DETECTION OF *CAMPYLOBACTER SPP* IN FAECES

Ring trial 16th of May 2018

Sciensano

Infectious diseases in humans - Foodborne pathogens

June 2018 • Elsene • Belgium

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The present trial was organised upon request of FAVV-AFSCA. The study focused on the detection of *Campylobacter* spp. in caeca according to ISO-10272-1 (Horizontal method for detection and enumeration of *Campylobacter* spp. – part 1).

1. PARTICIPATING LABORATORIES

Four laboratories participated to the study.

Nr. of participating laboratory
11
17
21
32

2. PLANNING OF THE STUDY

Study - March 2018:
27/03/2018: sample preparation and transport to the participating laboratories
28/03/2018: start of the study for all laboratories
20/04/2018: deadline submission of results

Repeat study - May 2018:
16/05/2018: sample preparation and transport to the participating laboratories
16/05/2018: start of the study for all laboratories
06/06/2018: deadline submission of results

3. MATERIALS AND METHODS

Study - March 2018:

Strain used:

- *Campylobacter coli* TIAC 3731

The strain used in the study is a well-characterized and confirmed reference strain received from the EURL-AR.

Tested matrix: chicken faeces (S17FP09303)

The faecal samples provided to the laboratories initially consisted of a pool of faeces that was thoroughly mixed, divided in smaller aliquots and stored at -20°C in our laboratory until usage.

Spiking of the samples:

- Sample preparation: four samples were prepared for each participating laboratory, e.g. 4 samples of 5g chicken faeces supplemented with 5ml of physiological water in a sterile recipient.
- Inoculum preparation: the strain was grown in Bolton broth at 41.5°C for 48h under microaerophilic conditions.
- Contamination of the samples: three samples (5g) were respectively, spiked with 1000µl of the stock solution, the 10^{-1} and 10^{-2}-fold dilution. One sample (10g) was considered as blank for *Campylobacter spp.* as it was not spiked (Table 1).
- The four samples were sent to the participating laboratory.
- Determination of the inoculum concentration: the inoculum concentration was determined by plating in triplicate on Columbia blood agar plates.

Table 1: Sample composition

Sample nr.	Matrix	Strain
C6	5 g of chicken faeces	TIAC 3731
C7	5 g of chicken faeces	/
C8	5 g of chicken faeces	TIAC 3731
C9	5 g of chicken faeces	TIAC 3731

Method: ISO-10272-1, Horizontal method for detection and enumeration of *Campylobacter* spp. – part 1.

Study - May 2018:

Strain used:

- *Campylobacter jejuni* S18FP03276

The strain used in the study is a well-characterized and confirmed strain isolated from chicken faeces.

Tested matrix: bovine faeces (S17FP04867)

The faecal samples provided to the laboratories initially consisted of a pool of faeces that was thoroughly mixed, divided in smaller aliquots and stored at -20°C in our laboratory until usage.

Spiking of the samples:

- Sample preparation: four samples were prepared for each participating laboratory, e.g. 4 samples of 5g bovine faeces in a sterile recipient.
- Inoculum preparation: the strain was grown in Bolton broth at 41.5°C for 24h under microaerophilic conditions.
- Contamination of the samples: three samples (5g) were respectively, spiked with 1000µl of the stock solution, the 10^{-1} and 10^{-2}-fold dilution. One sample (10g) was considered as blank for *Campylobacter spp.* as it was not spiked (Table 2).
- The four samples were sent to the participating laboratory.
- Determination of the inoculum concentration: the inoculum concentration was determined by plating in triplicate on Columbia blood agar plates.

Table 2: Sample composition

Sample nr.	Matrix	Strain
C1	5 g of bovine faeces	S18FP03276
C2	5 g of bovine faeces	/
C3	5 g of bovine faeces	S18FP03276
C4	5 g of bovine faeces	S18FP03276

Method: ISO-10272-1, Horizontal method for detection and enumeration of *Campylobacter* spp. – part 1.

4. RESULTS

Study - March 2018:

Inoculum concentration

The concentration of the inoculum is presented in table 3. No bacterial growth was observed.

Table 3: Inoculum concentration

C. coli TIAC 3731	Results			Mean (CFU/100µl)	SD
	plate 1	plate 2	plate 3		
Dil -4	0	0	0	0	0
Dil -5	0	0	0		
Dil -6	0	0	0		

Study results
As expected, none of the participating laboratories detected *Campylobacter spp.* in the three spiked samples and the blank sample.

Study - May 2018:

Inoculum concentration
The concentration of the inoculum is presented in table 4. The samples (5g) were spiked, respectively, with $1{,}1x10^{8}$ CFU/1000µl, $1{,}1x10^{7}$ CFU/1000µl and $1{,}1x10^{6}$ CFU/1000µl for the stock solution, the 10^{-1} and 10^{-2}-fold dilution.

Table 4: Inoculum concentration

C. coli S18FP03276	Results			Mean (CFU/100µl)	SD
	plate 1	plate 2	plate 3		
Dil -4	NC	NC	NC		
Dil -5	62	134	137	111	42
Dil -6	14	9	16		

Study results
The expected results of the present study are presented in table 5.

Table 5: Expected results
(D: detected or ND: not detected)

Sample nr.	*Campylobacter spp.*
C1	D
C2	ND
C3	D
C4	D

All participating laboratories detected *Campylobacter spp.* in the three spiked samples (Table 6). *Campylobacter* was not detected on the fourth blank sample.

Table 6: Observed results
(D: detected or ND: not detected)

Nr. of laboratory	Sample nr. (*Campylobacter spp.*)			
	C1	C2	C3	C4
11	D	ND	D	D
17	D	ND	D	D
21	D	ND	D	D
32	D	ND	D	D

5. CONCLUSION

As expected, for the study of March 2018, all laboratories did not detect *Campylobacter* spp. in chicken faeces. For the study of May 2018, all laboratories detected *Campylobacter spp.* in bovine faeces. No false-positive resultats were obtained for the blank samples.

6. REFERENCES

ISO-10272-1, Horizontal method for detection and enumeration of *Campylobacter* spp. – part 1.

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