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172-PT

PROFICIENCY TESTING 2016

Paratuberculosis (PTU)

Detection of PTU-specific antibodies in bovine serum and/or bovine milk by Enzyme Linked Immunosorbent Assay (ELISA)

CODA-CERVA-UCCLE

DATE BEGIN PT: 05 DECEMBER 2016

DATE REPORT: 02 FEBRUARI 2017

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure PRO/2.5/01 'Beheer van de proficiency testen op het CODA-CERVA-Ukkel/Gestion des essais d'aptitude au CODA-CERVA-Uccle', 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 PTU-specific antibodies in bovine serum and/or bovine milk by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum and/or reference milk samples must be analyzed by means of a PTU antibody ELISA test. The procedures for these ELISA tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. Reference serum samples

Replicates of 6 reference serum samples of bovine origin, either free from detectable PTU-specific antibodies (n = 2; coded 'PT2016PTUSERNS1' and 'PT2016PTUSERNS2') or containing detectable PTU-specific antibodies (n = 4; coded 'PT2016PTUSERPS1', 'PT2016PTUSERPS2', 'PT2016PTUSERPS3' and 'PT2016PTUSERPS4'), were used. In total, 180 aliquots were distributed to 9 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2016PTUSERNS1, PT2016PTUSERNS2, PT2016PTUSERPS1 and PT2016PTUSERPS3, and 4 aliquots of the reference serum samples PT2016PTUSERPS2 and PT2016PTUSERPS4. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 4).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained by the IDEXX Paratuberculosis Screening Antibody Test Kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET (pre-verification). The reference serum samples PT2016PTUSERNS1 and PT2016PTUSERNS2 were PTU antibody negative field sera, and the reference serum samples PT2016PTUSERPS1, PT2016PTUSERPS2, PT2016PTUSERPS3 and PT2016PTUSERPS4 were PTU antibody positive field sera from 4 different animals of the same positive farm. For 2 of these samples (PT2016PTUSERPS3 and PT2016PTUSERPS4) the animals were shown to be shedders by PCR analysis on faeces samples. For each reference serum sample, the same qualitative result was obtained with both ELISA kits used. Taken together, the reference serum samples PT2016PTUSERNS1 and PT2016PTUSERNS2 were considered as negative sera, and the reference serum samples PT2016PTUSERPS1, PT2016PTUSERPS2, PT2016PTUSERPS3 and PT2016PTUSERPS4 were considered as positive sera.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the IDEXX Paratuberculosis Screening Antibody Test Kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample with both ELISA kits. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of PTU-specific antibodies in bovine serum. In addition, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the IDEXX Paratuberculosis Screening Antibody Test Kit from IDEXX Montpellier SAS.

III.2.2. Reference milk samples

Replicates of 6 reference milk samples of bovine origin, either free from detectable PTU-specific antibodies (n = 2; coded 'PT2016PTUSERNM1' and 'PT2016PTUSERNM2') or containing detectable PTU-specific antibodies (n = 4; coded 'PT2016PTUSERPM1', 'PT2016PTUSERPM2', 'PT2016PTUSERPM3' and 'PT2016PTUSERPM4'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference milk samples PT2016PTUSERNM1, PT2016PTUSERNM2, PT2016PTUSERPM1 and PT2016PTUSERPM3 and 4 aliquots of the reference milk samples PT2016PTUSERPM2 and PT2016PTUSERPM4. The positions of the reference milk samples in the sent blocks were randomized for each participant (Table 5).

For each reference milk sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference milk samples was based on (i) the historical background of the animals and (ii) the results obtained by the IDEXX Paratuberculosis Screening Ab Test kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET (pre-verification). The reference milk samples PT2016PTUSERNM1 and PT2016PTUSERNM2 were derived from 2 different brands of commercial defatted milk, whereas the reference milk samples PT2016PTUSERPM1, PT2016PTUSERPM2, PT2016PTUSERPM3 and PT2016PTUSERPM4 were obtained from animals that were PTU antibody positive. For 3 of these samples (PT2016PTUSERPM2, PT2016PTUSERPM3 and PT2016PTUSERPM4) the animals were shown to be shedders by PCR analysis on faeces samples. For each reference milk sample, the same qualitative result was obtained with both ELISA kits used. Taken together, the reference milk samples PT2016PTUSERNM1 and PT2016PTUSERNM2 were considered as negative milk samples, and the reference milk samples PT2016PTUSERPM1, PT2013PTUSERPM2, PT2016PTUSERPM3 and PT2016PTUSERPM4 as positive milk samples.

After aliquoting the different reference milk samples, a homogeneity check was performed on 10 aliquots of each reference milk sample using the IDEXX Paratuberculosis Screening Ab Test kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample with both ELISA kits. Consequently, all reference milk samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of PTU-specific antibodies in bovine milk. In addition, reference milk sample were tested once after the PT in order to confirm their stability and status (post-verification) using the IDEXX Paratuberculosis Screening Ab Test kit from IDEXX Montpellier SAS.

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 either 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 either 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 CODA-CERVA-Uccle.

