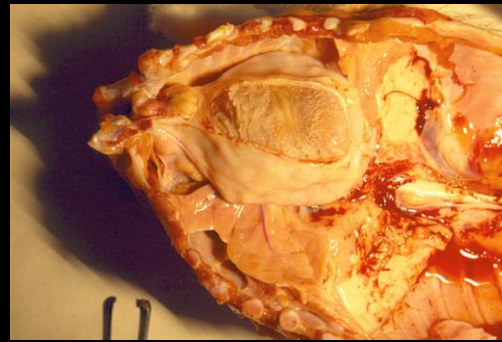


Haemophilus parasuis

G. Barigazzi



- *Glaesser's disease*
- *Grande diffusione*
- *Caratteristiche del batterio*
- *Prima descrizione 1910 (Glaesser)*



- Piccolo bacillo Gram -
- Chiamato *H. parasuis* negli anni '70
- Genere Pasteurellacee
- Classificazione biochimica ancora incerta
- Grande variabilità della specie

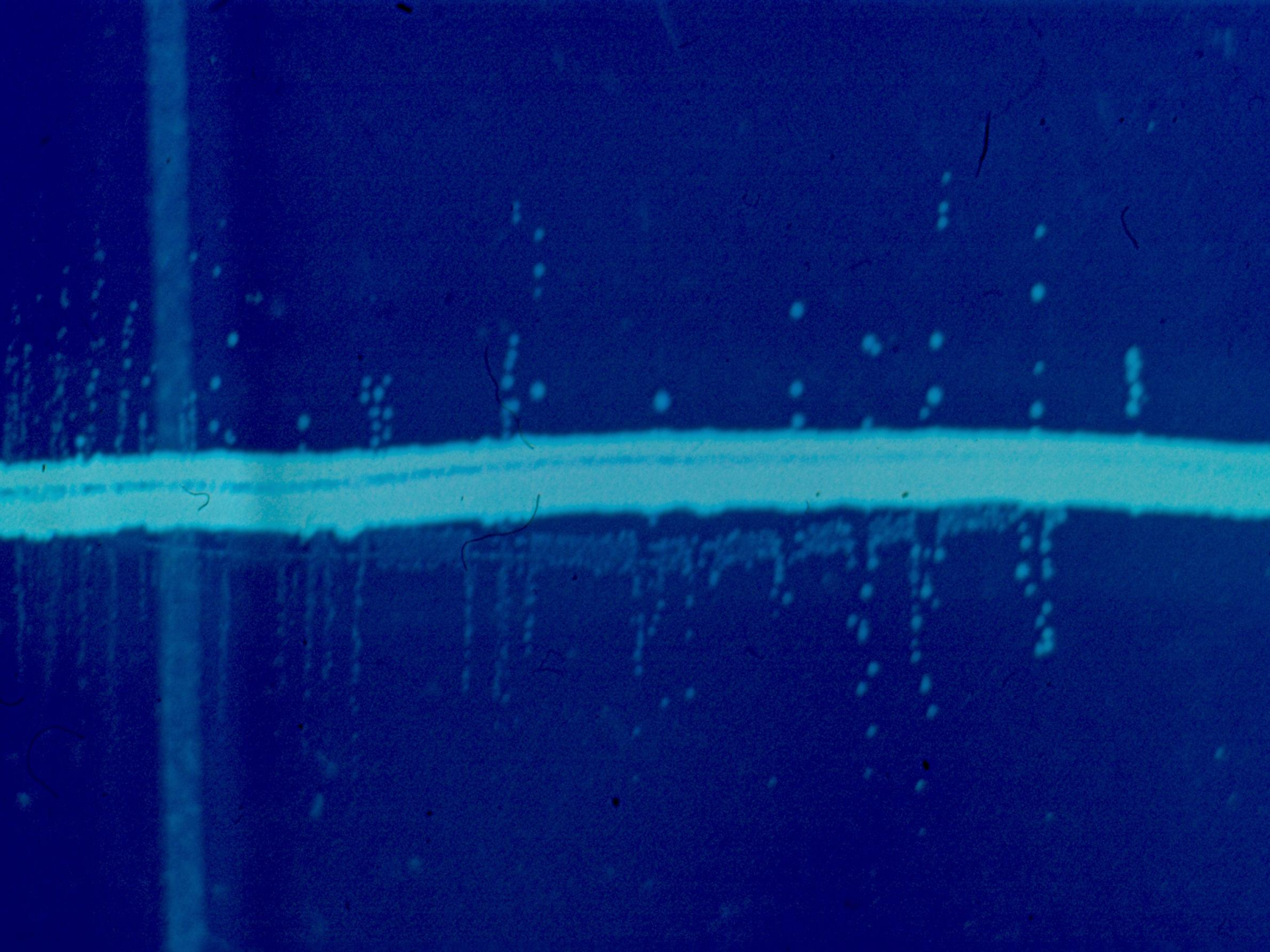
Table 40.3. Differential biochemical reactions of swine NAD-dependent *Pasteurellaceae*

