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**Workshop: Infectious Bronchitis (IB)
in the Brazilian Poultry Industry**

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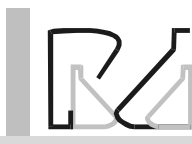
■ **Keywords**

Chicken, genotyping, infectious Bronchitis,
PCR, vaccination, variants.

Infectious Bronchitis in Brazilian Chickens: Current Data and Observations of Field Service Personnel

ABSTRACT

The infectious bronchitis virus (IBV) was detected for the first time in Brazil by Hipólito in 1957 in chickens sold live in the municipal market of Belo Horizonte, MG, when commercial poultry production was just starting in that country. The Massachusetts (Mass) serotype was identified. However, the clinical disease was only observed in 1975, when poultry production was intensely growing. The extensive outbreak produced the classical condition in layers and breeders, affecting egg production and quality, whereas broilers presented respiratory and "nephritis-nephrosis" signs. The disease rapidly spread to all poultry-producing regions in the country, and in 1979, both the imports and the manufacturing of live vaccines against IB strains Mass, H120 and H52, were licensed. In 1980, inactivated vaccines were introduced. Molecular techniques, particularly PCR, started to be used in the identification of IBV. A retrospective analysis showed that, up to 1989, the main IBV strain circulating in Brazil was Mass. However, other studies show the presence of a wide diversity of IBV strains in Brazil since the first strains were isolated, even before vaccination was introduced. Most researchers agree that the incidence of IBV different from Mass has increased, including of exclusively Brazilian genotypes, different from those described in other countries. Indeed, during the last few years, the number of genotypical variants has been much higher than that of the classical Mass serotype. Clinically, in addition to the classic presentations, atypical forms such as testicular atrophy and stones in the epididymis associated to low fertility have been described. Serological techniques started to be used in vaccination monitoring and as a diagnostic tool. Serological response standards were developed, and have shown to be very useful to determine the expected profile in vaccination programs and when clinical disease is suspected. However, the immuno-enzymatic test ELISA is the most frequently used around the world due to its convenience. These situations led service people working in the field to suspect that vaccination programs using Mass strains were not providing the required protection because of the presence of variant strains. Some argue that this was expected, particularly in layers and breeders, because Mass-type vaccines have been used for a long time, whereas most agree that the emergence of variants is the primary cause of the increasing severity of the disease in the field. This is supported by the results using IBV genotyping as a diagnostic tool, independently of phenotype (pathotype x immune system x environment). Other arguments state that broiler carcass downgrading rates in processing plants are not consistent with the increase in IB clinical severity. Seroconversion in non-vaccinated flocks is acknowledged, but it occurs sporadically and not necessarily correlated with disease outbreaks. There is a general agreement that IBV has shown high variability in Brazil in terms of genotype, pathotype, and serotype. However, research should



emphasize IBV phenotypical characteristics using birds as biological model.

FIRST DETECTION OF THE INFECTIOUS BRONCHITIS VIRUS IN CHICKENS IN BRAZIL

The Infectious Bronchitis virus of chickens was detected in Brazil in a very conventional way. Osmane Hipólito - at that time, professor of the discipline of infectious diseases in School of Veterinary Medicine (EV) of the Federal University of Minas Gerais (UFMG), located in Belo Horizonte (BH) - had returned from his doctorate in the US in 1956, where he had worked with the IBV strain Massachusetts (Mass), which was the only recognized and existing at that time. He had brought the strain adapted to embryonated chicken eggs (ECE). As IB was endemic and severe in the US, and had not been yet reported in Brazil, Hipólito started to search for antibodies against Mass IBV as soon as he returned, and found them in the serum of chickens sold in the central market of BH, using seroneutralization test (SN) in ECE. Subsequently, samples collected from these birds and inoculated in ECE showed IBV signs. And therefore, in 1957, the presence of IBV was recognized in Brazil. At that time, Brazilian poultry production was mostly backyard production.

After IBV was first identified, many years went by with no clinical manifestation of the disease.

In 1968, Hipólito, who had retired from the University of Minas Gerais, moved to the city of São Paulo, where he took the position of Head Professor in the School of Veterinary Medicine and Animal Science (FMVZ) of the University of São Paulo (USP). In this university, he created the first Poultry Pathology mandatory discipline in the curriculum of the Veterinary Medicine, which also included extension services to poultry farmers.

