Some news on *Actinobacillus pleuropneumoniae*

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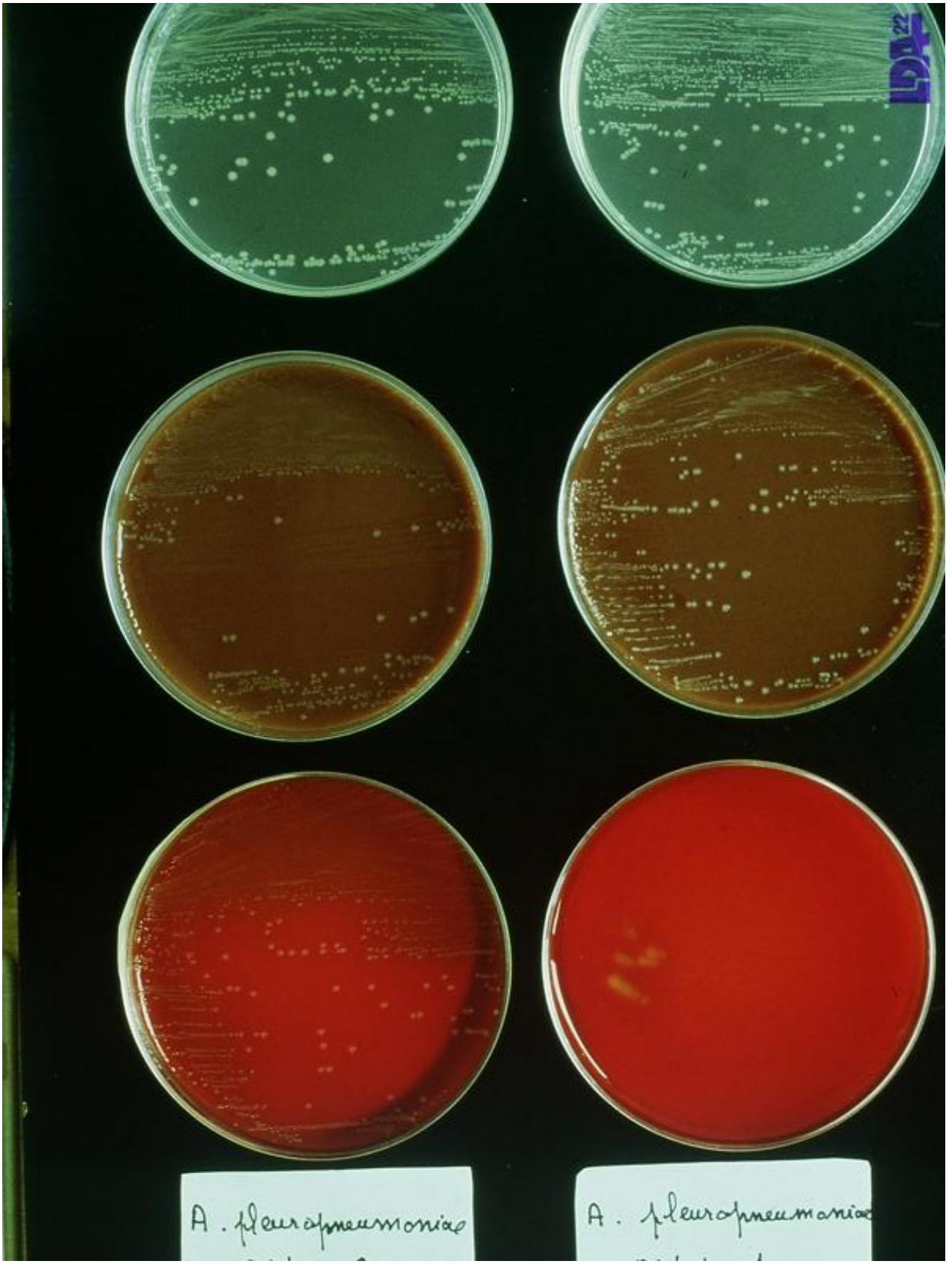
Actinobacillus pleuropneumoniae