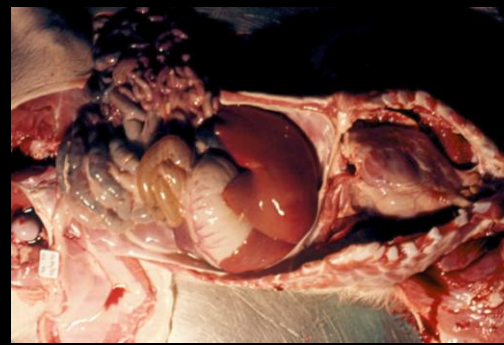
Biochemical Characteristic	Other NAD-dependent <i>Pasteurellaceae</i>					
	<i>Haemophilus parasuis</i>	<i>Actinobacillus pleuropneumoniae</i>	<i>Actinobacillus minor</i>	<i>Haemophilus Taxon C</i>	<i>Actinobacillus porcinus</i>	<i>Actinobacillus indolicus</i>
Urease	-	+	+	-	-	-
Hemolysis	-	+	-	-	-	-
Indole	-	-	-	-	-	+
Fermentation of						
Glucose	+	+	+	+	±	+
Lactose	-	-	+	-	±	±
Sucrose	+	+	+	+	±	+
Mannitol	-	+	-	-	±	±
Xylose	-	+	±	-	±	±
L-Arabinose	-	-	-	+	±	-
Raffinose	-	-	+	+	±	+

Sources: Møller and Kilian 1990; Rapp-Gabrielson and Gabrielson 1992; Møller et al. 1996; Kielstein et al. 2001.

Note: *A. minor* was formerly known as *Haemophilus* taxon "Minor Group"; *A. porcinus* was formerly known as *Haemophilus* sp. taxons D or E; *A. indolicus* was formerly known as *Haemophilus* sp. taxon F. Taxon C has infrequently been isolated from swine.

Key: + indicates greater than 90% of isolates are positive; -, less than 10% of isolates positive; ±, variable reactions among isolates.





- Difficoltà di crescita/identificazione in laboratorio
- Dipendenza dal NAD (crescita satellitare)
- Diversità sierologica (15 sierotipi)
- Lipopolisaccaride della capsula (fattore di virulenza?)

Designation of 15 Serovars of *Haemophilus parasuis* on the Basis of Immunodiffusion Using Heat-Stable Antigen Extracts†

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Received 23 August 1991/Accepted 24 January 1992

Previous independent investigations of the serotyping of *Haemophilus parasuis* strains have led to the designation of serovars A to D, 1 to 7, Jena 6 to Jena 12, and ND1 to ND5. Heat-stable antigen preparations from the reference strains for these serovars were tested by immunodiffusion with rabbit hyperimmune antisera. The existence of 15 distinct serologic groups was apparent, for which we propose the designations serovars 1 to 15. Examination of 290 field isolates from swine in the former German Democratic Republic indicated a prevalence of serovars 4 and 5, which together accounted for 41% of the isolates examined. However, 26.2% of the isolates were nontypeable with this test procedure and available antisera. Intraperitoneal inoculation of specific-pathogen-free pigs with cells representing the 15 serovars indicated differences in virulence which may be serovar related. Cells of strains representing serovars 1, 5, 10, 12, 13, and 14 were the most virulent, causing death or moribundity in inoculated pigs. Cells of serovars 2, 4, 8, and 15 caused polyserositis, but not death, in inoculated pigs. However, inoculation of cells of strains representing serovars 3, 6, 7, 9, and 11 resulted in no clinical symptoms or lesions indicative of *H. parasuis* infection.

TABLE 2. Virulence of strains representing *H. parasuis* serovars 1 to 15 in primary SPF swine

Serovar	No. of strains tested		No. of pigs inoculated ^a	Virulence ^b
	Reference	Field		
1	1	1	5	++
2	1	5	15	+ ^c
3	1	1	6	0
4	1	2	7	+ ^d
5	1	1	18	++ ^e
6	1	0	3	0
7	1	0	3	0
8	1	0	3	±
9	0	2	6	0
10	1	1	6	++
11	0	2	6	0
12	1	1	6	++
13	1	0	3	++
14	1	0	3	++
15	1	0	3	+

^a Pigs were inoculated intraperitoneally with an 18-h broth culture containing approximately 5×10^8 CFU.

^b Virulence was scored as follows: ++, death of pigs within 96 h postinoculation; +, clinical symptoms and systemic gross lesions of polyserositis and arthritis at necropsy; ±, mild clinical symptoms or gross lesions at necropsy; 0, no clinical symptoms or gross lesions at necropsy.

