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081-PT

PROFICIENCY TESTING 2018

Paratuberculosis (PTU)

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

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE BEGIN PT: 22 OCTOBER 2018

DATE REPORT: 28 JANUARY 2019

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 '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 PTU-specific antibodies in individual bovidae 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 milk samples must be tested by means of a PTU antibody ELISA test. The procedures for the 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 5 reference serum samples of bovidae origin, either free from detectable PTU-specific antibodies (n=2; coded 'PT2018PTUSERNS1' and 'PT2018PTUSERNS2') or containing detectable PTU-specific antibodies (n=3; coded 'PT2018PTUSERPS1', 'PT2018PTUSERPS2' and 'PT2018PTUSERPS3'), were used. In total, 100 aliquots were distributed to 5 participating laboratories. All participants received 4 aliquots of each reference serum sample, i.e. 20 aliquots in total. The identification numbers of the reference serum samples were randomized for all participants (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 PT2018PTUSERNS1 and PT2018PTUSERNS2 were PTU antibody negative commercial serum and field pooled sera respectively, and the reference serum samples PT2018PTUSERPS1, PT2018PTUSERPS2 and PT2018PTUSERPS3 were PTU antibody positive sera from an experimentally infected animal and 2 different field animals from different farms. For sample PT2018PTUSERPS1, presence of M. *paratuberculosis* was detected by culture in lymph node after slaughter, for sample PT2018PTUSERPS2 and PT2018PTUSERPS3, the animals were shown to be shedders by PCR analysis on faeces and milk samples respectively. For each reference serum sample, the same qualitative result was obtained with both ELISA kits used. Taken together, the reference serum samples PT2018PTUSERNS1 and PT2018PTUSERNS2 were considered as negative sera, and the reference serum samples PT2018PTUSERPS1, PT2018PTUSERPS2 and PT2018PTUSERPS3 were considered as positive sera.

After aliquoting the different reference serum samples, a homogeneity check was performed on 5 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 5 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 ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET.

III.2.2. Reference milk samples

Replicates of 5 reference milk samples, either free from detectable PTU-specific antibodies (n=2; coded 'PT2018PTUSERNM1' and 'PT2018PTUSERNM2') or containing detectable PTU-specific antibodies (n=3; coded 'PT2018PTUSERPM1', 'PT2018PTUSERPM2' and 'PT2018PTUSERPM3'), were used. The reference milk samples PT2018PTUSERNM1 and PT2018PTUSERNM2 were bovine tank milk and commercial milk whereas PT2018PTUSERPM1 was spiked commercial milk with positive serum and PT2018PTUSERPM2, PT2018PTUSERPM3 were 2 positive field milk samples from 2 different animals of the same positive farm.
In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 6 aliquots of the reference milk sample PT2018PTUSERPM2 and PT2018PTUSERPM3, 4 aliquots of the reference milk sample PT2018PTUSERPM1 and 2 aliquots of the reference milk sample PT2018PTUSERNM1 and PT2018PTUSERNM2 , i.e. 20 aliquots in total.The identification numbers of the reference milk samples were randomized for all participants (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 sample PT2018PTUSERNM1 was bovine tank milk and PT2018PTUSERNM2 was a commercial defatted milk, whereas the reference milk samples PT2018PTUSERPM1 derived from a commercial deffated milk spiked with positive serum. PT2018PTUSERPM2 and PT2018PTUSERPM3 were obtained from animals that 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 PT2018PTUSERNM1 and PT2018PTUSERNM2 were considered as negative milk samples, and the reference milk samples PT2018PTUSERPM1, PT2018PTUSERPM2 and PT2018PTUSERPM3 as positive milk samples.

