complexes identifies porcine kobuvirus as an important enteric virus. Sci Rep 8:9830
15. Bokma J, Vereecke N, Pas ML, Chantillon L, Vahl M, Weesendorp E, Deurenberg RH, Nauwynck H, Haesebrouck F, Theuns S, Boyen F, Pardon B (2021) Evaluation of nanopore sequencing as a diagnostic tool for the rapid identification of *Mycoplasma bovis* from individual and pooled respiratory tract samples. J Clin Microbiol 59:e0111021
16. Lauwers M, De Baere S, Letor B, Rychlik M, Croubels S, Devreese M (2019) Multi LC-MS/MS and LC-HRMS methods for determination of 24 mycotoxins including major phase I and II biomarker metabolites in biological matrices from pigs and broiler chickens. Toxins 11:171
17. Papatsiros VG (2011) Exploration of the connection between porcine necrotic ear syndrome and PCV2 infection. J Anim Vet Adv 10:185–187
18. Kureljušić B, Savić B, Milićević V, Jezdimirović N, Radanović O, Zutić J, Weissenbacher-Lang C (2021) Investigation of possible aetiological/triggering factors in porcine ear necrosis syndrome at a farrow-to-feeder pig system. Acta Vet Hung 69:161–168
19. Weissenbacher-Lang C, Voglmayr T, Waxenecker F, Hofstetter U, Weissenböck H, Hoelzle K, Hoelzle LE, Welle M, Ogris M, Bruns G, Ritzmann M (2012) Porcine ear necrosis syndrome: a preliminary investigation of putative infectious agents in piglets and mycotoxins in feed. Vet J 194:392–397
20. Weissenbacher-Lang C, Voglmayr T, Weissenböck H, Pyrek R, Waxenecker F, Hofstetter U, Hoelzle K, Hoelzle LE, Welle M, Bruns G, Ritzmann M (2013) Untersuchung möglicher Ursachen von Ohrrandnekrosen: unterschiedliche studien designs—vergleichbare resultate? Berl Munch Tierarztl Wochenschr 126:378–387 (in German)
21. Prunier A, Averos X, Dimitrov I, Edwards SA, Hillmann E, Holinger M, Ilieski V, Leming R, Tallet C, Turner SP, Zupan M, Camerlink I (2020) Review: early life predisposing factors for biting in pigs. Animal 14:570–587
22. Miłek D, Woźniak A, Guzowska M, Stadejek T (2019) Detection patterns of porcine parvovirus (PPV) and novel porcine parvoviruses 2 through 6 (PPV2–PPV6) in polish swine farms. Viruses. 11:474
23. Kim SC, Kim JH, Kim JY, Park GS, Jeong CG, Kim WI (2022) Prevalence of porcine parvovirus 1 through 7 (PPV1–PPV7) and co-factor association with PCV2 and PRRSV in Korea. BMC Vet Res 18:133
24. Lager KM, Mengeling WL (1994) Porcine parvovirus associated with cutaneous lesions in piglets. J Vet Diagn Invest 6:357–359
25. Whitaker HK, Neu SM, Pace LW (1990) Parvovirus infection in pigs with exudative skin disease. J Vet Diagn Invest 2:244–246
26. Ghosh S, Soto E, Illanes O, Navarro R, Aung MS, Malik YS, Kobayashi N, Fuentealba C (2018) High detection rates of Torque teno sus virus in co-infection with important viral pathogens in porcine kidneys on St. Kitts Island, Lesser Antilles. Transbound Emerg Dis 65:1175–1181
27. Subramanyam V, Hemadri D, Kashyap SP, Hiremath J, Barman NN, Ralte EL, Patil SS, Suresh KP, Rahaman H (2019) Detection of torque teno sus virus infection in Indian pigs. Vet World 12:1467–1471
28. Kekarainen T, Segalés J (2012) Torque teno sus virus in pigs: an emerging pathogen? Transbound Emerg Dis 59:103–108
29. Blomström AL, Belák S, Fossum C, McKillen J, Allan G, Wallgren P, Berg M (2009) Detection of a novel porcine boca-like virus in the background of porcine circovirus type 2 induced postweaning multisystemic wasting syndrome. Virus Res 146:125–129
30. Aryal M, Liu G (2021) Porcine bocavirus: a 10-year history since its discovery. Virol Sin 36:1261–1272
31. Gunn L, Collins PJ, Fanning S, McKillen J, Morgan J, Staines A, O'Shea H (2015) Detection and characterisation of novel bocavirus (genus *Bocaparvovirus*) and gastroenteritis viruses from asymptomatic pigs in Ireland. Infect Ecol Epidemiol 5:27270
32. Thomas F, Hehemann JH, Rebuffet E, Czjzek M, Michel G (2011) Environmental and gut bacteroidetes: the food connection. Front Microbiol 2:93
33. Nagaraja TG, Narayanan SK, Stewart GC, Chengappa MM (2005) Fusobacterium necrophorum infections in animals: pathogenesis and pathogenic mechanisms. Anaerobe 11:239–246
34. Buboltz JB, Murphy-Lavoie HM (2023) Gas gangrene in: StatPearls. StatPearls Publishing, Treasure Island (FL)
35. Kobisch M, Friis NF (1996) Swine mycoplasmoses. Rev Sci Tech 15:1569–1605
36. Sokoli A, Groebel K, Hoelzle K, Amselgruber WM, Mateos JM, Schneider MK, Ziegler U, Felder KM, Hoelzle LE (2013) *Mycoplasma suis* infection results endothelial cell damage and activation: new insight into the cell tropism and pathogenicity of hemotrophic mycoplasma. Vet Res 44:6
37. Stadler J, Ade J, Hermanns W, Ritzmann M, Wentzel S, Hoelzle K, Hoelzle LE (2021) Clinical, haematological and pathomorphological findings in *Mycoplasma suis* infected pigs. BMC Vet Res 17:214
38. Oh SI, Kim JW, Jung JY, Chae M, Lee YR, Kim JH, So B, Kim HY (2018) Pathologic and molecular characterization of *Streptococcus dysgalactiae* subsp. *equisimilis* infection in neonatal piglets. J Vet Sci 19:313–317
39. Amass SF, Clark LK, Knox K, Wu CC, Hill MA (1996) *Streptococcus suis* colonization of piglets during parturition. J Swine Health Prod 4:269–272
40. Park J (2011) Investigations of exudative epidermitis and ear necrosis in pigs. PhD Thesis, Department of Population Medicine, University of Guelph, Guelph
41. Mirt D (1999) Lesions of so-called flank biting and necrotic ear syndrome in pigs. Vet Rec 144:92–96
42. Oregon Department of Human Services (2000) Health effects information ammonia. Technical bulletin, Portland
43. Thurston-Enriquez JA, Gilley JE, Eghball B (2005) Microbial quality of runoff following land application of cattle manure and swine slurry. J Water Health 3:157–171

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