^c Five of the six strains tested were virulent, and one (Bakos strain A9) was nonvirulent.

^d Reference strain SW124 was mildly virulent (±).

^e Reference strain Nagasaki was moderately virulent (+).

Table 40.1. Prevalence of *Haemophilus parasuis* serovars

<i>H. parasuis</i> serovar	Percent Frequency									
	Japan 1990 ^b	Canada & USA1992	Germany 1992	Germany 1998	USA 2003	Canada & USA 2004 ^c	Australia 1996,2000	Spain 1999	Spain 2003 ^c	Denmark 2004
1	3	2	4	7	7	3	1	3	9	1
2	6	8	6	11	4	8	6	9	6	2
4	9	16	17	11	39	27	7	16	20	13
5	14	23	24	9	2	15	36	18	23	36
7 or 10 ^a	—	5	5	4	2	11	5	5	11	3
12	—	7	3	6	7	8	4	3	9	3
13	—	11	5	4	1	13	13	8	3	21
14	—	9	2	0	3	3	0	3	2	1
3,6,8,9,11 or 15	—	4	10	17	8	2	3	6	11	6
Nontypeable	68	14	26	31	27	10	28	29	8	15

Sources: Morikoshi et al. 1990; Rapp-Gabrielson and Gabrielson 1992; Kielstein and Rapp-Gabrielson 1992; Blackall et al. 1996; Kielstein and Wuthe 1998; Rúbies et al. 1999; Rafiee and Blackall 2000; del Rio et al. 2003; Oliveira et al. 2003; Tadjine et al. 2004a; Angen et al. 2004.

^aDifferences between the type strains and field isolates for serovars 7 and 10 have been reported and these serovars cannot always be distinguished by ID (Rapp-Gabrielson 1995, unpublished; Blackall et al. 1996; Rafiee and Blackall 2000; Tadjine et al. 2004).

^bOnly tested for *H. parasuis* serovars 1-7.

^cTyped by Indirect Hemagglutination (IHA).

Sierotipo	Totali	%
1	40	4,0
2	66	6,6
4	175	17,4
5	200	19,9
7	51	5,1
12	50	5,0
13	79	7,8
14	23	2,3
3,6,8,9,11,15	67	6,7
NT	256	25,4
TOT	1007	100,0

Table 40.2. Virulence of strains representing *H. parasuis* serovars for SPF swine

<i>H. parasuis</i> Serovar	No. of Strains Evaluated	Virulence ^a
1, 5, 10, 12, 13, 14	10	Death within 96 hours
2, 4, 15	10	Severe polyserositis and arthritis at necropsy
8	1	Mild clinical signs and gross lesions
3, 6, 7, 9, 11	8	No clinical signs or gross lesions

Source: Kielstein and Rapp-Gabrielson 1992.

^aSwine inoculated intraperitoneally with 5×10^8 colony-forming units.

Table 1. Microscopic lesions in pigs inoculated with *H. parasuis*. Pigs from the control group did not have lesions.

Pig no.	Broncho- pneumonia	Polyserositis				Catharral rhinitis
		Pleuritis	Pericarditis	Peritonitis	Meningitis	
1	-	+	-	-	-	-
2	+	+	+	+	+	-
3	-	-	-	-	-	-
4	+	+	-	-	+	+
5	-	+	-	-	-	-
6	-	-	-	-	-	-
7	+	+	-	-	+	-
8	-	+	-	+	-	-
9	+	-	-	-	+	-
10	+	+	+	+	+	-
Total no. affected pigs	5	7	2	3	5	1

+ = presence of lesion; - = absence of lesion.

Experimental reproduction of *Haemophilus parasuis* infection in swine: clinical, bacteriologic, and morphologic findings

John L. Vahle, Joseph S. Haynes, John J. Andrews

Experimental *H. parasuis* infection in swine

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Table 1. Bacteriologic findings in pigs inoculated with *Haemophilus parasuis*.*

Pig no.	Hours postinoculation	Necropsy specimens†											
		B1	Na	To	Tr	Lu	Pc	Pl	Pt	Jt	Mn	Li	Sp
1	12	0	+	0	0	0	0	0	0	0	0	0	0
2	12	0	+	0	+	0	0	0	0	0	0	0	0
3	36	+	+	0	0	0	0	0	0	+	0	0	0
4	36	+	0	0	0	0	0	+	+	0	0	+	+
5	84	0	0	0	0	0	0	0	0	+	0	0	0
6	84	0	+	0	+	0	0	+	+	+	+	0	0
7	108	0	0	0	0	0	0	0	0	+	0	0	0
8	108	0	0	0	0	0	0	0	0	+	0	0	0

*+ = Isolation of *H. parasuis*; 0 = Negative culture.

