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The Production Impact of Q Fever Vaccination in a Large Scale Hungarian Dairy Farm

A Q-láz elleni védőoltás termelésre gyakorolt hatása egy nagyüzemi magyar tejelő tehenésztben

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2023

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Table of Acronyms

AI	Artificial Insemination
BHV-1	Bovine Herpes Virus-1
CCV	Coxiella-Containing Vacuole
ELISA	Enzyme-Linked Immunosorbent Assay
ICD	Isocitrate Dehydrogenase
IFA	Immunofluorescence Assay
IL-12	Interleukin-12
LCV	Large Cell Variant
LPS	Lipopolysaccharide
MLVA	Multi-Locus Variable-number tandem-repeat Analyses
MST	Multi-Spacer Typing
PCR	Polymerase Chain Reaction
SCV	Small Cell Variant
TNF	Tumour Necrosis Factor

1. Introduction

In recent years, the global agricultural industry has faced significant challenges due to emerging infectious diseases, which pose substantial threats to the health and productivity of livestock populations. One such disease is Q fever, caused by the bacterium *Coxiella burnetii*, which has garnered increasing attention in the context of dairy farming. Q fever is zoonotic, making it a concern for both public health and the agricultural sector.

The economic implications of Q fever on dairy farms are profound, with potential repercussions extending far beyond the direct impact on animal health. As an infectious disease capable of causing abortions, reduced milk production, and reproductive disorders in dairy cattle, Q fever poses a significant risk to the overall profitability and sustainability of dairy operations. Moreover, its zoonotic nature raises concerns about the health and safety of farm workers, veterinarians, and individuals living in close proximity to infected animals.

This thesis will examine the economic impact of a Q-fever vaccination on a large dairy farm in the North-Western region of Hungary. The analysis involves examining various production indicators and factors to understand how vaccinations influence different aspects of the economy;

i.   Herd Health outcomes: Vaccinations can prevent the spread of infectious diseases and reduce their severity, leading to improved overall health. By reducing illness, vaccinations can positively impact the overall productivity of a population.

ii. Healthcare costs: Vaccinations can potentially lower healthcare costs by preventing costly veterinary treatments associated with Q-fever, and long-term costs relating to illnesses caused by reduced immune system due to Q-fever.

iii. Workforce productivity: Vaccinations contribute to maintaining a healthy workforce.

iv. Supply chains: Vaccinations can play a role in stabilising supply chains by reducing disruptions caused by outbreaks.

v.   Farm/Government expenditures: Analysing the economic impact of vaccinations also involves assessing the costs associated with implementing vaccination programs, including vaccine procurement, distribution, administration, and farm health campaigns. This analysis considers the budgetary implications for governments/farms and the potential return on investment from reduced healthcare costs and improved economic outcomes.

2. Literature Review

2.1. Discovery

The microorganism recognised as *Coxiella burnetii* was initially documented in the 1930's. This discovery occurred in parallel, with Herald Rea Cox identifying it in the United States while investigating an outbreak among guinea pigs, and Edward Derrick encountering it in Australia while studying patients afflicted with "Q fever," an abbreviation for "query fever"[1, 2]. The genus name "Coxiella" was derived from Dr. Cox's surname, who isolated the bacterium.

2.2. Taxonomy

Taxonomically it is positioned in the domain of *Bacteria*. It falls within the phylum *Proteobacteria*, and class, *Gammaproteobacteria*. *C. burnetii* is further placed within the order *Legionellales*, a designation that provides insight into its obligate intracellular status and its relatives. Within this order, the family *Coxiellaceae* distinguishes it from other genera, emphasising its unique genomic and biological features. The genus *Coxiella* encapsulates *burnetii* as its flagship species [1]. Scientific investigations suggest that there might be potential relatives or even new species closely related to *C. burnetii*, particularly from environmental samples and arthropod vectors [3].

2.3. Virulence Variants

There have been two antigenic variants identified: Phase I and Phase II. Phase I bacteria are considered the virulent form, while the phase II is an avirulent form generated during laboratory passage [4]. Phase I, characterised by its high infectivity, is the natural phase found in infected animals, arthropods, or humans. Chronic Q fever is characterised by the presence of anti-phase I antibodies [5].

The genotypic variability, identified through multi-spacer typing (MST), was studied by BEARE et al. to identify and classify different genotypes of *C. burnetii* [6]. These genotypic variations can play a role in influencing the bacterium's virulence. Studies by SZYMAŃSKA-CZERWIŃSKA et al. and PINERO et al. have identified Genotype I and J as prevalent genotypes [7, 8].

2.4. Structure

C. burnetii is pleomorphic however it is generally found in rod-shaped form. It is a Gram-negative bacterium having a thin lipopolysaccharide (LPS) layer. It employs various immune evasion strategies that are intrinsically linked to the structure of its LPS. The virulent phase I of *C. burnetii* has an LPS with a complete O antigen, granting it serum resistance. This

resistance is manifested by its moderate activation of the complement system and its ability to prevent the deposition of the complement factor C3b on its surface [9]. Phase I *C. burnetii* LPS, termed as "smooth LPS," inhibits bacterial recognition by the pattern recognition receptor TLR2. This is due to the O antigen in the LPS that masks TLR2 ligands on the bacterial surface. Contrarily, the avirulent phase II produces a "rough LPS," devoid of the terminal O antigen sugars, making it easily recognisable by TLR2. This recognition prompts the production of interleukin-12 (IL-12) and tumour necrosis factor (TNF), subsequently activating macrophages for bacterial clearance. In addition to this, the lipid A of *C. burnetii* behaves as a TLR4 antagonist [9]. These structural qualities contribute to the bacterium's ability to avoid eliciting a strong host inflammatory response upon infection, facilitating its long-term survival in the host.

2.5. Morphology

Morphologically, *C. burnetii* exhibits a biphasic life cycle, transitioning between two primary forms: the small cell variant (SCV) and the large cell variant (LCV). The SCV, often considered the spore-like form, is characterised by its dense cytoplasm and resistance to environmental stresses such as heat, desiccation, and common disinfectants, making it the predominant form outside host cells. The SCV is typically rod-shaped. They range from 0.2μm to 0.3μm in diameter [10]. SCVs are compact and possess a thick cell wall which contributes to their desiccation resistance.

On the other hand, the LCV is larger, approximately 0.5μm to 1.0μm and is metabolically active [10]. They are more pleomorphic with a dispersed filamentous chromatin [11]. This phase is predominantly seen during the bacterium's intracellular replication phase. The unique morphological adaptations of *C. burnetii*, particularly the resilient SCV form, have implications not only for its survival in varied environments but also for its transmission and infection dynamics.

2.6. Lifecycle

C. burnetii is an obligate intracellular pathogen. Upon infecting a host cell, it localises within a phagolysosome-like compartment termed the Coxiella-containing vacuole (CCV). Inside the bacterium utilises the acidic conditions within the phagolysosome to foster its replication and differentiation. The transition between spore-like SCV and metabolically active LCV is crucial for intracellular survival of *C. burnetii*. As SCVs enter host cells, they differentiate into LCVs, multiply, and then revert to SCVs before being released to infect other cells. Environmental

factors, including pH and nutrient availability within the host cell, influence the differentiation and replication. Acidic conditions in particular promote the bacterium's replication.

2.7. Host Range

C. burnetii has a broad host range and has been isolated in humans as well as various animal species. Livestock are recognised as primary reservoirs [4]. In an Eastern European surveillance study by DOBOS et al., dairy cattle seroprevalence ranged from 100.00% in Croatia, 98.55% in Czech Republic, 97.61%in Hungary ,70.83% in Serbia ,90.56% in Slovakia and 62.50% in Slovenia [12]. These animals often serve as the main source of human infections, particularly through the inhalation of aerosols contaminated with the bacterium. While livestock can remain asymptomatic, they can shed the bacteria in milk, faeces, urine, and especially birthing products. Several species of wildlife have also been identified as carriers of *C. burnetii*. This includes a diverse array of mammals, birds, and ticks, some of which have been involved in outbreaks of Q fever in humans. Dogs and cats can also harbour the bacteria and surprisingly, marine mammals, including seals and whales, suggesting a wide environmental distribution.

