Booklet of abstracts

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INTRODUCTION

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Juan Muñoz Bielsa Corporate Ruminants Marketing Director 1

Calf health: foundations for a healthy start



Efficacy of an oral solution of paromomycin for the treatment of newborn calves with cryptosporidiosis in European farms

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Objective

Cryptosporidiosis is a frequent parasitological infection of mammals including calves. The objective of this work is to provide insights from two large field studies conducted in Europe about the efficacy and safety of paromomycin used orally at a dosage of 150 mg/kg BW per day for 5 consecutive days against cryptosporidiosis (*C. parvum*) in naturally infected pre-ruminant calves.



Materials and methods

Two independent multicenter European field studies involving 44 dairy and suckling beef farms were carried out following a parallel, controlled, randomized, blinded design. Farms were selected based on recent history of C. parvum infection in calves, absence of concomitant preventive treatments against cryptosporidiosis, absence of recent or current BVD infections, and no recent history of diarrhoea due to coronavirus, E. coli and rotavirus, or alternatively dedicated vaccination. To be enrolled, newborn calves were required to meet the following criteria: age between 3-14 days on first treatment, faecal score \geq 2 (loose to watery), negative rapid test for E. coli, coronavirus, rotavirus, and positive rapid test for C. parvum. Any calf that presented with diarrhoea for > 24 hours or that has been previously exposed to antibiotics, parasiticides or probiotics was excluded. Animals were randomly allocated to two treatment groups. Calves in group 1 received 150 mg paromomycin sulfate/kg b.w. [Gabbrovet Multi®, Ceva], once daily, for 5 days by oral route while calves in group 2 were orally administered 100 µg halofuginone/kg b.w. [Halocur®, MSD], once daily, for 7 days. Several clinical and parasitological parameters were monitored at fixed times during the 21 days of the study: faecal score (0-3), general health observation (0-3), hydration score (0-3), and oocyst counts (number of oocysts per gram of dry faeces). Percentages of calves clinically cured on day 8 was considered a pivotal outcome to evaluate the efficacy of the interventions. Calves were considered cured if for a minimum of two consecutive observations all their clinical scores were equal to zero, to the exception of faecal score that could be equal to zero or 1. Other criteria such as time to clinical cure, number of days when calves were asymptomatic or considered in relapse from Day 0 to Day 8 and the parasitological cure at day 7 (oocyst count = 0) were also assessed. Safety (adverse events, serious adverse events) was evaluated at any time during the studies. The statistical unit was the calf.

Five hundred twenty-five calves, with a mean age of 9 days and a mean body weight of 44 kg were enrolled. Sixty percent were from Holstein-Friesians breed, with remaining calves from various beef or crossbred breeds (Aubrac, Blonde, Limousin, Charolais, Fleckvieh). Sixty-nine percent of calves were female. 260 calves were allocated to treatment group 1, 265 to the treatment group 2. At inclusion, the treatment groups were found comparable for clinical parameters and oocysts count. 93.4% (242/260) of calves in group 1 and 80.6% (212/265) in group 2 were considered clinically cured by day 8. Clinical cure rate in group 1 was found superior to those observed in group 2 (p<0.0001). In addition, calves in group 1 were found to be cured faster in comparison to calves in group 2.

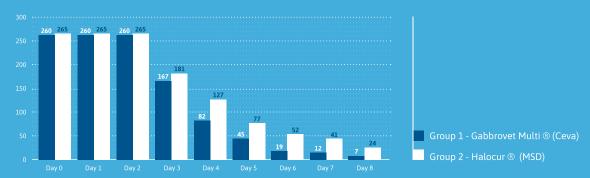


Figure 1: Calves at risk of not being cured

Hazard ratio (group 1 vs. group 2) was 1.40, indicating a higher likelihood (+40%) of being cured in group 1 at any time (best model). Relapses from day 2 to day 8 were low in calves from group 1 and 2 (2.7 and 2.9% respectively). The parasitological cure on day 7 was similar between the 2 groups, with a value of 77.4% in group 1 and 78.8% in group 2. The oocyst count decreased steadily after treatment in calves of group 1 from an initial mean log of 11.8 to a mean log of 2.9 on day 7. At least one adverse event was reported in 9.6 and 13.6% of calves in group 1 and 2 respectively. While none of these adverse events were considered possibly related to paromomycin, it was possibly related to halofuginone in two calves.



In these European field studies, daily oral treatment with 150 mg/kg of paromomycin (Gabbrovet Multi®) for 5 days was found safe and highly effective to cure sick dairy and beef calves with cryptosporidiosis and to control their oocyst burden. In addition, this treatment regimen was found superior to treatment based on halofuginone.

Impact of 150 mg/kg dosage of paromomycin on gut microbiota in healthy calves

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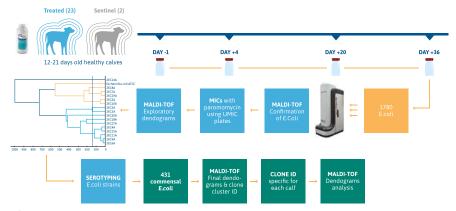
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Objective

This study aimed to evaluate the effect of oral paromomycin administration over five days on resistance development in the intestinal commensal microbiota of healthy calves.



Materials and methods

Twenty-five healthy calves aged 12 to 21 days, originating from seven French farms, were included in the study. They were housed collectively, fed milk replacers twice daily, and randomized into a treatment group (Gabbrovet Multi®, Ceva Santé Animale, 150 mg/kg daily for five days; n=23) or a control group without treatment (n=2). Daily monitoring included fecal consistency, depression scores, and appetite (evaluated on a 0-2 scale). Fecal samples were collected at four time points: before treatment (Day-1), during treatment (Day+4), and after treatment (Day+20, Day+36). Samples were immediately frozen at -80°C then transferred to the microbiology lab for isolation and microbiological analysis of commensal *Escherichia coli* strains. For each fecal sample, 20 purified and randomly selected *E. coli* colonies were selected. To manage the high number of strains, related strains were grouped using mass spectrometry (MaldiTof Biotyper Compass Explorer software). Minimum inhibitory concentrations (MICs) for paromomycin were determined using a customized microdilution method (UMIC), along with aminoglycoside antibiograms following CLSI guidelines. **Resistance evolution was tracked by comparing strain data at different times relative to Day-1**, using the CA-SFM kanamycin breakpoint for Enterobacteriaceae.

A total of 1,780 *E. coli* strains were isolated and analyzed. On Day-1, commensal *E. coli* populations comprised 69% susceptible and 31% resistant clones. **Treatment with paromomycin did not induce resistance in previously susceptible** *E. coli* **clones. Instead, the treatment eliminated a substantial proportion of susceptible** *E. coli***, resulting in a temporary predominance of preexisting resistant clones within the intestinal microbiota. This disruption was short-lived, as susceptible clones began to reappear post-treatment**. Indeed, by Day+36, susceptible *E. coli* accounted for 40% of the population, compared to 7% on Day+20, 10% on Day+4.