After aliquoting the different reference milk samples, a homogeneity check was performed on 5 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 5 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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

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 only participated in the PT serum and hence received 20 aliquots of reference serum samples. LAB6 and LAB7 only participated in the PT milk and hence received 20 aliquots of reference milk samples.
Frozen reference serum samples (100 aliquots in total) and lyophilized reference milk samples (120 aliquots in total) were sent to the 7 participating laboratories by national courier on the 22th of October 2018. All participating laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 22th of October 2018 and 5th of November 2018 (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 30th of October 2018 and 12th of November 2018 (Table 1). All participating laboratories, except LAB2, respected the deadline of 9th of November 2018 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 SERUM	Start of analysis MILK	Submission of the results (Excel file)
LAB1	22/10/2018	23/10/2018	25/10/2018	02/11/2018
LAB2	22/10/2018	25/10/2018	25/10/2018	**12/11/2018**
LAB3	22/10/2018	26/10/2018	26/10/2018	30/10/2018
LAB4	22/10/2018	30/10/20180 IDEXX + 05/11/2018 IDVET	05/11/2018 IDEXX + IDVET	07/11/2018
LAB5	22/10/2018	22/10/2018 IDVET + 02/11/2018 IDEXX	NA	08/11/2018
LAB6	22/10/2018	NA	26/10/2018	31/10/2018
LAB7	22/10/2018	NA	30/10/2018	06/11/2018

Legend: NA = not applicable

IV.3. Compliance with the procedure

All participating laboratories, except LAB2, 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:

(i) For the detection of PTU-specific antibodies in **serum**, all 5 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and thus achieved 100% of agreement (Table 2).

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

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

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

	LABNR						
	1	**2**	**3**	**4.1**	**4.2**	**5.1**	**5.2**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

Table 3. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference **milk** samples assigned by the PTU 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**	**4.1**	**4.2**	**6**	**7**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

IV.4.2. Variability among participating laboratories

(i) For the detection of PTU-specific antibodies in **serum**, no variability between laboratories could be observed since all participants correctly identified all reference serum samples. LAB4 and LAB5 obtained identical qualitative results using ELISA kits from 2 different producers.

(ii) For the detection of PTU-specific antibodies in **milk**, no variability between laboratories could be observed since all participants correctly identified all reference milk samples. LAB4 obtained identical qualitative results using ELISA kits from 2 different producers.