2.8. Epidemiology

Environmental conditions play a significant role in the transmission of *C. burnetii*. Dust and wind can spread the bacterium across vast areas. Dry and windy conditions enhance the risk of transmission among cattle and from animals to humans. Overcrowding, inadequate ventilation, and poor waste management enhance the risk of spreading the infection within the farm. The birthing process releases vast amounts of the bacterium, so calving areas are of particular concern. Introduction of new cattle from farms with known infection can serve as a source of infection to otherwise clean farms. Regular movement of cattle for trade or breeding can increase the risk of transmission. Ticks represent another dimension of the epidemiology of this bacterium. Various species, such as *Dermacentor* and *Amblyomma*, have been identified as vectors.

In cattle, the primary clinical sign of *C. burnetii* infection is abortion. However, other reproductive disorders such as stillbirth and weak offspring have also been reported. Not all infected animals show clinical symptoms, but they can still shed the bacterium.

C. burnetii has a worldwide distribution with the exception of New Zealand [13]. Within a farm, the distribution may not be uniform. Areas of calving, milking, and manure storage often act as hotspots. There is also evidence of certain cows with persistent shedding patterns, suggesting the existence of heavy-shedder cows [14].

2.9. Pathogenesis

2.9.1. Reproductive Tract

After primary exposure, typically through inhalation or ingestion of contaminated materials, *C. burnetii* circulates in the bloodstream and specifically targets the reproductive organs. CARBONERO et al. found that the bacterium's ability to target trophoblast cells, which leads to placental inflammation, can compromise oxygen and nutrient supply to the foetus, resulting in fetal distress and consequently, stillbirth.

The placenta harbours the highest concentration of *C. burnetii*. Sandoz et al. found that bacterial loads can reach upwards of 10^9 genome equivalents per gram of placental tissue [15]. Such high concentrations underscore the potential risks of environmental contamination during parturition. The tropism of *C. burnetii* for the placenta can be attributed to its preference for replicating within the trophoblast cells as AMARA et al. found the trophoblast cells create a protective environment for *C. burnetii* to grow [16]. The shedding of *C. burnetii* in placental materials and birth fluids provides an efficient mechanism for environmental contamination. Once introduced into the environment, the bacterium can survive and remain infectious for extended periods, owing to its resilient spore-like form. Other cattle can be exposed either by direct contact with contaminated birth materials or by inhaling aerosolised bacteria from the environment. The substantial shedding of *C. burnetii* from the placenta emphasises the importance of proper management of birthing materials. Efficient disposal or treatment of contaminated materials can significantly reduce the risks of transmission within herds and to humans.

The detailed pathogenesis of *C. burnetii* in bulls is less understood. However, there are reports of the bacterium colonising the testes, epididymis, and seminal vesicles. KRUSZEWSKA et al. investigated the presence of *C. burnetii* in the semen of bulls. Out of a sample of 57 bulls used for artificial insemination, 15% tested positive. The results support the idea that Q fever can be sexually transmitted, since the infections in cattle were noted after artificial insemination [17]. The ability of *C. burnetii* to colonise the reproductive tract and be shed in genital secretions has profound implications for cattle reproduction and herd health. Effective control measures, such as vaccination and culling, have been proposed to manage the infection and prevent its spread.

2.9.2. Mammary Glands

C. burnetii has shown a readiness to colonise the mammary glands of infected cattle. Following the initial infection, there can be hematogenous spread to the heavily vascularised tissue of the mammary glands. The target cell in the mammary tissue has not been resolved in situ, but in

vitro experiments have revealed that *C. burnetii* replicates in bovine mammary gland epithelial cells [18, 19]. These cells provide a conducive environment for its replication and survival. The shedding of *C. burnetii* in milk can vary among individual cows and over time. This infected milk can pose significant transmission risks within herds and to humans consuming unpasteurised milk. The transmission of *C. burnetii* to calves primarily occurs through the ingestion of contaminated colostrum or milk from an infected cow. Calves that consume contaminated milk excrete the bacterium through their urine and faeces, thus promoting the dissemination of the infection in the environment [20]. The infection of the mammary glands therefore influences the transmission to other livestock, impacts the environment, and poses potential risks to food safety.

2.9.3. Excreta

While *C. burnetii* primarily targets the mammary glands, placentas, and reproductive organs in cattle, GUATTEO et al. found that the bacteria were also shed via the faeces, however scarcely and sporadically [14]. There is little research on the presence of the bacteria in the urine however MAURIN et al. stated that mice injected intranasally or intraperitoneally with *C. burnetii* develop granulomatous lesions with mononuclear cells in the spleen, liver, kidneys, and adrenals [5]. The ability of the bacteria to infect the kidney indicates the potential for transmission via the urine. The resilience of *C. burnetii* in the environment means that even low levels of the bacteria in faeces or urine can be significant for transmission. When other cattle come into contact with contaminated pasture or water sources, they risk ingesting the bacterium, perpetuating the cycle of infection.

2.9.4. Transmission to the Environment

Aerosolisation of *C. burnetii* plays a pivotal role in the environmental transmission of the bacterium. Aerosolisation of *C. burnetii* refers to the process by which this bacterium becomes suspended in the air in tiny particles or droplets, making it airborne and capable of being inhaled. There are several factors that play a contributing role to the aerosolisation process. Farms with higher cattle density, more frequent births, or a lack of isolation for birthing animals can increase the concentration of aerosolised *C. burnetii* particles and thus facilitates a faster spread of the disease. Handling and disposal methods of manure significantly influence *C. burnetii* environmental transmission. While dry, windy conditions aid in the dispersal of aerosolised bacteria and contaminated dust from dry manure. According to the epidemiological data gathered from the outbreak in the Netherlands, aerosols dispersed by the wind can spread the disease 5 km or more downwind from the infected sites [21]. Wet manure can lead to

groundwater contamination. Soils with higher permeability can further propagate the transmission to deeper water bodies and water bodies further away. The pH, flow rate, temperature and presence of organic matter can influence the persistence of the bacteria in the water. Whitney et al. states that *C. burnetii* in vitro, both low ($1x10^{-6}$) and high ($1x10^{-3}$) concentrations of *C. burnetii* can survive for >100 days in tap water at 18oC–22°C and for >140 days at 4°C [22].

A study by HERMANS et al. focused on the potential role of contaminated land-applied goat manure in human Q-fever. It was concluded that the chronological sequence of events, beginning with lambing, followed by the field application of manure, and subsequently leading to the onset of illness in human cases, aligns with and implies a potential contributory role of manure, considering the typical incubation period of human Q-fever [23].

2.10. Clinical Symptoms

2.10.1. Abortion

Establishing the potential of *C. burnetii* to cause abortions is challenging because this organism is frequently found in the placenta, birthing materials, and vaginal mucus following both abortions and normal parturitions. Confirmation of an association between lesions and presence of the organism is therefore mandatory to confirm *C. burnetii* as the cause of foetal disease [24]. Several studies have utilised PCR (Polymerase Chain Reaction) to investigate the potential involvement of *C. burnetii* in bovine abortion. PARISI et al. and Clemente et al. found 17.2% and 11.6% PCR positive animals among cattle that had aborted, respectively [24–26].

