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ABSTRACT BOOK

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Alternative Methods for Veterinary Clostridia Vaccine Quality Control

Biologicals are products derived from biological sources, including immunological (such as vaccines and sera), hormones, antibodies and blood products. These types of products undergo strict quality control before applied to humans or animals.

Vaccines are recognized as a highly cost effective tool for preventing infectious diseases. They are derived from biological sources and due to the complexity of composition and heterogeneity of products, vaccine lots undergo legally required quality control before they are released.

Traditionally, laboratory animals have played a major role in quality control of vaccines, and still, many animals are used for this purpose.

Over the last decades, 3Rs methods to classical animal tests have been developed by control authorities, academia and vaccine manufacturers, successfully validated and implemented.

New generations of vaccines have been and are being developed which are better defined and allow the use of non-animal methods for their quality control.

Moreover, the concept of vaccine quality control is changing as emphasis is being put on ensuring the consistency of production of a vaccine and not regarding each batch produced as a unique product.

The characteristics of a new batch of a vaccine should be similar to those of a batch which has been shown to be safe and efficacious.

Clostridia bacteria are widely distributed in the environment, in addition to being present in the gut of animals. The bacteria are responsible for a large proportion of the unexplained sudden livestock deaths that most farms experience.

Tetanus is the most well-known of the clostridial diseases, and is especially problematic for horses. Infected horses usually die or require euthanasia. Additional clostridial bacteria include C. chauvoei (cause of blackleg), C. novyi (causes black disease), and C. haemolyticum (causes bacterial redwater). Other clostridial bacteria causing sudden death in cattle and sheep include C. sordelli and C. perfringens. Treatment of clostridial diseases with antitoxins and large doses of antibiotics is expensive and generally not successful, nor does it follow the principles of responsible use of antibiotics. The use of vaccines is required to give animals protection against these diseases, thereby avoiding the use of antibiotics.

Clostridial vaccines are usually multi component products containing up to 11 different clostridial strains/serotypes. They are also often combination vaccines against additional disease pathogens for example E. coli, Equine influenza virus, Mannheimia haemolytica, and Pasteurella trehalosi.

Their pharmaceutical quality is controlled by vaccine manufacturers in accordance with the specifications of the European Pharmacopoeia (Ph. Eur.) monographs for clostridial veterinary vaccines and with their market authorisation dossiers. For many of these vaccines both the toxin and toxoid bulk (obtained by detoxification

of toxin and used to produce the final vaccine batches) are currently controlled by animal-based tests. This is the case for toxicity and antigenicity in-process controls which are performed in mice by using the minimum lethal dose (MLD) and the total combining power (TCP) tests, respectively.

Alternative tests

Modified toxin-binding inhibition (ToBI) test for epsilon antitoxin test is in vitro methods in order to verify the potency of epsilon toxicoid in comparison with the in vivo TCP method.

For the ToBI test, the correlation ratio of 96.76, obtained in the curve pattern, demonstrates the effectiveness of the curve to be used in the epsilon toxoid evaluation. The correlation ratio between the titration degrees of toxoids obtained through TCP and ToBI tests was higher than 90%. The ToBI test can be used as a screening method for it is sensitive and effective to detect epsilon toxicoid produced by C. perfringens type D.

The potency of the final vaccine is then assessed by Toxin Neutralisation Test (TNT). All of these tests use mice and have lethal endpoints. Development of alternatives for potency testing was based on ELISAs able to measure antibody levels to the specific toxins relative to a standard serum with a defined unitage. These alternative assays were shown to correlate with the relevant TNTs and have been accepted by European Regulatory Authorities as batch release potency tests.

Two in vitro methods based on monoclonal antibodies (mAb) have been developed for the determination of specific antibodies against C. perfringens β-toxin (capture ELISA) and ε-toxin (competitive ELISA) in these sera. Both test systems show high specificity and good reproducibility.

Cell culture assays, using the MDCK cell line was confirmed as being sensitive to the C. perfringens epsilon toxin and VERO cell line to the C. novyi type B alpha toxin. Cell culture assays using these cells were developed and the test conditions were standardised. The antitoxin titres of rabbit antisera were calculated and compared with those of the manufacturers. The correlation coefficients between in vitro and in vivo method were calculated and were significant (99.73%). The cell culture assay offers a valid in vitro alternative to the animal experiments for the titration of sera generated in the course of potency tests of clostridial vaccines.

For Clostridium chauvoei, an alternative approach using a validated ELISA method and an in vitro replacement test for Clostridium hemolyticum utilizing toxin-neutralizing antibodies with the characterized protective antigen, is described.

