https://doi.org/10.48047/AFJBS.7.4.2025.422-443

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Bovine Viral Diarrhea Virus Circulation and detection of Persistently Infected Animals in Dairy Farms in Eastern Algeria

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Volume 7, Issue 4,Apr 2025

Received: 15 Feb 2025

Accepted: 05 Mar 2025

Published: 28 Apr 2025

doi:10.48047/AFJBS.7.4.2025.422-443

Abstract: Bovine Viral Diarrhea (BVD) is an endemic infection, responsible for economic losses in beef and dairy herds worldwide. This comprehensive study aims to dissect the circulation dynamics of the BVDV in dairy cattle, while identifying risk factors and persistently infected (PI) carrier animals, particularly during the infection of seronegative pregnant females. For that, 60 serum samples were collected from animals from seven farms and all the aspects relating to age, sex, productive system, parity, history of abortions, breeding type, diarrhea, stillbirths, and hygienic conditions were considered. An ELISA Antibody Competition Kit was used to evaluate the seroprevalence. Data showed that the BVDV was present within the studied farms, exhibiting an overall prevalence of 32%. For those boasting highly hygienic conditions, the seroprevalence was estimated at 20% with CI at 95% [2.5-37.5, p=0.170] whereas in less hygiene conditions, the seroprevalence was estimated at 37.5% with CI at 95% [22.5-52.5]. Age emerged as a crucial risk factor. Young cattle aged 6 to 12 months were found to be seronegative, exposing them to an elevated risk of becoming PI, which poses a significant threat to the sustainability of the herds. Conversely, animals aged 2 to 4 years (72%) and those aged 4 to 6 years (73%) demonstrated notable seropositivity, while those over 6 years (71%) returned to seronegative status, highlighting a complex immunity dynamic. Moreover, a concerning 82% of primiparous females were seronegative, thus exposing their offspring to a heightened risk of persistent infection. Regardless of the farming type, BVDV circulation was consistently observed, with prevalence rates of 31% and 32% respectively. The circulation of BVDV is confirmed and the risk of contamination is present since no vaccine is used, thus a high likelihood of infection among herds exists.

KEYWORDS: Breeding, BVD, persistently infected offspring, risk factors, seroprevalence

Introduction

Bovine Viral Diarrhea (BVD) was first reported in 1946 [1]. It is an important endemic infection [2]. The significant economic impacts of bovine viral diarrhoea (BVD) virus have prompted many countries worldwide [3].

Bovine Viral Diarrhea Virus (BVDV) belongs to the genus *Pestivirus* [4] and the family of *Flaviviridae*. The virus has a single-stranded positive-sense RNA genome [5] that is a production-limiting pathogen of cattle with global distribution [6]. Cattle are considered the natural host of BVDV [7], but serological evidence of infection has also been found in sheep, goats and eland antelope [8]. Bovine pestiviruses have also been shown to spread due to indirect contact with infected animals through contaminated bedding, fomites, equipment, machinery and amongst personnel, including veterinarians [3,9].

Bovine Viral Diarrhea Virus infection is in most instances subclinical, but the virus can also cause respiratory signs, diarrhea, reproductive failure, congenital malformations, pyrexia, depression, inappetence, nasal discharge, and erosion of oral mucosa [7]. BVD shows some characteristic clinical signs, whereby some animals are often asymptomatic carriers; therefore, a conclusive diagnosis is not always possible based on a mere physical examination including clinical symptoms of the animal [9]. The most serious outcome of infection is the development of persistently infected fetuses, which renders the animal more susceptible to secondary infections [8].

BVDV infections are sustainable and infamous within cattle populations. The persistently infected (PI) animals are considered the primary mode of transmission to susceptible ones and their role in maintaining BVDV infections in cattle populations was reported [10]. PI calves showed growth disorders and may excrete variable amounts of the virus throughout their lives, spreading the infection to the herd. They usually die during the first two years of life from mucosal disease or due to other illnesses [11]. Exploring the various conditions that favor the

survival of bovine pestiviruses, as well as knowing how long they can be maintained outside the host, will help in understanding the risk of indirect sources of BVD within and between herds. While PI animals are considered the major source of infection in a herd, transiently infected animal (TI) are also, for a short period, infective to susceptible individuals [3].

BVD was placed on the OIE's list of notifiable diseases, mostly due to its potential for international spread. While there are currently no formal reporting requirements for BVD, countries with national or regional control programs may have certain regulations for affiliated farmers that effectively restrict trade with animals of the positive suspect or unknown BVD status [12]. In Algeria, very few epidemiological studies have been performed. Hence, authentic data providing the seroprevalence and the risk factors are not available.