Multiple factors can influence the rate of abortions observed. Newly introduced cattle into the herd, either as replacements or due to trade, can introduce a *C. burnetii* bacterial infection. Furthermore, herd size and management practices, such as synchronisation of breeding, can amplify the intensity of birth rates in a condensed time frame leading to increased risk of birth materials in the environment resulting in increased transmission. Seasonal variation in abortion risk has not been registered, but the prevalence of seropositive cows seems to be highest in the autumn [24]. The exact reasons behind this are not fully understood but might be associated with stressors such as high temperatures or increased insect vectors.

Various infectious agents, including bacteria, viruses, and fungi, have been implicated in bovine abortions. One of the leading causes of abortions in cattle is the protozoan *Neospora caninum*. It is important to acknowledge that abortions associated with *N. caninum* are more prevalent in herds possessing antibodies to *C. burnetii* than in those herds devoid of such antibodies [24]. The heightened incidence of abortion is more plausibly attributed to *N. caninum* as opposed to

C. burnetii, given the established role of *N. caninum* as a principal causative agent of abortion in cattle [24].

Other significant pathogenic agents to note are Bovine herpesvirus 1 (BHV-1), Bluetongue virus, *Brucella abortus*, *Leptospira spp*. and *Aspergillus spp*. It is thus imperative to underscore the necessity for comprehensive diagnostic evaluations when investigating the causative agent of the abortions. This includes employing laboratory techniques such as PCR, assessing the timing of the abortion relative to the gestation period, and observing clinical manifestations.

2.10.2. Stillbirths and Weak Progeny

Stillbirths, defined as the death shortly before or during the process of parturition and weak offspring, often characterised by their inability to stand, feed, or exhibit normal neonatal behaviour have been associated with *C. burnetii* infections in several studies. A review by AGERHOLM et al. critically evaluated the hypothesis that *C. burnetii* causes a range of reproductive diseases. The review establishes a clear link between *C. burnetii* infection and various reproductive failures like abortion, premature delivery, stillbirth, weak offspring, metritis and infertility in cows [24]. It is important to mention that while *C. burnetii* can be a primary driver behind these adverse reproductive outcomes, other environmental and management-related factors can exacerbate the situation. Factors such as inadequate nutrition, crowded conditions, and simultaneous infections can intensify the effects of the bacteria, resulting increased abortions, still-births and weak progeny.

2.11. Pathology

One of the most pronounced pathological outcomes of *C. burnetii* infection in dairy cattle is within the reproductive system. In dairy cattle, the most characteristic lesion due to *C. burnetii* infection is placentitis. ARRICAU-BOUVERY et al. observed that placental lesions are marked by intercotyledonary necrosis, thickening, and a yellowish discharge [4]. The cotyledons themselves may appear necrotic and thickened. These findings are not specific to a *C. burnetii* infection, however. The mammary gland is another primary site impacted by *C. burnetii* infection. This pathology is linked to reduced milk yield and altered milk composition, presenting both health and economic concerns.

The primary Q fever infection initiates a systemic cell-mediated immune reaction and the formation of granulomas. These granulomatous lesions, characterised by a central open area surrounded by a fibrin ring, are commonly known as doughnut granulomas [27]. An enlargement of the lymph nodes, particularly the spleen and mesenteric lymph nodes, has been noted in some infected animals [5]. While the uterus and mammary glands of females are sites

of chronic C. burnetii infection, the bacteria can disseminate to other organs such as the liver and spleen [5, 28].

SANCHEZ et al. studied the pathology of experimental *C. burnetii* in foetuses through the infection of pregnant goats. He found that bacterial DNA was present in foetal liver and spleen on post inoculation day 26 and also in the lung, abomasal content and peritoneal fluid on post inoculation day 40 and in abortion cases [29]. AGERHOLM et al. stated that while the infection might predominantly be localised within the placenta, there exists a potential for its transmission to the foetus via the amniotic-oral pathway. This occurs if bacteria breach the placental barrier, contaminate the amniotic fluid, and are subsequently aspirated or ingested by the foetus [24]. Under these circumstances, the bacteria colonise the intestinal tract and have the potential to access the lungs through the trachea-bronchial pathway, subsequently leading to bronchopneumonia [24]. While the reproductive tract is the primary site of concern in *C. burnetii* infections in dairy cattle, the systemic spread highlights the necessity for comprehensive diagnostic approaches and may offer insights into transmission routes and potential control measures.

2.12. Prevention

2.12.1. Biosecurity and Hygienic Measures

Establishing stringent biosecurity measures is crucial in minimising the spread of infectious agents and safeguarding health of livestock and personnel working in the dairy industry. In PLUMMER et al. consensus statement for the American College of Veterinary Internal Medicine on managing *C. burnetii* infection in livestock, biosecurity measures were categorised into low, intermediate, and high-risk categories for patients. For low risk, personnel should recognise Q fever symptoms, seek medical attention if symptoms appear, and follow hospital biosecurity practices such as dedicated attire and frequent handwashing. Intermediate risk measures, in addition to the low-risk ones, emphasise protective gear when handling reproductive fluids, quick disposal of contaminated tissues, and medical vigilance for those exposed to high-risk animals. The highest risk protocols, building upon the previous tiers, necessitate isolating patients likely to shed *C. burnetii*, using respiratory protection after proper training, and maintaining daily temperature logs for those at the greatest risk, urging them to seek medical guidance when symptoms manifest [21].

2.12.2. Vaccination

Vaccination has been acknowledged as a robust preventive measure. Vaccinations in dairy cattle have the dual benefit of protecting the herd and reducing the zoonotic risk to farmworkers. Phase I and II vaccines exist, with Phase I vaccines primarily being used for animal vaccination and Phase II for humans. CEVA-Phylaxia Zrt. (CEVA Sante Animale, Libourne, France) is the marketing authorisation holder of the vaccine COXEVAC (containing inactivated *C. burnetii*, strain Nine Mile), with the target species cattle and goats [12]. Phase I Vaccines are derived from virulent strains of *C. burnetii* and express the full-length Phase I LPS on their surface. When administered, these antigens prime the immune system of the dairy cattle, leading to the formation of both humoral and cellular immune responses [30]. In general, Phase I vaccines are more immunogenic and tend to offer superior protection against the disease. Phase II Vaccines are prepared from avirulent strains with truncated LPS. Phase II vaccines were primarily developed for human vaccination.

The "Coxevac" vaccine is given as two injections under the skin, three weeks apart. Nine months later, two additional injections should be given to cattle, again three weeks apart [31]. "Coxevac is the commercial vaccine used primarily in Europe, especially in the wake of the Dutch Q-fever epidemic, to vaccinate goats, sheep and cattle.

2.12.3. Introduction of New Animals

The introduction of new animals into a herd, or the movement of animals between different livestock groups on a farm, can pose a significant biosecurity threat to the preservation of a herd's disease status. These risks can be mitigated through the use of quarantining or laboratory testing using serological or PCR methods.

In 2006, KLEE et al. formulated and assessed real-time PCR assays utilising TaqMan technology, which focused on the unique icd gene (isocitrate dehydrogenase) and the transposase gene within the IS1111a element, which is found in multiple copies within the *C. burnetii* genome. The assays demonstrated minimal variability, signifying a high level of reproducibility for both tests [32]. Employing meticulous laboratory diagnostic techniques such as those developed by KLEE et al. coupled with strategic quarantine protocols offers a formidable strategy to bolster biosecurity measures.

2.12.4. Ventilation

Effective ventilation lessens the microbial presence within dairy farm environments, leading to enhanced air quality and consequently benefiting the health of the animal [33]. Proper

ventilation will reduce the presence of the bacteria and thus decrease the risk of infection. It will also impact the temperature and humidity distribution across the barn, factors upon which the proliferation of the bacteria is dependent.

