The ID Screen® Brucella suis Indirect ELISA is a valuable supplement for serological diagnosis of porcine brucellosis

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Der ID Screen® Brucella suis Indirect ELISA ist eine wertvolle Ergänzung für die serologische Diagnose der Schweinebrucellose

Brucellose bei Hausschweinen wird hauptsächlich durch *Brucella (B.) suis* verursacht und wurde in vielen europäischen Ländern ausgerottet. Die Hausschweinpopulation wird mittels serologischer Methoden auf Seuchenfreiheit überwacht. Antikörper, die mit dem vorgeschriebenen diagnostischen glatten Lipopolysaccharid (sLPS) Antigen kreuzreagieren, können jedoch aufgrund der Antigenähnlichkeit mit anderen Bakterien, wie z. B. *Yersinia*-Arten, zu falsch positiven serologischen Ergebnissen (FPSRs) führen. Hausschweine sind häufig mit *Y. enterocolitica* besiedelt. Der ID Screen® Brucella suis Indirect ELISA, der auf sLPS und rauem LPS (rLPS) Antigen basiert, wurde entwickelt, um FPSR in Schweineseren zu bestätigen.

Die Zuverlässigkeit des ID Screen® Brucella suis Indirect ELISA wurde anhand von 143 Serumproben von Hausschweinen mit unterschiedlichem Brucellose-Status analysiert: Gruppe 1: Brucellose-freie Schweine aus einer Besamungsstation (n = 65); Gruppe 2: Schweine aus Brucellose-freien Beständen, die in verschiedenen sLPS-basierten serologischen Tests seropositiv waren und bei denen der Verdacht auf FPSR bestand (n = 53); Gruppe 3: Schweine aus Beständen mit bestätigter Brucellose (n = 25). Zusätzlich wurden die Seren mit dem Pigtype® Yersinia Ab ELISA getestet.

Die Seren der Gruppe 1 konnten mit dem ID Screen® Brucella suis Indirect ELISA als serologisch negativ für Brucellose bestätigt werden. In Gruppe 2 wurden 51 von 53 Seren (96 %) mit dem ID Screen® Brucella suis Indirect ELISA als FPSRs bestätigt. Neunzehn Seren (76 %) der Gruppe 3 testeten serologisch positiv auf Brucellose. Die Ergebnisse bestätigen die gute Unterscheidungskraft des ID Screen® Brucella suis Indirect ELISA für serologisch falsch positive Brucellose-Befunde. Darüber hinaus bestätigten unsere Daten, dass kreuzreaktive *Yersinia*-Antikörper die wahrscheinlichste Ursache für FPSRs sind.

Schlüsselwörter: *Brucella suis*, Komplementbindungstest (CFT), FPSRs, Rose-Bengal-Test (RBT), Schweine

Summary

Brucellosis in domestic pigs, mainly caused by Brucella (B.) suis, has been eradicated in many European countries and the domestic pig population is monitored for freedom of disease by serological methods. However, antibodies cross-reacting with the prescribed smooth lipopolysaccharide (sLPS) diagnostic antigen, might lead to false positive serological results (FPSRs) due to antigen similarity with other bacteria, such as Yersinia species. Domestic pigs are frequently colonised with Y. enterocolitica. The ID Screen® Brucella suis Indirect ELISA, based on sLPS and rough LPS (rLPS) was developed to confirm FPSRs in porcine sera.

The performance of the ID Screen® Brucella suis Indirect ELISA was analysed using 143 field sera from domestic pigs with different brucellosis status: group 1: brucellosis-free pigs kept in an artificial insemination unit (n=65); group 2: brucellosis-free herds showing seropositivity for brucellosis in different sLPS-based serological tests, suspected to be FPSRs (n=53); group 3: herds with confirmed porcine brucellosis (n=25). Additionally, sera were tested with Pigtype® Yersinia Ab ELISA.

Sera of group 1 were confirmed as truly serological negative for brucellosis with the ID Screen® Brucella suis Indirect ELISA. In group 2, 51 out of 53 sera (96 %) were confirmed as FPSRs by the ID Screen® Brucella suis Indirect ELISA. Nineteen sera (76 %) of group 3 tested truly serological positive for brucellosis. The results confirm the good discriminatory power of the ID Screen® Brucella suis Indirect ELISA for false serological brucellosis positivity. Moreover, our data confirmed that cross-reactive Yersinia antibodies are the most likely cause of FPSRs.

Keywords: *Brucella suis*, complement fixation test (CFT), FPSRs, Rose Bengal test (RBT), swine