Evolution of the % of resistant (white) and susceptible (blue) E. Coli commensal clones during the study (n=431)

MIC levels of E.coli commensal clones present at Day-1

Calf Clone N°	Day-1 Before trt	Day+4 During trt	Day+20 After trt	Day+36 After trt	Evolution
2-6				_	
2-11	32	32	32	32	\rightarrow
3-15	2	2	-	-	\rightarrow
3-17	2	2	-	-	\rightarrow
3-20	1	1	-	-	\rightarrow
5-26	512	512	-	_	\rightarrow
6-29	1024	-	1024	-	\rightarrow
7-30			-	-	
7-31	>1024	>1024	-	-	\rightarrow
8-32	>1024	>1024	-	>1024	\rightarrow
9-34	2	-	2	-	\rightarrow
9-35	2	2	2	2	\rightarrow
10-47	4	4	4	4	\rightarrow
13-52	2	2	-	-	\rightarrow
14-55	4	4	-	-	\rightarrow
15-56	2	2	2	2	\rightarrow
18-58	128	*	128	-	\rightarrow
18-59	128	128	-	-	\rightarrow
19-61	2	2	2	-	\rightarrow
19-62	4	-	4	-	\rightarrow
19-63	2	2	2	-	\rightarrow
20-65	2	2	-	-	\rightarrow
21-71	4	-	4	-	\rightarrow
22-77	4	-	4	-	\rightarrow
25-90	8	8	-	-	\rightarrow
25-92	4	-	4	-	\rightarrow

[&]quot;-" relates to the absence of detection for a given clone at a given time

CONCLUSION

No evidence of resistance acquisition in commensal *E. coli* was observed during this study. MIC values for individual clones remained stable across sampling points. The paromomycin treatment effectively disrupted the microbiota by favoring preexisting resistant clones, but the flora gradually reverted to its initial composition after treatment cessation. The high bactericidal dosage used in this study likely played a role in preventing resistance development. However, caution is advised for lower dosages (e.g., prophylactic regimen), which are known to increase the likelihood of resistance emergence.

Efficacy of a fixed combination of florfenicol and meloxicam to address lung lesions in veal calves with BRD

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Objectives

Bovine respiratory disease (BRD) is a syndrome affecting up to 61 % of veal calf herds. The diagnosis in field and treatment decisions are generally based on examination for BRD clinical signs. However, the low sensitivity and specificity can delay diagnosis and caused treatment failure. In contrast, lung ultrasonography is recognized as a more performant tool to detect BRD and to assess the lesions' healing process. To better characterize the BRD prevalence in veal calf herds and the healing process of lung lesions after treatment, a field study was performed using lung ultrasonography as screening tool and a combination of florfenicol and meloxicam for treatment.

Materials and methods

Animal care and procedures were in accordance with the European Directive 2010/63/EU and the national law D.L. 2014/26. A total of 84 veal calves of 30 ± 9 days and 53 ± 5 Kg were enrolled in the trial. Animal clinical and ultrasonography examinations were performed twice weekly for the first 60d and then weekly until 180d. Lungs were collected at the slaughterhouse to evaluate gross lesion prevalence. Clinical examinations were used to establish Wisconsin (BRD \geq 4) and California (BRD \geq 5) scores. The lung ultrasonography was used to establish ultrasonography score (US; 0-5 points score) and modified lung lesion score (LLS; BRD \geq 10.5). Cranial lesions depth (cm) was measured for each lesion of both lung sides. Animals with the US \geq 3 or cranial lesion depth \geq 3cm and at least 1 clinical sign were treated with one subcutaneous administration of a fixed combination of florfenicol and meloxicam (40mg/Kg+0.5mg/Kg;

Zeleris®, Ceva Santé Animale). Treated animals (Flor-Mel group) was monitored at +1, +3, +5, +7, +9, +11, and +14d post-treatment. The non-treated animals during the production cycle were classified as control group. The success (animals that improved after treatment), relapse (animals that required retreatment), chronicity (animals without improvements and with more than three treatments), and mortality (dead animals for BRD) rates of the treatment were evaluated until 45 days after treatment (45-day rates). Differences over time in the Flor-Mel group were assessed by PROC GLIMMIX procedure of S.A.S.-software using the effect of time, sex, breeds, and animal (random and repeated effect). A post-hoc pairwise comparison was performed using Bonferroni correction. Groups comparisons in disease prevalence and lung gross lesion were assessed by chi-square test. The average daily gain (ADG) was evaluated by one-way ANOVA for group effect. A *p-value*<0.05 was accepted.



Flor-Mel and Control groups enrolled respectively 36 and 48 calves. The BRD prevalence was low and similar between groups at arrival (Control: 2.1% for Wisconsin score, 6.3% for California score, 0% for US, and 0% for cranial lesions depth; Flor-Mel: 0% for Wisconsin score, 2.7% for California score, 0% for US, and 0% for cranial lesions depth). No differences were also observed at the end of the production cycle in disease prevalence or ADG (Control: 0% for Wisconsin score, 0% for California score, 22.9% for US, 10.1% for cranial lesions depth, ADG 1.50Kg/d; Flor-Mel: 0% for Wisconsin score, 5.6% for California score, 22.2% for US, 20.7% for cranial lesions depth, ADG 1.44 Kg/d). Prevalence of lung gross lesion was also similar (Control: 20.8%; Flor-Mel 27.7%; General prevalence: 23.8%). Treatment success 45d-rate was 97.1%, chronicity 45d-rate was 2.9%, mortality 45d-rate was 0%, and relapse 45d-rate was 0%. Both clinical scores increased after treatment with a peak at +5d. Afterwards, both clinical scores decreased within 2 to 4 days (+7 to +9d after treatment). The US score was 4.7±0.3 at treatment and decrease at +3d (US=3.64), +5d (US=2.41), +11d (US=1.68) after treatment. The LLS was 15.6±1.9 at treatment and decrease at +1d (LLS=12.52), +5d (LLS=6.98), +7d (LLS=4.33) after treatment. The right cranial lesion depth was 5.0 cm at treatment and decrease at +3d (3.9 cm; -22%), +5d (2.1 cm; -58%), +11d (0.9 cm; -82%) after treatment. The left cranial lesion depth was 4.5 cm at treatment and decrease at +3d (2.5 cm; -40%), and +5d (1.3 cm; -71%) after treatment.