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 internal identification of the reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the PTU 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	PT2018PTUSERPS1	POS	POS	1
2	1	2	PT2018PTUSERNS2	NEG	NEG	1
3	1	3	PT2018PTUSERPS2	POS	POS	1
4	1	4	PT2018PTUSERNS1	NEG	NEG	1
5	1	5	PT2018PTUSERPS2	POS	POS	1
6	1	6	PT2018PTUSERPS1	POS	POS	1
7	1	7	PT2018PTUSERNS2	NEG	NEG	1
8	1	8	PT2018PTUSERPS3	POS	POS	1
9	1	9	PT2018PTUSERNS1	NEG	NEG	1
10	1	10	PT2018PTUSERNS2	NEG	NEG	1
11	1	11	PT2018PTUSERPS2	POS	POS	1
12	1	12	PT2018PTUSERNS2	NEG	NEG	1
13	1	13	PT2018PTUSERPS3	POS	POS	1
14	1	14	PT2018PTUSERNS1	NEG	NEG	1
15	1	15	PT2018PTUSERPS1	POS	POS	1
16	1	16	PT2018PTUSERPS2	POS	POS	1
17	1	17	PT2018PTUSERPS3	POS	POS	1
18	1	18	PT2018PTUSERNS1	NEG	NEG	1
19	1	19	PT2018PTUSERPS1	POS	POS	1
20	1	20	PT2018PTUSERPS3	POS	POS	1
21	2	1	PT2018PTUSERPS2	POS	POS	1
22	2	2	PT2018PTUSERNS1	NEG	NEG	1
23	2	3	PT2018PTUSERPS1	POS	POS	1
24	2	4	PT2018PTUSERNS2	NEG	NEG	1
25	2	5	PT2018PTUSERPS1	POS	POS	1
26	2	6	PT2018PTUSERNS1	NEG	NEG	1
27	2	7	PT2018PTUSERPS2	POS	POS	1
28	2	8	PT2018PTUSERNS2	NEG	NEG	1
29	2	9	PT2018PTUSERPS2	POS	POS	1
30	2	10	PT2018PTUSERPS1	POS	POS	1
31	2	11	PT2018PTUSERNS1	NEG	NEG	1
32	2	12	PT2018PTUSERPS3	POS	POS	1
33	2	13	PT2018PTUSERNS2	NEG	NEG	1
34	2	14	PT2018PTUSERPS2	POS	POS	1
35	2	15	PT2018PTUSERPS1	POS	POS	1
36	2	16	PT2018PTUSERPS3	POS	POS	1
37	2	17	PT2018PTUSERNS1	NEG	NEG	1
38	2	18	PT2018PTUSERPS3	POS	POS	1
39	2	19	PT2018PTUSERNS2	NEG	NEG	1
40	2	20	PT2018PTUSERPS3	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2018PTUSERPS1	POS	POS	1
42	3	2	PT2018PTUSERNS2	NEG	NEG	1
43	3	3	PT2018PTUSERPS2	POS	POS	1
44	3	4	PT2018PTUSERNS1	NEG	NEG	1
45	3	5	PT2018PTUSERPS2	POS	POS	1
46	3	6	PT2018PTUSERPS1	POS	POS	1
47	3	7	PT2018PTUSERNS2	NEG	NEG	1
48	3	8	PT2018PTUSERPS3	POS	POS	1
49	3	9	PT2018PTUSERNS1	NEG	NEG	1
50	3	10	PT2018PTUSERNS2	NEG	NEG	1
51	3	11	PT2018PTUSERPS2	POS	POS	1
52	3	12	PT2018PTUSERNS2	NEG	NEG	1
53	3	13	PT2018PTUSERPS3	POS	POS	1
54	3	14	PT2018PTUSERNS1	NEG	NEG	1
55	3	15	PT2018PTUSERPS1	POS	POS	1
56	3	16	PT2018PTUSERPS2	POS	POS	1
57	3	17	PT2018PTUSERPS3	POS	POS	1
58	3	18	PT2018PTUSERNS1	NEG	NEG	1
59	3	19	PT2018PTUSERPS1	POS	POS	1
60	3	20	PT2018PTUSERPS3	POS	POS	1
61	4.1	1	PT2018PTUSERPS2	POS	POS	1
62	4.1	2	PT2018PTUSERNS1	NEG	NEG	1
63	4.1	3	PT2018PTUSERPS1	POS	POS	1
64	4.1	4	PT2018PTUSERNS2	NEG	NEG	1
65	4.1	5	PT2018PTUSERPS1	POS	POS	1
66	4.1	6	PT2018PTUSERNS1	NEG	NEG	1
67	4.1	7	PT2018PTUSERPS2	POS	POS	1
68	4.1	8	PT2018PTUSERNS2	NEG	NEG	1
69	4.1	9	PT2018PTUSERPS2	POS	POS	1
70	4.1	10	PT2018PTUSERPS1	POS	POS	1
