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081-PT

PROFICIENCY TESTING 2019

Q-FEVER (QFV)

Detection of QFV-specific antigens in milk

by Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE START PT: 25 NOVEMBER 2019

DATE REPORT: 28 JANUARY 2020

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', which is summarized in the 'Manual for the participant'.

II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of QFV-specific antigens in milk of bovidae origin by RT-qPCR.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference milk samples must be analyzed by means of a RT-qPCR. The procedure for the RT-qPCR assay must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. Reference samples

Twenty reference milk samples of small ruminants origin, either free from detectable QFV-specific antigens (n=2; coded 'PT2019QFVBACMN1' and 'PT2019QFVBACMN2') or containing detectable QFV-specific antigens (n=3; coded 'PT2019QFVBACMP1', 'PT2019QFVBACMP2' and 'PT2019QFVBACMP3') were used.

In total, 60 aliquots were distributed to 3 participating laboratories. All participants received 20 aliquots of reference samples. The identification numbers of the reference samples were randomized for all participants (Table 3).

For each reference sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference samples was based on (i) the historical background of the animals/farm and (ii) the results obtained by analytical tests (pre-verification). The pre-verification tests consisted of two extraction protocols, SOP/BAC/PRE/03 (in-house) and the QIAGEN DNeasy Blood & Tissue Kits. All extracted DNAs were subsequently analyzed using an in-house developed RT-qPCR assay (SOP/BAC/ANA/15), the ADIAVET™ *Coxiella* real time (Adiagene) and the VetMAX™ *C. burnetii* Relative Quant Kit (Thermo Fisher). Totally, all samples were characterized by a combination of two different extraction protocols and three RT-qPCR assays. For these reference samples, the same qualitative results were obtained with all assays used.

The reference milk samples were obtained from bulk tank milks of five different sheep/goat/bovine farms some participating to the trimestrial screening and resulting free of infection since 2013 (PT2019QFVBACMN1 and PT2019QFVBACMN2) or naturally infected (PT2019QFVBACMP1, PT2019QFVBACMP2 and PT2019QFVBACMP3) with *C.burnetii*. Milks, infected or not, were inactivated (internal procedure) and lyophilized.

Taken together, the reference milk sample PT2019QFVBACMN1 and PT2019QFVBACMN2 were considered as negative sample, the reference milk samples PT2019QFVBACMP1, PT2019QFVBACMP2 and PT2019QFVBACMP3 were considered as positive samples.

A homogeneity check on the aliquoted reference milk samples had been performed as in the context of PTs under the procedure SOP 25/01. Indeed, 10 aliquots of each reference sample were analysed using the in-house developed RT-qPCR assay (SOP/BAC/ANA/15), hereby obtaining the same qualitative result for all 10 aliquots of the same reference sample. Consequently, all reference samples were considered as reliable samples in order to evaluate the ability of the participating laboratories to correctly identify the absence or presence of QFV-specific antigens in milk. In addition, all

reference samples were tested once after the PT in order to confirm their stability and status (post-verification) using the in-house developed RT-qPCR assay (SOP/BAC/ANA/15).

III.3. Classification of results, level of agreement and threshold for qualification

III.3.1. Classification of results

Results provided by the participating laboratories are categorized as *success* when the reported result matches with the assigned status or *failure* when the reported result does not match with the assigned status.

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 20 aliquots of reference samples used for this PT.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 aliquots of reference samples used for this PT is at least 90%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the Scientific Directorate Infectious Diseases in Animals of Sciensano.

IV.1. Transfer and start of the analyses of the reference samples

The lyophilized reference milk samples were sent to the participants by national or international courier on 25th of November 2019. LAB1 and LAB2 acknowledged receipt of the samples on the same day, whereas LAB3 received the samples on 27th of November 2019.

Analyses were started between 28th of November and 3rd of December 2019 (Table 1).

IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano between 5th and 9th of December 2019 (Table 1). All participants respected the deadline of 13th of December 2019 for submission of the results.

Table 1. Overview of the dates on which (i) the reference samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	25/11/2019	28/11/2019	06/12/2019
LAB2	25/11/2019	29/11/2019	09/12/2019
LAB3	27/11/2019	03/12/2019	05/12/2019

IV.3. Compliance with the procedure

All participants provide a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that:

All participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement) (Table 2).

Table 2. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference milk samples assigned by the QFV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference milk samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	2	3
failure	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)

A quantitative data analysis (box plots) is shown for educational purposes in Annex 1.

IV.4.2. Variability among participating laboratories

Since all participating laboratories reached 100% of agreement for the identification of QFV-specific antigens in milk, no variability between the participants could be observed. Additional information regarding the Ct/Cp levels can be found in Annex 1.

For each participating laboratory, the obtained results and the assigned statuses for the reference milk samples are shown in Table 3.