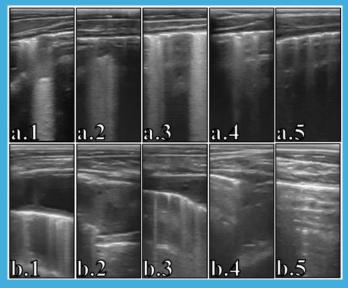


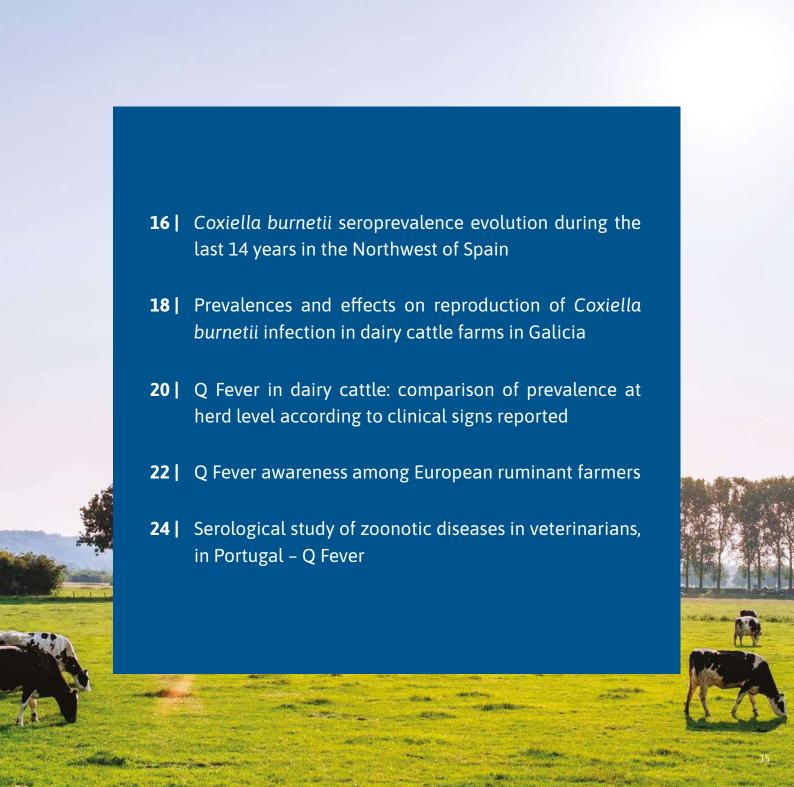
Figure 1: Examples of healing evolution processes of lobular lesion liver-like or hepatization and fluid bronchogram with B-line (a.1–a.5) and severe pleurisy with fibrinous layers and septa and comet tails (b.1–b.5). (a.1) Day of diagnosis and treatment; (a.2) +1 day after treatment; (a.3) +5 days after treatment; (a.4) +7 days after treatment; (b.2) +3 days after treatment; (b.3) +5 days after treatment; (b.4) +7 days after treatment; (b.7) +3 days after treatment $\frac{1}{2}$

$\stackrel{\square\!\!\!\square}{\smile}$ CONCLUSION

In this field study in veal calves, the frequent lung scan coupled with clinical examination led to early detection of BRD clinical cases, 5 days before most frequently used clinical scoring tools. **Prompt treatment with a fixed combination of florfenicol and meloxicam (Zeleris®) was associated with a high success rate, an absence of relapse, a rapid healing of lung lesions, and a similar growth to control calves.** In conclusion, targeted treatments following periodic clinical and ultrasonographic monitoring allowed an early diagnosis of active bronchopneumonia with relevant reduction in the prevalence of lung lesions at slaughter.

2

Q Fever: signs and control



Coxiella burnetii seroprevalence evolution during the last 14 years in the Northwest of Spain

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Objectives

Coxiella burnetii is the etiological agent of Q Fever in humans and coxiellosis in animals (Trachsel et al., 2023). Its environmental resistance made C. burnetii a highly infectious pathogen presents in every country around the world, with the exception of New Zealand and the Antarctica (Trachsel et al., 2023). Coxiellosis in cattle is often asymptomatic, but a possible relationship with various reproductive problems has been suggested. Due the zoonosis ability and the reproductive disorders associated, C. burnetii has been studied for the last decade. **Spain is one of the European countries**

with the highest Q Fever incidence. However, there is limited epidemiological data in cattle in Galicia (Northwest of Spain). In fact, there is only one study made about the prevalence of this bacterium in dairy cattle in this region (Yáñez et al., 2024). Therefore, the aim of this study was to determine the seroprevalence in the Northwest of Spain of C. burnetii using ELISA bulk milk testing and the evolution of the bacterium seroprevalence in bulk tank milk samples (BTMS) between the years 2010 and 2024.

Materials and methods

Thus, 50 BTMS were collected during the year 2010 and 36 BTMS were collected from January to June of 2024. All the BTMS were collected in 50 ml sterile Falcon tubes after mixing the bulk tank milk and were refrigerated (4-8 °C) after the collection at the farm. At the same time, the BTMS were analysed using ELISAi. ELISAi was carried out with the commercial kit "ID Screen® Q Fever Indirect Multi-species" (IDvet, Grabels, France). The reading of the ELISAi were done with the spectrophotometer "Multiskan EX" (Thermo Scientific®, Waltham, MA, EE. UU.) and a 450 nm wavelength. Absorbances were converted to a optic density ratio between the sample and the control (S/P). S/P= (Sample optic density-Negative control optic density) (Positive control optic density-Negative control optic density) *100. The S/P titter was categorized negative (S/P \leq 30), doubtful (30 < S/P \leq 40) and positive (S/P > 30).

In total, **97,3** % (**35/36**) of farms showed antibodies (> 30 S/P) against the bacterium in 2024, in comparison to data obtained in 2010, when **60%** (**30/50**) of the dairies were positive (>**30 S/P**) (Figure 1). Likewise, 62.5% of the regions in 2010 showed antibodies against *C. burnetii* (Lugo = 53.9% and A Coruña = 71.4%). As expected, in the ELISA bulk milk testing done in 2024 the 100.0% of the regions showed antibodies against this bacterium. **These results show a high presence, exposure, and geographical distribution of** *C. burnetii* **in dairy cattle farms in this region, and it also suggests that** *C. burnetii* **has increased the prevalence in both provinces studied during the last 14** years. On the other hand, a study carried out in 2018 concluded that in Basque Country the seroprevalence in sheep flocks did not significantly vary from 2005 (40.3%) to 2015 (32.1%), on the other hand the seroprevalence diminished during this period (Álvarez-Alonso *et al.*, 2018).

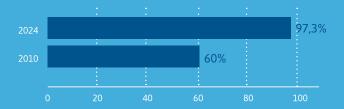


Figure 1: Presence of antibodies against Coxiella burnetii

CONCLUSION

References:

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This study suggests the **high exposure and presence of** *C. burnetii* **in Galician cattle farms and the possibility of its involvement in reproductive disorders in farms with specific epidemiological profiles**. Finally, it is important to consider that the high presence of *C. burnetii* in dairy farms could pose a risk to public health, therefore genotyping the strains circulating in herds in Galicia would be interesting to characterize the epidemiology.



Prevalences and effects on reproduction of Coxiella burnetii infection in dairy cattle farms in Galicia

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Objective

Coxiella burnetii is the etiological agent of coxiellosis in animals and Q Fever in humans. It is a highly infectious pathogen with high environmental resistance (Trachsel et al., 2023). In cattle, coxiellosis is often asymptomatic, but a possible relationship with various reproductive problems has been suggested (Yáñez et al., 2024). Dairy cattle in Galicia (Northwest of Spain) represents the 40% of the dairy cows in Spain (Agricultura & ALIMENTACIÓN Edita, n.d.). However, there is limited coxiellosis epidemiological data in this region (Yáñez et al., 2024). Therefore, the aim of this study was to determine the prevalence of C. burnetii and its effects on reproductive parameters in dairy cattle farms in Lugo and A Coruña.

Materials and methods

36 bulk tank milk samples (BTMS) were collected and analyzed using real time quantitative polymerase chain reaction (qPCR). Furthermore, reproductive data from the past six months for each farm were obtained to study their relationship with the presence of *C. burnetii*. All the BTMS were collected in 50 ml sterile Falcon and were refrigerated (4-8 °C) after the collection at the farm. DNA extraction was made with the commercial kit High Pure PCR Template Preparation Kit" (Roche® Mannheim, Alemania). qPCR was performed using the commercial kit EXOone *Coxiella burnetii* - oneMIX qPCR kit 100 tests" (Exopol S.L., Zaragoza, España) for gene IS1111 detection.

