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Review article

Vaccination paradox in bluetongue control

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Abstract

The bluetongue virus (BTV) is the causative agent of bluetongue, a non-contagious viral disease affecting ruminants. It is transmitted by insects of the *Culicoides* genus. The disease's complexity is reflected in the large number of recognized serotypes. Bluetongue can be particularly severe in sheep, causing symptoms such as fever, facial edema, cyanosis of the tongue, oral lesions, lameness, and high mortality. Cattle serve as a subclinical reservoir. Controlling BTV is a crucial challenge in livestock farming, requiring the identification of innovative vaccination strategies that induce effective immunity against the various circulating serotypes. In this context, the "vaccine paradox" of BTV emerges, revealing a discrepancy between the proven immunological efficacy of available vaccines and their inability to ensure stable epidemiological control. This paper investigates the reasons for this discrepancy and clarifies the structural limitations that prevent current vaccination strategies from achieving this.

Keywords: Bluetongue, Cattle, Sheep, Vaccine, Vector, Virus

Introduction

Bluetongue virus (BTV) is a member of the *Reoviridae* family with a double-stranded RNA genome organized into ten distinct segments [1, 2]. These segments are involved in the synthesis of specific proteins, which are categorized as either structural (VP1-VP7) or non-structural (NS1-NS4). VP2 and VP5 are responsible for forming the outer capsid [3]. Due to its high variability, VP2 is responsible for defining the viral phenotype [4]. VP5 is responsible for releasing the viral core into the cytoplasm by forming pores in the endosomal membrane. VP3 contributes to the formation of the icosahedral structure, creating an environment essential for various enzymatic functions. VP1 acts as an RNA-dependent RNA polymerase, synthesizing mRNA and replicating the genome, while VP4 is an enzyme exhibiting phosphohydrolase, methyltransferase, and guanylyltransferase activities [5]. In contrast, VP6 has helicase activity that is crucial for unraveling the double-stranded viral RNA and preventing its reassembly. VP7 is responsible for forming the intermediate layer of the capsid [6]. The BTV protein complement is completed by non-structural proteins, which represent the virus's operating system. The most abundant component is the NS1 protein, which forms microtubules essential for the synthesis of various viral proteins. NS2 is responsible for forming cytoplasmic microenvironments in which the recognition and sorting of genomic segments occur. Finally, NS3 and NS3A regulate the release of newly formed particles. VP1-VP7 play crucial roles in defining the core and capsid, while NS1-NS4 are involved in replication processes [7].

Culicoides insects are the vectors responsible for transmitting the virus [8]. Transmission does not occur through direct contact between animals, and BTV is not a zoonotic disease. BTV primarily affects sheep, cattle, and goats, but can also infect various species of wild ruminants, including deer, fallow deer, roe deer, elk, and other bovids such as mouflons and ibex [9]. Cattle act as reservoirs of infection due to their prolonged viremia, which is often asymptomatic. However, reduced milk production, transient respiratory problems, hypersalivation, or mild mucosal lesions may be observed in some cases. Sheep, on the other hand, are more susceptible to infection. BTV causes a complex clinical picture in sheep, characterized by high fever, loss of appetite, a swollen muzzle, conjunctivitis, ulcers, and lesions in the oral cavity (Figure 1). Foot lesions and interdigital hyperemia cause lameness and impaired mobility. Tongue cyanosis occurs in more advanced cases and is the pathognomonic sign.

Mortality is significantly influenced by factors such as the genetic susceptibility of the affected population, viral serotype, immune status, and environmental conditions that can affect vector competence. Initially, BTV was a disease that was typically confined to tropical and subtropical regions. However, since the early 2000s, different serotypes have spread to southern Europe and, due to climate change, have since expanded into central and northern Europe.

The most significant European epidemics, caused by BTV serotypes 1, 4, and 8, have demonstrated the virus's and vector's capacity for rapid adaptation to new ecosystems [10]. Currently, at least twenty-seven BTV serotypes are recognized, although classification remains under discussion [11].