± B1 = blood culture; Na = nasal turbinate swab; To = tonsil; Tr = tracheal swab; Lu = lungs; Pc = pericardial swab; Pl = pleural swab; Pt = peritoneal swab; Jt = joint swab; Mn = meningeal swab; Li = liver; Sp = spleen.



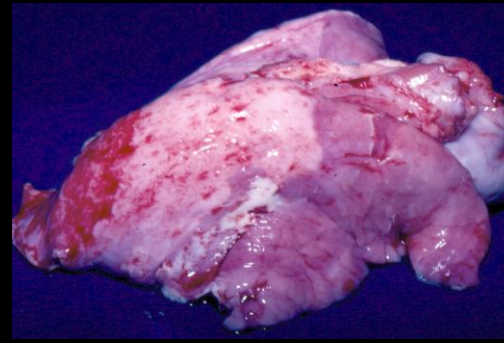
- La > parte degli studi negli anni 2000
- PCR
- EAI
- C'è ancora molto da studiare
- E da capire

Table 2. Immunohistochemical results for tissues from pigs inoculated with *H. parasuis*. Tissues from noninoculated control pigs were negative.

Pig no.	Meninges	Lung	Pleura	Pericardium	Liver serosa	Spleen serosa	Peritoneum	Tonsil
1	-	+	+	-	-	+	+	-
2	+	+	+	+	+	+	+	+
3	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+
5	-	-	+	-	-	-	+	+
6	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	-
8	-	+	-	-	-	-	-	+
9	+	+	-	-	-	-	-	+
10	+	+	+	+	-	+	+	-
Total no. affected pigs	5	7	6	4	3	5	6	5

* Immunolabeled cells (monocytes) inside vessels.

† Immunolabeled cells were macrophagelike cells associated with lymphoid tissue of spleen.



- Suino: solo ospite naturale
- Apparato respiratorio
- Ospite comune delle cavità nasali
- Allevamento:
 - ◆ convenzionale
 - ◆ elevata qualità sanitaria
- **Diverse manifestazioni patologiche**



- **Allevamento convenzionale**
 - ◆ Presenza di infezione e anticorpi
 - ◆ Malattia "condizionata"
 - ◆ Forma subacuta/cronica = polisierosite
- **Allevamento elevata qualità sanitaria**
 - ◆ No infezione, no anticorpi
 - ◆ Malattia sistemica (setticemia)
 - ◆ Promiscuità animali
 - ◆ Grave quadro clinico



- Coinvolgimento nella PRDC
 - ◆ Agente predisponente
 - ◆ Agente secondario
 - ◆ Agente primario
- Ruolo (ri)emergente
 - ◆ PRRS
 - ◆ *M. hyopneumoniae*
 - ◆ Influenza
 - ◆ PCV2



- Diversità tra gli isolati (naso/sistemici)
- Associazione sierotipo/polisierosite
- Differenze di virulenza
- Interazioni LPSS-sierotipo-fenotipo-virulenza
- Nuove possibilità con le biotecnologie
- Rapporto tra clinica e sierotipo



- Ruolo determinante immunità materna
 - ◆ Infezione sperimentale solo in SG
- Colonizzazione apparato respiratorio
 - ◆ No tonsille
- Polisierosite
- Polmonite
- Setticemia