52.3% of farms showed active shedding of the bacterium, with high prevalences in both provinces (Lugo = 53.9% and A Coruña = 71.4%). In contrast, other studies found either lower (Algeria, 26.6%, Agag *et al.*, 2024) or higher (Lombardy, Italy, 60.0%, Vicari *et al.*, 2013) shedding in BTMS. But, our results showed similar prevalence in these provinces than other regions of Spain, as Basque Country (51.7%) (Astobiza *et al.*, 2012). Regarding reproductive parameters, **the abortion rate and the incidence of endometritis were significantly higher (***p-value* **< 0.05) in farms highly positive for C. burnetii (Ct < 32)**. The abortion rate was also higher in these farms than in negative ones (5.0% vs 3.0%) as found in 77.0% of the articles analysed in a systematic review (Gisbert *et al.*, 2024). The percentage of positive farms was higher in those with endometritis (35.0% vs 29.0%). This finding aligns with a 2024 study showing higher endometritis in seropositive farms compared to seronegative ones (Yáñez *et al.*, 2024).

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CONCLUSION

This study confirms the high exposure and presence of *C. burnetii* in Galician cattle farms and suggests the possibility of its involvement in reproductive disorders in farms with specific epidemiological profiles. Finally, it is important to consider that the high presence of *C. burnetii* in dairy farms could pose a risk to public health.



Prevalence of Q Fever in dairy cattle farms with reproductive disorders

Coxiella burnetii is an intracellular gram-negative bacterium responsible for Q fever in many animal species and humans. The disease occurs worldwide, with the exception of New Zealand, where it has never been described. Among animals, ruminants are the most affected. They are also the source of most human cases. Clinically, in small ruminants, Q fever is dominated by abortions. However, in cattle, in addition to abortions, other clinical signs such as metritis, retained placenta, and fertility problems may occur (Gisbert et al., 2024).

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Objective

The aim of this study was to compare the prevalence of Q Fever infection at herd level between farms reporting abortions and those reporting only other reproductive disorders.

- Abortions / premature delivery / stillbirth / weak born offspring (APSW complex).
- Metritis / endometritis.
- Retained placenta.
- Infertility.

Materials and methods

PCR testing of bulk tank milk (BTM) is a simple and affordable way of testing for the presence of Coxiella burnetii in dairy herds. In 2020, Ceva and Qualyse developed QTest to facilitate the collection and transport of samples to the laboratory. QTest combines an easy sampling medium (FTA® card) that inactivates micro-organisms while preserving nucleic acids, with real-time PCR analysis carried out in a single laboratory in France. The use of QTest increases the sensitivity of BTM analyses (Treilles et al., 2021).

Each QTest is accompanied by a sample identification form. On this form, the breeder or veterinarian sending the sample indicates any reproductive disorders present on the farm. It is possible to tick several boxes:

The objective of QTest is to identify the presence of Coxiella burnetii in herds exhibiting reproductive dysfunction. In the present study, the results obtained over a four-year period, from October 2020 to September 2024, were subjected to analysis. The herds were classified into two groups. The first group comprised herds that had reported the APSW complex, while the second group consisted of herds that had reported any of the other reproductive disorders. As multiple clinical signs could be reported, it was decided to assign to the APSW complex group, herds where both APSW complex and at least one other clinical sign were reported.

The results of the two groups were then compared using the chi-square test.

During the study period **2014 samples were collected from farms** where at least one reproductive disorder had been reported. The geographical origin is shown in Figure 1.

APSW complex was reported on 1261 farms (62.6%). The presence of Coxiella burnetii in BTM was detected in 44.0% of them (n = 555). 753 farms (37.4%) reported only other reproductive disorders. Of these, 40.4% were positive for Coxiella burnetii in BTM (n = 304). The difference in prevalence was not statistically different between the two categories (p=0.11, Chi-square test) (Table 1).

	563 UK			7 <u>±=</u> 35	* 32 CAN	31 IRE	BEL 22
19 UKR	15 ROM	© 11	6 POI	6 NED	C∗ 2	1	

Figure 1: Geographical origin of the samples collected

Clinical signs	Number of farms	Number of infected farms	Chi-square test	
APSW complex	1261	555 (44%)	011	
Other reproductive disorders			p=0.11	

Table 1: Prevalence of infected farms depending on reproductive disorders declared

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Gisbert, P., Garcia-Ispierto, I., Quintela, L. A., & Guatteo, R. (2024). Coxiella burnetii and reproductive disorders in cattle: a systematic review. Animals, 14(9), 1313.

Treilles, M., Charollais, P., Guatteo, R., Azevedo, C., Achard, D., Munoz-Bielsa, J., & Gisbert, P. (2021). QTest: A new way to easily sample, store, and ship samples to perform Q Fever PCR analysis on bulk tank milk. JDS communications, 2(6), 409-414.



These results suggest that the presence of Coxiella burnetii in dairy cattle can be associated without difference with major reproductive disorders (abortions, premature delivery, stillbirth, weak born calves) or other reproductive disorders (metritis/endometritis, retained placenta, infertility). Testing for Coxiella burnetii should therefore be considered in herds with reproductive problems, even in the absence of a high abortion rate.

Q Fever awareness among European ruminant farmers

Raphaël Guatteo¹, George Valiakos², Vincent Dedet³ and the members of the European Q fever committee⁴

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Objective

In the context of the One Health approach and the management of zoonosis, a **European Q Fever Committee was established in 2024, bringing together veterinary and medical experts from various disciplines and countries.** As a first preliminary step, an unprecedented and large survey of ruminant (cattle, sheep and goats) farmers was conducted in eight European countries, to assess their degree of awareness and level of knowledge on Q Fever, and their related practices in hygiene and medical control of the disease.

Materials and methods

This survey was conducted by an independent company for opinion polling. Ruminant farmers were targeted in each country (Germany, France, Spain, Italy, UK, Belgium, Netherlands and Poland). The questionnaire used for a similar study conducted in France in 2019 was translated into English, and then into the respective local languages. Interviews were conducted using the Computer-Assisted Telephone Interview technology. Each interview was recorded and lasted 20 to 25 minutes. Interviews took place over the summer of 2024. As the data collected were dispersed over several countries and referred to various animal species, only descriptive statistical analysis was performed.