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

LAB1, LAB2, LAB3 and LAB4 participated in both the PT serum and the PT milk and hence received 40 aliquots: 20 aliquots of reference serum samples and 20 aliquots of reference milk samples. In contrast, LAB5, LAB6, LAB7, LAB8 and LAB9 only participated in the PT serum and hence received 20 aliquots of reference serum samples, whereas LAB10 and LAB11 only participated in the PT milk and thus received 20 aliquots of reference milk samples. The reference serum samples (180 aliquots in total) and reference milk samples (120 aliquots in total) were sent frozen (dry ice) to the 11 participating laboratories by national or international courier on the 5th of December 2016. LAB1, LAB2, LAB3, LAB5, LAB10 and LAB11 acknowledged receipt of the samples on the same day, whereas the other participants acknowledged receipt of the samples on 6th of December 2016.
LAB11 asked to resent the milk samples. These milk samples were resent by national courier on 16th of December 2016. LAB11 acknowledged receipt of the samples on the same day.
Analyses were performed between 7th and 20th of December 2016, both for the PT serum and the PT milk (Table 1).

IV.2. Dates at which results were returned to the CODA-CERVA-Uccle

Results were submitted to the CODA-CERVA-Uccle between 13th and 21th of December 2016 (Table 1).

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 CODA-CERVA-Uccle.

Laboratory	Reference samples received	Start of analysis serum	Start of analysis milk	Submission of the results (Excel file)
LAB1	05/12/2016	07/12/2016	07/12/2016	14/12/2016
LAB2	05/12/2016	08/12/2016	08/12/2016	16/12/2016
LAB3	05/12/2016	08/12/2016	13/12/2016	15/12/2016
LAB4	06/12/2016	15/12/2016	12/12/2016	15/12/2016
LAB5	05/12/2016	(#) 09/12/2016 15/12/2016	NA	16/12/2016
LAB6	06/12/2016	08/12/2016	NA	14/12/2016
LAB7	06/12/2016	07/12/2016	NA	21/12/2016
LAB8	06/12/2016	08/12/2016	NA	13/12/2016
LAB9	06/12/2016	08/12/2016	NA	13/12/2016
LAB10	05/12/2016	NA	13/12/2016	16/12/2016
LAB11	05/12/2016 16/12/2016	NA	20/12/2016	21/12/2016

Legend: NA = not applicable; (#) = this laboratory tested ELISA kits from 2 different producers

IV.3. Compliance with the procedure

Nine out of 11 participating laboratories have provided 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:

For the detection of PTU-specific antibodies in **serum**, 8 out of 9 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples and thus achieved 100% of agreement. LAB5 used 2 ELISA kits from 2 different producers. With one of the ELISA kit LAB5 (LAB5.1) misclassified 3 reference serum samples (corresponding to the PT2016PTUSERPS2, weak positive) and hence reached 85% of agreement. Whereas with the other ELISA kit LAB5 (LAB5.2) achieved 100% of agreement (Table 2).

For the detection of PTU-specific antibodies in **milk**, the 6 participating laboratories provided qualitative results that were in full agreement with the true status of the reference milk samples and thus achieved 100% of agreement (Table 3).

A quantitative data analysis is shown for educational purposes in Annex 1.

Table 2. Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the PTU reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR									
	1 (N=20)	**2 (N=20)**	**3 (N=20)**	**4 (N=20)**	**5.1 (N=20)**	**5.2 (N=20)**	**6 (N=20)**	**7 (N=20)**	**8 (N=20)**	**9 (N=20)**
Failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**3 (15.0)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sucess	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	**17 (85.0)**	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

Table 3. Agreement between results generated by the participating laboratories (LABNR) and the status of the reference milk samples assigned by the PTU reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference milk samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR					
	1 (N=20)	**2 (N=20)**	**3 (N=20)**	**4 (N=20)**	**10 (N=20)**	**11 (N=20)**
Failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sucess	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

Only a small variability between laboratories could be observed at the qualitative data level:

For the detection of PTU-specific antibodies in reference **serum** samples, no variability between qualitative laboratory results could be observed for LAB1, LAB2, LAB3, LAB4, LAB6, LAB7, LB8 and LAB9 since these participants correctly identified all reference serum samples. In contrast, LAB5.1 misclassified 3 out of 4 aliquots of the positive reference serum sample PT2016PTUSERPS2, weak positive (NI instead of POS) whereas LAB5.2 correctly identified all reference serum samples. LAB5 was divided into LAB5.1 and LAB5.2 since it used 2 ELISA kits from 2 different producers

For the detection of PTU-specific antibodies in reference **milk** samples, no variability between qualitative laboratory results could be observed since all participant correctly identified all reference milk samples.

For each participating laboratory, the obtained results and the assigned statuses for the reference samples are shown in Table 4 for the PT serum and in Table 5 for the PT milk.

