Prevalence of *Bordetella* Infection in a Hospital Setting in Niamey, Niger

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Summary

***Bordetella pertussis* still poses an important health threat in developing countries. In Niger, notified pertussis cases are few despite the low diphtheria–tetanus–pertussis/pentavalent vaccine coverage. We aimed to estimate the prevalence of *B. pertussis* in children aged <5 years consulting at a pediatric ward. A 5-month study in 2011 recruited 342 children with respiratory symptoms at the National Hospital of Niamey. Nasopharyngeal aspirates were tested by culture and real-time polymerase chain reaction. Overall, 34 (11.2%) of the 305 available nasopharyngeal aspirates tested by real-time polymerase chain reaction were positive for a *Bordetella* spp., with an estimated prevalence of 8.2 cases per 1000 children aged <5. None was notified to the surveillance network. A single specimen was positive on culture. This study, the first to provide laboratory-confirmed data on pertussis in Niger, highlights the need to sensitize health care personnel to actively notify clinical cases and to integrate laboratory diagnosis in the existing surveillance system.**

Key words: pertussis, prevalence, diagnosis, Niger

Introduction

Despite the availability of effective vaccines against *Bordetella pertussis*, the etiologic agent of pertussis (whooping cough) still poses a serious health threat, especially in infants aged <1 year [1–3]. Vaccination has reduced the burden in many countries worldwide, with global vaccine coverage of the three primary doses that increased from approximately 20% in 1980 to 81% in 2007 [4, 5]. Despite this progress, a resurgence of whopping cough has been observed in the past decades, with regular epidemics every 2–5 years. Increased incidence among adolescents and young adults, who are responsible for transmission of the disease to children too young to be completely immunized, has also been observed [6, 7].

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In low-income countries where 90% of cases are reported, the burden of pertussis is still difficult to estimate due to unavailable and sometimes imprecise surveillance data and the absence of laboratory diagnosis. This is further complicated by the fact that even the clinical diagnosis routinely used is non-specific for pertussis, especially in young children, where the only manifestation of the disease could be in the form of apnea and cyanosis [8, 9].

Recent published data on pertussis epidemiology in sub-Saharan Africa are lacking. Préziosi *et al.* [10] reported a crude incidence of 183 per 1000 child-years in children aged <5 years in Senegal, with a case-fatality rate of 2.8%. A 4-month study in Mali in 1989 [11] identified 17 cases among 83 children aged between 0 and 13 years (20.5%), while in Abeokuta, Nigeria, 33 (16%) cases were identified by culture among 209 infants with early suspicion of pertussis [12].

In Niger, no published data exist on pertussis, which is a notifiable disease. Reporting is solely based on the World Health Organization's (WHO) clinical definition of cases and laboratory diagnosis

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was inexistent. WHO's clinical definition of pertussis is a case diagnosed as pertussis by a physician, or a person with a cough lasting ≥2 weeks with at least one of the following symptoms: paroxysms (i.e. fits) of coughing, inspiratory 'whooping' or posttussive vomiting (i.e. vomiting immediately after coughing) without other apparent cause [4].

Furthermore, the vaccine coverage of diphtheria–tetanus–pertussis (DTP)/pentavalent is suboptimal and below WHO-recommended levels [13]. In 2006, 58% of children aged 12–23 months received their first dose of DTP, 49% their second dose and 39% their third dose [14]. A 3-month study in 2010 in Niger presented by Tchidjou *et al*. (2011) [15] documented that 97% of the children recruited received the first dose of DTP–hepatitis B virus/haemophilus influenzae b (DTP1) vaccine, but only 19% received the second dose and 14% the third dose. Although these vaccine coverage estimates are insufficient, the number of notified pertussis cases in the national surveillance system is also low. In the region of Niamey, comprising approximately 1 million inhabitants, only 82 clinical cases were notified in 2008 by the surveillance system, 58 of whom were children aged <5 years. Similarly, 102 989 cases of coughs and colds of unknown etiology were notified and represent the second cause of morbidity and mortality nationwide [16].

