

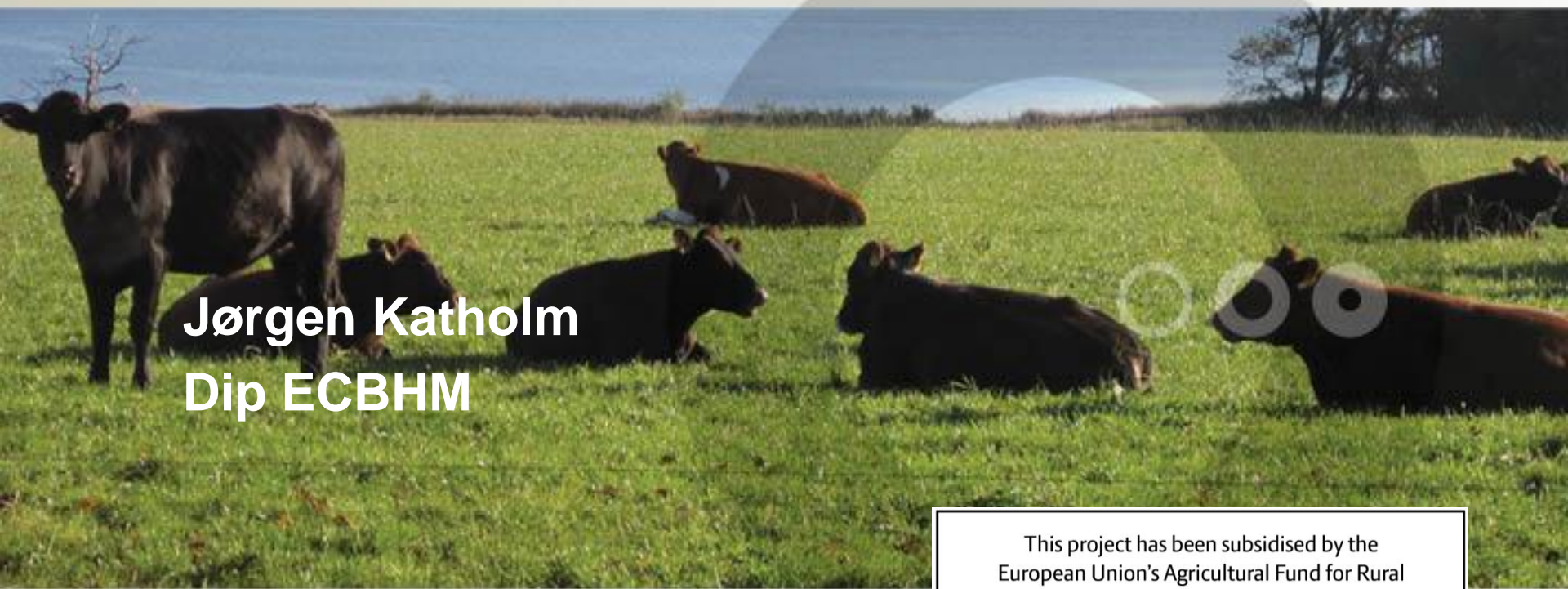


KNOWLEDGE CENTRE FOR AGRICULTURE



Our Milk
- a pure pleasure

Use of Pathoproof™ in Danish Dairy Herds

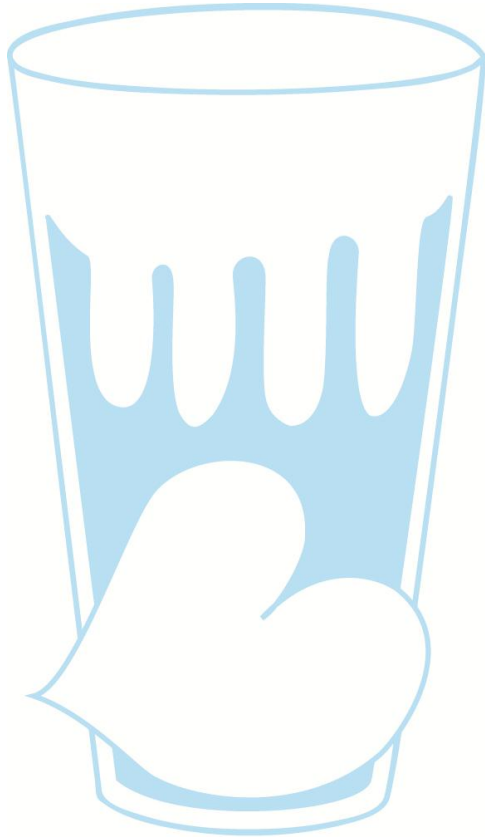


Jørgen Katholm
Dip ECBHM

This project has been subsidised by the European Union's Agricultural Fund for Rural Development and the Danish Ministry of Food, Agriculture and Fisheries.

Highly industrialised Dairy Industry

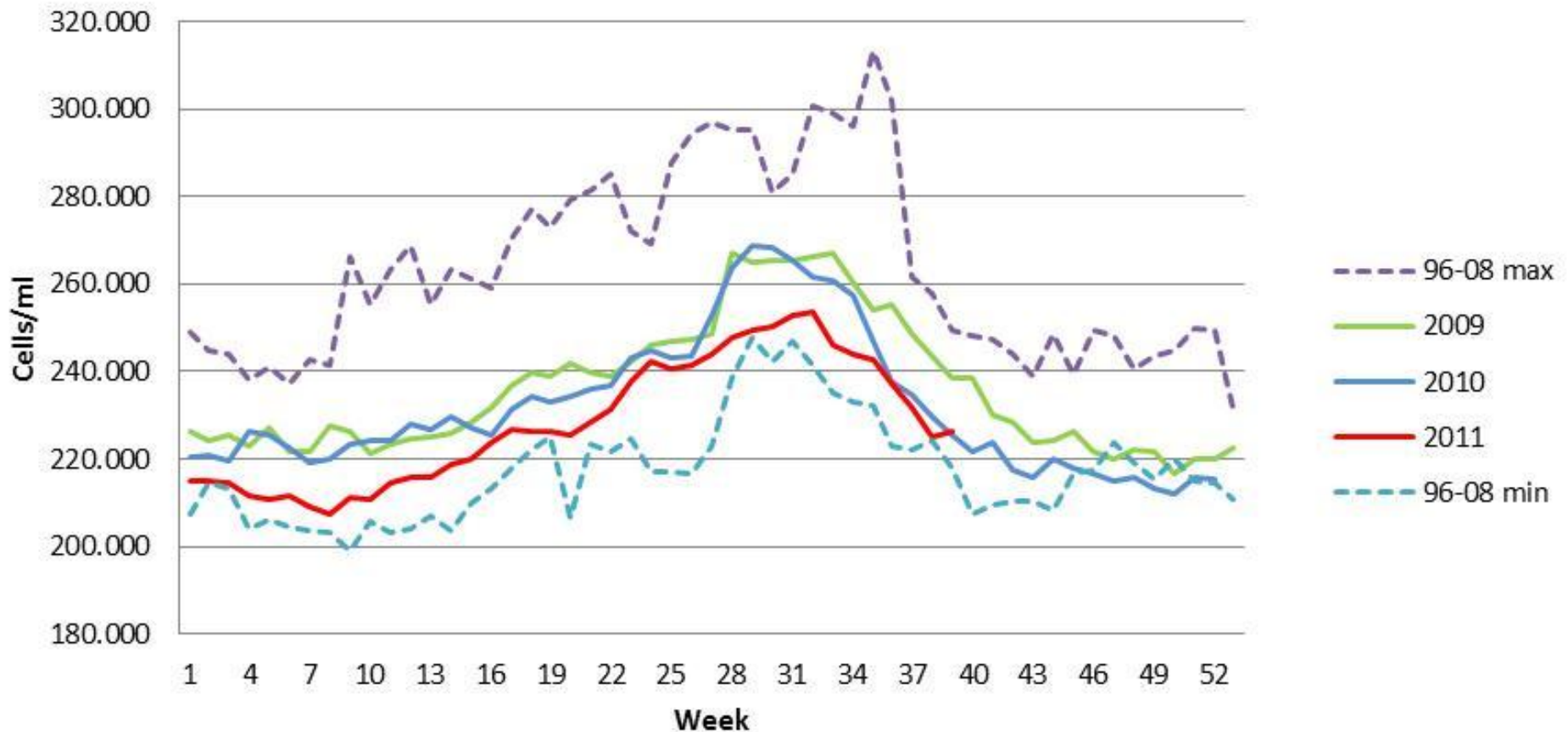




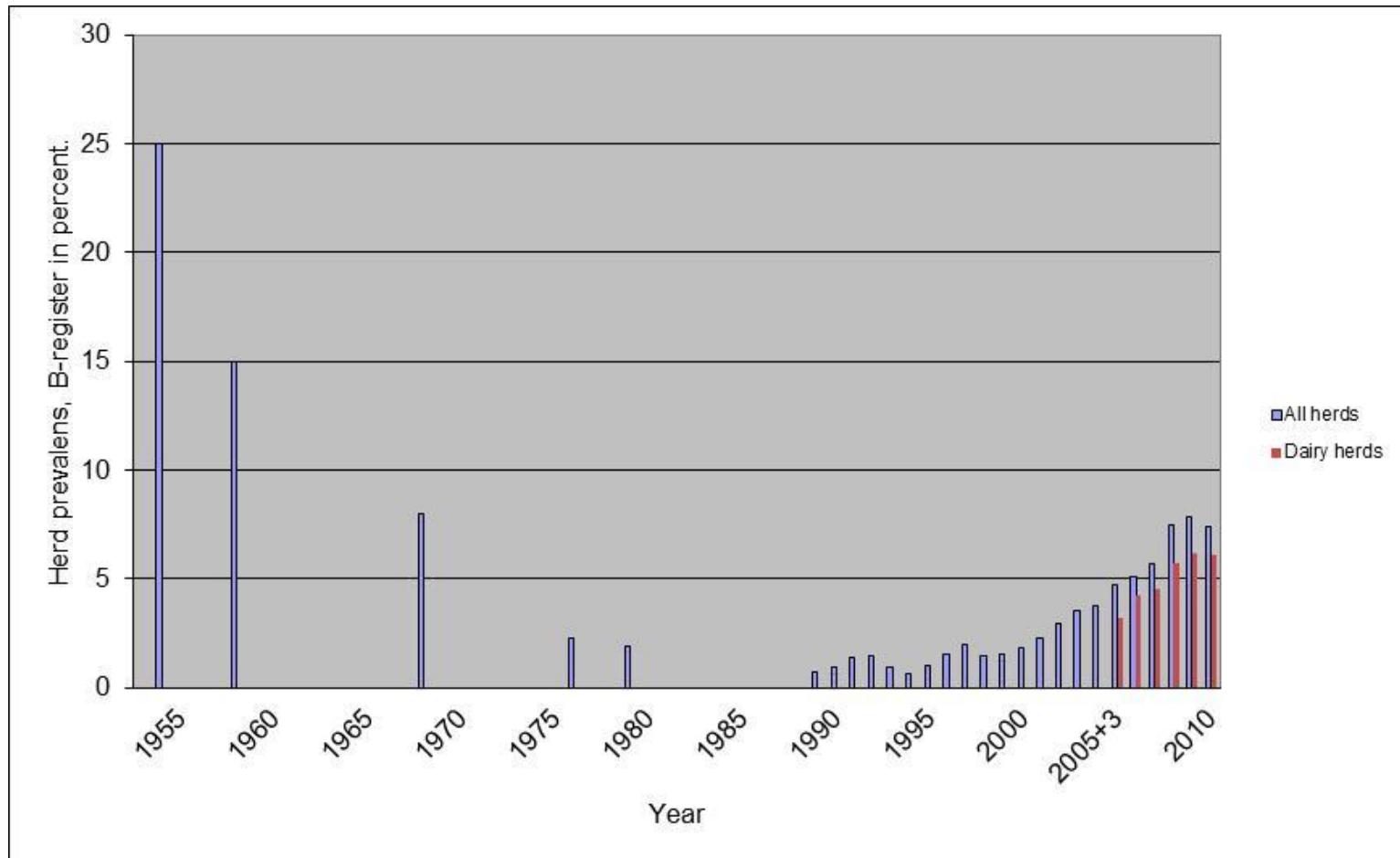
Our Milk

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Geometrisk tankcelletal for hver uge 2009-2011 og max og min for 1996-2008