Table 4. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference serum samples (SAMPLE), the positions of the reference serum samples as placed in the block (LABPOSIT), and the status assigned by the PTU reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive; NI: non-interpretable.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016PTUSERPS1	POS	POS	1
2	1	2	PT2016PTUSERPS4	POS	POS	1
3	1	3	PT2016PTUSERPS3	POS	POS	1
4	1	4	PT2016PTUSERNS1	NEG	NEG	1
5	1	5	PT2016PTUSERPS2	POS	POS	1
6	1	6	PT2016PTUSERPS3	POS	POS	1
7	1	7	PT2016PTUSERNS2	NEG	NEG	1
8	1	8	PT2016PTUSERPS4	POS	POS	1
9	1	9	PT2016PTUSERNS1	NEG	NEG	1
10	1	10	PT2016PTUSERPS1	POS	POS	1
11	1	11	PT2016PTUSERPS2	POS	POS	1
12	1	12	PT2016PTUSERPS4	POS	POS	1
13	1	13	PT2016PTUSERNS1	NEG	NEG	1
14	1	14	PT2016PTUSERPS2	POS	POS	1
15	1	15	PT2016PTUSERPS4	POS	POS	1
16	1	16	PT2016PTUSERPS1	POS	POS	1
17	1	17	PT2016PTUSERNS2	NEG	NEG	1
18	1	18	PT2016PTUSERPS3	POS	POS	1
19	1	19	PT2016PTUSERNS2	NEG	NEG	1
20	1	20	PT2016PTUSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2016PTUSERNS1	NEG	NEG	1
22	2	2	PT2016PTUSERNS2	NEG	NEG	1
23	2	3	PT2016PTUSERPS2	POS	POS	1
24	2	4	PT2016PTUSERPS4	POS	POS	1
25	2	5	PT2016PTUSERPS3	POS	POS	1
26	2	6	PT2016PTUSERPS1	POS	POS	1
27	2	7	PT2016PTUSERNS1	NEG	NEG	1
28	2	8	PT2016PTUSERPS3	POS	POS	1
29	2	9	PT2016PTUSERNS2	NEG	NEG	1
30	2	10	PT2016PTUSERPS4	POS	POS	1
31	2	11	PT2016PTUSERPS2	POS	POS	1
32	2	12	PT2016PTUSERPS1	POS	POS	1
33	2	13	PT2016PTUSERNS2	NEG	NEG	1
34	2	14	PT2016PTUSERNS1	NEG	NEG	1
35	2	15	PT2016PTUSERPS2	POS	POS	1
36	2	16	PT2016PTUSERPS4	POS	POS	1
37	2	17	PT2016PTUSERPS3	POS	POS	1
38	2	18	PT2016PTUSERPS2	POS	POS	1
39	2	19	PT2016PTUSERPS1	POS	POS	1
40	2	20	PT2016PTUSERPS4	POS	POS	1
41	3	1	PT2016PTUSERPS1	POS	POS	1
42	3	2	PT2016PTUSERPS4	POS	POS	1
43	3	3	PT2016PTUSERPS3	POS	POS	1
44	3	4	PT2016PTUSERNS1	NEG	NEG	1
45	3	5	PT2016PTUSERPS2	POS	POS	1
46	3	6	PT2016PTUSERPS3	POS	POS	1
47	3	7	PT2016PTUSERNS2	NEG	NEG	1
48	3	8	PT2016PTUSERPS4	POS	POS	1
49	3	9	PT2016PTUSERNS1	NEG	NEG	1
50	3	10	PT2016PTUSERPS1	POS	POS	1
51	3	11	PT2016PTUSERPS2	POS	POS	1
52	3	12	PT2016PTUSERPS4	POS	POS	1
53	3	13	PT2016PTUSERNS1	NEG	NEG	1
54	3	14	PT2016PTUSERPS2	POS	POS	1
55	3	15	PT2016PTUSERPS4	POS	POS	1
56	3	16	PT2016PTUSERPS1	POS	POS	1
57	3	17	PT2016PTUSERNS2	NEG	NEG	1
58	3	18	PT2016PTUSERPS3	POS	POS	1
59	3	19	PT2016PTUSERNS2	NEG	NEG	1
60	3	20	PT2016PTUSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2016PTUSERNS1	NEG	NEG	1
62	4	2	PT2016PTUSERNS2	NEG	NEG	1
63	4	3	PT2016PTUSERPS2	POS	POS	1
64	4	4	PT2016PTUSERPS4	POS	POS	1
65	4	5	PT2016PTUSERPS3	POS	POS	1
66	4	6	PT2016PTUSERPS1	POS	POS	1
67	4	7	PT2016PTUSERNS1	NEG	NEG	1
68	4	8	PT2016PTUSERPS3	POS	POS	1
69	4	9	PT2016PTUSERNS2	NEG	NEG	1
70	4	10	PT2016PTUSERPS4	POS	POS	1
71	4	11	PT2016PTUSERPS2	POS	POS	1
72	4	12	PT2016PTUSERPS1	POS	POS	1
73	4	13	PT2016PTUSERNS2	NEG	NEG	1
74	4	14	PT2016PTUSERNS1	NEG	NEG	1
75	4	15	PT2016PTUSERPS2	POS	POS	1
76	4	16	PT2016PTUSERPS4	POS	POS	1
77	4	17	PT2016PTUSERPS3	POS	POS	1
78	4	18	PT2016PTUSERPS2	POS	POS	1
79	4	19	PT2016PTUSERPS1	POS	POS	1
80	4	20	PT2016PTUSERPS4	POS	POS	1
81	5.1	1	PT2016PTUSERPS1	POS	POS	1
82	5.1	2	PT2016PTUSERPS4	POS	POS	1
83	5.1	3	PT2016PTUSERPS3	POS	POS	1
84	5.1	4	PT2016PTUSERNS1	NEG	NEG	1
85	5.1	5	PT2016PTUSERPS2	POS	POS	1
86	5.1	6	PT2016PTUSERPS3	POS	POS	1
87	5.1	7	PT2016PTUSERNS2	NEG	NEG	1
88	5.1	8	PT2016PTUSERPS4	POS	POS	1
89	5.1	9	PT2016PTUSERNS1	NEG	NEG	1
90	5.1	10	PT2016PTUSERPS1	POS	POS	1
91	5.1	11	PT2016PTUSERPS2	**POS**	**NI**	**0**
92	5.1	12	PT2016PTUSERPS4	POS	POS	1
93	5.1	13	PT2016PTUSERNS1	NEG	NEG	1
94	5.1	14	PT2016PTUSERPS2	**POS**	**NI**	**0**
95	5.1	15	PT2016PTUSERPS4	POS	POS	1
96	5.1	16	PT2016PTUSERPS1	POS	POS	1
97	5.1	17	PT2016PTUSERNS2	NEG	NEG	1
98	5.1	18	PT2016PTUSERPS3	POS	POS	1
99	5.1	19	PT2016PTUSERNS2	NEG	NEG	1
100	5.1	20	PT2016PTUSERPS2	**POS**	**NI**	**0**

