Immunization of calves and herd immunity to Bovine Respiratory Disease Complex (BRDC)

S. CAVIRANI

Department of Veterinary Medicine Science - University of Parma - Via del Taglio, 10 - 43100 Parma (Italy)

SUMMARY

Vaccination practice is one of the main tools to control Bovine Respiratory Disease Complex (BRDC). Since wide spreading and serious damages related to BRDC, immunization to respiratory pathogens should be considered a *core* vaccination in either dairy or beef cattle operations. Vaccination to BRDC should be planned following a program of immunization. Systematic immunization of the animals towards respiratory pathogens is pivotal to control BRDC reducing clinical signs and pathogen spreading. Pathogens target of vaccination, type of vaccine, vaccination timing and route of vaccine administration should be of concern to plan an effective vaccination program aimed at getting herd immunity to BRDC agents. The percentage of animal to be vaccinated at obtaining herd immunity to a specified pathogen is named critical proportion and it depends from the vaccine impact (efficacy) and the basic reproduction ratio of the pathogen. Calves are highly susceptible to BRDC agents, therefore to get herd immunity it's necessary to set up a vaccination program including immunization of young animals. Since vaccination is a preventive measure, the pathogen calves will encounter firstly should be the target of the first vaccine treatment. Both diagnostic and herd history offer an opportunity to make a correct choice. It's known that maternally derived antibodies trigger a strong interference to get a robust adaptive immunity through vaccination. Therefore, the optimal age for the first vaccination depends from the duration of maternal immunity, assuming an exponential decay in the fraction maternally protected as host age, and the mean host lifespan in years. Once got herd immunity, it must be kept overtime by adopting the repeat-pulse vaccination strategy. In accordance to duration of immunity elicited by the vaccine, the period of time that elapses between two consecutive vaccination can be calculated assuming the mean lifespan of the population to be vaccinated, the vaccine impact and the basic reproduction ratio of the pathogen. At any rate, the golden rule remains to set up a*n immunization program tailored for a herd*. At the aim it's necessary collaboration of farmer and veterinarian, as regards the instructions of the vaccine producer.

KEY WORDS

Calves, vaccination, bovine respiratory disease complex (BRDC).

INTRODUCTION

A long time ago Girolamo Fracastoro (Italian Physician, 1476-1553) in the De Contagione et Contagiosis Morbis, wrote: *"it's possible to win syphilis but not the genital disease"*. Similarly nowadays we can assert: *"it's possible to win IBR but not the respiratory disease"*. Nevertheless, we must keep fighting and hoping for.

Why Bovine Respiratory Disease is a complex? Because different respiratory signs and lesions are caused by different pathogens, namely viruses and bacteria.

What do the pathogens involved in BRDC share? High prevalence amongst the herds and wide spreading amongst animals of infected herd, and in some cases (e.g. BVDV), genetic and antigenic variability.

Besides the microbial factors, it's noteworthy that bovine is a species particularly predisposed to respiratory disease. Anatomy and physiology of bovine respiratory tracts are different from the other mammals (e.g. horse). Bovine lung anatomy (Fig. 1) undermines microbial clearance and it justifies difficulties in BRDC recovery as well as onset of frequent relapses despite an antibiotic treatment during the acute phase of disease[1]. In addition, body mass of bovine (Fig. 2) shows a strong imbalance between body weight/lung volume and oxygen consumptions[19]. To compensate the gap, bovine reacts with a *"physiological polypnoeic status"* (18-28 respiratory acts/min. of bovine *vs.* 16 ones of horse) predisposing to respiratory distress. Still, huge productive performances worsen the gap between oxygenating surface (lung alveolar epithelium) and body mass to be oxygenated. Lastly, in some areas, as the Po Valley in Northern Italy, high temperature associated to high humidity, typical of the summer season, triggers a further respiratory distress favouring microbial pathogenic activity. Sometimes remedy is worse than damage. Cooling systems, namely shower by using micro-droplets spray, cause an *"aerosol effect"*: environmental microbes are conveyed deeply into lung parenchyma through water micro-droplets and over there microbial pathogens fully express their pathogenic attitude. Therefore, it is not accidental the onset of severe BRDC outbreaks (Fig. 3) in summer season[4].