The objective of the current study is to estimate the viral circulation of BVD in dairy cattle. The aim of this study was to determine the risk factors favouring the introduction and maintenance of the virus by lifetime shedding animals (PI) during the infection of seronegative pregnant females. To the best of our knowledge, this study is the first one to be conducted in Algeria. Moreover, it has allowed us to highlight the presence of BVD in the local farms.

1. Material and Methods

1.1. Study area and animals

The study was carried out on dairy farms in the East region of the country (Latitude / Longitude: 36° 45' 59" N / 3° 28' 38" E) between April and December of 2019. The livestock consisted of seven farms with a total of sixty heads. All animals were screened for brucellosis.

1.2. Data collection

Sixty serum samples were collected from the above-mentioned farms together with all the pertinent data needed for follow-up analysis (age, sex, production system, stage of gestation, history of abortions, breeding type, and presence of diarrhea, stillbirths, and hygienic conditions of farms (Table 1).

Table 1. Breeding's Information

Breeding number	Breeding type	Number of animals	Hygienic conditions	Positive cases
B1	Intensive	2	Bad	0
B2	Semi-intensive	9	Bad	2
B3	Semi-intensive	9	Bad	7
B4	Intensive	8	Bad	4
B5	Intensive	12	Bad	3
B6	Intensive	7	Bad	2
B7	Semi-intensive	13	Good	1
Total	---	60	---	19

1.3. Sampling and laboratory investigation for BVDV

Blood samples were taken from the jugular vein, kept at +4 °C and transported to the laboratory and subsequently centrifuged for 5 minutes at 3,000 rpm. Afterward, the sera were preserved at –20°C until serological testing was performed.

Serum samples were tested with ELISA, ID Screen® BVD p80 Antibody Competition Kit to detect antibodies anti-p 80/125. The samples densities were measured at a wavelength of 450 nm.

1.4. Statistical analysis

Statistical analysis was carried out with the XLSTAT Software version 2016.02.28451. All statistical comparisons were made by Pearson's chi-square significance test with a confidence interval of 95%. The differences were considered statistically significant with a probability of $p \leq 0.05$.

2. Results

2.1. Estimation of breeding Conditions

Of the seven farms, four (57%) practice intensive farming while poor hygienic conditions were observed in 86% (6/7) of the total; only 2 farms presented a satisfactory state corresponding to a percentage of 29%. At intensive farms, 3 presented a poor state of hygiene

(43%); whereas in semi-intensive farms, 2 showed a poor state of hygiene (29%). Concerning good conditions, we noted one farm where semi-intensive mode is practiced (14%).

2.2. Prevalence estimation

Global seroprevalence

Global seroprevalence was estimated at 32% (19/60) (Fi. 1) with CI at 95% [19.9-43.4].