https://doi.org/10.17236/sat00471

Eingereicht: 04.06.2025
Angenommen: 13.01.2026

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Introduction

Among the three major *Brucella* (*B.*) species affecting livestock namely *B. abortus, B. melitensis* and *B. suis*, porcine brucellosis is caused mainly by biovars 1–3 of *B. suis*.[4] The domestic pig population in most European countries is considered free of the disease and the status of this notifiable zoonosis is surveyed mainly by serology.[8] In this epidemiological situation false positive serological results create significant problems for many stakeholders. Serological methods commonly used for porcine brucellosis and prescribed for international trade of animals include Rose Bengal test (RBT), Complement Fixation Test (CFT) and a number of enzyme-linked immunosorbent assays (ELISA).[10,28] All these methods are based on the smooth cell wall lipopolysaccharide (sLPS) fraction of the *B. abortus* strain 99, *B. abortus* strain 1119–3 or *B. melitensis* 16M strain.[28] However, due to very high similarity of the O-polysaccharide compound in the LPS of different *Brucella* species with other gram-negative bacteria, such as *Yersinia* (*Y.*) *enterocolitica* O:9, cross-reacting antibodies can lead to false positive serological reactions (FPSRs) in the above-mentioned tests.[28] This diagnostic interference is pronounced in pigs as they are frequently colonised by *Y. enterocolitica* in their oro-pharyngeal mucosa.[15,24,26] As *B. suis* is present in many wild boars and brown hares in Europe (mainly *B. suis* biovar 2) that can constitute a reservoir for infection of domestic pigs, the specificity of the serological test used for domestic pigs is particularly important.[9,11,12,13,16,17,19,23] Reliable serological detection of porcine brucellosis is even more important in countries such as Switzerland, where the pig production system differs greatly from other European countries with large and vertically integrated systems. Swiss pig production is highly decentralised, and made up of many small, independent farms with frequent outdoor access and highly fragmented transport systems, which constitute a general higher risk for diseases transmission,[25,29] including interactions with wild boars that harbour biotype 2.[2] False positive serological reactions have been a severe problem especially for breeding farms and stations for artificial insemination in Switzerland. In the past, potential breeding boars with high genetic potential have repeatedly showed false positive results in the RBT, the CFT and the ID Screen® Brucellosis Serum Indirect multi-species ELISA (Innovative Diagnostics, Grabels, France) during quarantine testing. The measures taken, such as large-scale follow-up examinations or blocking these boars for breeding, led to relevant breeding- and financial losses (pers. communication).

A study by McGiven et al. (2012) described a new approach to overcome the problems with FPSRs in porcine brucellosis.[20] This approach was based on the parallel testing for both, sLPS and rLPS of *B. abortus* and *B. ovis,* respectively.[5,7,20,27] It has been shown that FPSRs does not cross-react with rLPS in the majority of cases. Further studies focusing on alternative antigens than the prescribed sLPS of *B. abortus* or *B. melitensis* for serological detection of *Brucella* antibodies resulted in the development of new commercially available products such as the ID Screen® Brucella suis Indirect ELISA (Innovative Diagnostics).[5,7,20,27] This test system is based on the parallel testing for both, sLPS of *B. abortus* RB51 and rLPS of *B. ovis* REO198 to differentiate between FPSRs and true serological positive reactions.

Besides a recent study from Germany testing serum samples from wild boar,[18] we are not aware of any other peer-reviewed publication characterising the performance of the ID Screen® Brucella suis Indirect ELISA side by side with officially recognised serological methods for porcine brucellosis. Therefore, we tested the newly introduced ID Screen® Brucella suis Indirect ELISA in comparison to the RBT, CFT and ID Screen® Brucellosis Serum Indirect multi-species ELISA using field sera from Swiss domestic pigs with different brucellosis status. In addition, unlike in the recently published study on serum samples from wild boar,[18] we tested the sera with the Pigtype® Yersinia Ab ELISA (Indical Bioscience) for antibodies against *Yersinia* species to identify a possible source of FPSRs.

Material and methods

Serum samples

Diagnostic porcine sera submitted from 2016 to 2022 to the diagnostic unit of the Institute of Veterinary Bacteriology (Vetsuisse Faculty, University of Bern, Bern) and archived at -20°C have been used for this study. All sera were grouped based on previous serological test results and the epidemiological brucellosis status of the herd of origin. Sera were categorised in the following groups: group 1: Random sera (n= 65) from boars kept in an artificial insemination unit, which are proven free of brucellosis and routinely monitored as negative by RBT; group 2: Sera (n= 53) from boars tested for admission to the artificial insemination unit, kept in herds supposed to be brucellosis-free but had shown seropositivity (FPSRs) in at least one serological test (RBT, CFT, ID Screen® Brucellosis Serum Indirect multi-species ELISA); group 3: Sera (n= 25) from an outbreak of porcine brucellosis in 2009. This outbreak comprised pigs of the breed Mangalitza from three farms following an initial case of a boar with peritonitis and orchitis. *B. suis* biovar 2 was confirmed by culture and molecular methods in ten pigs derived from all three farms.[1]

Serological tests

All sera were tested with the following four methods.

1. The RBT was performed with *B. abortus* Weybridge 99S antigen coated latex Pourquier Rose Bengal Ag (IDEXX, Westbrook, Maine, USA) according to protocol of the World Organization for Animal Health (WOAH).[28] In each test run Pourquier brucellosis negative control (IDEXX) and Pourquier brucellosis positive control (IDEXX) were included. Any visible agglutination within four minutes was considered positive.

2. The CFT was performed according to the protocol of WOAH.[28] Complement (Serion GmbH, Würzburg, Germany), *Brucella* antigen (*B. abortus* CFT antigen (APHA Scientific, Addlestone, UK), the haemolytic system composed of 1 % erythrocyte suspension (Serion GmbH) and amboceptor (Serion GmbH) were adjusted in pretests accordingly. Each test run was proven by positive and negative control sera (*Brucella melitensis* positive caprine serum, APHA Scientific, Pourquier brucellosis negative control, IDEXX). Sera above 20 International Units per milliliter (IU/ml) were considered as positive. The ID Screen® Brucellosis Serum Indirect multi-species ELISA (Innovative Diagnostics, Grabels, France) is based on the sLPS of *B. abortus* RB51, which detects antibodies for *B. suis/melitensis/abortus*. The protocol follows manufacturer's instructions. S/P ratios ≥120 % were interpreted as positive, S/P ratios between 111 % and 119 % as suspicious and S/P ratios ≤110 % as negative according to the manufacturer's instructions.