BRDC IN CALVES

Body targets of microbial pathogens during the first six months of life are recorded in Table 1. Findings outline that

Autore per la corrispondenza:
Sandro Cavirani (sandro.cavirani@unipr.it).

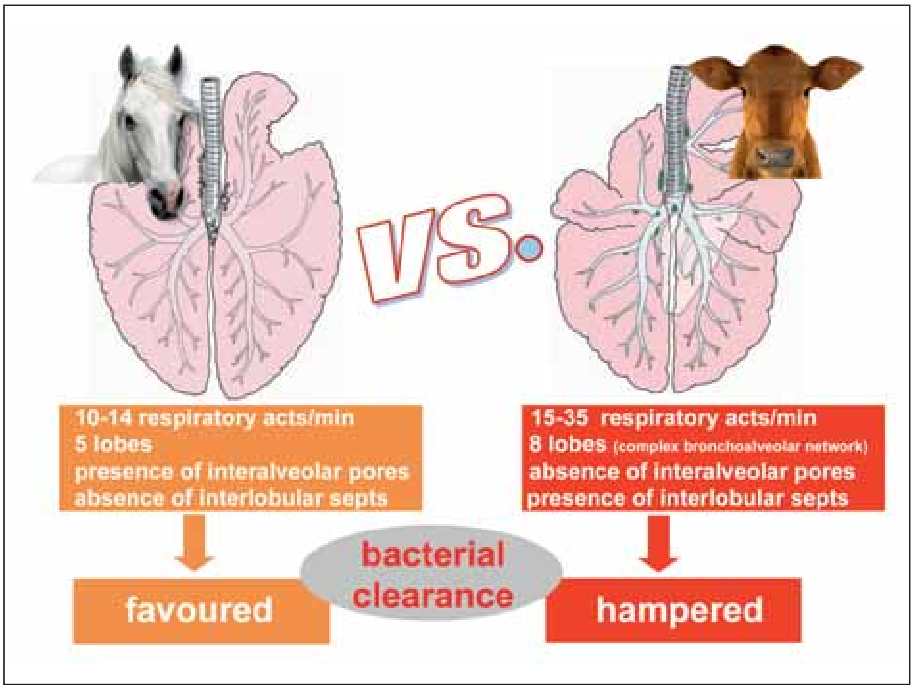

Figure 1 - Lung anatomy differences between horse and bovine.

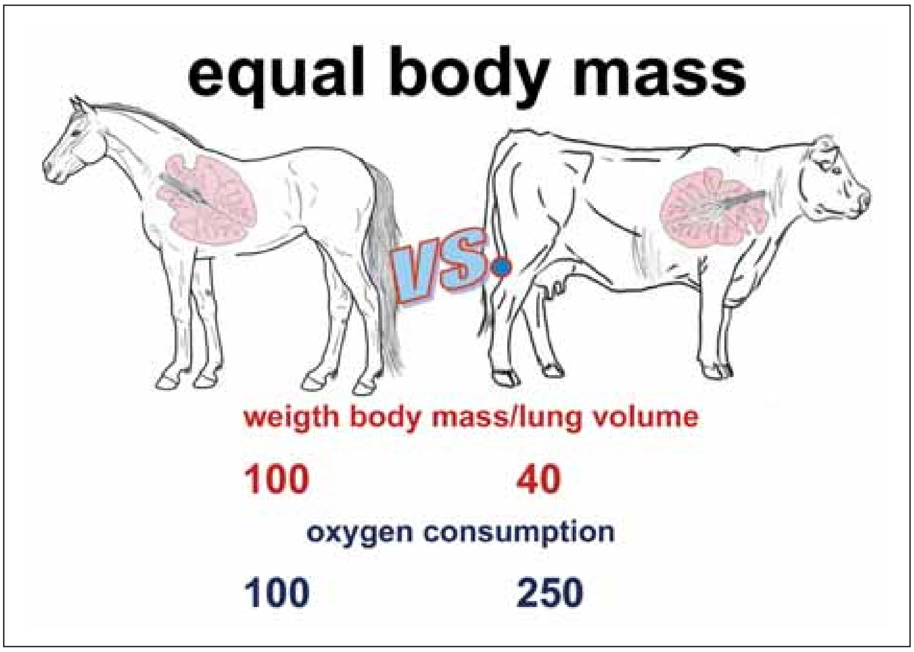

Figure 2 - Lung phisiology differences between horse and bovine.

