ORIGINAL RESEARCH PEER REVIEWED

Virulence genes of *Escherichia coli* vaginal isolates associated with postpartum dysgalactia syndrome in sows

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Summary

Objective: Identify the occurrence of certain virulence genes of *Escherichia coli* vaginal isolates associated with postpartum dysgalactia syndrome (PDS) in sows.

Materials and methods: Two hundred and two sows from 5 Macedonian pig farms were clinically examined for PDS 12 to 24 hours after farrowing. Vaginal swabs for bacteriological testing were taken from PDS-affected (PDSA, n = 47) and PDS-unaffected (PDSU, n = 155) sows. In total, 74 isolates of *E coli* were tested by multiplex polymerase chain reaction for the presence of virulence genes related to specific pathogenic strains.

Results: Genes associated with extraintestinal pathogenic *E coli* (ExPEC) strains were the most prevalent among all tested *E coli* isolates. The most dominant gene among all *E coli* isolates was *fimC*. The *iss* gene was more prevalent in PDSA sows compared to PDSU sows ($P = .02$). Multivariable logistic regression showed that lower parity sows ($P \leq .001$) and presence of the *iss* ($P = .003$) and *astA* genes ($P = .03$) were correlated with the occurrence of PDS.

Implications: Lower parity sows vaginally infected with *E coli* associated with particular ExPEC strains are at higher risk of developing PDS. Positive vaginal swabs for *E coli* and *iss* gene found early after farrowing were associated with PDS in sows. Classification of *E coli* into specific ExPEC pathotype was not possible by virulence genotyping only.

Keywords: swine, *Escherichia coli*, virulence genes, sows, postpartum dysgalactia syndrome

Received: October 30, 2020
Accepted: March 24, 2021

Resumen - Genes de virulencia de aislamientos vaginales de *Escherichia coli* asociados con el síndrome de disgalaxia posparto en cerdas

Objetivo: Identificar la incidencia de ciertos genes de virulencia de aislamientos vaginales de *Escherichia coli* asociados con el síndrome de disgalaxia posparto (PDS) en cerdas.

Materiales y métodos: Se examinaron clínicamente doscientas dos cerdas de 5 granjas de cerdos de Macedonia para detectar el PDS entre 12 y 24 horas después del parto. Se tomaron hisopos vaginales para pruebas bacteriológicas de cerdas afectadas por PDS (PDSA; n = 47) y no afectadas (PDSU; n = 155). En total, 74 cepas de *E coli* se analizaron mediante la reacción en cadena de la polimerasa multiplex para detectar la presencia de genes de virulencia relacionados con cepas patógenas específicas.

Resultados: Los genes asociados con cepas de *E coli* patógenas extraintestinales (ExPEC) fueron los más prevalentes entre todos los aislados de *E coli* analizados. El gen más dominante entre todos los aislados de *E coli* fue *fimC*. El gen *iss* fue más prevalente en las cerdas PDSA en comparación con las cerdas PDSU ($P = .02$). La regresión logística multivariable mostró que las cerdas de menor paridad ($P \leq .001$) y la presencia de los genes *iss* ($P = .003$) y *astA* ($P = .03$) se correlacionaron con la aparición de PDS.

Implicaciones: Las cerdas de menor paridad infectadas por vía vaginal con *E coli* asociadas con cepas específicas de ExPEC tienen un mayor riesgo de desarrollar PDS. Los hisopos vaginales positivos para *E coli* y el gen *iss* encontrados poco después del parto se asociaron con el PDS en las cerdas. La clasificación de *E coli* en un patotipo específico de ExPEC no fue posible mediante genotipificación de virulencia únicamente.

Résumé - Gènes de virulence des isolats vaginaux d'*Escherichia coli* associés au syndrome de dysgalactie post-partum chez les truies

Objectif: Identifier la présence de certains gènes de virulence d'isolats vaginaux d'*Escherichia coli* associés au syndrome de dysgalactie post-partum (PDS) chez les truies.

Matériel et méthodes: Deux cent deux truies de cinq élevages de porcs macédoniens ont été examinées cliniquement pour le PDS 12 à 24 heures après la mise bas. Des écouvillons vaginaux pour les tests bactériologiques ont été prélevés sur des truies atteintes de PDS (PDSA; n = 47)

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This article is available online at **http://www.aasv.org/shap.html**.

Angjelovski B, Atanasov B, Kjosevski M. Virulence genes of *Escherichia coli* vaginal isolates associated with postpartum dysgalactia syndrome in sows. *J Swine Health Prod*. 2021;29(6):302-308.

et non atteintes de PDS (PDSU; n = 155). Au total, 74 isolats d'*E coli* ont été testés par réaction d'amplification en chaîne par la polymérase multiplex pour la présence de gènes de virulence liés à des souches pathogènes spécifiques.

