Detection of *Streptococcus agalactiae* in Danish Dairy Herds
Work package 1

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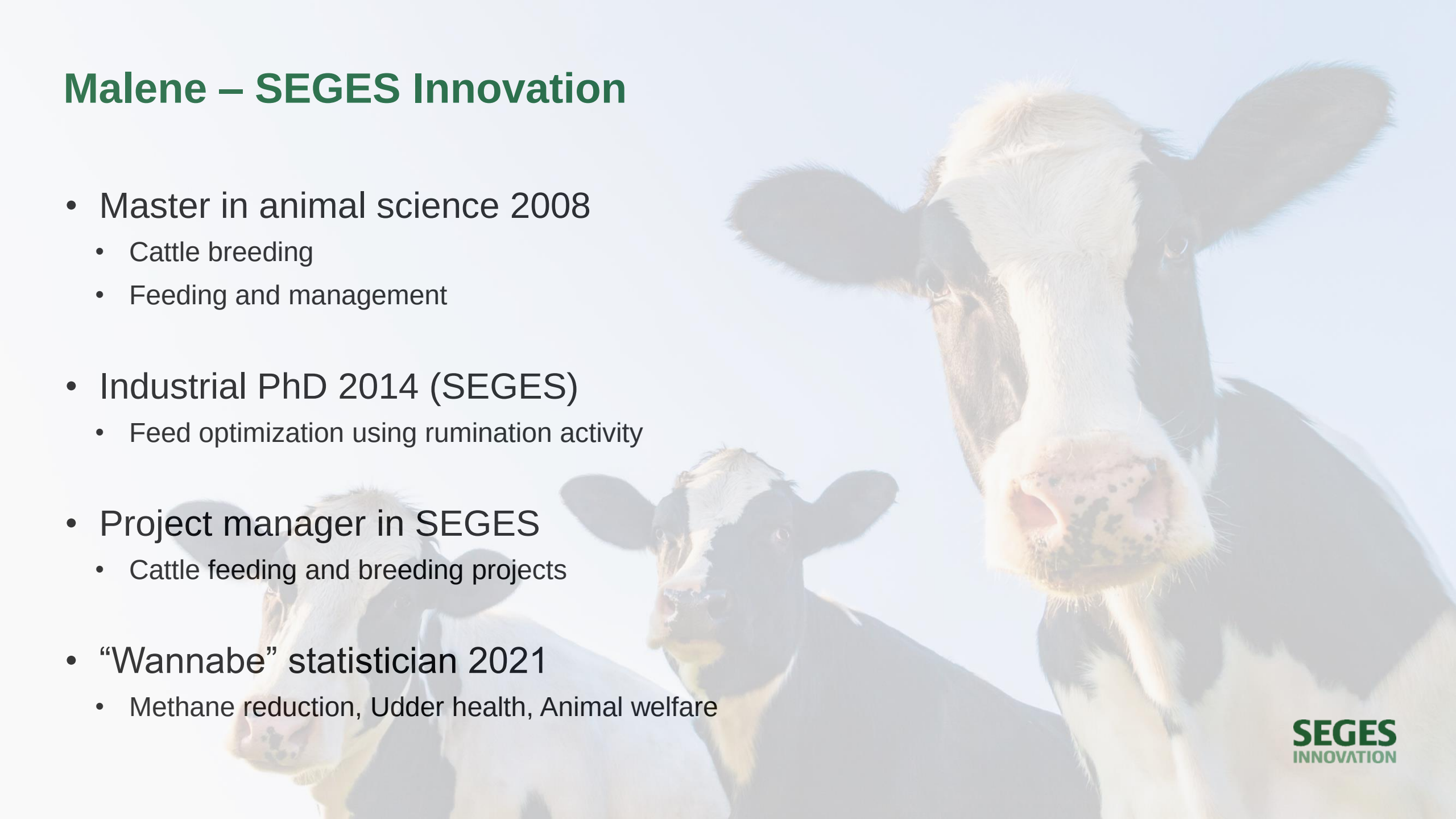
Malene – SEGES Innovation

- Master in animal science 2008
  - Cattle breeding
  - Feeding and management
- Industrial PhD 2014 (SEGES)
  - Feed optimization using rumination activity
- Project manager in SEGES
  - Cattle feeding and breeding projects
- “Wannabe” statistician 2021
  - Methane reduction, Udder health, Animal welfare



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Background – WP 1

- *Streptococcus agalactiae* (*S. agalactiae*) is a contagious pathogen
  - Causing subclinical mastitis and high somatic cell count
  - Production losses and impaired milk quality

- Persistent infection, however fluctuating or cyclic pattern of shedding
  - Challenging the monitoring of prevalence and infection rate

- Mandatory surveillance program of *S. agalactiae* in Denmark
  - How are we doing with the current surveillance program?
  - Changes in strategy and detection methods?

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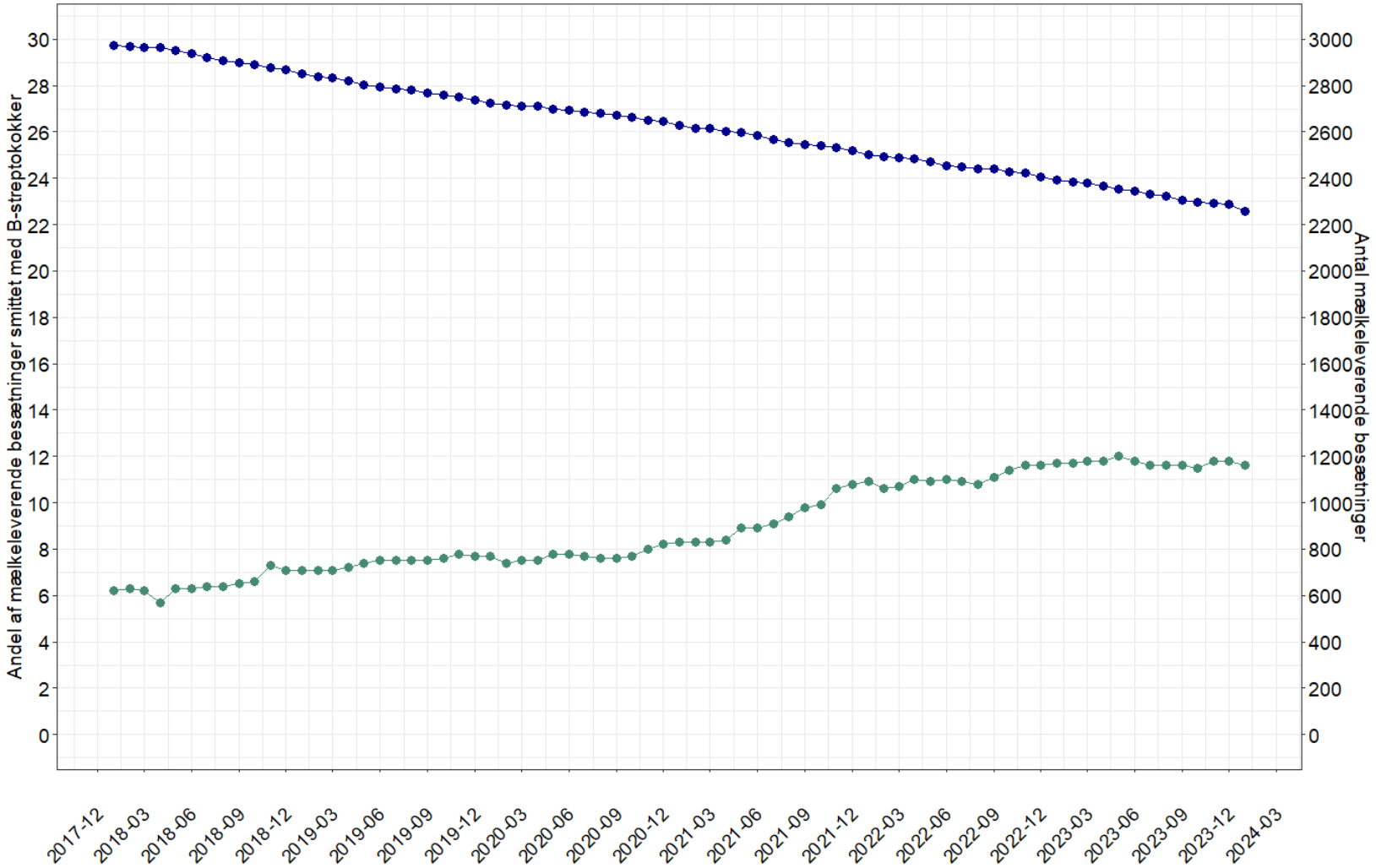
Surveillance of *Streptococcus agalactiae*