3. The ID Screen® Brucella suis Indirect ELISA (Innovative Diagnostics) is a bi-well differential iELISA based on parallel testing of the serum on sLPS (*B. abortus* RB51) and rLPS (*B. ovis* REO198). This ELISA is designed to confirm FPSRs in swine brucellosis serology with 97 % specificity according to the manufacturer's instructions. The protocol follows manufacturer's instructions. Only S/P ratio combination of ≥60 % for sLPS and ≥45 % for rLPS were interpreted as truly positive for brucellosis, whereas other S/P ratio combinations were interpreted as not truly positive for brucellosis according to the manufacturer's instructions.

4. The Pigtype® Yersinia Ab ELISA (Indical Bioscience, Leipzig, Germany) is coated with recombinant Yersinia outer proteins (Yops) and detects antibodies against pathogenic *Yersinia* species in porcine serum. The protocol follows manufacturer's instructions. S/P ratios ≥30 % were accepted as positive and <30 % as negative result.

Data analysis

The statistical significance of the differences between results of the different groups for the Pigtype® Yersinia Ab ELISA was calculated with Fischer's Exact Test (p<0,01) in an integrated tool of the Windows 11 software Excel spreadsheet (Microsoft Corporation, Redmond, USA). Figures were generated with Windows 11 software Power Point Program (Microsoft Corporation). Confidence intervals were calculated with an open access software (Kohn MA, Senyak J. Sample Size Calculators, UCSF CTSI. 12 October 2024. Available at https://www.sample-size.net/ [Accessed 24 October 2024]).

The sensitivity and specificity were calculated according to Altman DG & Bland JM (1994).[3]

$$\text{Specificity (Sp) for FPSRs} = \frac{\text{number of true negatives}}{\text{number of true negatives + false positives}} = \text{x } 100\ (\%)$$

$$\text{Sensitivity (Se)} = \frac{\text{number of true positives}}{\text{number of affected animals}} \text{ x } 100\ (\%)$$

Results

All sera in group 1 (n=65, 100 %) were negative in the three sLPS based serological tests (RBT, CFT, ID Screen® Brucellosis Serum Indirect multi-species ELISA) as well as in the ID Screen® Brucella suis Indirect ELISA as expected (Table 1). Though, it must be mentioned that by using the ID Screen® Brucella suis Indirect ELISA two sera showed S/P ratios of >45 % for rLPS and another three sera showed S/P ratio >60 % for sLPS (Table 1, Figure 1). Of these five sera, the serum with 148 % S/P ratio for sLPS was positive in the Pigtype® Yersinia Ab ELISA as well.

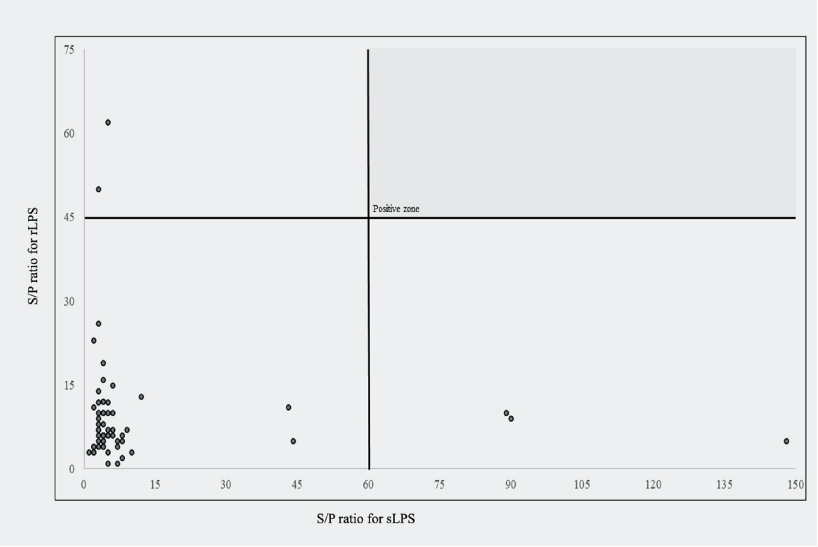

Figure 1: Optic density ratios of the samples versus positive control (S/P ratio) in the bi-well ID Screen® Brucella suis Indirect ELISA for smooth and rough lipopolysaccharide (sLPS and rLPS) of porcine sera in group 1 (Brucella-free farms). Manufacturer's thresholds for positivity are 45% for rLPS and 60% for sLPS, highlighted as grey rectangle.