71	4.1	11	PT2018PTUSERNS1	NEG	NEG	1
72	4.1	12	PT2018PTUSERPS3	POS	POS	1
73	4.1	13	PT2018PTUSERNS2	NEG	NEG	1
74	4.1	14	PT2018PTUSERPS2	POS	POS	1
75	4.1	15	PT2018PTUSERPS1	POS	POS	1
76	4.1	16	PT2018PTUSERPS3	POS	POS	1
77	4.1	17	PT2018PTUSERNS1	NEG	NEG	1
78	4.1	18	PT2018PTUSERPS3	POS	POS	1
79	4.1	19	PT2018PTUSERNS2	NEG	NEG	1
80	4.1	20	PT2018PTUSERPS3	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4.2	1	PT2018PTUSERPS2	POS	POS	1
82	4.2	2	PT2018PTUSERNS1	NEG	NEG	1
83	4.2	3	PT2018PTUSERPS1	POS	POS	1
84	4.2	4	PT2018PTUSERNS2	NEG	NEG	1
85	4.2	5	PT2018PTUSERPS1	POS	POS	1
86	4.2	6	PT2018PTUSERNS1	NEG	NEG	1
87	4.2	7	PT2018PTUSERPS2	POS	POS	1
88	4.2	8	PT2018PTUSERNS2	NEG	NEG	1
89	4.2	9	PT2018PTUSERPS2	POS	POS	1
90	4.2	10	PT2018PTUSERPS1	POS	POS	1
91	4.2	11	PT2018PTUSERNS1	NEG	NEG	1
92	4.2	12	PT2018PTUSERPS3	POS	POS	1
93	4.2	13	PT2018PTUSERNS2	NEG	NEG	1
94	4.2	14	PT2018PTUSERPS2	POS	POS	1
95	4.2	15	PT2018PTUSERPS1	POS	POS	1
96	4.2	16	PT2018PTUSERPS3	POS	POS	1
97	4.2	17	PT2018PTUSERNS1	NEG	NEG	1
98	4.2	18	PT2018PTUSERPS3	POS	POS	1
99	4.2	19	PT2018PTUSERNS2	NEG	NEG	1
100	4.2	20	PT2018PTUSERPS3	POS	POS	1
101	5.1	1	PT2018PTUSERPS1	POS	POS	1
102	5.1	2	PT2018PTUSERNS2	NEG	NEG	1
103	5.1	3	PT2018PTUSERPS2	POS	POS	1
104	5.1	4	PT2018PTUSERNS1	NEG	NEG	1
105	5.1	5	PT2018PTUSERPS2	POS	POS	1
106	5.1	6	PT2018PTUSERPS1	POS	POS	1
107	5.1	7	PT2018PTUSERNS2	NEG	NEG	1
108	5.1	8	PT2018PTUSERPS3	POS	POS	1
109	5.1	9	PT2018PTUSERNS1	NEG	NEG	1
110	5.1	10	PT2018PTUSERNS2	NEG	NEG	1
111	5.1	11	PT2018PTUSERPS2	POS	POS	1
112	5.1	12	PT2018PTUSERNS2	NEG	NEG	1
113	5.1	13	PT2018PTUSERPS3	POS	POS	1
114	5.1	14	PT2018PTUSERNS1	NEG	NEG	1
115	5.1	15	PT2018PTUSERPS1	POS	POS	1
116	5.1	16	PT2018PTUSERPS2	POS	POS	1
117	5.1	17	PT2018PTUSERPS3	POS	POS	1
118	5.1	18	PT2018PTUSERNS1	NEG	NEG	1
119	5.1	19	PT2018PTUSERPS1	POS	POS	1
120	5.1	20	PT2018PTUSERPS3	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	5.2	1	PT2018PTUSERPS1	POS	POS	1
122	5.2	2	PT2018PTUSERNS2	NEG	NEG	1
123	5.2	3	PT2018PTUSERPS2	POS	POS	1
124	5.2	4	PT2018PTUSERNS1	NEG	NEG	1
125	5.2	5	PT2018PTUSERPS2	POS	POS	1
126	5.2	6	PT2018PTUSERPS1	POS	POS	1
127	5.2	7	PT2018PTUSERNS2	NEG	NEG	1
128	5.2	8	PT2018PTUSERPS3	POS	POS	1
129	5.2	9	PT2018PTUSERNS1	NEG	NEG	1
130	5.2	10	PT2018PTUSERNS2	NEG	NEG	1
131	5.2	11	PT2018PTUSERPS2	POS	POS	1
132	5.2	12	PT2018PTUSERNS2	NEG	NEG	1
133	5.2	13	PT2018PTUSERPS3	POS	POS	1
134	5.2	14	PT2018PTUSERNS1	NEG	NEG	1
135	5.2	15	PT2018PTUSERPS1	POS	POS	1
136	5.2	16	PT2018PTUSERPS2	POS	POS	1
137	5.2	17	PT2018PTUSERPS3	POS	POS	1
138	5.2	18	PT2018PTUSERNS1	NEG	NEG	1
139	5.2	19	PT2018PTUSERPS1	POS	POS	1
140	5.2	20	PT2018PTUSERPS3	POS	POS	1