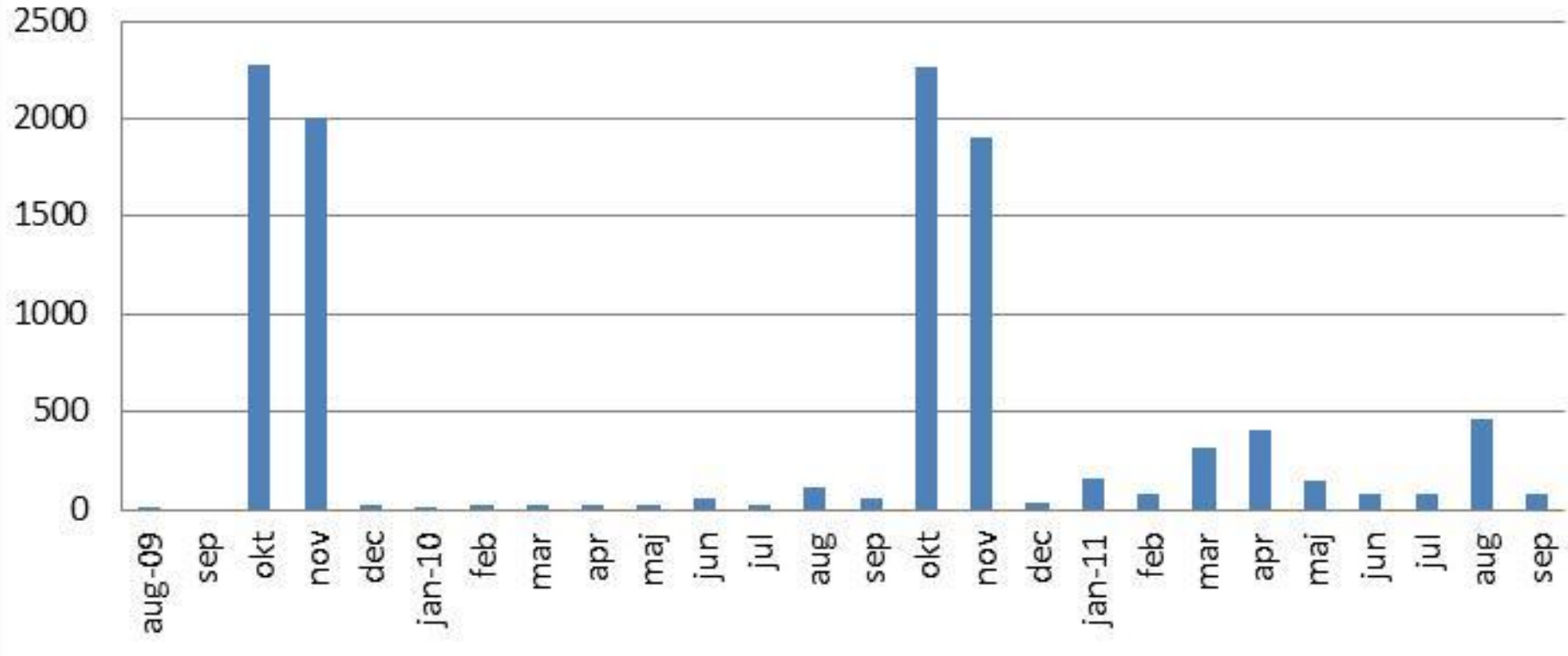


Herds in the Danish B-register from 1954 to 2010 in percent of all herds and from 2005 of active dairy herds





Bulk tank PCR



2009 - 4306

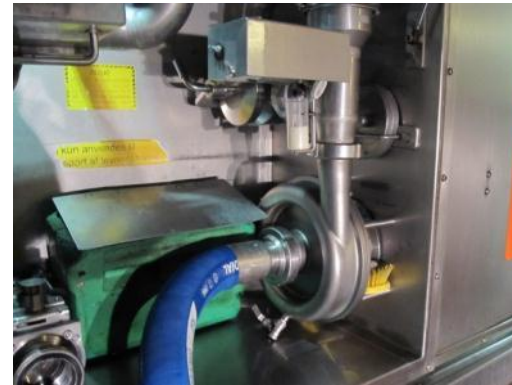
2010 - 4558

2011 - 1813

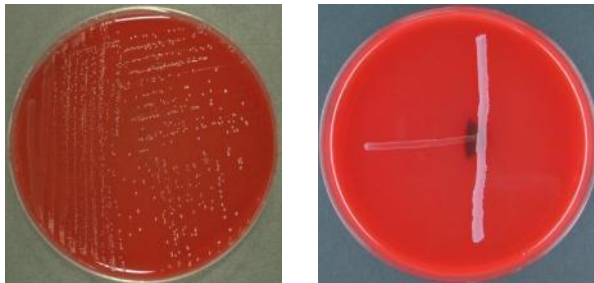
Bulk tank milk



Collection and sampling



Culture Streptococcus agalactiae + / -



PCR – real time Ct value for 12 gene

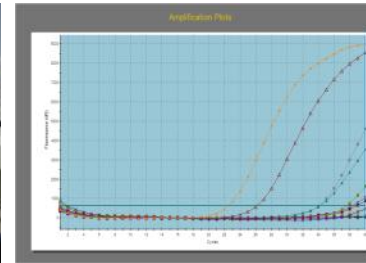


Table 1. Culture and PCR for *Streptococcus agalactiae* from all 4258 Danish dairy herds in 2009

Test for GBS	PCR	
	Positive	Negative
Culture		
Positive	178	20
Negative	132	3928

Table 2: Number of dairy herds PCR and culture positive for *Streptococcus agalactiae* 2009 and 2010

Year	herds	PCR GBS-positive	Culture GBS-Positive
2009	4258	301 (7.3 %)	198 (4.7 %)
2010	4093	271 (6.6 %)	141 (3.4 %)



Table 3: Results of PCR test PathoProof™ in BTM samples from 4258 herds in 2009

Bacteria/gen	% NoCt	Lowest	10% Percentile
<i>Staph aureus</i>	9	20	29
<i>Staph. Sp</i>	0	18	27
Beta-lactam	22	22	31
<i>Str. agalactiae</i>	93	17	26
<i>Str. dysgalactiea</i>	14	16	28
<i>Str. uberis</i>	5	14	26
<i>Coryne. bovis</i>	10	25	32
<i>Enterococcus</i>	22	21	30
<i>E.coli</i>	39	18	30
<i>Klebsiella</i>	87	19	31
<i>S. marcescens</i>	98	25	34
<i>A.pyo./ P. ind</i>	37	19	32

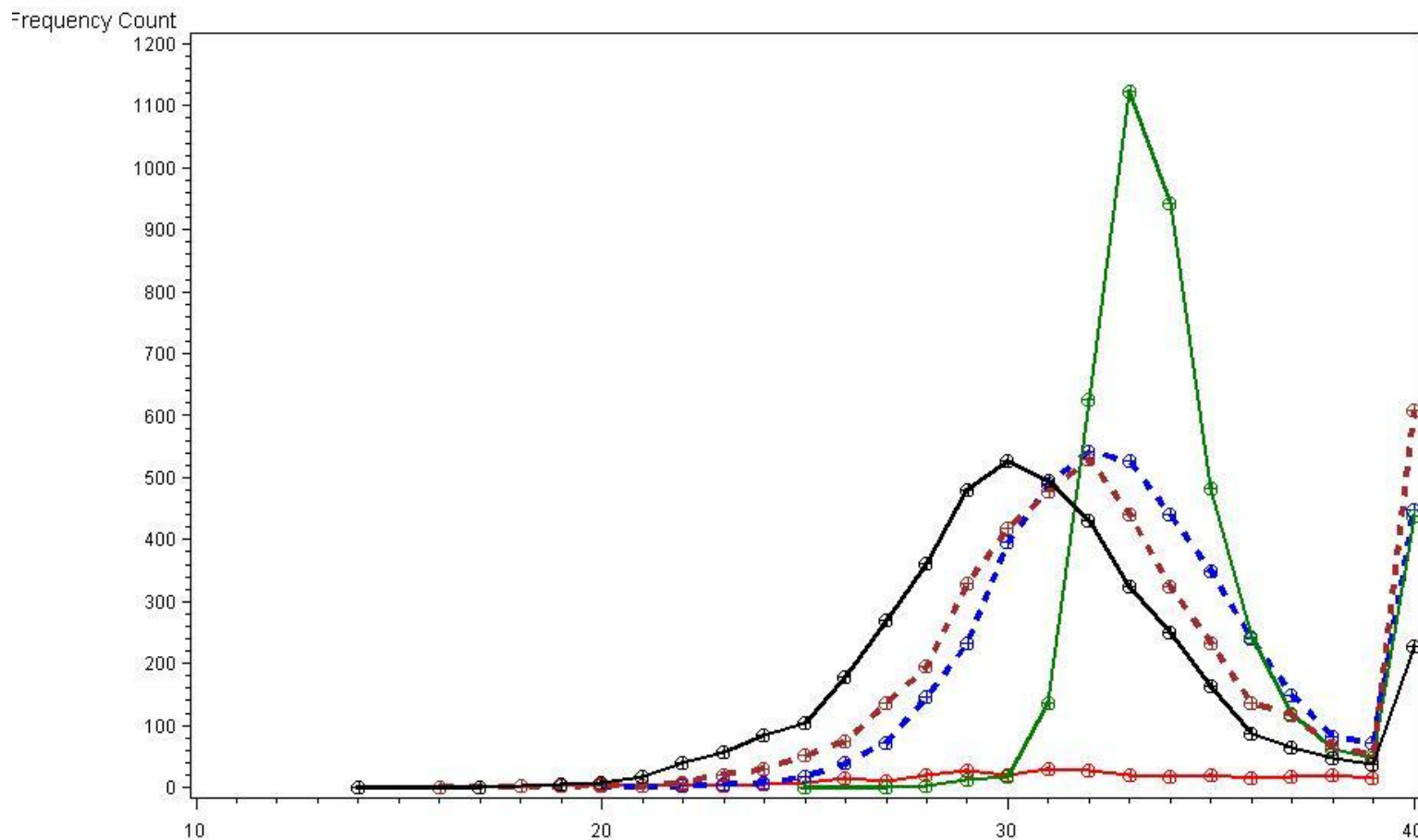
Conclusion

- Streptococcus agalactia is an increasing problem in Denmark
- PCR is more sensitive than culture
 - Sensitivity 94%
- PCR on BTM samples can be used as surveillance of other mastitis pathogens in dairy herds, and give farmers good information on further prophylactic actions against udder infections