Table 1: Serological results as percentage positivity (%) for porcine brucellosis and anti-Yersinia antibodies of three sera groups with different brucellosis status

Sera Group+ (n*)	RBT[1] (%, n)	CFT[2] (%, n)	ID Screen® Brucellosis Serum Indirect multi-species ELISA (%, n)[3]	ID Screen® Brucella suis Indirect ELISA (%, n)	Pigtype® *Yersinia* Ab ELISA (%, n)
1 (n=65)	0	0	0	0	40 (n=26)
2 (n=53)	85 (n=45)	26 (n=14)	49 (n=26)	4 (n=2)	70 (n=37)
3 (n=25)	96 (n=24)	100 (n=25)	100 (n=25)	76 (n=19)	76 (n=19)

*numbers; + group 1; sera from a brucellosis-free station; group 2; sera with false positive serological reactions, group 3; sera from a confirmed brucellosis outbreak
[1]Rose Bengal Test, 2Complement Fixation Test, 3 Includes suspicious results

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In group 2 (n=53), the highest proportion of positivity among the sera was diagnosed by the RBT (n=45, 85 %), followed by the ID Screen® Brucellosis Serum Indirect multi-species ELISA (including suspicious results) (n=26, 49 %) and by CFT (n=14, 26 %) (Table 1). Twenty-three sera reacted positive only in the RBT, while reacting negative in the other tests applied (Table 2). Seven sera were positive only in the ID Screen® Brucellosis Serum Indirect multi-species ELISA (Table 2). In the ID Screen® Brucella suis Indirect ELISA, 51 sera out of 53 (96 %) tested negative and were thereby confirmed as FPSRs. Altogether, only two sera were categorised as positive with S/P ratio combination of ≥60 % for sLPS and ≥45 % for rLPS (Figure 2). These two sera reacted positive in all the other sLPS-based tests (RBT, CFT, ID Screen® Brucellosis Serum Indirect multi-species ELISA). Among nine sera, which tested positive in all sLPS based test (RBT, CFT, ID Screen® Brucellosis Serum Indirect multi-species ELISA (Table 2), seven could be confirmed as FPSRs in the ID Screen® Brucella suis Indirect ELISA. Not surprisingly, within 29 sera with S/P ratios of ≥60 % for sLPS in this test (Figure 2), 23 sera (79 %) were also positive or suspicious in the sLPS based ID Screen® Brucellosis Serum Indirect multi-species ELISA. Two sera had a positive S/P ratio of ≥45 % for rLPS, but a negative S/P ratio of ≤60 % on sLPS in the bi-well ID Screen® Brucella suis Indirect ELISA as well as a negative S/P ratio of ≤110 %in the sLPS ID Screen® Brucellosis Serum Indirect multi-species ELISA.

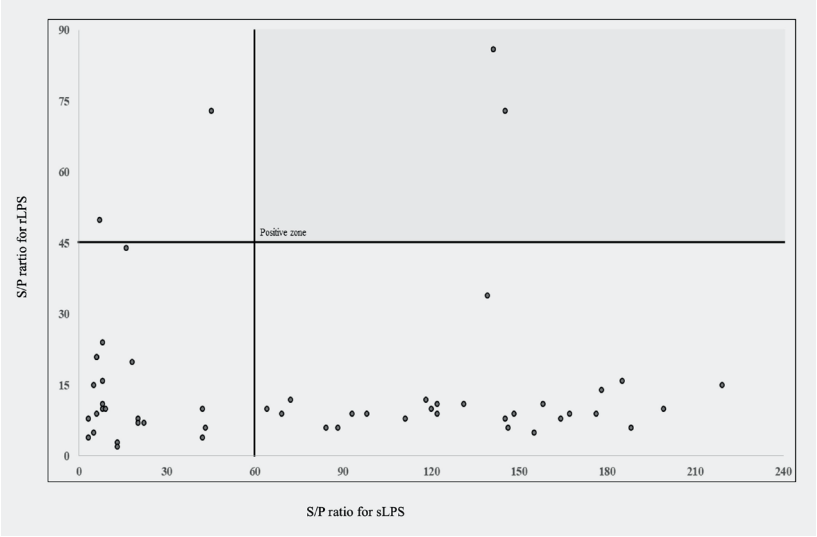

Figure 2: Optic density ratios of the samples versus positive control (S/P ratio) in the bi-well ID Screen® Brucella suis Indirect ELISA for smooth and rough lipopolysaccharide (sLPS and rLPS) of the sera in group 2 (porcine sera with false positive serological reactions (FPSRs)). Manufacturer's thresholds for positivity are 45 % for rLPS and 60 % for sLPS, highlighted as grey rectangle.

In group 3, all sera (n=25) tested positive in CFT (100 %) and positive or suspicious in the ID Screen® Brucellosis Serum Indirect multi-species ELISA (100 %), whereas 24 sera tested positive in RBT (96 %). In the ID Screen® Brucella suis Indirect ELISA 19 sera (76 %) tested positive for both, sLPS and rLPS, but six sera reacted negative for the rLPS (Table 1, Figure 3).

The diagnostic specificity for FPSRs and sensitivity for the ID Screen® Brucella suis Indirect ELISA in this study was determined as 96 % (95 %CI 87–99) and 76 % (95 %CI 55–91), respectively (Table 3a, Table 3b).

With the Pigtype® Yersinia Ab ELISA 26 sera out of 65 sera (40 %) of group 1 were positive (Table 1). In group 2 (37 sera out of 53 sera (70 %), p=0,0016), (Table 1) and group 3 (19 sera out of 25 sera (76 %), p=0,042), (Table 1) significantly higher rates of positivity in comparison to group 1 were observed (Figure 4). The difference between group 2 and group 3 was not significant (p=0,788) (Figure 4).