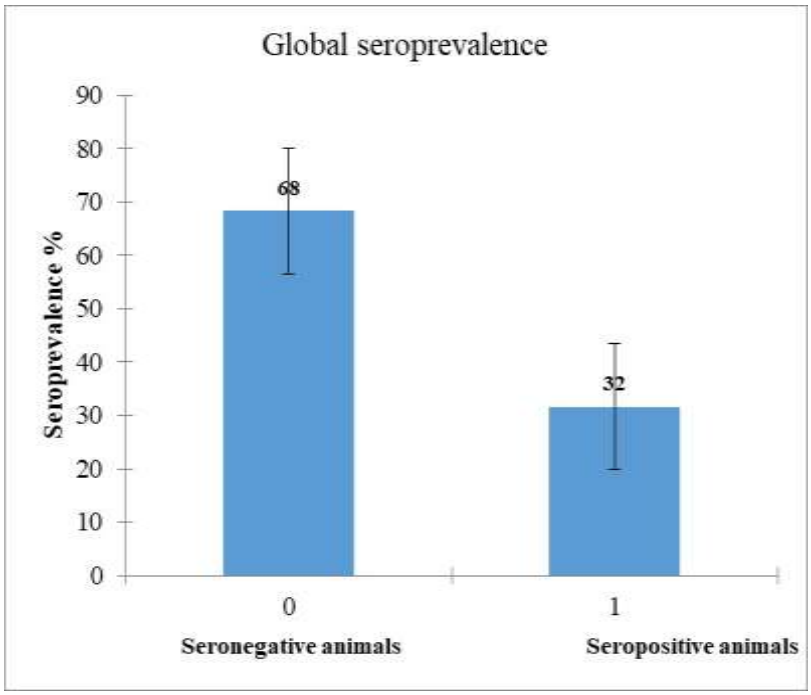

Figure 1. Global seroprevalence

Seroprevalence at breeding level

At the breeding level (Fig. 2), global seroprevalence was found at 31.7%. The higher seroprevalence was registered in breedings B3 (77.8%) with CI at 95% [50.6-100.0] and B4 (50%) with CI at 95% [15.4-84.7], whereby animal numbers corresponded respectively to 9 and 8 heads. While in the breeding containing 12 and 13 heads (B5 and B7), the seroprevalence is estimated at 25% and 7.7% with CI at 95% [0.5-49.5], [0.0-22.2], respectively. In breeding B1, the sera were all negative (0%), whereby the farmer announced that the animals had aborted. In the second breeding B2, two animals were seropositive with a seroprevalence estimated at 22% (2/9) with CI at 95% [0.0-49.4] (a female aged 6 years old and a male aged 6 months). In

breeding B3, a seroprevalence of 77.8% with CI at 95% [50.6-10.0] was recorded. Five females aged between 5 and 9 years were seropositive. In breeding B4, 50% (4/8) with CI at 95% [15.4-84.6] of the animals are seropositive. These are two adult females aged 3 and 5 years. Concerning the young animals, they are aged 2 and 3 months old. In farm B5, seroprevalence was estimated at a rate of 25% (3/12) with CI at 95% [0.5-49.5]. Regarding farm B6, two females, both aged 4 years old, are seropositive corresponding to a seroprevalence of 28.5 % with CI at 95% [0-62]. In farm B7, one (1) seropositive case was detected out of a possible 13 animals with a prevalence of 7.7% with CI at 95% [0.0-22.2] on a female-aged 11 years old.

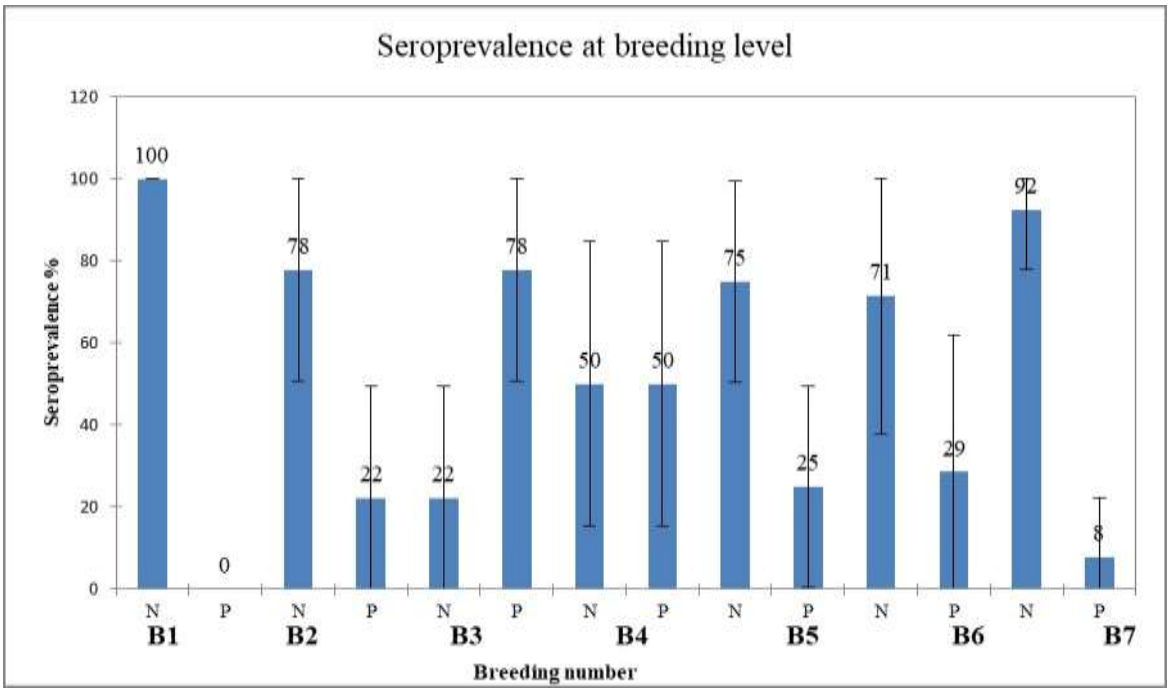

Figure 2. Seroprevalence at breeding level (N=Negative, P=Positive).

2.3. Seroprevalence according to livestock type

According to livestock type (Fig. 3), data revealed that the prevalence in intensive and semi-intensive livestock is estimated at 31% with CI at 95% [14.2-47.9] and 32% with CI at 95% [15.8-48.7], respectively.