Résultats: Les gènes associés aux souches d'*E coli* pathogènes extra-intestinaux (ExPEC) étaient les plus répandus parmi tous les isolats d'*E coli* testés. Le gène le plus dominant parmi tous les isolats d'*E coli* était le *fimC*. Le gène *iss* était plus répandu chez les truies PDSA que chez les truies PDSU ($P = .02$). La régression logistique multivariée a montré que les truies de parité plus faible ($P \leq .001$) et la présence des gènes *iss* ($P = .003$) et *astA* ($P = .03$) étaient corrélées à l'apparition de PDS.

Implications: Les truies à parité inférieure infectées par voie vaginale par *E coli* associées à des souches d'ExPEC particulières courent un risque plus élevé de développer un PDS. Des prélèvements vaginaux positifs pour *E coli* et le gène *iss* trouvés tôt après la mise bas ont été associés à la PDS chez les truies. La classification d'*E coli* en pathotype ExPEC spécifique n'a pas été possible uniquement par le génotypage de virulence.

Postpartum dysgalactia syndrome (PDS) in sows is one of the main health concerns characterized by fever, endometritis, and insufficient colostrum and milk production during the first days after farrowing.[1-3] The syndrome decreases growth rate and increases mortality in preweaning piglets due to reduced lactation performance of sows in the first 48 to 72 hours post partum.[1,4,5] It is associated with huge financial losses and negatively affects animal welfare in intensive pig production worldwide.[5,6] The incidence at herd level depends on criteria used to assess the occurrence of PDS and is estimated to vary between 0.5% and 60%, with an average of 13%.[4-8]

Although it is considered a multifactorial disease, coliform bacteria such as *Escherichia, Enterobacter, Citrobacter,* and *Klebsiella* play a major role in the etiology of the infection.[6,8-11] The importance of *Escherichia coli* in the clinical manifestation of PDS has been reported in few experimental studies.[10-12] Recent studies demonstrated that endometritis in sows shortly after farrowing is considered to be a risk for development of PDS.[1,3] Additionally, the dominance of *E coli* in the genital tract of affected sows highlights its potential role in the clinical manifestation of the syndrome.[6,8,13,14] However, to our knowledge, *E coli* isolates obtained from the vaginal swabs of PDS diseased sows have not been analyzed for presence of virulence genes.

Based on location, clinical diseases, and virulence characteristics, strains of *E coli* can be classified into intestinal pathogenic *E coli* (IPEC), extraintestinal pathogenic *E coli* (ExPEC) and commensal *E coli*.[15] Enterotoxigenic *E coli* (ETEC) and shiga toxin-producing *E coli* (STEC) pathotypes of IPEC in pigs are well demonstrated as etiological agents for diarrhea and edema diseases in piglets.[16] Additionally, urogenital infections caused by uropathogenic *E coli* (UPEC) and septicemia in pigs are correlated with ExPEC pathotypes.[17,18] In the study of Gerjets et al,[19] *E coli* isolates from milk samples of healthy sows and sows with coliform mastitis were examined for the presence of virulence genes associated with ExPEC, ETEC, and other pathogenic *E coli*. Nevertheless, there is a lack of information about the virulence genes of *E coli* recovered from the genital tract of sows and the occurrence of PDS.

The aim of this study was to determine the presence of virulence genes related to ExPEC and ETEC in vaginal isolates of sows with clinical PDS.

Animal care and use

This work was performed in accordance with the Macedonian Legislation on protection and welfare of animals and approved by the Faculty of Veterinary Medicine, Ss. Cyril and Methodius University in Skopje, Macedonia (Decision No. 0202-418/6).

Materials and methods

Animals

The study was carried out between July 2014 and August 2016 on 5 commercial pig farms in Macedonia. In total, 202 sows with available reproductive data of different parities (1-9) and different genetic lines (Landrace-Yorkshire F1 and Dalland hybrid) were recruited for this study. Sows with unknown reproductive records (parity number and time of farrowing completion) were excluded from the study.

All sows and their litters were clinically examined for the presence of PDS 12 to 24 hours after farrowing (8 AM to 10 AM) based on predetermined clinical signs (Table 1). During clinical inspection, sows were defined as PDS-affected (PDSA) when they showed pathological vulvar discharge or mastitis and had at least one or more clinical signs listed in Table 1.

After clinical assessment, 47 sows were identified as PDSA, while 155 sows were declared as healthy, or PDS-unaffected (PDSU), and showing none of the clinical signs previously described. On the same day following clinical examination, vaginal swabs were taken for bacteriological testing. In total, 202 samples were taken from 47 PDSA and 155 PDSU sows. Before sampling, the vulva was cleaned and disinfected with 10% iodine solution. Vaginal swabs were taken using sterile metal speculums with 3-cm external diameter and 30 to 40 cm in length, deeply inserted into the vagina, by thorough contact with the ventral mucosa for at least 10 seconds. Swabs were stored at 4°C and transported to the laboratory within 2 hours.