905 farmers completed the interview, providing exploitable answers. Cattle (n=571), sheep (n=198) and goat (n=177) farmers were represented (some were farming several species) (Figure 1). In the Netherlands, only goat farmers (n=20) and in Germany, Belgium and Poland, only cattle farmers (n=80, n=60 and n=80, respectively) were interviewed. Respondents were mostly men (77%); 70% were over 40 years of age, which is comparable to the current situation in Europe. The average number of heads of animals per farm was 103 for cattle, 234 for sheep and 236 for goats. Overall, answers demonstrated a significant lack of farmers' awareness about the disease, regardless of the country: approximately 75% of all farmers were either unaware of the disease or only familiar with its name in all countries (Figure 2). Veterinarians and digital channels were identified as the two main sources of information for animal health, both in comparable importance across countries, and even among the youngest farmers (25-35 years of age). Personal experience with Q Fever is very limited (2-5% of respondents) in most countries, but is higher in the Netherlands (12%) and France (13%). In the Netherlands, the largest zoonotic outbreak, linked to goat farming, occurring in 2007-2009, might explain this score. The French results might be ascribed to the awareness campaigns set up by the French Q Fever committee over the last decade. Dutch and French farmers were also those who rated the risk of introduction of Q Fever on their farm as the highest (> 20 % of respondents consider this risk as high or very high). Less than 10% of other respondents perceived this risk as moderate. Most farmers aware of the disease know that the bacterium can be transported by wind over several kilometers and is transmissible from animals to humans. There was less consensus





Figure 1: Numbers of farmers interviewed per species bred

Q FEVER AWARENESS



Only 2-5% have personal experience with the disease

Figure 2: Q fever awareness among farmers

about the impact of Q Fever on the performance of breeding stock: e.g. among cattle farmers, the awareness rate in Spain was nearly twice that of France (57% vs. 31%, respectively). Even though the majority of respondents aware of the disease mention abortion as a manifestation of the disease, other clinical signs were rarely acknowledged. Following Q Fever diagnosis on a farm, at least 90% of farmers mention hygiene measures and manure management as priority control measures. Vaccination and treatments are equally considered by about 25% of farmers, except in Italy where vaccination is only mentioned by 6% of farmers and in Germany where it is not mentioned at all. In contrast, 50% of British cattle farmers and 38% of Spanish cattle farmers refer to vaccination over other treatments. In all countries, apart from the Netherlands (100%) and France (27%), vaccination rates against Q Fever are generally low (89-95% of respondents do not vaccinate), a puzzling fact since those farmers are aware of its zoonotic trait. However, among farmers with personal experience with Q Fever, the vaccination rate is higher (29-50%, and even 71% in France).

Even though in some countries only farmers of a specific ruminant species were interviewed, to the authors' knowledge, this is the largest survey on the perception of Q Fever by ruminant farmers in Europe. Although some countries present particular profiles, it should be noted that awareness of the disease is limited among farmers, including infection control and prevention of transmission. The established European Q Fever committee intends to use the reported results to tailor specific awareness/information campaigns for each country.

Serological study of zoonotic diseases in veterinarians, in Portugal - Q Fever

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Objective

The "One Health" concept is gaining increasing relevance and remains a subject of continuous study due to its role in promoting ecosystem balance and integrating human, animal, and environmental health. Emerging and re-emerging zoonotic diseases strongly exemplify the interconnectedness of the "One Health" pillars, representing significant threats to both human and animal health. The resurgence of certain diseases reflects the impact of environmental changes, highlighting the need for collective, interdisciplinary strategies for their prevention and control, alongside the use of basic, straightforward

measures, such as the active implementation of simple biosecurity practices. **Q Fever is a globally distributed disease caused by a bacterium known as Coxiella burnetii.** Ruminants are recognized as the primary reservoirs, shedding the agent into the surrounding environment through secretions and/or excretions. The main objectives of this study were to **identify risk factors associated with Coxiella burnetii infection and to assess the implementation of biosecurity measures among veterinarians working in livestock species.** Additionally, it aimed to determine the seroprevalence of Q Fever infection in this population.

Materials and methods

In this research, the anti-*C. burnetii* antibody titers were quantified in serum samples from two groups: the test group, consisting of veterinarians (n=92), and the control group, composed of blood donors (n=184). The analysis was conducted using a commercial FLISA test.

The results showed that 33.7% of the samples in the test group tested positive for IgG anti-Coxiella burnetii, corresponding to 31 out of the 92 veterinarians tested, suggesting exposure to the agent. In comparison, the control group had a seropositivity rate of 17.4%. Statistical analysis revealed a significant difference between the two groups (P=0.0023), suggesting a significantly higher exposure risk in the test group. This study demonstrated that veterinarians in the test group had a greater

likelihood of exposure to the agent (OR=2.41) compared to the control group.

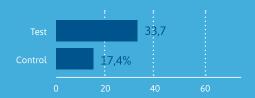


Figure 1: Seropositivity to Coxiella burnetii in veterinarians in Portugal

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CONCLUSION

The study of occupational diseases among veterinarians involved in livestock production is a relatively underexplored topic, considering the risks they face during their professional activities. This is one of the first studies conducted in Portugal to demonstrate the exposure risk of veterinarians to Coxiella burnetii – a risk that could be mitigated through biosecurity measures, which are often overlooked in the daily practice of field veterinarians.

3

Udder health: mastitis control using fewer antibiotics

Ketoprofen as the sole initial treatment for mild and moderate bovine mastitis: efficacy and antibiotic reduction

Ketoprofen as the sole initial treatment for mild and moderate bovine mastitis: efficacy and antibiotic reduction

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Targeted mastitis treatment concepts are the most scientifically validated approaches to comply with the prudent use of antimicrobials. However, this approach is still a challenge for many farmers who often lack experience with it. Another option for more prudent use of antibiotics in mastitis therapy could be the initial treatment with a non-steroidal anti-inflammatory drug (NSAID) for non-severe mastitis.

Objective

The aim of this study was to compare the treatment of mild and moderate mastitis with ketoprofen to the usual treatment with intra-mammary antibiotics.

Materials and methods

A randomized, multi-herd, non-inferiority study was carried out to compare two treatments of non-severe mastitis cases. The animals in the studied group were treated with systemic NSAID (ketoprofen (KE); KE group; n=104), followed by antibiotic treatment only in cases without clinical improvement, while the animals in the reference group (AB group) received an intramammary antibiotic (n=118) (Figure 1). The study included 222 cases of non-severe clinical mastitis on three conventional dairy farms in Northern Germany between November 2022 and November 2023. Study outcomes were clinical cure at day 5, bacteriological cure, CM recurrence in a period of 60 days and new infection risk. Mixed logistic regression was used to analyze the effect of treatment on outcomes. The non-inferiority assumption was checked with the confidence interval of the treatment difference for clinical cure at day 5, calculating using the least square means and their standard deviations.





With regard to the clinical cure on day 5, the treatment in the KE group was inferior. Clinical cure [84.7% (100/118 and 61.5% (64/104)] and bacteriological cure [79.3% (73/92) and 61.2% (41/67)] were significantly higher in the AB group than in the KE group. The risks for recurrent cases [7.3% (8/109) and 15.7% (14/89)] were significantly lower in the AB group than in the KE group. The new infection risk did not differ significantly between the treatment groups [6.8% 50 (8/118) in the AB group and 6.7% (7/104) in the KE group]. In 87% of the cases (n=90) of the KE group, a subsequent antibiotic treatment was not necessary to reach clinical cure on day 5 (Figure 1).