- Increasing percentage of infected herds – decreasing number of herds



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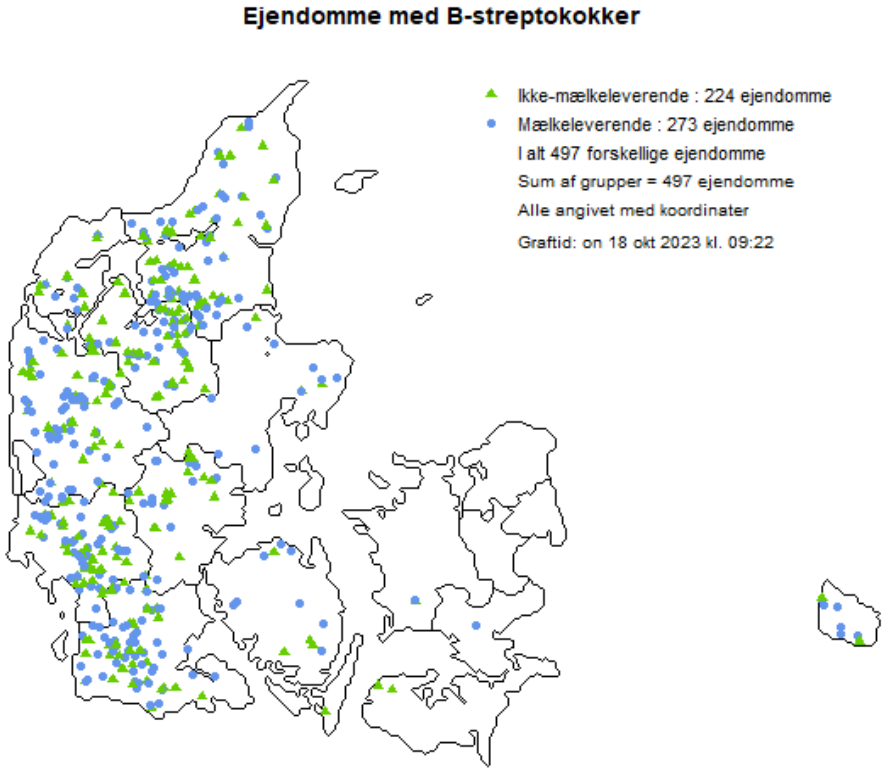
Surveillance of *S. agalactiae* – status to infected

- Currently, the status regarding *S. agalactiae* can be changed from “not infected” to ”infected” based on:
  - PCR Ct value below 40 on the bi-annually mandatory bulk tank sample and thereafter followed by at least one out of two bulk tank samples below Ct value 40, drawn within 30 days from the first sample, with at least 8 days between.
  - Purchasing livestock from infected herd or placing livestock together with livestock from an infected herd (e.g. farm shows, grazing etc.)
  - PCR Ct value below 30 for *S. agalactiae* from voluntary routinely drawn milk samples from dry cows or detection of *S. agalactiae* in milk samples from clinical mastitis cases (since June 2021)



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Surveillance of *S. agalactiae*

- Regaining status ”Not infected” of *S. agalactiae*
  - Four bulk tank samples with Ct value of 40 with an interval of minimum 30 to maximum 45 days between the samples.
  - Either analysis of quarter samples from all cows or analysis of quarter samples gathered into one from all cows in the herd – within the same day – all negative results.

*Ved undersøgelse på enkeltdyr kan status fri først godkendes, når der foreligger et negativt resultat fra køer behandlet med antibiotika eller golde på prøvetidspunktet. Køer behandlet med antibiotika skal undersøges ved enkeltkirtelundersøgelse eller samleprøve mindst 4 uger efter sidste behandling. Goldkøer (både goldbehandlede og ikke behandlede) skal undersøges ved enkeltkirtelundersøgelse 5-7 dage efter kælvning

Kilde: Vejledning om B-streptokok mastitis hos kvæg https://www.retsinformation.dk/eli/retsinfo/2018/9110

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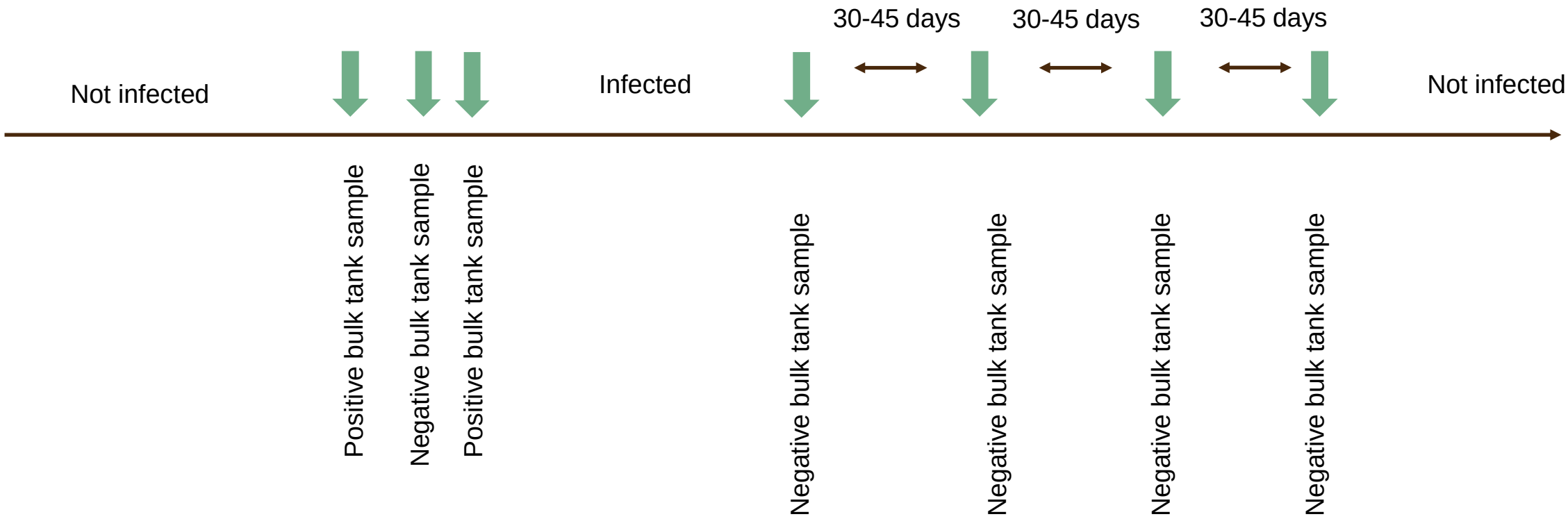
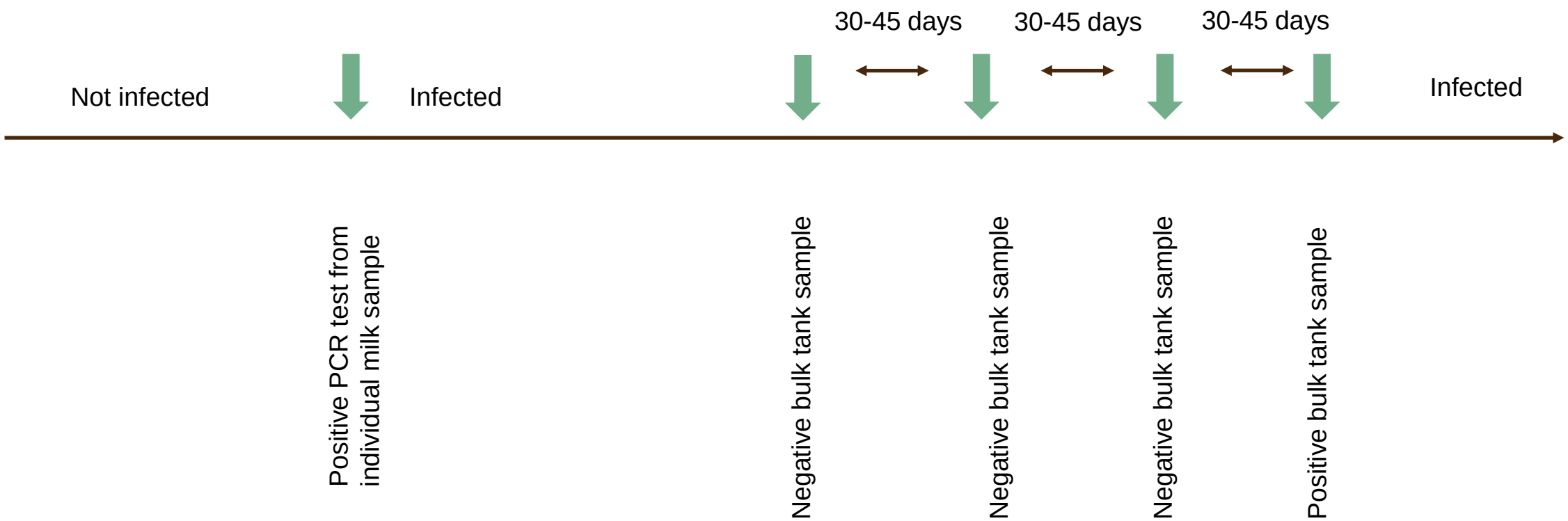
Case herds regarding status – not infected