- **Very well known disease**
- **Clinically important in different countries (Mexico, Brazil, France, Spain, Eastern Europe, Asia)**
- **Relatively well controlled in USA and Canada**
- **In these two countries, $$ and effort:**
  - **To keep herds free from subclinical infection**
  - **To monitor absence of App in herds**
- **High impact of diagnostic laboratories**

CAHLN-2013

NEWS ON APP:
BIOTYPES AND SEROTYPES


LDA 22
A. pleuropneumoniae
A. pleuropneumoniae

App: biotypes and serotypes

- **Biotype I**
  - «Typical» App
  - Best known serotypes: 1 to 12 and 15
  - Four toxins produced: ApxI, ApxII, ApxIII and Apxiv

- **Biotype II**
  - «Atypical» App, similar (bacteriology) to *A. suis*
  - Some serotypes are similar to biotype I App: 2, 4, 7
  - Serotypes 13 and 14 as described in Europe
  - These biotypes are either almost absent…or we are not doing a good job at the lab
  - Serotype 13 in Canada: biotype I (classical); cross-reactions in serotyping and serology with serotype 10

Different serotypes and biotypes of App in Canada (old table)

App serotype	Biotype	Presence in Canada	Serology available in NA
1	I	Yes	Yes
2	I and II	Only biotype I	Yes
3	I	Yes	Yes
4	I and II	Only biotype I*	Yes
5	I	Yes	Yes
6	I	Yes	Yes
7	I and II	Only biotype I	Yes
8	I	Yes	Yes
9	I and II	No	Yes
10	I	Yes	Yes
11	I	No	Yes
12	I	Yes	Yes
13	I and II	Only biotype I	Yes
14	II	No	Yes**
15	I	Yes	Yes

*only from healthy pigs

**not validated in the field

App: serotypes present in Canada

- **Based on what I presented the last table???**
- **Isolation (confirmed)?**
- **Isolation (oral/written, very old reports)?**
- **Data from laboratories in Canada and USA using very different techniques?**
- **A little bit of everything…**

Serotypes detected in the ELISA test in a Canadian context: serology

- **No (important) cross reactions**
  - 1, 2, 5, 12
- **Old known cross-reactions**
  - 4 and 7; 3, 6 and 8
- **Recently described cross-reactions**
  - 3/6/8 and 15
  - 10 and 13 (North American strains)

Serotypes detected in a Canadian context: serotyping

- **Serological techniques good enough for serotypes 1, 2, 5 and 7**
- **With problems, we may be able to identify serotypes 4 (monoclonal antibodies) and 12**
- **Very difficult to differentiate serotypes 10 and 13**
- **We cannot differentiate serotypes 3, 6 and 8**
- **Very difficult to differentiate serotypes 3/6/8 and 15**
- **We routinely receive strains from ISU that could not be serotyped by them**

Different serotypes and biotypes of App in Canada

App serotype	Biotype	Presence in Canada	Serology available in NA
1	I	Yes	Yes
2	I and II	Only biotype I	Yes
3	I	?	Yes
4	I and II	Only biotype I*	Yes
5	I	Yes	Yes
6	I	?	Yes
7	I and II	Only biotype I	Yes
8	I	?	Yes
9	I and II	No	Yes
10	I	?	Yes
11	I	No	Yes
12	I	Yes	Yes
13	I and II	Only biotype I	Yes
14	II	No	Yes**
15	I	?	Yes

*only from healthy pigs

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Different serotypes and biotypes of App in Canada (2013)

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2	I and II	Only biotype I	Yes
3	I	?	Yes
4	I and II	Only biotype I*	Yes
5	I	Yes	Yes
6	I	?	Yes
7	I and II	Only biotype I	Yes
8	I	?	Yes
9	I and II	No	Yes
10	I	?	Yes
11	I	No	Yes
12	I	Yes	Yes
13	I and II	Only biotype I	Yes
14	II	No	Yes**
15	I	?	Yes