Table 5. 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 PTU 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	PT2018PTUSERPM2	POS	POS	1
2	1	2	PT2018PTUSERPM1	POS	POS	1
3	1	3	PT2018PTUSERPM3	POS	POS	1
4	1	4	PT2018PTUSERPM1	POS	POS	1
5	1	5	PT2018PTUSERPM3	POS	POS	1
6	1	6	PT2018PTUSERNM1	NEG	NEG	1
7	1	7	PT2018PTUSERPM3	POS	POS	1
8	1	8	PT2018PTUSERPM2	POS	POS	1
9	1	9	PT2018PTUSERPM2	POS	POS	1
10	1	10	PT2018PTUSERNM1	NEG	NEG	1
11	1	11	PT2018PTUSERPM3	POS	POS	1
12	1	12	PT2018PTUSERPM2	POS	POS	1
13	1	13	PT2018PTUSERNM2	NEG	NEG	1
14	1	14	PT2018PTUSERPM2	POS	POS	1
15	1	15	PT2018PTUSERPM1	POS	POS	1
16	1	16	PT2018PTUSERPM3	POS	POS	1
17	1	17	PT2018PTUSERNM2	NEG	NEG	1
18	1	18	PT2018PTUSERPM2	POS	POS	1
19	1	19	PT2018PTUSERPM1	POS	POS	1
20	1	20	PT2018PTUSERPM3	POS	POS	1
21	2	1	PT2018PTUSERPM1	POS	POS	1
22	2	2	PT2018PTUSERPM2	POS	POS	1
23	2	3	PT2018PTUSERNM1	NEG	NEG	1
24	2	4	PT2018PTUSERPM2	POS	POS	1
25	2	5	PT2018PTUSERPM3	POS	POS	1
26	2	6	PT2018PTUSERPM1	POS	POS	1
27	2	7	PT2018PTUSERNM1	NEG	NEG	1
28	2	8	PT2018PTUSERPM2	POS	POS	1
29	2	9	PT2018PTUSERPM3	POS	POS	1
30	2	10	PT2018PTUSERPM2	POS	POS	1
31	2	11	PT2018PTUSERNM2	NEG	NEG	1
32	2	12	PT2018PTUSERPM3	POS	POS	1
33	2	13	PT2018PTUSERPM1	POS	POS	1
34	2	14	PT2018PTUSERPM3	POS	POS	1
35	2	15	PT2018PTUSERNM2	NEG	NEG	1
36	2	16	PT2018PTUSERPM3	POS	POS	1
37	2	17	PT2018PTUSERPM2	POS	POS	1
38	2	18	PT2018PTUSERPM3	POS	POS	1
39	2	19	PT2018PTUSERPM2	POS	POS	1
40	2	20	PT2018PTUSERPM1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2018PTUSERPM2	POS	POS	1
42	3	2	PT2018PTUSERPM1	POS	POS	1
43	3	3	PT2018PTUSERPM3	POS	POS	1
44	3	4	PT2018PTUSERPM1	POS	POS	1
45	3	5	PT2018PTUSERPM3	POS	POS	1
46	3	6	PT2018PTUSERNM1	NEG	NEG	1
47	3	7	PT2018PTUSERPM3	POS	POS	1
48	3	8	PT2018PTUSERPM2	POS	POS	1
49	3	9	PT2018PTUSERPM2	POS	POS	1
50	3	10	PT2018PTUSERNM1	NEG	NEG	1
51	3	11	PT2018PTUSERPM3	POS	POS	1
52	3	12	PT2018PTUSERPM2	POS	POS	1
53	3	13	PT2018PTUSERNM2	NEG	NEG	1
54	3	14	PT2018PTUSERPM2	POS	POS	1
55	3	15	PT2018PTUSERPM1	POS	POS	1
56	3	16	PT2018PTUSERPM3	POS	POS	1
57	3	17	PT2018PTUSERNM2	NEG	NEG	1
58	3	18	PT2018PTUSERPM2	POS	POS	1
59	3	19	PT2018PTUSERPM1	POS	POS	1
60	3	20	PT2018PTUSERPM3	POS	POS	1
61	4.1	1	PT2018PTUSERPM1	POS	POS	1
62	4.1	2	PT2018PTUSERPM2	POS	POS	1
63	4.1	3	PT2018PTUSERNM1	NEG	NEG	1
64	4.1	4	PT2018PTUSERPM2	POS	POS	1
65	4.1	5	PT2018PTUSERPM3	POS	POS	1
66	4.1	6	PT2018PTUSERPM1	POS	POS	1
67	4.1	7	PT2018PTUSERNM1	NEG	NEG	1
68	4.1	8	PT2018PTUSERPM2	POS	POS	1
69	4.1	9	PT2018PTUSERPM3	POS	POS	1
70	4.1	10	PT2018PTUSERPM2	POS	POS	1