The study results have demonstrated that cell-based assays can be more sensitive and accurate, have high reproducibility, can shorten the duration of tests and provide huge advantages in terms of saving animal lives and reducing costs, while ensuring safety and public health are not compromised.

Potentially, all the Clostridium protective antigens could be evaluated by antigen quantification methods, such as quantitative ELISAs, after the protective antigen has been identified by gene cloning or after rights to the protective gene have been obtained from sources that have cloned the genes for the purpose of developing reference standards. Based upon the published literature and available regulatory methods, replacement of the toxin-neutralization test for specific Clostridium spp. vaccines is a realistic goal but will require the global recognition of reference vaccines and the identification of the target antigens for these vaccines.

In addition, because of their potentially higher sensitivity and precision, in-vitro assays may offer better tracking of production consistency and allow more accurate vaccine blending.

In the control of veterinary vaccines we have moved away from routine batch testing of abnormal toxicity and we are also now moving away from routine testing for target animal species toxicity. So now the most important thing will be replacement of animal challenge tests by alternatives such as serological tests, cell-based testing or in vitro tests.

Within the last years, considerable efforts have been made to reduce the number of animal experiments in this field, lower the number of animals, and decrease the suffering of the animals during testing. Several research projects for the development and validation of alternative methods have been initiated. Furthermore, the 3R Concept (Replacement, Reduction and Refinement) is increasingly taken into consideration when developing

or revising legal provisions. This led to various improvements regarding animal welfare in the quality control of clostridial vaccines

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Fluorescence light energy in the management of lacerated and contused wound in a horse

Traumatic wounds are frequently faced by the equine practitioner. Although primary closure is preferred, often wounds heal by second intention, a time-consuming process which may take several weeks with possible unsatisfactory functional results. Fluorescent light energy (FLE) represents an additional option in wound management and has been applied in small animal to manage different skin conditions including wounds and canine perianal fistulas. An 11-years-old Arabian stallion was presented to the Veterinary Teaching Hospital as first opinion consultation for a right hindlimb blunt-force contusion framed by a scalloped laceration, at the level of third metacarpal. The horse received systemic antibiotic and anti-inflammatory therapy for ten days with a limited response. Owing this, such treatments were discontinued and FLE (Phovia® Vetoquinol) management was started. It consisted of applying a roughly 2 mm layer of gel on the lesion and illuminating with the blue LED device that delivers noncoherent blue light with peak wavelength between 440 and 460 nm and a power density of between 55 and 129 mW/cm2, for 2 min, at approximately 5 cm distance. After illumination, the gel was gently removed using sterile gauzes and a second cycle performed soon after. After FLE session, a protective bandage was applied. Phovia was administered twice weekly and after four FLE applications (2 weeks) granulation tissue fulfilled the laceration. Within 10 additional weeks (5 weeks) wound showed a >90% improvement in the extent of re-epithelization and FLE was discontinued after 7 weeks in total. This fluorescence-generating system is an innovative and non-invasive wound care device that promotes healing of wounds besides allowing regular monitoring of medical advancements. The present is only a case report but the obtained results suggest a possible role of FLE in daily practice for the management of wounds in horses which deserves to be better explored.

Biography:

Andrea Marchegiani, Researcher. Marilena Bazzano, Research fellow. Fulvio Laus, Professor.

The main field of interest and research concerns equine medicine and the application of fluorescent light energy in veterinary medicine. AM, MB, and FL are authors of publications in both national and international scientific journals and speakers in several national and international conferences.

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Hyperechoic periportal cuffing as an ultrasonographic inflammatory sign in the abdominal pathology of canines

Introduction

The hyperechoic appearance of the portal vein and its branches can be seen in different pathologies described in veterinary and human medical literature. Although periportal cuffing and the hyperechoic aspect of the perivascular portal tributaries can be connected with gastrointestinal disorders, the ultrasonographic sign can also be related with vascular disorders, representing an important inflammatory sonographic marker.

Material and Methods

Medical records were analyzed retrospectively in 60 dogs of different breeds and of both sexes, with different gastrointestinal signs (primary or associated with other diseases), between November 2020 and April 2022. Hyperechoic periportal cuffing highlighted by abdominal ultrasonography was an inclusion criteria. Confirmed diagnoses included 22 cases of acute pancreatitis; 3 cases of necrotizing pancreatitis; 8 cases of acute hepatitis; 5 cases of cholecystitis; 3 cases with portosystemic shunts (multiple acquired and congenital); 12 cases of portal hypertension; 7 cases with gastroenteritis.