Distribution of Ct values



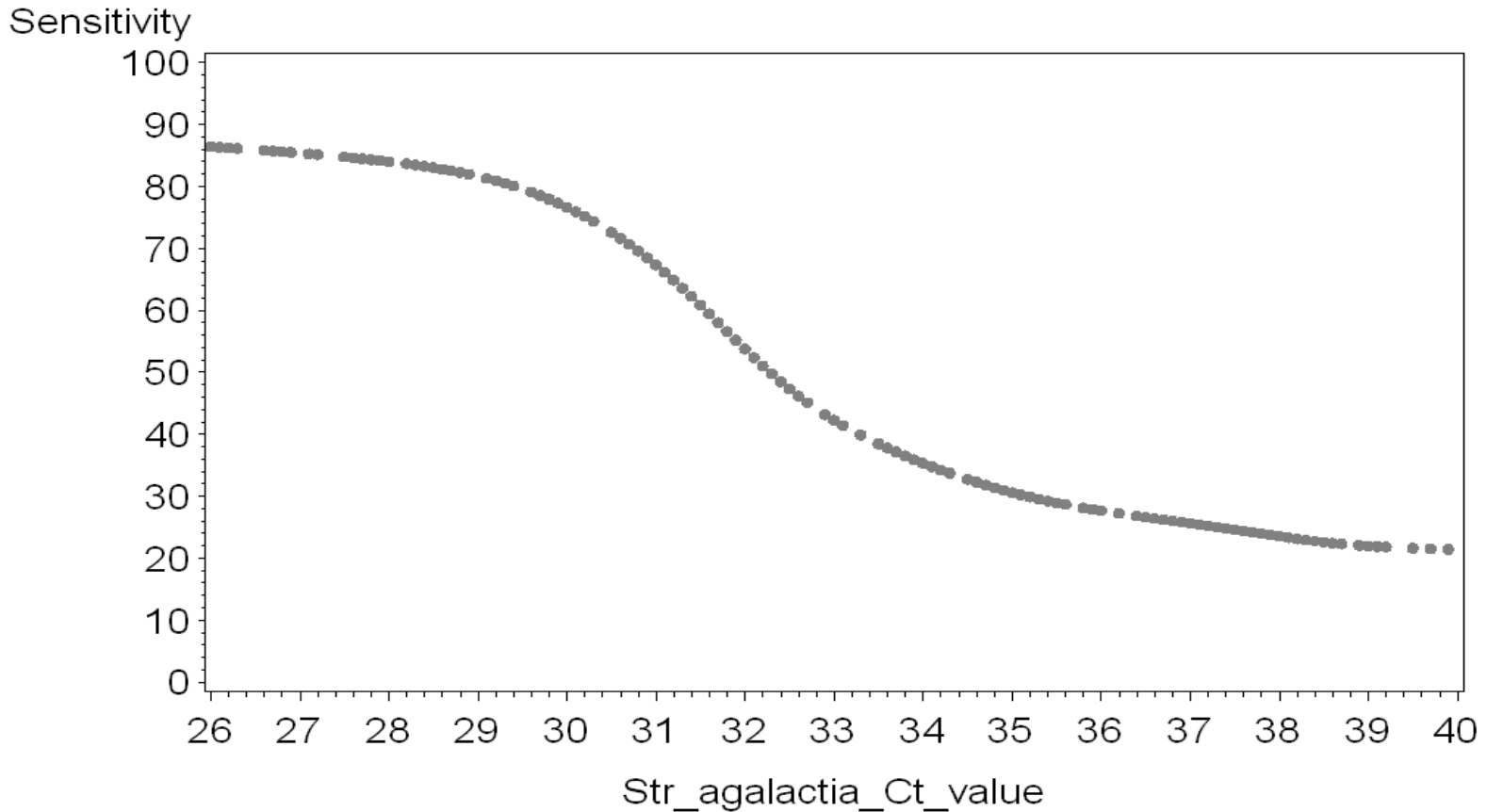
Our Milk
- a pure pleasure



type agal aureus cbovis dysg uberis

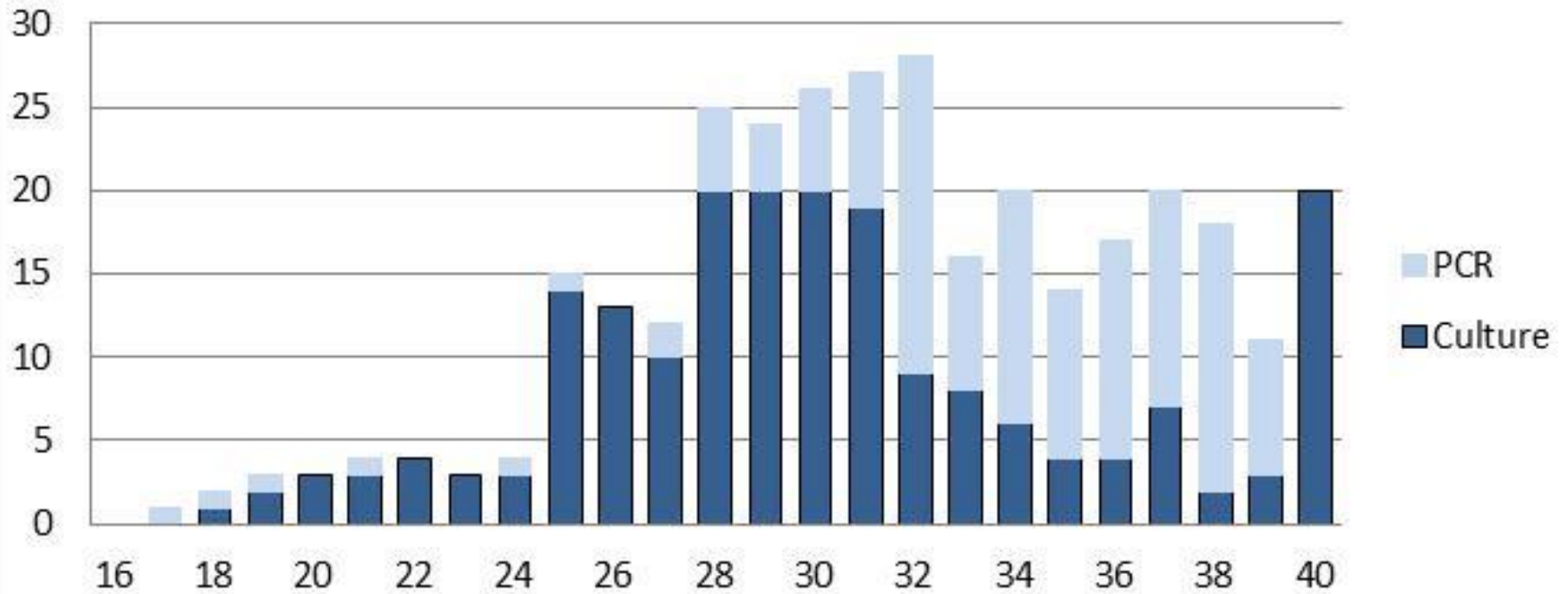
(Ct=40 for *Str. agalactiae* = 3928 outside axis)

The sensitivity of traditional culture compared to real-time PCR highly related to the Ct-value (correlated to bacterial concentration)

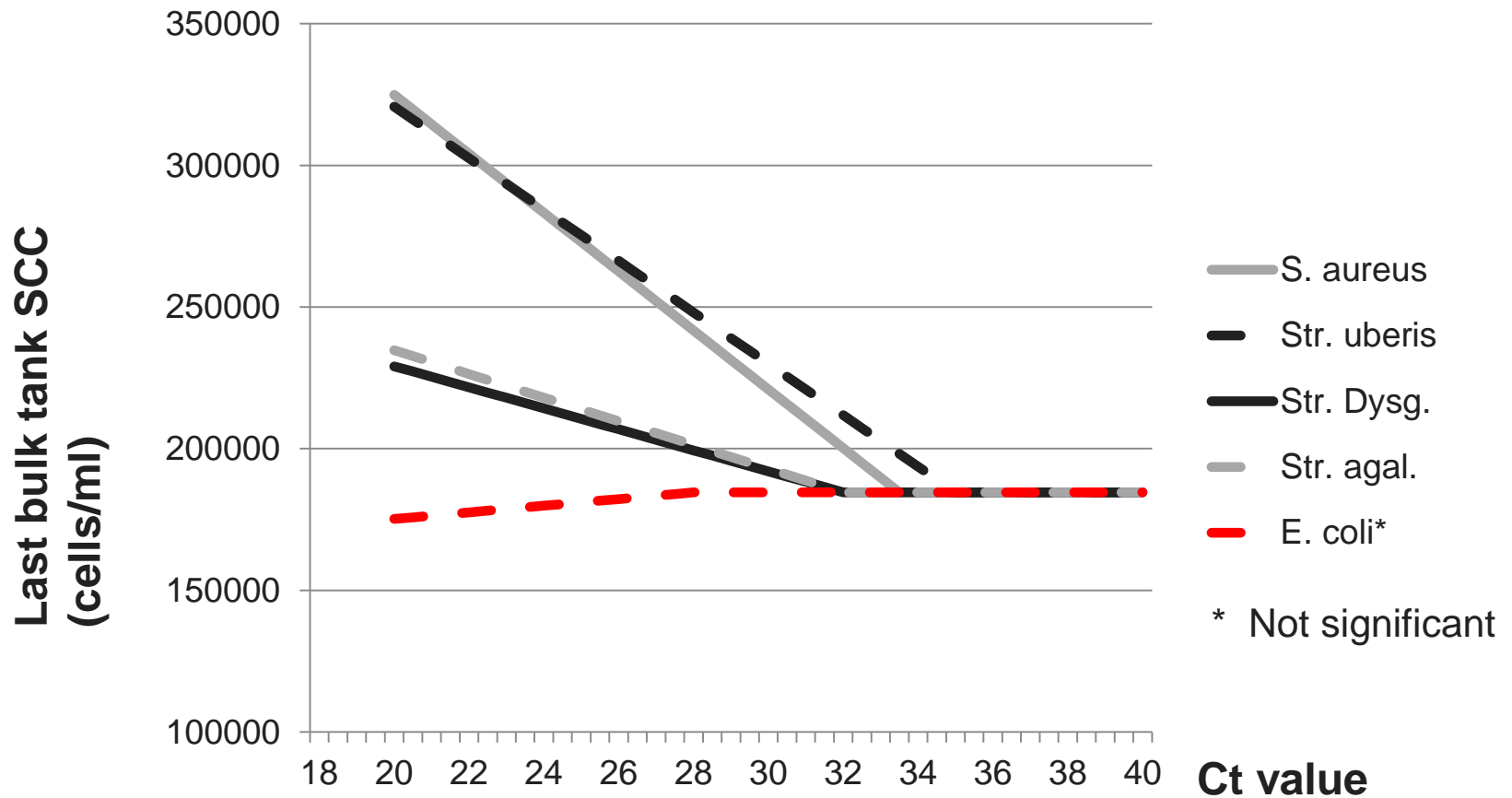


(Curve smoothed using a Generalized additive model)