European Union (EU) Regulation 2016/429, known as the Animal Health Law, identifies bluetongue (BT) as a notifiable disease, highlighting its economic, commercial, and health impact [12]. The disease has significant economic and epidemiological consequences worldwide. It affects livestock productivity, international trade, healthcare costs, and the need for increasingly advanced surveillance systems. The emergence of new outbreaks leads to the implementation of measures to restrict animal movement, resulting in serious economic repercussions, reduced trade, and lower livestock productivity. Recent data from 2025 confirms the significant relevance of this topic, with cases recorded in several European countries, including France, Germany, Austria, Italy, and Denmark. Specifically, 6,321 cases of the BTV-3 serotype were recorded in France, compared to over 290 in Switzerland. For the BTV-8 serotype, 1,104 cases were recorded in France, compared to over 400 in Italy [13]. The recent emergence and rapid spread of the BTV-3 serotype in Europe have highlighted the continent's vulnerability to new viral introductions. The fact that it has spread to multiple countries in a single season confirms the limitations of current surveillance and vaccination systems, and reinforces the need for more adaptive, multiserotype strategies. These elements emphasize the importance of identifying more flexible, multiserotype vaccination strategies, strengthening surveillance systems, and implementing immunity planning that considers vector dynamics. In this context, the need to identify increasingly effective strategies against BTV is urgent.

This review aims to analyze the multiple factors that influence the effectiveness of vaccination strategies against BTV, evaluating how their interaction contributes to outlining the vaccine paradox.

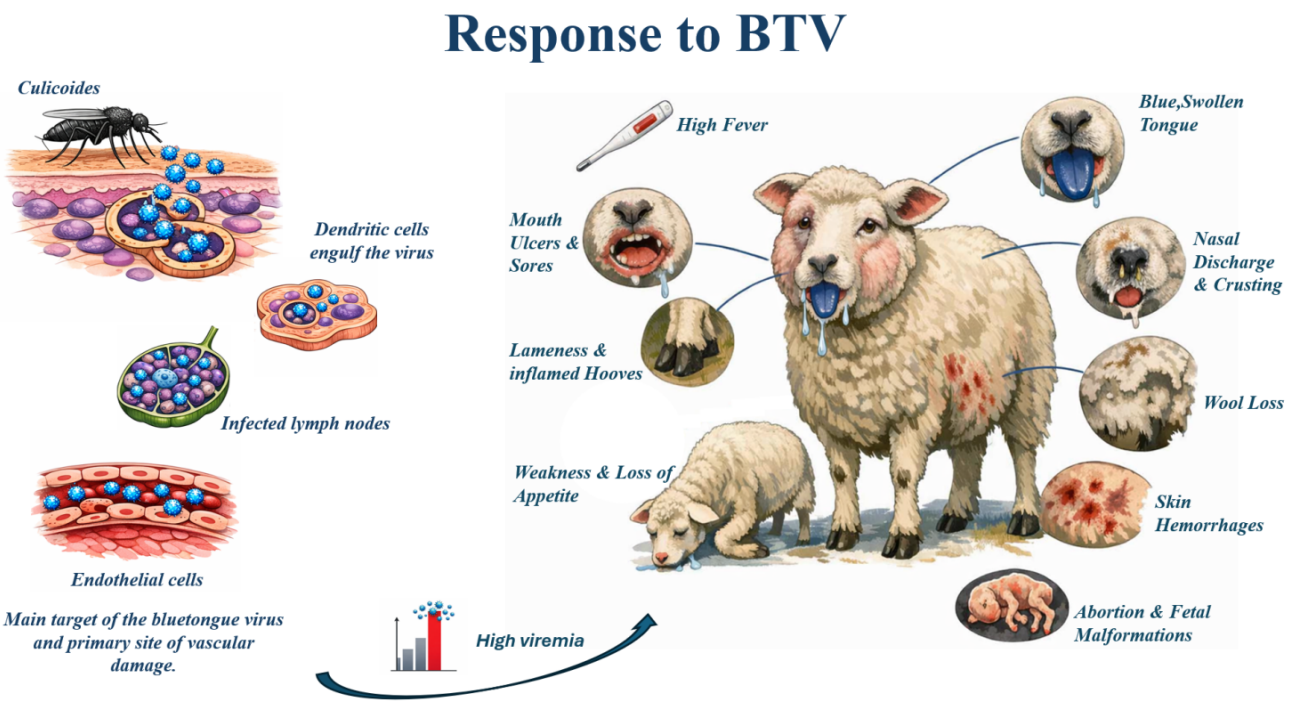

Figure 1: The figure illustrates the most common signs observed in sheep affected by BTV.