2.13. Laboratory Detection

Several methodologies have been developed over the years for the detection of *C. burnetii*. Serological methods, primarily indirect immunofluorescence assay (IFA), remain a gold standard for the diagnosis of Q fever, allowing the identification of phase-specific antibodies [34]. As mentioned earlier, for direct detection, PCR-based assays targeting the IS1111a transposase gene of *C. burnetii* offer a rapid and highly sensitive method. Moreover, modern techniques like multi-locus variable-number tandem-repeat analyses (MLVA) and MST are used for genotyping and epidemiological studies to understand transmission patterns and bacterial source tracking [32].

2.14. Q- Fever in humans

Q-Fever has posed recurrent challenges to public health since its discovery in the 1930's, especially in livestock-intensive regions due to its zoonotic nature. Globally, Q-Fever has been reported in almost every country. While the disease is reported across all European nations, it appears to have a lower incidence or be less diagnosed in northern countries. There are reports on Q-Fever from Sweden, Finland, Poland, the Czech Republic, Slovakia, and Romania [35]. The 2007-2010 epidemic in the Netherlands, where over 4,000 human cases were identified, predominantly linked to dairy goats, remains one of Europe's largest documented outbreaks. In the wake of this epidemic, the EU formulated legislations, such as the (EU) 2016/429 regulation, which emphasised enhanced surveillance, data collection, and disease management for transmissible animal diseases like Q-Fever [36].

Upon human infection, primarily through inhalation of aerosolised bacteria, *C. burnetii* targets the alveolar macrophages. It subsequently replicates within, leading to cell destruction and bacterial dissemination. Histopathologically, the acute form of the disease often depicts granulomatous inflammation, while the chronic version exhibits multi organ involvement, predominantly endocarditis, also hepatitis and less frequently, vascular lesions [5].

The treatment options can vary depending on whether the patient is suffering from acute or chronic Q-fever. Doxycycline is the first-line treatment for acute Q-fever in adults. Typically, it's prescribed at 100 mg taken orally twice daily for about 2 to 3 weeks. Chronic Q-fever is more challenging to treat and can be life-threatening. It's mainly characterised by endocarditis, vascular infections, or persistent localised infections. A combination of doxycycline and

hydroxychloroquine is usually prescribed for long durations, often extending to 18 months or longer [5].

Q fever prevention strategies including hand hygiene, wearing protective clothing and shoes, eye goggles and face shields, and respiratory protection using a face mask or N95 respirator when the risk of exposure is high such as handling birth fluid/placenta [37]. Along with these measures, a vaccine is also available. The vaccine comprises whole-cell inactivated *C. burnetii*. A significant challenge was that the Q-Vax® vaccine, Q-VAX, Commonwealth Serum Laboratories, Parkville, Australia, could induce adverse reactions in individuals who had been previously exposed to the bacterium. To mitigate this risk, a skin test to detect prior exposure, similar to the tuberculin test for TB, was recommended before vaccination. Those with a positive skin test were typically not vaccinated because of the risk of severe reactions. According to DELSING et al., given the potential side effects and the limited supply of Q-Vax®, the vaccine's use in the Netherlands during the 2007 to 2010 epidemic was restricted to specific high-risk groups. The Health Council of the Netherlands also issued advice on vaccinating patients with increased risk of chronic Q fever. The target population of 'increased risk groups' has been defined as patients with underlying cardiac conditions, as well as patients with a known aortic aneurysm or vascular prosthesis [38].

The worldwide prevalence of Q- Fever, coupled with significant outbreaks such as the 2007-2010 epidemic in the Netherlands, highlights the importance of vigilant surveillance and robust disease management.

3. Materials and Methods

3.1. Farm Livestock and Infrastructure Overview

The investigations were executed at a large-scale dairy farm situated in the North-Western region of Hungary. The farm operates as a dairy cattle facility, housing cows, heifers, and calves of the Purebred Holstein-Friesian cattle breed. The origin of the farm's livestock dates back to 2003 when 44 pregnant heifers were procured from Szeghalom. The following year, in 2004, a total of 117 animals were relocated to the farm as part of a liquidation process in Bácsalmás. In 2005, the livestock was expanded by 56 cows and 57 heifer calves, sourced from the nearby Dénesfa. Subsequently, in 2006, the farm welcomed 30 pregnant heifers and 50 heifer calves from Dalmand. The procurement of animals from diverse origins resulted in a significant rise in infectious diseases, attributed to varying immune statuses. Procurement of animals from external sources was halted until 2013. In 2013, 127 pregnant heifers were purchased. An additional 60 animals were acquired from Bogyoszló and Farád in 2015. Further expansion was facilitated through internal breeding strategies. The numerical growth in productive animals demonstrated a continuous upward trend, with averages of 797 (2015), 860 (2016), 859 (2017), 904 (2018), 977 (2019), 1045 (2020) and 1040 (2021).

The farm housing comprises several deep-bedded barns with designated areas for pregnant heifers, culled cows, close-up heifers, close-up cows, dry cows, lame cows, mastitis cows, and a calving/hospital barn. Additionally, two other buildings house four resting stall barns, including groups of 2 fresh cows, 4 high-producing cows, and 2 cows at the end of lactation. The animals are strategically split based on the number of lactations, with a preference for maintaining first-calf heifers and cows that have calved multiple times in separate groups throughout the entire lactation period. In total, the productive animals are divided into 12 milking groups.

The milking parlour features a BouMatic rotary milking machine with 50 stalls. The milking system is managed through the Smart Dairy farm management program. There are three milkings per day requiring 3 farm workers.

3.2. The Purpose of the Examination

The primary objective of this analysis is to assess the production impact of the Q-fever vaccination and to compare the financial investments in the vaccines against the potential economic benefits of improved reproductive performance and decreased treatment costs.

3.3. Vaccination Strategy

The farm implemented a vaccination strategy following a series of diagnostic tests. These included testing bulk milk for antibodies (Enzyme-Linked Immunosorbent Assay, ELISA) and antigen (PCR), as well as analysing blood samples from aborted cows for antibodies (ELISA) and antigen (PCR). The presence of antibodies in both milk and blood indicated a post-infection state, while the absence of antigens in both mediums suggested no active infection. The identification of the pathogen, coupled with its zoonotic importance, substantiated the need for vaccination.

The selected vaccine is Coxevac (Ceva-Phylaxia), administered subcutaneously at a dosage of 4 ml. Two distinct vaccination strategies were considered:

1. Herd-Level Immunisation: This approach involves administering two vaccine shots spaced 3-4 weeks apart.
2. Continuous Immunisation: Vaccination occurs consistently at predetermined intervals during lactation or at a specific age.

The farm opted for the second method. All cows underwent the initial vaccination during the drying-off period, followed by the second shot upon arrival at the close-up barn three weeks before calving. Pregnant heifers received their first shot at seven months into pregnancy and the second shot upon arrival at the close-up barn four weeks prior to calving.

3.4. Approach to the Economic Analysis

The purpose of the calculations is to quantify the economic aspects of Q Fever vaccination, allowing for an objective assessment of whether the vaccination program was beneficial for the farm. The Q-fever vaccination was initiated in September 2019. This study employs an approach wherein the initial four months subsequent to the commencement of the vaccine (September 2019 to December 2019) are treated as an extension of the "before vaccination" period. This adjustment is made to accommodate the potential latency in the vaccine's impact, recognising the necessity of allowing sufficient time for the immunisation to exert its effects. Summarily, this study endeavours to conduct a thorough 48-month analysis, encompassing data from January 2018 to December 2019 (pre-vaccination) and January 2020 to December 2021 (post-vaccination). In the economic analysis we used the partial budgeting method.

3.5. Vaccination Programme Evaluation

In the applied partial budgeting method, benefit of the vaccination programme can be divided into three main categories:

- Reduced costs stemming from fewer days open leading to reduced feeding expenses.
- Reduced costs arising from decreased number of treatments of retained placenta and other reproductive failures.
- Reduced cost from decreased semen and synchronisation medication doses.