Bacteriological analysis

Bacteriological testing was performed by using routine diagnostic procedures. The initial inoculation of the samples was performed on 5% sheep blood agar (blood agar base; Merck) and selective media for gram-negative bacteria (Xylose Lysine Deoxycholate, MacConkey, and Tryptone Bile X-Glucuronide Agar; Merck). After 24 hours of aerobic incubation at 37°C, the grown bacteria were distinguished by their morphology, hemolysis on blood agar catalase reaction, Gram staining, and growth on selective media. Selected colonies were subcultivated on blood agar for another 24 hours at 37°C to obtain pure cultures. The final identification was performed by automated system VITEK2 Compact (BioMérieux). Obtained *E coli* isolates were selected for further investigations.

Bacterial DNA of *E coli* strains was prepared by dissolving 2 to 3 colonies in 200 µL of distilled water. After 30 minutes of heating at 95°C, 2.5 µL of the supernatant was used for polymerase chain reaction (PCR) analyses. In total, 74 *E coli* isolates were examined using multiplex PCR assays for the presence of 27 virulence genes encoding virulence factors associated with ExPEC, ETEC, and STEC strains as described by Ewers et al[15] and Casey and Bosworth.[16] The complete list of targeted virulence genes and primer sequences used for amplification procedures are shown in Table 2. Avian pathogenic *E coli* (APEC) strain IMT 2470, UPEC

Table 1: Frequency of clinical signs observed in PDSA sows (n = 47)

Clinical sign	Description	PDSA sows, No. (%)
Pathological vulvar discharge	Copious purulent vulvar discharge	30 (63.8)
Mastitis	Warm, painful, swollen, and firm mammary glands	20 (42.6)
Fever	Increased rectal temperature (≥ 39.5°C)	17 (36.2)
Reduced appetite	Consumed less than half the quantity of feed provided	16 (34.0)
Hypogalactia	Reduced milk flow (drops of milk)	30 (63.8)
Depression in sow	Lethargy and sternal recumbency	28 (59.6)
Altered piglet behavior	Lethargy, restlessness, vigorous nursing efforts	32 (68.1)

PDSA = postpartum dysgalactia syndrome-affected.

strains[15] IMT7920 and IMT9267, and ETEC strains[16] IMT204, IMT19, IMT4830, and IMT3838 served as controls for molecular assays and were kindly provided by the Institute of Microbiology and Epizootics of the Free University Berlin.

Statistical analysis

Statistical analyses were performed using STATISTICA (version 8.0; StatSoft, Inc). The prevalence of *E coli* was calculated at sow level and descriptive statistics (Mean [SD]) were applied for the parity of sows positive for *E coli*. The Mann-Whitney test was used to detect significant differences between the parity of PDSA and PDSU sows positive for *E coli*. Frequency of detected virulence genes was calculated for *E coli* isolates in both PDSA and PDSU sows. A Chi square-test and the Fisher's exact test were preformed to find the differences in the frequency of the *E coli* virulence genes between PDSA and PDSU sows. The results were considered statistically significant at $P < .05$.

Multivariable logistic regression was applied to parity and the presence of *E coli* virulence genes as independent variables with regard to the PDS status. The dependent variable was the occurrence of PDS as a binary trait (PDSA sows or PDSU sows). The presence of the virulence genes and parity with $P \leq .25$ were selected to be used in the multivariable logistic model. The final logistic model was developed by following a forward stepwise approach using parity and virulence genes as predictors for PDS. The final model consisted of significant variables with $P < .05$. The strength of relationships was expressed using odds ratio (OR).

Results

The prevalence of *E coli* was significantly higher ($\chi^2_4 = 16.287$, $P < .001$) in the PDSA sows (57.45%; 27 of 47) compared to the PDSU sows (26.45%; 41 of 155).The PDSA sows positive for *E coli* had significantly lower parity (3.18 [1.94]) than the parity observed in PDSU sows (5.21 [2.53]) positive for *E coli* ($U = 293.00$; $P < .001$). From PDSA sows, 33 *E coli* isolates were recovered from the vaginal swabs, while 41 isolates were detected in vaginal swabs of PDSU sows. Of the 74 *E coli* isolates, 70 isolates had at least one virulence gene. From the 32 *E coli* isolates from PDSA sows, 113 virulence genes were identified compared with 138 virulence genes detected in 38 isolates from PDSU sow samples.