Serotype specificity

The antigenic complexity of the BTV is undoubtedly one of the main obstacles to developing effective vaccination strategies. Although the immunological efficacy of the vaccine is influenced by several factors, the presence of many serotypes is crucial in determining the quality and breadth of the protective response [14, 15]. Each of the various serotypes has unique characteristics in terms of virulence, capacity for diffusion, and epidemiological impact, resulting in highly complex and diverse conditions that amplify the difficulty in managing them using vaccines characterized by selective protection against specific serotypes. Available vaccines, particularly inactivated ones, offer serotype-specific protection almost exclusively. Vaccination strategies often take a 'serotype-by-serotype' approach, which can be problematic when tackling a multiserotype, constantly evolving pathogen such as the BTV. As shown in Table 1, inactivated vaccines consistently induce a strong antibody response and a significant reduction in viremia and clinical signs.

However, this protection is strictly limited to the serotypes involved in the vaccine and does not extend to heterologous serotypes. The marked serotype diversity of BTV directly contributes to the vaccination paradox, as each serotype requires a specific and non-cross-reactive immune response. The co-circulation of multiple serotypes and the emergence of variants not included in available vaccines can rapidly undermine population immunity. This explains why vaccination, although effective at the individual level, does not always ensure stable epidemiological control.

Table 1: Vaccines available against the bluetongue virus classified according to type, serotype, and animal species.

	Vaccines (Manufacturer)	Serotype	Species	Evidence	Ref
Live attenuated	BTVAC bivalent (Biopharma)	BTV-1, BTV-4 *(Bivalent)*	Sheep, goats	Rapid Ab response	[16]
Live attenuated	OBP multivalent A/B/C (Onderstepoort)	Multiple attenuated strains (BTV-1–14, BTV-19)	Sheep, goats	Broad seroconversion	[17]
Inactivated vaccines	Bluevac-4	BTV-4	Cattle, sheep	Strong Ab response, ↓ viremia	[18, 19]
Inactivated vaccines	Bluevac-8	BTV-8	Cattle, sheep	Clinical protection	[18, 20]
Inactivated vaccines	Bluevac-3	BTV-3	Cattle, sheep	↓ viremia, ↓ mortality	[21]
Inactivated vaccines	BTVPUR ALSAP 8	BTV-8	Cattle, sheep	Clinical protection	[22]
Inactivated vaccines	Zulvac (specific formulations)	BTV-1 / 4 / 8	Cattle, sheep	↓ viremia	[18, 23]
Inactivated vaccines	Bovilis BTV8	BTV-8	Cattle, sheep	Strong Ab response	[20]
Inactivated vaccines	Syvazul BTV 3	BTV-3	Cattle, sheep	↓ viremia, ↓ mortality	[24]
Inactivated vaccines	Bultavo 3	BTV-3	Cattle, sheep	↓ clinica, ↓ lesioni	[25]

BTV = Bluetongue virus; Ab = antibody; ↓ = decrease

An additional element of complexity is the simultaneous circulation of different serotypes, which requires the concurrent use of multiple vaccines. The clear prevalence of circulating serotypes can change rapidly, especially due to factors such as animal movements and climate change. Furthermore, vaccination campaigns could influence the emergence of antigenic variants, although current evidence for vaccine-driven selection in BTV remains limited. The use of live attenuated vaccines, while inducing a rapid and robust immune response, is associated with a series of risks related to possible genetic reassortment. Although such events are rare, their potential severity has led many European countries to recommend inactivated vaccines, which, however, offer more limited protection.