Case herds regarding status – infected – not infected



Case herds regarding status – infected – still infected



Surveillance of *S. agalactiae* – bulk tank samples

- After each round of bulk tank sampling following is checked:
  - Are the any samples that needs to be re-run – non-interpretative results?
  - Any herds with two or more samples? The first results is included.
  - Herds that lack sampling
  - Distribution between not infected and infected samples on status of the herd at the day of sampling

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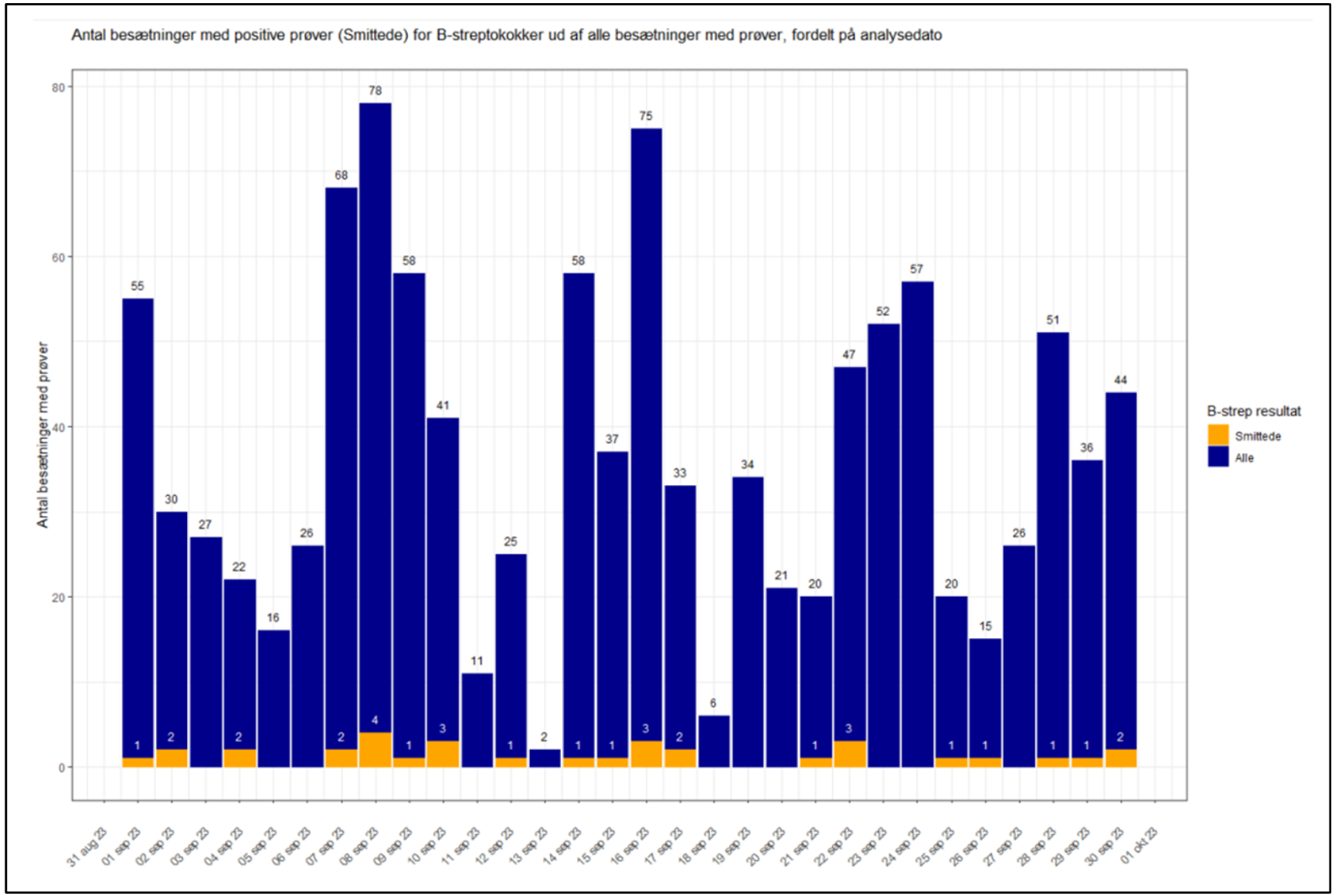
Surveillance of *S. agalactiae* – individual samples



Monthly analysis of individual samples from voluntary individual dry cow samples – in co-operation with yield control compagnie (RYK)

Purpose: To evaluate whether or not positive samples are found within specific dates – due to cross contamination at the lab.