Table 2: Results of the sera in group 2 (53 porcine sera with false positive serological reactions (FPSRs)) for different serological methods for brucellosis based on smooth lipopolysaccharide (LPS) antigen and the rough LPS based ID Screen® *Brucella suis Indirect* ELISA and anti-*Yersinia* antibodies based on the Pigtype® *Yersinia* Ab ELISA

Number of sera (n)	RBT[1]	CFT[2]	ID Screen® Brucellosis Serum Indirect multi-species ELISA	ID Screen® Brucella suis Indirect ELISA	Pigtype® *Yersinia* Ab ELISA
23	positive	negative	negative	negative	12 positive
9	positive	positive	positive	7 negative	positive
9	positive	negative	positive	negative	7 positive
7	negative	negative	positive	negative	5 positive
4	positive	positive	negative	negative	3 positive
1	negative	positive	positive	negative	positive

[1]Rose Bengal Test, 2Complement Fixation Test, [3]including suspicious results

Of the 37 sera positive for *Yersinia* antibodies in group 2 (FPSRs), (Table 1), 84 % (n=31) were also positive in RBT, 59 % (n=22) were also positive in the ID Screen® Brucellosis Serum Indirect multi-species ELISA and 35 % (n=13) were also positive in CFT. In contrast, only two sera (5 %) react positive in ID Screen® Brucella suis Indirect ELISA (Table 2). On the other hand, of the sera in group 3 (n=25), the vast majority (n=19, 76 %) reacted positive in the Yersinia Ab ELISA, too (Table 1). All of these sera were positive in RBT, ID Screen® Brucellosis Serum Indirect multi-species ELISA, CFT and 16 sera (84 %) additionally in the ID Screen® Brucella suis Indirect ELISA.

Discussion

In general, in a population of *Brucella*-free swine, sLPS based serological tests like RBT, CFT and ID Screen® Brucellosis Serum Indirect multi-species ELISA have a good diagnostic performance.[28] This was also confirmed in our study, as all sera of swine that were *Brucella*-free and belonged to group 1 showed negative results in all serological *Brucella* tests used in this study. However, the sLPS-based serological tests for brucellosis are hampered in their ability to avoid false-positive results due to cross-reactive antibodies against other gram-negative bacteria.[28] The results from the suspected FPSRs samples (group 2) confirmed that the three sLPS based assays reacted false positive when cross reacting antibodies were present in sera tested, as 85 % of the sera reacted positive in RBT, 49 % reacted positive with the ID Screen® Brucellosis Serum Indirect multi-species ELISA and 26 % gave positive results with CFT. An interesting observation in this study was the different number of sera reacting positive solely in one or the other *Brucella* sLPS based method in group 2. It was found that RBT, CFT and ID Screen® Brucellosis Serum Indirect multi-species ELISA method each had individual FPSRs, most probable due to different antigenic confirmation and presentation, resulting in different antibody affinities.

Testing porcine serum samples on both, *Brucella* sLPS and rLPS with ID Screen® Brucella suis Indirect ELISA, should help to differentiate between false versus true serological positive reaction. In contrast to the sLPS, the rLPS antigen consists only of the Lipid-A and core polysaccharide whose structures are very different in *Brucella*, leading to less cross-reactivity with antibodies against other gram-negative bacteria.[20] Our results for the rLPS based ID Screen® Brucella suis Indirect ELISA in the FPSRs group 2 confirmed this hypothesis. This assay provided negative serological results for all sera of the non-infected group 1 and 96 % of the FPSRs group 2 population. Only two sera from the FPSRs group 2 reacted positive on both, the sLPS and the rLPS antigens. As expected, it could be shown that cross reactivity with *Brucella* was mainly originating from the antibodies reacting to sLPS antigen. Utilisation of rLPS as

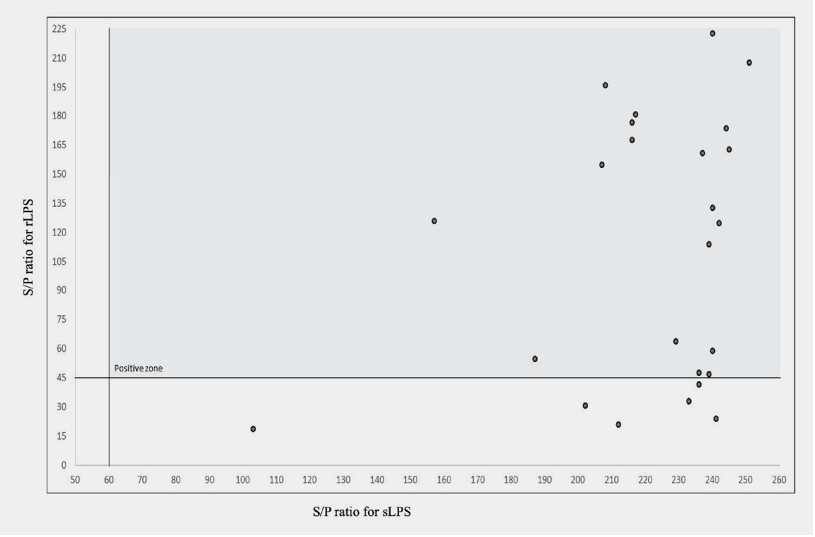

Figure 3: Optic density ratios of the samples versus positive control (S/P ratio) in bi-well ID Screen® Brucella suis Indirect ELISA for smooth and rough lipopolysaccharide (sLPS and rLPS) of the sera in group 3 (porcine sera from a *Brucella suis* outbreak). Manufacturer's thresholds for positivity are 45 % for rLPS and 60 % for sLPS, highlighted as grey rectangle.