The analysis incorporated financial data from 2021 for the cost of feed per cow per day, the calculation of retained placenta costs, and the vaccine costs for all studied years. The daily cost of feed per cow was 2046 HUF or €5.40 in 2021. The drug Centraureo, employed in treating retained placenta, required two doses, with each dose costing 121 HUF or €0.32. The individual cost of each vaccine dose was 438 HUF or €1.16. The average cost of semen and hormone synchronisation treatment was determined by considering the pre- and post-vaccination annual average costs. In the partial budgeting the assessment of these benefits (savings) will be compared to the total cost of the vaccines. In concluding the comprehensive economic analysis of the vaccine programme, the net savings will be determined as the margin between the benefits and costs of the vaccination program.

The number of services per conception, along with the conception rate, conception rate following the first AI treatment and number of abortions will also be analysed. These parameters assess the comprehensive impact of the vaccination program, as improved reproductive outcomes contribute to enhanced animal health and foresee a reduction in future costs, emphasizing the long-term economic benefits.

4. Results and Discussion

Table 1: Comparative Analysis of Reproductive Parameters Before and After Vaccination

	Pre- Vaccination Period	Post Vaccination Period	Difference
Average herd number (cows, heifers/ year)	941	1043	+ 102 (↑10.8%)
Average number of inseminated animals (cows, heifers/ year)	227	220	- 7 (↓3.2%)
Average number of pregnant animals (cows, heifers/ year)	63	69	+ 6 (↑9.37%)
Average number of services per conception (number of services per conception/ year)	2.96	2.59	- 0.375 (↓12.67%
Average overall conception rate (%)	28 ∓0.06	31 ∓ 0.05	+ 3 (↑10.71%)
First AI average conception rate (%)	33 ∓ 0.08	35 ∓ 0.05	+ 2 (↑6.06%)
Average number of open days (days/ year)	124.38	117.96	-6.42 (↓5.16%)
Average number of retained placenta (retained placenta/ herd/ year)	39	24	- 15 (↓38.5%)
Prevalence of retained placenta per pregnant cow (%)	5.1	2.8	-
Average number of abortions (abortions /herd/ year)	7	14	7 (↑100%)
Average cost of semen and hormone synchronisation treatment (HUF/ herd/ year)	25,143,791	24,290,931	-852,860 (↓3.39%)
Total number of vaccines administered (number of vaccines)	-	4,341	-

Table 1 shares a comparative analysis of reproductive parameters before and after vaccination, with graphical representation integrated throughout the ensuing discussion. The data demonstrates a 12.67% improvement in the average services per conception, highlighting the potential of Q fever vaccination in enhancing reproductive efficiency in dairy cattle (**Figure 1**).

Figure 1. Services Per Conception
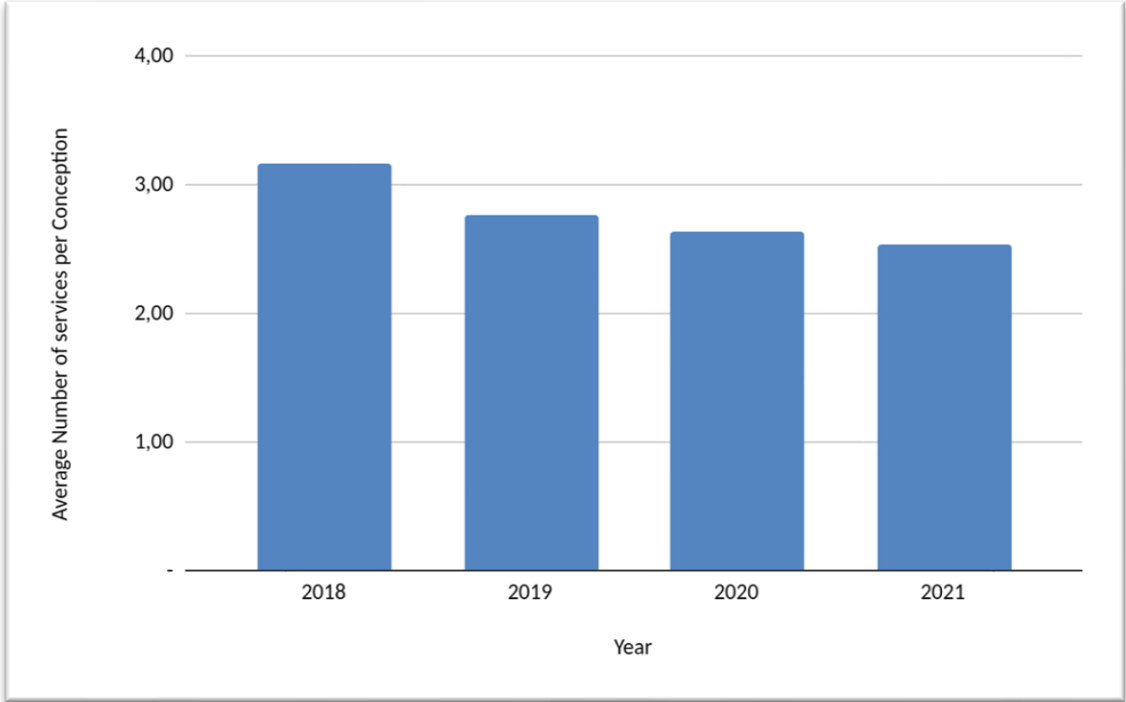
Examining the conception rate, as presented in Table 1 and depicted in **Figure 2**, the average overall conception rate during the pre-vaccination period is relatively stable, with some mild fluctuations. The post-vaccination period shows an increase of 10.71% in the average overall conception rate compared to the pre-vaccination period, with less variability. Table 1 and **Figure 3** show the average conception rate following the first AI treatment in the post-vaccination period is reflects the trend of the average overall conception rate, indicating a 6.06% increase. Similarly, the standard deviation decreases during the post-vaccination period, reflecting a reduced variability in the data.
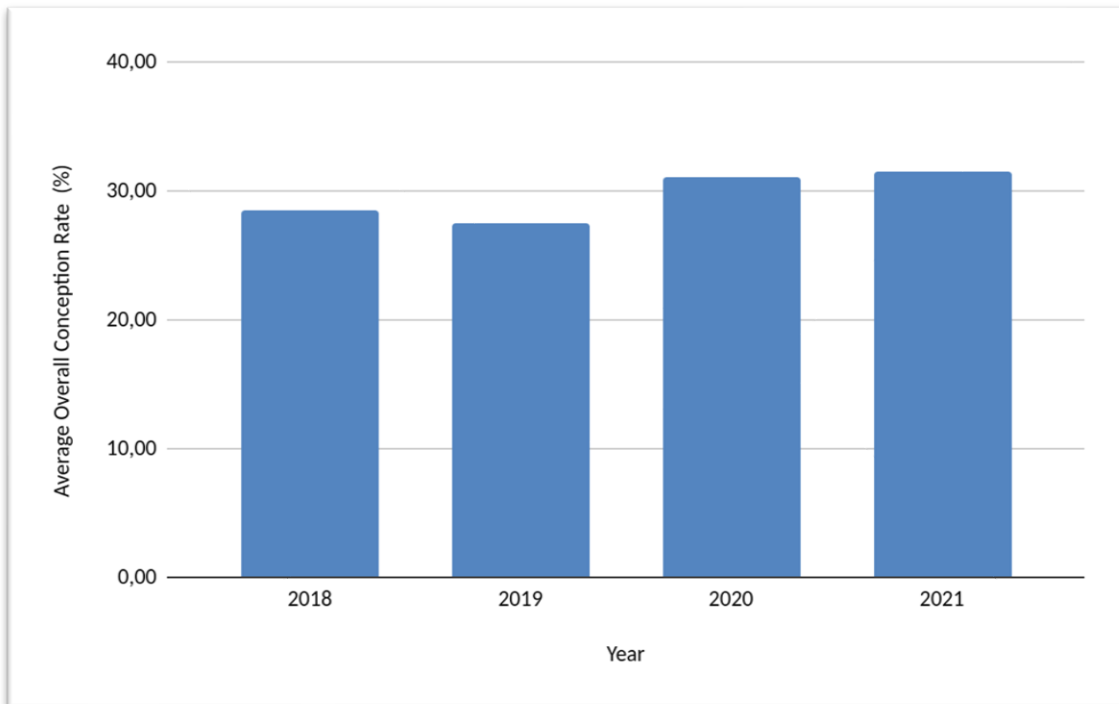
Figure 2. Conception Rate