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
101	5.2	1	PT2016PTUSERPS1	POS	POS	1
102	5.2	2	PT2016PTUSERPS4	POS	POS	1
103	5.2	3	PT2016PTUSERPS3	POS	POS	1
104	5.2	4	PT2016PTUSERNS1	NEG	NEG	1
105	5.2	5	PT2016PTUSERPS2	POS	POS	1
106	5.2	6	PT2016PTUSERPS3	POS	POS	1
107	5.2	7	PT2016PTUSERNS2	NEG	NEG	1
108	5.2	8	PT2016PTUSERPS4	POS	POS	1
109	5.2	9	PT2016PTUSERNS1	NEG	NEG	1
110	5.2	10	PT2016PTUSERPS1	POS	POS	1
111	5.2	11	PT2016PTUSERPS2	POS	POS	1
112	5.2	12	PT2016PTUSERPS4	POS	POS	1
113	5.2	13	PT2016PTUSERNS1	NEG	NEG	1
114	5.2	14	PT2016PTUSERPS2	POS	POS	1
115	5.2	15	PT2016PTUSERPS4	POS	POS	1
116	5.2	16	PT2016PTUSERPS1	POS	POS	1
117	5.2	17	PT2016PTUSERNS2	NEG	NEG	1
118	5.2	18	PT2016PTUSERPS3	POS	POS	1
119	5.2	19	PT2016PTUSERNS2	NEG	NEG	1
120	5.2	20	PT2016PTUSERPS2	POS	POS	1
121	6	1	PT2016PTUSERNS1	NEG	NEG	1
122	6	2	PT2016PTUSERNS2	NEG	NEG	1
123	6	3	PT2016PTUSERPS2	POS	POS	1
124	6	4	PT2016PTUSERPS4	POS	POS	1
125	6	5	PT2016PTUSERPS3	POS	POS	1
126	6	6	PT2016PTUSERPS1	POS	POS	1
127	6	7	PT2016PTUSERNS1	NEG	NEG	1
128	6	8	PT2016PTUSERPS3	POS	POS	1
129	6	9	PT2016PTUSERNS2	NEG	NEG	1
130	6	10	PT2016PTUSERPS4	POS	POS	1
131	6	11	PT2016PTUSERPS2	POS	POS	1
132	6	12	PT2016PTUSERPS1	POS	POS	1
133	6	13	PT2016PTUSERNS2	NEG	NEG	1
134	6	14	PT2016PTUSERNS1	NEG	NEG	1
135	6	15	PT2016PTUSERPS2	POS	POS	1
136	6	16	PT2016PTUSERPS4	POS	POS	1
137	6	17	PT2016PTUSERPS3	POS	POS	1
138	6	18	PT2016PTUSERPS2	POS	POS	1
139	6	19	PT2016PTUSERPS1	POS	POS	1
140	6	20	PT2016PTUSERPS4	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
141	7	1	PT2016PTUSERPS1	POS	POS	1
142	7	2	PT2016PTUSERPS4	POS	POS	1
143	7	3	PT2016PTUSERPS3	POS	POS	1
144	7	4	PT2016PTUSERNS1	NEG	NEG	1
145	7	5	PT2016PTUSERPS2	POS	POS	1
146	7	6	PT2016PTUSERPS3	POS	POS	1
147	7	7	PT2016PTUSERNS2	NEG	NEG	1
148	7	8	PT2016PTUSERPS4	POS	POS	1
149	7	9	PT2016PTUSERNS1	NEG	NEG	1
150	7	10	PT2016PTUSERPS1	POS	POS	1
151	7	11	PT2016PTUSERPS2	POS	POS	1
152	7	12	PT2016PTUSERPS4	POS	POS	1
153	7	13	PT2016PTUSERNS1	NEG	NEG	1
154	7	14	PT2016PTUSERPS2	POS	POS	1
155	7	15	PT2016PTUSERPS4	POS	POS	1
156	7	16	PT2016PTUSERPS1	POS	POS	1
157	7	17	PT2016PTUSERNS2	NEG	NEG	1
158	7	18	PT2016PTUSERPS3	POS	POS	1
159	7	19	PT2016PTUSERNS2	NEG	NEG	1
160	7	20	PT2016PTUSERPS2	POS	POS	1
161	8	1	PT2016PTUSERNS1	NEG	NEG	1
162	8	2	PT2016PTUSERNS2	NEG	NEG	1
163	8	3	PT2016PTUSERPS2	POS	POS	1
164	8	4	PT2016PTUSERPS4	POS	POS	1
165	8	5	PT2016PTUSERPS3	POS	POS	1
166	8	6	PT2016PTUSERPS1	POS	POS	1
167	8	7	PT2016PTUSERNS1	NEG	NEG	1
168	8	8	PT2016PTUSERPS3	POS	POS	1
169	8	9	PT2016PTUSERNS2	NEG	NEG	1
170	8	10	PT2016PTUSERPS4	POS	POS	1
171	8	11	PT2016PTUSERPS2	POS	POS	1
172	8	12	PT2016PTUSERPS1	POS	POS	1
173	8	13	PT2016PTUSERNS2	NEG	NEG	1
174	8	14	PT2016PTUSERNS1	NEG	NEG	1
175	8	15	PT2016PTUSERPS2	POS	POS	1
176	8	16	PT2016PTUSERPS4	POS	POS	1
177	8	17	PT2016PTUSERPS3	POS	POS	1
178	8	18	PT2016PTUSERPS2	POS	POS	1
179	8	19	PT2016PTUSERPS1	POS	POS	1
180	8	20	PT2016PTUSERPS4	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
181	9	1	PT2016PTUSERPS1	POS	POS	1
182	9	2	PT2016PTUSERPS4	POS	POS	1
183	9	3	PT2016PTUSERPS3	POS	POS	1
184	9	4	PT2016PTUSERNS1	NEG	NEG	1
185	9	5	PT2016PTUSERPS2	POS	POS	1
186	9	6	PT2016PTUSERPS3	POS	POS	1
187	9	7	PT2016PTUSERNS2	NEG	NEG	1
188	9	8	PT2016PTUSERPS4	POS	POS	1
189	9	9	PT2016PTUSERNS1	NEG	NEG	1
190	9	10	PT2016PTUSERPS1	POS	POS	1
191	9	11	PT2016PTUSERPS2	POS	POS	1
192	9	12	PT2016PTUSERPS4	POS	POS	1
193	9	13	PT2016PTUSERNS1	NEG	NEG	1
194	9	14	PT2016PTUSERPS2	POS	POS	1
195	9	15	PT2016PTUSERPS4	POS	POS	1
196	9	16	PT2016PTUSERPS1	POS	POS	1
197	9	17	PT2016PTUSERNS2	NEG	NEG	1
198	9	18	PT2016PTUSERPS3	POS	POS	1
199	9	19	PT2016PTUSERNS2	NEG	NEG	1
200	9	20	PT2016PTUSERPS2	POS	POS	1