So far – no accumulation of positive samples from ”not infected” herds at specific dates

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Introduction to analysis of detection methods

- *Detection methods of S. agalactiae* since July 2021
  - Detection of *S. agalactiae* in bi-annually collected bulk milk samples tested with PCR
  - Detection of *S. agalactiae* from clinical mastitis cases (quarter level)
  - Detection of *S. agalactiae* in voluntary cow-level milk samples, collected prior to dry off
  - Herds purchasing livestock from herds infected with *S. agalactiae,* will be given the status "infected"
- Including additional detection methods has the potential to improve the ability to find infected herds
- Aim
  - To evaluate the distribution of detection methods related to herds shifting status to "infected".
  - Explore the extent *S. agalactiae* can be rediscovered in the standard mandatory bulk milk samples

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Materials and Methods

- Data from the Danish Cattle Database
- Data from July 2021 – June 2023
- Records of *S. agalactiae* status and dates of change in status
- Status either “infected” or “not infected”
- Data on:
  - Findings of *S. agalactiae* in bulk milk samples,
  - Results from PCR or bacteriology - presence of *S. agalactiae* from individual cow- or quarter milk samples
  - Status on *S. agalactiae* of herds from where cows were being moved from

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Results

- Distribution of detection methods
- For all herds found infected within the period
  - 56 % changed to “infected” based on findings of *S. agalactiae* in individual cow milk samples (PCR or bacteriology)
  - 18 % changed status to “infected” based on findings of *S. agalactiae* in bulk milk samples

- Rediscovering *S. agalactiae* in bulk milk samples
  - In herds where *S. agalactiae* was detected due to individual milk samples (PCR or bacteriology), the pathogen was only rediscovered in the subsequent bulk milk sample, in approximately 11 % of the cases.

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Conclusion

- Conclusion of the study
  - Combining different methods of detecting *S. agalactiae* in dairy herds, might improve the detection of infected herds
  - *Streptococcus agalactiae* was only rediscovered in bulk milk samples in a small proportion of infected herds detected by individual sample
  - Hypothesized, that this can be due to
    - Infected cows are dry or slaughtered
    - Dilution of the pathogen in bulk milk of large herds

- Further research
  - Further research is needed to establish how the within herd prevalence of *S. agalactiae* and volume of the bulk milk of infected herds, affects the ability to correctly identify infected herds.

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Frequent bulk tank samples

- Fluctuating pattern of shedding of *S. agalactiae*

- Analysis of frequently drawn bulk tank samples
  - Ct value below 40 – ”infected” otherwise ”not infected”
  - Two periods of frequent sampling - during summer and fall 2023
  - 3 herds

- Compare with individual milk sample
  - PCR below 30 – ”infected” otherwise ”not infected”

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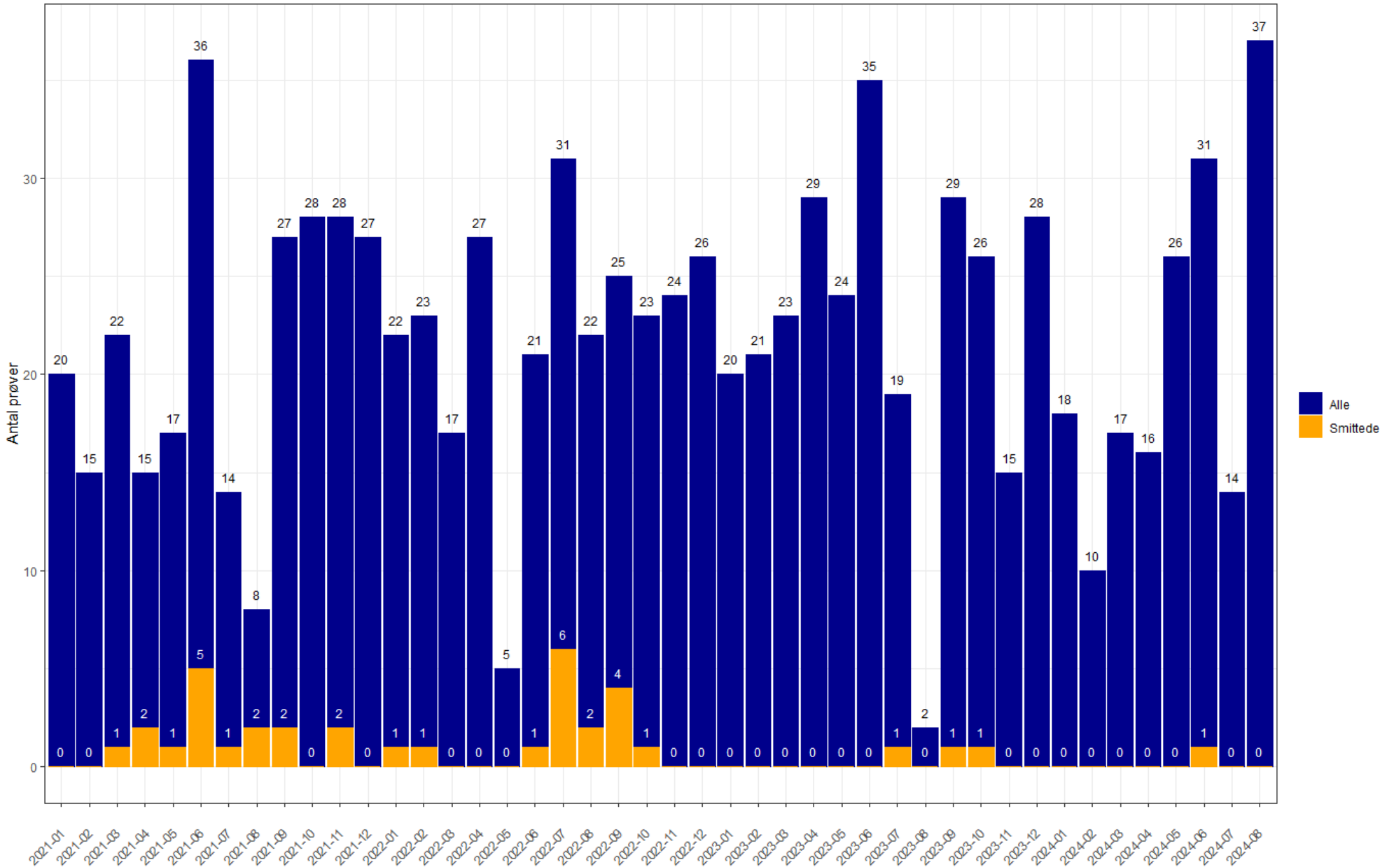

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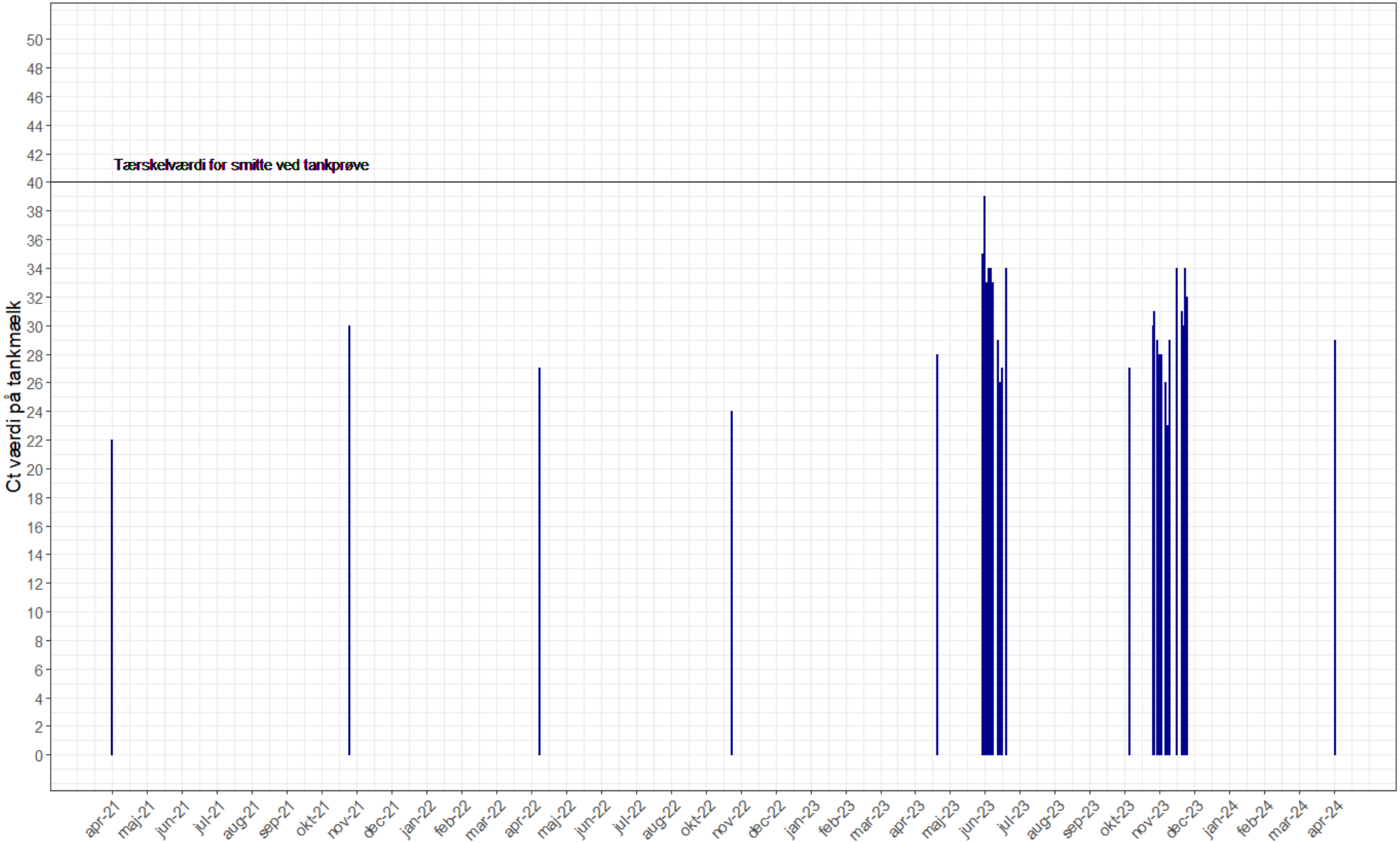