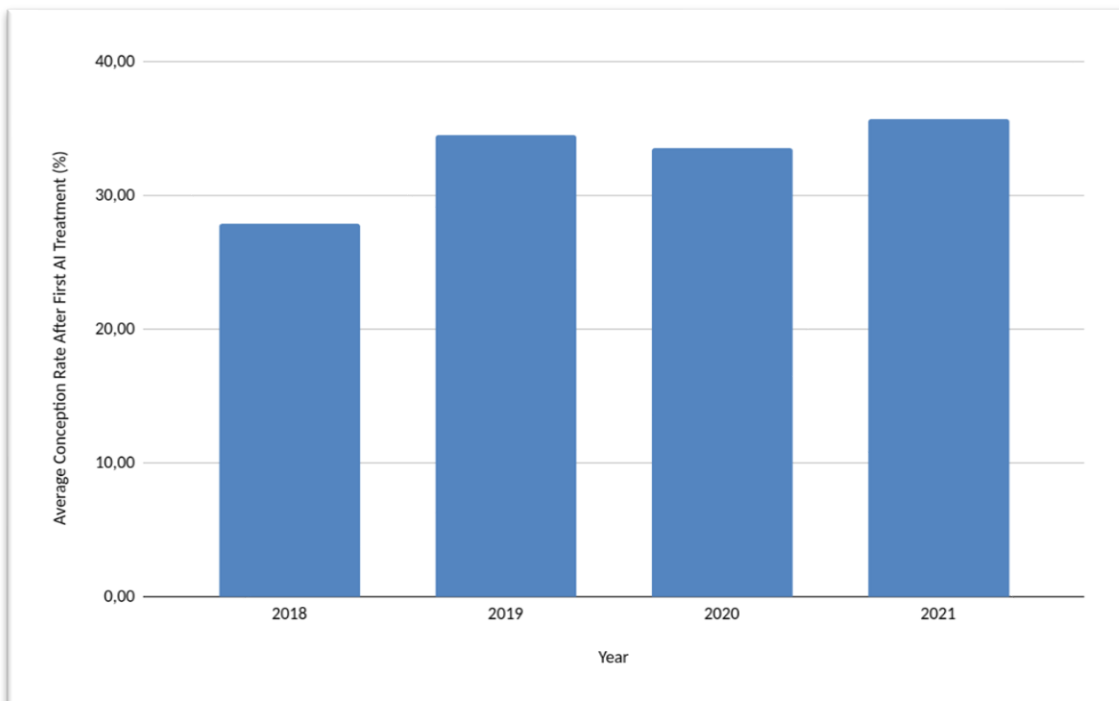*Figure 3. Conception Rate After First AI Treatment*

Table 1 details a post-vaccination decrease of approximately 6.4 days in average open days, reflecting a 5.16% improvement in reproductive efficiency. This reduction is visually represented in **Figure 4**, suggesting improved reproductive efficiency and potentially enhanced overall herd health.
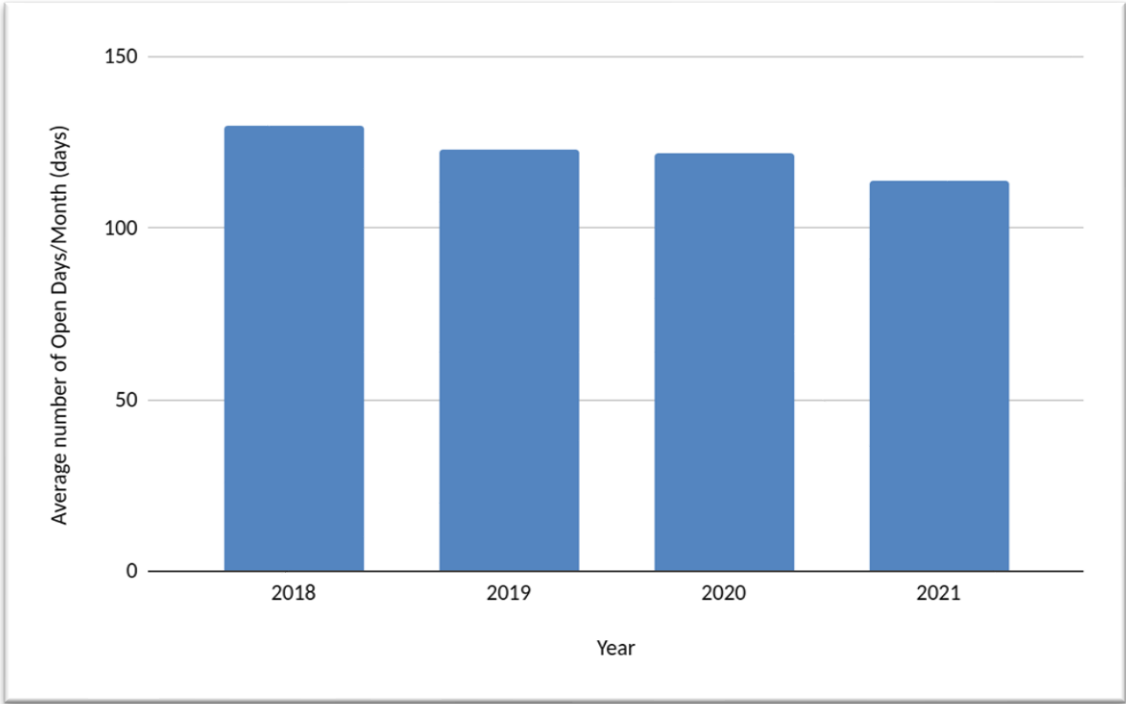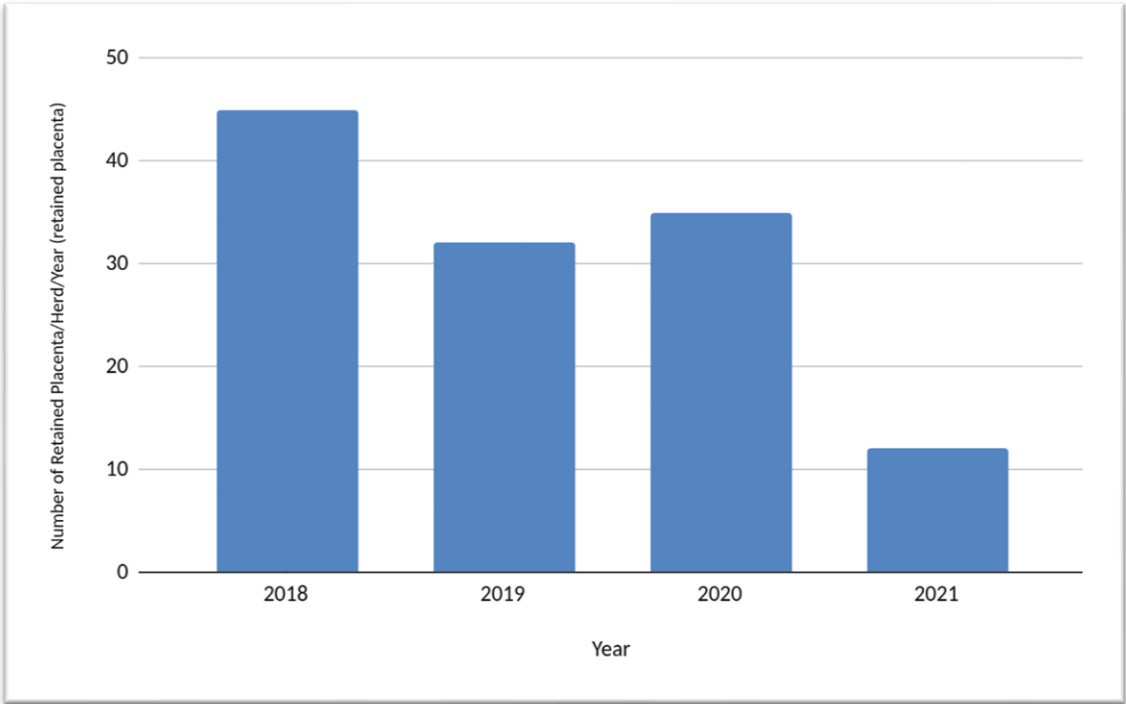
Figure 4. Average Number of Open Days