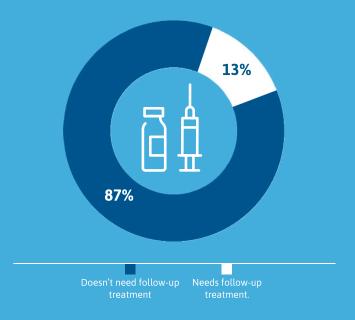


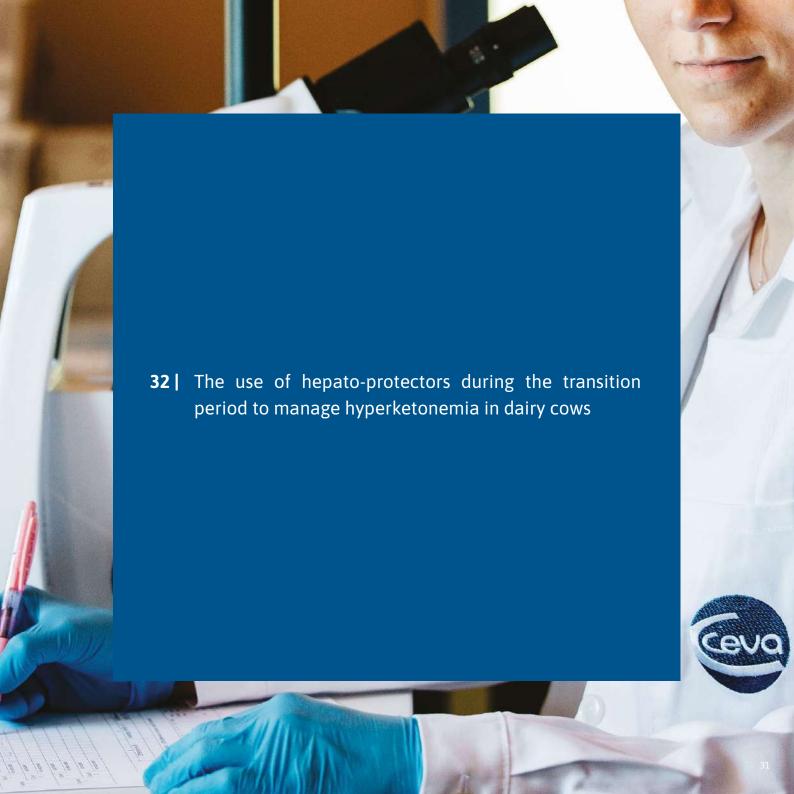
Figure 2: Mild and moderate mastisis cases treated with ketoprofen (KE Group)



Treatment of mild and moderate mastitis with ketoprofen represents an alternative that promoted the deliberate and selective use of antibiotics. Furthermore, as ketoprofen has no withdrawal period for milk, less milk was discarded in the ketoprofen group than in the antibiotic group. However, some limitations with regard to treatment efficacy in Gram-positive mastitis were observed. A targeted mastitis concept based on the identification of the bacterium involved could help to select cases to be treated with ketoprofen alone and therefore to overcome this limitation.

4

Metabolic disorders: transitioning with care



The use of hepato-protectors during the transition period to manage hyperketonemia in dairy cows

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Objective

Hyperketonemia (increased blood level of β -hydroxybutyrate or BHB) is a major metabolic disease in dairy cows negatively affecting animals' health. A proper functioning of the energy metabolism (gluconeogenesis and Krebs-cycle) is essential to reduce the risk of hyperketonemia during post-partum period. For these reasons, hepato-protectors composed by precursors of energy metabolism pathways (as propionate, gluconeogenic amino acids (AA), vitamins and co-factors) are usually used in dairy cows during the transition period. A field study was performed to better characterize the efficacy of hepato-protectors to reduce the risk of hyperketonemia in dairy cows.



Materials and methods

Animal care and procedures received ethical approval (n.204359/2023). A total of 445 Holstein-Friesian dairy cows were enrolled at the begging of the dry period, and randomly and equally divided into three groups: CTR (control without treatment); BOG-ERB (treated with Bograss® and Erbacolina PLUS®, CEVA Animal Health); MON (treated with monensin, Kexxtone). The BOG-ERB received 2 ml of Bograss®/10 kg BW, and 70 mL/animal/treatment of Erbacolina PLUS® at 12d before calving and 6d after calving; while the MON received 35.2 g/animal of monensin at 21d before calving. Blood sampling was performed at -21 and -7d before calving, at the day of calving, and at +7, +14, +28, and +55d after calving. All animals were evaluated for NEFA and BHB levels, while a complete biochemical analysis was performed for 45 animal per group (30 pluriparous, and 15 primiparous). Differences in biochemical parameters were assessed with a linear mixed-effects model. A post hoc pairwise comparison was performed using Bonferroni correction. A logistic regression analysis was performed using blood BHB values (healthy, BHB<1.2 mmol/L; hyperketonemia, BHB≥1.2 mmol/L). The model considered the risk factors of treatment, time, parity, and season. A p-value<0.05 was considered significant, whereas a 0.05≤p-value<0.10 was considered a trend.

Hyperketonemia prevalence in BOG-ERB group was between 2.9% to 11.1% during the post-partum period. In contrast, the prevalence was 6.1% to 24.1% in CTR, and was 5.3% to 16% in MON. In fact, the logistic regression identified for BOG-ERB an OR of 0.43 (CI: 0.3 to 0.8) compared to the CTR, and of 0.44 (CI: 0.1 to 0.8) compared to MON. The BOG-ERB cows showed the lower risk of hyperketonemia during the post-partum period considering a lowest level of NEFA from +7d to +28d, and BHB from +14d to +55d. Moreover, the CTR incurred in hypoglycemia at +7d. Regarding the liver, the AST and GGT concentrations were lowest in BOG-ERB from calving until the end of the study. The albumin level was greatest in BOG-ERB from +14d to +55d in association with the lowest level of urea. Serum ALP concentration was greatest in BOG-ERB from -7d before calving to +55d associated with greater level of Ca up to +14d. Furthermore, serum Cl and Na were greater in

the same group around calving. Regarding cows' performances, the BOG-ERB had the greatest pregnancy/artificial insemination (BOG-ERB: 55.7; CTR: 49.8; MON: 49.9), and a greater milk production compared to CTR group without differences with MON group (BOG-ERB: 43±0.5 Kg/day; CTR: 42±0.5 Kg/day; MON: 43.5±0.5 Kg/day). Finally, the MON group produced more milk during the first 150 DIM, while the BOG-ERB produced more milk after 210 DIM.

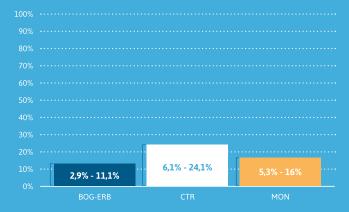


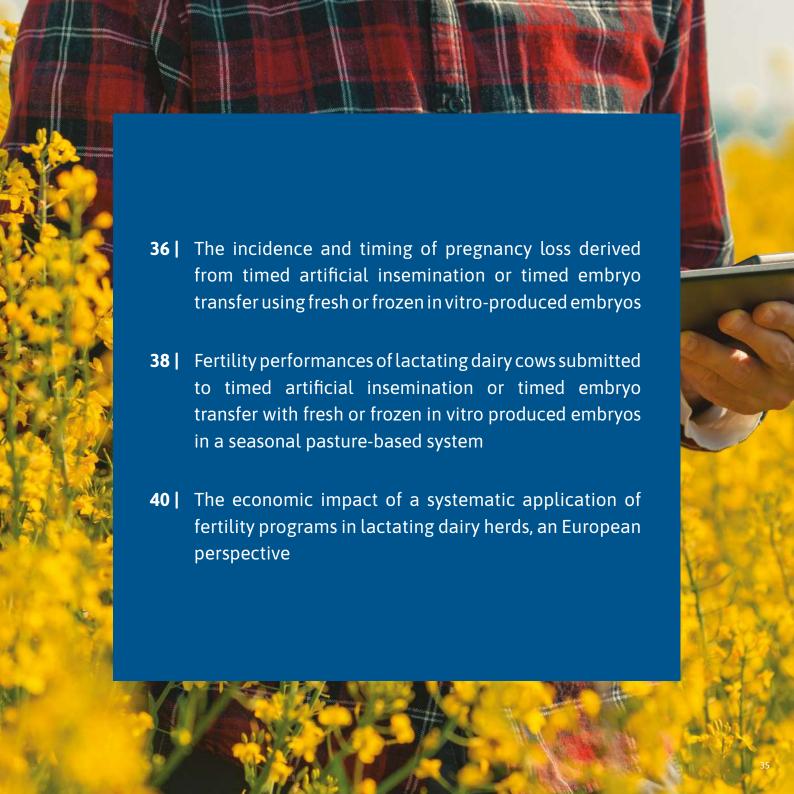
Table 1: Hyperketonemia prevalence during post-partum period.