PCR and culture positive samples in relation to PCR Ct value

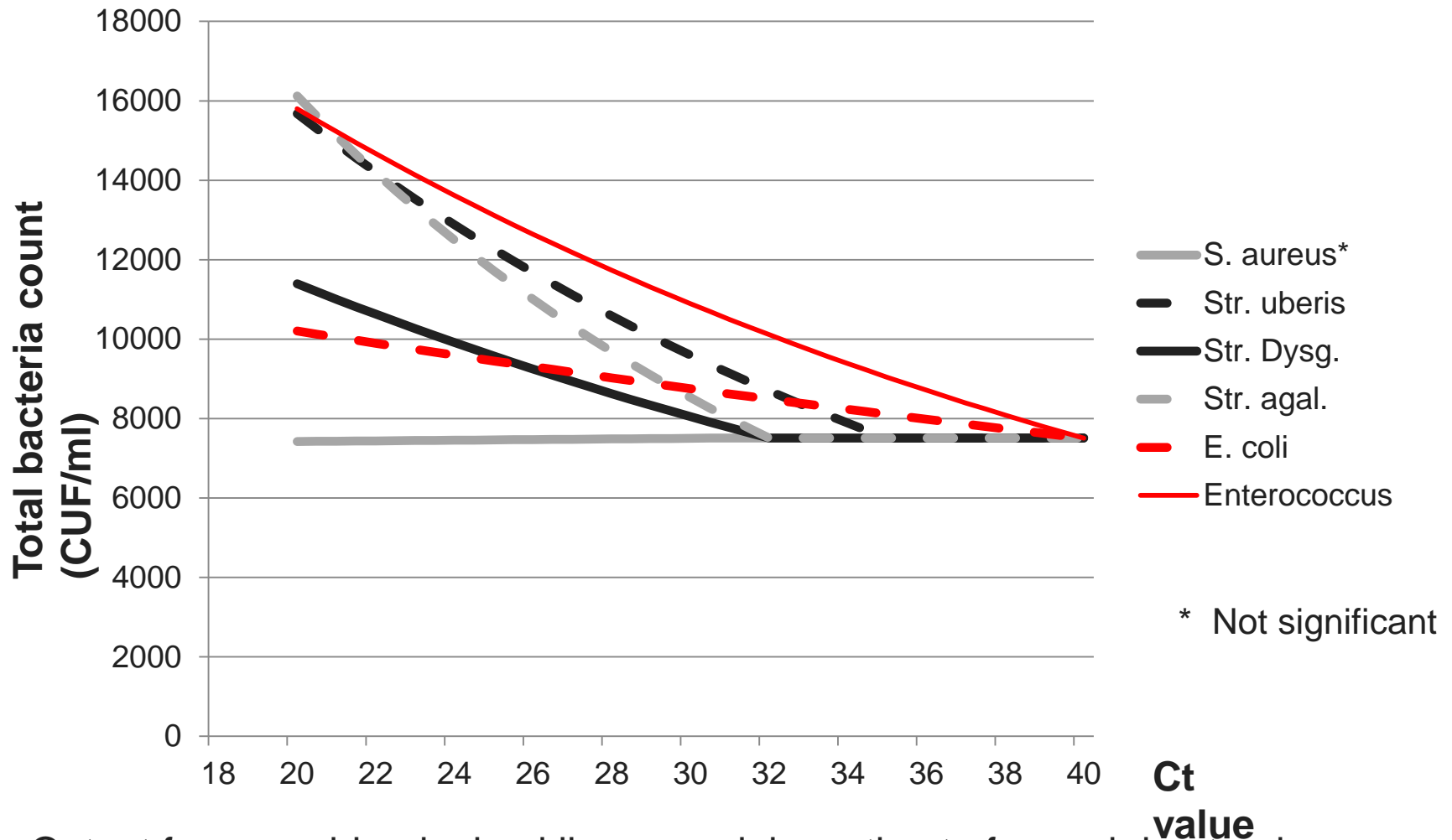


Low Ct values was associated with higher Bulk tank somatic cell count



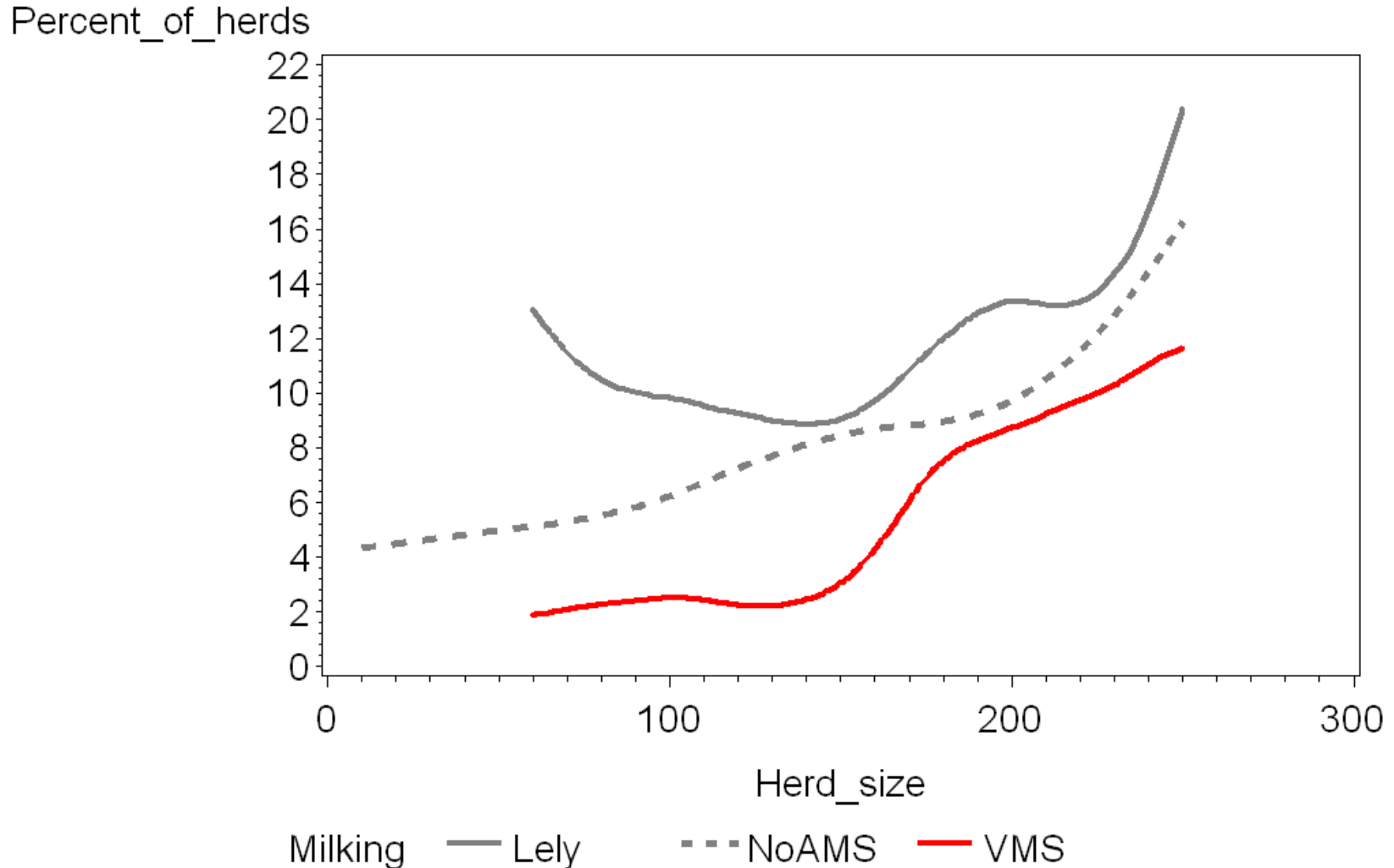
Output from combined mixed linear model – estimate for each bacteria

Low Ct values was associated with higher total bacteria count



Output from combined mixed linear model – estimate for each bacteria

Prevalence of *Str. agalactiae* increased with herd size depending on milking system.



(Curve smoothed using a Generalized additive model)

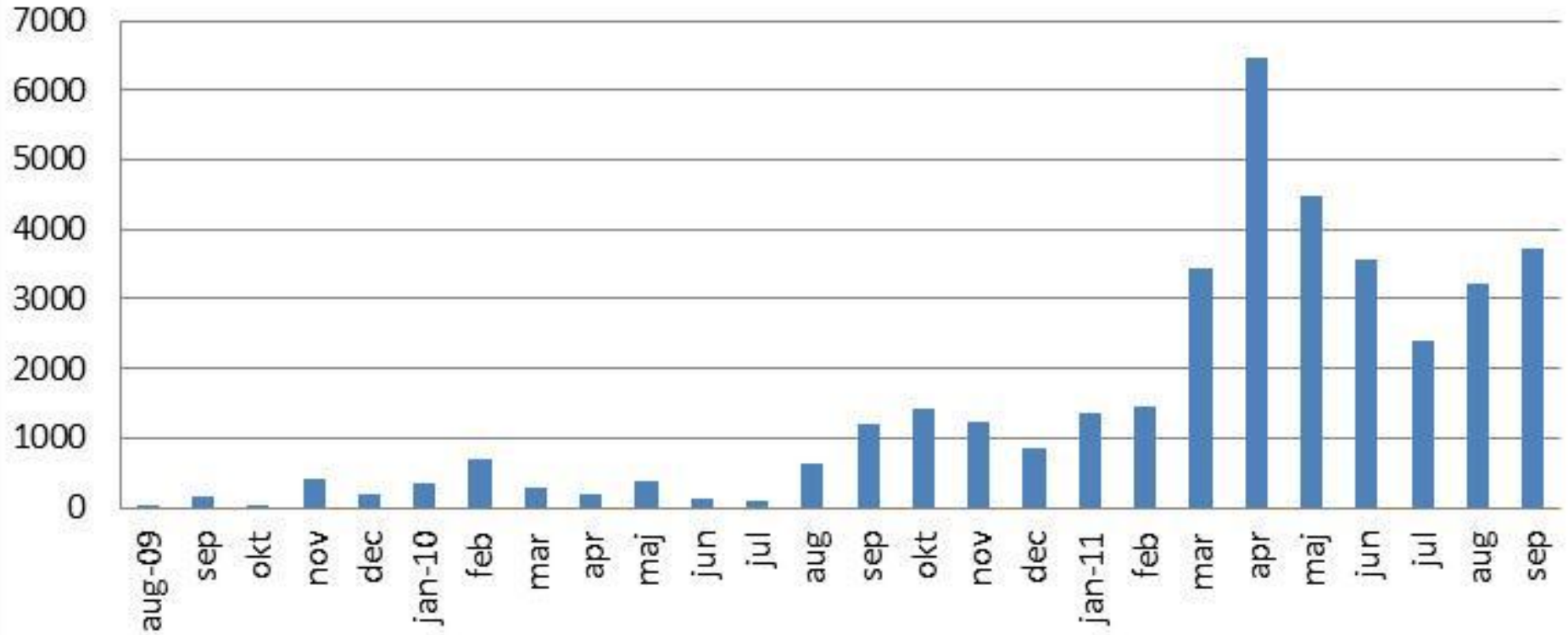


Use of Real time PCR on bulk tank milk

Low ct values of major mastitis pathogens are related to reduced milk quality

Real time PCR are more sensitive than bacteriological culture in detecting *Str. agalactiae* in the bulk tank

Individual cow PCR



2009 – 847

2010 – 7468

2011 – 30092



Test at the same milksample - 99 samples

PCR

Culture

Blood agar
Eurofins

Selektiv agar
Foulum

Pos 27 (10)

10

3

Samples of individual cows at DHI

DYREREGISTRERING • Kvæg, Får og Geder Brugers-MF jka

Hovedmenu Redigér Vis Funktioner Opsætning Hjælp

Kvæg Ydelseskontrol

Sundhed

Bestil veterinær analyser

Staldregistrering Produktion Sundhed Y Besætning Dyr Egen udskrift Ins.plan Dataudtræk Masseindtastning

Sygdom Medicin Klinisk/velfærdsreg Besætningsdiagnoser Symptomreg Behandlinger Dokumenter J Bluetongue Vet. analyser

Seneste kontrollering med udtagning af ParaTB-prøver: 26-11-2009
 Næste planlagte ydelseskontrol med udtagning af ParaTB-prøver: . . .
 Næste planlagte ydelseskontrol: 03-02-2010
 Forventet kælvdato er beregnet pr. 16-01-2010

Søgekriterier:
 CTV > [] Lakt > [] [Søg]

Bestilling til hele besætningen: Paratuberkulose Salmonella [Bestil]