The number of virulence genes per isolate ranged from 1 to 10 and 96.41% (242 of 251) of genes were associated with ExPEC strains. The most dominant virulence gene in all *E coli* isolates was *fimC* (94.29%; 66 of 70) with prevalence of 90.63% (29 of 32) in isolates of PDSA sows and 97.37% (37 of 38) in isolates from vaginal swabs of PDSU sows. The lowest prevalence of 1.42% was found for *K88 (F5)* and *kpsMTII*, while genes *irp2*, *papC*, *vat*, *F18*, *Stx2e*, *STb*, *LTI*, *987P (F6)*, *K99 (F4)*, and *afa/draB* were not found in any of the isolates. Concerning the prevalence of virulence genes between the two groups of sows positive for *E coli*, *fimC* was the most dominant gene in both groups of sows, while significance was observed only for *iss* gene with higher prevalence found in PDSA sow samples ($P = .02$; Table 3).

Parity and presence of virulence genes *iss, iucD, astA*, *hlyA*, *sfa/foc*, *pic*, and *iha* with $P \leq .25$ were included in the logistic regression. The multivariable logistic regression model showed that parity associated with the presence of virulence genes *iss*, *sfa/foc*, *astA*, and *hlyA* were significantly associated with PDS occurrence ($R^2 = 0.373$; $P < .001$; Table 4).

Discussion

In this study, *E coli* isolates from vaginal tracts of PDSA and PDSU sows were compared to get information on differences in the *E coli* virulence genes regarding PDS. We found significantly lower parity for PDSA sows positive for *E coli* in contrast to parity for PDSU sows positive for *E coli*. This finding is in accordance with the study conducted by Bostedt et al,[14] where *E coli* was the predominant bacterium in the genital tract of 78 gilts suffering from puerperal septicemia.

Virulence genes belonging to ExPEC strains were the most frequent genes detected in all *E coli* isolates similar to the results reported by Gerjets et al.[19] Uropathogenic *E coli* strains, members of ExPEC, are the main causative agents associated with urogenital tract infections (UGTI).[15,20] Colonization of the urogenital tract by UPEC strains are associated with certain virulence genes encoding virulent capsule antigens, iron acquisition systems, adhesions, and secreted toxins.[20] The *fimC* gene, described as a urovirulence factor playing an important role in urinary tract infection,[20] was also detected in a high percentage in our study. This finding is in agreement with other studies.[19,21] In a survey conducted by Gerjets et al,[19] *fimC* was found in 84.7% of the isolates from the milk of sows with coliform mastitis and in 82.3% of the isolates from the milk of healthy sows. Similarly, high prevalence of *fimC* (91.3%) was found in *E coli* isolates recovered from sows with UGTI.[21] In another study, *fimC* was highly prevalent in *E coli* isolates obtained from

Table 2: Primers used for detection of 27 virulence genes associated with ETEC, STEC, and ExPEC strains

Virulence factor	Forward primer	Reverse primer	Product size	Pathotype	Reference
STb	TGCCTATGCATCTACACAAT	CTCCAGCAGTACCATCTCTA	113	ETEC	16
STaP	CAACTGAATCACTTGACTCTT	TTAATAACATCCAGCACAGG	158	ETEC	16
LT	GGCGTTACTATCCTCTCTAT	TGGTCTCGGTCAGATATGT	272	ETEC	16
Adhesins					
K99 (F4)	AATACTTGTTCAGGGAGAAA	AACTTTGTGGTTAACTTCCT	230	ETEC	16
F18	TGGTAACGTATCAGCAACTA	ACTTACAGTGCTATTCGACG	313	ETEC	16
987P (F6)	AAGTTACTGCCAGTCTATGC	GTAACTCCACCGTTTGTATC	409	ETEC	16
K88 (F5)	GTTGGTACAGGTCTTAATGG	GAATCTGTCCGAGAATATCA	499	ETEC	16
F41	AGTATCTGGTTCAGTGATGG	CCACTATAAGAGGTTGAAGC	612	ETEC	16
afa/draB	TAAGGAAGTGAAGGAGCGTG	CCAGTAACTGTCCGTGACA	810	ExPEC	15
iha	TAGTGCGTTGGGTTATCGCTC	AAGCCAGAGTGGTTATTCGC	609	ExPEC	15
fimC	GGGTAGAAAATGCCGATGGTG	CGTCATTTTGGGGGTAAGTGC	477	ExPEC	15
sfa/foc	GTCCTGACTCATCTGAAACTGCA	CGGAGAACTGGGTGCATCTTA	1242	ExPEC	15
hra	TCACTTGCAGACCAGCGTTTC	GTAACTCACACTGCTGTCACCT	537	ExPEC	15
tsh	ACTATTCTCTGCAGGAAGTC	CTTCCGATGTTCTGAACGT	824	ExPEC	15
papC	AAGCCAGAGTGGTTATTCGC	TGATATCACGCAGTCAGTAGC	501	ExPEC	15
Protectins					
neuC	GGTGGTACATTCCGGGATGTC	AGGTGAAAAGCCTGGTAGTGTG	676	ExPEC	15
kpsMT II	CAGGTAGCGTCGAACTGTA	CATCCAGACGATAAGCATGAGCA	280	ExPEC	15
cvi/cva	TCCAAGCGGACCCCTTATAG	CGCAGCATAGTTCCATGCT	598	ExPEC	15
iss	ATCACATAGGATTCTGCCG	CAGCGGAGTATAGATGCCA	309	ExPEC	15
Iron acquisition					
Irp2	AAGGATTCGCTGTTACCGGAC	TCGTCGGGCAGCGTTTCTTCT	413	ExPEC	15
iucD	ACAAAAAGTTCTATCGCTTCC	CCTGATCCAGATGATGCTC	714	ExPEC	15
Toxins					
hlyA	GTCCATTGCCGATAAGTTT	AAGTAATTTTTGCCGTGTTTT	352	ExPEC	15
astA	TGCCATCAACACAGTATATCC	TAGGATCCTCAGGTCGCGAGTGACGGC	116	ExPEC	15
vat	TCCTGGGACATAATGGCTAG	GTGTCAGAACGGAATTGTC	981	ExPEC	15
Stx2e	AATAGTATACGGACAGCGAT	TCTGACATTCTGGTTGACTC	733	STEC	16
Miscellaneous					
malX	GGACATCCTGTTACAGCGCGCA	TCGCCACCAATCACAGCCGAAC	922	ExPEC	15
pic	ACTGGATCTTAAGGCTCAGG	TGGAATATCAGGGTGCCACT	409	ExPEC	15