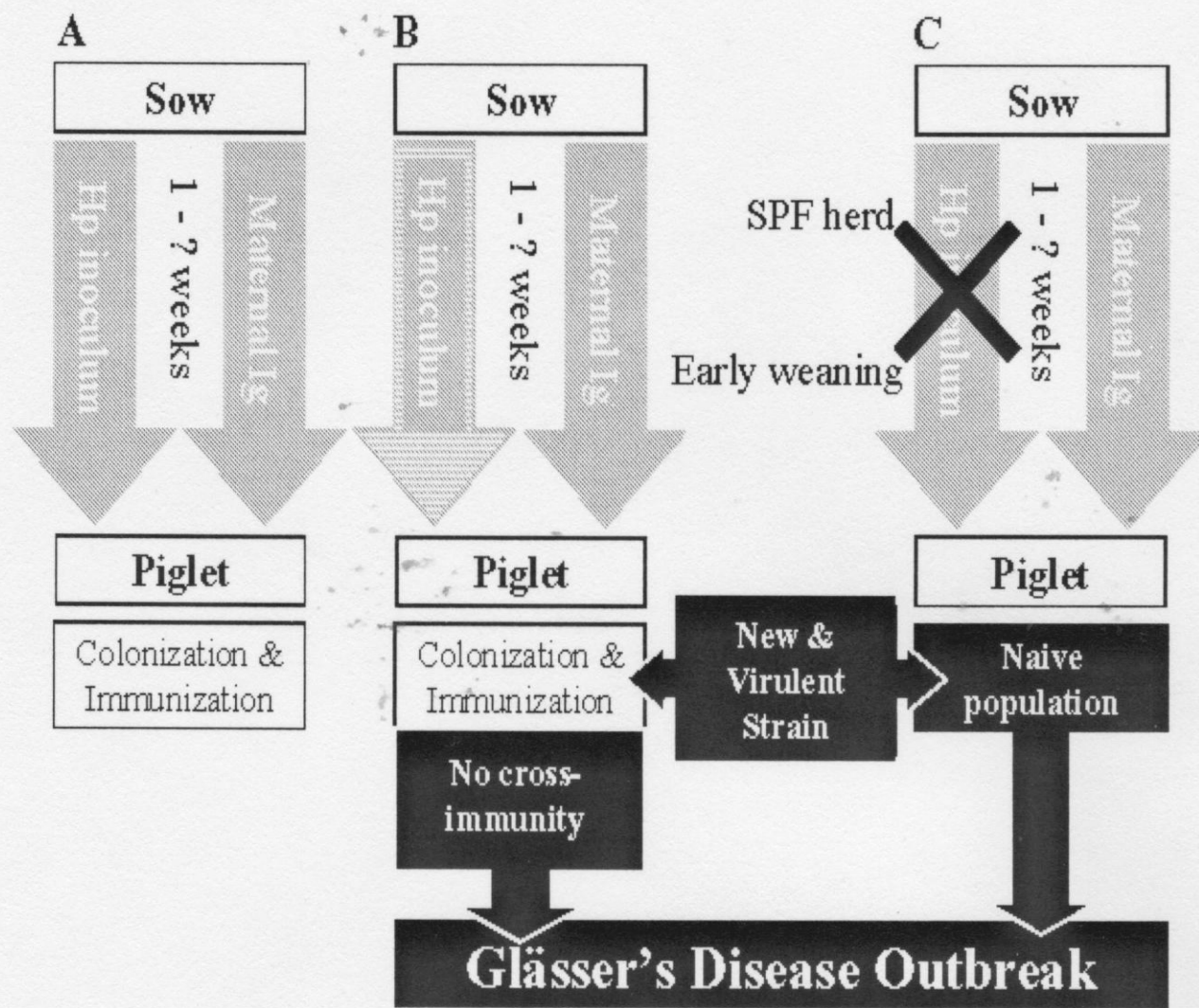


Fig 1. Diagram of the relationship between: colonization, natural immunity and Glässer disease outcome. **A.** Equilibrium between colonization and immunity acquisition. **B.** Disease outcome due to the entry of a new strain. **C.** Disease outcome due to the elimination of the bacteria from the population and the subsequent introduction of a virulent strain

CLINICA

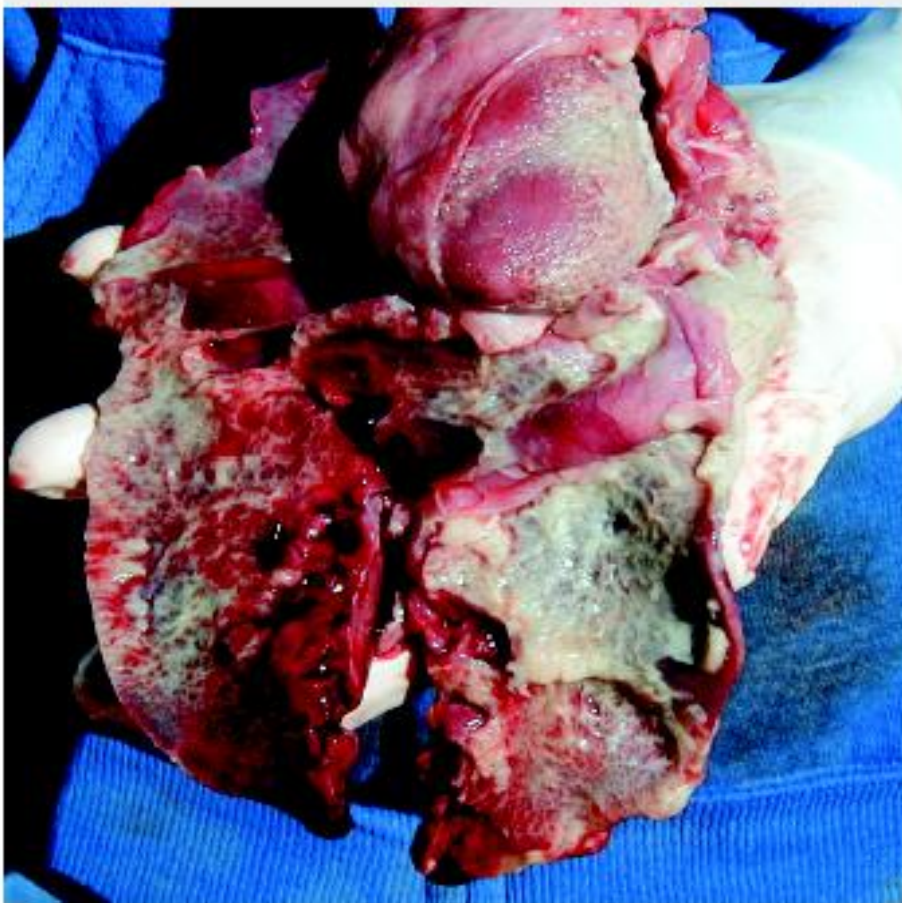
- **Allevamento convenzionale**
 - ◆ Tosse, dispnea, scolo nasale, anoressia, zoppia, perdita di peso, pelo ispido, edema sottocutaneo, scarti, mortalità anche improvvisa
- **Allevamento elevata qualità sanitaria**
 - ◆ Segni di setticemia; febbre apatia, inappetenza, dispnea, cianosi, mortalità improvvisa