Table 3a: Specificity (Sp) for False Positive serological Reactions (FPSRs) of the ID Screen® Brucella suis indirect ELISA based on results of the sera in group 2 (53 sera with FPSRs) from *Brucella suis*-free herds

	ID Screen® Brucella suis indirect ELISA result		
RBT+/CFT*/ multispecies i-ELISA result		positive	negative
	positive	2	51
	negative	0	0

+Rose Bengal Test; *Complement Fixation Test
Specificity (Sp) for FPSRs = 51/53 = 0,96 = 96 %

Table 3b: Sensitivity (Se) of the ID Screen® Brucella suis indirect ELISA with Complement Fixation Test as gold standard based on results of the sera in group 3 (25 sera from a *Brucella suis* outbreak)

	CFT* result		
ID Screen® Brucella suis indirect ELISA result		positive	negative
	positive	19	0
	negative	6	0

*Complement Fixation Test
Sensitivity (Se) = 19/25 = 0,76 = 76 %

an additional target antigen is useful for a higher specificity, which does however not reach 100 %, as two sera in group 1 and 2 each reacted above the threshold for rLPS. These sera demonstrated that cross-reacting antibodies for rLPS antigen are present, too, although to a much lower extent. In summary, the ID Screen® Brucella suis Indirect ELISA was useful in confirming FPSRs in 96 % of the FPSRs samples (Table 3a). These results are in accordance with data provided by the manufacturer.

Within the last years in Switzerland several false positive results with RBT, CFT and/or ID Screen® Brucellosis Serum Indirect multi-species ELISA occurred while testing boars on nucleus farms or in quarantine of stations for artificial insemination (pers. communication). In accordance with the Swiss legislation, in cases where several pigs within one farm react positive for *Brucella* in all three serological sLPS based tests, the farm should be categorised as brucellosis-positive and blocked until the suspicion can be dispelled (Swiss ordinance of epizootics). While most of the boars showing FPSRs are single reactors one nucleus farm had multiple reactors on a regular basis. At that time, no serological test to confirm FPSRs was available and alternative testing procedures had to be implemented to prove the brucellosis negative status of the herd. As FPSRs is not permanent, repeated testing within several weeks might be helpful in confirming the previous FPSRs.[14,20] In the above-mentioned nucleus farm, serological retesting resulted again in FPSRs. Additionally, brucellin skin test is mentioned in WOAH guidelines to be useful for confirmation of FPSRs.[28] However, lack of standardisation in production and availability of brucellin antigen as well as the subjectivity in test interpretation are limiting factors for this approach.[6] Therefore, bacterial culture for *Brucella suis* turned out to be the last option for conformation of the *Brucella* free status of the nucleus farm. The herd was monitored by culture four times in six months by sending samples from all aborted placentas and fetuses as well as placentas from inconspicuous births and organs from slaughtered pigs. The results in conjunction with the epidemiological situation could confirm the brucellosis free status of the herd. However, this monitoring was very time consuming and costly for the farm and the competent authorities. Especially in brucellosis-free countries, neither culture nor brucellin skin testing are therefore suitable for confirming a suspected FPSRs. In situations where a rapid diagnosis is required, e. g. import or quarantine of live animals, an alternative, rapid and reliable serological method is needed to confirm FPSR in porcine brucellosis. Therefore, the ID Screen® Brucella suis Indirect ELISA was validated in the Swiss national reference laboratory for brucellosis and approved for official testing in Switzerland in 2020 as a confirmatory test for FPSRs. Since then, it has been successfully applied for single FPSRs.

On the other hand, the ID Screen® Brucella suis Indirect ELISA showed a low diagnostic sensitivity (76 %) in the outbreak group (group 3). In our study, six out of 25 sera in group 3 reacted positive to sLPS, but not to rLPS (Figure