Through a prospective study integrating laboratory diagnosis, we aimed to estimate the prevalence of *B. pertussis* in children aged <5 years consulting at a pediatric ward in Niamey, Niger. Secondary objectives were to estimate the proportion of confirmed vaccinated cases and compare confirmed versus notified cases.

Methods

Between 6 December 2010 and 30 April 2011, all children aged <5 years consulting at the pediatric screening unit of the National Hospital of Niamey with at least one of the following respiratory symptoms were recruited: persistent cough lasting 1 week or more, clinical suspicion of pertussis or pneumonia by a physician or a child with a cold having a family member with prolonged cough. Children with known or suspected tuberculosis infection were excluded. The National Hospital of Niamey is the main referral hospital but patients from both urban and rural areas come directly for consultation instead of going through the peripheral hospitals and health centers. All care for children aged <5 is provided free of charge. The following demographic and clinical data were collected for each child in a case report form: gender, age, duration of cough, clinical symptoms associated with pertussis, previous antibiotic intake, vaccination history with the three primary doses of DTP/pentavalent vaccine and information on household members having prolonged cough. From each child, a nasopharyngeal aspirate (NPA) specimen was collected into a sterile tube by a trained nurse and sent to the hospital laboratory for detection of *Bordetella* spp. by culture. The remaining specimen was conserved at −20°C and later transported under refrigerated conditions to the Centre de Recherche Médicale et Sanitaire (CERMES) Niamey for *Bordetella* spp. detection by real-time polymerase chain reaction (RT-PCR). Each NPA specimen collection was performed by the study nurse under the supervision of the head nurse of the pediatric unit, who is well-versed with NPA collection and also coordinates the unit's NPA collection for the national influenza surveillance program in Niger.

The definitions of a clinical and a laboratory-confirmed case were those recommended by the WHO. A clinical case was defined as a cough lasting at least 2 weeks with at least one of the following symptoms: coughing paroxysms, inspiratory whooping or posttussive vomiting. A laboratory-confirmed case was defined as a positive culture or a positive RT-PCR.

The study protocol was approved by the National Ethical Committee of Niger. All caretakers/parents of the recruited children gave their signed informed consent after reading the information notice, written in French, Haussa or Zerma local languages. Parents who could neither read nor write had their information notice read to them by an impartial witness and signed using their fingerprint. All children received care free of charge irrespective of their participation in the study.

Bacterial strains

The most frequently circulating *Bordetella* strains, namely, *B. pertussis*, *Bordetella parapertussis*, *Bordetella holmesii* and *Bordetella bronchiseptica*, were used as reference strains. The *B. parapertussis* strain was isolated from patients by the microbiology laboratory of the Robert Debré Hospital in Paris, and the other three were from the microbiology laboratory of the 'Hôpital Intercommunal' Creteil, France. All the strains were confirmed by the Pertussis National Reference Center of the Pasteur Institute, Paris, France.

Culture technique for Bordetella spp.

The NPA from each child was inoculated within 1 h of collection into freshly prepared Bordet–Gengou medium (Difco) containing sheep blood with and without 40°mg/l cephalexin inhibitor as well as into charcoal agar with and without cephalexin (Oxoid). The inoculated plates were incubated for 3–10 days at 37°C and examined daily. Any suspected *Bordetella* colonies were subcultured on Bordet–Gengou and sheep blood agar, respectively. They were further examined by gram staining, oxidase

tests and other biochemical tests (urease, nitrate reductase). Fertility tests were performed regularly using reference strains of *B. pertussis* and *B. parapertussis* as control. Any isolate obtained was conserved at −80°C for confirmation on RT-PCR.

DNA extraction

Only 305 of the 342 NPAs were available for RT-PCR analysis, as 37 were lost during transportation.