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference milk samples (SAMPLE), the external identification of the reference milk samples (LABPOSIT), and the status assigned by the QFV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019QFVBACMP1	POS	POS	1
2	1	2	PT2019QFVBACMP3	POS	POS	1
3	1	3	PT2019QFVBACMN1	NEG	NEG	1
4	1	4	PT2019QFVBACMP2	POS	POS	1
5	1	5	PT2019QFVBACMN2	NEG	NEG	1
6	1	6	PT2019QFVBACMP1	POS	POS	1
7	1	7	PT2019QFVBACMN1	NEG	NEG	1
8	1	8	PT2019QFVBACMP3	POS	POS	1
9	1	9	PT2019QFVBACMP2	POS	POS	1
10	1	10	PT2019QFVBACMN1	NEG	NEG	1
11	1	11	PT2019QFVBACMP1	POS	POS	1
12	1	12	PT2019QFVBACMP3	POS	POS	1
13	1	13	PT2019QFVBACMP2	POS	POS	1
14	1	14	PT2019QFVBACMN2	NEG	NEG	1
15	1	15	PT2019QFVBACMP2	POS	POS	1
16	1	16	PT2019QFVBACMP1	POS	POS	1
17	1	17	PT2019QFVBACMN1	NEG	NEG	1
18	1	18	PT2019QFVBACMN2	NEG	NEG	1
19	1	19	PT2019QFVBACMN2	NEG	NEG	1
20	1	20	PT2019QFVBACMP3	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2019QFVBACMP2	POS	POS	1
22	2	2	PT2019QFVBACMN1	NEG	NEG	1
23	2	3	PT2019QFVBACMP1	POS	POS	1
24	2	4	PT2019QFVBACMP3	POS	POS	1
25	2	5	PT2019QFVBACMN2	NEG	NEG	1
26	2	6	PT2019QFVBACMP2	POS	POS	1
27	2	7	PT2019QFVBACMN2	NEG	NEG	1
28	2	8	PT2019QFVBACMP1	POS	POS	1
29	2	9	PT2019QFVBACMP3	POS	POS	1
30	2	10	PT2019QFVBACMN2	NEG	NEG	1
31	2	11	PT2019QFVBACMP2	POS	POS	1
32	2	12	PT2019QFVBACMN1	NEG	NEG	1
33	2	13	PT2019QFVBACMP1	POS	POS	1
34	2	14	PT2019QFVBACMP3	POS	POS	1
35	2	15	PT2019QFVBACMN1	NEG	NEG	1
36	2	16	PT2019QFVBACMN1	NEG	NEG	1
37	2	17	PT2019QFVBACMN2	NEG	NEG	1
38	2	18	PT2019QFVBACMP2	POS	POS	1
39	2	19	PT2019QFVBACMP3	POS	POS	1
40	2	20	PT2019QFVBACMP1	POS	POS	1
41	3	1	PT2019QFVBACMP1	POS	POS	1
42	3	2	PT2019QFVBACMP3	POS	POS	1
43	3	3	PT2019QFVBACMN1	NEG	NEG	1
44	3	4	PT2019QFVBACMP2	POS	POS	1
45	3	5	PT2019QFVBACMN2	NEG	NEG	1
46	3	6	PT2019QFVBACMP1	POS	POS	1
47	3	7	PT2019QFVBACMN1	NEG	NEG	1
48	3	8	PT2019QFVBACMP3	POS	POS	1
49	3	9	PT2019QFVBACMP2	POS	POS	1
50	3	10	PT2019QFVBACMN1	NEG	NEG	1
51	3	11	PT2019QFVBACMP1	POS	POS	1
52	3	12	PT2019QFVBACMP3	POS	POS	1
53	3	13	PT2019QFVBACMP2	POS	POS	1
54	3	14	PT2019QFVBACMN2	NEG	NEG	1
55	3	15	PT2019QFVBACMP2	POS	POS	1
56	3	16	PT2019QFVBACMP1	POS	POS	1
57	3	17	PT2019QFVBACMN1	NEG	NEG	1
58	3	18	PT2019QFVBACMN2	NEG	NEG	1
59	3	19	PT2019QFVBACMN2	NEG	NEG	1
60	3	20	PT2019QFVBACMP3	POS	POS	1

V. Discussion

The purpose of this PT was to assess performances of the participating laboratories when analyzing reference milk samples of bovidae origin for the detection of QFV-specific antigens by RT-qPCR.

All participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). (Table 2 and Table 3)

QFV extraction kits were from different producers: one from Qiagen (LAB1), one from Thermo Fisher (LAB2) and one from Life Technologies (LAB3).
QFV RT-PCR kits were from different producers: two from Life Technologies (LAB1 and LAB3) and one In house/Home made kit (LAB2).

