The virulence factors of *Escherichia coli* isolated from uterus could be used to predict metritis in the postpartum dairy cow.

by
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Introduction

Definition

Metritis:

- Severe inflammation of all layers of the uterine mucosa.
- Occurs between the 2nd and 4th week postpartum

Consequences: Decreased milk yield ($)

Decreased reproductive performances

Diagnosis approach

Observation of clinical signs

- a fetid, watery, red-brown uterine discharge,
- 2 to 3 weeks after calving

Severe cases:

- signs of systemic illness (fever > 39°C, dullness....)

These clinical signs are the work of the bacteria which are present in the uterus

Palmer(2003)

Bacterial species

Table 1: Bacterial species most frequently isolated from metritis cases

Bacterial species	%
Fusobacterium necrophorum *Prevotella melaninogenicus*	80
Trueperella pyogenes	65
Escherichia coli	36

(*Huszencza et al., 1999, Dohmen et al., 2000; Földi et al., 2006)*

- *E coli* may facilitate the development of other bacterial species
- Together they act synergistically to induce the clinical signs of metritis

Problematic

- When clinical signs appear, bacteria have already caused enough damage so that treatment will not eliminate the losses in milk and reproduction

Possible solution

Improve early diagnostic method

This will help farmers to:

- Identify cows predisposed to develop metritis
- Anticipate a preventive treatment
- Limit the losses in milk and reproduction

Such a method could be based on bacteriology

Hypothesis

The bacterial species present in uterus of the cow in the 1st week postpartum and the *E. coli* virulence factors may help to predict the risk of development of metritis during the 2^{nd}, 3^{rd} and 4^{th} week post-partum.

Overall objective

Presence intra-uterine

- *T. pyogenes*
- *F. necrophorum*
- *P. melaninogenica*
- *E. coli*
  - *virulence genes*

1st week post-partum

Association

?

Expression of clinical signs of metritis

2nd , 3rd and 4th weeks post-partum

Specific objectives

Step 1

- **Determine, in the first week postpartum, the intrauterine prevalence of:**

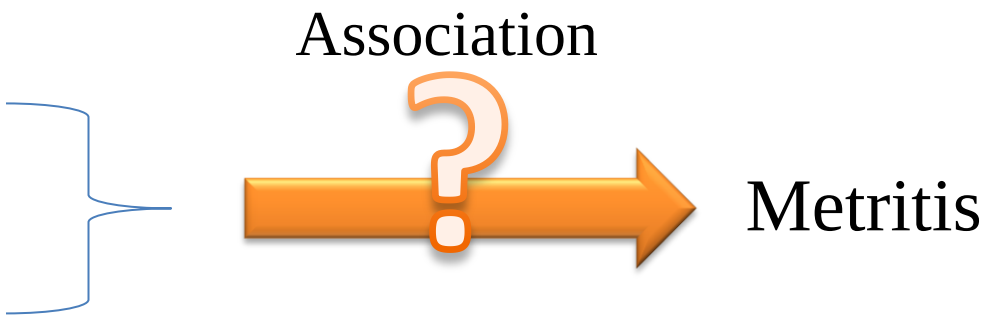
  1- different bacterial species (*T. pyogenes*, *F. necrophorum*, *P. Melaninogenicus*, *and E. coli*)

  2- virulence genes of *E. coli*

Step 2

- **Determine associations with metritis**

- bacterial species
- virulence genes



Materials and Methods

Experimental design

- Study period: November 2011 – August 2012
- Sample size: 497 postpartum Holstein cows from 6 farms

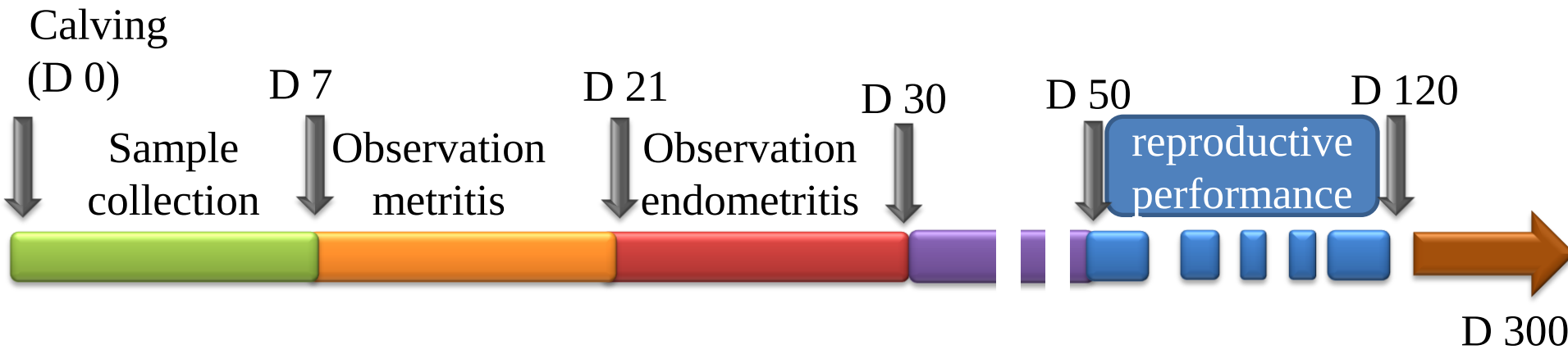


Figure 1: Chronology of the project activities

Step 1: Identification of bacterial species present in samples

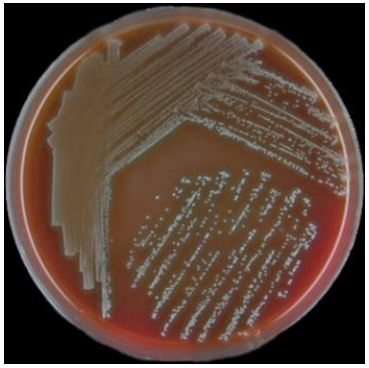
E. coli

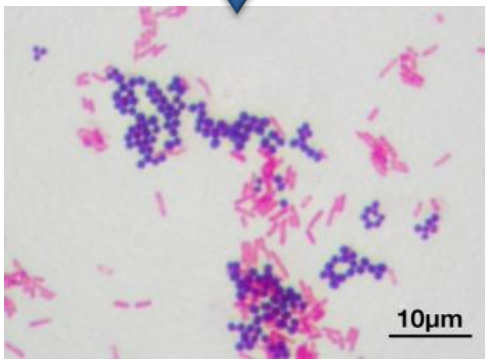
Lactose-positive colonies (pink) on Maconkey

5 isolates of *E. coli*

Tested for virulence genes

T. pyogenes





Gram positive, coryneform rods catalase negative

F. necrophorum
P. melaninogenica

Aero	Anae	API 20A
-	+	*F. necrophorum* *P. melaninogenica*

Figure 2 : Bacterial culture

Step 1: Identify the virulence genes of *E. coli*

Method used: radioactive probe hybridization (32P)

Table 2: Virulence genes tested (40)

Virulence genes	Function
fimH, papC, tsh, ibeA, aidA, bmaE, f17, afa, afaD8, paa, f5, eae	Colonisation and adhesion -Fimbriae - adhesins
sitA, iucD, iutA, fepC, iroN, irp1, irp2, fyuA	Iron capture - Siderophores
hlyA, cdtB, cnf1, cnf2, stx1, stx2, LT, sta, stb, cvaC, hlyE	Production of toxins
iss, kpsMTII	Bacterial resistance - Bacterial capsule
malX, neuC, hra1, cs31A, usp	Diverse functions