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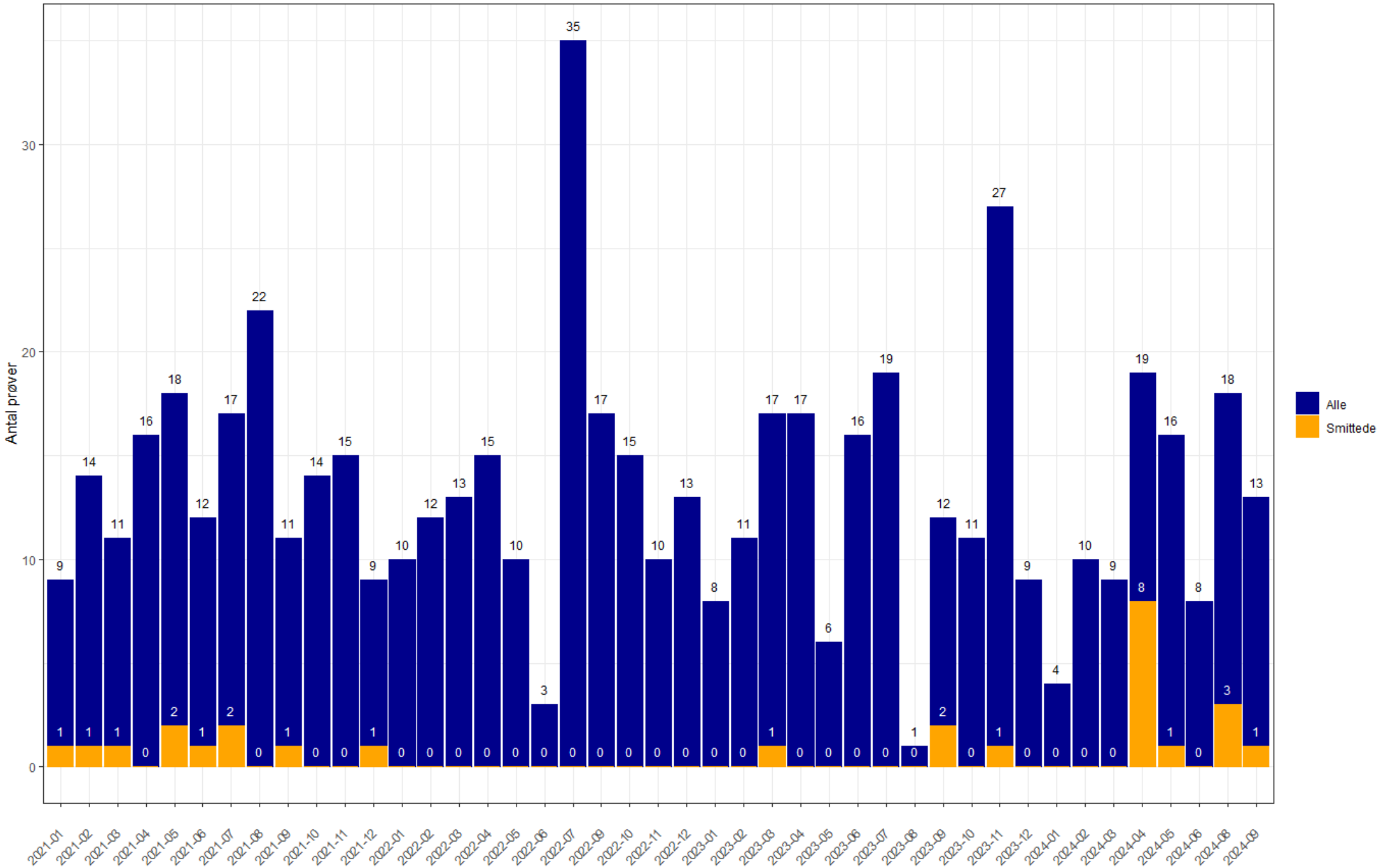

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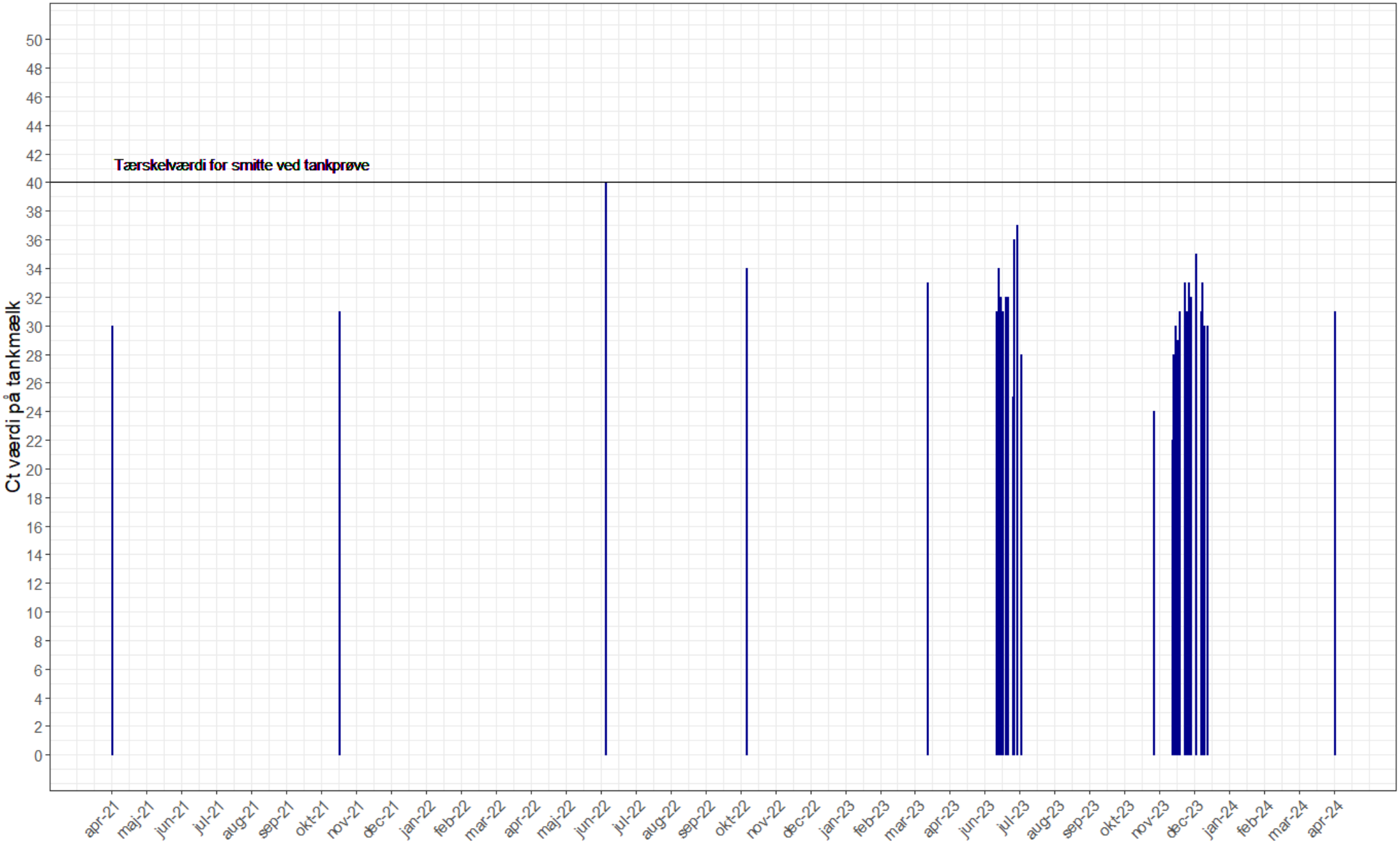