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference milk samples assigned by the QFV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the detection of QFV-specific antigens by RT-qPCR in reference milk samples.

Coordinator proficiency tests

Katia Knapen and Bernard China

Appendix

Name of the participating laboratories

Association Régionale de Santé et d'Identification Animales (ARSIA) (Ciney, Belgium)
Laboratoire National de Contrôle des Reproducteurs (LNCR / ACSEDIATE (Maisons-Alfort, France)
Sciensano (Ukkel, Belgium)

Annex 1: Quantitative data analysis (Box plots)

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs R (box plots).

Box plots represent the minimum and maximum value that are not considered as outliers, the 25th and 75th percentile (respectively P25 and P75), the median (P50), and possible outliers per sample and per laboratory. Values lower than (P25-1.5(P75-P25) and higher than (P75+1.5(P75-P25)) are considered as outliers. Note that due to the low number of data available, outliers cannot be detected when the number of data is smaller than 5 and P25=minimum and P75=maximum when the number data is 2.

<u>The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.</u>

Box plots of the Ct/Cp values per positive reference milk sample and per participating laboratory are shown in Figure 1

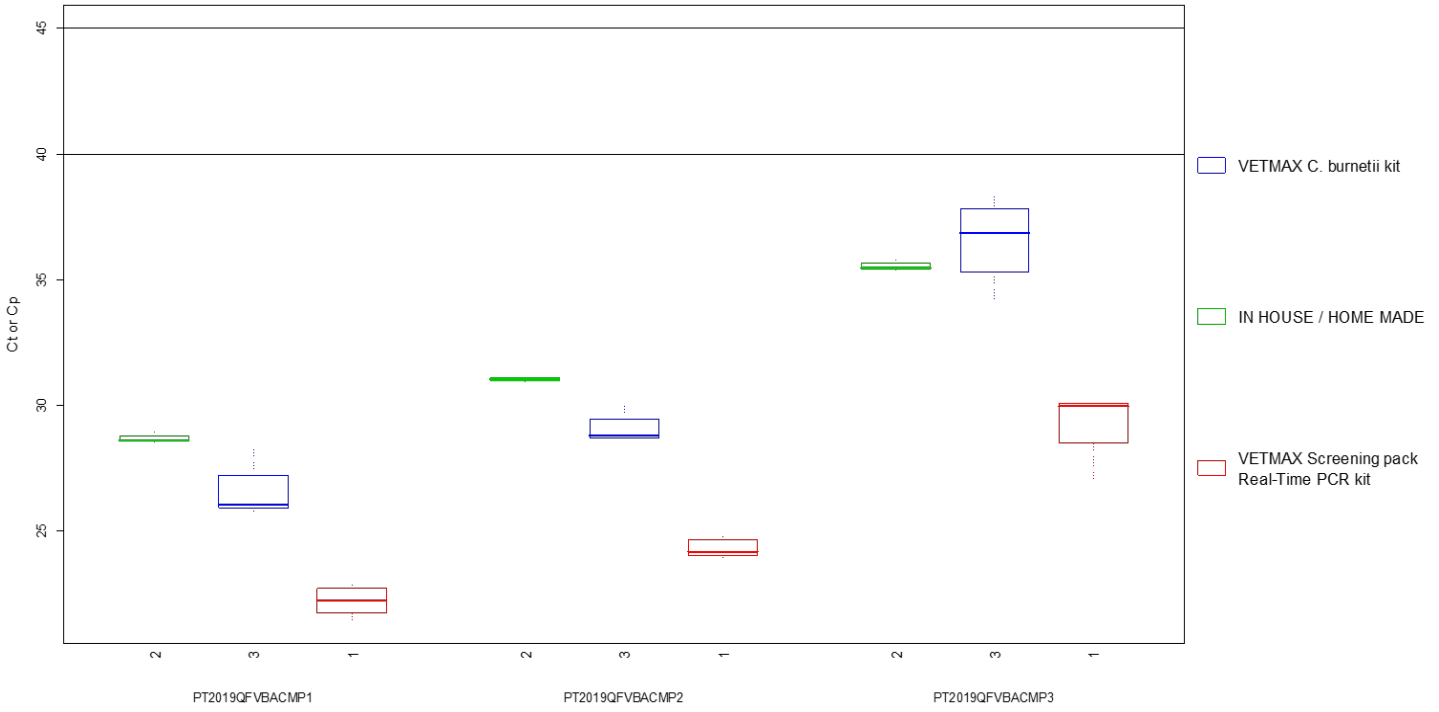

Figure 1. Box plots showing the Ct/Cp values per positieve reference milk and per participating laboratory.
Cut-off values are shown by horizontal lines (Home made and VETMAX Screening pack 40 and VETMAX C.brunetti 45).