Methodology

Step 2: Determine associations with metritis

- **bacterial species**
- **virulence genes of *E. coli***

Association

Metritis

pearson’s Chi-square

- Alpha 5%

RESULTS

Results

Step 1: Identification of bacterial species present in samples

Table 3: Prevalence of the bacterial species isolated from the cow uterus in the first week pospartum

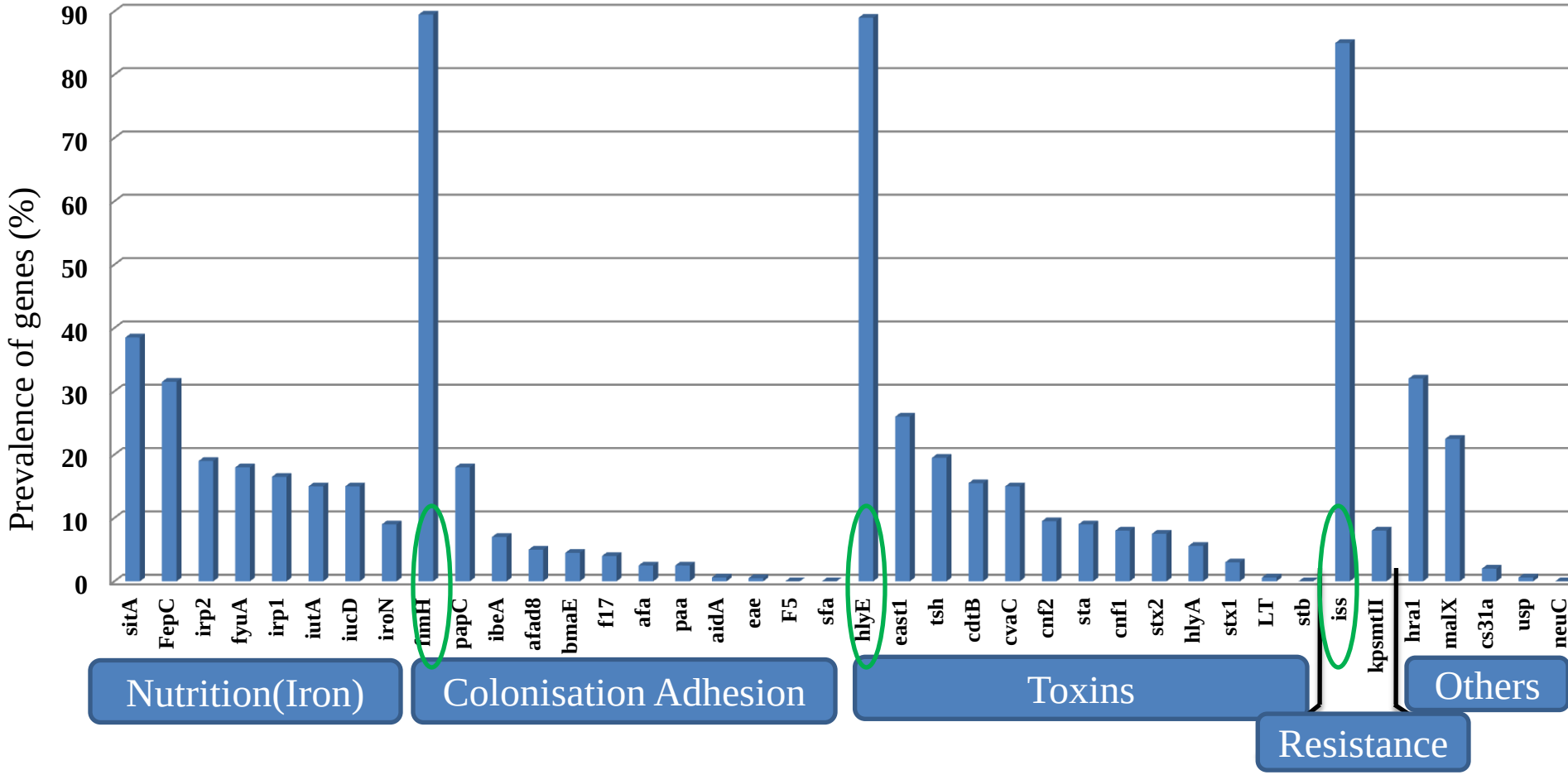
Farm	Samples				Metritis	
	n	positive for (%)			n	%
		E. coli	*T. pyogenes*	*F. necrophorum*		
F1	178	88 (49)	69 (39)	9 (5)	40	22
F2	149	78 (52)	44 (29)	4 (2,6)	16	11
F3	137	75 (55)	49 (36)	3 (2,5)	8	6
F4	12	7 (58)	3 (25)	0	1	8
F5	12	11 (91)	4 (33)	0	4	33
F6	9	3 (33)	1 (11)	0	0	0
Total	497	261 (52)	149 (30)	16 (3)	69	14

- Werner et al, 2012, Germany: at D10 PP, *E. coli* (25%) > *T. p* (10%)

Results

Step 1: Identify the virulence genes of *E. coli*

Figure 3: Prevalence of the virulence genes of *E. coli* isolated from the cow uterus in the first week pospartum