ETEC = Enterotoxigenic *Escherichia coli*; STEC = shiga toxin-producing *E coli*; ExPEC = extraintestinal pathogenic *E coli*; *STb* = Thermo stable toxin b; *STaP* = Thermo stable toxin b; *LT* = Thermo labile toxin; *K99* (*F4*) = Fimbrial adhesin F4; *F18* = Fimbrial adhesin *F18*; *987P* (*F6*) = Fimbrial adhesin F6; *K88* (*F5*) = Fimbrial adhesin *F5*; *F41* = Fimbrial adhesin F41; *afa/draB* = Afimbrial/Dr antigen-specific adhesin; *iha* = Iron-regulated-gene-homologue adhesin; *fimC* = Type 1 fimbriae (d-mannose-specific adhesin); *sfa/foc* = S fimbriae (sialic acid-specific) and F1C fimbriae; *hra* = Heat-resistant agglutinin; *tsh* = Temperature-sensitive haemagglutinin; *papC* = Pilus associated with pyelonephritis; *neuC* = K1 capsular polysaccharide; *kpsMT II* = Group II capsule antigens; *cvi/cva* = Structural genes of colicin V operon (microcin ColV); *iss* = Increased serum survival; *Irp2* = Iron-repressible protein (yersiniabactin synthesis); *iucD* = Aerobactin synthesis; *hlyA* = Hemolysin A; *astA* = EAST1 (heat-stable cytotoxin associated with enteroaggregative *E coli*); *vat* = Vacuolating autotransporter toxin; *Stx2e* = Shiga-like toxin II; *malX* = Pathogenicity-associated island marker CFT073; *pic* = Serin protease autotransporter.

Table 3: Prevalence of *Escherichia coli* virulence genes in PDSA (n = 27) and PDSU sows (n = 41)

Virulence gene	PDSA sows, No. (%)	PDSU sows, No. (%)	*P**
fimC	23 (85.18)	36 (87.80)	.75
iss	18 (66.66)	16 (39.02)	.02
iucD	14 (51.85)	15 (36.58)	.21
cvi/cva	9 (33.33)	15 (36.58)	.78
hra	9 (33.33)	12 (29.26)	.72
astA	9 (33.33)	7 (17.07)	.12
tsh	7 (25.92)	10 (24.39)	.88
malX	4 (14.81)	6 (14.63)	.98
neuC	2 (7.40)	2 (4.87)	.66
F41	1 (3.70)	4 (9.75)	.39
hlyA	0 (0.00)	4 (9.75)	.09
STaP	1 (3.70)	2 (4.87)	.81
sfa/foc	0 (0.00)	3 (7.31)	.15
iha	0 (0.00)	3 (7.31)	.15
pic	0 (0.00)	2 (4.87)	.24
kpsMT II	1 (3.70)	0 (0.00)	.21
K88 (F5)	0 (0.00)	1 (2.43)	.41

* The *P* value for the prevalence of *E coli* virulence genes between PDSA and PDSU sows was obtained using Chi square-test. Level of significance is $P < .05$.