Dyrnr	Lakt. nr.	Forventet			ParaTB					Salmonella			PCR				Godkendt	Åjournført		
		kælvning	kælvning	goldning	1	2	3	4	Inf. gp.	Prøve	1	2	Prøve	1	2	3		Prøve	Dato	Af
01345	6				0,0		0,0	0,0	0	<input type="checkbox"/>			<input type="checkbox"/>	5	5		<input type="checkbox"/>	<input type="checkbox"/>		
01349	6	03-07-2010	168	126	0,0		0,1		0	<input type="checkbox"/>			<input type="checkbox"/>	1	2	1	<input type="checkbox"/>	<input type="checkbox"/>		
01370	5	02-06-2010	137	95	0,0		0,1	0,1	0	<input type="checkbox"/>			<input type="checkbox"/>	3	2	3	<input type="checkbox"/>	<input type="checkbox"/>		
01441	5	15-07-2010	180	138	0,2				0	<input type="checkbox"/>			<input type="checkbox"/>	1	2	1	<input type="checkbox"/>	<input type="checkbox"/>		
01446	5	13-08-2010	209	167	0,0				0	<input type="checkbox"/>			<input type="checkbox"/>	5	5	5	<input type="checkbox"/>	<input type="checkbox"/>		
01459	5	18-05-2010	122	80	0,0		0,1	2,7	5	<input type="checkbox"/>			<input type="checkbox"/>	2	2	2	<input type="checkbox"/>	<input type="checkbox"/>		
01460	4	24-01-2010	8		0,0		0,0	0,0	0	<input type="checkbox"/>			<input type="checkbox"/>		3	1	<input type="checkbox"/>	<input type="checkbox"/>		
01470	4	09-07-2010	174	132	0,0		0,0	0,0	0	<input type="checkbox"/>			<input type="checkbox"/>	4	5	1	<input type="checkbox"/>	<input type="checkbox"/>		
01557	4	10-07-2010	175	133	0,0				0	<input type="checkbox"/>			<input type="checkbox"/>	2	3	1	<input type="checkbox"/>	<input type="checkbox"/>		
01558	3	25-07-2010	190	148	0,0				0	<input type="checkbox"/>			<input type="checkbox"/>	2	2	3	<input type="checkbox"/>	<input type="checkbox"/>		
01584	4	17-08-2010	213	171	0,0				0	<input type="checkbox"/>			<input type="checkbox"/>	1	3	2	<input type="checkbox"/>	<input type="checkbox"/>		
01587	4				0,0		0,0	0,0	0	<input type="checkbox"/>			<input type="checkbox"/>	2	1	1	<input type="checkbox"/>	<input type="checkbox"/>		
01601	3				0,0		0,1	0,0	0	<input type="checkbox"/>			<input type="checkbox"/>	3	4	4	<input type="checkbox"/>	<input type="checkbox"/>		
01604	4						0,1	0,1	0	<input type="checkbox"/>			<input type="checkbox"/>	1			<input type="checkbox"/>	<input type="checkbox"/>		
01633	4						0,0	0,1	3	<input type="checkbox"/>			<input type="checkbox"/>	5		1	<input type="checkbox"/>	<input type="checkbox"/>		
01634	4	23-07-2010	188	146	0,0			0,1	3	<input type="checkbox"/>			<input type="checkbox"/>	1	1	4	<input type="checkbox"/>	<input type="checkbox"/>		

Antal prøver ialt: ParaTB 0 Salmonella 0 PCR 0
 Seneste bestilling: Dato [] Kl [] Af RYY663

[Opdater forventet kælvdato] [Tidligere bestillinger] [Slet alle bestillinger] [Godkend bestilling]

Skals



Sundhedsstatus

Overvågning tankmælk

Tilknyttede bes.nr | Staldopdeling | Indlæs | Udlæs | Øremærkebestilling | Sundhedsstatus

Prøvetype: PCR

Sygdom | Overvåg enkeltdyr | Overvåg tankmælk | Bøkt. fund | Overvåg slagteblod | KVR | Journal | ParaTB oversigt | ParaTB tilmeld | PCR tilmeld

Prøvemateriale		Udtagningsdato	Modtaget dato	Resultat			Status	Gyldig	Mejerinr	Leverandørnr	Art		Ajourført	
Kode	Tekst			Prøve	Kode	Tekst					Kode	Tekst	Af bruger	Dato
3	Mælk	30-08-2011	30-09-2011				K	<input checked="" type="checkbox"/>	1		11	Årlig Tankmælk	H6601	30-09-

- Ny prøve
- Ret prøve
- Slet Ctrl+D
- Fortryd række Ctrl+Z
- Klip felt Ctrl+X
- Kopier felt Ctrl+C
- Indsæt felt Ctrl+V
- Vis PCR-analysedata

Vis flere



Sundhedsstatus

Overvågning tankmælk

Tilknyttede bes.nr Staldopdeling Indlæs Udlæs Øremærkebestilling Sundhedsstatus

Prøvetype: PCR

Sygdom Overvåg enkeltdyr Overvåg tankmælk

Prøvemateriale		Udtagningsdato	Modtaget dato
Kode	Tekst		
3	Mælk	30-08-2011	30-09-2011

PCR tilmeld

Code	Tekst	Ajourført	
		Af bruger	Dato
1	Årlig Tankmælk	H6601	30-09-2011

PCR resultater

Ejendom: [redacted] Udtagsdato: 30-08-2011

Bakterietype / gen	Resultat	Ajourført	
		af bruger	dato
C. bovis	31,0	H6601	30-09-2011
Mycop bovis	40,0	H6601	03-10-2011
Beta-lactam	32,0	H6601	30-09-2011
E. coli	39,0	H6601	30-09-2011
Strep dysgalactiae	32,0	H6601	30-09-2011
Mycop sp.	40,0	H6601	03-10-2011
Staph sp	30,0	H6601	30-09-2011
B-strep	33,0	H6601	30-09-2011
Strep uberis	30,0	H6601	30-09-2011
Alger	40,0	H6601	03-10-2011
Klebsiella sp	40,0	H6601	30-09-2011
S. marcescens	40,0	H6601	30-09-2011
A. pyogenes+P. ind.	40,0	H6601	30-09-2011
Gær	40,0	H6601	03-10-2011
Staf. aureus	35,0	H6601	30-09-2011
Enterococcus sp	34,0	H6601	30-09-2011

Luk

Vis flere

Vejleder X

Ejendom W

Besætning Q

Bedrift 1



Dansk Kvæg	Malkekvæg	PCR - Besætningsudskrift
	Bes-nr Kontrol dato 22.01.10 4	Udskrevet 08.02.10 15.32 Side 1 Jørgen Katholm 87 31 20 00 9985

Tankmælksundersøgelser

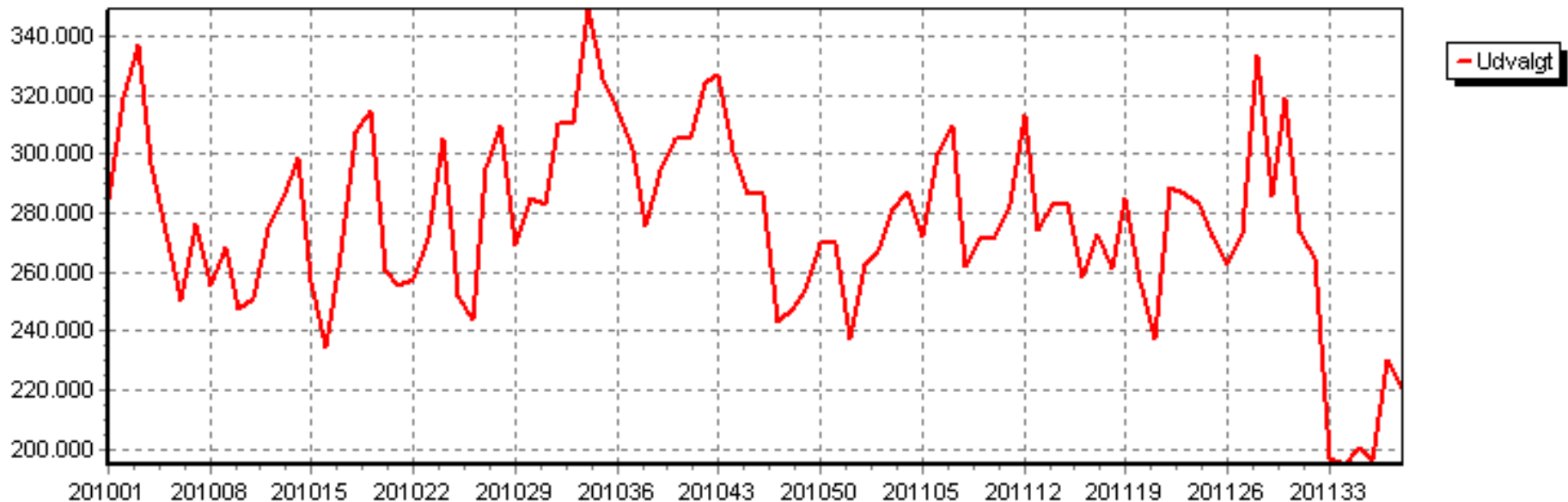
Dato	Staf a	Staf sp	Lac-tam	B-str	Str d	Str u	Ent	C. Bovis	E. Coli	Kle	S. Mac	A. pyo
20.01.2010	34,5	33,2	35,8	40	40	31,7	40	32	39,9	40	40	39,6

Enkeltdyrundersøgelser

CKRdyrn Dato	DEK	Staf a	Staf sp	Lac-tam	B-str	Str d	Str u	Ent	C. Bovis	E. Coli	Kle	S. Mac	A. pyo
-00023 2010	191	40	29,3	34,6	37,4	36,3	34,6	40	40	40	40	40	37,5
-00337 2010	415	40	24,8	26,2	40	40	40	40	26,1	40	40	40	40
-00549 2010	479	40	29,9	37,3	33,6	40	40	40	32,3	40	40	40	40
-00790 2010	194	40	27,4	30,1	40	40	39,4	40	27,7	40	40	40	40
-00830 2010	51	40	27,6	32,9	40	40	40	40	28,5	37,9	40	40	35
-01003 2010	374	40	37,4	40	40	40	40	40	28,4	40	40	40	40
-01031 2010	298	27,2	24,9	31,7	40	40	40	40	32,7	39,7	40	40	40
-01075 2010	176	27,2	24,7	26,6	40	40	38,9	40	30,2	40	40	40	40
-01077 2009	200	40	40	40	40	40	15,9	40	40	33,1	40	40	40
-01092	642	29,1	22,4	24,7	22,8	40	40	28,6	23,5	40	40	40	36

Segregation Segregation Segregation

- 31 marts 2011 18 GBS positive of 193 tested
- Isolated with *S aureus* cows in total 32 cows and treated in groups
- PCR tank pos may june neg july sept



Segregation segregation segregation

- Newinfected beginning of 2009. After 6 months prevalence 34%
- 22/4 2011 new test 22 cows PCR positive 7%