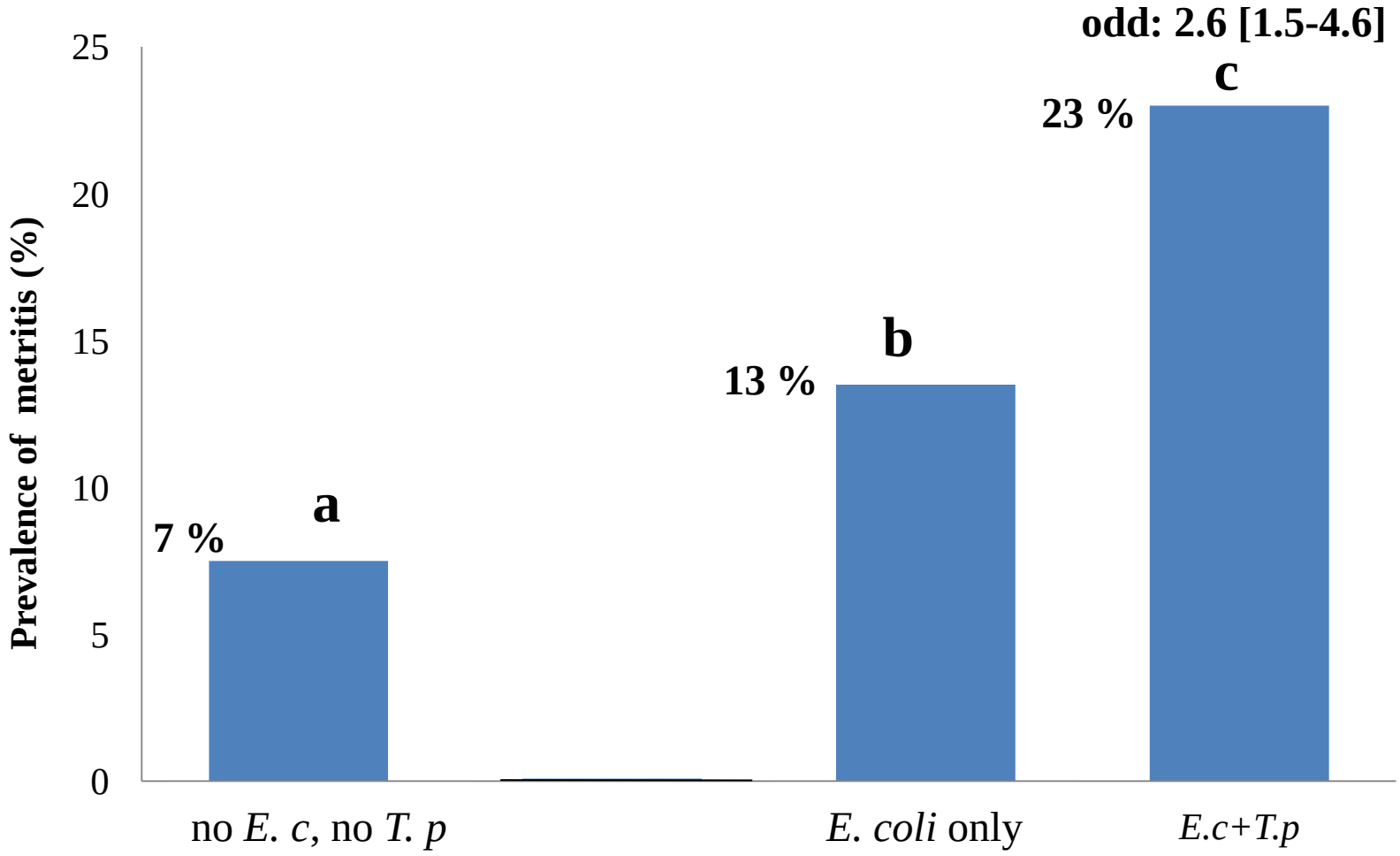


- Bicalho (2010), Silva (2009) *fimH*, *hlyE*, : most prevalent genes

Results

Step 2: Association between bacterial species and metritis

Figure 4: Prevalence of metritis in relation to the presence and absence of bacterial species

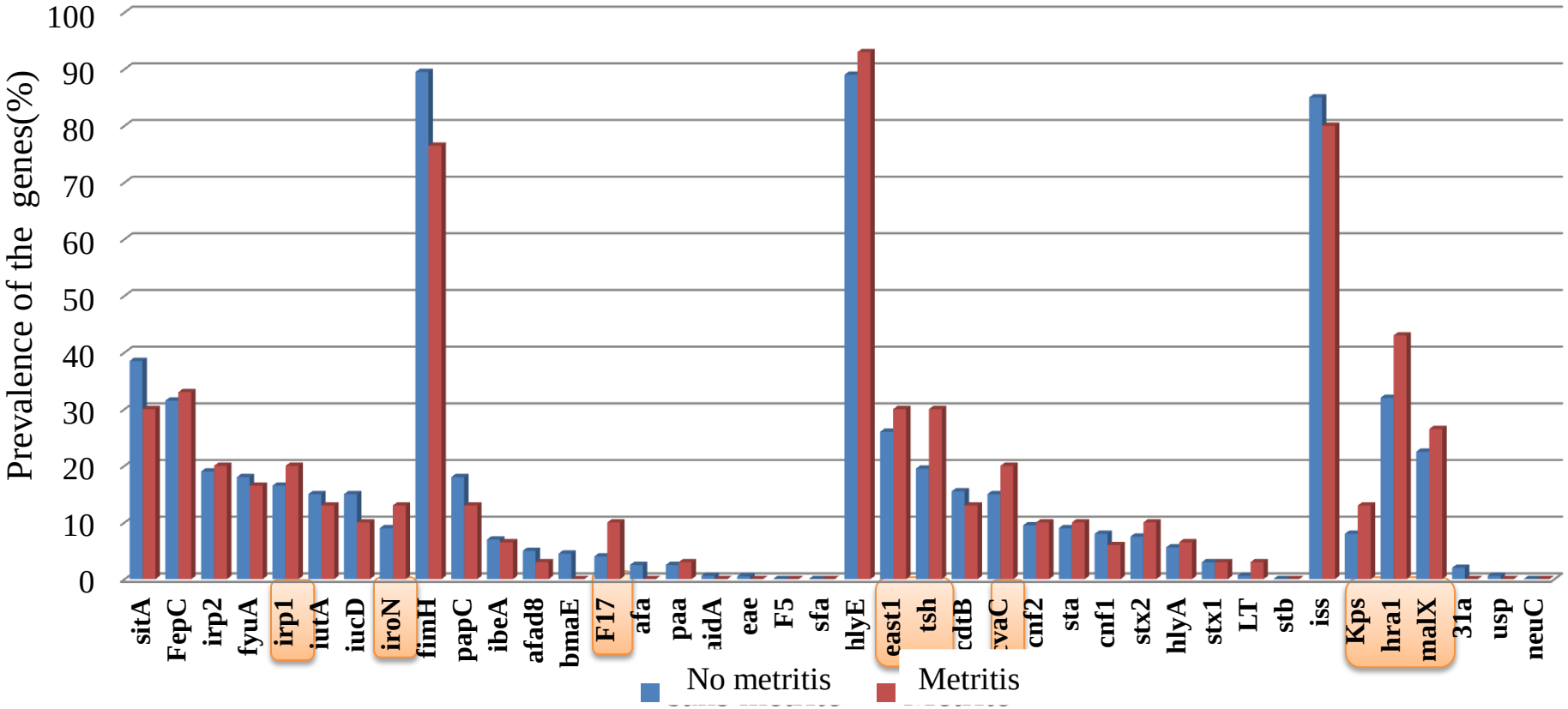


- The simultaneous presence of *E. coli* and *T. pyogenes* in the uterus of cows in the 1st week postpartum increases 2.6 times the risk of development of metritis in the weeks that follow.

