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ANTI-ANTHRAX VACCINATION IMPACTS ON IMMUNITY IN EXTENSIVELY RAISED DAIRY COWS

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Abstract

Anti-anthrax vaccination is stressful for animals, with a potential negative impact on some immune effectors. This research aimed to investigate the extent of those and estimate the effectiveness of vaccination. Twenty-three extensively raised dairy cows and 11 calves were sampled before and two weeks after the anthrax vaccination (R 1190 Stamatin strain). Blood samples were collected, subjected to blood counts and the N/L ratio was calculated as a stress index. The total Ig (24%o zinc sulphate precipitation) and circulating immune complexes (CIC) (4.2% polyethylene glycol precipitation) were quantified from the serum samples.
The N/L ratio was of 0.79 ± 0 .59 before and 0.58 ± 0.45 after the vaccination in adult animals, while in calves it increased significantly (0.61 ± 0.25 and 1.11 ± 0.68, respectively). The total Ig concentrations supported a lesser immunization of the calves than in dairy cows (6.95 ± 2.09 versus 12.10 ± 6.68 Vernes degrees, respectively) supporting the more stressful effect of the primary vaccination than of the booster one. Nevertheless, the antibody clearance was enhanced in the younger animals (5.4 ± 0.25 versus 1.0 ± 0.1 ODU, respectively). Repeated stimulation is the substrate for an enhanced adaptive response to vaccination in cattle.

Key words*: dairy cows, anthrax, vaccination, immunity, extensive raising.*

INTRODUCTION

Within the category of farmed, cattle have a major socio-economic, health, biological and ecological importance in the economy of numerous states, having a positive economic impact on the rural environment and representing a substantial source of income for many communities around the world (Thornton, 2010). Still, farmers involved in cattle breeding, in spite of providing support to the local markets and jobs for other members of the community (veterinarians, technologists/ mechanics and distributors of animal products), have to face numerous difficulties, such as those imposed by expanding environment protection and animal health and welfare legislation. The carbon fingerprint limitations are also likely to influence the cattle farming (Herrero et al., 2013; Rotz et al., 2013).
The societal development will trigger further progress in breeding technologies, improved nutrition strategies and perfected preventive measures applied to farmed animal diseases to intensify the production potential of bovine. Human health concern based on a nutritional opinion diverted from red meat or cow milk consumption could still heavily moderate the increase in numbers and size of bovine farms. Cattle-farming is one of the main branches of animal production, which provides people and the processing industry with particularly valuable products and by-products such as milk and meat as food products, and hides, bones, hooves, horns and manure as by-products (Pawlak and Kołodziejczak, 2020), especially in developing countries.
The historically-supported close proximity of people and animals, bovine included, leading to domestication, has also enhanced the progression of a new host-pathogen relationship and jump over species for various microbes (Schiffman et al., 2002). Thus, numerous diseases of bovine could show a zoonotic character.
Anthrax is considered to be a non-contagious zoonotic disease, albeit the soil represents the main source for *Bacillus anthracis*.

Nevertheless, there are risk categories of farmers, caretakers, veterinarians, slaughterhouse workers or personnel involved in handling carcases or by-products, which are exposed to anthrax infection from animal sources (Rume et al., 2020).

Achieving high-performance production is conditioned by the health of the animals, which are exposed to a multitude of technological factors on the farm, sometimes inducive of undesirable effects. One of the most widespread operations to support health is immune prophylaxis, performed through vaccination operations. Depending on the epidemiological situation, the vaccination protocols applied in cattle farms are diversified, some of them being included in the national strategic program.

Although vaccinations have long been considered a revolutionary means of preventing disease (Turnbull, 1991; Misra, 1996), more and more recent research reveals adverse effects attributed to these vaccinations, especially repeated ones, caused either by the included antigenic structures (hypersensitivity, allergy, anaphylactic shock) or adjuvants used (local inflammatory reactions, anaphylactic shock) (Fasanella et al., 2001).

All these types of reactions are based on an exaggerated reactivity on the part of the immune system.

This research aimed at investigating the stress levels, expressed by neutrophile/lymphocyte ratios and humoral immune responses (total Ig and circulating immune complexes' levels) and estimating the effectiveness of vaccination.

MATERIALS AND METHODS

Biological material. The experiment was performed on dairy cows (n = 23) and young bovine, both males and females (n = 11) over two month of age, reared extensively. The animals were vaccinated according to the Strategic Program, with a live vaccine containing the attenuated R 1190 Stamatin strain, acapsulogenic, oedematogenic and immunogenic.

Venous blood samples were collected on EDTA, for blood smears and on a clotting agent, for serum collection, before and one month after the vaccination.

N/L ratio (Chung et al., 2015). The use of the leukogram, more precisely the calculation of the N/L ratio as a stress indicator (Davies et al., 2008, Hickman, 2017) was used by multiple researchers in medicine and ecology to monitor stress levels in different species. The method is more precise than investigation the level of corticosteroid levels, which make the baseline measurements difficult under stressful field conditions.