Table 5. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference milk samples (SAMPLE), the positions of the reference milk samples as placed in the block (LABPOSIT), and the status assigned by the PTU reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive; NI: non-interpretable.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016PTUSERPM2	POS	POS	1
2	1	2	PT2016PTUSERPM4	POS	POS	1
3	1	3	PT2016PTUSERPM1	POS	POS	1
4	1	4	PT2016PTUSERNM1	NEG	NEG	1
5	1	5	PT2016PTUSERPM2	POS	POS	1
6	1	6	PT2016PTUSERPM3	POS	POS	1
7	1	7	PT2016PTUSERNM2	NEG	NEG	1
8	1	8	PT2016PTUSERPM4	POS	POS	1
9	1	9	PT2016PTUSERPM2	POS	POS	1
10	1	10	PT2016PTUSERNM2	NEG	NEG	1
11	1	11	PT2016PTUSERPM1	POS	POS	1
12	1	12	PT2016PTUSERPM2	POS	POS	1
13	1	13	PT2016PTUSERPM3	POS	POS	1
14	1	14	PT2016PTUSERNM1	NEG	NEG	1
15	1	15	PT2016PTUSERNM2	NEG	NEG	1
16	1	16	PT2016PTUSERPM4	POS	POS	1
17	1	17	PT2016PTUSERNM1	NEG	NEG	1
18	1	18	PT2016PTUSERPM1	POS	POS	1
19	1	19	PT2016PTUSERPM3	POS	POS	1
20	1	20	PT2016PTUSERPM4	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2016PTUSERPM3	POS	POS	1
22	2	2	PT2016PTUSERPM2	POS	POS	1
23	2	3	PT2016PTUSERNM1	NEG	NEG	1
24	2	4	PT2016PTUSERNM2	NEG	NEG	1
25	2	5	PT2016PTUSERPM4	POS	POS	1
26	2	6	PT2016PTUSERPM1	POS	POS	1
27	2	7	PT2016PTUSERPM3	POS	POS	1
28	2	8	PT2016PTUSERNM2	NEG	NEG	1
29	2	9	PT2016PTUSERPM4	POS	POS	1
30	2	10	PT2016PTUSERPM2	POS	POS	1
31	2	11	PT2016PTUSERNM1	NEG	NEG	1
32	2	12	PT2016PTUSERPM4	POS	POS	1
33	2	13	PT2016PTUSERPM1	POS	POS	1
34	2	14	PT2016PTUSERNM1	NEG	NEG	1
35	2	15	PT2016PTUSERPM3	POS	POS	1
36	2	16	PT2016PTUSERPM2	POS	POS	1
37	2	17	PT2016PTUSERNM2	NEG	NEG	1
38	2	18	PT2016PTUSERPM2	POS	POS	1
39	2	19	PT2016PTUSERPM4	POS	POS	1
40	2	20	PT2016PTUSERPM1	POS	POS	1
41	3	1	PT2016PTUSERPM2	POS	POS	1
42	3	2	PT2016PTUSERPM4	POS	POS	1
43	3	3	PT2016PTUSERPM1	POS	POS	1
44	3	4	PT2016PTUSERNM1	NEG	NEG	1
45	3	5	PT2016PTUSERPM2	POS	POS	1
46	3	6	PT2016PTUSERPM3	POS	POS	1
47	3	7	PT2016PTUSERNM2	NEG	NEG	1
48	3	8	PT2016PTUSERPM4	POS	POS	1
49	3	9	PT2016PTUSERPM2	POS	POS	1
50	3	10	PT2016PTUSERNM2	NEG	NEG	1
51	3	11	PT2016PTUSERPM1	POS	POS	1
52	3	12	PT2016PTUSERPM2	POS	POS	1
53	3	13	PT2016PTUSERPM3	POS	POS	1
54	3	14	PT2016PTUSERNM1	NEG	NEG	1
55	3	15	PT2016PTUSERNM2	NEG	NEG	1
56	3	16	PT2016PTUSERPM4	POS	POS	1
57	3	17	PT2016PTUSERNM1	NEG	NEG	1
58	3	18	PT2016PTUSERPM1	POS	POS	1
59	3	19	PT2016PTUSERPM3	POS	POS	1