*only from healthy pigs

**not validated in the field

App: serotype 10

- « Originally » described as one of the most virulent serotypes
- Experimental infections with the reference strains: we could not reproduce disease; others did
- Almost no report from natural clinical cases worldwide
- We could not find any strain belonging to this serotype in Canada (we looked in all boxes…)
- Strains of this serotype have not been isolated in Minnesota and Iowa (or at least, they could not find any strain)
- Availability of anti-serotype 13 (from Canadian/US) origin is relatively recent: we did know now that this serotype cross-react with serotype 10
- I wonder if we have ever had serotype 10 in North America

Different serotypes and biotypes of App in Canada (old table)

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1	I	Yes	Yes
2	I and II	Only biotype I	Yes
3	I	?	Yes
4	I and II	Only biotype I*	Yes
5	I	Yes	Yes
6	I	?	Yes
7	I and II	Only biotype I	Yes
8	I	?	Yes
9	I and II	No	Yes
10	I	?	Yes
11	I	No	Yes
12	I	Yes	Yes
13	I and II	Only biotype I	Yes
14	II	No	Yes**
15	I	?	Yes

*only from healthy pigs

**not validated in the field

App: serotypes 3-6-8-15

- **Arrival of PCR to differentiate serotypes 3-6-8**
- **Serotype 3 usually considered as a low virulent serotype with the exception of the UK**
- **So, they decided to really verify if the have a high prevalence of serotype 3…**
- **Serotyping vs PCR**
- **Results:**

App: serotyping in the UK

	Percentage of isolates from diseased animals
Serovar	**Immunological**
2	6
3	51
6	3
7	8
8	30
12	1.5

App: serotyping in the UK

	Percentage of isolates from diseased animals	
Serotype	**Immunological**	**PCR**
2	6	3
3	51	1.5
6	3	3
7	8	9
8	30	82
12	1.5	1.5

App: serotypes 3-6-8-15

- **Strains isolated in Canada and USA during the last 5 years**
- **PCR 3-6-8**
- **If strong positive for anti-App 15 (in addition to 3, 6, and/or 8) but negative by PCR: considered as serotype 15**

App 3, 6, 8 or 15: North America

	Strains isolated in Canada or USA	
Serotype	**Number of strains**	**Percentage**
3	1	1%
6	11	13,5%
8	57	69.5%
15*	13	16%

*Strains that strongly reacted with anti-serotype 15 (and presented some reactions to serotypes 3, 6 or 8), but negative by the 3-6-8 PCR

Different serotypes and biotypes of App in Canada (2013)

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1	I	Yes	Yes
2	I and II	Only biotype I	Yes
3	I	Yes (+/-)	Yes
4	I and II	Only biotype I*	Yes
5	I	Yes	Yes
6	I	Yes (+)	Yes
7	I and II	Only biotype I	Yes
8	I	Yes (+++)	Yes
9	I and II	No	Yes
10	I	No	Yes
11	I	No	Yes
12	I	Yes	Yes
13	I and II	Only biotype I	Yes
14	II	No or ?	Yes**
15	I	Yes (+)	Yes

*only from healthy pigs

**not validated in the field

Which serotype is the most « prevalent » in our country?

- There are two different types of prevalence for App
  - Serotypes most frequently isolated from diseased pigs (mostly virulent serotypes)
  - Serotypes most frequently present in swine herds (independently of the presence of the disease): usually (not always) low virulent serotypes
    - Serology (usually)
    - PCR (less common) from tonsils
  - Not necessarilly the same serotypes

App from clinical cases (2011-2013*)

	85 Strains received at our laboratory	
Serotype	**Number of strains**	**Percentage**
1	3	3,5 %
2	4	5 %
5	**35**	**41 %**
6	1	1 %
7	**26**	**30,5 %**
8	9	10,5 %
12	7	8 %

*Only 4 months of 2013

App: Prevalence by serology

- It is influenced by the distribution of different (low virulent?) serotypes that subclinically infect conventional herds
- Attention: it is also influenced by the « **infectivity** » of the serotype
  - High infectivity: serotypes 3 ,6, 8,15; 12
  - Low infectivity: serotypes 1, 5, 10, 13
- Testing 20 samples of a subclinically infected herd may present clear positive results if it is a serotype 12, but negative results if it is a serotype 5
- Prevalences from serological studies should be taken with caution