Results

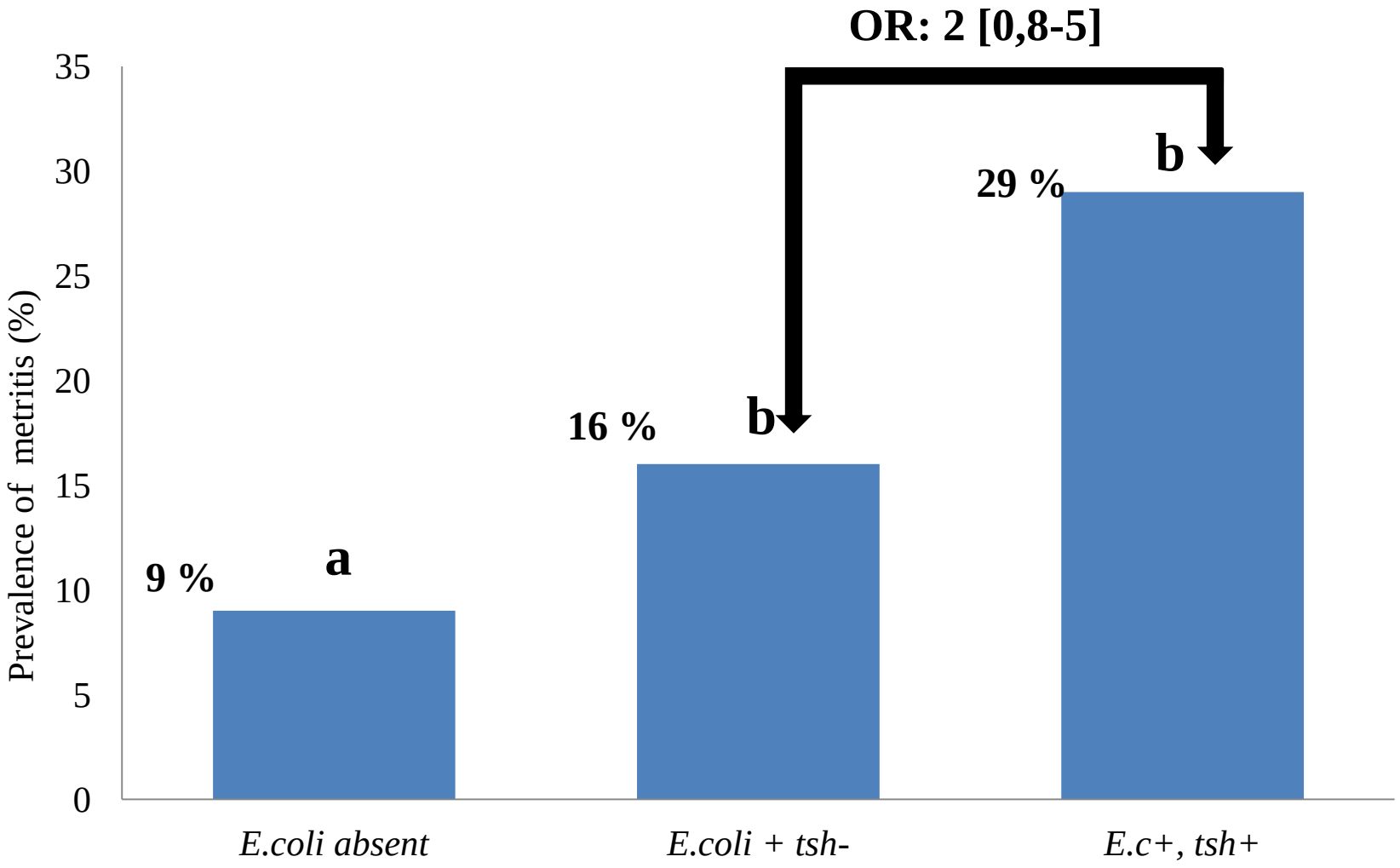
Step 2: Association between virulence genes and metritis

Figure 5: Prevalence of the virulence genes of *E.coli* in cows with metritis and without metritis



Step 2: Association between virulence genes and metritis

Figure 5: Effect of *tsh* on the prevalence of metritis

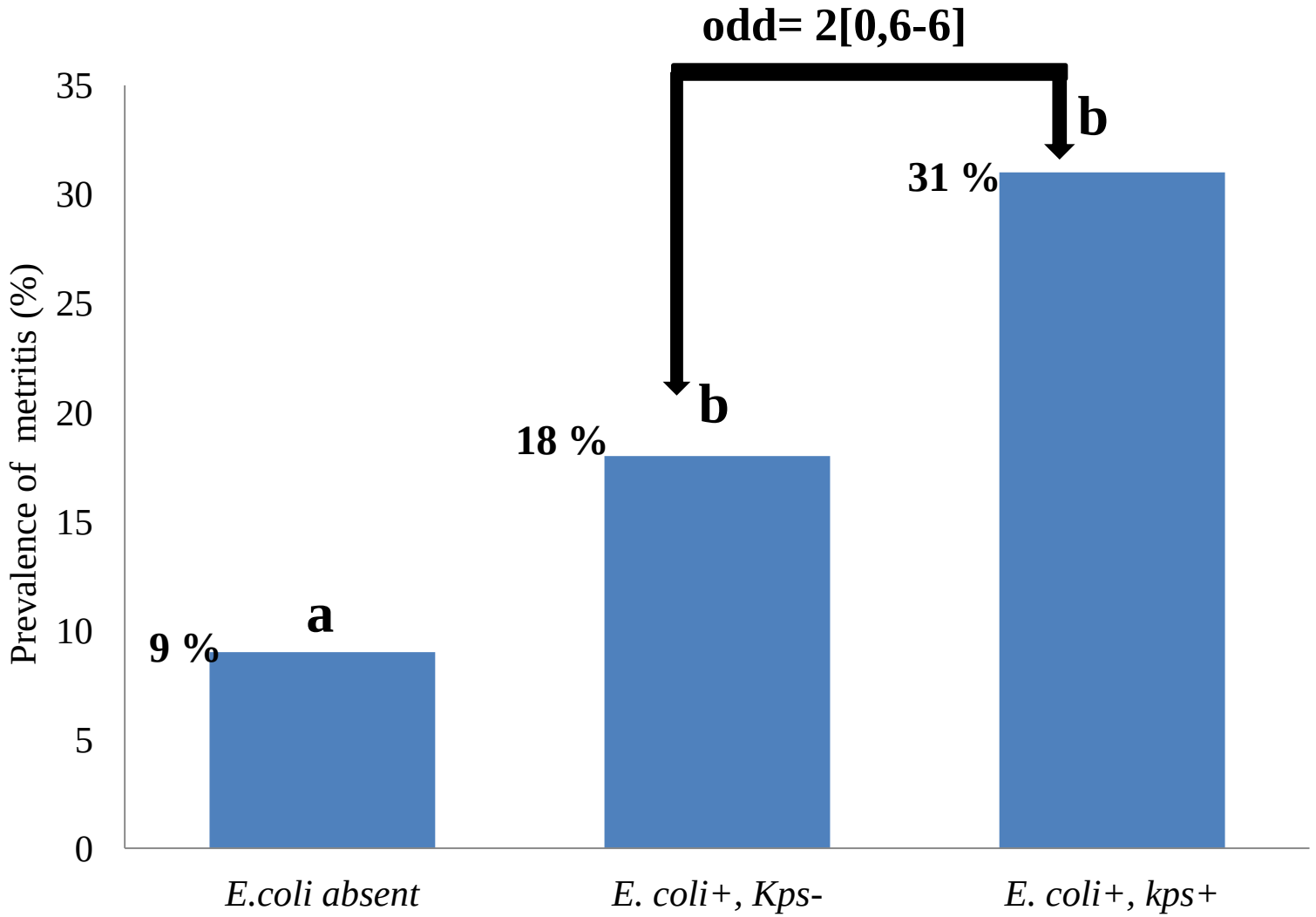


- The presence of *tsh* increases 2 times the risk of development of metritis

Results

Step 2: Association between virulence genes and metritis

Figure 6: Effet of *kps* on the prevalence of metritis



- The presence of the gene *kps* increases 2 times the risk of development of metritis

Results

Step 2: Association between virulence genes and metritis

Table 4: Virulence genes of *E. coli* associated with metritis

Virulence gene	n	% Metritis			odd1 [95%IC]	odd2 [95%IC]
		E.c-	*E.c* + gene -	*E.c*+ gene+	*E.c-* / *E.c* + gene +	*E.c* + gene - / *E.c* + gene +
f17	7	9	18	43	9 [2-42]	3 [0,8-14]
tsh	31	9	16	29	4 [2-9]	2 [0,8-5]
kpsMTII	13	9	18	31	6 [2-19]	2 [0,6-6]
hlyE	141	9	12	20	3[2-7]	2[0,5-9]
hra1	51	9	16	25,5	3,5 [2-8]	1,5 [0,6-5]
iroN	14	9	18	28,5	4 [2-12]	2[09-6]
malX	36	9	18	22	3 [2-13]	1,7[0,5-4]

Conclusion

- Factors associated with metritis
  - The simultaneous presence of *E. coli* and *T. pyogenes*
  - Virulence genes: *F17*, *tsh*, *kps*, *hra1*, *iroN*, *malX*

- These factors could be used as a predictive value for postpartum metritis in dairy cattle.