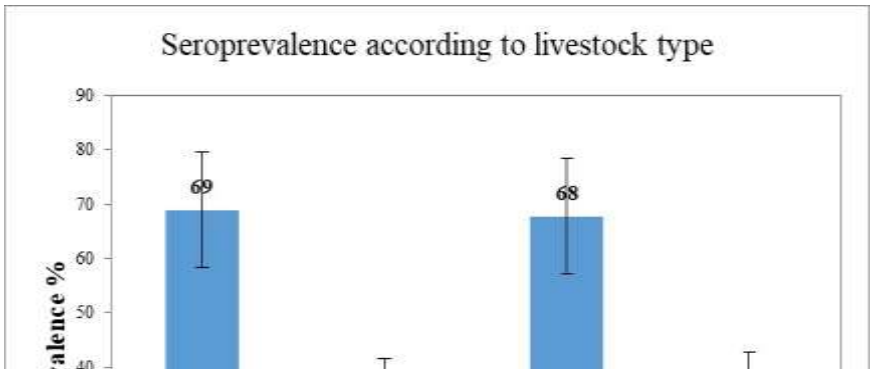

Figure 3. Seroprevalence according to livestock type (*p-value*= 0.919, khi^2= 0.01).

2.4. Seroprevalence according to age

The results showed that 55% with CI at 95% [25.1-84.0] of the animals aged between 2-6 months are seropositive, 29% with CI at 95% [7.8-51.0] of seropositive animals were aged over six (6) years old (Fig. 4). For animals aged between 2-4 years, 28% with CI at 95% [7.1-48.5] are seropositive and finally, 18% with CI at 95% [1.0-54.0] of seropositive animals were aged between 4-6 years. Data additionally revealed that no animal aged between 6 months to 1 year was detected as seropositive. Concerning the animals aged between 6 months to 1 year (3 animals), all are seronegative and may be PI. Concerning the females aged between 2-4 years, 72% with CI at 95% [51.5-93.0] were seronegative (13/18) while three animals (3) were seropositive (3/48). Regarding the females aged between 4 to 6 years, the seronegative prevalence rate corresponded to 73.0% with CI at 95% [46.4-99.0] whereby the seropositivity rate corresponded to 27.2% with CI at 95% [0.9-53.5]. For females aged over 6 years, the seronegative prevalence is estimated to be 71% with CI at 95% [49.0-92.2] and the seropositivity rate corresponded to 29% with CI at 95% [7.8-51.1] (Fig.4).

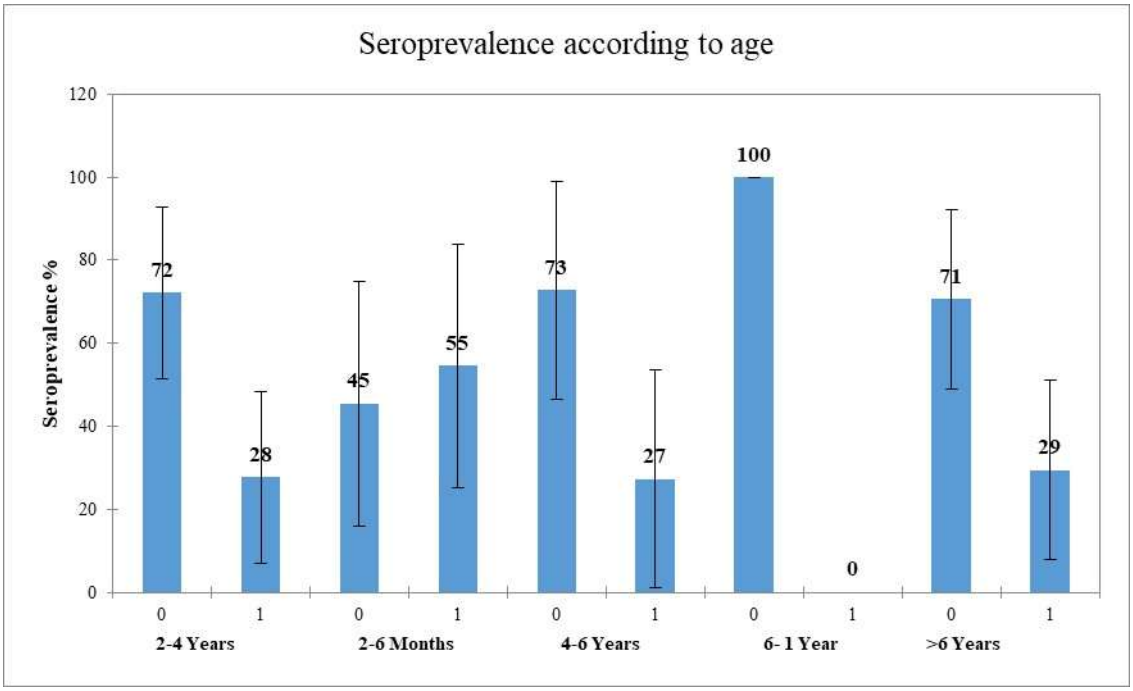

Figure 4. Seroprevalence according to age (*p-value* = 0.727, Khi ^{2} = 0.010, P >0.05).