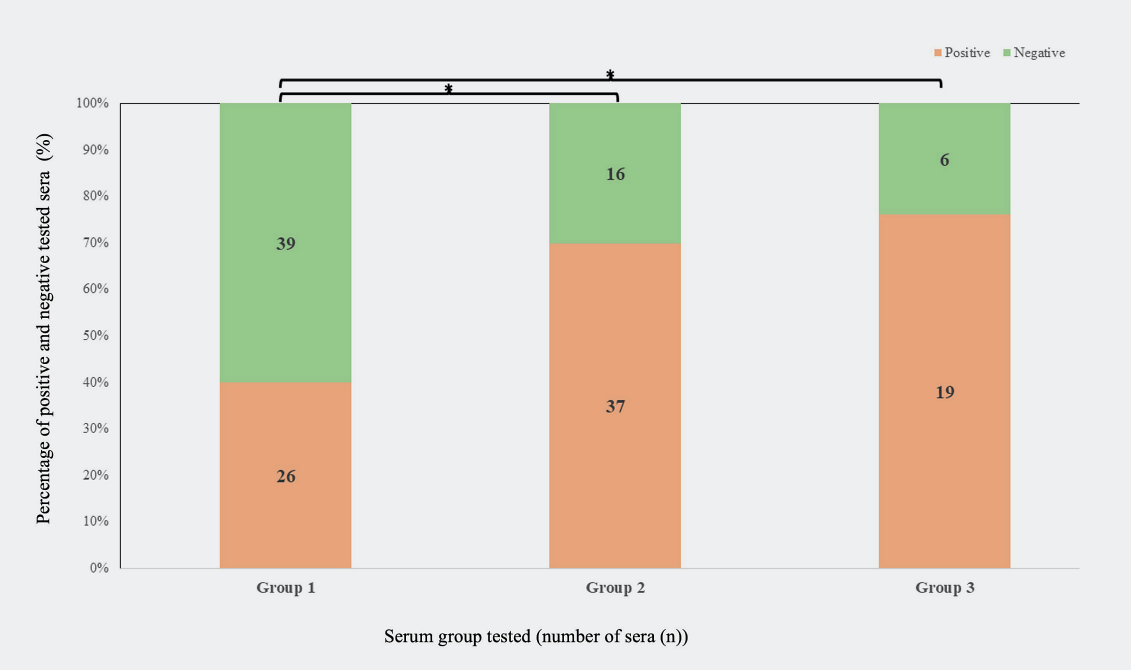

Figure 4: Results of porcine sera in groups 1–3 with the Pigtype® Yersinia Ab ELISA. Numbers within the columns indicate the number of sera with associated test result. Diagram shows the percentages of the results in each group. *Significant difference at p <0,01.

3), which results in a negative result according to the manufactures instructions. The same applies to the results of larger groups of sera tested by the manufacturer (pers. communication). Moreover, a low sensitivity of 66,7 % for this bi-well ELISA has also been demonstrated in a recent study from Germany investigating serum samples from wild boar.[18] This test is therefore not suitable for the reliable detection of brucellosis. This is in line with the manufacturer's intention to use this test exclusively as a confirmatory test for FPSRs. As a confirmatory test for porcine brucellosis, the sLPS-based tests showed much better sensitivity, with the CFT performing best. The presence of non-*Brucella* specific, cross-reactive antibodies as possible source for FPSRs could be confirmed with the ID Screen® Brucella suis Indirect ELISA. To rule out the role of cross-reactive antibodies against *Yersinia* species, Pigtype® Yersinia Ab ELISA (Indical Bioscience) was used. The diagnostic sensitivity and specificity of the latter test has been reported as 75 % and 98 %, respectively.[15] The significantly higher positivity rate of antibodies against *Yersinia* species in sera with FPSRs (group 2 (70 %)) compared to sera without FPSRs (group 1 (40 %)) indicates, that indeed antibodies reacting to *Yersinia* antigen are highly responsible for cross-reactivity to *Brucella* sLPS. However, 16 sera in group 2 reacted negative in the *Yersinia* iELISA, which demonstrated that antibodies due to colonisation or infection with other gram-negative bacteria than pathogenic *Yersinia* could lead to cross-reactive antibodies, too.[14,20] The Pigtype® Yersinia Ab ELISA is based on *Yersinia* outer proteins (Yops) as antigen. They represent a specific target for pathogenic *Yersinia* species and should distinguish and exclude antibodies against other Enterobacterales according to the manufacturer's instructions. On the other hand, not every pig colonised with *Yersinia* species, revealed antibodies cross-reacting with *Brucella* sLPS, as 26 sera of group 1 were positive for *Yersinia* antibodies but reacted negative in the sLPS *Brucella* tests. In addition to *Y. enterocolitca* serotype O:9, which is described as the main cause of cross-reactive antibodies, pigs can also be colonised with other *Y. enterocolitca* serotypes whose antibodies are detectable with this Yersinia ELISA, but might not cross-react with sLPS.[22] Further studies would be necessary for clarification.

The compelling differences in positivity rates observed with the Pigtype® Yersinia Ab ELISA between the three groups might be associated with the husbandry of the animals. Serum samples comprised in group 1 were obtained from pigs confined in an artificial insemination station, kept at high hygienic conditions and being substantially older, they are expected to have less recent *Yersinia* colonisation and therefore lower anti-*Yersinia* antibody titers.[14] It should also be mentioned in this context that these pigs are pre-selected, as animals with cross-reacting antibodies were not included in the station. Pigs in group 2, on the other hand, are younger and come from pig breeding or fattening farms; they are more likely to have antibodies against *Yersinia* species.[15] The high positivity in group 3 can also be related to husbandry conditions as they are pigs raised in outdoor piggeries with very low biosecurity and hygiene measurements, which might lead to high *Yersinia* colonisation rates.

Conclusions

In general, sLPS based serological tests are suitable for routine screening purposes of porcine brucellosis, but these tests do not distinguish between positive and false-positive results that occur when cross-reacting antibodies are present. The ID Screen® Brucella suis Indirect ELISA has a higher discrimination than the other serological tests tested and is therefore a valuable addition to the serological toolbox, greatly reducing the constraints and obstacles for a farm and the stakeholders in the surveillance of freedom of disease. We confirmed that most false positive results occurred from sera, that harboured *Yersinia* specific antibodies.

Acknowledgments

We thank the team of the diagnostic laboratory of the Institute of Veterinary Bacteriology, which hosts the Swiss National Reference Laboratory for Brucellosis. This study was financed by core funding of the University of Bern and by the Federal Food Safety and Veterinary Office, Bern (grant no 33–837).