DNA was extracted from 200 µl of the NPA specimens using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Extracts were tested undiluted.

Real-time PCR

DNA samples from *B. pertussis*, *B. parapertussis*, *B. holmesii*, *B. bronchiseptica* (positive controls) and a no-template (negative) control were used at each run. Three single RT-PCRs were performed on each specimen and in duplicate, using primers and probes targeting the insertion sequence IS*481* [17, 18], the pertussis toxin promoter (*ptxA*-Pr) specific for *B. pertussis* [19] and IS*1001* target specific for *B. parapertussis* [17].

An average crossing threshold (Ct) value was calculated on the two RT-PCR assays to give a final value, and a positive result was defined when a visible fluorescence curve and a Ct value of <40 were observed.

The algorithm for interpreting the results was as follows: a *B. pertussis*-positive specimen was positive on both IS*481* and *ptxA-Pr* but negative on IS*1001*, whereas a *B. parapertussis*-positive specimen was positive solely on IS*1001*. Specimens positive uniquely on IS*481* were interpreted as a *Bordetella* spp [20] (Table 1).

Any culture-positive isolate was later confirmed by RT-PCR following two serial dilutions directly amplified with the three primers and fluorescent probes.

Statistical analysis

Descriptive analysis was performed using Stata 11 (Stata Corporation, College Station, TX). Means and standard deviations were calculated. A *p*-value of <0.05 was considered significant. A univariate analysis (Fisher's exact test) was performed to determine any association between positive PCR results and the clinical variables. Variables with *p*-values <0.3 (age, vaccinated with three doses, coughing paroxysm or whoop, suffocation, presence of WHO criteria and cough >2 weeks) were included in a multiple logistic regression using backwards selection. Only the variable 'presence of WHO criteria' was maintained, as 'cough >2 weeks' is an integral part of the criteria. The model was verified using the Hosmer–Lemeshow test.

Results

Study population

From 6 December 2010 to 30 April 2011, 342 children aged <5 years were recruited. The majority were male ($n = 189$, 55.3%) and the median age was 11 months (interquartile range: 6–23 months).

At inclusion, the majority of children presented with nasal congestion, suffocation and pneumonia (Table 2). The physician did not confirm a clinical suspicion of pertussis in any child, but on investigation with the parents/caretakers, 63 (18.4%) presented with paroxysms or whoop.

Almost all children had received at least one dose of either DTP or pentavalent vaccine, and 201 (58.8%) reported complete vaccination with the three primary doses. The majority of parents (77.2%) consulted with their child's vaccination card. Vaccine coverage was lowest in children aged 0–6 months, some of whom by virtue of their age at inclusion had not received the three primary doses or no vaccine at all.

In all, 236 children (69%) had taken antibiotics in the week before consultation (cotrimoxazole syrup in 64.8% of cases), 80 children (23.4%) were hospitalized and radiography revealed complicated/severe pneumonia in seven children.

Culture

A *Bordetella* species was identified on only one of the 342 specimens (0.3%) cultured. The specimen belonged to an unvaccinated 18-month-old boy who

TABLE 1
Interpretation criteria of RT-PCR results

RT-PCR results for the three targets	IS481+ (Ct <35)	IS481+ (35 ≤ Ct < 40)	IS481− (Ct ≥40)
ptxA-Pr+ (Ct <40)	*B. pertussis*	*B. pertussis*	To be retested
ptxA-Pr− (Ct ≥40)	*Bordetella* spp.	Likely *Bordetella* spp.	Negative
IS*1001*+ (Ct <35)	To be retested for co-infection	To be retested for co-infection	*B. parapertussis*
IS*1001*+ (35 ≤ Ct < 40)	To be retested for co-infection	To be retested for co-infection	Likely *B. parapertussis*

TABLE 2
Frequency of clinical symptoms and vaccine history by age-group at inclusion (n = 342)