App: Prevalence by serology

- It is influenced also by the serological test used

Test	Sensitivity	Specificity
CFT	46 %	90 %
LPS-ELISA	74 %	100 %
ApxI/Tbp2 ELISA	13 %	100 %
ApxIV ELISA	13 %	100 %

*Opriessnig, Gottschalk et al., 2012

App from healthy animals (serology)

Serotype	Percentage
1	2 %
2	4%
5	6 %
3/6/8/15	**15 %**
7	**26%**
12	**17 %**

MacInnes et al. 2008

Comparison of different tests with sera from vaccinated animals (bacterin)*

Test	Sensitivity
CFT	40 %
LPS-ELISA	50 %
ApxI/Tnb ELISA	0 %
ApxIV ELISA	0 %

*Opriessnig et al., 2012

App: present/future work

- Serotype 14: present in Canada?
- Developping quantitative real-time PCR for direct detection of serotype-specific App from tonsils
- Detection of App from live clinically healthy animals (tonsils)
  - Biopsies, swabs, brushes, etc.
- Characterization of untypable strains
- Serology in oral fluid (collaboration with JZ)
- Development of a PCR for serotype 15
- Development of an ELISA test for the detection of antibodies against *Mycoplasma hyopneumoniae*

Streptococcus suis

- Still one of the most important bacterial swine pathogen worldwide
- 35 different serotypes
- Difficult to control
- No effective vaccine available
- Serotype 2: Important zoonosis in some part of the world
  - Some cases described in Canada and USA
  - Many in Europe
  - A lot in Asia

Distribution (%) of the 7 serotypes of *S. suis* most frequently recovered from diseased in Canada and USA (2011) between 2007 and 2012

Capsular type	USA	2007	2008	2009	2010	2011	2012
2	17	**17**	**25**	**13**	**11**	**18**	**13**
1/2	8	5	8	11	6	9	6
3	20	12	14	14	13	10	10
4	9	7	6	5	4	5	6
7	13	5	5	5	4	4	2
8	11	7	5	9	8	8	5
22	2	3	2	4	7	7	3
NT	4	16	18	14	20	17	23