60	3	20	PT2016PTUSERPM4	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2016PTUSERPM3	POS	POS	1
62	4	2	PT2016PTUSERPM2	POS	POS	1
63	4	3	PT2016PTUSERNM1	NEG	NEG	1
64	4	4	PT2016PTUSERNM2	NEG	NEG	1
65	4	5	PT2016PTUSERPM4	POS	POS	1
66	4	6	PT2016PTUSERPM1	POS	POS	1
67	4	7	PT2016PTUSERPM3	POS	POS	1
68	4	8	PT2016PTUSERNM2	NEG	NEG	1
69	4	9	PT2016PTUSERPM4	POS	POS	1
70	4	10	PT2016PTUSERPM2	POS	POS	1
71	4	11	PT2016PTUSERNM1	NEG	NEG	1
72	4	12	PT2016PTUSERPM4	POS	POS	1
73	4	13	PT2016PTUSERPM1	POS	POS	1
74	4	14	PT2016PTUSERNM1	NEG	NEG	1
75	4	15	PT2016PTUSERPM3	POS	POS	1
76	4	16	PT2016PTUSERPM2	POS	POS	1
77	4	17	PT2016PTUSERNM2	NEG	NEG	1
78	4	18	PT2016PTUSERPM2	POS	POS	1
79	4	19	PT2016PTUSERPM4	POS	POS	1
80	4	20	PT2016PTUSERPM1	POS	POS	1
81	10	1	PT2016PTUSERPM2	POS	POS	1
82	10	2	PT2016PTUSERPM4	POS	POS	1
83	10	3	PT2016PTUSERPM1	POS	POS	1
84	10	4	PT2016PTUSERNM1	NEG	NEG	1
85	10	5	PT2016PTUSERPM2	POS	POS	1
86	10	6	PT2016PTUSERPM3	POS	POS	1
87	10	7	PT2016PTUSERNM2	NEG	NEG	1
88	10	8	PT2016PTUSERPM4	POS	POS	1
89	10	9	PT2016PTUSERPM2	POS	POS	1
90	10	10	PT2016PTUSERNM2	NEG	NEG	1
91	10	11	PT2016PTUSERPM1	POS	POS	1
92	10	12	PT2016PTUSERPM2	POS	POS	1
93	10	13	PT2016PTUSERPM3	POS	POS	1
94	10	14	PT2016PTUSERNM1	NEG	NEG	1
95	10	15	PT2016PTUSERNM2	NEG	NEG	1
96	10	16	PT2016PTUSERPM4	POS	POS	1
97	10	17	PT2016PTUSERNM1	NEG	NEG	1
98	10	18	PT2016PTUSERPM1	POS	POS	1
99	10	19	PT2016PTUSERPM3	POS	POS	1
100	10	20	PT2016PTUSERPM4	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
101	11	1	PT2016PTUSERPM3	POS	POS	1
102	11	2	PT2016PTUSERPM2	POS	POS	1
103	11	3	PT2016PTUSERNM1	NEG	NEG	1
104	11	4	PT2016PTUSERNM2	NEG	NEG	1
105	11	5	PT2016PTUSERPM4	POS	POS	1
106	11	6	PT2016PTUSERPM1	POS	POS	1
107	11	7	PT2016PTUSERPM3	POS	POS	1
108	11	8	PT2016PTUSERNM2	NEG	NEG	1
109	11	9	PT2016PTUSERPM4	POS	POS	1
110	11	10	PT2016PTUSERPM2	POS	POS	1
111	11	11	PT2016PTUSERNM1	NEG	NEG	1
112	11	12	PT2016PTUSERPM4	POS	POS	1
113	11	13	PT2016PTUSERPM1	POS	POS	1
114	11	14	PT2016PTUSERNM1	NEG	NEG	1
115	11	15	PT2016PTUSERPM3	POS	POS	1
116	11	16	PT2016PTUSERPM2	POS	POS	1
117	11	17	PT2016PTUSERNM2	NEG	NEG	1
118	11	18	PT2016PTUSERPM2	POS	POS	1
119	11	19	PT2016PTUSERPM4	POS	POS	1
120	11	20	PT2016PTUSERPM1	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum and/or reference milk samples of bovine origin for the detection of PTU-specific antibodies by ELISA.