	Age-group in months				
	0–6 $n = 95$ (%)	7–12 $n = 77$ (%)	13–23 $n = 86$ (%)	>23 $n = 84$ (%)	Total $n = 342$ (%)
Gender					
Male	59 (31.2)	48 (25.4)	40 (21.2)	42 (22.2)	189 (55.3)
Female	36 (23.5)	29 (18.9)	46 (30.1)	42 (27.4)	153 (44.7)
Clinical symptoms					
Fever	24 (25.5)	22 (28.6)	25 (29.1)	33 (39.3)	104 (30.4)
Coughing paroxysms/whoop	20 (21.1)	12 (15.6)	17 (19.8)	14 (16.7)	63 (18.4)
Nasal congestion	65 (68.4)	52 (67.5)	69 (80.2)	70 (83.3)	256 (74.8)
Vomiting	58 (61.1)	53 (68.8)	49 (57.0)	52 (62.0)	212 (62.0)
Suffocation	76 (80.6)	64 (83.1)	67 (77.9)	64 (76.2)	271 (79.2)
Suspicion of pneumonia	94 (98.9)	77 (100)	86 (100)	84 (100)	341 (99.7)
Vaccination history					
No vaccination proof[a]	20 (21.1)	5 (6.5)	20 (23.3)	33 (39.3)	78 (22.8)
Not vaccinated	4 (4.2)	2 (2.6)	1 (1.2)	0 (0)	7 (2.1)
Vaccinated with one dose	27 (28.4)	4 (5.2)	2 (2.3)	1 (1.2)	34 (9.9)
Vaccinated with two doses	18 (18.9)	0 (0)	4 (4.7)	0 (0)	22 (6.4)
Vaccinated with three doses[b]	26 (27.4)	66 (85.7)	59 (68.6)	50 (59.5)	201 (58.8)

[a]These are children who did not present their vaccination cards at consultation. Only the mother's declaration was documented (63 vaccinated, 14 not vaccinated and 1 unknown).
[b]In Niger, the three primary doses of DTP/pentavalent vaccines are recommended for administration at 6–10–14 weeks.

TABLE 3
Distribution of the 34 Bordetella cases detected by RT-PCR and by age-group

Strain	Age-group in months				
	0–6 *n* (%)	7–12 *n* (%)	13–23 *n* (%)	>23 *n* (%)	Total *n* (%)
Bordetella spp.	9 (75)	9 (81.8)	4 (80)	4 (100)	28 (82.3)
B. pertussis	3 (25)	0 (0)	1 (20)	0 (0)	4 (11.8)
B. parapertussis	0 (0)	2 (18.2)	0 (0)	0 (0)	2 (5.9)
Total	12 (35.3)	11 (32.3)	5 (14.7)	6 (17.7)	34 (100)

had taken cotrimoxazole but did not have paroxysms/whooping and did not meet the WHO clinical criteria for notification. All the fertility and sterility tests were valid.

Real-time polymerase chain reaction

Overall, 34 (11.1%) of the 305 available NPAs tested by RT-PCR were positive for a *Bordetella* species, with an estimated prevalence of 8.2 cases per 1000 children aged <5 consulting at the national hospital of Niamey during the study period. Twenty-three of the 34 positive cases were children aged <1 year (67.6%; Table 3).

Specifically, four (1.3%) were positive for *B. pertussis* and two (0.7%) for *B. parapertussis*. The four *B. pertussis* cases were aged 1, 3, 5 and 18 months (culture-positive case). All four cases had nasal congestion, pneumonia and suffocation and had received cotrimoxazole. None presented with fever and none were completely vaccinated with the three primary vaccine doses: two had received two vaccine doses each and two had not been vaccinated at all. Three of the four children had a household member with cough lasting more than 1 week. Only one child presented with a paroxysm/whoop and met the WHO criteria for notification.