2.5. Seroprevalence according to parity

The seven farms had forty-six females represented by eleven primiparous (18%) and thirty-five multiparous females (58%). Our results showed that 18% (2/11) with CI at 95% [0.0-40.9] of the primiparous females are seropositive, while the remaining 82% (9/11) with CI at 95% [59.0-100.0] are seronegative (Fig. 5). Concerning multiparous females, 31% (11/35) with CI at 95% [16.1-46.8] are seropositive. Conversely, 69% (24/35) of females are seronegative with CI at 95% [53.2-84.0].

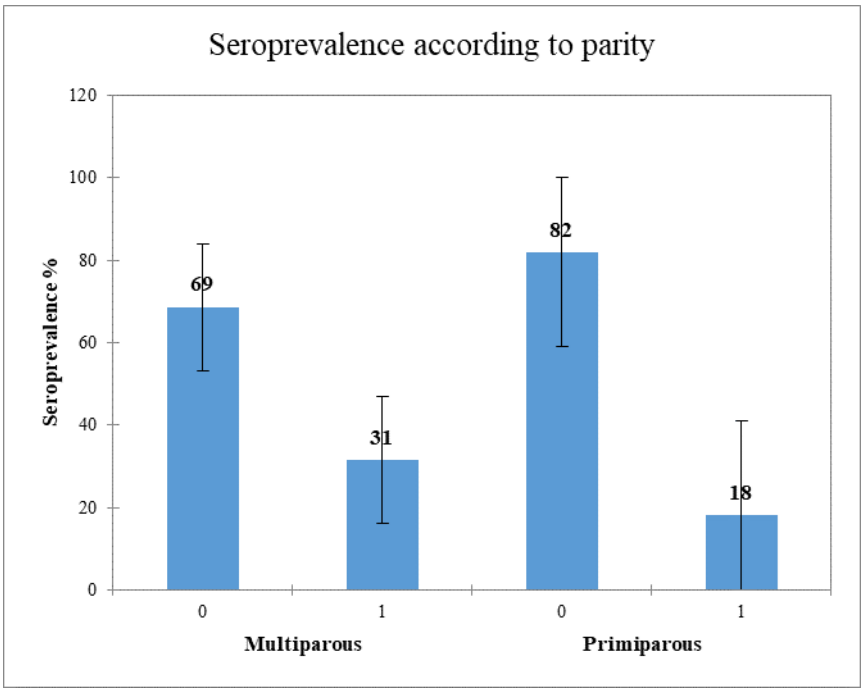

Figure 5. Seroprevalence according to parity.

2.6. Seroprevalence according to the hygienic conditions

Regarding the study of seroprevalence according to the hygienic conditions (Fig. 6), data show that in good hygiene conditions, the seroprevalence is estimated at 20% (4/20) with CI at 95% [2.5-37.5] while in poor hygiene conditions, the seroprevalence is estimated at 37.5% (15/40) with CI at 95% [22.5-52.5]. In the poor conditions, the risk of contamination is significant with OR estimated at 0.01 and the *p-value* at 0.05.

