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Clonal analysis and virulence-associated traits of native *Escherichia coli* from urethra of gilts and natural/artificial pregnant sows.

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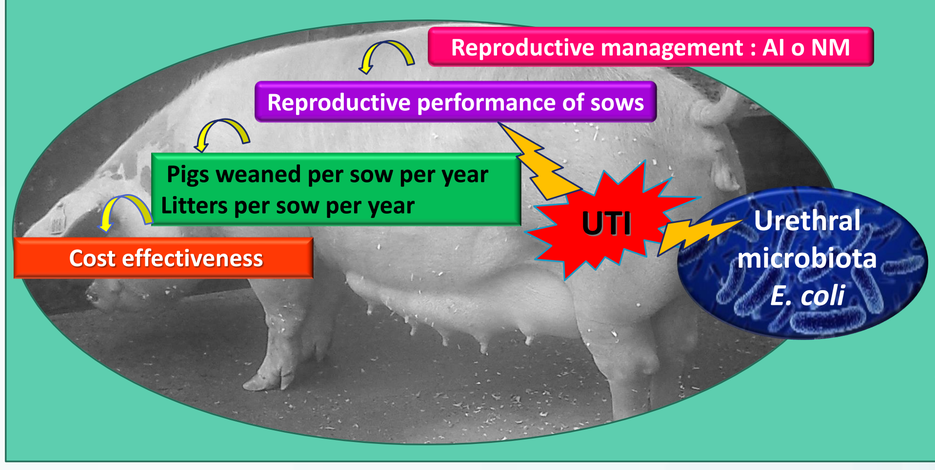
BACKGROUND

The reproductive performance of sows is a key factor in herd´s productivity (1). Urinary tract (UT) infections are a common problem in swine females (2), causing repeat breeding with a delayed return to estrus, which reduces the animal´s welfare and the litter performance; Escherichia coli being associated to these infections (3,4). Recent studies described a unique microbiota in the UT in females (5,6). Others authors concluded that the composition of the UT bacterial communities could have an important role in the health condition of the host (6,7).



Conditions in the reproductive management (AI or NB) affect the characteristics of the UT microbiota by favoring the prevalence of pathogenic microorganisms and thus, decreasing the reproductive performance in sows. UTI, Urinary Tract Infections; AI, Artificial Insemination; NB, Natural Breading.

OBJECTIVE

The purpose of this study was

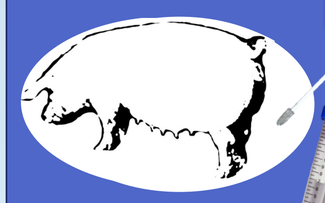
- The isolation and clonal association of *E. coli* from the urethral microbiota of sows. healthy gilts (HG) and pregnant sows by natural breading (NB) or artificial insemination (AI).
- Study the virulence factors relevant for pyelonephritic strains

METHODOLOGY

Isolation of *E. coli* from the urethral microbiota of:

Pregnant sows

Healthy Gilts (HG) n=9 | by Natural Breading (NB) n=11 | by Artificial Insemination (AI) n=11



cytobrush was collected in 1 mL of phosphate buffered saline solution (PBS) pH 7.0 and kept refrigerated until processing

Quantitative colony counts: mesophilic bacteria, enterobacteria

LAPTg agar, blood agar, Mac Conkey's agar

Lactose positive colonies
Gram staining and standard biochemical tests

E. coli

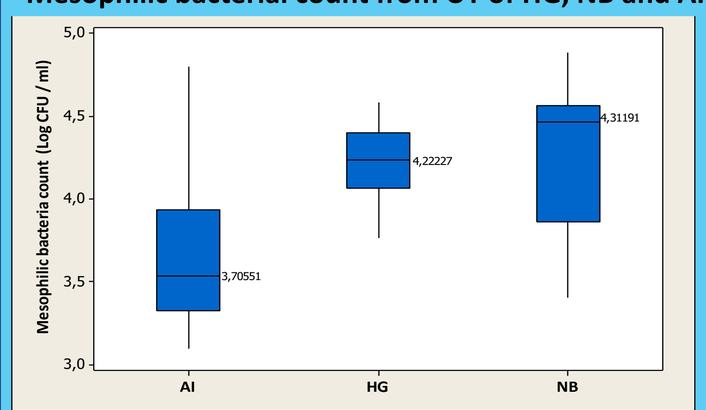
Extraction and purification of genomic DNA (QIAamp DNA Mini Kit)