Only three (5.8%) of the 52 available NPAs from children presenting with paroxysms or whoop were positive by RT-PCR, compared with 31 positives (12.3%) among the 253 children without paroxysms/whoop.

From the univariate analysis, we observed a greater number of laboratory-positive *Bordetella* cases among children meeting the WHO clinical

TABLE 4
Association between presence of Bordetella spp. and the different clinical variables

Variable	$N = 34$	Percent positive by RT-PCR	Univariate analysis p	Logistic regression model (multivariate analysis)
Age in months[a]				
0–6	12	14.3 [6.7–21.8]		
7–12	11	16.9 [7.7–26.1]		
13–23	5	6.4 [0.9–11.9]		
>23	6	7.7 [1.7–13.7]	0.125	
Sex				
Male ($n = 161$)	16	9.9 [5.2–14.6]		
Female ($n = 144$)	18	12.5 [7–17.9]	0.478	
Household contact with cough lasting more than 1 week				
Yes ($n = 233$)	24	10.3 [6.8–14.2]	0.398	
No ($n = 72$)	10	13.9 [5.8–22]		
Vaccination history				
Not vaccinated ($n = 21$)	3	14.3 [0–29.7]		
Vaccinated (one or more dose) ($n = 284$)	31	10.9 [7.3–14.6]	0.636	
Complete vaccination (three doses)[a]				
Yes ($n = 173$)	23	13.3 [8.2–18.4]		OR = 1.4/1 [0.7–3.1]
No ($n = 132$)	11	8.3 [3.6–13.1]	0.173	$p = 0.353$
Coughing paroxysm or whoop[a]				
Yes ($n = 52$)	3	5.6 [0–12.2]		
No (253)	31	12.2 [8.2–16.3]	0.176	
Colds (nasal congestion)				
Yes ($n = 230$)	9	12.0 [4.6–19.4]		
No ($n = 75$)	25	10.9 [6.9–14.9]	0.787	
Cough >2 weeks[a]				
Yes ($n = 272$)	26	9.6 [6.0–13.1]		
No ($n = 219$)	8	24.2 [9.3–39.1]	0.011	
Suffocation[a]				
Yes ($n = 239$)	29	12.2 [8-16.4]		
No ($n = 67$)	5	7.5 [1.1-13.8]	0.278	
Presence of WHO criteria[a]				
Yes ($n = 24$)	6	25.0 [7.2–42.8]		OR = 4.75/1 [1.5–14.8]
No ($n = 281$)	28	10.0 [6.4–13.5]	0.025	$p = 0.007$
Hospitalization				
Yes ($n = 66$)	7	10.6 [3.1–18.1]		
No ($n = 239$)	27	11.3 [7.2–15.3]	0.875	
Previous consultation				
Yes ($n = 201$)	23	11.4 [7–15.9]		
No ($n = 104$)	11	10.6 [4.6–16.5]	0.820	

[a]Included in the logistic regression model
Hosmer–Lemeshow chi^2 (2) = 0.66 Prob > chi^2 = 0.7197

criteria for notification, and also in children with a cough lasting more than 2 weeks, compared with those who did not. After adjusting for sex, age and coughing paroxysm or whoop, only the presence of the WHO clinical criteria was significantly linked to PCR positivity. Twenty-four (7.9%) children recruited in the study fulfilled the WHO clinical criteria for pertussis notification to the surveillance system; yet, none was notified by the health care personnel. Six of the 24 children (25%) were positive for *Bordetella* spp. by RT-PCR compared with 28 (10%) among the 281 cases not meeting the WHO criteria.

Statistically, PCR-positive cases were 4.75 times more likely to fulfill the WHO criteria for notification (Table 4).

Although we did not find an association between vaccination history and positive RT-PCR, it is worth

noting that 31 of the 34 *Bordetella* laboratory-positive cases were among the 284 children who had received at least one vaccine dose (10.9%), whereas three were among the 21 non-vaccinated children (14.3%).