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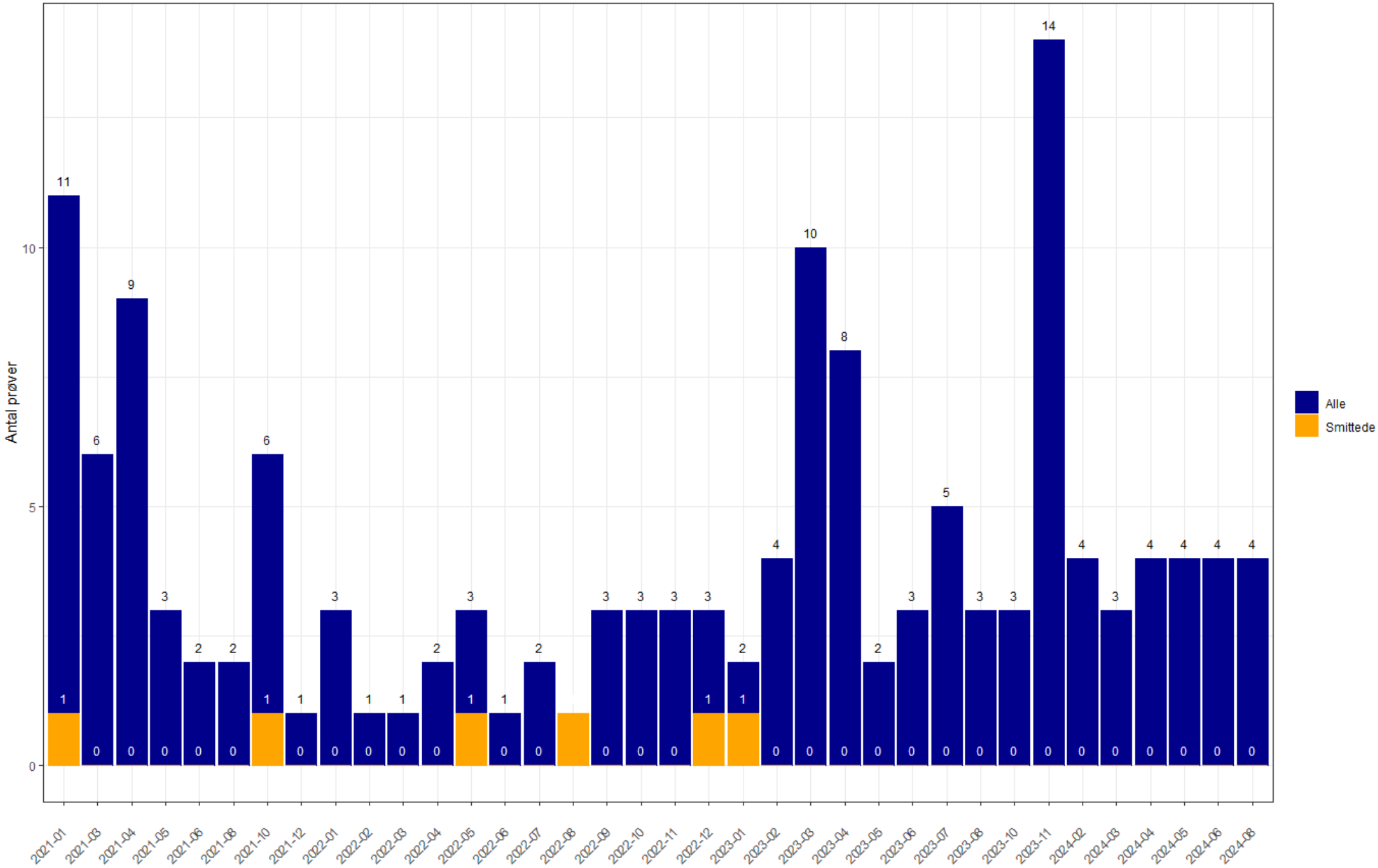

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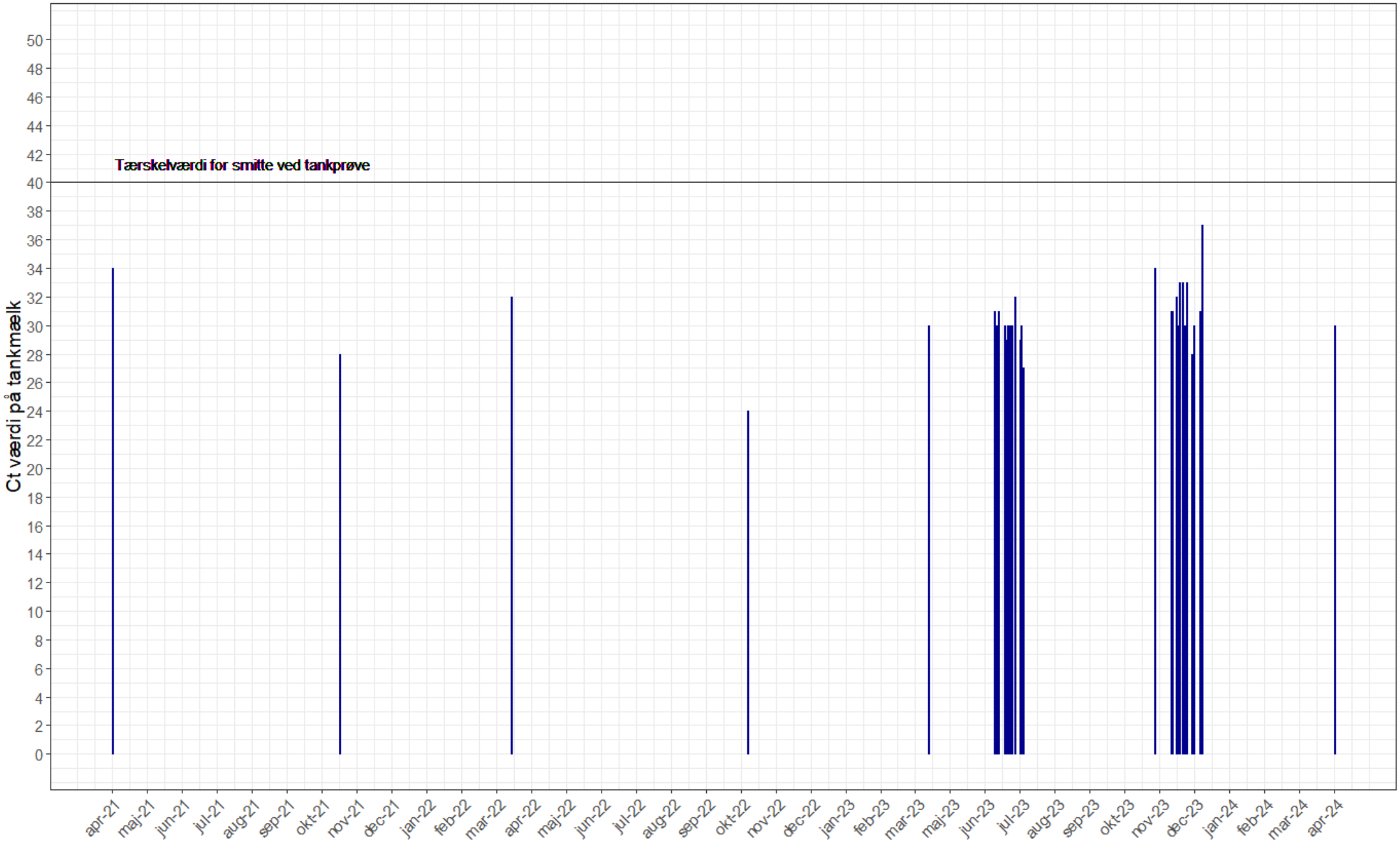