Acknowledgments

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Thanks

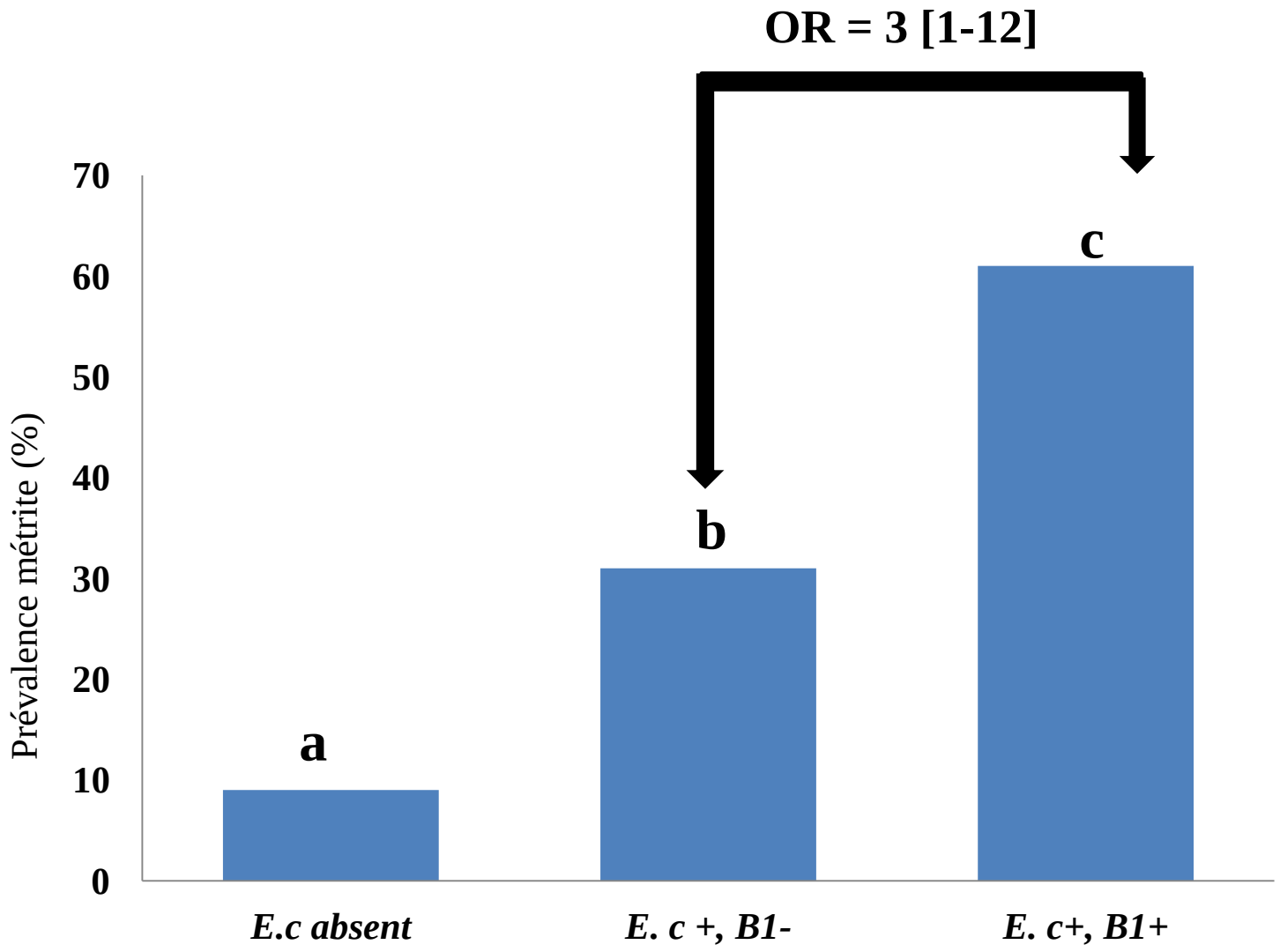
Perspectives

- **Develop a kit to predict the risk of development of metritis from the first week postpartum**

Table2: Association entre les gènes de virulence de *E. coli* et le développement de la métrite à la 2ème et 3ème semaine postpartum

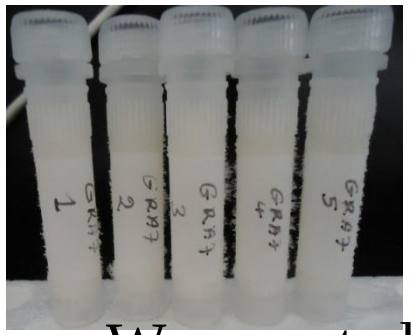
Gènes de virulence	n	% Métrite		*RR [95%IC]*	*p*
		(+)	(-)		
E. coli	262	17	9	1,8 [1,1-3,5]	0,01
f17	7	43	18	3,5 [0,8-14]	0.01
tsh	31	29	16	1,8 [0,8-4]	0,1
kpsMTII	13	31	18	1,7 [0,6-6]	0,25
hlyE	141	20	12	1,6[0,4-7]	0,4
hra1	51	25,5	16	1,5 [0,8-4]	0,15
iroN	14	28,5	18	1,5 [0,5-5]	0,33
malX	36	22	18	1,2 [0,5-3]	0,5

Figure 14: Association entre groupe phylogénétique et la métrite

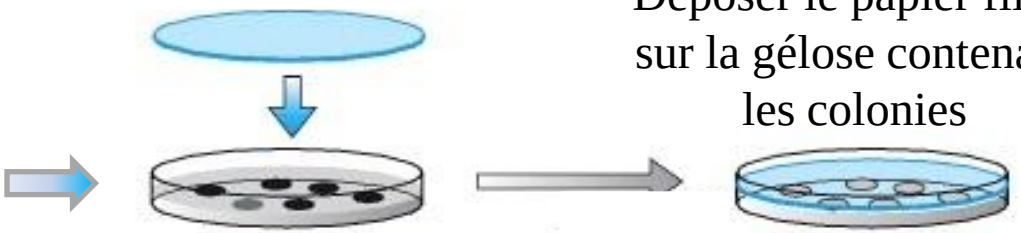


- La présence de *E. coli* du groupe B1 augmente de 3 fois le risque de développement de la métrite

Objectif 2: Identifier les gènes de virulence de *E. coli*



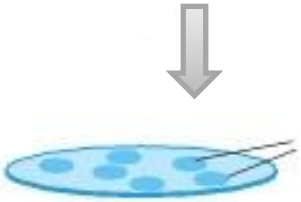
Papier filtre Whatman



Déposer le papier filtre sur la gélose contenant les colonies

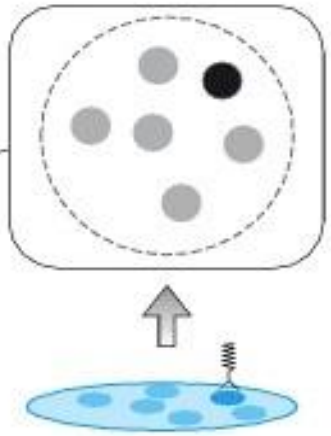
- Werner et al, 2012, Germany: à J10 PP, *E. coli* (25%) > *T. p* (10%)
- Dohmen et al., 2000; Földi et al., 2006:
  F. n et *P. m* (80%) > *T.p* (65%) > *E. coli* (36%)