Clonal association: rep-PCR fingerprinting
The primers evaluated in this study were :
ERIC2 (5'AAGTAAGTGACTGGGGTGAGCG 3 ')
BOX A1R (5' CTACGGCAAGGCGACGCTGACG 3')

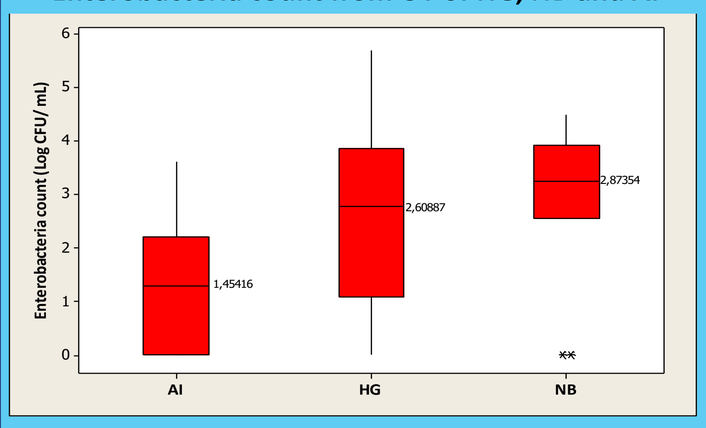
Evaluation for pyelonephritic virulence factors by PCR: *hlyA, cnf1, ibeA, iutA, kpsMT II, fimH, papC, sfa/focD, afa/draBC, traT, agn43, csgA.*

RESULTS

Mesophilic bacterial count from UT of HG, NB and AI

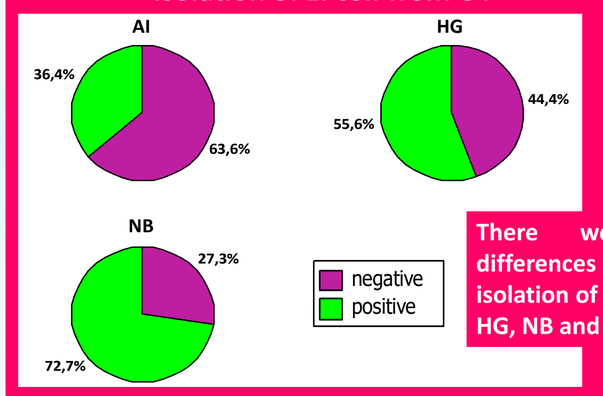


Enterobacteria count from UT of HG, NB and AI



Cultures revealed a slightly minor mesophilic microorganisms count (CFU/mL) for AI (3.7±0.59) group compared to HG (4.2±0.24) and NB (4.3±0.44). However, there were no differences for enterobacteria count (CFU/mL): 1.45±1.36, 2.87±1.53 and 2.61±1.84, for HG, NB and AI sows .