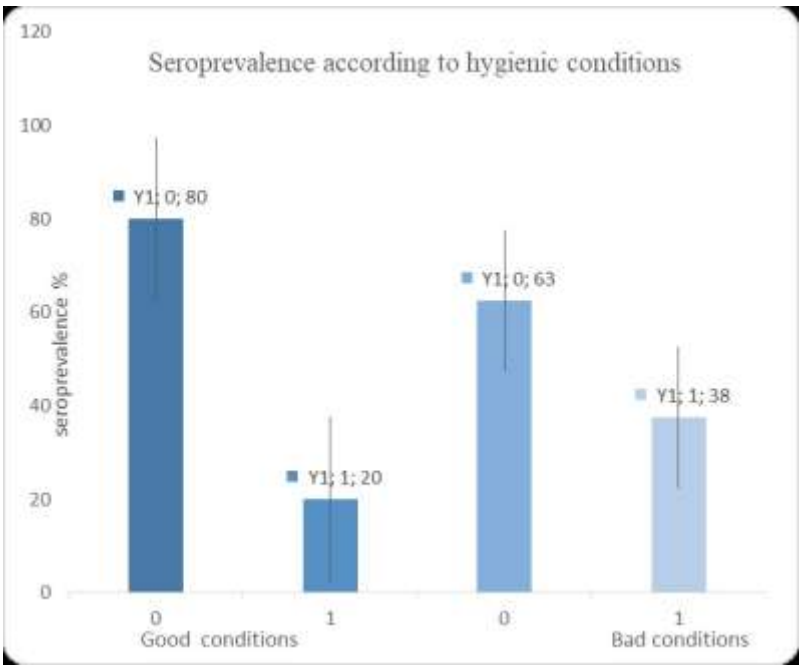

Figure 6. Seroprevalence according to hygienic conditions (*p-value*= 0.170, khi2= 1.887, P>0.05).

3. **Discussion**

Eighteen of the nineteen positive animals (18/19) lived in poor conditions corresponding to a rate of 94.7%; this result joined other data reporting that poor farm hygiene is the leading cause of BVDV installation [13].

Global seroprevalence was estimated at 32%. This rate is lower than those obtained in previous works in Algeria; 77.8% [5], 89.49% [14], 79.1% [15], 60% [16], 1991), 50.9% [1], and 42.5% [13]. Meanwhile, a seroprevalence of 28 to 53% has been reported in another work [7]. Interestingly, our data is higher than that obtained by Almeida *et al*. [17] in Brazilia (26%) and closer to those obtained by Rypuła *et al*. [18] in Poland (33%), Ularamu *et al*. [19] in South Africa with a rate varying between 37% to 100%, and Hyera *et al*. [20] in Tanzania (34%).

BVD infection is mainly associated with dairy cattle [13]. It was endemic in all countries where no control has been carried out [21]. As no official data are available on BVDV circulation in Algeria, and since the disease is not included in mandatory control programs, we have no sufficient data to draw any conclusion about the epidemiological situation there.

In the resent study, the global seroprevalence was found at 31.7%. This rate is lower than that reported in previous studies with a seroprevalence of 95% [22] and of 65.5% [23]. Khodakaram-Tafti and Farjanikish [24] mentioned that demographic factors such as herd size and density are significant predictors for the prevalence of infection in populations where BVDV is endemic.

Concerning seroprevalence found in breeding B3, B4, B5 and B7, our results do not agree with those found previously; 77.8% [13] and 43% [25]. The difference may be due to the size

of herds.

In breeding B1, the sera were all negative (0%), whereby the farmer announced that the animals had aborted. This result is not reported in seronegative cattle while abortion was registered at 84.2% in seropositive females [15]. We can suppose a possible viral circulation as cases of repetitive abortions were seen, particularly in female1. BVDV leads to immunosuppression, which enhances the probability that cattle are secondarily infected by other pathogens [2] responsible for abortions such as brucellosis. However, BVDV was the most common pathogen responsible for abortion in earlier studies [15]. The absence of a seropositive result does not exclude a viral presence because the second animal present on the farm may be a PI. Virological analyses were necessary to confirm our hypothesis.

In the second breeding B2, where seroprevalence was estimated at 22% in a female-aged 6 years old and a male aged 6 months; the progeny of the seropositive cow is also positive, which means that colostral immunity is ensured and therefore the risk of developing PI animals is eliminated [26]. Concerning the positive male aged 6 months, the risk to be PI is ruled out. It can therefore be considered a transiently infected animal (TI) and has developed antibodies post-infection. TI cattle are (for a short period) infective to susceptible individuals [1]. TI animals can be positive (antigens) at first but none on the second time because they become seropositive and protected by antibodies [27]. Regarding seronegative animals, a PCR test is necessary for the parents as well as their offspring, especially if they are found to be permanent seronegative and supported by the presence of defects in their states [6]. Concerning the possible presence of PI animals, an antigen-ELISA test can detect them at all points in time [27].

In breeding B3, a seroprevalence of 77.8% considered high compared to the other farms studied. Five females aged between 5 and 9 years were seropositive; as noted previously, antibody prevalence is generally higher in adults compared to young animals [28,29].

Furthermore, their offspring are also seropositive (2 calves aged 2 and 4 months plus a calf aged 5 months). These animals are protected, and the younger ones cannot be PI. The remaining animals (2) are a male and a female, both aged 4 months and seronegative. Thus, a permanent seronegativity of the animals can be translated by a PI status. If that is not the case, it can be a free status [1] with the absence of a viral passage (animals resistant to the infection), which is perhaps the case of subject number 5 (female of 4 years old). However, this last one can be a subject of seroconversion in case of immunodeficiency.