Trempage successif
- SDS 10%
- NaOH + NaCl
- Tris + NaCl



ADN

- Hybridation
  -ADN spécifique marqué à ^{32}P
- Lavage
- Autoradiographie

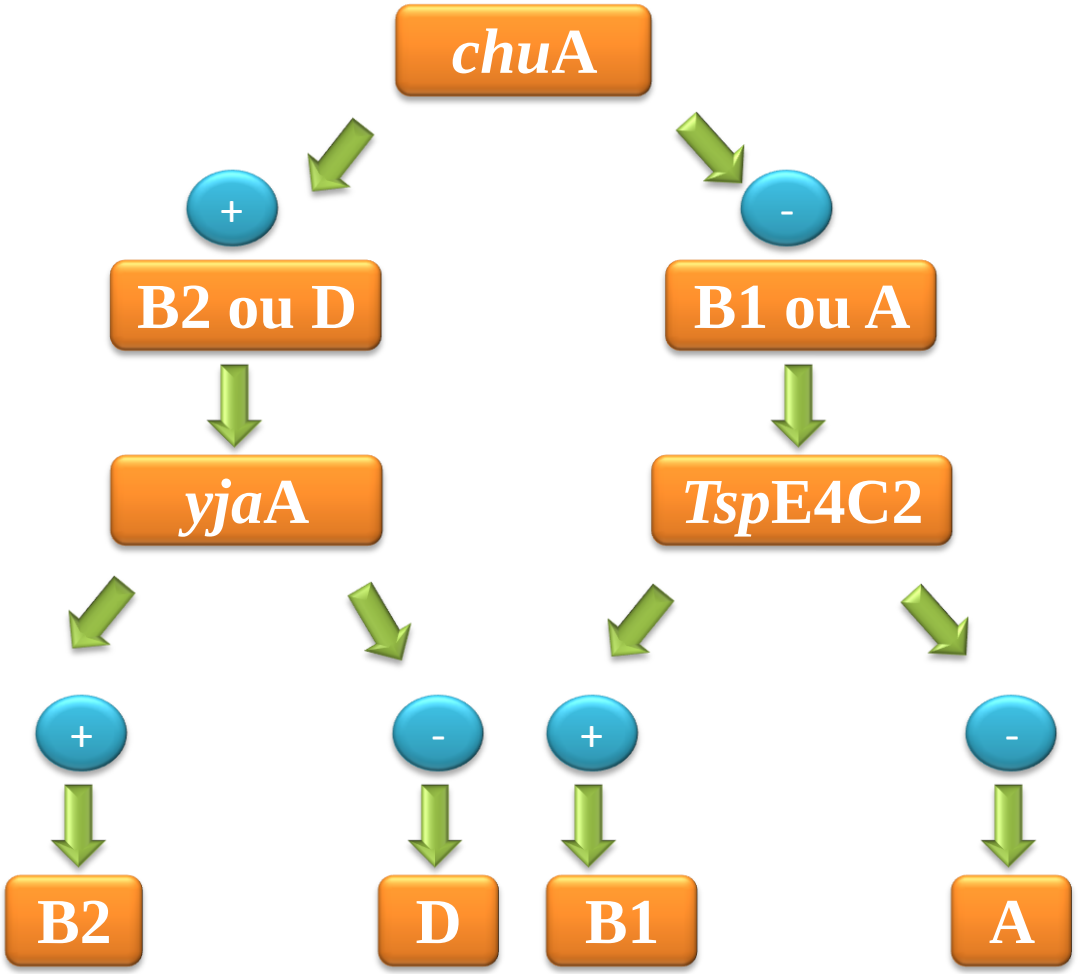


Film de rayon X

Figure 8: Hybridatic
des sondes (32P)

Objectif 3: Déterminer des groupes phylogénétiques

A B1 B2 D



PCR multiplex

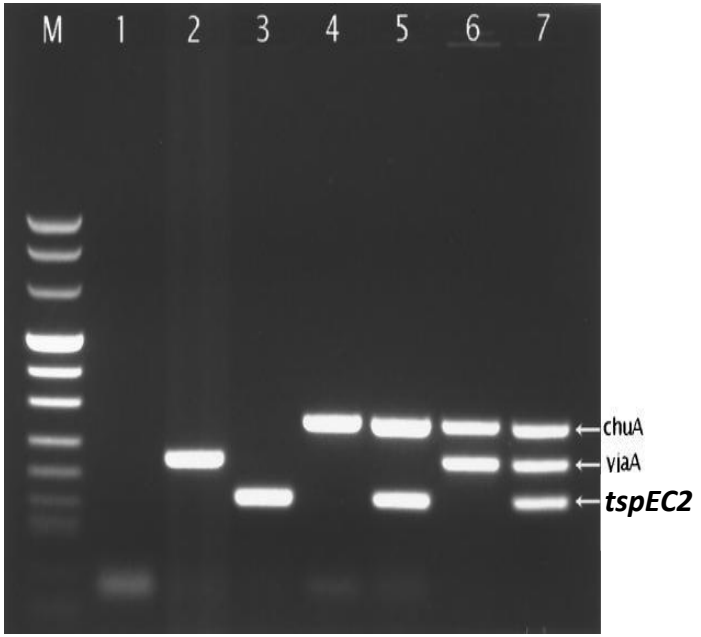
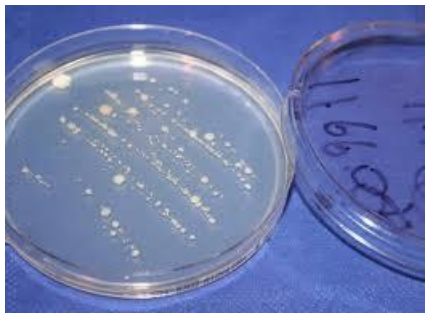


Figure 11: Arbre de décision pour déterminer les groupes phylogénétiques de *E. coli*

Clermont 2000

Objectif 3: Déterminer les sérogroupes « O »