Figure 5. Number of Retained Placenta Cases

The reduction in the number of retained placenta, as indicated in Table 1 and visually depicted in **Figure 5**, presents a compelling indication of the potential impact of Q fever vaccination on dairy cows. The number of retained placenta dropped from 39 retained placenta per herd per year pre-vaccination to 24 retained placenta per herd per year post-vaccination. Additionally, there is a notable decrease in retained placenta prevalence per pregnant cow, with the rate dropping from 5.1% retained placenta per pregnant cow pre-vaccination to 2.8% retained placenta per pregnant cow post vaccination.

Figure 6. Number of Abortions
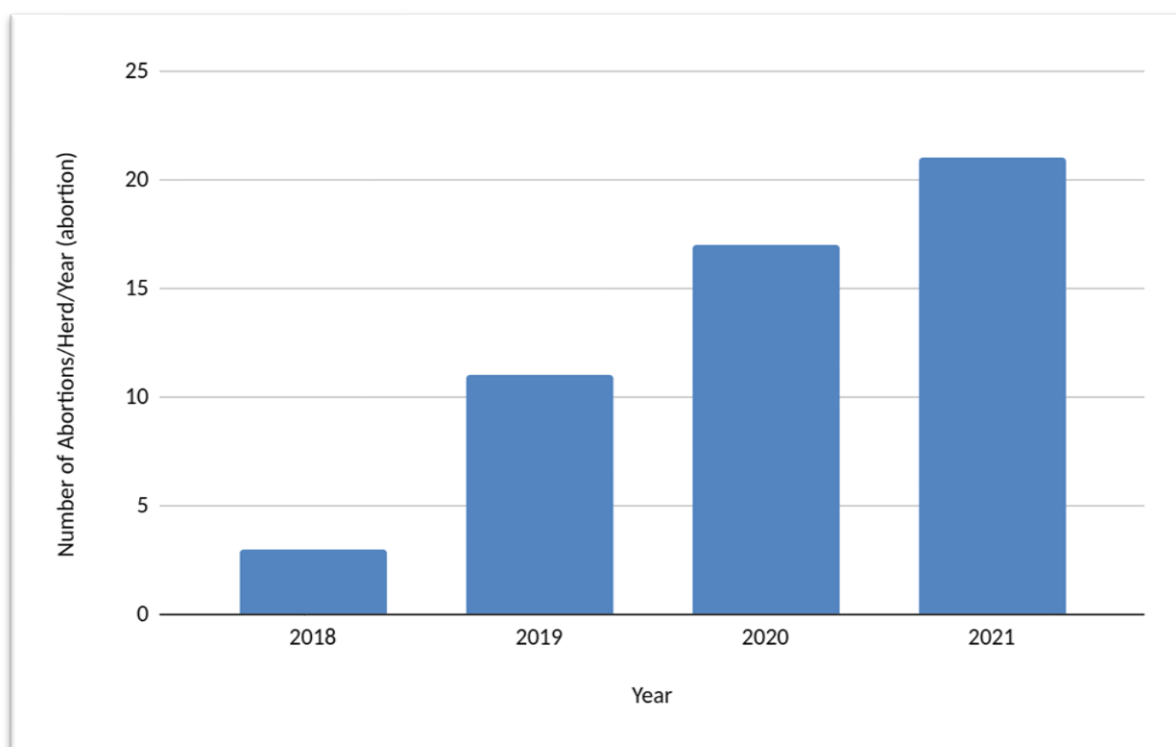
Unexpectedly, Table 1 and **Figure 6** reveal an increase in abortion rates post-Q fever vaccination. The pre-vaccination average of 7 abortions per herd per year rises to 14 abortions per herd per year post-vaccination. Given this unexpected outcome, further in-depth analysis is warranted. The presence of bacterial agents such as *Leptospira spp.*, fungal infections such as *Aspergillus spp.*, protozoal agents such as *Neospora caninum* and mycotoxins should be investigated. Throughout the analytical period, spanning both pre- and post-vaccination phases, the farm lacked specific and sensitive testing methodologies for prevalent mycotoxins known to induce abortion, such as Ergot Alkaloids. The existing testing protocols focused exclusively on fundamental toxins, notable for their common occurrence and relevance to food hygiene, namely aflatoxin, Deoxynivalenol (DON), Zearalenone, and T-2 Mycotoxin. Instances of mortality and morbidity were noted, aligning with findings indicative of mycotoxin-related

complications, including hepatic impairments and haemorrhagic events. A comprehensive examination will offer insights into potential co-factors contributing to reproductive outcomes post-vaccination. The interaction among these various factors complicates the analysis highlighting the need a more comprehensive investigation to gain a holistic understanding.

The pre-vaccination costs of the annual cost of semen and hormone synchronisation treatment for the herd are conveyed in Table 1. These amounted to 25,143,791 HUF average per year pre-vaccination, decreasing to 24,290,931 HUF average per year post-vaccination.

Table 2. Reduced costs in the post-vaccination period

INDICATORS	HUF	€
Reduced feeding cost due to fewer days open per herd per year	-13,695,129	- 35,945.22
Reduced retained placenta cost per herd per year	- 4,636	-12.17
Reduced cost of semen and hormone synchronisation treatment per herd per year	- 852,860	- 2,238.48
Total	-14,552,625	- 38,195.87

Note: 1 € = 381 HUF

Table 2 outlines the cost reductions resulting from improved efficiencies due to vaccination. This encompasses diminished expenses linked to the number of open days, lowered costs attributed to a decreased number of treatments necessitated by reduced instances of retained placentas, and diminished expenses associated with semen and hormone treatments owing to enhanced fertility.

A reduction in days open signifies a more efficient breeding cycle, potentially yielding increased productive days per cow and subsequent cost savings in feed during non-productive periods. The cost savings of feed resulting from the decrease in the number of open days is 13,695,129 HUF/herd/year. Additionally, the observed 38.5% reduction in retained placentas equates to an annual cost savings of 4,636 HUF/herd/year. Furthermore, the annual cost reduction for semen and hormone synchronisation treatment is -852,860.20 HUF/herd/year, reflecting a 3.39% decrease. Beyond immediate savings, improved reproductive outcomes contribute to the long-term productivity and sustainability of dairy operations.