1 test 95 positive

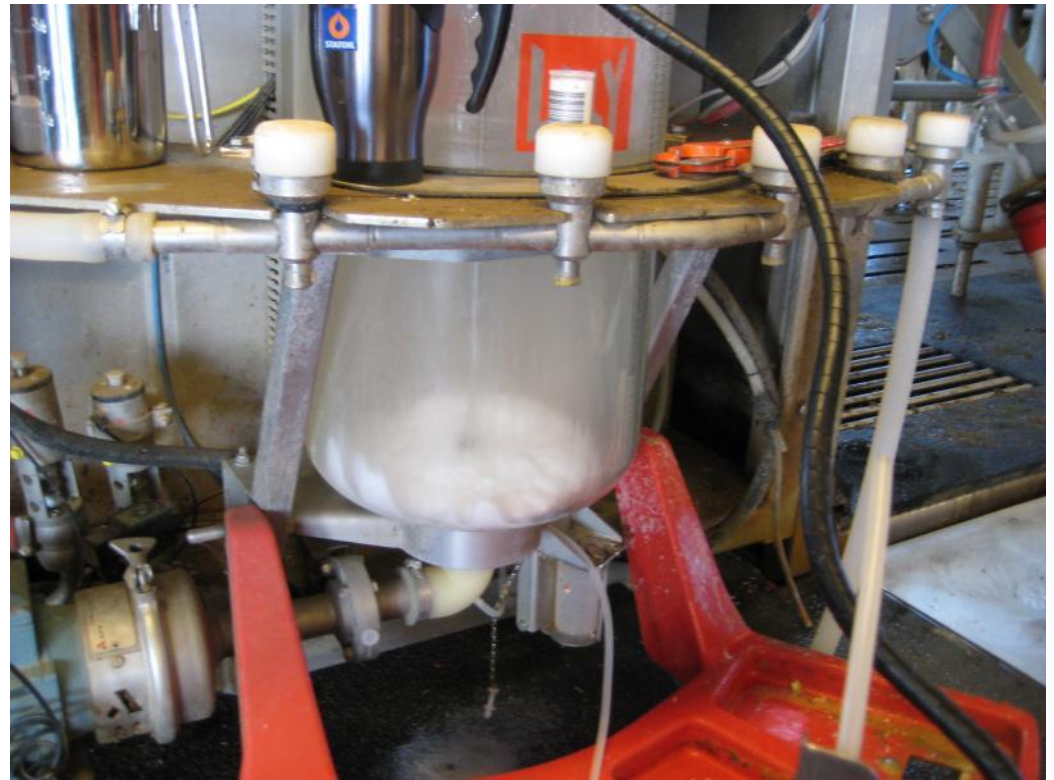
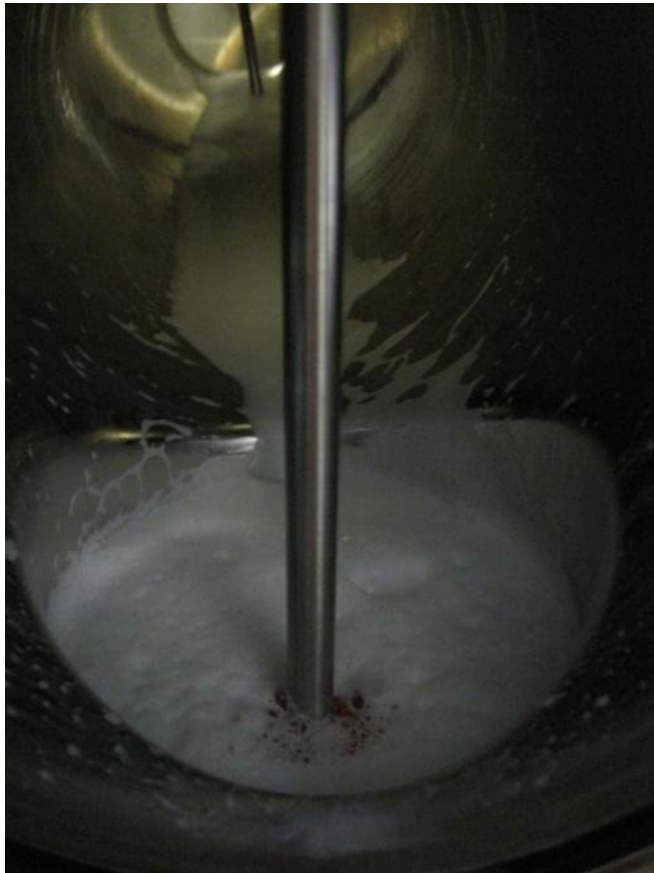
65 retested, 13 positive

Blitz Therapy

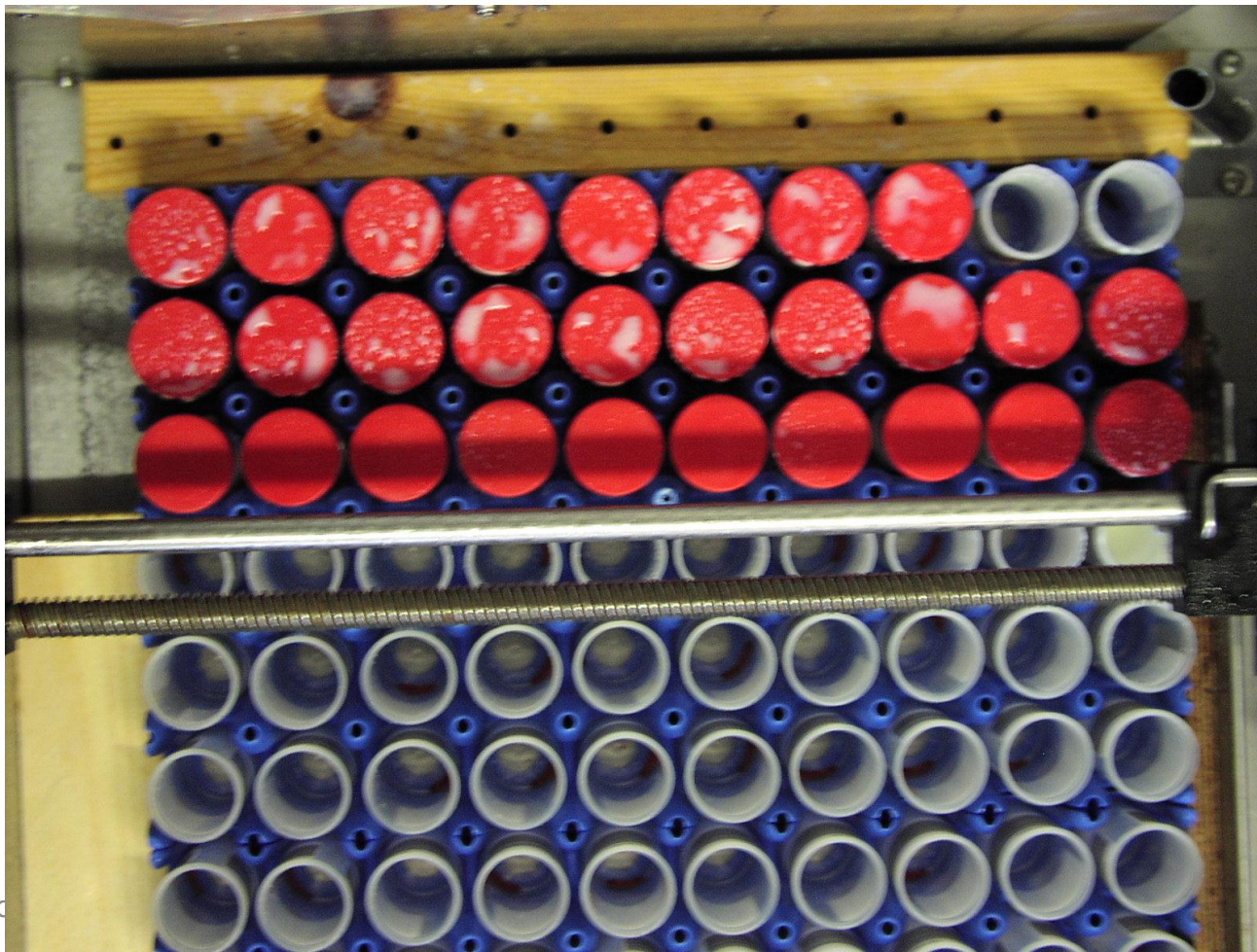
- It Works
- 87 GBS cows in 104 cows all treated 16 sep 2010
- Retested 8/10 2010 14 positive in 100 cows
- 19/10 2010 Negative culture Negative PCR



Focus area reduktion of carry over



Carry over – Somthing to think about New results VMS – 7,3%



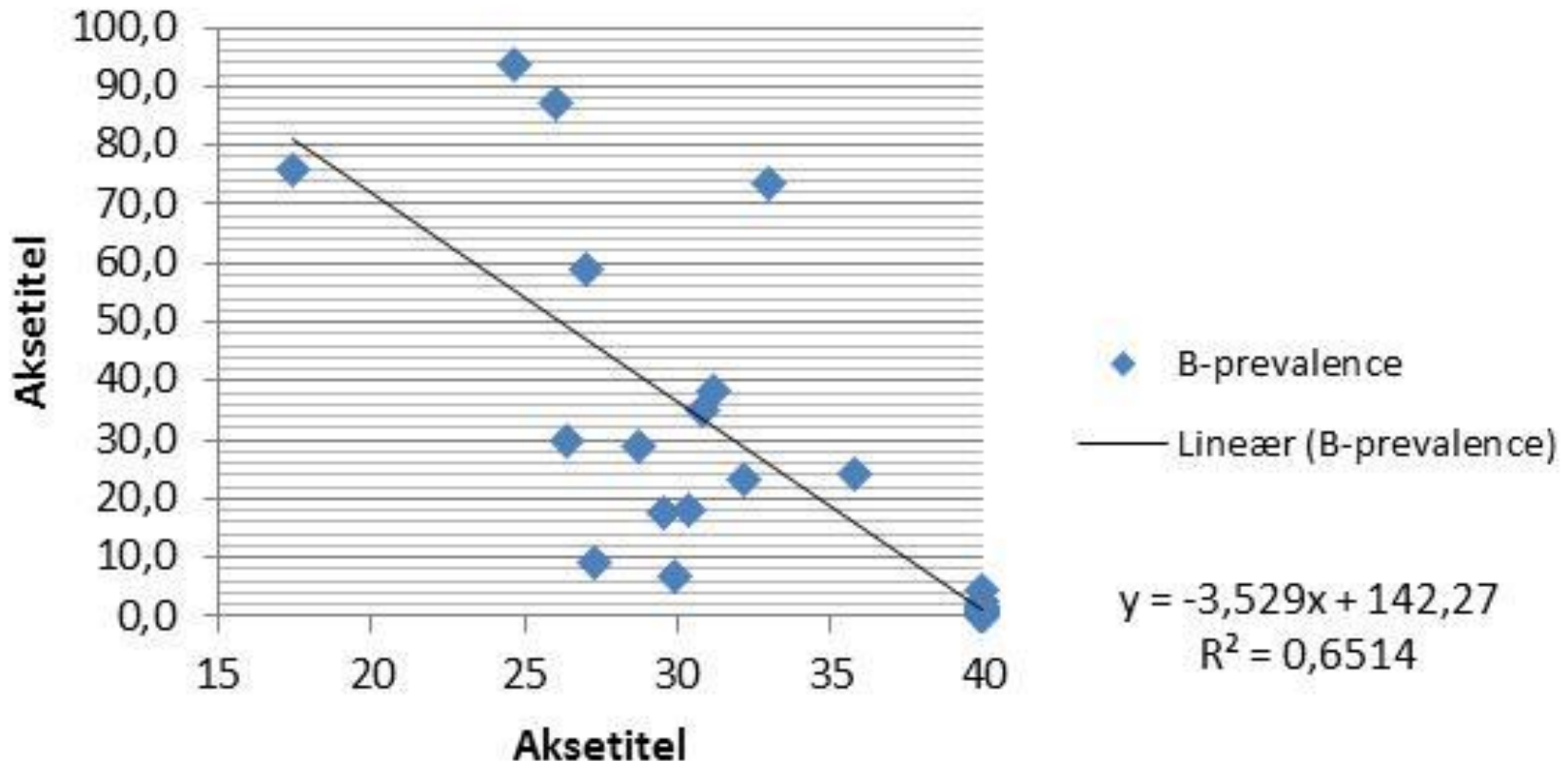


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chnr	I alt	<20	B<30	B>30<37	B>37	neg	Prævalens	Ct tank	Positive tank
1	303		3	50	16	234	23,0	32,2	14 ud af 16
2	168		9	48	2	109	35,0	30,8	12 ud af 17
3	257		46	158	20	33	87,0	26	19 ud af 19
4	111					111	0,0	40	0 ud af 19
5	98		4	13	12	69	30,0	26,4	17 ud af 17
6	212					212	0,0	40	0 ud af 24
7	128	1	4	67	22	34	73,4	33	16 ud af 17
8	317		21	48	19	229	28,8	28,7	41 ud af 41
9	114					114	0	40	0 ud af 30
10	171			1	1	169	1	40	0 ud af 30
11	295	1	4	15	2	273	7	29,9	22 ud af 22
12	54						0	40	0 ud af 15
13	147				1	146	0,7	40	1 ud af 30
14	206				1	205	0,5	40	4 ud af 20
15	160			1	1	158	1,3	40	5 ud af 40
16	120				1	119	0,8	40	2 ud af 25
17	138			1	1	137	1,5	40	1 ud af 16
18	94				4	90	4,2	40	1 ud af 20
19	55						0	40	1 ud af 43
20	97						0	40	0 ud af 17
21	154		4	23	10	117	24	35,8	9 ud af 11
22	193		2	12	4	175	9,3	27,3	14 ud af 17
23	120			2	1		2,5	40	0 ud af 17
24	100	4	17	48	7	24	76	17,4	26 ud af 26
25	126				1	125	0,8	40	0 ud af 19
26	251		3	30	11	207	17,5	29,6	17 ud af 17
27	60					60	0	40	0 ud af 18
28	116			2		114	1,7	40	0 ud af 22
29	123		7	11	4	101	17,9	30,4	20 ud af 22
30	157		26	46	21	64	59	27	36 ud af 36
31	113						0	40	0 ud af 15
32	68			22	4	42	38,2	31,2	33 ud af 33
33	166	2	46	99	9	10	93,8	24,7	38 ud af 38
34	77			1			1,3	40	0 ud af 15