Inactivated vaccines, on the other hand, provide good protection and significantly reduce viremia, but only against the specific serotype. Their excellent safety profile makes them suitable for large-scale field campaigns, although their strict serotype specificity reduces flexibility in multiserotype or rapidly evolving scenarios. This process means that in the presence of a new emerging serotype, the entire vaccinated population is susceptible again, necessitating the development and authorization of a new vaccine.

Finally, promising alternatives to live attenuated and inactivated vaccines for controlling BTV include recombinant and virus-like particle vaccines. These allow for the inclusion of more conserved antigens, potentially extending protection beyond a single serotype [26]. Their main disadvantage is the current lack of widespread commercial availability, which restricts their immediate deployment despite their promising immunological profile. Distinguishing Infected from Vaccinated Animals (DIVA)-compatible vaccines are also an essential tool for integrating vaccination and surveillance, allowing the distinction between vaccinated and infected animals. However, the commercial availability of these technologies remains limited, and their widespread application requires further validation.

Species specificity

The host immune response to BTV is crucial for understanding disease evolution, as it highlights profound differences in immunological processes among species. In this context, it is essential to consider that cattle, despite exhibiting mild or no clinical signs, serve as amplifying reservoirs, sustaining viral circulation for much longer than sheep and significantly influencing the epidemiology of bluetongue [27]. Consequently, vaccination strategies oriented almost exclusively to sheep should be rethought to include cattle also, whose immunity differs profoundly in its dynamics, intensity, and duration. One of the most significant differences in BTV pathogenesis between sheep and cattle lies in the behavior of the vascular endothelium, the virus's primary target [28]. Although BTV can infect endothelial cells of both species, the responses these cells mount differ and determine the outcome of infection [29]. In sheep, the endothelium exhibits significant sensitivity to the cytopathic effects of the virus, resulting in accelerated viral replication and increased viral RNA production, leading to early metabolic stress. This leads to the intense activation of pro-apoptotic pathways involving caspases, the endoplasmic reticulum, and oxidative stress

mechanisms. The consequence is an early loss of endothelial barrier integrity, resulting in edema, hemorrhages, vasculitis, and widespread tissue damage. One factor that may contribute to this vulnerability is the comparatively slower and less efficient interferon response observed in ovine endothelial cells, as suggested by experimental studies.

The *Culicoides* bite inoculates the virion into the dermis [30]. This is characterized by the introduction of the virus into the dermis, accompanied by a series of immunomodulatory molecules present in the saliva of *Culicoides*. These molecules include Kunitz-type inhibitors, D7-like proteins, and antigen 5-like proteins, which modulate the local inflammatory response by increasing vasodilation and reducing coagulation, making dermal cells more permeable [31]. In the dermis, BTV is taken up by dermal dendritic cells via endocytosis. Within the endosome, acidification activates the VP5 protein, which undergoes a pH-dependent conformational change [32]. After activation, the VP5 protein forms pores in the endosomal membrane, enabling the viral core to be released into the cytoplasm for initial replication. Dendritic cells then migrate to the draining lymph nodes, where the virus further replicates before it enters the bloodstream and establishes viremia. During this stage, BTV encounters endothelial cells, the primary pathogenic target. In both sheep and cattle, entry occurs via receptor-mediated endocytosis, and release into the cytoplasm is mediated by VP5 via the same pH-dependent mechanism. In the endothelial cytoplasm, double-stranded viral RNA is primarily recognized by melanoma differentiation-associated protein 5 (MDA5), with minor contributions from retinoic acid-inducible gene I (RIG-I) [33, 34]. This activates the Mitochondrial Antiviral-Signaling protein (MAVS) – TANK-binding kinase 1 (TBK1) – Interferon regulatory factor 3 (IRF3) cascade, and the production of type I interferon [35, 36]. However, available data indicate that basal expression and activation thresholds of these receptors may differ between sheep and cattle, which potentially contributes to a slower or less intense type I interferon response in sheep. Consequently, the signaling cascade that leads to the activation of the transcription factors IRF3 and IRF7 is slower. Epigenetic regulation also contributes to this delay; interferon genes in sheep have more compact chromatin, which takes longer time to become accessible for transcription. Consequently, the production of type I interferon, which is essential for limiting viral replication in the early stages, begins only once the virus reaches high levels and begins to damage the endothelium.