71	4.1	11	PT2018PTUSERNM2	NEG	NEG	1
72	4.1	12	PT2018PTUSERPM3	POS	POS	1
73	4.1	13	PT2018PTUSERPM1	POS	POS	1
74	4.1	14	PT2018PTUSERPM3	POS	POS	1
75	4.1	15	PT2018PTUSERNM2	NEG	NEG	1
76	4.1	16	PT2018PTUSERPM3	POS	POS	1
77	4.1	17	PT2018PTUSERPM2	POS	POS	1
78	4.1	18	PT2018PTUSERPM3	POS	POS	1
79	4.1	19	PT2018PTUSERPM2	POS	POS	1
80	4.1	20	PT2018PTUSERPM1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4.2	1	PT2018PTUSERPM1	POS	POS	1
82	4.2	2	PT2018PTUSERPM2	POS	POS	1
83	4.2	3	PT2018PTUSERNM1	NEG	NEG	1
84	4.2	4	PT2018PTUSERPM2	POS	POS	1
85	4.2	5	PT2018PTUSERPM3	POS	POS	1
86	4.2	6	PT2018PTUSERPM1	POS	POS	1
87	4.2	7	PT2018PTUSERNM1	NEG	NEG	1
88	4.2	8	PT2018PTUSERPM2	POS	POS	1
89	4.2	9	PT2018PTUSERPM3	POS	POS	1
90	4.2	10	PT2018PTUSERPM2	POS	POS	1
91	4.2	11	PT2018PTUSERNM2	NEG	NEG	1
92	4.2	12	PT2018PTUSERPM3	POS	POS	1
93	4.2	13	PT2018PTUSERPM1	POS	POS	1
94	4.2	14	PT2018PTUSERPM3	POS	POS	1
95	4.2	15	PT2018PTUSERNM2	NEG	NEG	1
96	4.2	16	PT2018PTUSERPM3	POS	POS	1
97	4.2	17	PT2018PTUSERPM2	POS	POS	1
98	4.2	18	PT2018PTUSERPM3	POS	POS	1
99	4.2	19	PT2018PTUSERPM2	POS	POS	1
100	4.2	20	PT2018PTUSERPM1	POS	POS	1
101	6	1	PT2018PTUSERPM2	POS	POS	1
102	6	2	PT2018PTUSERPM1	POS	POS	1
103	6	3	PT2018PTUSERPM3	POS	POS	1
104	6	4	PT2018PTUSERPM1	POS	POS	1
105	6	5	PT2018PTUSERPM3	POS	POS	1
106	6	6	PT2018PTUSERNM1	NEG	NEG	1
107	6	7	PT2018PTUSERPM3	POS	POS	1
108	6	8	PT2018PTUSERPM2	POS	POS	1
109	6	9	PT2018PTUSERPM2	POS	POS	1
110	6	10	PT2018PTUSERNM1	NEG	NEG	1
111	6	11	PT2018PTUSERPM3	POS	POS	1
112	6	12	PT2018PTUSERPM2	POS	POS	1
113	6	13	PT2018PTUSERNM2	NEG	NEG	1
114	6	14	PT2018PTUSERPM2	POS	POS	1
115	6	15	PT2018PTUSERPM1	POS	POS	1
116	6	16	PT2018PTUSERPM3	POS	POS	1
117	6	17	PT2018PTUSERNM2	NEG	NEG	1
118	6	18	PT2018PTUSERPM2	POS	POS	1
119	6	19	PT2018PTUSERPM1	POS	POS	1
120	6	20	PT2018PTUSERPM3	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2018PTUSERPM1	POS	POS	1
122	7	2	PT2018PTUSERPM2	POS	POS	1
123	7	3	PT2018PTUSERNM1	NEG	NEG	1
124	7	4	PT2018PTUSERPM2	POS	POS	1
125	7	5	PT2018PTUSERPM3	POS	POS	1
126	7	6	PT2018PTUSERPM1	POS	POS	1
127	7	7	PT2018PTUSERNM1	NEG	NEG	1
128	7	8	PT2018PTUSERPM2	POS	POS	1
129	7	9	PT2018PTUSERPM3	POS	POS	1
130	7	10	PT2018PTUSERPM2	POS	POS	1
131	7	11	PT2018PTUSERNM2	NEG	NEG	1
132	7	12	PT2018PTUSERPM3	POS	POS	1
133	7	13	PT2018PTUSERPM1	POS	POS	1
134	7	14	PT2018PTUSERPM3	POS	POS	1
135	7	15	PT2018PTUSERNM2	NEG	NEG	1
136	7	16	PT2018PTUSERPM3	POS	POS	1
137	7	17	PT2018PTUSERPM2	POS	POS	1
138	7	18	PT2018PTUSERPM3	POS	POS	1
139	7	19	PT2018PTUSERPM2	POS	POS	1
140	7	20	PT2018PTUSERPM1	POS	POS	1