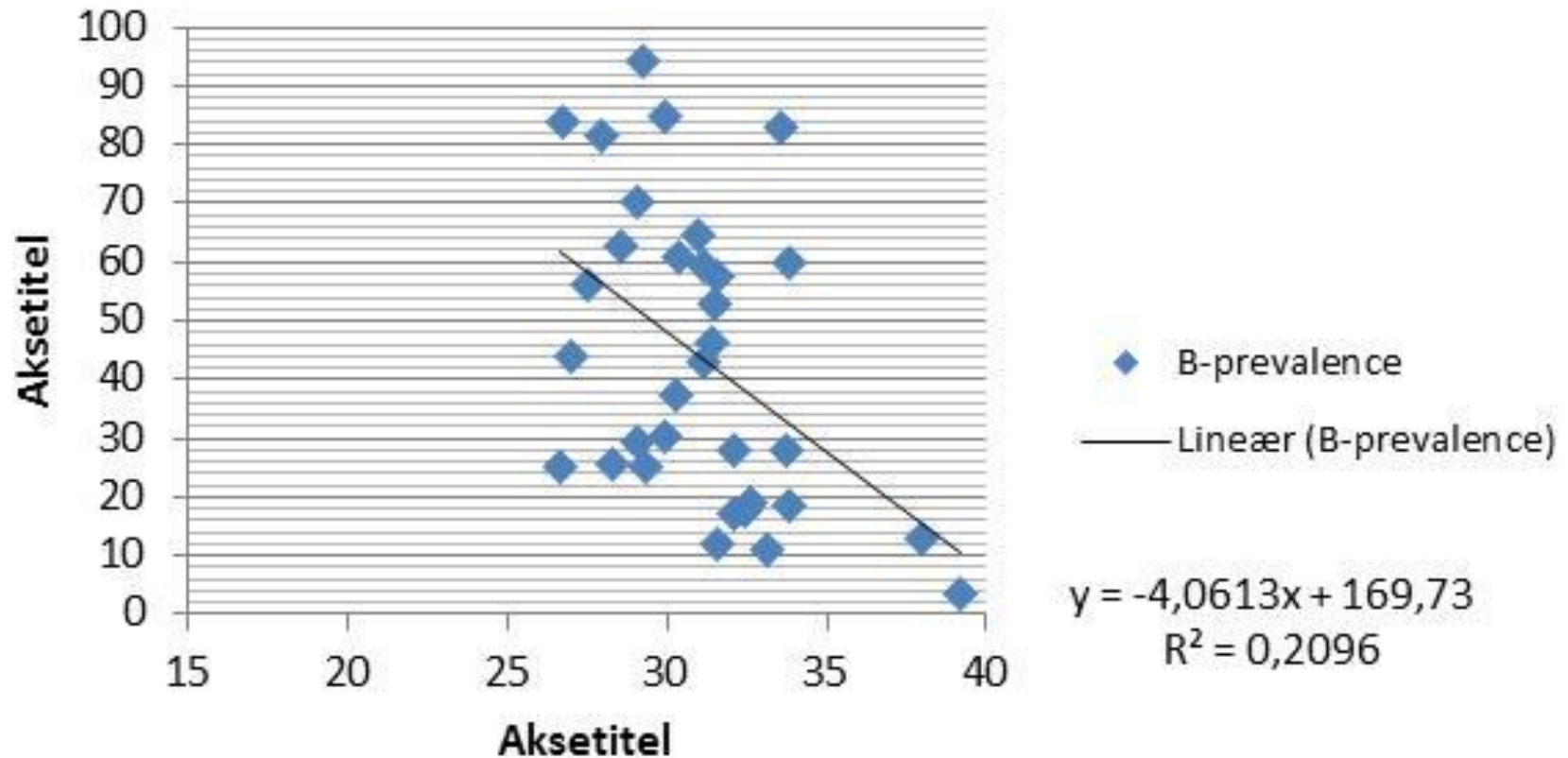
Bulk tank milk Ct værdi and cow prævalens

B-prevalence



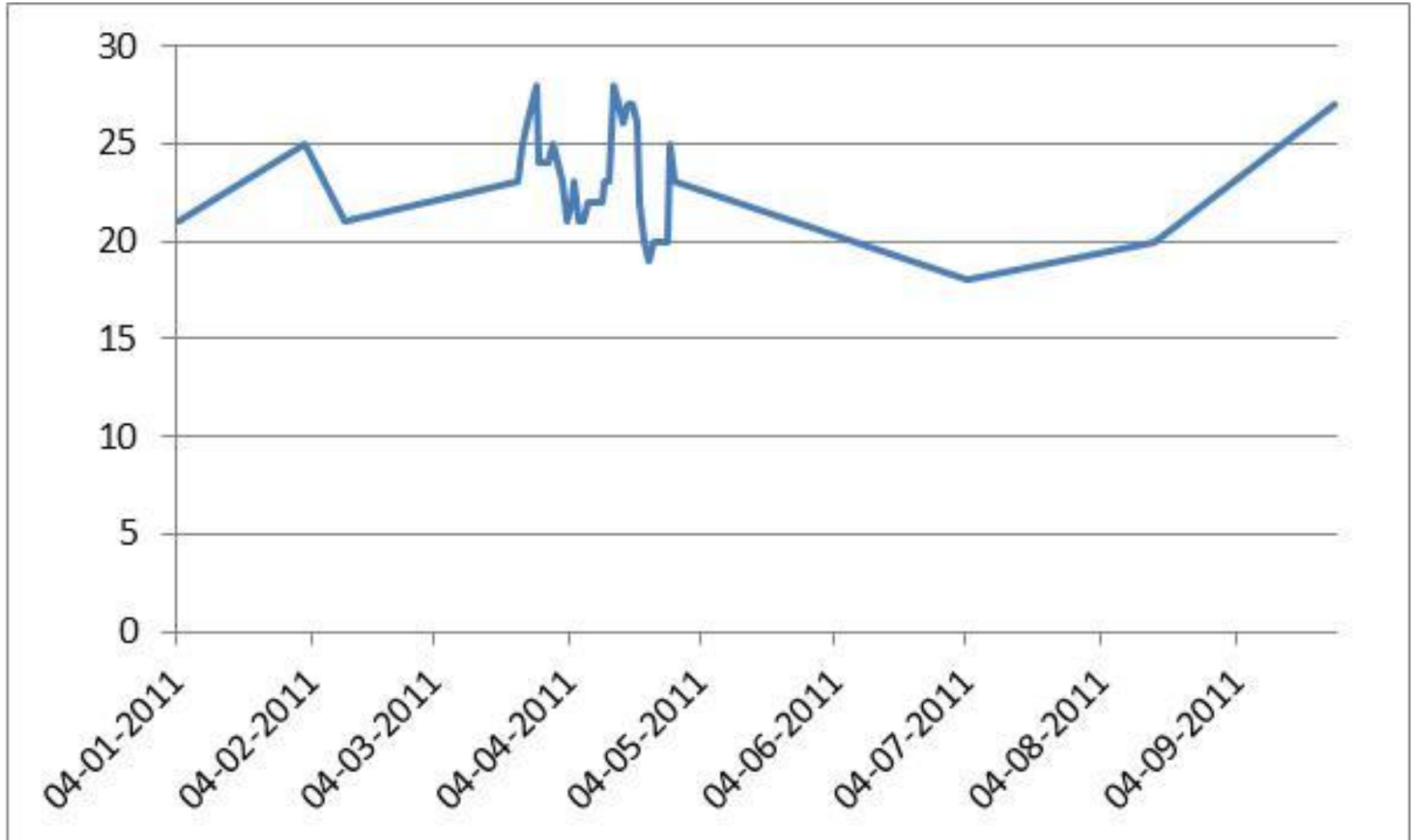
Bulk tank milk Ct værdi and cow prævalens