LESIONI

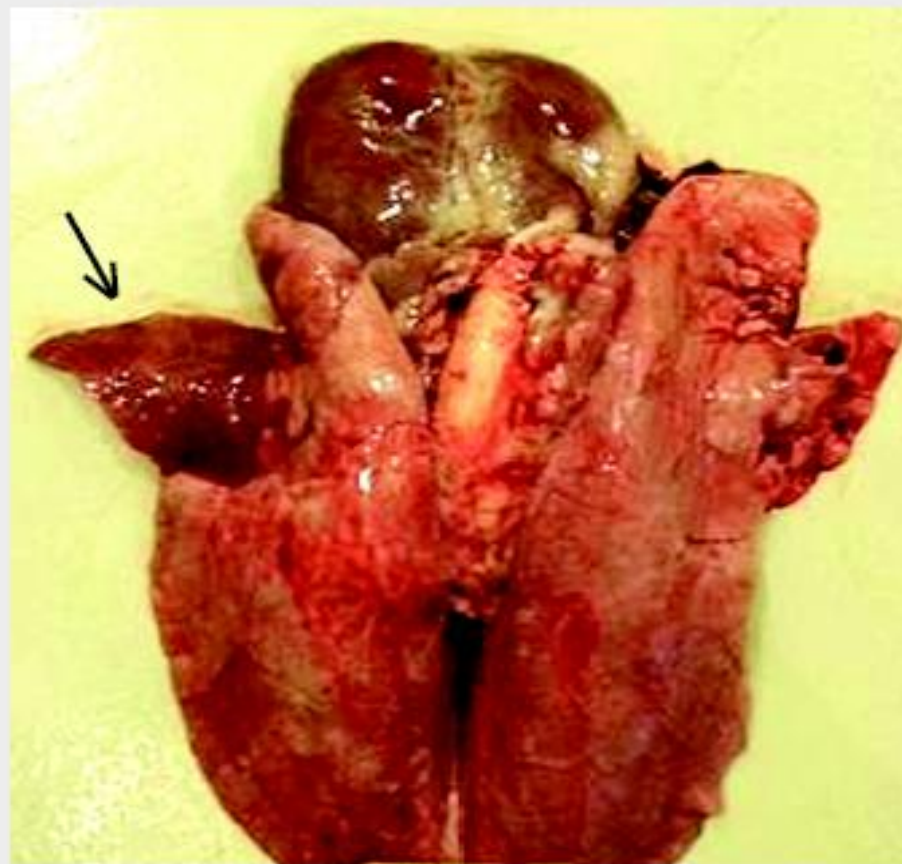
- Polisierosite siero-fibrinosa (fibrinosa)
 - ◆ Pleurite, pericardite, peritonite, artrite, POLMONITE
- Non sempre evidenti
 - ◆ Edema polmonare
 - ◆ Congestione meningeae
 - ◆ Necrosi epatiche
 - ◆ Splenomegalia
 - ◆ Meningite

Figure 1: Systemic infection with *Haemophilus parasuis* is usually characterized by development of fibrinous polyserositis. However, some animals may develop only pneumonia. A: Fibrinous pleuritis and pericarditis in a field case; B: Pneumonia (arrow) after experimental infection.

