Detection of *Actinobacillus pleuropneumoniae* (App) serotype 1 in pigs by real-time quantitative PCR

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Introduction

- *Actinobacillus pleuropneumoniae* = swine pleuropneumonia
- Still clinically important in many countries
- Historically, serotypes 1, 5 and 7 mostly recovered from clinical outbreaks in Canada
- USA and Canada: sub-clinical infections
- In these two countries, $$ and effort:
  - To keep herds free from subclinical infection with virulent serotypes
  - To monitor absence of App in breeding herds
- Laboratory testing: key issue; main concern

Diagnosis of sub-clinically infected herds

- Serology
  - Excellent results in routine cases
  - Unexpected positive reactions are rarely observed; however: these reactions are difficult to interpret for breeder farms
  - Indirect diagnosis

- Detection of App in tonsils
  - Direct diagnosis of the presence of the pathogen
    - Isolation by standard culture
    - Isolation by magnetic beads
    - PCR

Detection of App in tonsils

- Isolation by standard culture
  - Fastidious; many contaminants
  - Poorly sensitivity
- Isolation by magnetic beads
  - Better sensitivity
  - Some contaminants are still present
  - Expensive and time consuming
- PCR
  - Good sensitity, mainly with after-culture samples
  - Fast
  - More difficult when done directly from tonsils

Available PCR for direct detection of App from tonsils

- Some conventional PCR tests for qualitative detection of App species directly from tonsils: results difficult to interpret
- One real-time PCR for direct quantitative detection of App species from tonsils
- For serotype 1, only conventional PCR tests (for CPS) to be used with pure cultures
  - not tested for direct App serotype 1 detection from tonsils

Goal of the project

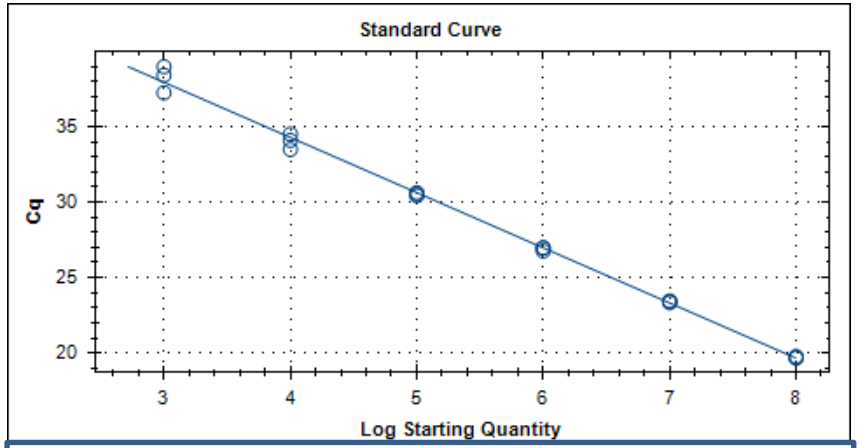
- Since no sensitive, fast and affordable test is available for detection of App serotype 1 in sub-clinically infected animals, the goal of this project was to develop a quantitative real-time PCR test for the direct detection of App serotype 1 in sub-clinically infected animals.

qPCR conditions

	LPS1-9-11 PCR	CPS1 PCR
Gene	ORF17 of LPS-O antigen biosynthesis region of serotypes 1, 9 and 11	CPS1c of capsular polysaccharide biosynthesis region
Product length	148 bp	200 bp
Primers	200 nM	200 nM
Probe	160 nM Cy5-BHQ1 (quencher)	200 nM FAM-BHQ1 (quencher)
Annealing Temperature	58°C	

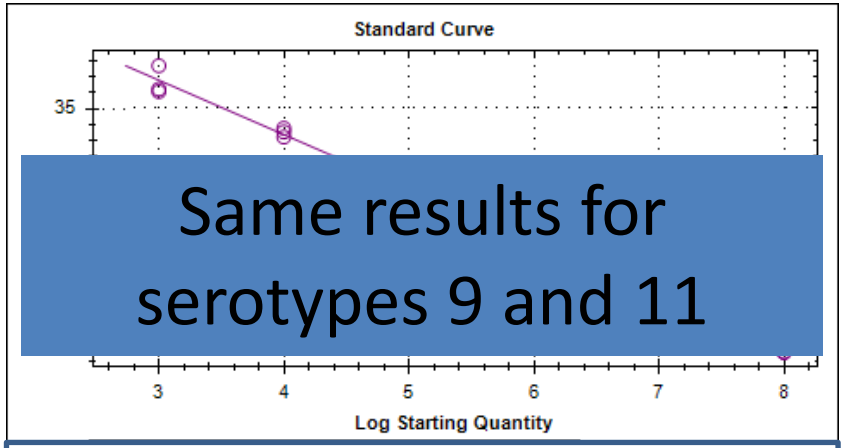
Efficiency

CPS1
with serotype 1



Efficiency: 87.7%; R^2: 0.996; slope: -3.656

LPS1-9-11
with serotype 1



Same results for serotypes 9 and 11

Efficiency: 95.4%; R^2: 0.997; slope: -3.437

Good primers and probes

Specificity

- Reference strains of App serotypes 1 to 15

CPS1
positive:
only serotype 1

LPS1-9-11
positive:
serotypes 1, 9 and 11

Other bacterial species:

- *Actinobacillus porcitonsillarum* (3)
- *Actinobacillus suis* (2)
-
-
-
- *Actinobacillus lignieresii (1)*
- *Actinobacillus porcinus* (1)

All negative with both PCR

Analytical sensitivity

PCR	Strain used	CFU/ PCR reaction	CFU/ml
CPS1	Serotype 1	6	1.6×10^3
LPS1-9-11	Serotype 1	6	1.6×10^3
	Serotype 9	0.6	1.4×10^2
	Serotype 11	0.7	1.8×10^2

Specificity: App field strains

232 strains tested

Isolated from 1990 to 2013 in Canada, USA, France, Brazil, Argentina and Mexico

Serotype	Number of strains tested	positive strains	
		LPS1-9-11	CPS1
1	45	40	45
2	4	0	0
3	14	0	0
4	13	0	0
5	30	0	0
6	9	0	0
7	35	0	0
8	38	0	0
9	2	2	0
12	12	0	0
13	13	0	0
15	15	0	0
NT	3	0	0

Atypical strains

- Strains isolated from herds seropositive for App serotype 7, but negative for App serotype 1.
- Tested with different mAb recognizing CPS and LPS of the serotypes 1 and 7.

Strains	CPS		LPS	
	Serotype 1	Serotype 7	Serotype 1	Serotype 7
Amy91-781f-26b	+	Neg	Neg	+
A05-1768-1	+	Neg	Neg	+
A04-1499	+	Neg	Neg	+
A08-013	+	Neg	Neg	+
A05-0339-1	+	Neg	Neg	+

Strains K1:O7 (CPS 1 and LPS 7)

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BRIEF COMMUNICATIONS

Atypical *Actinobacillus pleuropneumoniae* isolates that share antigenic determinants with both serotypes 1 and 7

M. Gottschalk, A. Lebrun, S. Lacouture, J. Harel, C. Forget, K. R. Mittal

Abstract. In the present study, the characterization of 3 atypical isolates of *Actinobacillus pleuropneumoniae* is presented. Two isolates (1B and 27E) showed positive reactions in coagglutination, immunodiffusion, and indirect hemagglutination tests for serotypes 1 and 7, whereas the third isolate (26B) reacted with antisera to serotypes 1, 4, and 7. These atypical isolates of *A. pleuropneumoniae* possessed a capsular polysaccharide (CPS) antigenically related to serotype 1 as well as an O-chain lipopolysaccharide antigenically related to serotype 7 or to serotypes 4 and 7, as shown by the use of monoclonal antibodies. Results of toxin profile and virulence assays for mice and pigs showed them to be more related to *A. pleuropneumoniae* serotype 7 field isolates. All 3 isolates induced antibodies mainly against serotype 7/4 O-long-chain lipopolysaccharide (LC-LPS) and, to a lesser extent, to the CPS of serotype 1, in experimentally infected pigs. Diagnostic laboratories that use a LC-LPS-based enzyme-linked immunosorbent assay (ELISA) for serodiagnosis of *A. pleuropneumoniae* infection in swine would probably diagnose herds infected with these atypical isolates as being infected by *A. pleuropneumoniae* serotypes 7 or 4, whereas those that use a CPS-based ELISA would probably consider them as infected by *A. pleuropneumoniae* serotype 1.

Sensitivity in tonsils

- DNA was extracted from suspensions of negative tonsil spiked with 10^7 to 10^3 CFU of serotype 1/ml.

PCR	CFU/ PCR reaction	CFU/0.1 g tonsil
CPS1	64	1×10^4
LPS1-9-11	6	1×10^3

Validation in tonsils

- Negative: herd serologically negative to all serotypes of App.

All the tonsils tested so far are negative

- Positive: herd with clinical signs of pleuropneumonia,
  - serotype 1 isolated in lungs

10 tonsils have been tested so far

CPS1: 6 positive tonsils

LPS1-9-11: 8 positive tonsils

$2X10^5$ to $2X10^8$ CFU/g of tonsil

Conclusion

- CPS1 PCR specific for serotype 1
- LPS1-9-11 PCR specific for serotypes 1, 9 and 11
- Both PCR have good sensitivity
- Both PCR can be used to detect quantitatively App serotype 1 in tonsils
- Used together and in combinaison with mAb, good tool for atypical strains.
- When validated, PCR LPS1-9-11, highly useful to be used in Europe or to test animals to be imported to Canada
  - Serotype 1 almost absent in Europe
  - Serotypes 9 and 11 absent in NA

Future work

- To complete the validation in tonsils
- To test LPS1-9-11 PCR with more strains of serotypes 9 and 11 (collaboration with AFSSA Ploufragan)
- To test both PCR with nasal and tonsil swabs as well as tonsil biopsies (samples from live animals)

  (collaboration with CReSA, Barcelona)

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