Supporting animal metabolism through the use of hepato-protectors decreases lipomobilization, metabolic stress, and protein catabolism. This condition reduces the risk and prevalence of hyperketonemia during the post-partum period with greater reproductive performances and a more constant milk production.

5

reprodAction: reproductive management and economic impact



The incidence and timing of pregnancy loss derived from timed artificial insemination or timed embryo transfer using fresh or frozen in vitro-produced embryos

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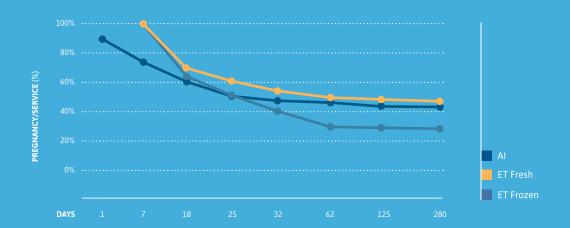
Objective

This study aimed to **characterize the incidence and timing of pregnancy loss from the service event** (timed artificial insemination or timed embryo transfer) to parturition.

Materials and methods

Lactating Holstein-Friesian cows were randomly assigned to receive either timely artificially inseminated (TAI, n = 243) or timely embryo transferred (TET, n = 863) with a fresh or frozen in vitro-produced blastocyst following a protocol consisting off on day 0, cows received a 2-mL intramuscular injection of GnRH analogue (Ovarelin, 100 μ g of gonadorelin diacetate tetrahydrate; Ceva Sante Animale, Libourne, France) and a progesterone-releasing intravaginal device (PRID Delta; 1.55 g Progesterone, Ceva). On day 7, a 5-mL intramuscular injection of PGF2 α (Enzaprost, 25 mg of dinoprost trometamol; Ceva) was administered, followed by a second 5-mL injection of PGF2 α on day 8 and removal of the PRID. On day 9.5 (32 hours after PRID removal), a second intramuscular injection of GnRH was give, and cows were either inseminated 32h later (TAI) or received a blastocyst 7,5 days after the last GnRH injection. The blastocysts were derived from oocytes collected from the ovaries of elite dairy donors (14 Holstein Friesian and 8 Jersey) and elite beef donors (21 Angus) using transvaginal ovum pick-up. Additionally, oocytes were collected from the ovaries of beef heifers of known pedigree following slaughter at a commercial abattoir (n = 119). Blood samples were collected on day 7 from all cows to determine progesterone concentration, and from subsets of cows on day 18 (n = 524) and day 25 (n = 378) to determine mRNA abundance of interferon-stimulated gene-15 and pregnancy-specific protein B concentration, respectively, for early pregnancy diagnosis. Transrectal ultrasonography was conducted to determine pregnancy status on days 32, 62, and 125 after synchronized ovulation. Parturition dates were recorded for all cows that reached term delivery.

The predicted probability of pregnancy (%) varied at each time point (days 7, 18, 25, 32, 62, 125, parturition) depending on treatment (Al: 77.0, 60.2, 52.3, 48.8, 47.0, 44.6, 44.0; fresh ET: 100.0, 69.5, 60.3, 56.1, 48.4, 46.8, 45.5; frozen ET: 100.0, 61.7, 52.2, 41.6, 32.9, 31.8, 30.2). Regardless of treatment, the largest proportion of pregnancy loss occurred from the service event (Al on day 0 or ET on day 7) to day 18, with minimal loss occurring between day 62 and parturition (Al: 1.8%, fresh ET: 1.9%, frozen ET: 3.5%). Treatment differences in the predicted probability of pregnancy per service event were detected between fresh ET vs. frozen ET on day 32, and both Al and fresh ET vs. frozen ET on days 62, 125, and at parturition. There was a greater probability of pregnancy loss between days 32 and 62 following ET (Fresh: 11.3%, Frozen: 18.0%) than Al (4.0%). The percentage of cows that calved following the transfer of a fresh embryo (45.5%) was similar to Al (44.0%), but lower when a frozen embryo was transferred (30.2%).





In conclusion, AI and fresh ET led to a higher probability of cows becoming pregnant and maintaining the pregnancy to term compared to frozen ET. Cows that were still pregnant on day 62 had a very high likelihood of maintaining the pregnancy to full-term parturition, regardless of treatment. Further work is needed to improve the likelihood of pregnancy establishment and reduce embryonic and fetal mortality following the transfer of a cryopreserved in vitro-produced embryo.

Fertility performances of lactating dairy cows submitted to timed artificial insemination or timed embryo transfer with fresh or frozen in vitro produced embryos in a seasonal pasture-based system

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Objective

This study aimed to compare pregnancy per service (P/S) in lactating dairy cows following timed artificial insemination (TAI) or timed embryo transfer (TET) using either fresh or frozen in vitro produced (IVP) embryos.

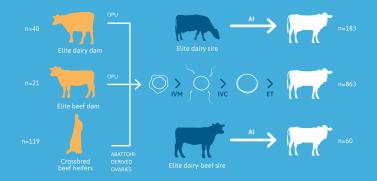
Materials and methods

Oocytes were collected weekly for up to 9 weeks using transvaginal ovum pick-up from elite dairy donors (ET-DAIRY; n = 40; Holstein-Friesian and Jersey) and elite beef donors (ET-ELITE-BEEF; n = 21; Angus). Both heifers and cows were included in these donor groups. Additionally, oocytes were collected from the ovaries of beef heifers of known pedigree following slaughter (ET-COMM-BEEF; n = 119). After in vitro maturation, fertilization, and culture, single Grade 1 blastocysts were transferred either fresh or after freezing and on-farm thawing into lactating Holstein-Friesian dairy cows synchronized with a 10-day PRID-Ovsynch protocol. On day 0, cows received a 2-mL intramuscular injection of GnRH analogue (Ovarelin, 100 μ g of gonadorelin diacetate tetrahydrate; Ceva Sante Animale, Libourne, France) and a progesterone-releasing intravaginal device (PRID Delta; 1.55 g Progesterone, Ceva). On day 7, a 5-mL intramuscular injection of PGF2 α (Enzaprost, 25 mg of dinoprost trometamol; Ceva) was administered, followed by a second 5-mL injection of PGF2 α on day 8 and removal of the PRID. On day 9.5 (32 hours after PRID removal), a second intramuscular injection of GnRH was given. On day 10, 243 cows received AI (16 hours after the second GnRH), and 863 cows were assigned to receive ET on day 17. Blood samples were collected on day 17 to evaluate serum P4 concentrations. Pregnancy rates were determined between days 32-35 after synchronized ovulation using transrectal ultrasonography. Pregnancy was confirmed between days 62-65 to determine embryonic loss. Pregnancy data were analyzed using generalized linear mixed models, with service treatment (TAI vs. TET) as a fixed effect.