SCENARIO OF POULTRY HEALTH PROBLEMS BETWEEN 1970-80

In the beginning of the 1970s, Brazilian poultry production started to exponentially grow, particularly in the state of São Paulo and in the west of Santa Catarina. Whole-broiler frozen carcasses started to be exported to Middle East countries in the middle of the 1970s, driven by the energy crisis and the need of having exportable commodities to pay the Brazilian "oil account".

These exports broke a paradigm in the poultry industry. During that time, poultry farms - which

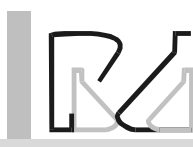
produced almost exclusively older broilers - were experiencing severe losses due to health problems. Good biosecurity practices and single-age farm were very rare. Lymphoid leucosis occurred in all farms rearing long-cycle birds. Marek's disease was clinically evident in broilers, resulting in dramatic losses, despite the introduction of vaccines. Endemic coccidiosis was treated with countless anticoccidial programs. Respiratory complex diseases freely circulated: mycoplasmosis due to *Mycoplasma gallisepticum* was present in almost every farm, together with infectious coriza, and the most severe and lethal form of velogenic Newcastle disease. However, diseases such as infectious bursal disease, infectious laryngotracheitis, pneumovirus, infectious anemia, and reovirus were not observed.

BEGINNING OF CLINICAL IB IN BRAZIL

It was in the above environment that, after 1975, the first clinical cases of IB were observed. Huge numbers of IB cases, coming from all over Brazil, were submitted to the Poultry Pathology clinic of FMVZ-USP. The previous experience of Professor Hipólito helped in the rapid and long-expected recognition of the disease.

Virtually every clinical IB presentation were observed during that period: the classical condition in layers and breeders, with sudden drop in egg production, and thin, deformed, porous and wrinkled eggshells with irregular calcium carbonate deposits in the surface and color loss, eggs with no shells, etc. The external egg changes were always concomitant with internal changes, with thin albumen. Respiratory symptoms were not always observed. There were also asymptomatic flocks, with little or no change in egg quality, but which egg production did not reach the expected level. Gross atrophy changes were observed in these birds, which were called 'false layers'. In broilers, the respiratory form was very evident. Broiler flocks with respiratory signs also presented wetter feces, which caused litter caking. At necropsy, there were respiratory lesions and an almost common form of enlarged kidneys, with the presence of urate in the lobules, characterizing a pathology called 'nephritis-nephrosis'.

According to Katayama *et al.* (2006), between 1977-78, 22.5% (25/111 birds) of clinical respiratory cases of IB submitted to the Poultry Pathology clinic of FMVZ, USP/SP were confirmed by isolation and identification. Almost every case (83.3% to 100%), coming from different parts of the country, were suspected of IB due to their history.



During that period, the serological prevalence of IB was 78% in commercial layers, 46% in breeders, and 28% in broilers.

Licensing of the use of vaccination of chickens against IB in Brazil

IB was an emerging problem in poultry health in Brazil, and the FMVZ-USP team submitted a study with the title "Chicken Infectious Bronchitis - The disease in Brazil" and won the 3rd Dow Veterinary Award in 1979 (Hipólito *et al.*, 1979).

In the 1978 World Poultry Congress held in Rio de Janeiro, IB in Latin America was the theme of a round table. The disease was recognized in almost every Latin-American country, but there were heated arguments as to the prevalent serotypes. There were few diagnostic tools available, and the data were mostly empirical.

After long discussions, the Brazilian Ministry of Agriculture approved and licensed in 1979 both the imports and the local manufacturing of live IB vaccines against the Mass strains H120 and H52 to be used in all chicken flocks in Brazil. In 1980, inactivated vaccines using the same strains were introduced.

IB virus classification and terms

Before moving forward, it is important to describe IBV classification and terms used in this article.

Serotype: classification based on the serological reactions of a determined sample.

Pathotype: isolate capacity to cause lesions in determined cell types or tissues.

Genotype (or genotype): classification based on genetic characteristics.

Protectotype: virus contained in a vaccine that promotes *in-vivo* protection against the challenge by a field virus with the same serotype or different serotypes of that contained in the vaccine.