A



B



DIAGNOSI

- Anamnesi ++
- Clinica +
- Anatomia patologica +++
- Batteriologica ++++
 - ◆ Non facile
 - ◆ Tecniche PCR

DIAGNOSI

- Necroscopica
 - ◆ Animale vivo
- Numerosità del campione
- Invio al laboratorio
 - ◆ Difficoltà del laboratorio

DIAGNOSI DIFFERENZIALE

- Streptococcus suis
- Mal Rosso
- Salmonella
- E. coli
- Altri agenti infettivi
 - ◆ PRRS
 - ◆ Influenza
 - ◆ PCV2

TRATTAMENTO

- Profilassi e matafilassi
 - ◆ Poco successo
- Trattamenti i. m.
 - ◆ Penicilline
 - ◆ Cefalosporine
 - ◆ Florfenicolo
 - ◆ Chinoloni
- Buona sensibilità agli antibatterici

Antibiotici: attività % nei confronti di *H. parasuis* (K.B.)

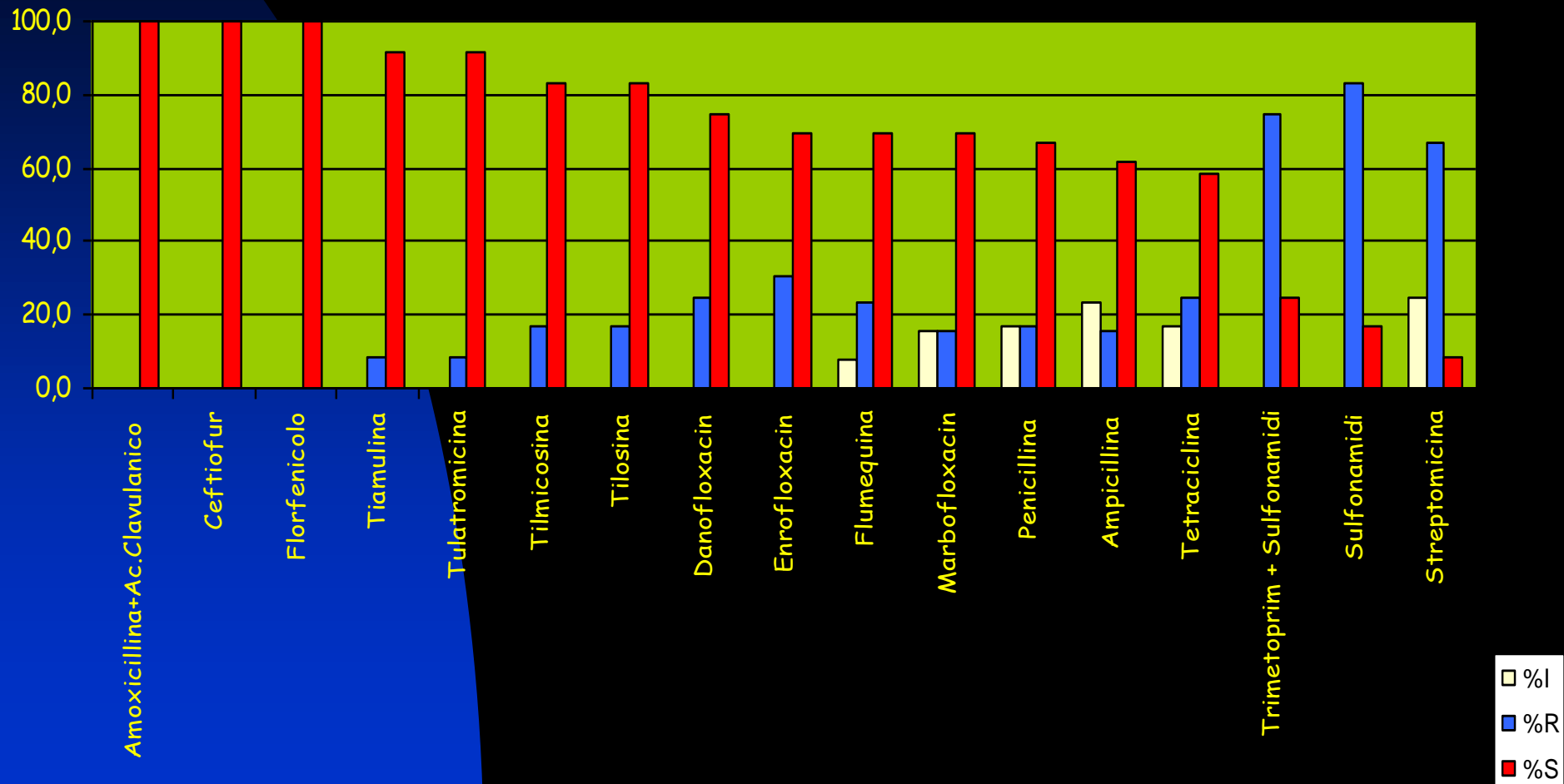
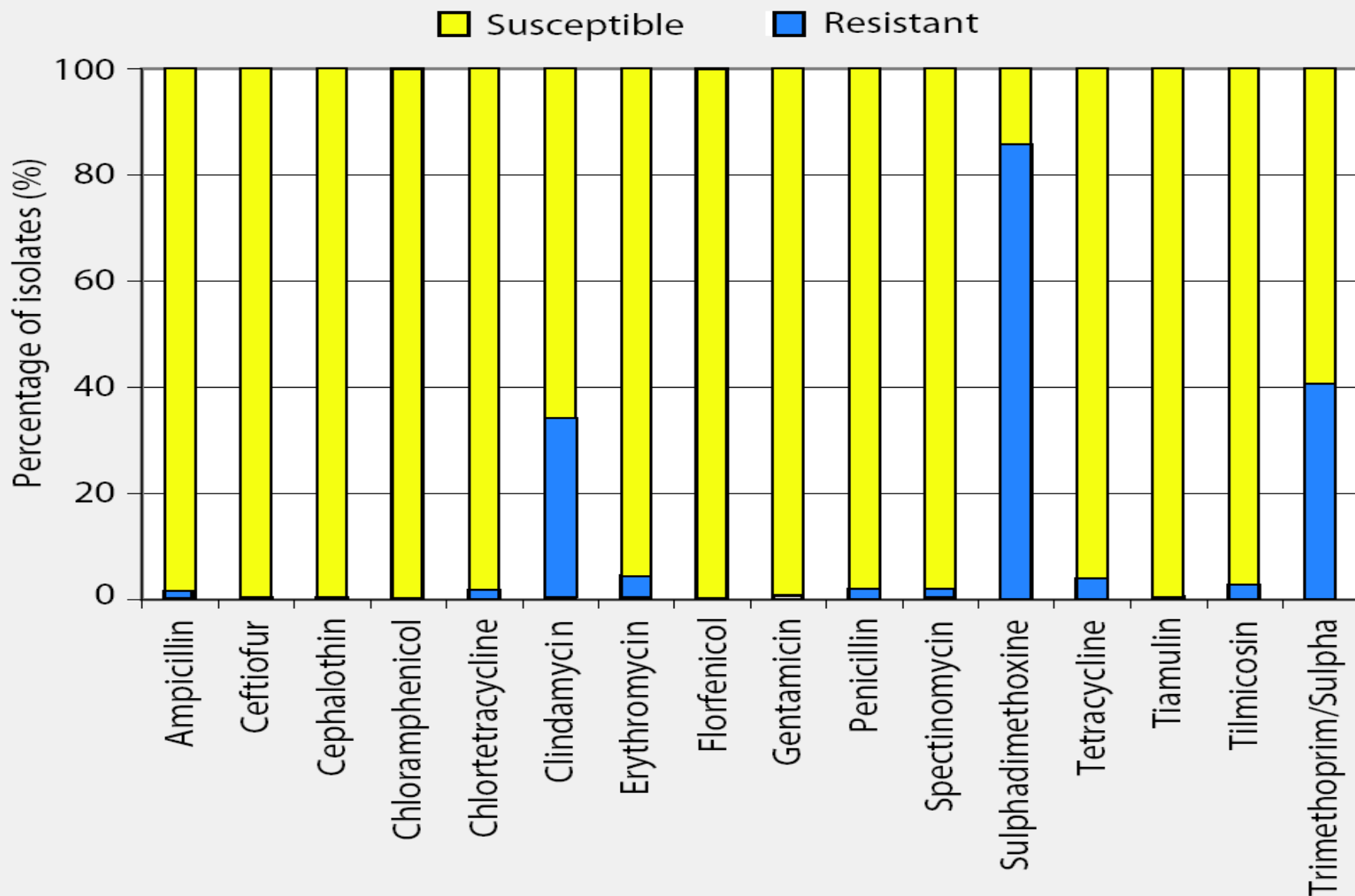


Figure 1: *Haemophilus parasuis* antimicrobial susceptibility profiles obtained at the University of Minnesota Veterinary Diagnostic Laboratory during the fiscal year of 2006.



PROFILASSI

■ Norme di conduzione

- ◆ Soggetti di dubbia provenienza (portatori sani)
- ◆ «Tutto pieno-tutto vuoto», integrato dalla pulizia e disinfezione delle strutture e delle attrezzature
- ◆ Mantenere un elevato standard igienico e sanitario in allevamento e nelle manipolazioni.
- ◆ Evitare i fattori stressanti e immunodepressivi

Conclusioni

- H. parasuis realtà importante
- Molto diffuso negli allevamenti
- Al di là dell'evidenza?
- Patogeno complesso
- Patogeno di un complesso
- Patogeno primario

Domani

- Glaesser 1910!!
- Stregua di altri patogeni
- Allevamento elevato standard sanitario
- Pig flow
- Vaccinazione
- Glaesser 1910

FINE

Grazie per l'attenzione