PDSA = postpartum dysgalactia syndrome-affected; PDSU = postpartum dysgalactia syndrome-unaffected; *fimC* = Type 1 fimbriae (d-mannose-specific adhesin); *iss* = Increased serum survival; *iucD* = Aerobactin synthesis; *cvi/cva* = Structural genes of colicin V operon (microcin ColV); *hra* = Heat-resistant agglutinin; *astA* = EAST1 (heat-stable cytotoxin associated with enteroaggregative *E coli*); *tsh* = Temperature-sensitive haemagglutinin; *malX* = Pathogenicity-associated island marker CFT073; *neuC* = K1 capsular polysaccharide; *F41* = Fimbrial adhesin F41; *hlyA* = Hemolysin A; *STaP* = Thermo stable toxin b; *sfa/foc* = S fimbriae (sialic acid-specific) and F1C fimbriae; *iha* = Iron-regulated-gene-homologue adhesin; *pic* = Serin protease autotransporter; *kpsMT II* = Group II capsule antigens; *K88* (*F5*) = Fimbrial adhesin F5.

Table 4: Association of virulence genes ($P < .05$) and sow parity ($P < .001$) with occurrence of PDS in sows

Variable	β	OR (95% CI)	SE	*P**
Parity number	-0.43	0.65 (0.53-0.79)	0.10	< .001
iss gene	0.39	1.48 (1.21-1.80)	0.10	< .001
sfa/foc gene	-0.30	0.73 (0.60-0.89)	0.10	.003
astA gene	0.23	1.25 (1.02-1.52)	0.10	.02
hlyA gene	-0.22	0.79 (0.66-0.96)	0.10	.03

* The *P* values were obtained using multivariable logistic regression with a binary trait of PDS as dependent variable and sow parity and virulence genes as independent variables. In the logistic regression PDSA group was coded 1 and PDSU group was coded 0.

PDS = postpartum dysgalactia syndrome; OR = Odds ratio; *iss* = Increased serum survival; *sfa/foc* = S fimbriae (sialic acid-specific) and F1C fimbriae; *astA* = EAST1 (heat-stable cytotoxin associated with enteroaggregative *Escherichia coli*); *hlyA* = Hemolysin A; PDSA = PDS-affected; PDSU = PDS-unaffected.

both septicemic and healthy chickens.[22] Weak association of the prevalence of *fimC* and occurrence of PDS was also confirmed in our study where high prevalence (97.4%) of this fimbrial gene was also identified in PDSU sows.

Bacterial serum resistance has been reported as an important virulence factor since it enables bacteria to avoid the bactericidal effect of a serum.[23] Without this virulence factor, bacteria are being lysed by complement, which is more often activated through surface bacterial antigens via an alternative pathway.[24] The C3b component plays an important part in the alternative pathway mechanism enabling adherence of bacteria to C5 to C9 bacteriolytic membrane attack complex (MAC).[24]

Higher prevalence of *iss* gene in PDSA sows in our research confirms the findings of other researchers.[23-26] Pedersen Mörner et al[23] found that serum resistance virulence factor was more frequently detected in *E coli* isolates obtained from sows with coliform mastitis. Moreover, this virulence factor was often detected in strains isolated from the milk of mastitic cows.[25] Kassé et al[26] found that the *iss* gene was detected in 70% of *E coli* isolates found in dairy cows with postpartum metritis. The *iss* gene is more frequently distributed in APEC strains and is closely associated with large transmissible R plasmids or ColV plasmids in APEC (pAPEC-O1, pAPEC-O2-ColBM and pTJ100).[27] However, some studies have documented contradictory findings regarding the prevalence of *iss* between different *E coli* populations. In the study conducted by Rodriguez-Siek et al,[28] *iss* gene was found in 81% of APEC and in 60% of the UPEC isolates. In addition, this gene was confirmed in 56% of the newborn meningitis *E coli* (NMEC) strains[15] and in a few human fecal commensal *E coli* isolates.[29] Thus, more frequent presence of *iss* gene in ExPEC strains may be related to their ability to survive in extraintestinal conditions.[29]

The virulence genes related to APEC strain IMT2470 that we frequently detected in PDSA sows were found in UPEC strains too.[15,30] In the research of Ewers et al[30] at least one virulence gene related with APEC strain IMT2470 was found in all five UPEC isolates. In the survey of Ewers et al,[15] a substantial number of APEC associated genes (*iss, iucD, iroN, traT*) were also detected in UPEC strains. However, we could not categorize the detected *E coli* strains into specific ExPEC pathotype by virulence genotyping only. Many virulence features such as iron-uptake systems, protectins, fimbriae, and other adhesins are essential for fitness properties of the bacteria to enable them to efficiently adapt and colonize the host rather than their classical virulence factors primarily included in infection.[31]

In our study, we found that the presence of *iss* and *astA* and lower parity increased the clinical manifestation of PDS in sows. Moreover, the adjusted R^2 of 0.373 obtained for lower parity, presence of *iss* and *astA*, and absence of *sfa/foc* and *hlyA* were associated with the occurrence of PDS. Scientific data regarding the effect of parity as a risk factor on the occurrence of PDS are inconsistent. While Baer and Bilkei[32] reported that higher (> 4) parity sows had increased risk from recidiving mastitis-metritis-agalactia syndrome, other authors found greater risk of postparturient disorders for lower parity sows.[14,33,34]

We also found that younger sows vaginally infected by *E coli* were more prone to disease. According to Hoy,[35] higher parity sows have a more developed immune system than lower parity sows primarily due to their wider contact with microbiological agents during their lifetime. Nevertheless, our results could lead to potential biases, such as not having representative samples of virulence factors for the general sow population. This is certainly a weakness of the current study and additional research with adequate sample size is required to determine the biological associations of detected virulence genes and PDS in sows.