Furthermore, of the 173 children reporting having received three doses of vaccine, 23 were positive for a *Bordetella* spp. (13.3%). All 23 positive children presented their vaccination cards during consultation. Twenty-two of the 23 children had been administered the pentavalent vaccine and one child the DTP vaccine. On the contrary, only 11 (8.3%) of the 132 incompletely or un-vaccinated children had positive RT-PCR, nine of whom presented their vaccination cards.

Discussion

This study, the first in Niger, aimed to estimate the prevalence of *B. pertussis* within the pediatric screening unit, which captured all children aged <5 presenting at the National Hospital of Niamey. Overall, of the 305 children with available NPAs, RT-PCR permitted the detection of 34 cases (11.2%) positive for at least a *Bordetella* species, four for *B. pertussis* and two for *B. parapertussis*, with an estimated prevalence of 8.2 cases per 1000 children aged <5. Most of the laboratory-confirmed cases (23/34) were children aged <1 year, and three of the four *B. pertussis* cases were <6 months old [2]. Studies in neighboring countries have documented similar proportions of confirmed *Bordetella* spp. [10–12].

The insertion sequence IS*481* was detected in 28 of the 34 positive cases with Ct values >30 and <40, thereby confirming the high sensitivity of this target and indicating very probably the presence of *Bordetella* spp. [21]. Only one child was diagnosed by culture and later confirmed as *B. pertussis* by RT-PCR, consistent with data from Zouari, *et al* (2012) [22]. All *Bordetella* cases were identified by RT-PCR, confirming the sensitivity of this technique compared with culture [3, 23]. Estimating the accuracy of RT-PCR for pertussis diagnosis remains difficult and has always been hampered by the low sensitivity of culture, the gold-standard test, and by the constant evolution of targets in RT-PCR assays [24]. Some authors have, however, tried to reduce this bias by using statistical approaches such as the composite reference standard or latent class analysis [25].

The fact that most of the children in our study had taken an antibiotic before consultation could render the isolation of *Bordetella* even more difficult and improbable. In Niger, most health centers are in rural settings and underequipped to perform laboratory diagnosis. Despite several limitations of culture for *Bordetella* isolation, its importance in analyzing the spatiotemporal evolution of *Bordetella* within a surveillance system has been documented [23]. North–South collaborations should be reinforced to ensure external quality control, improve on the efficiency of laboratory diagnosis and train technicians to permit the implementation of adequate diagnostic measures in low-resource settings.

There was no evident association between the presence of *Bordetella* and the classical clinical symptoms of pertussis such as coughing paroxysms or inspiratory whoop. This suggests the non-specificity of symptoms associated with pertussis responsible in part for the underestimation of the disease frequency, aggravated by the absence of laboratory diagnosis and the passive awareness of health care personnel. It is important to note that coughs and colds are not specific signs, especially in Niger, where the dry and dusty desert winds could have aggravated their frequency during the study period.

In Niger, as in many African countries, the epidemiological situation of pertussis is poorly known, despite its status as a notifiable disease. In this study, 24 children fulfilled the WHO clinical criteria for notification to the surveillance system but not a single case was declared by the health personnel, signaling the need to strengthen surveillance. Wendelboe *et al*. [26] showed that close household contacts are responsible for 76–83% transmission of the disease to the vulnerable child. In the event of an identified suspected case, household members should be solicited to identify the source of transmission and be immunized and/or treated to prevent further spread. Catch-up campaigns of young adults may also be considered [2].