BRDC signs usually occur after 15 days of life and the prevalence skyrockets over time.

A causal link between neonatal enteritis and respiratory disease in calves is of concern. Data (Table 2) pointed out that calves experiencing respiratory disease within the first 60 days of life (group 1) exhibit a significantly higher (chi-square test: P = 0.00742) prevalence of neonatal enteritis than ones showing respiratory disease later (group 2). Relationship between neonatal enteritis and respiratory disease is further demonstrated by a significantly higher (chi-square test: P = 0.00872) isolation rate of *Escherichia coli* from lung of died calves belonging to group 1 than group 2. Bacteria isolated from lung of calves, died for respiratory disease during the first 60 days of life, are of enteric origin likely. Phenomenon has to be referred to the "*microbial translocation*". Impairment of enteric barrier leads to translocation of microbial pathogens, mainly bacteria, from gut to lung parenchyma by means of the lympho-haematogenic route[7].

WHAT ARE THE PILLARS OF BRDC CONTROL?

Biosafety, Antibiotic and Vaccine

In field conditions, biosafety suffer from two main criticisms: workers education and farm logistics. In conclusion: due to the current condition of our cattle operations, may we realistically trust biosafety? And regarding antibiotics? Today we are speaking of responsible use of antibiotics, tomorrow we will speak of ban of important antimicrobial molecules. In conclusion: since Italian - but not only - livestock industry makes extensive use of antibiotics, a worrying scenario awaits us.

Therefore, in accordance to World Health Organization and World Veterinary Association, vaccine is and will be, even more, the main tool to guarantee human and animal health, BRDC control included. Unfortunately, in some cases good principles crash towards the harsh wall of reality. Italian current conditions highlight that, in front of more than 120 microbial pathogens, potentially undermining our cattle herds, less than 25 can be controlled by vaccination. In addition, we are coping a persistent gap: lack of a combo vaccine containing BoHV-1 marker antigen makes difficult to plan effortless vaccination programs for immunization to BRDC microbial agents by and large. Moreover, in the near future there are not pleasing perspectives regarding new vaccines availability. High costs and long times for registration procedures hamper development of new vaccines. Just recently, European Authorities realized the need to make easier the registration procedures for new and innovative vaccines. The will to discuss and to solve the problem is displayed in the "Report on requirement for authorization of vaccine within the EU - 10 July 2015" edited by the European Medicine Agency (EMA). This could be read as a signal that "*the climate is changing*", nevertheless at present it remains a declaration of intent only. What's next?

Figure 3 - Mortality and lesions in a BRDC outbreak occurred in a dairy herd during the summer season.

Table 1 - Body targets of microbial pathogens in calves during the first six months of life.
(Diagnostic data from Unit of Infectious Disease of Animals, Department of Veterinary Medicine Science - University of Parma, ITALY.)

Body target	1-15 days	15-60 days	60-150 days
Gastro-enteric tract	65%	48%	20%
Respiratory tract	5%	15%	36%
Others	30%	37%	44%

Table 2 - Relationship between enteric and respiratory diseases occurred in dairy calves during the first 4 months of life.
(Diagnostic data from Unit of Infectious Disease of Animals, Department of Veterinary Medicine Science - University of Parma, ITALY.)

GROUP	Calves showing BRDC	Time of BRDC occurrence	Calves experiencing neonatal enteritis
1	420	7-60 days	227 (68%)
2	397	61-120 days	75 (19%)

Herd Immunity to BRDC pathogens and vaccination practice

In accordance to principles of traditional medicine, vaccination is aimed at protecting the immunized individual against a specified pathogen. Otherwise, in population medicine, vaccination is aimed at developing the so called *"herd immunity"* against a specified pathogen[8].
What does it mean? Protection of a population from a specified infectious agent occurs when a high percentage of individuals shows an immune response, acquired by either previous exposition or vaccination to the pathogen. Herd immunity makes difficult for an infectious agent to spread since there are so few susceptible individuals left to infect. In practice: the grater is the proportion of individuals in a community who are immune, the smaller will be the probability that those who are not immune will encounter the infectious agent.
A vaccinated individual protects himself and at the same time contributes to protect the unvaccinated one. On this principle, vaccination is an *"ethical practice"*[2]. Nevertheless, it's sad to realize that, till now, the role of vaccination is not correctly understood and, worst, vaccination practice is under attack, in human as well as in veterinary medicine.
BRDC is a multifactor syndrome involving a population, namely the herd. Therefore, the control needs to achieve herd immunity against the main pathogens involved[15].
At this point, the question is: how many animals should be vaccinated to get herd immunity? In general, epidemiologists state that a population gets herd immunity to a specified pathogen when at least 70% of individuals are immune. However, each infectious agent does have an own herd immunity. The percentage of population to be vaccinated at obtaining herd immunity is named Critical Proportion **(Cp)** and can be calculated by the following formula[14]:

$$Cp = 1/Vi\,(1- 1/Ro)$$

Vi: vaccine impact (efficacy of the vaccine to control the spread of the infection and it's related to fraction of protected animals and relative duration of protection); **Ro**: Basic Reproduction Ratio (pathogen spreading attitude expressed as number of secondary cases arising from a single primary case of disease).

An example regarding human medicine: for measles, **Cp** is 92%[3].
An example regarding buiatrics: for bovine viral diarrhea, **Cp** is 78% and raises to 97% in presence of persistently infected (PI) animals[16].

From calves immunization to BRDC herd immunity

Why do we decide to vaccinate for one or more respiratory pathogens? Undoubtedly, experiencing a severe infectious disease outbreak, BRDC included, represents the main reason to lead the farmer to set up or to implement a program of control by vaccination. It is too late, anyway.
Following the diktat, *"it's better to prevent than cure"*, preventive measures, as vaccination, are main tolls to fight infectious disease, in both humans and animals. At the aim of setting up a suitable program of immunization, before vaccination, it should be correct to carry out a serological screening to detect presence and prevalence of BRDC microbial agents in the herd. Serum-sampling should be performed following a stratified random method, including cows, heifers, calves (after six months of age) and bulls, if present[18].

If one or more infections are found out, vaccination must be carried out as a preventive measure despite lack of clinical signs. Nowadays, more than in the past, famers agree this approach to control BRDC risk.
Otherwise, in a free herd, the risk to introduce a specified pathogen, but not only, should be assessed. Epidemiological situation of the area of relevance and attitude to introduce animals from the market must be of concern to establish the correct risk assessment. Therefore, an educational training by veterinarian, elucidating the farmer about risk of disease introduction into his farm, is crucial to address the decision to vaccinate or not. At the aim, veterinarian must improve skills regarding immunization practice, namely type of vaccines, related route and timing of administration.
Following data are the results of diagnostic activity carried out in the Unit of Infectious Disease of Animal - Department of Veterinary Medicine Science, Parma University (Italy). Laboratory investigations showed the involvement of virus and bacteria in BRDC outbreaks occurred in dairy herds in 2012-2017. In comparison to data previously (2000-2012) obtained, direct diagnostic procedures (isolation, immunofluorescence, PCR) on respiratory tract specimens showed that *Bovine Respiratory Syncytial Virus* (BRSV) and *Mannheimia haemolityca* are the still the main respiratory agents. *Bovine Herpes virus -1* (BoHV-1) prevalence decreases, conversely *Bovine Viral Diarrhoea Virus* (BVDV), *Bovine Respiratory Coronavirus* (BRCoV) and *Histophilus somni* increase over time. An emerging pathogen is *Bibersteinia trehalosi* that, only recently, its own biochemical characters allowed to distinguish from *M. haemolytica*[6]. Likely, misidentification of the germ could be the reason of a low amount of isolation till now recorded. In *"BRDC pathogens ranking"*, *Mycoplasma bovis* was deliberately not included. As previously showed[17] and even if *M. bovis* has been detected in roughly 80% of BRDC outbreaks here submitted to laboratory investigations (isolation and PCR), a doubt still persists[13]: is *M. bovis* a primary or a secondary BRDC agent? To elucidate the issue, a serological survey (paired, acute vs convalescent, serum samples) was carried out and involved 125 acute BRDC outbreaks occurred in 2012-2017. Only in 7% of BRDC outbreaks a group seroconversion to *M. bovis* was demonstrated. Serological findings showed that *M. bovis*, even if widespread among the domestic dairy herds, in a limited number of cases could be the causal agent of the respiratory disease. Irrespective that and despite widespread of *M. bovis* infection, no vaccine is commercially available in Europe at present.
Calves are highly susceptible to BRDC agents therefore to get herd immunity, it is pivotal to set up a vaccination program including immunization of young animals. That beign said, the question is: what pathogen calves will encounter firstly? Obviously, it will be the target of the first immunization treatment. Both diagnostic findings and herd history offer an opportunity to make a correct choice.
Recent findings, referred form many diagnostic lab, showed that BoHV-1 infection seldom involves young animals in either free or infected herds. Nevertheless, early vaccination of calves to BoHV-1 continues to be a usual practice and often the first immunization treatment in calves. More correctly, we must take into account that in field condition, before BoHV-1, calves have more possibility to encounter BRSV, BVDV, BRCoV, *M. haemolytica* and others. Therefore, since vaccination is a preventive measure, one or more of the mentioned pathogens should be the target of early vaccination in calves.
By reference to herd immunity, it is a common opinion that maternally derived antibodies trigger a strong interference at getting adaptive immunity through vaccination practice. And it in force the *dogma*: the optimal age for first vaccination must be a trade-off between the accumulation of newly susceptible hosts as their maternal protection decays and the need to delay vaccination when maternal antibodies do not interfere with development of vaccine protection. However, it is not so dogmatically true. In presence of maternally derived antibodies, calves do not show a detectable antibody response after priming immunization treatment. Nevertheless, the onset of B-cell memory and cell-mediated immune response, induced by vaccination, was demonstrated[9]. After booster treatment, B-cells memory elicit the same humoral response in calves submitted to priming vaccination in absence or presence of maternal antibodies[12]. At any rate, users have to pay attention to vaccine manufacturer recommendations regarding the age of calves. Particularly for bacterial pathogens (e.g. *Mannheimia haemolytica* and *Histophilus somni*) vaccines, administration is usually recommended after 3 months of life since onset of severe adverse effects in younger animals. Moreover, remember that an earlier vaccination is a "*off label*" procedure by law.
The optimal age for the first vaccination **(Av)** results applying the following formula[14]:

$$\mathbf{Av = M\, \mathit{ln}\, (1 + L/M)}$$

M: duration of maternal immunity assuming an exponential decay in the fraction maternally protected as host age; **L**: mean host lifespan in years.

Traditionally, a common opinion is that maternal immunity protect calves during the 4-6 months of life. However, in field conditions maternal protection is shorter due to the phenomenon of *Failure of Passive Transfer* (**FPT**)[5]. FPT status is a deficit in transfer of colostrum antibodies from dam to calf. A survey, carried out in Italian dairy operations with high milk production (>9,000 kg/cow per year), recorded that FPT status is widespread, involving 35% of sampled calves (Table 3). No statistical difference has been detected in relationship to mother's parity.
Moreover, a kind of *"gender inequolity"* exists in dairy calves. Findings pointed out that FPT prevalence was significantly higher (chi-square test, P = 0.017882) in male calves than in female ones (Table 4). This should be of concern for health management, particularly in veal (white meat) calves operations that collect male calves mainly from dairy herds. Usually, these calves are not vaccinated to BRDC agents since the animals are traditionally believed to be protected by maternal immunity during the whole rearing cycle, about 6 months. Facing the widespread of FPT status occurring in male calves, lack of a specified vaccination program is opposite to a correct disease management. In addition, vaccination is able to reduce the use of antibiotics as preventive measure.
A further side effect, related to FPT status, concerns maternal vaccination for control of neonatal enteric pathogens by colostrum intake. Even if vaccination is an efficient tool to trigger a robust immune response in dam, the persistence of

Table 3 - FPT prevalence in 243 dairy herds with high milk production (>9,000 kg/cow per year) located in the Northern Italy.
(Diagnostic data from Unit of Infectious Disease of Animals, Department of Veterinary Medicine Science - University of Parma, ITALY.)

Mather's parity	Calf blood-samples	FPT positive samples
Fist-calving	380	131 (35%)
Repeat-calving	860	306 (36%)
Totals	1,240	437 (35%)

Table 4 - FPT prevalence in female and male calves from 22 dairy herds with high milk production (>9,000 kg/cow per year) located in the Northern Italy.
(Diagnostic data from Unit of Infectious Disease of Animals, Department of Veterinary Medicine Science - University of Parma, ITALY.)