Occurrence and dissemination of IB viruses in Brazil

The tool initially used to characterize IBVs was cross-seroneutralization in embryonated chicken eggs, which was labor- and time-consuming, and expensive. This technique determined the virus' serotype.

The standard serotype, and the first characterized in the world and in Brazil, was Mass. Samples with antigenically different from the original virus were considered variants or a new serotype. The use of molecular techniques in the study of IBVs greatly advanced the understanding the variations of these

viruses, and introduced the genotypes. However, the results derived from the use of this technique have been often misinterpreted, and wrongly correlated with pathotypes or protectotypes.

Molecular techniques have been extensively applied in the study of Brazilian samples. Miyaji (1996) reported that until 1989, the IBV circulating in the country was essentially of the Mass type in a universe of more than 20 different recognized serotypes, defined according to the epitopes present in the external globular portion of the glycoprotein of the envelope spike.

Nevertheless, several studies, particularly those that sequenced the S1 and S2 genes, have shown the occurrence of a huge diversity of IBVs on Brazil since the first strains were isolated, even before the vaccine was introduced. Most researchers agree that the incidence of IBV different from Mass has increased, including of exclusively Brazilian genotypes, different from those described in other countries (Abreu *et al.*, 2006-2008; Chacón *et al.*, 2007, 2008; D'Arce *et al.*, 2008; Di Fabio *et al.*, 2000; Montassier *et al.*, 2006; Villarreal *et al.*, 2006-2007). A recent article showed that Mass serotype was present in only 8.0% of the 75 studied IBV samples (Sandri *et al.*, 2008). This variability was shown by Chacón *et al.* (2007), whose results are presented in Table 1, and by the typification data obtained by Simbios biotechnology laboratory of Porto Alegre, RS (Table 2).

Table 1 - RT-PCR results of the analysis of 118 samples of IBV isolated in different types of chickens, carried out at FMVZ, USP, SP, between 2004 and 2006.

Type of chicken	IBV	IBV-Type Mass*	IBV-Variant
Breeders	44	9	35 (83.3%)
Broilers	62	20	42 (67.7%)
Layers	12	1	11 (91.7%)
Total	118	30	88 (74.6%)

(*) Strains 793B, D274, and H120. Chacón *et al.* (2007).

Table 2 - PCR results of the analysis of 454 samples of IBV isolated in different types of chickens, performed at Simbios biotechnology laboratory, between 2006 and 2009.

Total received	1,068 cases
Positive for IBV	454 (42.5 %)
Typed as Mass	126 (36.1%)
Negative	614 (57.5%)
Typed	349 cases
Genotypic variants	223* (63.9%)

(*) Not Mass. Source: Lunge (2009).

RARE IBV PATHOTYPES FOUND IN BRAZIL

Concurrently with the emergence of genetic variants, some atypical IB clinical cases started to be



described, such as testes atrophy and epididymis stones (Figure 1). Cockerels from 45-50-week-old broiler breeder flocks, with normal egg production rates, but high infertility rates, presented high anti-IB antibody titers, testis atrophy, and epididymus stones. A variant IBV was identified by PCR (Bernadino, 2009).

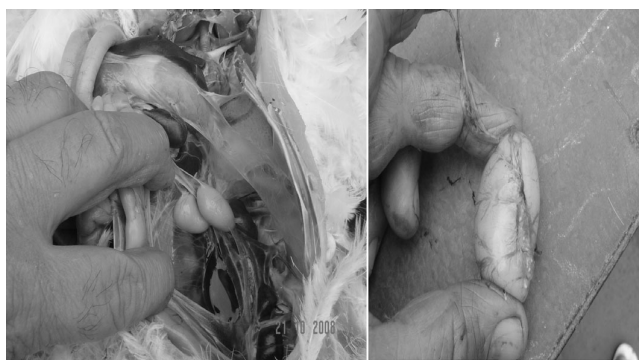


Figure 1 - Atrophic testes of adult cockerels. Source: Bernadino (2009).

A more detailed study of a case of infertility due to problems in 57-week-old cockerels presenting respiratory disease, face edema, and nephritis revealed the presence of IBV in the testis. The sample characteristics placed it close to the genotypes D274, Cal99, and Ark 99, and distant from vaccine serotypes M41, Ma5, Conn, DE072, and 793B. Moreover, Pneumovirus serotype A was isolated in the same material (Villarreal *et al.*, 2006).

