PERTUSSIS IN EMILIA ROMAGNA REGION (ITALY): MICROBIOLOGICAL DIAGNOSIS

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INTRODUCTION

Whooping cough is a bacterial respiratory infection caused by *Bordetella pertussis* and it is a significant cause of childhood morbidity and mortality. Microbiological diagnosis of pertussis is difficult to obtain due to the variations in the specificity and sensitivity of the different microbiological methods. The ECDC is currently addressing the improvement of pertussis diagnosis for surveillance, outbreak detection and monitoring in order to assure quality and comparability of data. Since 2013, our laboratory has been the designated Reference Laboratory for pertussis microbiological diagnosis in the Emilia Romagna region (ER, Italy), where there is an overall high level of healthcare service.

AIM

We describe the data obtained by microbiological and serological diagnosis performed on suspected pertussis cases reported in the ER region during December 2013 to November 2015.

MATERIALS AND METHODS

Seventy-eight suspected pertussis cases (mean age: 4.5 years; range 5 days – 48 years) were reported (Tab. 1).
The microbiological diagnosis was performed as follows:

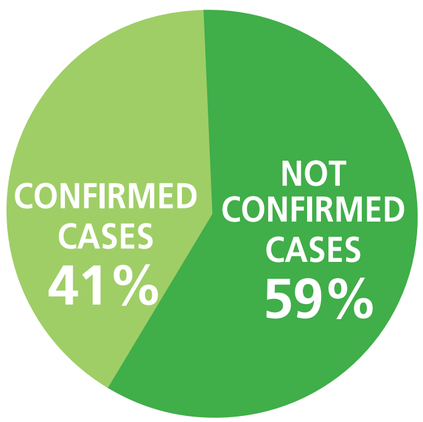
- in all cases the molecular diagnosis was carried out using commercial PCR method (BORDETELLA R-gene® bioMerieux and *illumigene*® Pertussis DNA Amplification Assay - Meridian);
- the culture test was performed in 54 (69.2%) cases;
- the serological test was performed in 32 (41%) cases using the IgG-PT and IgA-PT (NovaLisa, Bordetella pertussis toxin-ELISA, DiaSorin).

Table 1. Characteristics of population and samples examined

N. of suspected cases	**78**
Male	38 (48.7%)
Female	40 (51.3%)
Age mean	4.5 years
Vaccination status	
Vaccinated	32
1 dose	22
2 doses	4
3 doses	6
Not vaccinated	28
Unknown	18
N. of samples	**110**
Nasopharingeal aspirate	78
Serum	32

RESULTS

Figure 1. Confirmed cases (n=32)



Of the 78 cases, 32 (41%) resulted positive for at least one of the three microbiological methods used (Fig. 1).
The results obtained by molecular, culture and serological tests in the 78 suspected pertussis cases were summarized in Table 2.

Table 2. Microbiological results

N. of cases	Real time PCR	Culture test	ELISA test
6	+	+	/
6	+	/	/
6	+	-	-
6	+	-	+
4	-	-	+
4	-	/	+
12	-	-	-
20	-	-	/
14	-	/	/

+: positive; -: negative; /: not done; bold results boxed off in red: confirmed cases

The PCR method identified the most number of positive cases (24/32, 75%). The 8 cases (25%) resulted negative by PCR were confirmed as pertussis cases only by serological testing and 2 cases (25%) were diagnosed by IgA-PT detection. When the IgG-PT level does not allow a diagnostic interpretation, IgA-PT should be measured [1].
In the 8 cases, the median time between the onset of symptom and sampling was 28 days (range 24-33 days).

Table 3. Confirmed cases distributed by age group

	<1 year	1-10 years	>10 years
N. of cases	20*	10	2

*A fatal case of pertussis infection was reported in a 2 month old infant. After this episode (October 2015), we observed an increase of suspected cases reported.
Currently in our laboratory molecular diagnosis is carried out using the LAMP method (*illumigene*® Pertussis DNA Amplification Assay Meridian). Eighteen samples tested by PCR were retested with the LAMP method (Tab. 4).

Table 4. Comparison of results obtained by PCR vs LAMP methods

		PCR results		
		+	-	Total
LAMP results	+	15	0	15
	-	0	3	3
	Total	15	3	18

+: positive; -: negative

A good agreement (100%) was observed. Furthermore the LAMP method allows to test each specimen individually and in shorter time span than PCR testing (1h vs 3h)

CONCLUSIONS

Early microbiological diagnosis and treatment of pertussis might limit its spread to other susceptible people.
In order to assure a correct microbiological diagnosis for pertussis it is important that available diagnostic tests are used appropriately in relation to the timing of symptom onset.

REFERENCES

[1] Riffelmann et al. ECDC; 2012.
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