Calves sex	Number of calves	FPT prevalence
Female	200	26%
Male	200	38%
Totals	400	32%

Figure 4 - Intranasal and parenteral vaccination of a dairy calf.

factors, predisposing onset of FPT status in calves, jeopardizes protective effect of colostrum. An accountable feedback: usually farmers do not accept persistence of enteric problems following pregnant cow immunization, therefore they could lose confidence in that vaccine and, at worst, in the vaccination practice, by and large.

Keeping of herd immunity over time

Once got herd immunity, it must be kept over time by adopting the repeat-pulse vaccination strategy. In accordance to duration of immunity (**DOI**) of the vaccine, the period of time that elapses between two consecutive vaccinations is determined. That is named Critical interval (**Ci**) and it is calculated by using the following formula[14]:

$$Ci = (LxVi)/Ro$$

L: mean host life span in years; **Vi**: vaccine impact; **Ro**: basic reproduction ratio of the immunized pathogen.

Usually, for the commercially available vaccines Ci is included in the registration dossier and recorded in the vaccine datasheet.
When pathogens to be vaccinated and vaccination timing are established, the last step is to decide the route of vaccine administration. Inactivated or modified-live, monovalent or combo vaccines are administered by parenteral routes, namely subcutaneously (mainly inactivated vaccines) or intramuscularly (mainly modified-live vaccines) injection (Fig. 4), and that in accordance to the producer recommendations. Despite that, in many cases subcutaneous vaccines are injected intramuscularly and vice versa. Unfortunately, it's just not the same: efficacy of immunization and duration of immunity are adversely affected by an incorrect administration route. Once again, an incorrect administration is a *"off-label"* procedure by law.
Mucosal-intranasal vaccination (Fig. 4) is an alternative and effective method of immunization, for young animals in particular. Intranasal immunization elicits both mucosal and systemic immune response. Since the seventies, intranasal vaccination has been applied to immunize cattle against BoHV-1 and PI3 virus, by live-modified-termosensitive viral strains. Since bovine anatomy favours intranasal vaccination, at present live modified vaccines, namely BoHV-1 traditional or marker, BRS and PI3 viral antigens, are administered by this route[21]. The main targets of intranasal vaccination are lymphoid structures belonging to Waldeier's Ring that includes pharyngeal, tubal, soft palate, palatine and lingual tonsils. Traditionally, a lack of interference with mucosal immunization by maternally derived antibodies has been believed. Nevertheless, even in the case of intranasal vaccination, high titres of passive antibodies lead to a certain degree of interference to onset of systemic immunity[10]. The evidence cannot be undervalued in a program of immunization of young animals. Disrespecting producer recommendations, to get a robust immune response following an early intranasal immunization it's advisable to plan a booster treatment within 2 months of life, mainly by parenteral route. It's worth the Latin motto: *melius est abundare quam deficere* (it's better too much than not enough).
A final remark: traditional vaccines do not allow to assess effectiveness of a vaccination program through serological investigations. Only BoHV-1 (IBR) marker vaccine allows viral circulation to be detected in a vaccinated population by a

suitable serological test. Despite that, in field conditions, farmers usually consider lack of clinical signs as the expression of vaccine effectiveness. It is the main input for veterinarians to carry on and to improve the vaccination strategy to control-eradicate infectious diseases, BRDC microbial agents in particular.

CONCLUSIONS

- Herd immunity, obtained through a correct immunization practice, is a pillar for BRDC control and to minimize likelihood of disease onset.
- Immunization of calves is pivotal to get herd immunity to BRDC microbial agents.
- A feasible vaccination program must take into account epidemiological features of the herd or the area of relevance, vaccines available in the market and cost-effectiveness of the program.
- Regarding calves immunization, it's remarkable to mention sequence and timing of vaccination treatments by assessing maternal immunity and microbial risk.
- For companion animals, namely dogs and cats, guidelines by WSAVA (World Small Animal Veterinary Association) distinguish between *core*, *non-core* and *not recommended* vaccines, taking into account risk of contagion and damages related to each infectious pathogen[20]. Likewise, in buiatrics, vaccination to BRDC microbial agents should be retained a *core* practice in both dairy and beef industry.
- Full cooperation of veterinarian and farmer is essential to set up *"a vaccination program tailored for the herd"*[11].