Intestinal and kidney problems in broilers and layers were related to infection by IBVs grouped in a single cluster near serotype D274, and distant from Mass (Montassier *et al.*, 2006).

Another atypical clinical finding in chickens, which at first was believed to be related with IBV infection, was deep pectoral myopathy. However, IBV serotype 4/91 was outruled, as well as any other serotype (Brentano *et al.*, 2005, 2006).

SEROLOGICAL IB MONITORING

IB serological monitoring of poultry flocks at regular intervals is a common practice in Brazil, particularly in broiler breeder flocks. The results of the analyses depend on the employed technique, vaccination history, farm management, and the lab that performs the tests. Therefore, there is no general standard of interpretation of the results.

We present below some IB serological profiles resulting from the analyses of field samples obtained in routine monitoring.

The Laudo laboratory of Uberlândia, MG, using micro-seroneutralization in cell culture, considers that challenge is present during pullet development, when the response is the double of the standard response; alert (poor response), when response is 50% lower than the standard. During lay, geometric mean titers (GMT) higher than 1,000 indicate challenge (Botrel, 2009).

Indirect ELISA is the test most frequently used for IB serological monitoring. Almost all laboratories use internationally-manufactured kits and international standards. Here below are some results obtained by Mercolab, Cascavel, PR (Back, 2009).

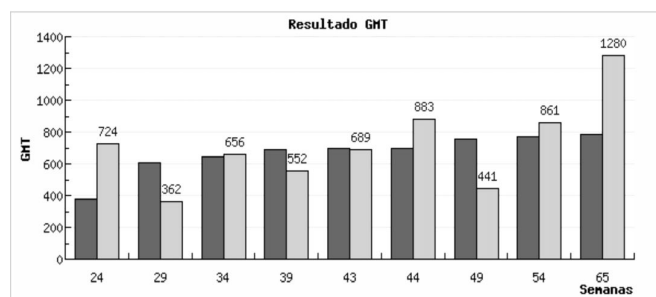
PERCEPTION OF SERVICE PEOPLE AS TO IB SITUATION IN THE FIELD

The main perception of service people working in the field of IB situation in Brazil is that vaccination programs using live and inactivated vaccines with Mass strains, independently of vaccine combination or program, is that poultry are not being receiving the necessary protection due to the occurrence of variant strains. The opinions of some service people presented below were selected under this perspective, and were added to the text as personal communication. We would like to thank all those that sent us their opinions.

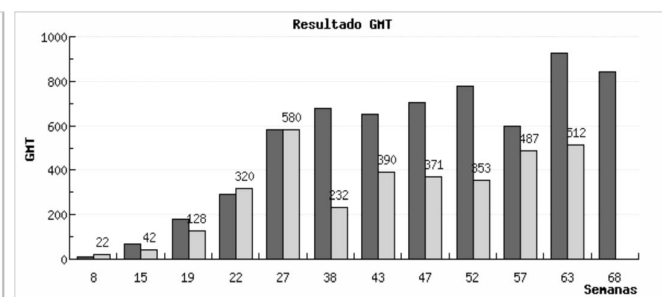
According to Botrel (2009), IB vaccination functions as a "filter", as it does not work against some strains as challenge pressure increases. Therefore, virtually all long-cycle flocks, such as breeders and layers, are challenged with IBVs, which are not necessarily variants.

Leffer (2009) points out that IB problems are becoming more acute in recent years, with respiratory and reproductive and/or kidney disease, regardless the vaccination program. Health surveillance and diagnostic method improvements have supported this view, showing high titers in serological profiles in current monitoring. Layers seem to be the most affected with clinical disease, with respiratory symptoms during pullet development, and later reduced egg production and eggshell quality issues, with the emergence of 'false' layers. In breeders, the main signs are eggshell quality problems (deformation, color), lower egg production and reduced fertility caused by the males. In broilers, IB is present even in vaccinated flocks. These IB cases are supposedly related to challenge with variants. In several affected flocks, symptoms and their consequences have been "controlled" with antibiotics.