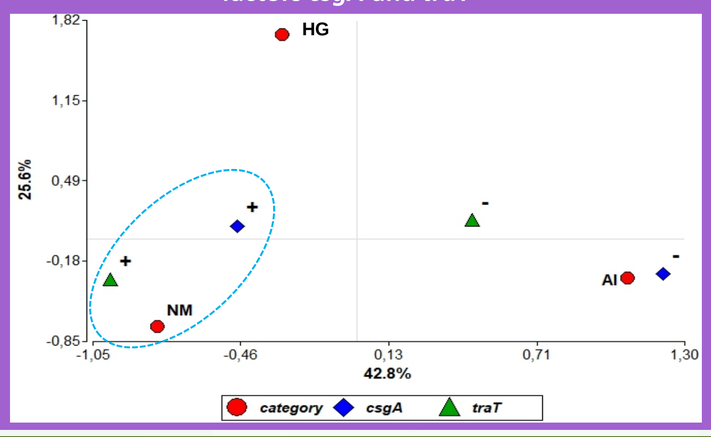
Isolation of *E. coli* from UT



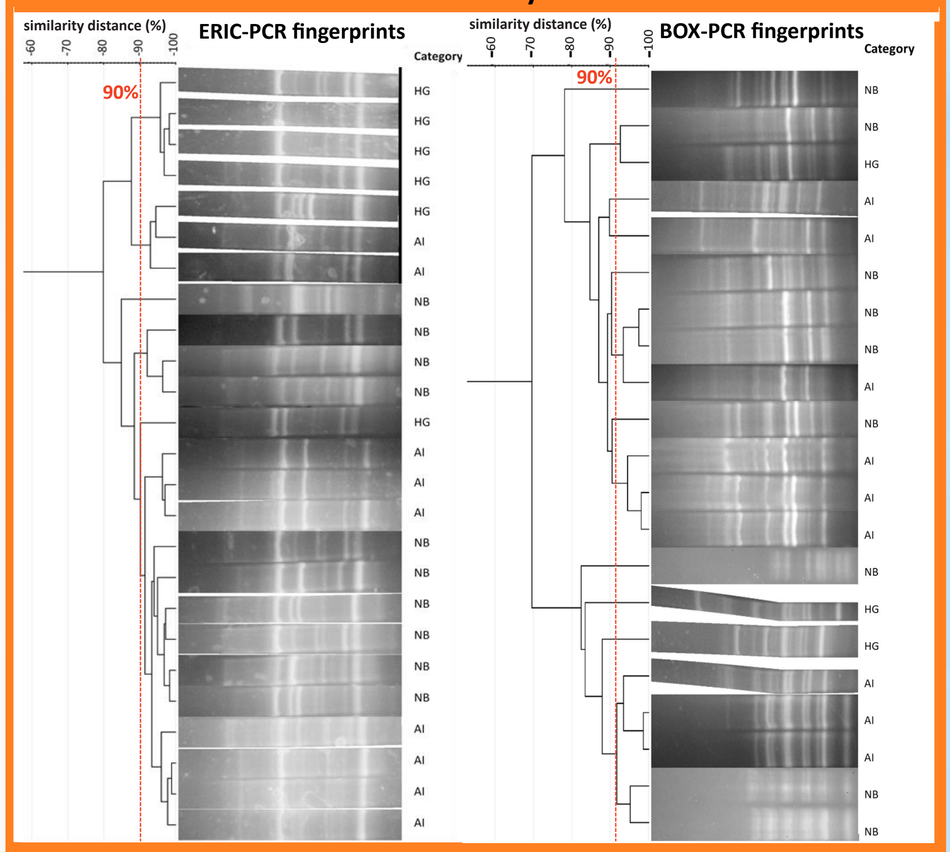
There were no differences in the isolation of *E. coli* for HG, NB and AI sows .

PCR Positive reaction was found for: *fimH* (76%), *agn43* (92%), *traT* (32%) and *csgA* (72%). The VF genes *traT* and *csgA* showed a differential prevalence and were associated with *E. coli* from NB sows All the isolates were negative for *hlyA, cnf, ibeA, iutA, kpsMT II, papC, sfa/focD,*

Correspondence analysis map between sows category and the virulence factors *csgA* and *traT*



Clonal analysis



The clusters, defined at 95% similarity level, were able to separate the *E. coli* isolates by category. However, the clonal analysis with both, Box or Eric primers, revealed high similarity (>90%) between *E. coli* isolates from the different animal groups.

CONCLUSION

These results indicate that the management conditions could affect the characteristics of the urethral microbiota in sows, particularly, *E. coli* populations; therefore, the risk for urinary tract diseases.

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