For the detection of PTU-specific antibodies in serum, 8 out of 9 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). LAB5.1 misclassified 3 out 4 aliquot of the positive reference serum sample PT2016PTUSERPS2 (85% of agreement) whereas LAB5.2 provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). It must be specified that LAB5 (divided in LAB5.1 and LAB5.2) used 2 ELISA kits from 2 different producers. Indeed the participating laboratories used PTU antibody ELISA kits from 2 different producers: the IDEXX Mycobacterium paratuberculosis antibody test kit was used by LAB1 (batch 6082), LAB2, LAB5.2 and LAB6 (batch 6061), LAB7 (batch 5163), LAB8 (batch 6042), and LAB9 (batch 5076) and the ID.VET ID Screen Paratuberculosis Indirect kit was used by LAB3, LAB4 and LAB5.1 (batch 800).

For the detection of PTU-specific antibodies in milk, all participating laboratories provided qualitative results that were in full agreement with the true status of the reference milk samples (100% of agreement). Also the participating laboratories used PTU antibody ELISA kits from 2 different producers: the IDEXX Mycobacterium paratuberculosis antibody test kit was used by LAB1 (batch 6082), LAB2 (batch 6061), LAB10 and LAB11 (batch 6112) and the ID.VET ID Screen Paratuberculosis Indirect kit was used by LAB3 and LAB4 (batch 800).

VI. Conclusions

According to the procedure currently in force, the performance of all participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference samples assigned by the PTU reference laboratory of CODA-CERVA-Uccle (see III.3.3.). As a consequence: (i) all laboratories that participated in the PT serum except LAB5.1 achieved a satisfactory performance for the detection of PTU-specific antibodies in reference serum samples, and (ii) all laboratories that participated in the PT milk achieved a satisfactory performance for the detection of PTU-specific antibodies in reference milk samples.

Coordinator proficiency tests
Katia Knapen

Appendix

Names of the participating laboratories

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) (Niort, France)

Association Régionale de Santé et d'Identification Animales (ARSIA) (Ciney, Belgium)

Comité du lait (Battice, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)

Laboratoire National de Contrôle des Reproducteurs (LNCR) / ACSEDIATE (Maisons-Alfort, France)

Laboratoire Départemental d'Analyses du Tarn (LDA81) (Albi, France)

Laboratoire Départemental d'Analyses du Lot (LDA46) (Cahors, France)

Laboratoire Vétérinaire Départemental du Tarn et Garonne (LVD82) (Montauban, France)

Melkcontrolecentrum Vlaanderen (MCC) (Lier, Belgium)

Veterinary and Agrochemical Research Center (CODA-CERVA) (Uccle, Belgium)

Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs R. All quantitative data analyses were performed on normalized data, namely the percentages blocking calculated according to the instructions of the PT provider: [OD sample - mean (OD negative kit controls) / mean (OD positive kit controls) - mean (OD negative kit controls)] * 100.

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.

Box plots of the normalized data according to the instructions of the PT provider per reference serum and milk samples and per participating laboratory were made using the statistical software R. The box plots for the (sub)laboratories participating in the PT ELISA serum and ELISA milk are shown in Figure 1 and Figure 2, respectively.

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.