V. Discussion

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

For the detection of PTU-specific antibodies in serum, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 2 and Table 4).
PTU antibody ELISA kits from 2 different producers and different batches from the same ELISA kit were used: IDEXX (3 batches: 7081, 7117 and 8089) and IDVet (1 batch: B19).

For the detection of PTU-specific antibodies in milk, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement) (Table 3 and Table 5).
PTU antibody ELISA kits from 2 different producers and different batches from the same ELISA kit were used: IDEXX (4 batches: 7081, 7117, 7162 and 8089) and IDVet (1 batch: B19).

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 samples assigned by the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). Consequently, all participants in the PT serum achieved a satisfactory performance for the detection of PTU-specific antibodies in reference serum samples and all participants 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

Name of the participating laboratories

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)

Lavetan NV (Turnhout, Belgium)

MCC-Vlaanderen (Lier, Belgium)

Sciensano (Uccle, 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.

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.

For the **antibody ELISA serum reference samples,** box plots of the normalized OD values according the PT provider per reference sample and per participating laboratory are shown in Figure 1.

Figure 1 (antibody ELISA serum reference samples)

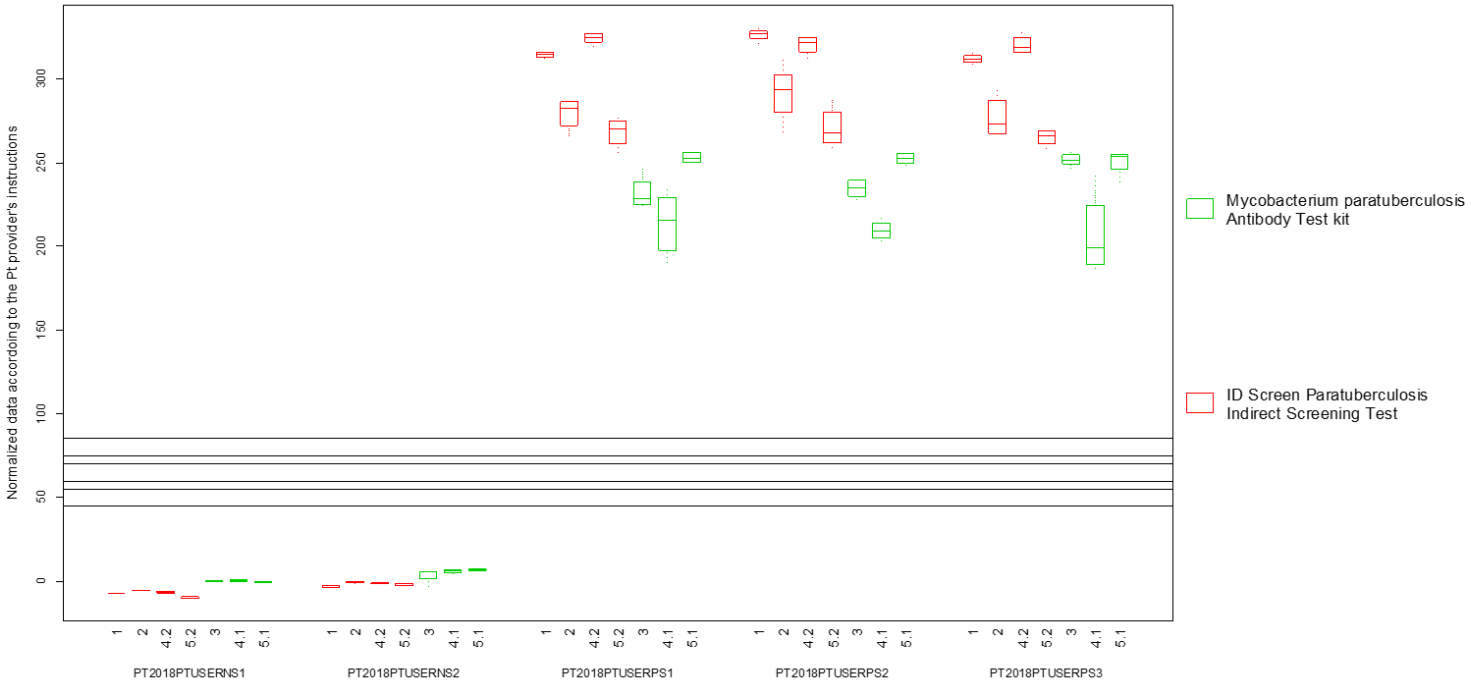

Figure 1. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory. PTU antibody ELISA kits from 2 different producers were used: IDEXX and IDVet. Cut-off values (IDEXX 45-55 and IDVET 60-70 or 75-85) are shown by horizontal lines.