In contrast, bovine endothelium displays greater intrinsic resistance to infection. Antiviral receptors are expressed more abundantly and are more sensitive; the IRF3/IRF7 cascade is activated more quickly, and interferon genes are more readily accessible. This enables early and robust interferon production, limiting viral replication and preventing the virus from reaching cytopathic levels. Furthermore, bovine endothelial cells are less susceptible to the immunosuppressive action of viral NS3 and NS4 proteins. These proteins are more effective at inhibiting the interferon response in sheep. In this context, the development of increasingly effective vaccination strategies is linked to the need to consider the differences in immunological responses across species. Vaccines, on the other hand, are developed following uniform strategies that overlap profoundly different biological systems. The evaluation of vaccine efficacy focuses almost exclusively on antibody production, neglecting the varying speeds at which species activate innate pathways, their varying sensitivities to antiviral signals, and their varying abilities to contain viral replication in the very early stage post-infection. As a result, the same vaccine formulation may be adequate in one species but insufficient in another, leading to uneven protection that does not reflect the hosts' true vulnerability. In this context, aspects of the endothelial response and early antiviral activation could affect vaccine efficacy, particularly in species with a slower innate response.

Under conditions of high vector pressure, it is plausible that a seroconverted animal with delayed interferon activation could still permit an initial phase of viral replication. These factors could therefore help to explain why the same vaccine formulation produces different results in different species.

Time

The timing of bluetongue vaccination is one of the factors contributing to the so-called vaccine paradox. While timing is recognized as a factor that can influence effectiveness, strategies are not always tailored to the specific characteristics of BT. In this regard, inactivated vaccines, currently considered the gold standard for BT control, the immune response often begins to develop a few days after the first dose, usually within 10 days [37]. In these models, seroconversion is followed by a marked increase in antibody levels after the booster, while full protection is achieved three weeks after the second dose. This temporal latency plays a key role in the dynamics of BT.

Bluetongue is transmitted by the *Culicoides* vector, the spread of which is closely linked to seasonality [38]. It is now known that rising temperatures play a key role in the spread of the vector, thereby reducing the timeframe during which vaccination is effective in both mitigating and preventing viral circulation. Carpenter et al. have demonstrated that infection levels are reached more rapidly at 30°C than at 20°C, thereby advancing the vector's ability to transmit the infection and increasing virogenesis [39]. Furthermore, increases in temperature

have been observed to influence the physiology of the vector by increasing oogenesis rates and reducing the interval between blood meals. This results in an increased biting rate and probability of transmission. In a study by Tugwell et al., it was observed that higher temperatures affected phototaxis, locomotion, and the ability of vectors to orient themselves towards a UV source within 24 hours [40].

Increased temperatures enhanced the sensory mechanisms that integrate light stimuli with motor activation, thereby affecting the flight ability of *Culicoides*. These phenomena are exacerbated by climate change, which has characterized recent decades, promoting exceptionally favorable conditions for transmission in regions considered temperate. These elements demonstrate that the timeframe for inducing vaccine immunity must be carefully evaluated based on periods of peak vector activity. If this is not calculated and climate change is not considered, the immune response may mature when infection pressure is already high, reducing the vaccine's effectiveness. This structural asynchrony between immunization and vector dynamics constitutes one of the elements of the vaccine paradox.

Conclusions and perspectives

Bluetongue virus control remains constrained by a mismatch between the speed of BTV emergence and the speed at which homologous vaccines can be selected, authorized, manufactured, distributed, and administered. Vaccination is indispensable, but it is not a stand-alone solution. Currently available conventional vaccines reduce disease and/or viremia when matched to the circulating serotype, but protection remains largely serotype-dependent; in addition, live attenuated vaccines raise safety concerns, while both live attenuated and inactivated commercial products have limited compatibility with serological DIVA surveillance [41, 42]. Consequently, the "vaccination paradox" should not be interpreted as vaccine failure, but as the result of applying serotype-specific and time-dependent tools to a virus whose ecology is shaped by reassortment, vector seasonality, animal movements, climate and species-dependent host responses [43].