In summary, this study found that virulence genes associated with ExPEC were the most frequently detected among *E coli* isolates recovered from the vaginal swabs of both PDSA and PDSU sows. Lower parity and certain virulence genes related to ExPEC strains were strongly associated with clinical PDS in sows. This study gives novel information about virulence genes of *E coli* isolated from the genital tract of sows and PDS. The number of sows selected for this research corresponded to the available reproductive data obtained by the commercial pig farms included in the study. However, further research with large and equal sample sizes should be conducted to identify whether specific virulence gene profiles of ExPEC strains recovered from the genital tract are in line with the clinical appearance of PDS. The prevalence of virulence genes from other coliform bacteria and PDS in sows should be considered in future studies.

Implications

Under the conditions of this study:

- Vaginal swabs positive for *E coli* and *iss* were associated with PDS in sows.
- Younger sows with certain ExPEC strains were more likely to have clinical PDS.
- *Escherichia coli* pathotypes could not be categorized by virulence genotyping.

Acknowledgments

Pig farms are acknowledged for their contribution to this study. The authors would like to thank the colleagues from the Laboratories of Molecular Diagnostics and Microbiology from the Faculty of Veterinary Medicine in Skopje for their technical support with the multiplex PCR assays. Dr Antina Lübke-Becker from the Institute of Microbiology and Epizootics of the Free University Berlin is greatly acknowledged for providing control strains for molecular analysis.

Conflict of interest

None reported.

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References

1. Angjelovski B, Radeski M, Djadjovski I, Mitrov D, Bojkovski J, Adamov N, Dovenski T. Prevalence and clinical signs of postpartum dysgalactia syndrome at the first day after farrowing in farmed sows in the Republic of Macedonia. *Mac Vet Rev.* 2019;42(1):79-86.

2. Gerjets I, Kemper N. Coliform mastitis in sows: A review. *J Swine Health Prod.* 2009;17(2):97-105.

*3. Peltoniemi OA, Björkman S, Oliviero C. Parturition effects on reproductive health in the gilt and sow. In: *Proceedings of ESDAR and AERA*. European Society for Domestic Animal Reproduction and Spanish Association for Animal Reproduction; 2016:36-47.

4. Kemper N, Bardehle D, Lehmann J, Gerjets I, Looft H, Preissler R. The role of bacterial pathogens in coliform mastitis in sows. *Berl Munch Tierarztl Wochenschr.* 2013;126(3-4):130-136.

5. Papadopoulus G, Vanderhaeghe C, Janssens GPJ, Dewulf J, Maes D. Risk factors associated with postpartum dysgalactia syndrome. *Vet J*. 2010;184:167-171.

6. Hirsch AC, Philipp H, Kleemann R. Investigation on the efficacy of meloxicam in sows with mastitis-metritis-agalactia syndrome. *J Vet Pharmacol Ther.* 2003;26:355-360.

7. Bäckström L, Morkoc AC, Connor J, Larson R, Price W. Clinical study of mastitis-metritis-agalactia in sows in Illinois. *J Am Vet Med Assoc.* 1984;185:70-73.

8. Angjelovski B, Cvetkovikj A, Mrenoshki S, Radeski M, Cvetkovikj I, Ratkova M, Dovenski T. Bacteria associated with clinical postpartum dysgalactia syndrome in farmed sows in the Republic of Macedonia. *Turk J Vet Anim Sci.* 2016;40(6):776-781.

9. Kemper N, Gerjets I. Bacteria in milk from anterior and posterior mammary glands in sows affected and unaffected by postpartum dysgalactia syndrome (PPDS). *Acta Vet Scand.* 2009;51:26. doi:**10.1186/1751-0147-51-26**

10. Bertschinger HU, Bürgi E, Eng V, Wegmann P. Lowering of the incidence of puerperal mastitis in the sow by protection of the mammae from contamination. Article in German. *Schweiz Arch Tierheilkd*. 1990;132:557-566.

11. Ross RF, Harmon RL, Zimmermann BJ, Young TF. Susceptibility of sows to experimentally induced *Escherichia coli* mastitis. *Am J Vet Res.* 1983;44:949-954.

12. Magnusson U, Mörner AP, Persson A, Karlstam E, Sternberg S, Kindahl H. Sows intramammarily inoculated with *Escherichia coli*: Influence of time of infection, hormone concentrations and leucocyte numbers on development of disease. *J Vet Med B Infect Dis Vet Public Health.* 2001;48:501-512.