Mean pregnancy per service at day 32 was similar between Al (48.8%) and ET (48.9%) and did not differ between dairy and beef embryos (50.3% vs. 48.1%, respectively). P/S was lower (P=0.003) following the transfer of frozen embryos compared to fresh embryos (41.6% vs. 56.1%, respectively). Pregnancy loss between days 32 and 62 was higher (P=0.003) for ET (15.1%) compared to Al (4.7%), with greater losses observed for frozen beef (18.5%), fresh beef (17.3%), and frozen dairy (19.2%) compared to fresh dairy (6.0%).

Serum P4 concentration on day 7 was associated with P/S at days 32 and 62. Cows in the lowest quartile for serum P4 concentrations (Q1) had a lower probability of being pregnant on day 32 (33.4%) compared to cows in the three upper quartiles (45.7%, 55.6%, and 61.2% for Q2, Q3, and Q4, respectively).



CONCLUSION

Pregnancy per service event on days 32-35 was similar for TAI and TET, although 9.6% of cows initially synchronized for ET were rejected. Of cows that were pregnant on day 32, pregnancy loss was greater for TET than for TAI. P4 concentrations on day 7 were positively correlated with pregnancy outcomes at days 32 and 65.



The economic impact of a systematic application of fertility programs in lactating dairy herds, a European perspective

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Objective

Hormone-based reproductive management programs can enhance dairy cows' reproductive performance. This study aimed to compare the economic impact of two types of reproductive management programs: one using systematic hormonal treatments for cows within a specific Days in Milk (DIM) range, and another using cow-specific hormonal treatments based on veterinary diagnoses of ovarian dysfunction during fertility checks.

Materials and methods

A dynamic, stochastic bio-economic simulation model, representing a 200-cow herd, was extended to include ovarian dysfunction and fertility inputs. Four hormone-based reproductive management programs were modeled:

Baseline Program: Reflecting current Dutch herd practices, cows are inseminated based on estrus detection, and non-cyclic cows receive hormone treatments based on veterinary diagnoses. Treatments include PRID-Synch for anoestrus cows, Ovsynch for cows diagnosed with cystic ovarian disease, and PGF2α administration to subestrous cows (Figure 1).

- **TAI only:** Systematic hormonal treatment for timed artificial insemination (TAI) using Double Ovsynch for 1st AI and resynchronization for all the cows found non-pregnant at pregnancy diagnosis 32±3 days after AI according to ovarian status either with or without PRID supplementation (Figure 2).
- **TAI + Estrus Detection (ED)**: Similar to the second program but includes estrus detection for all the cows not pregnant after TAI (Figure 3).
- **Estrus Detection + TAI:** Cows inseminated using ED after the VWP and if not detected in estrus are submitted to a PRID-Synch protocol (Figure 4).

All cows found non-pregnant at ultrasonographic pregnancy check performed at 32±3 after AI were re-synchronized based on the presence or absence of a corpus luteum (CL), cows lacking a CL received PRID-Synch cows with a CL received Ovsynch. The annual mean net economic return (NER) was calculated for each program.

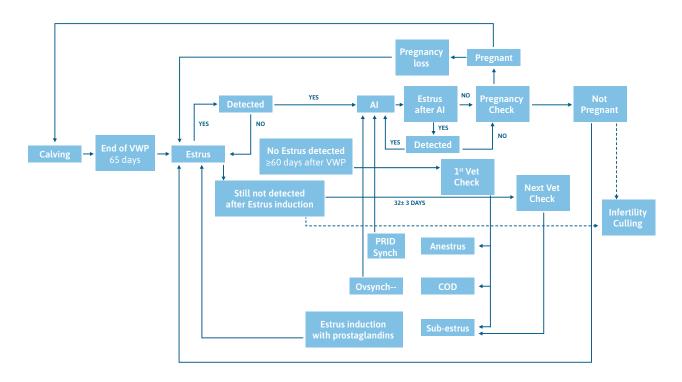


Figure 1: Baseline Schematic

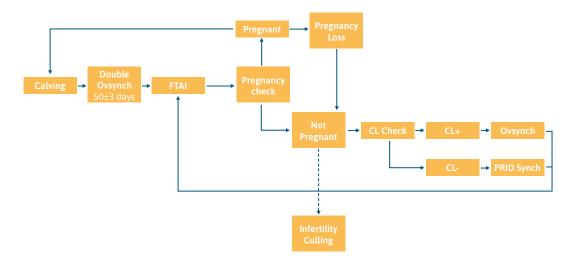


Figure 2: TAI Only Schematic

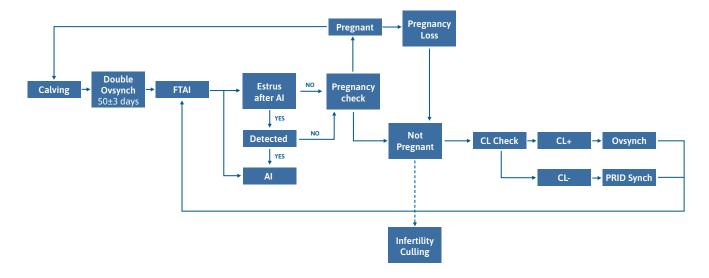


Figure 3: TAI + Estrus detection Schematic

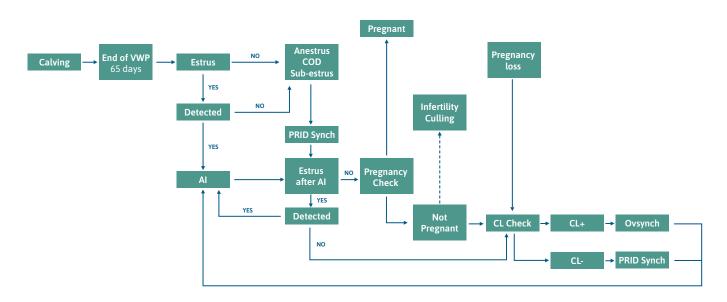


Figure 4: Estrus Detection + TAI Schematic

The highest NER was observed in the FTAI+ED program, with €23,764 higher net revenues compared to the default program, followed by the FTAI and ED+TAI programs with €19,550 and €14,314 higher net revenues, respectively. Although systematic hormone-based programs incurred higher costs due to more hormone use and increased calving and feed costs, the additional revenues from milk and calves outweighed these costs. For example, the FTAI+ED program had €8,953 higher total costs but generated €32,654 higher revenues annually.

	Total Costs (€)	Total Revenues (€)	Net Economic Return (NER) (€)
Baseline	_		Reference
TAI only	+7,104 EUR	+26,654 EUR	+19,550 EUR
TAI + Estrus Detection	+8,953 EUR	+32,654 EUR	+23,764 EUR
Estrus Detec- tion + TAI	+6,874 EUR	+21,188 EUR	+14,314 EUR

CONCLUSION

In summary, systematic hormone-based reproductive management programs provided economic advantages over the default program, where hormones are administered based on veterinary diagnoses during fertility checks.