The ID Screen® Brucella suis Indirect ELISA is a valuable supplement for serological diagnosis of porcine brucellosis

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Le test ELISA indirect ID Screen® Brucella suis est un complément précieux pour le diagnostic sérologique de la brucellose porcine

La brucellose chez les porcs domestiques, principalement causée par *Brucella* (*B.*) *suis*, a été éradiquée dans de nombreux pays européens et la population porcine domestique fait l'objet d'une surveillance sérologique afin de garantir l'absence de la maladie. Cependant, les anticorps qui réagissent de manière croisée avec l'antigène diagnostique lipopolysaccharide lisse (sLPS) prescrit peuvent entraîner des résultats sérologiques faussement positifs (FPSR) en raison de la similitude de l'antigène avec d'autres bactéries, telles que les espèces *Yersinia*. Les porcs domestiques sont fréquemment colonisés par *Y. enterocolitica*. Le test ID Screen® Brucella suis Indirect ELISA, basé sur le sLPS et le LPS rugueux (rLPS), a été développé pour confirmer les FPSR dans les sérums porcins.

Les performances du test ID Screen® Brucella suis Indirect ELISA ont été analysées à l'aide de 143 sérums provenant de porcs domestiques présentant différents statuts face à la brucellose : groupe 1 : porcs indemnes de brucellose élevés dans une unité d'insémination artificielle (n = 65) ; groupe 2 : troupeaux indemnes de brucellose présentant une séropositivité pour la brucellose dans différents tests sérologiques basés sur le sLPS, soupçonnés d'être des FPSR (n = 53) ; groupe 3 : troupeaux avec brucellose porcine confirmée (n = 25). De plus, les sérums ont été testés avec le test Pigtype® Yersinia Ab ELISA.

Les sérums du groupe 1 ont été confirmés comme étant véritablement séronégatifs pour la brucellose à l'aide du test ELISA indirect ID Screen® Brucella suis. Dans le groupe 2, 51 des 53 sérums (96 %) ont été confirmés comme étant des FPSR par le test ELISA indirect ID Screen® Brucella suis. Dix-neuf sérums (76 %) du groupe 3 se sont révélés véritablement séropositifs pour la brucellose. Les résultats confirment la bonne capacité de discrimination du test ELISA indirect ID Screen® Brucella suis pour les faux positifs sérologiques à la brucellose. De plus, nos données ont confirmé que les anticorps *Yersinia* à réaction croisée sont la cause la plus probable des FPSR.

Mots clés: *Brucella suis*, test de fixation du complément (CFT), FPSR, test de Rose Bengal (RBT), porc

L'ID Screen® Brucella suis Indirect ELISA costituisce un valido test complementare per la diagnosi sierologica della brucellosi suina

La brucellosi dei suini domestici, causata prevalentemente da *Brucella (B.) suis*, è stata eradicata in numerosi Paesi europei; di conseguenza, la popolazione suina viene sottoposta a programmi di sorveglianza sierologica per dimostrare lo stato di indennità. Tuttavia, l'impiego di antigeni diagnostici basati sul lipopolisaccaride liscio (sLPS) può determinare reazioni crociate anticorpali, con conseguenti risultati sierologici falsamente positivi (FPSR) a causa della somiglianza antigenica con altri batteri, come le specie del genere *Yersinia*. I suini domestici sono frequentemente colonizzati da *Y. enterocolitica*. L'ID Screen® Brucella suis Indirect ELISA, basato sull'impiego di sLPS e lipopolisaccaride ruvido (rLPS), è stato sviluppato per confermare la presenza di risultati sierologici falsamente positivi nei sieri suini.

Le prestazioni dell'ID Screen® Brucella suis Indirect ELISA sono state valutate analizzando 143 campioni di siero provenienti da suini domestici con diverso stato rispetto alla brucellosi, suddivisi in tre gruppi: gruppo 1, suini indenni da brucellosi allevati in un centro di inseminazione artificiale (n = 65); gruppo 2, allevamenti indenni da brucellosi che presentavano sieropositività alla brucellosi in diversi test sierologici basati su sLPS, sospettata come dovuta a risultati sierologici falsamente positivi (n = 53); gruppo 3, allevamenti con brucellosi suina confermata (n = 25). Inoltre, i sieri sono stati analizzati mediante Pigtype® Yersinia Ab ELISA.

Tutti i sieri del gruppo 1 sono risultati confermati come realmente sieronegativi per la brucellosi mediante l'ID Screen® Brucella suis Indirect ELISA. Nel gruppo 2, 51 su 53 campioni (96 %) sono stati confermati come FPSR mediante l'ID Screen® Brucella suis Indirect ELISA. Nel gruppo 3, 19 sieri (76 %) sono risultati realmente sieropositivi per la brucellosi suina. I risultati dimostrano l'elevata capacità discriminante dell'ID Screen® Brucella suis Indirect ELISA nel differenziare la vera sieropositività da *Brucella suis* dalle reazioni sierologiche falsamente positive. Inoltre, i dati confermano che la presenza di anticorpi anti-*Yersinia* a reattività crociata rappresenta la causa più probabile degli FPSR nei suini domestici.

Parole chiave: *Brucella suis*, test di fissazione del complemento (CFT), risultati sierologici falsamente positivi (FPSR), test di Rose Bengal (RBT), suini

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