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Statistical analysis

- Does the Ct value in bulk tank samples in infected herds depend on the quantity of milk delivered?
- In other words - Does the volume of the bulk milk of infected herds, affects the ability to correctly identify infected herds by bulk samples?

- Data
  - Relation between Delivered milk (bulk volume) and Ct-value in mandatory bulk tank sample
  - Infected herds – confirmed by continuous PCR samples at dry cow level
  - Delivered milk volume – usually every second day
  - Account for period of bulk tank sample – Spring and Fall
  - Account for percentage of infected cows in period between bulk tank samples

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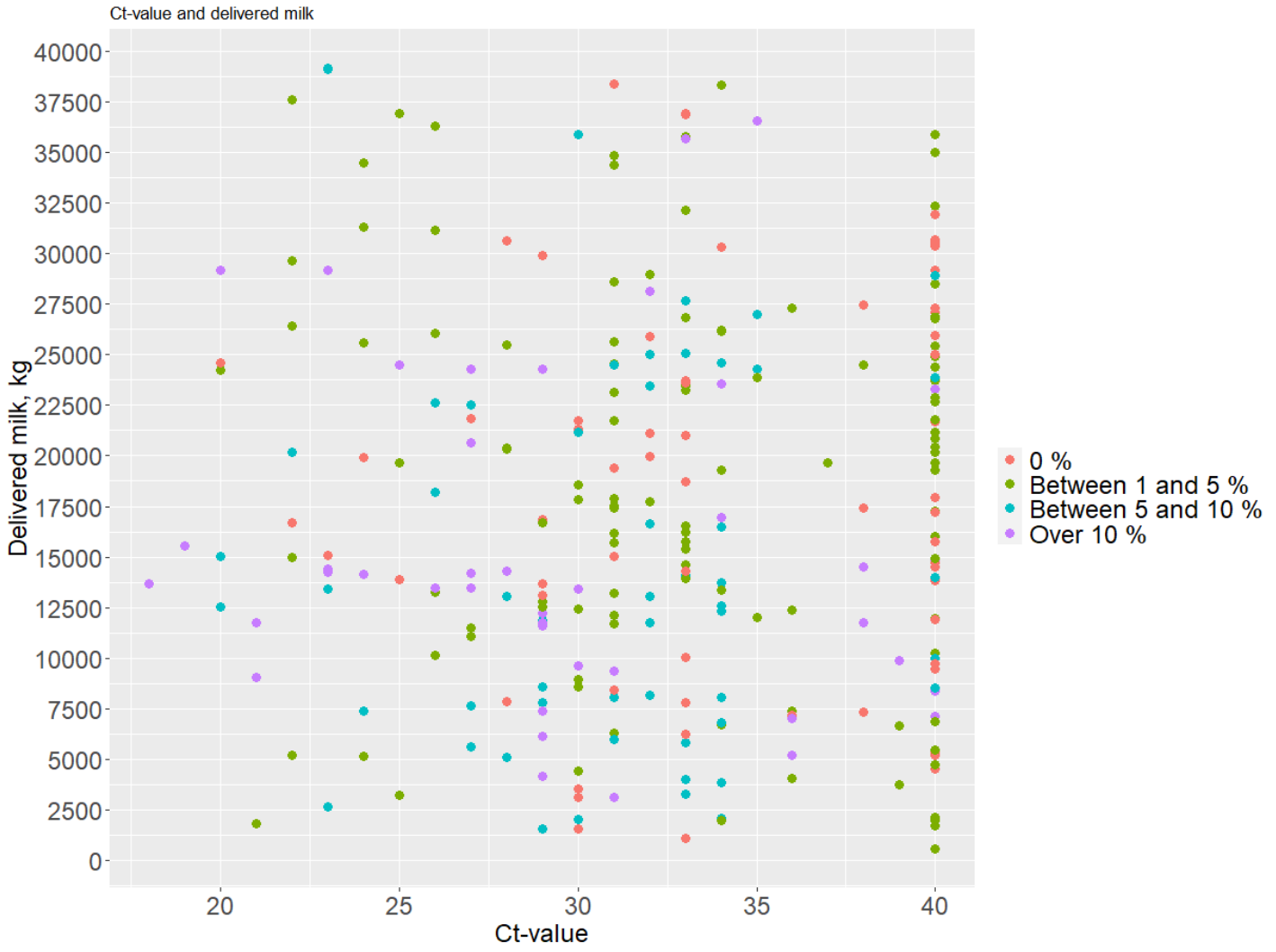
Bulk tank samples in infected herds?

- Does the volume of the bulk milk of infected herds, affects the ability to correctly identify infected herds?
- No specific pattern –
  - Relation between Ct value and Delivered milk
  - Percentage of infected cows found by PCR samples



Statistical analysis

Statistical model

Ct value ~ Delivered milk quantity + period + Percentage infected cows + (1|CHRNR)

Parameter	Estimate	Std. error	P-value
Intercept	33.4	1.10	<0.0001
Delivered milk, kg	-0.005	0.04	NS
Period (Spring (vs. Fall)	0.275	0.59	NS
Percentage infected 0-5 % (vs. 0 %)	-0.61	0.84	NS
Percentage infected 5-10 % (vs. 0 %)	-1.77	1.00	NS
Percentage infected above 10 % (vs. 0 %)	-2.49	1.18	NS