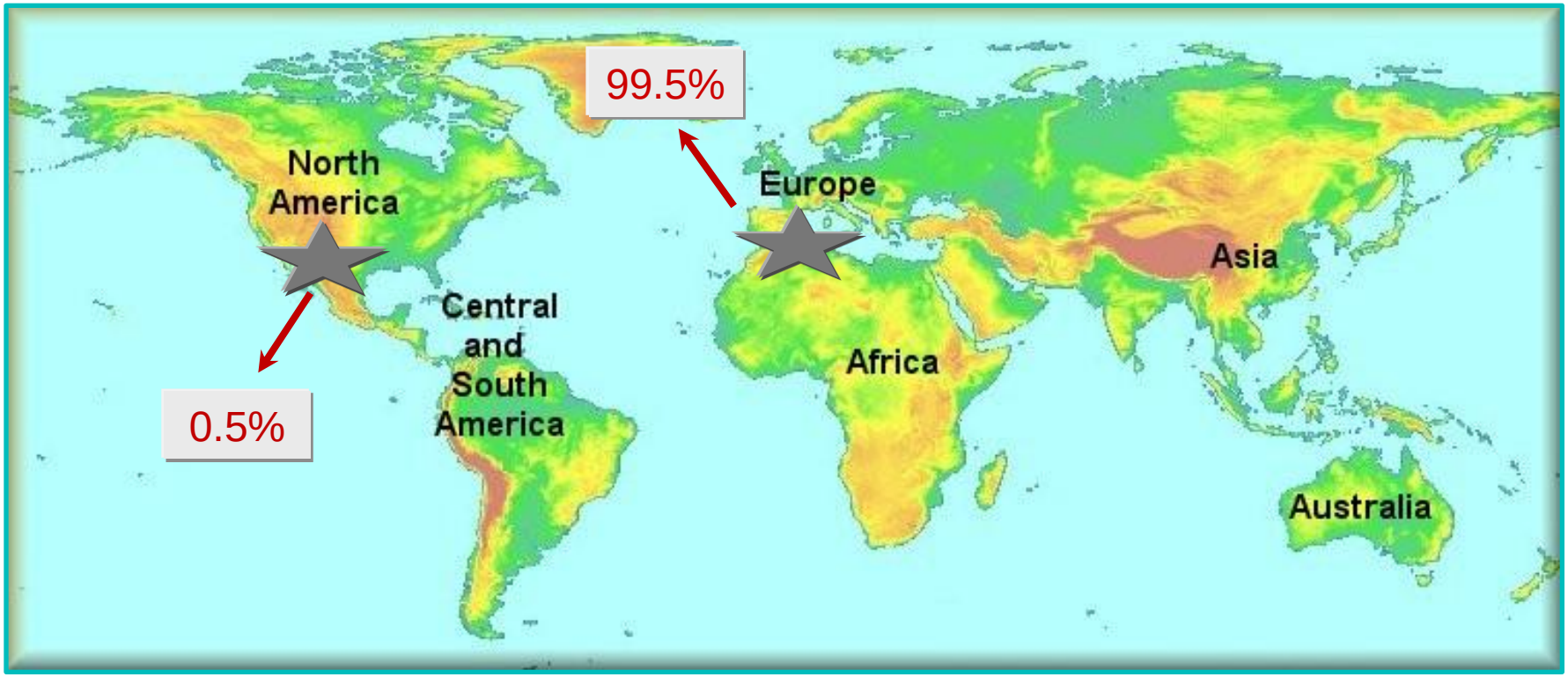
Distribution of *S. suis* serotype 2 in different countries

Country	Serotype 2 from clinical cases
France	70%
Spain	51%
Italy	31%
Netherlands/Belgium	36 to 49%
UK	35%
Brazil	39%
Canada/USA	**<20%**

Virulence of European and North American strains of *S. suis* serotype 2

	Strains		
	Virulent France	**Virulent Canada**	**Non virulent**
Fever	+++	+++	-
Locomotor problems	+++	+++	-
Nervous symptoms	+++	+	-
Mortality	+++	+	-
Meningitis	+++	+	-
Arthritis	+++	+++	-
Bacterial isolation (blood)	++	+++	+/-