Conclusion and Discussion

Periportal cuffing of the liver is an ultrasonographic sign seen more frequently in cases of abdominal inflammation, in which the diseases are heterogeneous and most commonly arise from the liver or the gastrointestinal tract. In this study, portal tributaries hyperechoic appearance and periportal hyperechogenicity in dogs, was also seen in cases of portal hypertension (hepatic, pre and post hepatic) and in cases congenital or acquired portosystemic shunts; however, these aspects were not described in the literature. The changes are potentially related with systemic inflammation, increased pressure within the portal lumen and vasculitis. In all the cases diagnosed with hyperechoic periportal cuffing, levels of C-reactive protein were moderately elevated, which can strengthen the association of the periportal cuffing as an inflammatory sonographic marker. The article aims to present changes in the portal vasculature seen in dogs with abdominal pathology and the importance of identifying hyperechoic periportal cuffing as an inflammatory marker of the vasculo-abdominal pathology.

Biography:

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Graduated from the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, Bucharest, Romania in 2018, currently working in UK at Lime Trees Veterinary Hospital. PhD student of the Faculty of Veterinary Medicine Bucharest, with major interest in veterinary imaging and portal system vascular diseases. Speaker for multiple conferences in veterinary medicine.

Published work:

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Microgeographical disparity in shell morphology of the tidal river periwinkle Pachymelania fusca (Gastropoda, Sorbeoconcha, Hemisinidae)

The tidal river periwinkle (Pachymelania fusca, Hemisinidae) is an important commercial and ecological species in the tropical shellfish fishery, but its population demographics are receiving minimal attention. This study adopted a conventional morphometric method to compute the disparity in shell morphology within a microgeographic scale. A total of 198 specimens from three mudflats (Nsidung [N], Esukutan [E], and Adiabo [A]) of the Calabar River, Nigeria were collected for three months (July–September 2021). The nine morphometric measurements obtained for each specimen were allometrically transformed and subjected to multivariate statistics. Results revealed that the first principal component accounted for 99.6% of the total variance produced, mostly by aperture outline, mid-body girth, and aperture base girth. 93.8% of cases were correctly classified as [N], 64.7% as [A], and 41.2% as [E] by the original discriminant function. Following that, 66.0% of the specimens were correctly classified as belonging to one of two distinct stocks ([A]-[E] and [N]). Based on these results, we postulate that the periwinkles of the Calabar River showed morphometrically different attributes within a few meters apart. We therefore call on relevant environmental agencies and other stakeholders to take urgent actions that could lead to the conservation of the genetic integrity of this species and the studied river.

Keyword: Calabar River, geographic scale, intertidal region, morphometric approach, mudflats, stock assessment.

James Kithuka
The Brooke - East Africa, Kenya

Agrovet Mentoring and its Contribution in Delivery of Veterinary Services in Developing Countries

There are about 10,000 agrovets operating in Kenya but only 4,000 of them are registered. Many of these are poorly equipped with technical knowledge to professionally dispense veterinary medicines despite 70% farmers visiting agrovets has their first reference when their animals gets sick. Agrovet regulation is weak and harmful non-evidence-based practice occurs thus weakening the animal health system.
50 agrovets were sampled and questionnaire administered to gauge their current perceptions and understanding of anti-microbial resistance, pain management, and disease surveillance. Of the operators interviewed 67% were men; 80% were between 26 – 45 years and only 18% had a degree in animal health.51% of the agrovets dispensed antimicrobials on prescription. However, none produced any prescriptions or clinical records.Agrovets (80%) indicated that profit was the motivation for not selling antimicrobials on prescription. All agrovets have heard of notifiable diseases, and all of them reported such suspected cases to the County veterinary authorities.Asked if they considered pain to be important for animal health and welfare, 89% of agrovet operators said yes but only 2% of the agrovets had pain relief in their stock.
To help improve quality of agrovet services, Brooke East Africa developed Agrovet Mentoring Framework (AMF). AMF outlines essential competencies required by agrovets and assesses current gaps in the animal health system in which they operate. The five areas assessed are; legal compliance, communication, pharmacological expert, shop content and governance. Trained mentors provide case-based support allowing Agrovet to demonstrate their knowledge and skill within their work place.
In 2017 AMF results indicated outstanding 10%, good 38% and unsatisfactory 52%; in 2020 performance was; outstanding 73%, good 24% and unsatisfactory 3%. Agrovet number mentored rose from 22 (2017) to 104 (2021). AMF results indicate that mentorship has positively improved competency levels as74.9% of mentored agrovets are now demonstrating competence in dispensing, treating and providing advice to livestock owners in addition to identifying animal health infrastructure challenges like availability of essential medicines that help policy stakeholders to address from point of information.
In conclusion: Agrovets play a pivotal role in primary animal healthcare. Brooke's AMF is an effective tool to build skills in the workplace and combat gaps within the animal health system.