There were surprisingly more RT-PCR-positive *Bordetella* cases among the children who were completely vaccinated with the three primary doses (23) than in the incompletely vaccinated and unvaccinated children (11). Although this difference was not statistically significant, it was, however, unexpected, as all 23 presented proof of vaccination indicating that 22 children had been administered the pentavalent vaccine and one the DTP vaccine. The probable reasons for this finding could be linked to vaccine effectiveness or difference in circulating strains compared with vaccine strains or to vaccine practices such as cold-chain conditions and vaccine administration by health personnel. Studies in South African infants have documented lower antibody titers to pertussis in HIV-exposed uninfected than in HIV-unexposed uninfected infants, and that HIV-exposed uninfected infants demonstrated stronger pertussis vaccine responses than HIV-unexposed uninfected after routine vaccination [27, 28]. Information on the HIV status of the children and their mothers could probably help to explain the immune responses we observed, but this information is lacking in our study, as it was not a part of our objectives.

Overall, the low yield of *Bordetella* infections could indicate vaccine efficacy, as more than 80% of the study population had received at least one vaccine dose. More in-depth studies (i.e. age-specific prevalence

of *B. pertussis*-specific antibodies in cross-sectional studies) are therefore necessary to investigate the protective effect of vaccines used within the Niger population. The auditing of vaccine practices and laboratory techniques is also recommended.

This study presented certain limitations. The objective was centered on the detection of *B. pertussis* but only four specimens were positive. Comparative analyses could therefore not be limited to *B. pertussis* alone, but on all 34 positive cases. Further, an analysis of the circulating *B. pertussis* strains was not possible because only one isolate was obtained by culture. The fact that children were not recruited before antibiotic use rendered culture even more difficult. This frequent use of auto-medication observed in sub-Saharan Africa also needs to be addressed. Although we present the first data from Niger, data collection spanned 5 months and additional information is needed. Considering that respiratory infections are highly seasonal, more studies to investigate any geographical or seasonal variability are needed.

In conclusion, this study is the first to provide laboratory-confirmed data on pertussis in Niger. The estimated prevalence was 8.2 cases per 1000 children aged <5, but most of the cases were children aged <1 year. Clinical diagnosis of whooping cough is problematic because of the wide range of non-specific manifestations and variable awareness among clinicians and health care workers. The results from this study highlight the need to sensitize health care personnel to actively notify clinical pertussis cases. This would facilitate the collection of robust information for burden of disease estimates, which in the present context is not possible. Moreover, testing for *Bordetella* should not be limited to very specific clinical symptoms such as coughing paroxysms or inspiratory whoop. The sensitivity and specificity of laboratory tests, especially culture, can be influenced by the use of antibiotics early in the course of infection. This was the first time that laboratory diagnosis for *Bordetella* was performed in Niger. North–South collaborations should be reinforced to ensure external quality control, improve on the efficiency of laboratory diagnosis and train technicians to permit the implementation of adequate diagnostic measures in developing countries.