For that, blood samples collected on EDTA where smeared at a 30 degree angle and then stained with a Panoptic method after drying (Kit for Fast Staining in Haematology (Fast Panoptic) for clinical diagnosis).

The staining involved the following steps:

a) Dipping the slide in a holder with the fixative for fast staining (Panoptic No. 1) 5 x 1 second each time followed by the drainage of the excess liquid over filter paper;

b) Submerging the slide in another holder with the Eosin (Panoptic No. 2) for fast staining, 5 x 1 second each time and draining;

c) Dipping the slide in a holder with Blue for fast staining (Panoptic No. 3), 5 x 1 second each time and draining;

d) Rinsing the smear with Buffer solution, pH = 7.2;

e) Finally, draining the slide and examining it under the microscope, magnification x 100.

The leukocyte populations were expressed as a % and the N/L ratio was calculated.

Circulating immune complex measurements (Khokhlova et al., 2004)

Circulating immune complexes (CIC) measurement allows the evaluation of the molecular clearance capacity of the physiological mechanisms of the host.

For this, sera were separated from the clotted blood by centrifugation at 2500 rpm for 10·min and kept at –20°C until tested. The CIC precipitating agent was represented by a 4.2% polyethylene glycol (PEG) solution in borate buffer, while buffer-treated samples served as controls for borate-induced precipitation. The reaction was performed in a 96-well-plate to enhance spectophotometrical readings. 196.7·µl of borate buffer and PEG solution, respectively, were mixed with 3.3·µl of each serum, in parallel wells, in duplicate. The samples were allowed to precipitate at room temperature (20-21°C) for 60·min, then read

spectrophotometrically (optical densities, OD) at a wavelength of 450·nm in the test plate (d = 0.5·cm) (multichannel spectrophotometer SUMAL PE2, Karl Zeiss, Jena, Germany). The calculation of CIC levels was performed according to the formula:

$$\mathbf{CIC\ (units) = (OD_{PEG} - OD_{borate}) \times 10^3}$$

Immunoglobulin measurements (Khokhlova et al., 2004). Opsonins or total immune-globulins, represent humoral effectors within the 'first line of defence' against aggressors. The colloidal stability of gamma globulins is lower than that of serum albumins at a pH·7.4, therefore low concentrations of metal salts precipitate the immunoglobulin (24‰). For this, a volume of 193.4·ml of a 0.024 mg/100 ml zinc sulphate solution in barbital buffer was mixed with 6.6·ml of serum and allowed to precipitate for 30·min at room temperature (20-21°C). Optical density (ODU) then was read spectrophotometrically (l = 475·nm, d = 0.5·cm) and used to calculate the concentration in Vernes degrees, by multiplying the optical density with 100.

Statistical processing of the data. Averages of CIC and total Ig for both samplings along with the standard deviations were calculated. The Excel program was used to calculate the significance of the differences between the two samplings by means of the t- Student test.

RESULTS AND DISCUSSIONS

Vaccination generally represents a beneficial method of positively influencing the immune system that has facilitated the eradication of numerous communicable diseases, due to the specificity of the response. The practical application of immune prophylaxis relying on antigenic components isolated from microorganisms is an approach based on understanding the pathogenetic mechanisms, on the analysis of the host's protective response to pathogens, and on the use of immune system regulation by stimulating responses on behalf of T and B lymphocytes.

Most vaccines are injected, a route that involves disadvantages, a practical and also an immunological one. Inoculations are painful and expensive requiring specific materials and a trained operator, and therefore mass vaccination procedure is laborious. Immunologically, this pathway does not always mimic the usual track of pathogens into the body and may not stimulate the immune system properly, for example, if the infection site is one of the mucosae. In addition, inoculation, through the pain caused and the negative reflex induced, exerts an immune suppression, thus, due to increase in corticosteroid levels, the expected result is not achieved.

The present study attempted to establish a relationship between systemic immune protection in extensively reared cows on a family farm by looking at how humoral and cellular immune effectors are affected by the routine anti-anthrax vaccination.

Thus, the non-specific humoral immune response (total immunoglobulins, CIC) as well as the status of leukocytes involved in the first line of defense was investigated in these animals, aiming at establishing the influence of a compulsory immune prophylactic operation with recognized stress inductive effects.

The results obtained showed that the N/L indicator allows estimate the degree of stress induced by the bovine vaccination against cattle (Figure 1).