Keywords: Agrovet; Mentoring; Framework; Antimicrobial; Animal health

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Nutraceuticals: how to enhance fertility in stallions

Stallion semen preservation has played a fundamental role in the success of artificial insemination (AI) and several attempts have been made to improve male fertility over years. Cooling and freezing storage of semen is not immune from deleterious effects on spermatic cells such as decrease in cell viability, motility, and fertility. Nutraceutical supplementation has been proposed as a tool to increase the quality of stallion semen and improve the reproductive efficiency of horses. Substances of vegetable origin, minerals, and vitamins are available on the market for the management of male infertility in both human and veterinary medicine. Several strategies and nutraceutical compounds have been explored to have a beneficial effect on stallion sperm quality. Polyunsaturated fatty acids (PUFAs) from different sources (pomegranate seed oil, heterotrophically grown microalgae, algae- and flaxseed-based supplements, linseed oil), carnitine, antioxidants and vitamins (selenium, carotene, tocopherol, ascorbic and folic acid, vitamins A, C, and E), botanical extracts (including herbs, fruits, vegetables, enriched plasmolysed yeast) has been added to stallions' diet, alone or as a mixture, in order to investigate the effects on semen characteristics and redox status. Starting from the last decade, the frame of research directed toward the use of nutritional supplements able to improve the ability of equine semen to cope with cryodamage and oxidative stress has produced remarkable results. The advantage of PUFAs as unique dietary supplement is fairly conflicting, minor improvements have been reported in some studies, whereas no improvement has been observed by other authors, probably related to redox imbalance following excessive dietary fatty acids intake, which has been recognized as a major cause of male infertility. Providing optimal levels of antioxidants resulted to be essential for the maintenance of normal reproductive functions and the most promising results have been obtained in those studies investigating the combination of PUFAs and antioxidants.

Biography:

Marilena Bazzano, Research fellow.

The main field of interest and research concerns equine medicine and reproduction, especially the application of innovative management modalities to increase male fertility. MB is author of publications in both national and international scientific journals and speakers in several national and international conferences.

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Vascular endothelial growth factor A improves quality of matured porcine oocytes and their subsequent development of in vitro fertilized embryos

The effects of vascular endothelial growth factor A (VEGFA) supplementation (5 ng/mL) in the culture medium on the development of porcine embryos were assessed in comparison with the control group without VEGFA supplementation during in vitro maturation (IVM) and in vitro culture (IVC). The addition of VEGFA in the IVM medium increased monospermic rates compared to those of the control groups (67 vs 57.7%,respectively; $P < 0.05$).Concomitantly, polyspermy reduced when oocytes were matured in the IVM medium supplemented with VEGFA compared to the control group (11.8 vs. 20%, $P < 0.05$). Supplementing both IVM and IVC media with VEGFA promoted the blastocyst rate compared to the control group (46% vs. 31%, $P < 0.05$). Also, the total cell number per blastocyst increased in the presence of VEGFA in the IVC (74.4 ± 10) or in both IVM and IVC (81.8 ± 8.2), compared to those in the control group (62 ± 11, $P < 0.05$). The apoptotic indices were lower in the group with VEGFA supplementation in both IVM and IVC media than that in the control group (2.4 ± 0.1 vs. $5.5 \pm 1.4\%$; $P<0.05$). Moreover, VEGFA also increased GSH level of oocytes by 1.4 to 3.4 folds, while concomitantly decreased ROS byhalf fold in matured oocytes, 4-celled embryos and blastocysts. Taken together, VEGFA exerts a positive effect on oocyte maturation and subsequent embryonic development. It functions as a growth or paracrine factor that helps maintaining the ROS-GSH homeostasis, likely, by acting as an antioxidant during oocyte maturation and early embryogenesis in porcine species.

Keywords: embryo; GSH; in vitro fertilization; porcine; ROS; VEGFA

Biography:

Michel KERE Assistant Professor, Institute of Rural Development, Nazi Boni University.

Dr. Michel KERE is anAssistant-Professor in the Institute of Rural Development, Nazi Boni University, Burkina Faso. He obtained a Master of Science and technology of Rural Development in animal husbandry at the Institute of Rural Development, Burkina Faso. He earned Ph.D. in Reproductive Biotechnology at Department of Animal Science, National Chung Hsing University, Taiwan, ROC. He serves as the head of research stations of Nazi Boni University. Dr. Michel KERE is interested in reproductive biotechnology, growth factors, antioxidants, molecular biology, animal feed, and Green House Gas emission. His present research focuses on effects of balanced rations on tropical animal reproduction, as well as draft animal training and management.