In breeding B4, 50% of the animals are seropositive. These are two adult females aged 3 and 5 years. Concerning the young animals, they are aged 2 and 3 months old. In this case, it is important to determine the infection's stage in these animals, knowing that this seropositivity is preceded by a transient viropositivity responsible for a transient horizontal transmission and a vertical transmission if the females contracted the virus during the first months of gestation [30]. A PCR test to confirm PI status is desirable for the animals corresponding to samples 25 and 26, in order to rule out any danger of permanent excretors. Concerning these samples, the animals are aged respectively 3 and 6 months old, so a PI confirmation test is necessary, knowing that these are high-risk animals because they are permanent shedders, since PI animals are considered the major source of infection in a herd [1]. The animals numbered 27 and 28, respectively aged 2 and 3 months are seropositive, testifying to the passage of a passive colostral immunity transmitted by immunized mothers (seropositive). Indeed, seropositive females transmit protective antibodies to newborn animals through the colostrum; if they have been infected outside the period of the risk of PI formation. Incidentally, these antibodies disappear only after the age of approximately 6 months [26], whereas the study subjects are 2 and 3 months old and therefore still retain their antibodies.

In farm B5, seroprevalence was estimated at a rate of 25% (3/12) with CI at 95% [0.5-49.5]. Seronegative animals (9) may be PI or free or incubating animals, however, we did not

confirm by virological examination in this study. Detection of three (3) seropositive animals, indicates a viral circulation and a risk for the others, thus, TI cattle are also, for a short period, infective to susceptible individuals [3]. For seronegative females in their first third of gestation, the risk of PI offspring is there, due to the existence of seropositive who may be TI and potential shedders of the virus during acute infection [31]. Infection of seronegative females who are in the first trimester of gestation (33, 34, 36, 39 and 40) is also a risk for a PI offspring via contact with the animals 29, 30, and 32 in the acute phase. The infection can also lead to abortion, along with several types of congenital anomalies and growth retardation [24].

Regarding farm B6, the rate (28.5%) is lower than that obtained in a previous work (33.2%) in females aged between 13 and 48 months. These females are pregnant in the last third stage. Even if a risk of a PI offspring is ruled out, abortions are likely as it was observed at 84.2% in seropositive females [15]. On the other hand, some researchers found that abortions may appear at any time during pregnancy and are not necessarily associated with the time of infection [32]. Concerning, seronegative females on this farm, the female numbered 47 (aged 4) was in the first third of gestation, so viral shedding by TI is possible and the risk of PI offspring was certain [24]. As for females 41, 42 and 43 respectively aged 18 years, 9 years, and 12 years, the risk of affecting a possible PI offspring is ruled out in light of the animal's age.

In farm B7, one female aged 11 years old was seropositive. Even if the prevalence is low (7.7%), the risk of infection exists for remaining animals especially if this female has been recently infected. Viral shedding will be short-lived (with a TI animal), ranging anywhere from a few days to a few weeks [3] with different amounts of viral excretion. Promiscuity will allow for virus dissemination and contagion of other animals and horizontal transmission occurs mainly by contact with virus-shedding animals [33]. Interestingly, given the age of animals (over 8 years), the probability of positive offspring is almost nil, and the risk of having PI is

null. Conversely, for the seronegative females aged between 3 and 7 years (50, 51, 53, 54, 56, 57, and 60), the probability of being infected still exists although the probability of having PI offspring is null because these females are not pregnant. Three young animals were seronegative. First is a female aged 2 months old in B3 together with two males aged 3 months and 5 months in B4 from mothers aged 3 years and 5 years, respectively. These animals might be PI, as authors reported that the presence of maternal antibodies in PI animals could inhibit the accurate detection of young PI animals [34].

The viral sources are represented by excretory animals (90%) and by the environment (10%), due to the low resistance of the virus within the external environment; PI animals account for 70% of excretory cattle. They are the key to virus transmission, due to their permanent virus excretion, and the viral load excreted overall is greater than that of infected transient animals [35].

According to livestock type, the statistical analysis showed that the type of livestock is not a risk factor related to BVD. These results confirmed that the nature of breeding does not influence circulation and propagation of BVDV whereas infection is rather conditioned by contact between herds, and according to animal density and proximity of pastures [36].

Data revealed that no animal aged between 6 months-1 year was detected as seropositive (0%). In this case, some studies showed a seroprevalence of 13.6% in animals aged between 8 months and one year on dairy farms [2].

