Identification of *Streptococcus suis* Meningitis by Direct Triplex Real-Time PCR, Burkina Faso

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Meningitis confirmation in Burkina Faso uses PCR for detecting *Streptococcus pneumoniae*, *Neisseria meningitidis*, or *Hemophilus influenzae*. We identified 38 cases of meningitis among 590 that were PCR-positive for 3 nonpneumococcal streptococcal pathogens, including 21 cases of *Streptococcus suis*. Among the country's 13 regions, 10 had *S. suis*–positive cases.

Streptococcus suis is a commensal organism of the upper respiratory tract of pigs that can occasionally cause severe invasive infections in these animals (*1*,*2*). The bacterium also can infect humans who have close contact with pigs or pork products, leading to serious diseases such as meningitis, endocarditis, and sepsis (*3*). *S. suis* can survive in dust, manure, and pig carcasses for days or even weeks under optimal conditions; therefore, the working environments in slaughterhouses and farms can be a source of human infection (*3*,*4*). Meningitis is the most common clinical manifestation of *S. suis* infection in humans and has an estimated case-fatality rate of 3% (*2*). Whereas this zoonotic pathogen is among the leading causes of adult meningitis in some countries of Southeast Asia (*5*), its incidence in Africa is largely unknown. We report 21 retrospectively confirmed cases of *S. suis* meningitis in Burkina Faso.

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The Study

Burkina Faso conducts nationwide case-based surveillance for bacterial meningitis. Cerebrospinal fluid (CSF) samples collected from patients with suspected meningitis are sent to 1 of 5 national laboratories for confirmation by culture and real-time PCR testing (*6*). Because of multiple challenges encountered by the laboratories in isolating bacteria from CSF, the confirmation of meningitis cases relies heavily on molecular detection (*6*). The real-time PCR assay currently used at the national laboratories only detects *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*. Between 2015 and 2018, the national laboratories tested 7,174 CSF samples from patients with suspected meningitis nationwide. Of these, 4,930 (68.7%) were PCR-negative for *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*. To further investigate suspected meningitis cases that were PCR-negative after testing for the 3 pathogens, a subset of specimens was selected for additional screening based on leukocytes counts ≥50 cells/mm^3. In total, 912 PCR-negative specimens were available for the study; among these, 590 fit the selection criteria. The specimens were retested by using a triplex direct real-time PCR that was designed for the simultaneous detection of *Streptococcus agalactiae* (group B *Streptococcus* [GBS]), *S. pyogenes* (group A *Streptococcus* [GAS]), and *S. suis*. The nucleotide sequences and final concentrations of the primers and probes used in this assay (Table 1) were identical to those used in singleplex real-time PCR methods (*7*–*10*), with the exception that FAM was replaced by HEX as the reporter dye for *cfb*-specific probes for the GBS target and by Cy5 as the reporter dye for *spy*-specific probes for the GAS target. Each reaction was prepared in a final volume of 25 μL, including 1 μL of each primer and probe, 2 μL of CSF as DNA template, 12.5 μL of PerfeCta MultiPlex qPCR ToughMix mastermix (QuantaBio, https://www.quantabio.com),

Table 1. Sequences and concentrations of primers and probes used to detect *Streptococcus suis*, *S. agalactiae*, and *S. pyogenes*, Burkina Faso*

Target gene	Forward primer, 5′ → 3′	Reverse primer, 5′ → 3′	Probe, 5′ → 3′	Concentration of oligo per reaction, nM†
cfb (*7*), GBS	GGGAACAGATTATGAAAAACCG	AAGGCTTCTACACGACTACCAA	AGACTTCATTGCGTGCCAACCCTGAGAC 5′-HEX; 3′-BHQ1	200/200/200
fbpS (*8*), *S. suis*	TCCRATRCTGCTCTGCCATT	TGATAGTAGAAGTCCAGCARACT	AATAGCCC"T"GAAAAMCAGCCACWYTTTGARA 5′-FAM; 3′-SpC6; "T" = BHQ1	200/200/100
spy (*9*), GAS	GCACTCGCTACTATTTCTTACCTCAA	GTCACAATGTCTTGGAAACCAGTAAT	CCGCAAC"T"CATCAAGGATTTCGTTACCA 5′-Cy-5; 3′- SpC6; "T" = BHQ2	300/300/100
RNaseP (*10*)	AGATTTGGACCTGCGAGCG	GAGCGGCTGTCCCACAAGT	TTCTGACCTGAAGGCTCTGCGCG 5′-FAM; 3′-BHQ1	400/400/100

*GAS, group A *Streptococcus* (*Streptococcus pyogenes*); GBS, group A *Streptococcus* (*S. agalactiae*); RNaseP, human ribonuclease P.
†Concentration of forward primer/reverse primer/probe.

and 1.5 µL of PCR-grade water. The thermal profile for the real-time PCR runs was 1 cycle of 55°C for 5 min, 1 cycle of 95°C for 10 min, then 40 cycles of 95°C for 15 s and 60°C for 1 min. All specimens were tested by real-time PCR for the presence of the human ribonuclease P (RNaseP) gene to check for the presence of inhibitors. A specimen was considered positive if the cycle threshold (C_t) for 1 of the bacterial targets was ≤35 and was considered negative if the C_t was ≥40 for all bacterial targets with C_t ≤35 for RNaseP. If C_t was between 35 and 40 for the bacterial targets and ≤35 for RNaseP, the specimen is retested; if the result was reproducible, it was considered positive. The lower limits of detection (LLDs) were determined as previously described (*11*). The LLD of the direct triplex assay (3 CFU/µL for *S. suis*, 28 CFU/µL for GBS, and 26 CFU/µL for GAS) were compared with the LLD of the triplex assay conducted on DNA extracted from the same serial dilutions (2 CFU/µL for *S. suis*, 5 CFU/µL for GBS, and 3 CFU/µL for GAS).

