

A retrospective study of the incidence of *Escherichia coli* adherence factors since the ban of zinc oxide as a veterinary medicinal product

L. Pérez,¹ DVM, MSc; M. Claver,¹ DVM, BSc; J. Bringas,¹ DVM, MSc

¹Elanco Animal Health, Avenida Bruselas 13, Alcobendas, Spain. Contact: lorena.perez@elancoah.com

Introduction and Objectives

The use of zinc oxide (ZnO) as a veterinary medicinal product in the EU in weaned piglet feed was withdrawn by the European Commission in 2022¹. It was the main tool together with antibiotics to fight against postweaning diarrhoea (PWD). This study aimed to investigate if the withdrawal of ZnO is related to an increase of the incidence of PWD caused by *Escherichia coli* (*E. coli*) in the postweaning period.

Material and Methods

A total of 733 Spanish pig farms with acute cases of PWD were included into the study. The farms were divided into two groups based on the use of ZnO as a medicinal product in feed resulting in 423 farms with ZnO and 310 farms without it. Animals were selected based on PWD clinical signs. An average of three rectal swabs were collected from three different pigs on each farm from four- to eight-week-old pigs within the first 24 hours of the acute phase of the disease. Swabs with fecal matter were submitted to the laboratory (Exopol; Zaragoza, Spain) for diagnosis. PCR was performed to detect adherence factor including F4 (K88), F18 and adhesin involved in diffuse adherence (AIDA-1). The statistical analysis was carried out using the statistical software package STATA 17.0 (STATA, USA). A homogeneity test using contingency tables was used to detect statistical associations.

Results

Results can be observed in table 1, 2 and 3. In the test of homogeneity, the results showed that the incidence of AIDA-1 was statistically higher ($X^2=14.45$; $p=0.000$) on farms where ZnO was not used anymore (78.39% vs 65.48%). Related to F4, it did not exist a statistical relation ($X^2=2.74$; $p=0.097$), although numerically an increase of this fimbria was observed on farms without ZnO (60.97% vs 54.85%). For F18, the results did not show any statistical association ($X^2=0.91$; $p=0.339$) resulting in 75.48% on farms without ZnO and 72.34% with it.

Table 1: Contingency table results for AIDA-1 adhesin gene.

| AIDA | ZnO | | Total |
|----------|------------------------|------------------------|------------------------|
| | absense | presence | |
| negative | 67 90.1 21.61 | 146 122.9 34.52 | 213 213.0 29.06 |
| positive | 243 219.9 78.39 | 277 300.1 65.48 | 520 520.0 70.94 |
| Total | 310 310.0 100.00 | 423 423.0 100.00 | 733 733.0 100.00 |

Pearson $\chi^2(1) = 14.4467$ Pr = 0.000
Likelihood-ratio $\chi^2(1) = 14.7470$ Pr = 0.000
Fisher's exact = 0.000
1-sided Fisher's exact = 0.000

Table 2: Contingency table results for F4 adherence factor gene.

| F4 | ZnO | | Total |
|----------|------------------------|------------------------|------------------------|
| | absense | presence | |
| negative | 121 132.0 39.03 | 191 180.0 45.15 | 312 312.0 42.56 |
| positive | 189 178.0 60.97 | 232 243.0 54.85 | 421 421.0 57.44 |
| Total | 310 310.0 100.00 | 423 423.0 100.00 | 733 733.0 100.00 |

Pearson $\chi^2(1) = 2.7428$ Pr = 0.098
Likelihood-ratio $\chi^2(1) = 2.7493$ Pr = 0.097
Fisher's exact = 0.112
1-sided Fisher's exact = 0.057

Table 3: Contingency table results for F18 adherence factor gene.

| F18 | ZnO | | Total |
|----------|------------------------|------------------------|------------------------|
| | absense | presence | |
| negative | 76 81.6 24.52 | 117 111.4 27.66 | 193 193.0 26.33 |
| positive | 234 228.4 75.48 | 306 311.6 72.34 | 540 540.0 73.67 |
| Total | 310 310.0 100.00 | 423 423.0 100.00 | 733 733.0 100.00 |

Pearson $\chi^2(1) = 0.9113$ Pr = 0.340
Likelihood-ratio $\chi^2(1) = 0.9154$ Pr = 0.339
Fisher's exact = 0.352
1-sided Fisher's exact = 0.192

Discussion and conclusion

This study demonstrated that a higher amount of *E. coli*'s virulence factors is present on farms since the ban of ZnO as a medicinal product. The non statistical association between ZnO and F18 can be explained as the percentage is considered as high for the two groups of farms. In conclusion, other tools are needed to control the increased incidence of postweaning diarrhoea caused by *E. coli* since ZnO as a medicinal product is not allowed and the use of antibiotics is being limited².

References

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