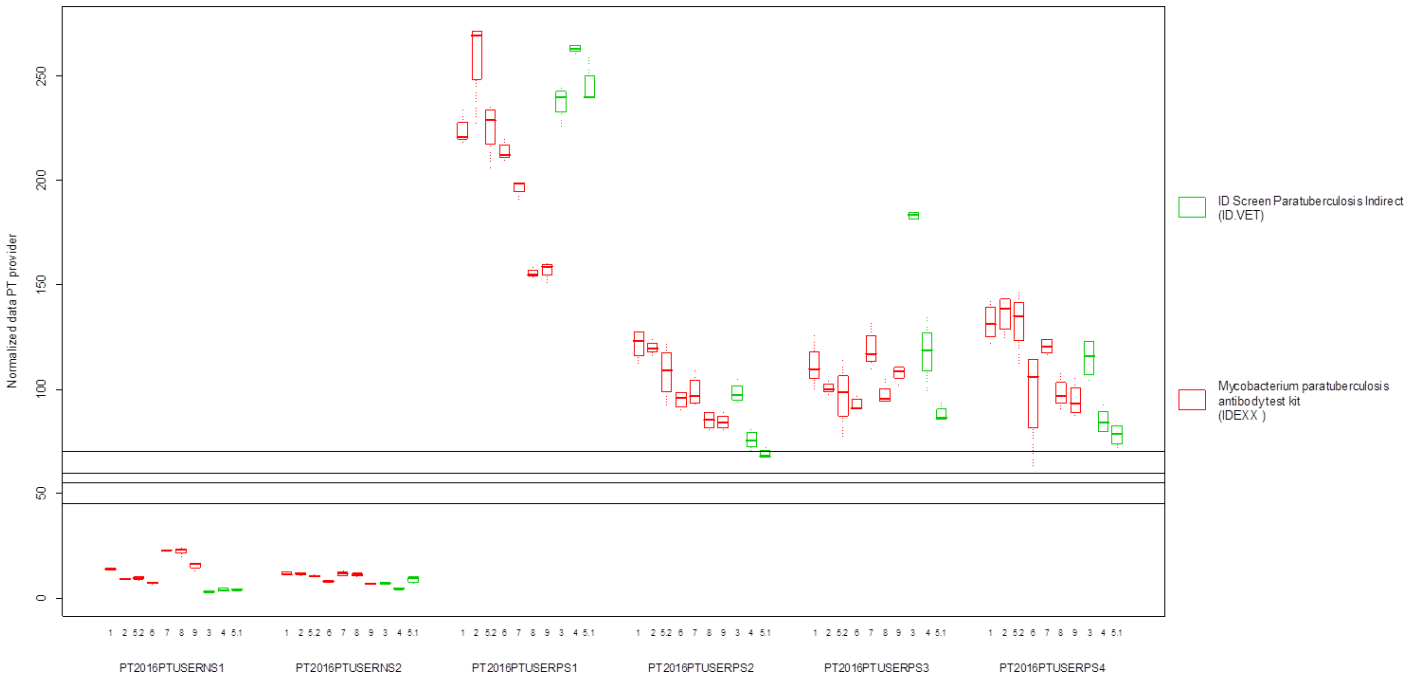

Figure 1. Box plots showing the percentage S/P ratio per reference serum sample and per participating laboratory. The participating laboratories used ELISA kits from 2 different producers: the IDEXX Mycobacterium paratuberculosis antibody test kit was used by LAB1 (batch 6082), LAB2, LAB5.2 and LAB6 (batch 6061), LAB7 (batch 5163), LAB8 (batch 6042), and LAB9 (batch 5076) and the ID.VET ID Screen Paratuberculosis Indirect kit was used by LAB3, LAB4 and LAB5.1 (batch 800).
Cut-off values for the *Mycobacterium paratuberculosis* antibody test kit from IDEXX (45-55%) and the ID Screen Paratuberculosis Indirect kit from ID.VET (60-70%) are shown by horizontal lines.

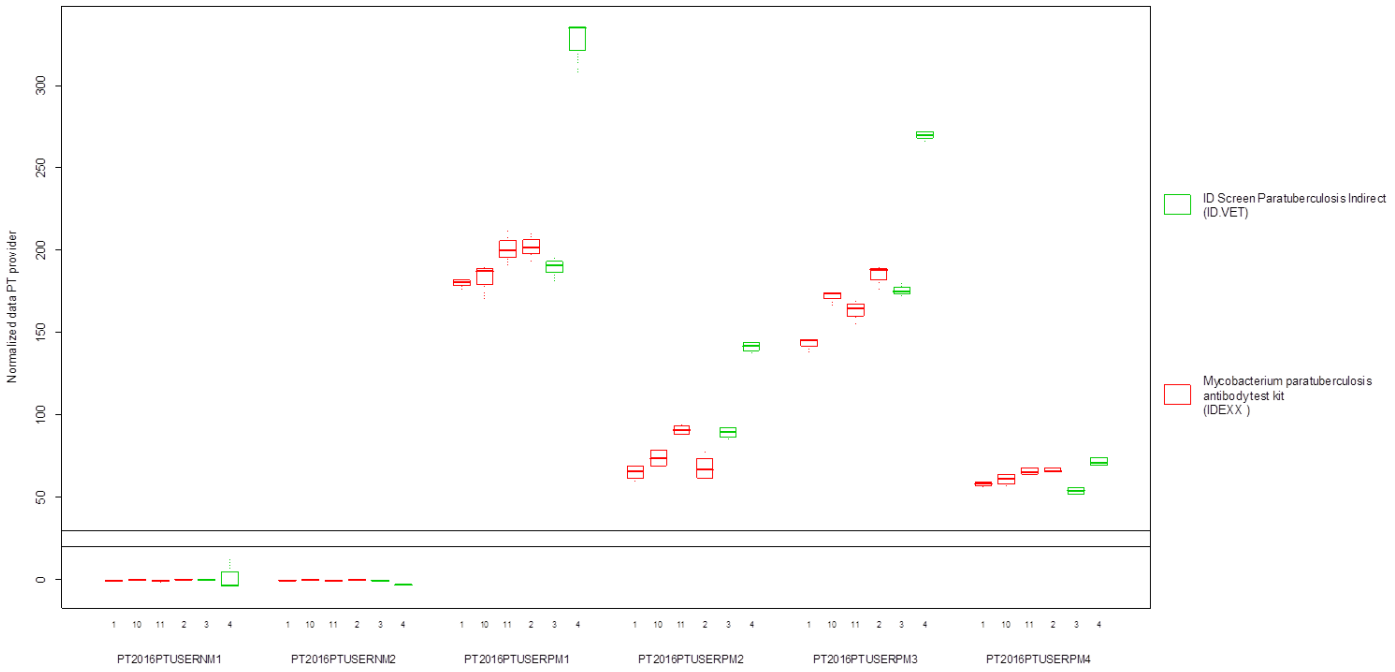

Figure 2. Box plots showing the percentage S/P ratio per reference milk sample and per participating laboratory. The participating laboratories used ELISA kits from 2 different producers: the IDEXX Mycobacterium paratuberculosis antibody test kit was used by LAB1 (batch 6082), LAB2 (batch 6061), LAB10 and LAB11 (batch 6112) and the ID.VET ID Screen Paratuberculosis Indirect kit was used by LAB3 and LAB4 (batch 800).
Cut-off values for the *Mycobacterium paratuberculosis* antibody test kit from IDEXX (20-30%) and the ID Screen Paratuberculosis Indirect kit from ID.VET (30%) are shown by horizontal lines.