S. suis: human disease (up to 2005)



Rembember: less cases in pigs due to serotype 2 in NA

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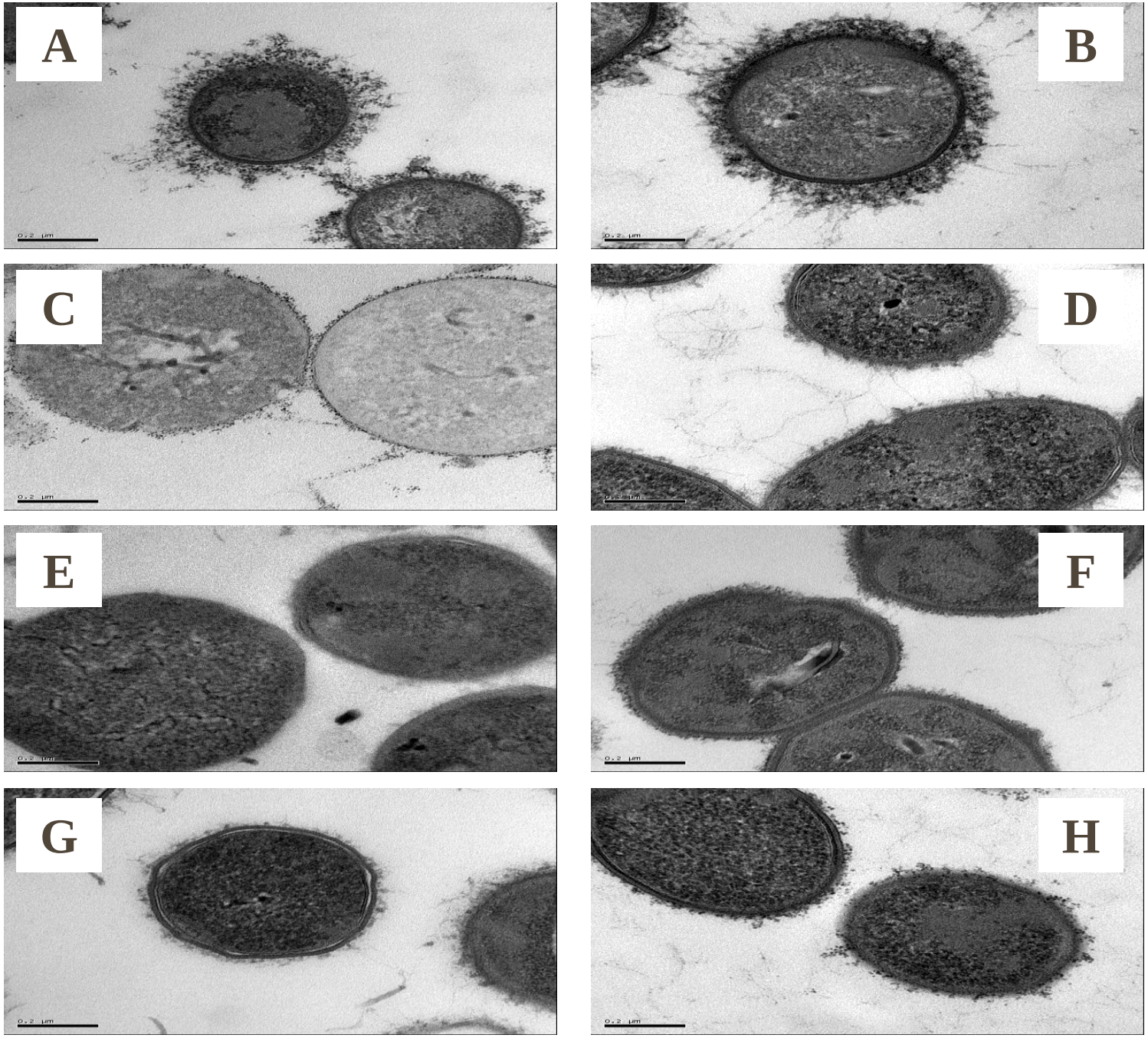
2	17	17	25	13	11	18	13
1/2	8	5	8	11	6	9	6
3	20	12	14	14	13	10	10
4	9	7	6	5	4	5	6
7	13	5	5	5	4	4	2
8	11	7	5	9	8	8	5
22	2	3	2	4	7	7	3
NT	4	16	18	14	20	17	23

Non typable strains

- Are these *S. suis*?
  - 99% of strains genetically confirmed as *S. suis*
- New serotypes?
- Non encapsulated strains?
  - Newly non described serotypes
  - Already known serotypes but with no capsule
- We performed hydrophobicity studies (to suggest the presence or not of a capsule) and electron microscopy
- Results


A
B
C
D
E
F
G
H

Non typable strains

- They are *S. suis*
- Most of them are non encapsulated
- We are developping with a Japanese team multiple PCR for complete serotyping of 35 serotypes of *S. suis*
- These strains will be tested…
- In the past: considered as non encapsulated = non virulent
- Recent results indicate an important role in endocarditis
- More studies to come

S. suis: present/future work (partial list)

- Characterization of non typable strains
- Characterization of Canadian serotype 2 strains
- Co-infection studies
  - *S. suis*/PRRSV
  - *S. suis*/SIV
  - *S. suis/M. hyorhinis*
- Multiple PCR for serotyping
- More basic research studies (collaboration with China)
- Vaccine candidates: we have many...so far, it seems that it is not interesting for the Canadian Swine Health Board and Swine Innovation Pork

Acknowledgements

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