Agglutination sur lame



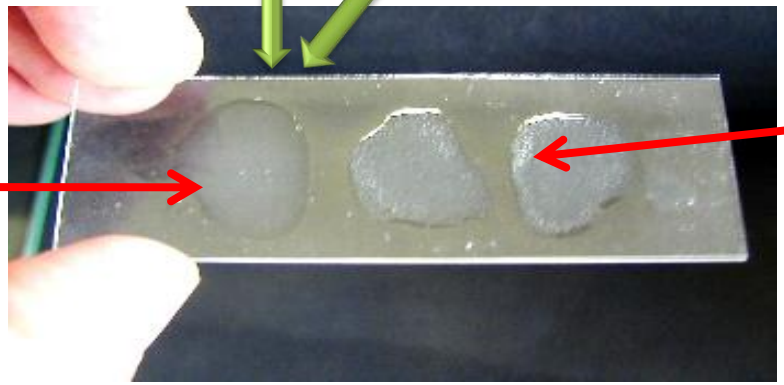
Culture sur TSA

autoclavé (2h)
dans du PBS

Anti sérum anti O

Une goutte

Une goutte

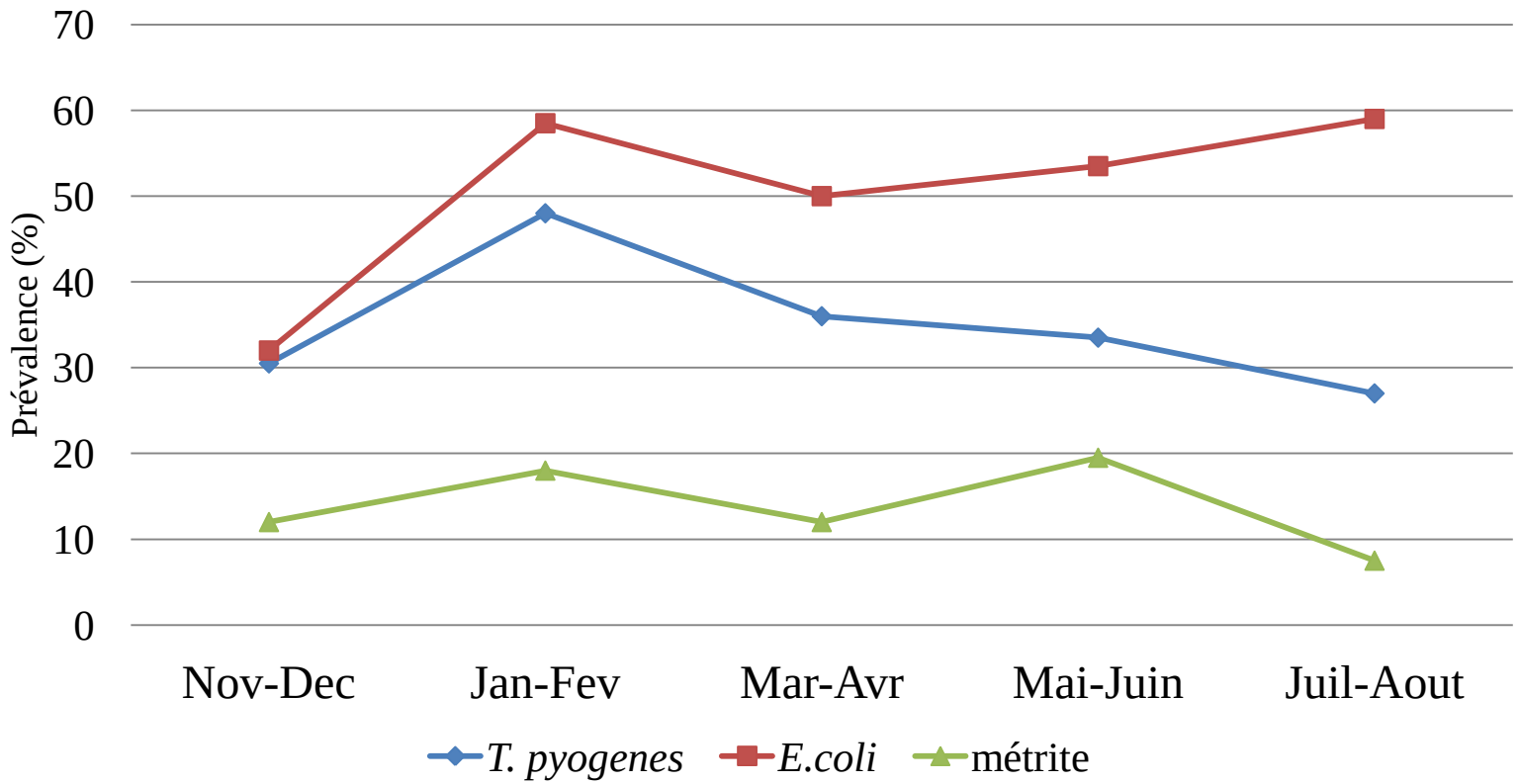


Pas
d'agglutination

Agglutination

Figure 10: Sérotypie par agglutination sur lame

Figure 6: Prévalence de la métrite et des espèces bactériennes par saison



Prévalence moyenne métrite: 13%

Autres études: [10% – 20%] moyenne: 15%

Table 7: Association entre la présence intra-utérines des espèces bactériennes à 1ère semaine postpartum et le développement de la métrite à la 2ème et 3ème semaine postpartum

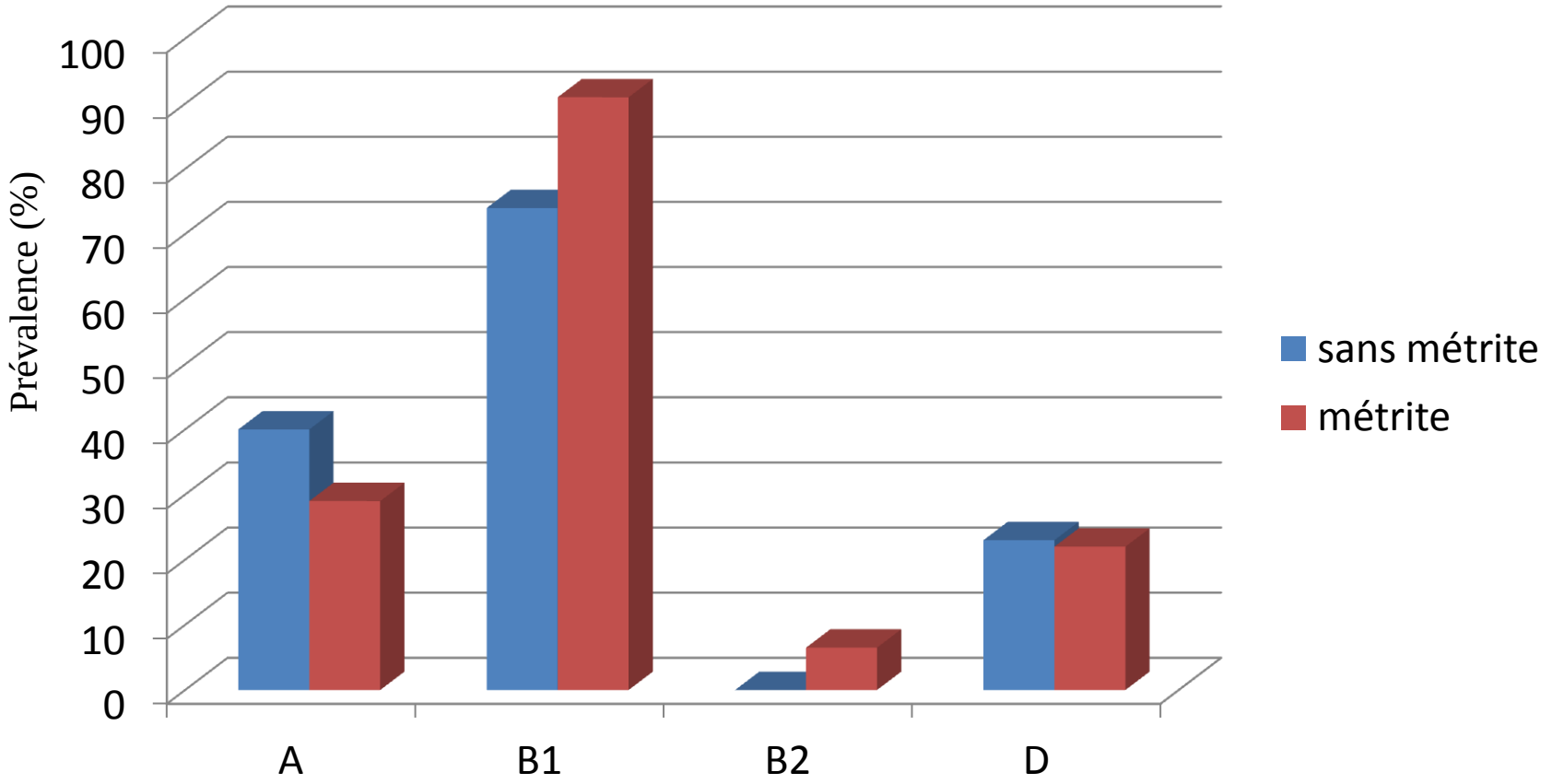
Pathogène	Pathogène (-)		Pathogène (+)		*RR [95%IC]*	*p*
	n	% métrite	n	% métrite		
E. coli seul	346	13,5	151	13	1 [0,5-1,5]	<0,9
T.p seul	438	13	59	10	0,7 [0,2-1,6]	<0,4
E.coli + *T.p*	390	11	107	23	2 [1,5-4,5]	<0,001
ni *E.c*, ni *T.p*	317	16,5	180	7,5	0,4	<0,005
Total			497			