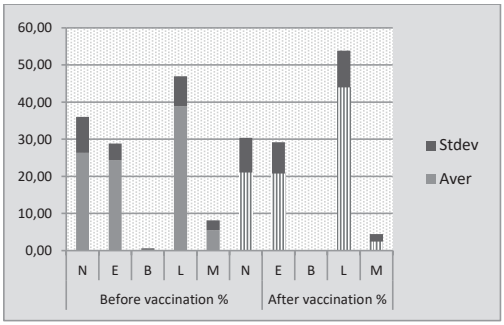

Figure 1. Changes of leukocyte populations subsequent to vaccination (x ± s) in adult animals, subject to repeated vaccination

It has been shown that there is an interrelationship between the N/L ratio (Figure 2) in young cattle in which the stressful effect of vaccination is obvious, with the adaptive cellular immune profile diminished in this category of animals.

Opsonin levels increased in adult animals significantly ($p<0.05$), probably due to the repeated vaccination procedure (Figure 4).

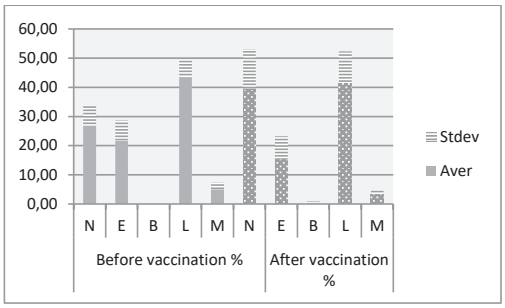

Figure 2. Changes of leukocyte populations subsequent to vaccination (x ± s) in animals subject to primary vaccination

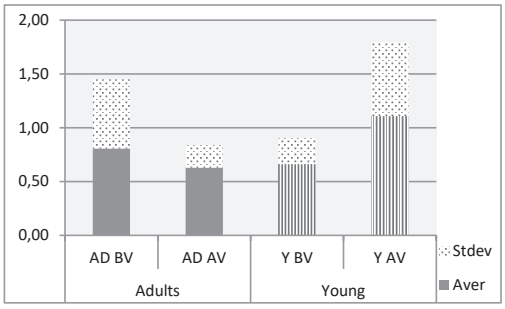

Figure 3. Variation of the N/L ratios (stress indicator) after the vaccination (x ± s)
Legend: AD-adults, Y-young, BV-before vaccination, AV-after vaccination

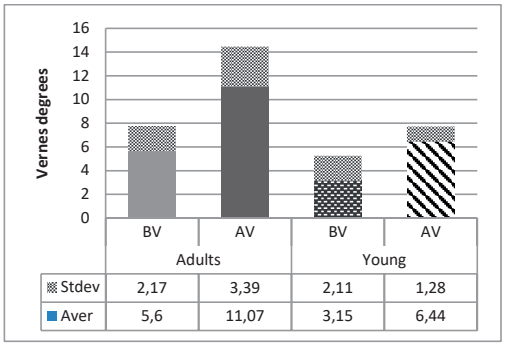

Figure 4. Changes in total Ig levels in the experimental groups after the vaccination (x ± s)
Legend: AD-adults, Y-young, BV- before vaccination, AV-after vaccination

The increase in the Ig levels in young animals, although less than in the adult ones, clearly underlines the possible stimulating effect of the vaccination on the immune system and the general immediate protection of these animals. The lesser values were supported by the higher stress levels indicated by the increased N/L ratios in young animals after vaccination.

Similarly, the detected CIC concentrations showed that there is no relevant increase in this parameter in adult animals, while in young animals, the increase of CIC levels was significant ($p<0.01$), corresponding to the increased possibility of deposition of those in target organs (Figure 5).

In the case of the young group, unlike in adults, the clearance capacity of CIC was statistically significantly decreased post-vaccination ($p<0.05$), probably due to the increased stress levels suggested by the N/L ratio values.

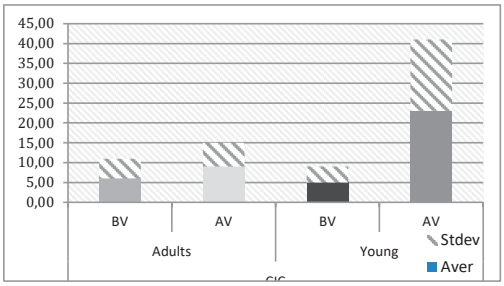

Figure 5. Changes in CIC levels in the experimental groups after the vaccination (x ± s)
Legend: AD-adults, Y-young, BV-before vaccination, AV-after vaccination

CONCLUSIONS

The significantly increased N/L ratios in calves indicated a more severe stress in the latter. In spite of this, there was an increase of the antibody levels but a decrease of their clearance (increased CIC levels) which could lead to pathogenic effects of the deposited complexes. Repeated anti-anthrax vaccination could therefore be the substrate for an enhanced adaptive response to vaccination in adult, but not in young cattle.

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*** Kit for Fast Staining in Haematology (Fast Panoptic) for clinical diagnosis

*** OIE Terrestrial Manual 2018, Section 3.1, Multiple species, Chapter 3.1.1. Anthrax, p. 311-320