The recent European experience with BTV-3 has made this paradox explicit. BTV-3/NET2023 was confirmed in the Netherlands in September 2023 by whole-genome sequencing after identification of clinical cases in sheep and cattle, and its source and route of introduction remained unresolved [44]. In 2024, Belgium reported a rapid country-wide spread infection associated with increased mortality, reduced births, and lower milk production, while delayed BTV-3 vaccine availability and a naive ruminant population were identified as factors that complicated control [45]. The detection of BTV-12 in the Netherlands in 2024, a serotype not previously reported in Europe, reinforces that vaccine plans built only around the last epidemic serotype may be rapidly outpaced by new introductions [46]. The authorization of BTV-3 vaccines, such as Bluevac-3 and Syvazul BTV-3, under exceptional circumstances represents a necessary emergency response, but it also underscores the need for post-authorization field effectiveness data and rapid updating of vaccine portfolios [19].

Future control should therefore move from reactive vaccination to anticipatory and adaptive vaccination. Vaccine choice should be directly linked to real-time serotyping and genomic surveillance, so that vaccine strain, vaccine coverage, and movement rules remain aligned with the serotypes demonstrated by surveillance [43]. Vaccination calendars must also be planned before expected *Culicoides* activity, because the interval between primary vaccination and protective immunity can leave animals exposed during the highest-transmission window. Campaigns should not focus only on clinically susceptible sheep. Cattle, goats, and other domestic or wild ruminants may show milder or subclinical infection but can contribute to virus maintenance and onward transmission; therefore, reservoir species should be included when the objective is to reduce viral circulation rather than only to prevent mortality [42, 43].

A major research priority is the development of vaccine platforms that are safe, DIVA-compatible, rapidly adaptable, and less dependent on strict serotype matching. Recombinant vectors, protein-based vaccines, virus-like particles, disabled infectious single-animal platforms, and heterologous prime-boost strategies can combine serotype-specific neutralizing antigens such as VP2 with more conserved proteins that stimulate T-cell responses [41]. Recent Modified Vaccinia Ankara candidates co-expressing VP2 with NS1 and NS2-Nt induced protection in sheep against homologous or heterologous challenge and substantially reduced viral replication, supporting T-cell-assisted cross-serotype protection as a realistic direction [47, 48]. The 2026 CVMP positive opinion for VeroBlue-3, a BTV-3 VP2-protein vaccine, also suggests that modular antigen-based products are entering the regulatory pipeline, although duration of immunity and field performance will require continued evaluation [21]. Surveillance must become equally integrated. Whole-genome sequencing is essential for identifying introductions, reassortment and vaccine-strain mismatch, but virological data alone are insufficient.

Future systems should combine syndromic reporting, pan-BTV and serotype-specific RT-qPCR, targeted serology, whole-genome sequencing, *Culicoides* trapping, and climate-based risk forecasting. Recent entomological data from the Netherlands detected BTV RNA in indigenous *Culicoides* species and suggested that local

vector competence may partly explain the rapid spread of BTV-3/NET2023; such findings underscore the need for region-specific data on vector abundance and competence. These data should feed directly into vaccine procurement, prioritization of high-risk areas, timing of animal movements, and farmer communication.

In conclusion, the central question is no longer whether vaccination is useful, but how vaccination can be embedded in an adaptive system able to anticipate BTV evolution and vector dynamics. The most realistic perspective is a layered control strategy: homologous inactivated vaccines for immediate risk reduction, systematic post-vaccination monitoring to measure field effectiveness, genomic and entomological surveillance to detect emerging serotypes early, and accelerated development of broad DIVA-compatible platforms. Only this integration can transform vaccination from an emergency response into a predictive tool for reducing disease incidence, economic losses, and viral circulation in a changing European epidemiological landscape. For decision-makers and veterinary services, this means adopting vaccination schedules based on seasonal risk, including reservoir species in immunization plans and systematically using sequencing to guide vaccine selection.

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