the **antibody ELISA milk reference samples,** box plots of the normalized OD values according the PT provider per reference sample and per participating laboratory are shown in Figure 2.

Figure 2 (antibody ELISA milk reference samples)

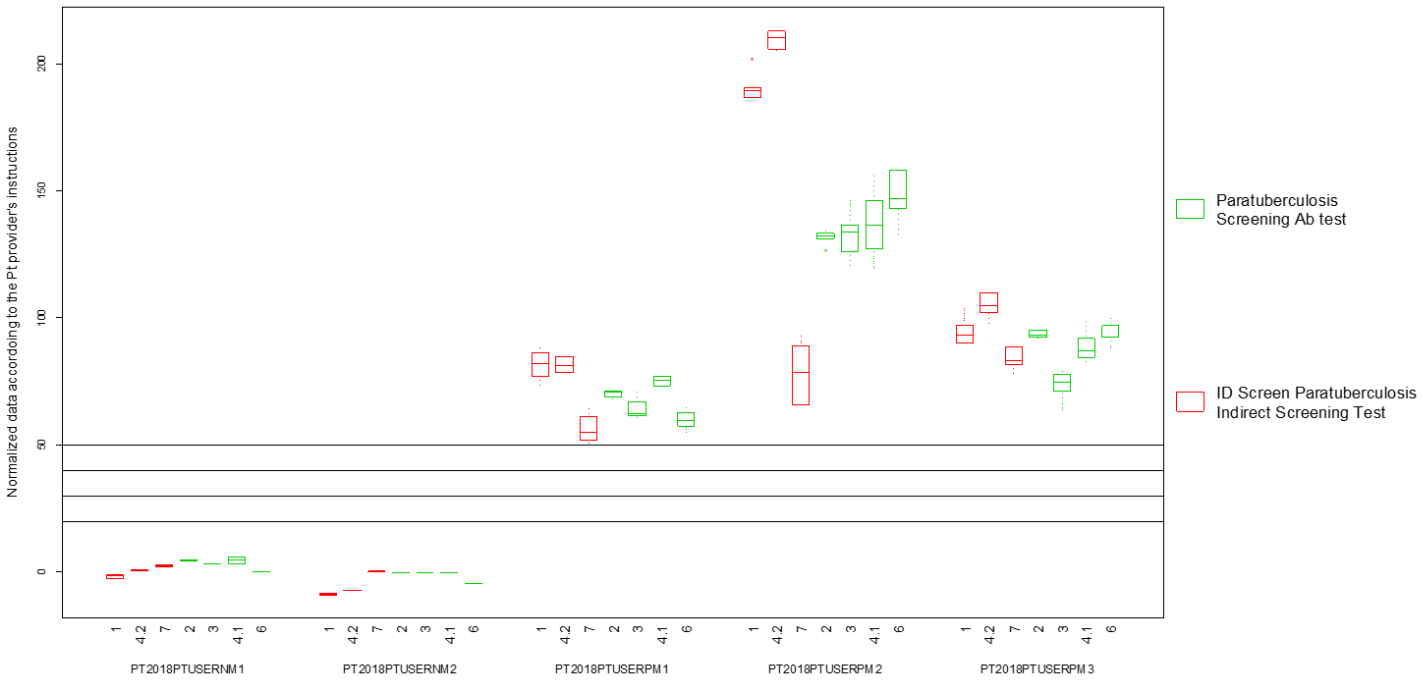

Figure 2. Box plots showing the normalized OD values according the PT provider per reference milk and per participating laboratory. PTU antibody ELISA kits from 2 different producers were used: IDEXX and IDVet. Cut-off values (IDEXX 20-30 or 30-40 or 30-50 and IDVET 30) are shown by horizontal lines.