References

1. Plotkin S, Orenstein W, Offit P. Vaccines. 5th edn. Philadelphia: Saunders, 2008.
2. Guiso N, Liese J, Plotkin S. The global pertussis initiative: meeting report from the fourth regional roundtable meeting, France, April 14-15, 2010. Hum Vaccin 2011; 7:481–8.
3. Zouari A, Smaoui H, Njamkepo E, *et al*. La réémergence de la coqueluche en Tunisie. Med Mal Infect 2011;41:97–101.
4. World Health Organization. WHO vaccine-preventable diseases: monitoring system. Geneva: WHO, 2008.
5. WHO, UNICEF, World Bank. State of the World's vaccines and immunization. 3rd edn. Geneva, WHO, 2009.
6. Wymann MN, Richard JL, Vidondo B, *et al*. Prospective pertussis surveillance in Switzerland, 1990–2006. Vaccine 2011;29:2058–65.
7. Schellekens J, von König CH, Gardner P. Pertussis sources of infection and routes of transmission in the vaccination era. Pediatr Infect Dis J 2005;24:S19–24.
8. Wood N, Mclntyre P. Pertussis: review of epidemiology, diagnosis, management and prevention. Paediatir Respir Rev 2008;9:201–12.
9. Cherry JD, Grimprel E, Guiso N, *et al*. Defining pertussis epidemiology: clinical, microbiologic and serologic perspectives. Pediatr Infect Dis J 2005;24: S25–34.
10. Préziosi MP, Yam A, Wassilak SG, *et al*. Epidemiology of pertussis in a West African community before and after introduction of a widespread vaccination program. Am J Epidemiol 2002; 155:891–6.
11. Ag Bendech M, Diarra N, Bougoudogo F, *et al*. Epidemiologic aspects of whooping cough in a village of the central district of Kolokani in Mali. Bull Soc Pathol Exot 1992;85:397–9.
12. Hoppe JE, Rockenstiehl A, Hagedorn HJ, *et al*. Bacteriological and serological study of pertussis in Abeokuta, Nigeria. Trop Geogr Med 1992;44: 219–24.
13. Anonymous. Pertussis vaccines—WHO position paper. Wkly Epidemiol Rec 2005;80:31–9.
14. UNICEF. Overview on child survival. UNICEF-Niger, Factsheet. 2008.
15. Tchidjou KH, Vescio MF, Busani L, *et al*. Low vaccine coverage among HIV infected children in Niamey, Niger. Poster n 20100203 presented at ESCAIDE 2011.
16. Institut National de la Statistique (INS). Annuaire des statistiques sanitaires du Niger. Disponible in 2008. http://www.snis.cermes.net/donnees.php.
17. Kösters K, Riffelmann M, Wirsing von König CH. Evaluation of a real-time PCR assay for detection of *Bordetella pertussis* and *B. parapertussis* in clinical samples. J Med Microbiol 2001;50:436–40.
18. Probert WS, Ely J, Schrader K, *et al*. Identification and evaluation of new target sequences for specific detection of *Bordetella pertussis* by real-time PCR. J Clin Microbiol 2008;46:3228–31.
19. Fry NK, Tzivra O, Li YT, *et al*. Laboratory diagnosis of pertussis infections: the role of PCR and serology. J Med Microbiol 2004;53:519–25.
20. Tatti KM, Wu KH, Tondella ML, *et al*. Development and evaluation of dual-target real-time polymerase chain reaction assays to detect *Bordetella* spp. Diagn Microbiol Infect Dis 2008;61:264–72.
21. Riffelmann M, von König CH, Caro V, *et al*. Nucleic acid amplification tests for diagnosis of *Bordetella* infections. J Clin Microbiol 2005;43:4925–9.
22. Zouari A, Smaoui H, Brun D, *et al*. Prevalence of *Bordetella pertussis* and *Bordetella parapertussis* infections in Tunisian hospitalized infants: results of a 4-year prospective study. Diagn Microbiol Infect Dis 2012;72: 303–17.

23. André P, Caro V, Njamkepo E, *et al*. Comparison of serological and real-time PCR assays to diagnose *Bordetella pertussis* infection in 2007. J Clin Microbiol 2008;46:1672–7.
24. Loeffelholz M. Towards improved accuracy of Bordetella pertussis nucleic acid amplification tests. J Clin Microbiol 2012;50:2186–90.
25. Baughman AL, Bisgard KM, Cortese MM, *et al*. Utility of composite reference standards and latent class analysis in evaluating the clinical accuracy of diagnostic tests for pertussis. Clin Vaccine Immunol 2008;15: 106–14.
26. Wendelboe AM, Njamkepo E, Bourillon A, *et al*. Transmission of *Bordetella pertussis* to young infants. Pediatr Infect Dis J 2007;26:293–9.
27. Jones CE, Naidoo S, De Beer C, *et al*. Maternal HIV infection and antibody responses against vaccine-preventable diseases in uninfected infants. JAMA 2011; 305:576–84.
28. Reikie BA, Naidoo S, Ruck CE, *et al*. Antibody responses to vaccination among South African HIV-exposed and unexposed uninfected infants during the first 2 years of life. Clin Vaccine Immunol 2013;20:33–8.