Min Yue
Zhejiang University, China

WGS aids ONE HEALTH surveillance

Genomic sequencing and corresponding big-data analytic toolkits are beginning to make a significant impact on food safety by providing a more precise platform for pathogen characterization, tracking, and streamline therapeutical treatment suggestions. Here, we focus on an emerging multi-drug resistance clone ST45 in Salmonella in China and many other countries. By using the 1800 genomics data from local sequenced strains and global datasets, with a well-characterized meta-data, we use the core SNPs from these datasets to build a global galaxy server for pathogen tracking and found it is efficient for tracking the origin and other phenotypical results in the context of the global food supply chain. Interestingly, We also found most of the multi-drug resistant strains showed a significant association with domesticated animals, mainly bovine, which was indicated as a probable reservoir for the multi-drug-resistant clone. We also use Random Forest, a machine learning toolkits, to prioritize the SNPs to associate with the antimicrobial resistance patterns in all the 1800 strains, and develop a molecular qPCR test, using the eight SNP markers, to predict the antimicrobial resistance pattern based on the newly established genotypes. This approach could be used to guide the risk assessments for the biohazard, i.e. Salmonella, along the food transmission chain and ultimately provide rational antimicrobial options for the clinicians. Together, our big-data platform provides an efficient way for Salmonella tracking and antimicrobial resistance pattern forecasting.

Keywords: Salmonella, Genomic epidemiology, Antimicrobial resistance, Molecular diagnostics

Biography:
Prof. Min Yue is interested in applying the integrative “Omics” approach to understand the functional diversity of bacterial virulence factors and mechanisms, particularly the impact of population genomics on diversity and evolution of Salmonella pathogenesis. He is also interested in adopting the above knowledge in the creation and development of predictive, preventive, or therapeutic measures. He has published over 70 SCI-index journal, including Nature communications. He serve as the Associate Editor for Frontiers in Veterinary Medicine, Frontiers in Veterinary Scinece, and editorial broad for Foodborne Pathogens and Disease, Animal Diseases.

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Selenium yeast alleviates deltamethrin-provoked cerebrum injury in quails via activation of the GPX4/TLR4 signaling pathway

Deltamethrin (DLM) is a member of pyrethroid pesticide widely applied for agriculture and aquaculture, and its residue in the environment seriously threatens the bio-safety. The cerebrum might be vulnerable to pesticide-triggered oxidative stress. Selenium (Se) possesses a number of biological activities. Our study focused on investigating the therapeutic potential of Se yeast (SY) on DLM-induced cerebral injury in quails after chronically exposing to DLM and exploring the underlying mechanisms. Forty male quails were distributed into 4 groups randomly: control, DLM (45 mg/kg body weight intragastrically), SY (0.4 mg/kg SY added in standard diet), and DLM + SY. We measured biomarkers of oxidative stress, determination of blood index, performed histopathological analysis, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay, and relative mRNA and protein levels. The results showed that SY supplementation ameliorated DLM-induced oxidative stress, apoptosis, and the release of inflammatory mediators in the quail cerebral. SY elevated the content of Se and increased glutathione peroxidase 4 (GPX4) level in the DLM-treated quail cerebrum. Furthermore, SY enhanced antioxidant defense system by upregulating nuclear factor-erythroid-2-related factor 2 associated members. Meanwhile, SY diminished the changes of apoptosis- and inflammation-associated proteins and genes including toll-like receptor 4 (TLR4). Our study demonstrates that SY attenuates DLM-induced cerebrum injury in quails via activation of the GPX4/TLR4 signaling pathway. GPX4 may be an effective therapeutic target for the treatment of DLM-induced cerebral injury. Furthermore, this study not only provides a novel insight for elucidating DLM-induced cerebrum toxicity, but also a practical foundation for the treatment of DLM-induced damage by dietary supplement of SY.

Biography:

Zhigang Zhang, Professor

Zhigang Zhang (https://orcid.org/0000-0002-4974-5850) is a professor of Northeast Agricultural University in China. He is a distinguished professor of the Chang Jiang Scholars Program, and won a prize of the Provincial Youth Science and Technology Award in 2022. He has published more than 100 papers in Chinese and international journals, including Journal of Hazardous Materials (IF= 14.224) and Journal of Advanced Research (IF= 12.822). He is also a member of the Chinese Association of Animal Science and Veterinary Medicine, and serves as editor for journals of Frontiers in Bioscience-Landmark and Separations.

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