References

1. Abutarbush SM, Schunicht OC, Wildman BK, Hannon SJ, Jim GK, Ward TI, Booker CW (2012). Comparison of enrofloxacin and ceftiofur sodium for the treatment of relapse of undifferentiated fever/bovine respiratory disease in feedlot cattle. Can. Vet J., 53: 57-62.
2. Anderson R and May R (1992). The concept of herd immunity and the design of community-based immunization programmes. Vaccine, 10: 928-935.
3. Bester J (2013). Vaccination is best for children. The argument for relaying on herd immunity fails. J. Biothec. Ing., 14: 375-378.
4. Cavirani S, Taddei S, Ghidini F, Chiapponi C, Campani A, Gentile A (2005). Coinvolgimento di BVDV e *Mannheimia heamolytica* in un grave episodio di malattia respiratoria in bovine da latte. Proceedings XXXVII Congresso S.I.B., 95-100.
5. Cavirani S, Taddei S, Cabassi CS, Donofrio G, Toni F, Ghidini F, Piancastelli C, Schiano E, Flammini CF (2005). Deficit of colostral IgG and of passive immunity transfer in dairy herds with high milk production. Large Animal Review, 11: 17-21.
6. Collins R (2011). Bibersteinia trehalosi in cattle - Another component of Bovine Repiratory Disease Complex. Cattle Pract. 19: 9-12.
7. D'Ettore G (2012). Microbial transolocation and infectious: what is the link? Int. J. Microbiol. Med., Vol. 12, Article ID356981.
8. De Jong MC and Bouma A (2014). Herd immunity after vaccination: how to quantify it and how to use it to halt diseases. Vaccine, 19: 2722-2728.
9. Ellis JA, West KH, Waldner C, Rhodes C (2005). Inhibition of priming for respiratory syncytial virus-specific protective immune response following parenteral vaccination of passively immune calves. Can Vet. J., 46: 155-16.
10. Ellis JA, Gow SP, Goji N (2010). Response to experimentally induced infection with bovine respiratory syncytial virus following intranasal vaccination of seropositive and seronegative calves. J.Am.Vet.Med.Ass., 236: 991-999.
11. Hjerpe CA (1990). Bovine vaccines and herd vaccination programs (1990). Vet. Clin. North Am. Food Anim. Pract., 6: 167-260.
12. Kimman TG, Westenbrink F, Straver PJ (1989). Priming for local and systemic antibody memory response to bovine respiratory syncytial virus: effect of amount of virus, viral replication, route of administration and maternal antibodies. Vet. Immunol. Immunopathol., 22: 145-160.
13. Maunsell FP and Donovan GA (2009). Mycoplasma bovis infections in young calves. Vet. Clin. North America: Food Anim. Pract., 25 (1), 139-177.
14. Pastoret PP (1997). Veterinary Vaccinology. Ed. Elsevier Science Pub Co. Chapter 14, Part 12, pp.: 565-573.
15. Phillip JI (1972). Bovine respiratory disease: is control possible? Vet. Rec., 13: 552-555.
16. Stokka GL (2010). Prevention of respiratory disease in cow/calf operation. Vet. Clin. North. Am. Food Anim., 26: 229-24.
17. Thomas A, Ball H, Dizier I, Trolin A, Bell C, Mainil J, Linden A (2002). Isolation of mycoplasma species to lower respiratory tract of healthy cattle and cattle with respiratory disease in Belgium. Vet. Rec., 151: 472-476, 2002.
18. Tunker P and Yesilbag K (2015). Serological detection of infection dynamics for respiratory viruses among dairy calves. Vet. Microbiol., 180: 180-185.
19. Veit H and Farrel R (1978). The anatomy and physiology of bovine respiratory system relating to pulmonary disease. Cornell Vet., 68: 555-81.
20. WSAVA (2016). Guidelines for vaccination of dogs and cats. J. Small Anim. Pract., 57: 1-33.
21. Xue W, Ellis J, Mattick D, Smith L, Brady R, Trigo E (2010). Immunogenity of a modified virus vaccine against bovine viral diarrhea virus type 1 and 2, infectious bovine rhinotracheitis virus, bovine parainfluenza-3 virus, and bovine respiratory syncytial virus when administered intranasally in young calves. Vaccine, 14: 3784-3792.