Among the specimens tested, 21 were positive for *S. suis*, 13 for GAS, and 4 for GBS (Table 2). Of the 21 *S. suis*–positive case-patients, 1 was 4 years of age, 3 were 5–14 years of age, 2 were 15–29 years of age, 9 were 30–49 years of age, and 6 were ≥50 years of age; 16 (76.2%) were male and 5 (23.8%) were female. Two (9.5%) patients died, 14 recovered, and the outcome for 5 was unknown. Eight (61.5%) of the GAS-positive case-patients were <5 years of age and 5 were 5–14 years of age; 1 (7.7%) patient died, 5 recovered, and the outcome for 7 was unknown. All the GBS-positive case-patients were ≤6 months of age and all recovered. Median time from symptom onset to CSF collection was 2 days (range 1–10 days) for all the patients.

S. suis–positive cases were found among specimens from rural districts in 10/13 regions of the country with 28% (6/21) from the Centre-Est Region and 19% (4/21) from the Plateau Central Region. All adults with *S. suis* were farmers. Because of the retrospective detection of the etiology, the extent of any direct exposure of the *S. suis*–positive patients to pigs or pork products was unknown and attempts to collect additional information from the patients or their families were unsuccessful.

Table 2. Characteristics of patients with meningitis caused by *Streptococcus suis*, *S. pyogenes*, and *S. agalactiae*, Burkina Faso, 2015–2018*

Characteristics	Patients infected, no. (%)			All patients, no. (%), n = 590
	S. suis, n = 21	*S. pyogenes*, n = 13	*S. agalactiae*, n = 4	
Age, y				
<5	1 (4.8)	8 (61.5)	4 (100)	307 (52)
5–14	3 (14.3)	5 (38.5)	0	168 (28.5)
15–29	2 (9.5)	0	0	64 (10.8)
30–49	9 (42.8)	0	0	37 (6.3)
≥50	6 (28.6)	0	0	14 (2.4)
Sex				
M	16 (76.2)	9 (69.2)	2 (50)	332 (56.3)
F	5 (23.8)	4 (30.8)	2 (50)	258 (43.7)
Year CSF collected				
2015	5 (23.8)	1 (7.7)	0	87 (14.7)
2016	2 (9.5)	2 (15.4)	0	104 (17.6)
2017	6 (28.6)	3 (23.1)	2 (50)	195 (33.1)
2018	8 (38.1)	7 (53.8)	2 (50)	204 (34.6)
Median leukocytes, cells/mm^3 (range)	400 (51–6,250)	1,000 (53–8,000)	1,100 (800–2,400)	920 (50–8,000)
Median C_t (range)	26.1 (18.13–35.52)	25.33 (14.78–34.71)	23.47 (17.24–24.23)	25.33 (14.78–35.52)

*CSF, cerebrospinal fluid; C_t, cycle threshold.

Small-scale pig farming is a major economic activity in most parts of Burkina Faso. Although porcine-related occupations, undercooked pork consumption, and exposure to pigs have been identified as major risk factors for *S. suis* infection (*5*), its occurrence in a wide range of animal species have been reported (*2*).

DNA extracted from the *S. suis*–positive specimens were used for a multiplex conventional PCR for serotype detection (*12*). All the specimens were serotype 2 or 1/2 because the method used here could not clearly differentiate serotypes 2 and 1/2. Bacterial isolates from these cases were not available for further characterization. Serotype 2 is believed to cause 74%–95% of human cases of *S. suis* infections reported worldwide, but serotype 1/2 has not been reported to cause disease in humans (*1*,*2*).

Conclusions

Limited data are available regarding the incidence of streptococcal infections in Africa, and the disease burden likely is underestimated, especially for disease caused by *S. suis*. Two reported studies conducted in Togo identified 16 human cases of *S. suis* meningitis during 2010–2015 caused by serotype 2 (*13*,*14*). Another recent study in Madagascar described 2 human cases of *S. suis* meningitis, also caused by serotype 2 (*15*).

Our study reports 21 cases of *S. suis* meningitis from 2015–2018 in Burkina Faso. *S. suis* infections more commonly are observed in adult males and are directly correlated with occupational exposure to pigs or pork products (*2*,*13*,*15*). All adult cases reported here were farmers, although their exposure to pigs or pork was unknown. The presence of children among the patients in this study and in previous studies (*13*) indicates that older children also should be considered at risk. Finding 21 cases of this outbreak-prone zoonotic pathogen in this study raises concern about the incidence of *S. suis* disease in Burkina Faso and in other parts of Africa where pigs are raised. Prospective surveillance for *S. suis* could help identify farming communities where measures to prevent disease and its potential socioeconomic damages are needed.

About the Author

Dr. Ouattara is a microbiologist in the *Streptococcus* Laboratory, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. His primary research interests include meningitis, invasive streptococcal diseases, bacterial pathogenesis, and global laboratory capacity building.

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