13. Waller CM, Bilkei G, Cameron RDA. Effect of periparturient diseases accompanied by excessive vulval discharge and weaning to mating interval on sow reproductive performance. *Aust Vet J*. 2002;80:545-549.

14. Bostedt H, Maier G, Herfen K, Hospen R. Clinical examinations on gilts with puerperalsepticaemia and toxaemia. Article in German. *Tierarztl Prax Ausg G Grosstiere Nutztiere.* 1998;26:332-338.

15. Ewers C, Li G, Wilking H, Kiessling S, Alt K, Antáo EM, Laturnus C, Diehl I, Glodde S, Homeier T, Böhnke U, Steinruck H, Philipp H-C, Wieler LH. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: How closely related are they? *Int J Med Microbiol.* 2007;297(3):163-176.

16. Casey TA, Bosworth BT. Design and evaluation of a multiplex polymerase chain reaction assay for the simultaneous identification of genes for nine different virulence factors associated with *Escherichia coli* that cause diarrhea and edema disease in swine. *J Vet Diagn Invest*. 2009;21(1):25-30.

17. Krag L, Hancock V, Aalbæk B, Klemm P. Genotypic and phenotypic characterisation of *Escherichia coli* strains associated with porcine pyelonephritis. *Vet Microbiol*. 2009;134(3-4):318-326.

18. Daigle F, Dozois CM, Jacques M, Harel J. Mutations in the f1651A and f1651E fimbrial genes and regulation of their expression in an *Escherichia coli* strain causing septicemia in pigs. *Microb Pathog.* 1997;22(4):247-252.

19. Gerjets I, Traulsen I, Reiners K, Kemper N. Comparison of virulence gene profiles of *Escherichia coli* isolates from sows with coliform mastitis and healthy sows. *Vet Microbiol.* 2011;152(3-4):361-367.

20. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Exp Mol Pathol*. 2008;85(1):11-19.

21. Schmidt A. *Molekulare epidemiologische Untersuchungen von klinischen* Escherichia-coli-*Isolaten unt erschiedlicher Wirtstierspezies* [*Molecular epidemiological studies from clinical Isolates of* Escherichia coli *indifferent species of host animals*]. PhD dissertation. Free University of Berlin; 2011.

22. Kawano M, Yaguchi K, Osawa R. Genotypic analyses of *Escherichia coli* isolated from chickens with colibacillosis and apparently healthy chickens in Japan. *Microbiol Immunol.* 2006;50(12):961-966.

23. Pedersen Mörner A, Faris A, Krovacek K. Virulence determinants of *Escherichia coli* isolated from the milk of sows with coliform mastitis. *Zentralbl Veterinarmed B.* 1998;45:287-295.

24. Taylor PW. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. *Microbiol Rev.* 1983;47(1):46-83.

25. Barrow PA, Hill AW. The virulence characteristics of strains of *Escherichia coli* isolated from cases of bovine mastitis in England and Wales. *Vet Microbiol.* 1989;20(1):35-48.

26. Kassé FN, Fairbrother JM, Dubuc J. Relationship between *Escherichia coli* virulence factors and postpartum metritis in dairy cows. *J Dairy Sci.* 2016;99(6):4656-4667.

27. Johnson TJ, Siek KE, Johnson SJ, Nolan LK. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. *J Bacteriol.* 2006;188(2):745-758.

28. Rodriguez-Siek KE, Giddings CW, Doetkott C, Johnson TJ, Fakhr MK, Nolan LK. Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology.* 2005;151(6):2097-2110.

29. Johnson TJ, Wannemuehler YM, Nolan LK. Evolution of the *iss* gene in *Escherichia coli*. *Appl Environ Microbiol.* 2008;74(8):2360-2369.

30. Ewers C, Janssen T, Kiessling S, Philipp HC, Wieler LH. Rapid detection of virulence-associated genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction. *Avian Dis*. 2005;49(2):269-273.

31. Dobrindt U. (Patho-) genomics of *Escherichia coli*. *Int J Med Microbiol.* 2005;295(6-7):357-371.

32. Baer C, Bilkei G. Ultrasonographic and gross pathological findings in the mammary glands of weaned sows having suffered recidiving mastitis-metritis-agalactia. *Reprod Domest Anim.* 2005;40:544-547.

33. Krieter J, Presuhn U. Genetic variation for MMA treatment. *Züchtungskunde.* 2009;81(3):149-154.

34. Gerjets I, Traulsen I, Reiners K, Kemper N. Assessing individual sow risk factors for coliform mastitis: A case-control study. *Prev Vet Med.* 2011;100(3-4):248-251.

35. Hoy S. The impact of puerperal diseases in sows on their fertility and health up to next farrowing. *Anim Sci*. 2006;82(5):701-704.

*Non-refereed reference.