Table 3. Overall Economic Analysis Data

INDICATORS	HUF	€
Total reduced costs per herd per year	14,552,625	38,195.87
Vaccination per herd per year	949,594	2,492.37
Profit per herd per year	13,603,031	35,703.50
Cost/benefit ratio	14.33	
ROI (%)	1432.5	

Note: 1 € = 381 HUF

Table 3 encompasses the comprehensive economic analysis data. The annual cost of the Q fever vaccine program amounts to 949,594 HUF/herd/year or €2,490.37/herd/year, serving as a crucial reference in the economic assessment. Despite the implementation cost, Q fever vaccination for dairy cows yields significant net benefits by reducing expenses related to retained placenta, semen and hormone synchronisation treatment, and feed costs. This results in an annual net savings of 13,603,031 HUF/herd/year or €35,703.50 /herd/year, highlighting the economic viability and positive financial impact of the vaccination initiative on reproductive efficiencies in dairy farming.

5. Conclusions

The economic analysis of the Q fever vaccination program on the dairy farm reveals a compelling case for its implementation. The notable improvements in reproductive efficiency, evidenced by a reduction in services per conception rate and open days, along with a decrease in retained placenta instances, emphasise the favourable influence on overall herd health and, consequently, the improvement in economic performance. The reduction in the annual cost of semen and hormone synchronisation treatment further contributes to the economic viability of the program. While the associated costs related to the reduction in days open and number of retained placentas contribute positively to the economic viability of the vaccination program, it is crucial to note an unexpected increase in abortion rates post-vaccination.

The interplay of variables such as temperature, humidity, feed quality, and nutritional deficiencies significantly influence reproductive performance. A holistic investigation into the intricate dynamics influencing reproductive performance, with specific attention to mycotoxins due to the post-mortem findings, would enhance the thoroughness of the analysis. Furthermore, conducting diagnostic testing to ascertain causation for retained placentas and abortions would substantially refine the data, determining whether *C. burnetii* is the underlying cause or co-factor of these issues. This clarification would subsequently impact the economic analysis.

In conclusion, the examination of this Q fever vaccination program highlights substantial economic gains resulting from the enhanced reproductive efficiency achieved through vaccination.

6. Summary

This thesis examines the production impact, particularly pertaining to reproductive performance, of Q fever vaccination on a large-scale dairy farm in the North-Western region of Hungary. The profound economic implications of Q fever on dairy farms encompass potential reproductive outcomes such as lower conception rate, increased numbers of retained placentas and increased number of abortions in dairy cattle. It therefore poses a considerable risk to the profitability and sustainability of dairy operations. The primary objective of this analysis is to assess the production impact of the Q-fever vaccination by comparing the financial investment in the vaccine against the potential economic benefits of improved reproductive performance and decreased treatment costs.

This study employed data from 2018 and 2019 as the pre-vaccination period and data from 2020 and 2021 as the post-vaccination period. The study revealed that post-vaccination, reproductive efficiency notably improves, with a 12.6% reduction in services per conception rate and a 10.71% increase in conception rate, indicating enhanced performance in dairy cattle. Additionally, a reduction in average open days by approximately 6.4 days leads to cost savings of 13,137 HUF/cow/year or €34.45/cow/year associated with feed during non-productive periods. The decrease in retained placenta instances per herd per year from 39 to 24 post-vaccination results in a cost reduction of 4,636 HUF/ herd/ year or €12.17/herd/year, supporting the positive impact of the vaccine on overall herd health and productivity. Furthermore, the reduction in annual costs related to semen and hormone synchronization of -852,860 HUF/herd/year or €2238.48/herd/year treatment further underscores the positive economic influence of the vaccination.

Accordingly, there is an overall reduced cost of 14,552,625 HUF or €38,195.87 annually. The total annual cost of the vaccine program is 949,594 HUF/year or €2492.37/year. This leads to an overall net savings of 13,603,031 HUF or €35,703.50 annually. Overall, the net savings achieved emphasise the economic viability of implementing Q fever vaccination in dairy farming for long-term sustainability and positive financial outcomes.

7. Összefoglalás

Ez a dolgozat a Q-láz elleni vakcinázás termelésre gyakorolt hatását vizsgálja, különös tekintettel a szaporodási teljesítményre, egy nagy tejgazdaságban, Magyarország északnyugati régiójában. A Q-láznak a tejgazdaságokra gyakorolt mélyreható gazdasági hatásai olyan lehetséges reprodukciós következményeket foglalnak magukban, mint az alacsonyabb fogamzási arány, a magzatburok-visszamaradás számának növekedése és a vetélések számának növekedése a tejelő szarvasmarháknál. Ezért jelentős kockázatot jelent a tejtermelő üzemek jövedelmezőségére és fenntarthatóságára nézve. Az elemzés elsődleges célja a Q-láz elleni vakcinázás termelési hatásának értékelése a vakcinázási költségnek a javuló reprodukciós teljesítményből és a kezelési költségek csökkenéséből származó potenciális gazdasági előnyök összehasonlításával.

Ez a tanulmány a 2018-as és 2019-es év adatait használta a védőoltás előtti időszakként, a 2020-as és 2021-es év adatait pedig a védőoltás utáni időszakként. A tanulmány eredményei azt mutatták, hogy a vakcinázás után a szaporodási hatékonyság jelentősen javult, a fertilitási index 12,6%-os csökkenésével és a fogamzási arány 10,71%-os növekedésével. Emellett az üres napok átlagos számának kb. 6,4 nappal való csökkenése 13.137 Ft/tehén/év vagy 34,45 €/tehén/év költségmegtakarítást eredményezett a nem produktív időszakok alatti takarmányozási költség-megtakarítás kapcsán. A magzatburok-visszamaradások éves számának 71-ről 47-re való csökkenése az oltást követően 4.636 Ft/állomány/év (12,17 €/állomány/év) költségcsökkenést eredményezett, ami alátámasztja a vakcina pozitív hatását az állomány általános egészségi állapotára és szaporaságára. Továbbá a sperma- és ivarzás-szinkronizálási költségek éves csökkenése (-852.860 Ft/állomány/év; (2.238,48 €/állomány/év) tovább erősíti a vakcinázás pozitív gazdasági hatását.

Ez összességében 14.552.625 Ft, azaz évi 38.195,87 € bevételt eredményezett. A vakcinázási program teljes éves költsége 949.594 Ft/év (2492,37 €/év) volt. Ez összesen 13.603.031 Ft, azaz évi 35.703,50 € nyereséget eredményezett Összességében a vakcinázási program jövedelmezősége alátámasztja a Q-láz elleni vakcinázás bevezetésének gazdasági életképességét és hosszú távú fenntarthatóságát a tejtermelő szarvasmarha telepek esetében.

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Acknowledgements

I would like to express my sincere gratitude to Dr. Gerda Kiss for her enthusiastic teaching and generous sharing of knowledge in dairy farm medicine. Beyond the insights gained through this thesis, my practical experiences on the farm in North-Western Hungary during my studies have provided me with an in-depth understanding of varied methodologies in veterinary medicine for dairy farming in Hungary. This exposure allows me to bring a broader perspective and undoubtedly inspire innovative ideas to contribute to my future veterinary practices in Ireland.

I would like to extend my gratitude to Dr. László Ózsvári. Our conversations regarding the approach to this economic analysis have been truly delightful. I sincerely value his unwavering commitment, systematic approach, and ability to prioritise and discern the key aspects of this study. His sense of humour, coupled with these qualities, has made concluding my final assignment of my studies a thoroughly enjoyable experience.

I would also like to express my heartfelt thanks to Katie Nixon. From the first night out in university to this very moment, she has been an exceptional friend. I am grateful to Katie for her valuable contributions of ideas to this thesis and support throughout.

Lastly, I want to extend my gratitude to my family and all my other friends. The small acts of kindness from each of them over the years have been the essential building blocks that have brought me to this significant point. I would like to specifically acknowledge my father and mother, Joesph and Jan Ryan, my brother, Eddie Ryan, and my friends, Cliabhna Fanning and Fiona Shannahan, for the individual ways in which they have provided encouragement and support.