Vertical transmission of BVD occurs when bovine pestiviruses infect susceptible, pregnant females and the virus crosses the placenta, establishing infection within the developing foetus [3]. Concerning the animals aged between 2-6 months, 55% are seropositive and consequently protected. For this age group, five (5) animals corresponding to a rate of 45% with CI at 95% [16.0-74.9] are seronegative; these animals could be considered as PI and the seronegative result may be explained by the fact that the presence of maternal antibodies in PI

animals can inhibit the detection of young PI animals. It is referred to as the "colostral antibody gap" [34] however, to what extent maternal antibodies have on the viral load and subsequent infectivity of PI animals remains unknown [3].

The animals aged between 6 months to 1 year are seronegative and may be PI. That is as reported by authors because of a colostral antibody gap and unknowing infectivity of PI [1,3,34].

Concerning the females aged between 2-4 years, the result shows the circulation of BVDV at this particular farm, putting the seronegative females at risk, which could be viro-positive. This hypothesis could be at the origin of the birth of a PI progeny.

Regarding the females aged between 4 to 6 years, the prevalence is lower than that found by the earlier studies whereby antibody prevalence is generally higher in adults compared to young animals [29]. Other studies have shown that individuals over 4 years of age have a high seroprevalence (53.0%) [13]. For females aged over 6 years, the results are not consistent with previous research showing that antibody prevalence is generally higher in adults [22,28,29]. In view of the high negative seroprevalence for animals aged 4 to 6 (and up) years, the risk of a PI progeny exists. Consequently, the maintenance of the infection on the farm is effective as PI animals are considered the major source of infection in a herd [3]. The statistical analysis also showed that it is not a risk factor for BVD.

The primiparous females are seropositive (18%), while the remaining 82% are seronegative. The latter represents a danger in case of infection because the offspring could be PI, especially since the virus circulates on the farm.

Concerning multiparous females, the risk of PI offspring is ruled out. Conversely, 69% are seronegative. The risk of PI offspring in the case of infection at the 1st stage is high with respect to the presence of seropositive on the farm [24]. However, there are no studies that relate the number of pregnancies or parity to the risk of infection and transmission of the virus.

Data show that in good hygiene conditions, the seroprevalence is estimated at 20%, while in poor hygiene conditions, the seroprevalence is estimated at 37.5%. Data showed a higher seroprevalence in farms with unsatisfactory conditions (37.5%), no other research has reported similar results. In sharp contrast, the existence of infected or excreted animals such as PI and/or TI on the farm is the main source of contamination [3,24]. The statistical analysis also showed that hygienic conditions were not a risk for BVDV circulation.

In fact, the multivariate ANOVA analysis revealed significant differences based on parity ($p < 0.05$), indicating that multiparous females exhibited a seroprevalence of 31% (11/35) with a 95% confidence interval (CI) of [16.1-46.8], whereas only 18% (2/11) of primiparous females were seropositive, with a 95% CI of [0.0-40.9]. Furthermore, hygienic conditions also demonstrated a significant influence on seroprevalence, with rates of 20% (4/20) under good conditions (95% CI [2.5-37.5]) compared to 37.5% (15/40) under poor conditions (95% CI [22.5-52.5]). These results indicate that multiparous females and those raised in poor hygienic conditions are more likely to be seropositive, underscoring the importance of management practices and hygiene in the prevention of infections.

4. Conclusions

The present study showed that the BVD virus circulates on the farms in the areas of study. Even if animals are protected because they have developed antibodies, the circulation of BVDV is confirmed and the risk of contamination is still present on these above-mentioned farms. Since there is no vaccine in use, a high likelihood of infection is expected among the herds in that area. For a better assessment of the risk on a national scale, a broader survey would be desirable in order to set up a control and prevention program in view of the risks involved, especially if PI animals are already present on the farms. Control and prevention must be based, as already done in other countries, on the elimination of PI animals and with full respect of biosecurity rules.

Author Contributions: Conceptualization, R.B.; Methodology, R.B., F.A.H. B.R. and N.M.; formal analysis, R.B., F.A.H. and N.M.; resources, D.K. and N.M.; writing-review and editing, supervision: R.B.; F.A.H., R.B., D.K., N.M.

Funding: No special funding.

Institutional Review Board Statement:

Informed Consent Statement: Written informed consent has been obtained from the farm owners.

Data Availability Statement: The datasets generated and analyzed during this study are available from the authors upon reasonable request.

Acknowledgments: Technical staff at SEGES Innovation P/S are gratefully acknowledged for collecting all the samples. The technical assistance of Margrethe Carlsen in performing the laboratory work is gratefully acknowledged.

Conflicts of Interest: The authors declare no conflicts of interest.

Acknowledgments: The authors would like to thank **CEVA** Santé Animale, Algeria, for their financial support and kind assistance, all our gratitude for their full help. We would like to thank also all the owners of the farms and breeders for their kind collaboration.

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