Staf a-prevalence





Day to day variation TG strep agalactiae

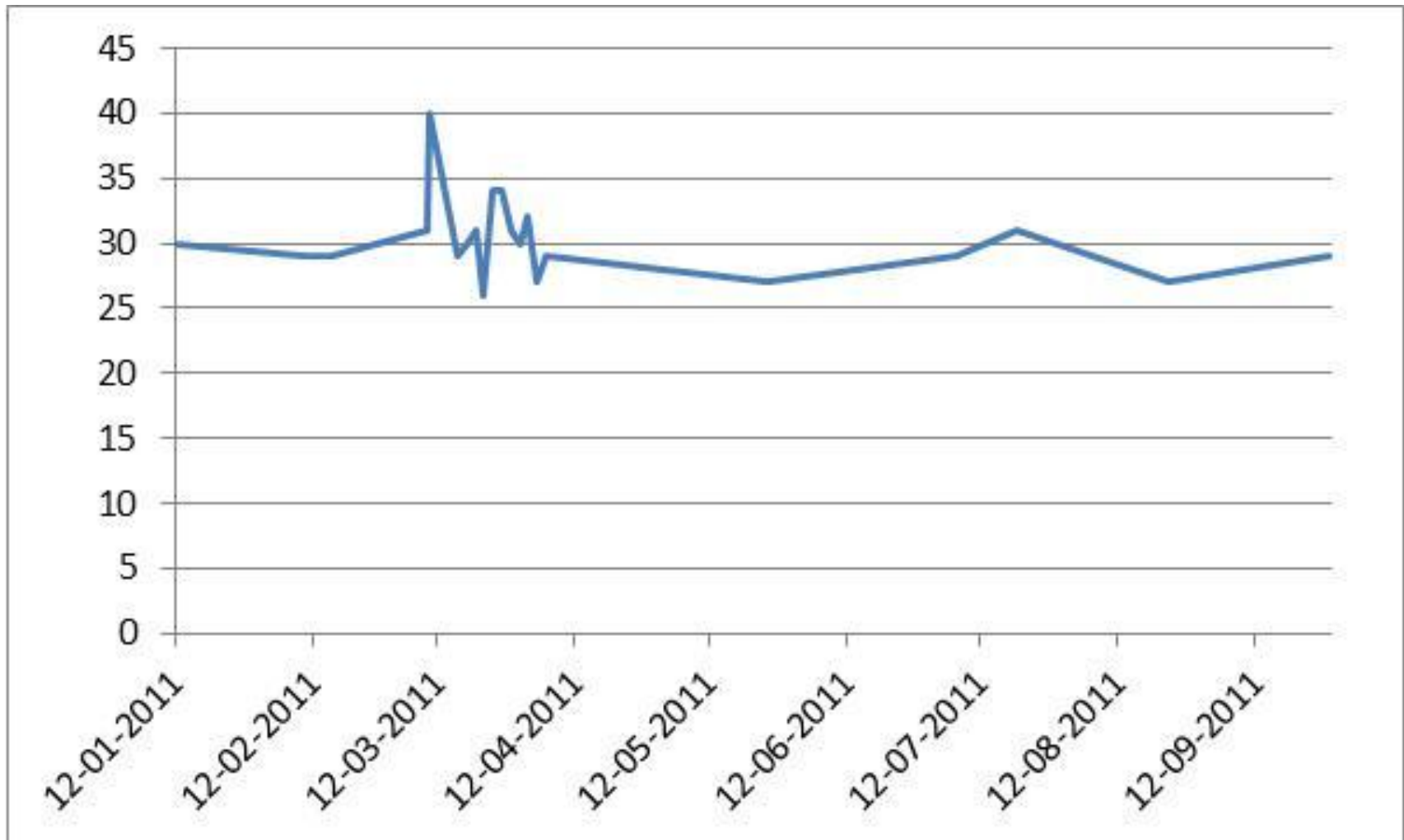


Day to day variation MIL strep agalactiae





Day to day variation MIL Staf aureus



Day to day variation MIL Strep uberis





Strep agalactiae eradications

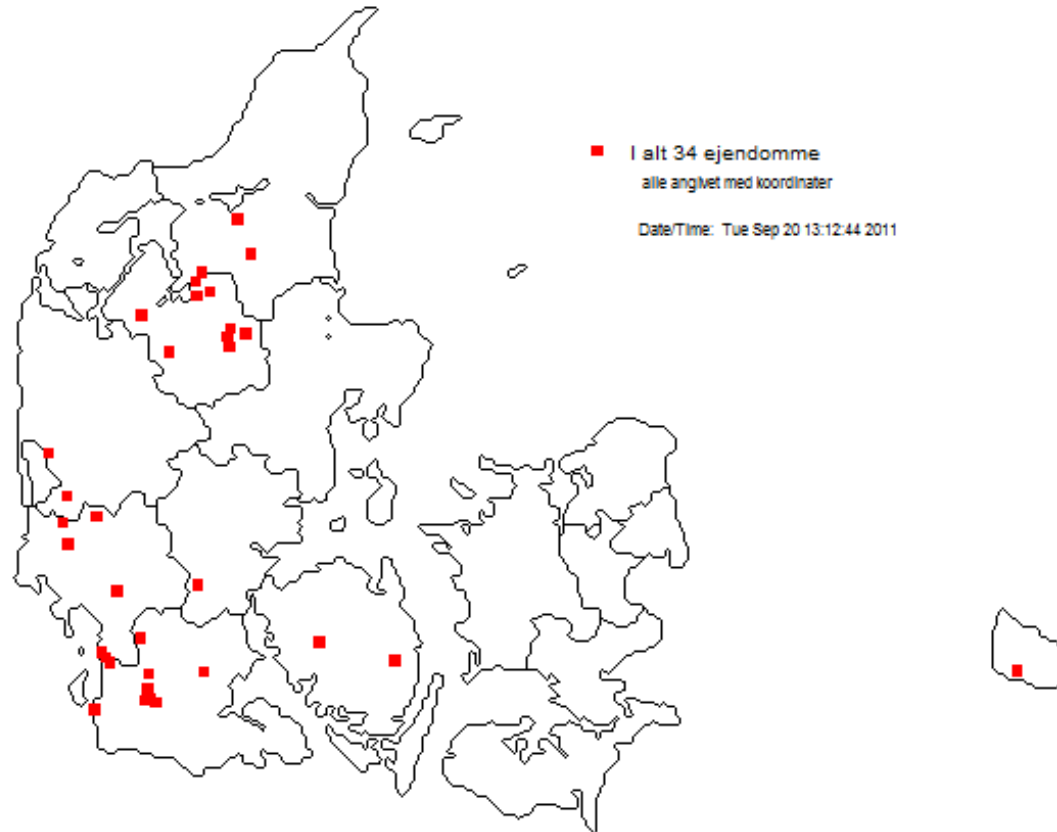
	Date for segregation	Milk tank prior to	Animals	Culture	PCR	positiv	Prevalens	Date	Result
Lely	19/1 2009	-	183			26culture/63 PCR	34	2/2 11	25
Sildeben	10/2 1909	-	135			5 culture/12 PCR	8	2/2 11	35,5
VMS	15/3 2009	-	126			18 culture/53 PCF	42	FRI 2/11 10	
Lely	19/8 2009	-	303	281		95	34	2/2 11	29,6
Lely	1/10 2009	-	175		159	29+13	26	8/11 10	27,7
Lely	25/11 2009	-	125		107	8+3	10	FRI 18/1 1	
	15/6 2010	27.3 6/5 2011		24 Pool in 120 cows		1 positive out of 60 c	26		
	20/09 2010	21.6 30/6 10				64 16+10	40		
Lely	16/12 2009	27.7 9/11	181		149	79+12	61	4/1 11	26,1
	23/12 2009	19.6 9/11	103		94	17+3	21	11/1 11	23,0
	26/1 2010	25.8 28/10	50		48	1	2	Fri 25/10 10	
	1/2 2010	30.1 28/10	42		Pool	3 pool - 3 cows	7		25-okt neg culture
	8/2 2010	32.3 9/11	75		68	0+2	3	1/11 10	pos dyrk
Herringbon	10/2 2011	40 1/11	71		Pool	pos pool 5 pos cows			
	18/2 2010	28.9 9/11	78		75	16	21	13/4 10	NoCt
	8/3 2010	34.5 12/11	301		54 Pool	15	5	FRI 2/11 11	
	3/3 2010	25.7 9/11	105		102	9	9	2/11 2010	neg culture
-	14-07-2010	30.3 11/9	85		Pool 17	sitiv of 45 single c	22		
Lely	8/3 2010	30.3 12/11 09	270		275	12+4	6	20/1 11	40
	1/11 10	31.4 2/9 10	330		pool 13	pos out of 65 anim	2		
	20/4 2010	30.8 9/11 09	105		3 out of 19 Pool	10 individual cows	4	FRI 26-08-2010	
	4/5 2010	37.8 9/11	158		11 out of 28 Pool	individual cows 5-	4		02-nov 29,7
	13/8 2010	26.1 20/10 09	122		104	77+10	84	19/10 10	neg culture
	05/8 2010	40.0 2/11 09	54			1	2	Fri	
	30/8 2010	30.1 3/11 09	86		76	4	5	Fri	
				Pool all neg			0	Fri	
	30/9 2010	22.2 20/10 09	122		104	95	91	18/10 10	påvist
Lely	27-09-2010	28.5 20/10 09			186	114	61		
Lely	29/10 10	20.8 2/6 10			327	103+51	47		
	07-12-2010	31.2 18/10 10	45		9 (from 43cows)	out of 15 single c	9	Fri	4/4 2011
	12-09-2010	26.1 17/8 10	146		120	40+8	33		11-nov påvist
	19-10-2010	30.6 9/9 2010	234		195	9+6	8		02-nov 40
	Kontrol 6/3		71		58	9+7	28	1/11 10	NoCt
	17/12 2010	25.5 24/11 10	82		70	7	10		
	10/9 2009	-	166	high cel		7	4	FRI 19/10 10	
Lely	21/10 10		189		104	9+1		17/11 10	2 culture n
	14-01-2011	27.1 24/11 10	47		Pool	7+1 out of 21	17	Fri	40
VMS	09-02-2011	27.6 16/1 11		113		40	35	Fri	
	21-03-2011	31.3 22/3 2011			195	20+13	17		
	14-mar		305		Pool	9 pool 11 cows	4	FRI	

Klinik – ledbetændelser forben (8/16)



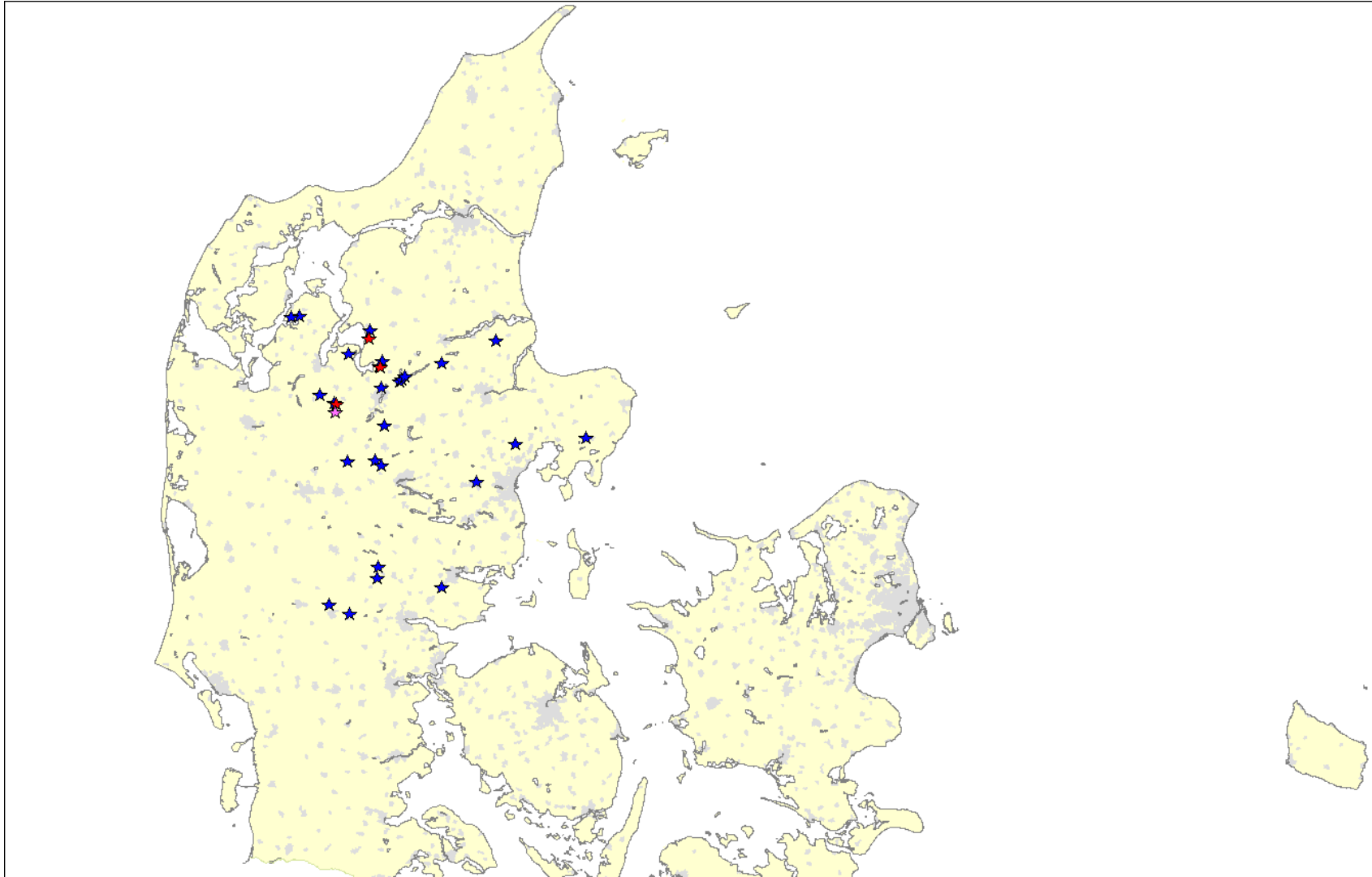
34 herds with clinical problems caused by "Mycoplasma"

Mycoplasma



2011- 09 test of 372 bulk tank samples Mycoplasma (16-Kit)

4 positive *Mycoplasma bovis*, 29 positive all *Mycoplasma*





Our Milk
- a pure pleasure