- Le risque de développement de la métrite est 2 fois plus élevé chez les vaches positives à *E. coli* et *T. pyogenes* à la 1ère semaine postpartum
- Werne et al, 2012: *E. coli* (RR=1,8), *T. pyogenes* (RR=1,8)

Autres façon d'analyser les Results

- Combinaison de gènes de virulence
- L'homogénéité des isolats d'un échantillon
- Le nombre de gène de virulence présent dans l'échantillon
- La charge bactérienne contenue dans les échantillons
- L'influence de la parité

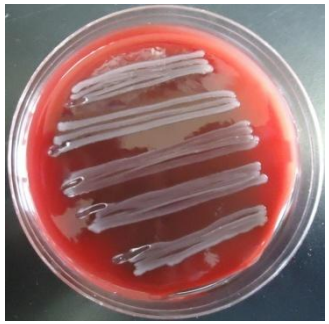
Figure 13: prévalence des groupes phylogénétiques chez les vaches avec métrite et sans métrite

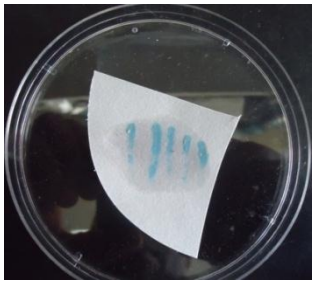


Confirmation tests for *E. coli*

Colonies of *E. coli* on MacConkey

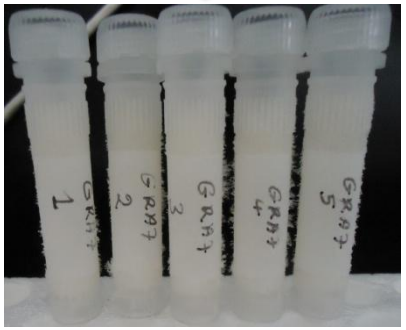
Isolation of 5 colonies on blood agar





Indole spot test

Simmons citrate test



Conservation in glycerol 30% at -80°C

Motility

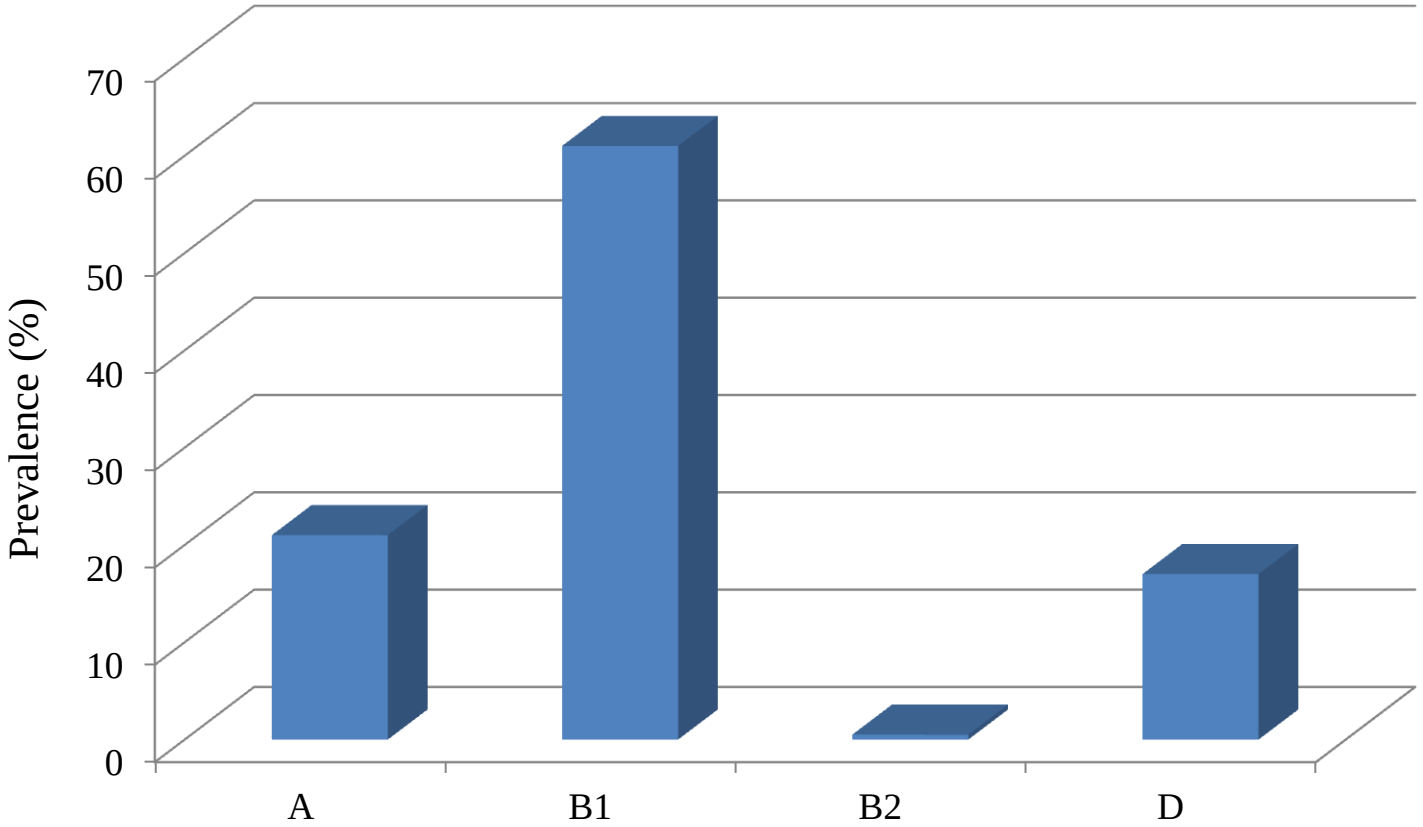
Colony Hybridation

Figure 5: Isolation of *E. coli*

Results

Objective 3: Determine the phylogenetic groups of *E. coli*

Figure 8: Prevalence of phylogenetic groups of *E. coli* isolated from cow uterus in the first week postpartum



B1 (61%) > A (21%) > D (17%) > B2 (0,5%)

Objective 3: Determine the phylogenetic groups of *E. coli*

- **Phylogenetic groups**

A **B1** **B2** **D**

Method: Multiplex PCR