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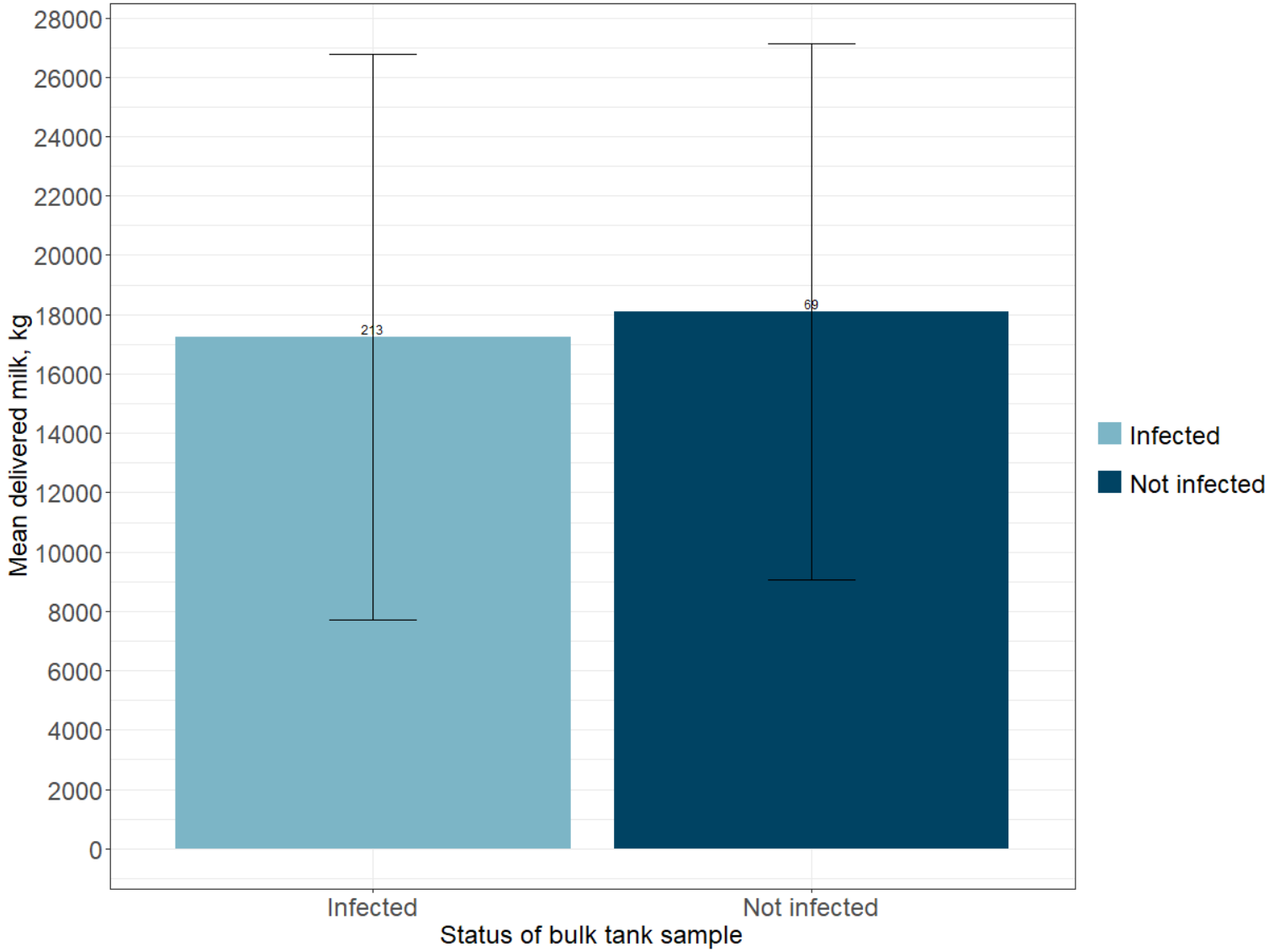
Bulk tank samples – all Ct values within infected period

- Delivered milk from infected herds
- All Ct value within infected period
- Large variation between herds
- No difference between average delivered milk in herds

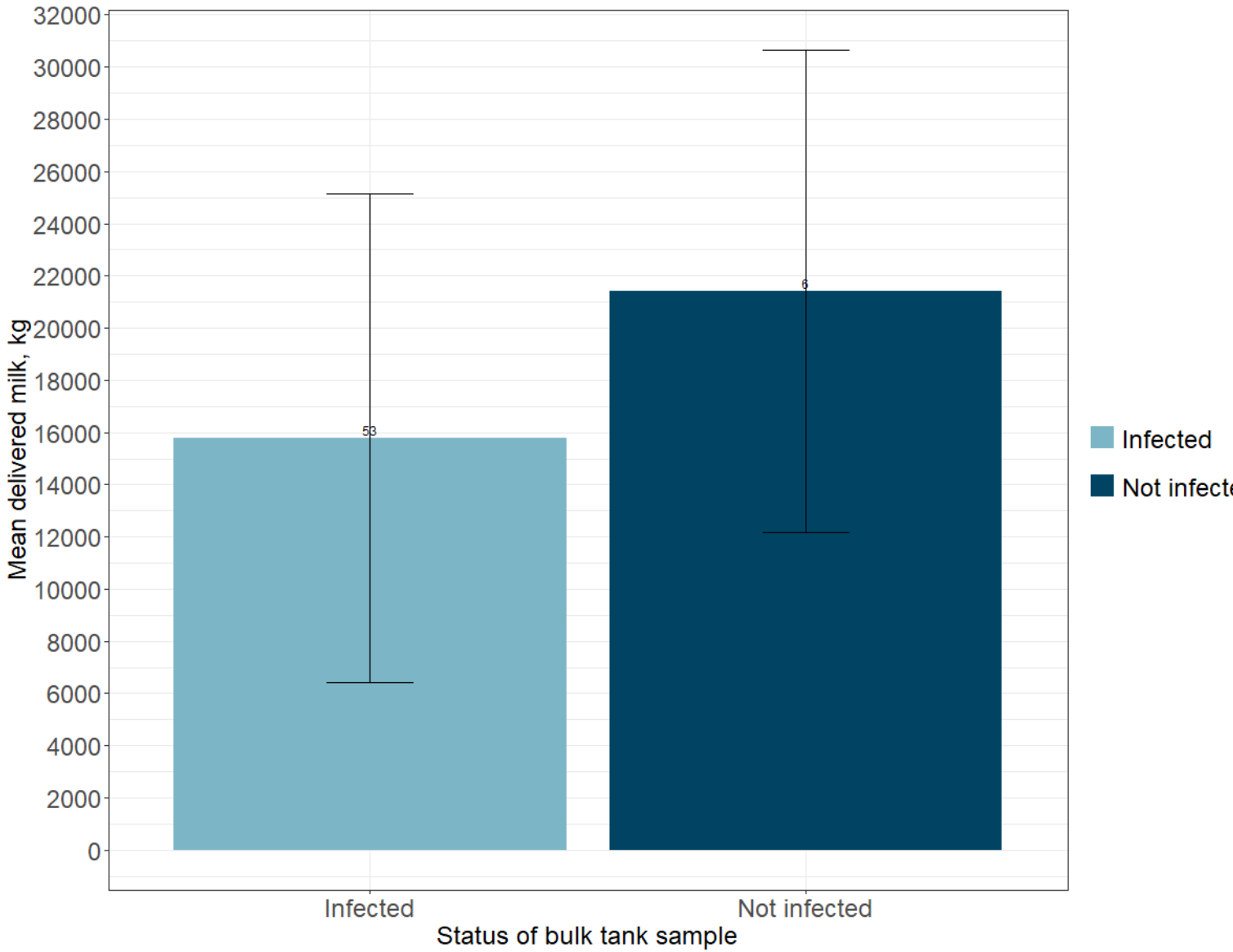
Status – bulk tank	N	Mean (SD), kg
Infected	213	17.244 (9376)
Not infected	69	18.095 (9036)



Bulk tank samples – lowest Ct value

- Delivered milk from infected herds
- Lowest Ct value within infected period
- Still large variation between herds
- Lowest numerical average delivered milk in herds with Ct value below 40

Status – bulk tank	N	Mean (SD), kg
Infected	53	15.787 (9376)
Not infected	6	21.416 (9236)




Thank you for your attention!
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Questions for discussion

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