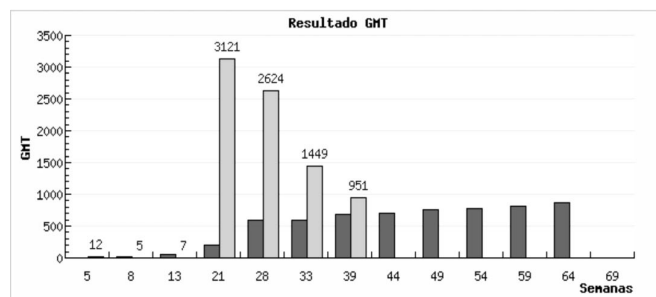
Zuanase (Zuanase 2009) observes that respiratory



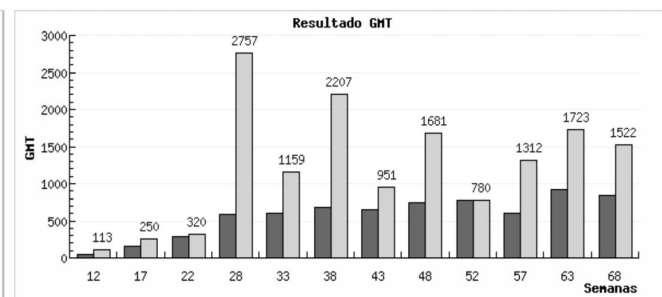
a) Uniform and expected serological response of a good vaccination program.



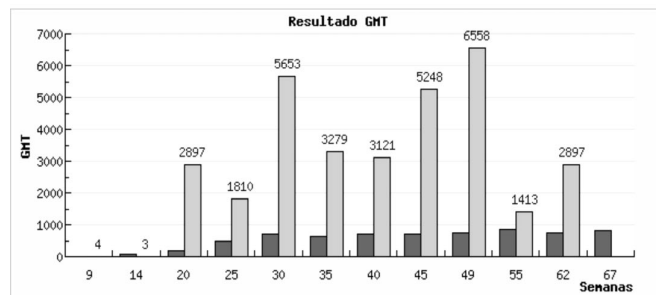
b) Serological response with no maintenance of vaccine titers after production peak.



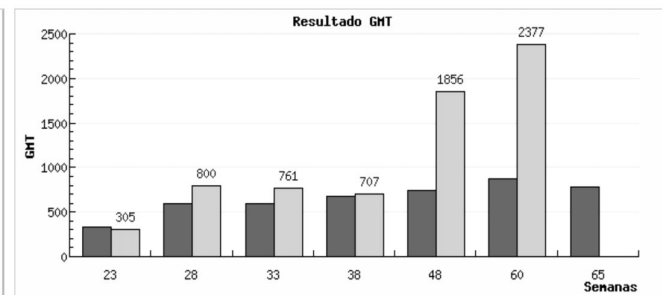
c) High serological response after inactivated vaccine and no challenge.



d) Irregular serological response, suggesting challenge during egg production.



e) Irregular serological response, indicating challenge during egg production.



f) Serological response, suggesting challenge at the end of the first egg production cycle.

Figura 2 (a-f) - IB serological profiles obtained by micro-seroneutralization test in cell in samples collected from broiler breeders. Yellow bar = tested flock; Blue bar = laboratory standard (Botrel, 2009).

and production problems reported in the field are not properly diagnosed, as they are usually attributed to infection with IBV variants, and genotyping of strains isolated in these cases have supported this belief. However, he notes, some of the genotypic variants found bear no relation with any clinical problem. He therefore recommends the standardization of the diagnostic methods to evaluate genotype x serotype x pathotype interactions. His opinion as to the unfortunate and undue use of IBV genotyping as a diagnostic tool is supported by Katayama (2009), who emphasizes the need, for diagnosis purposes, to take the phenotype (pathotype x immune system x environment) into account.

The fact is that the combination of IB with the infection by Pneumovirus is catastrophic for broiler breeders' egg production. This requires the vaccination also against Pneumovirus, with administration of a live vaccine on the 1st, 10th and 20th week of age via drinking water, plus injection of oil-inactivated vaccine at 35 weeks of age. Live vaccines against Pneumovirus during pullet development only prevent clinical disease when IBV challenge is low or absent. Similarly, in broilers, vaccination (even using two live vaccines) against IBV does not provide good protection when the challenge is high (Patrício, 2009). As an example, Patrício reports a clinical case that happened in the state of SP in 2008, when 45-week-old broiler breeders

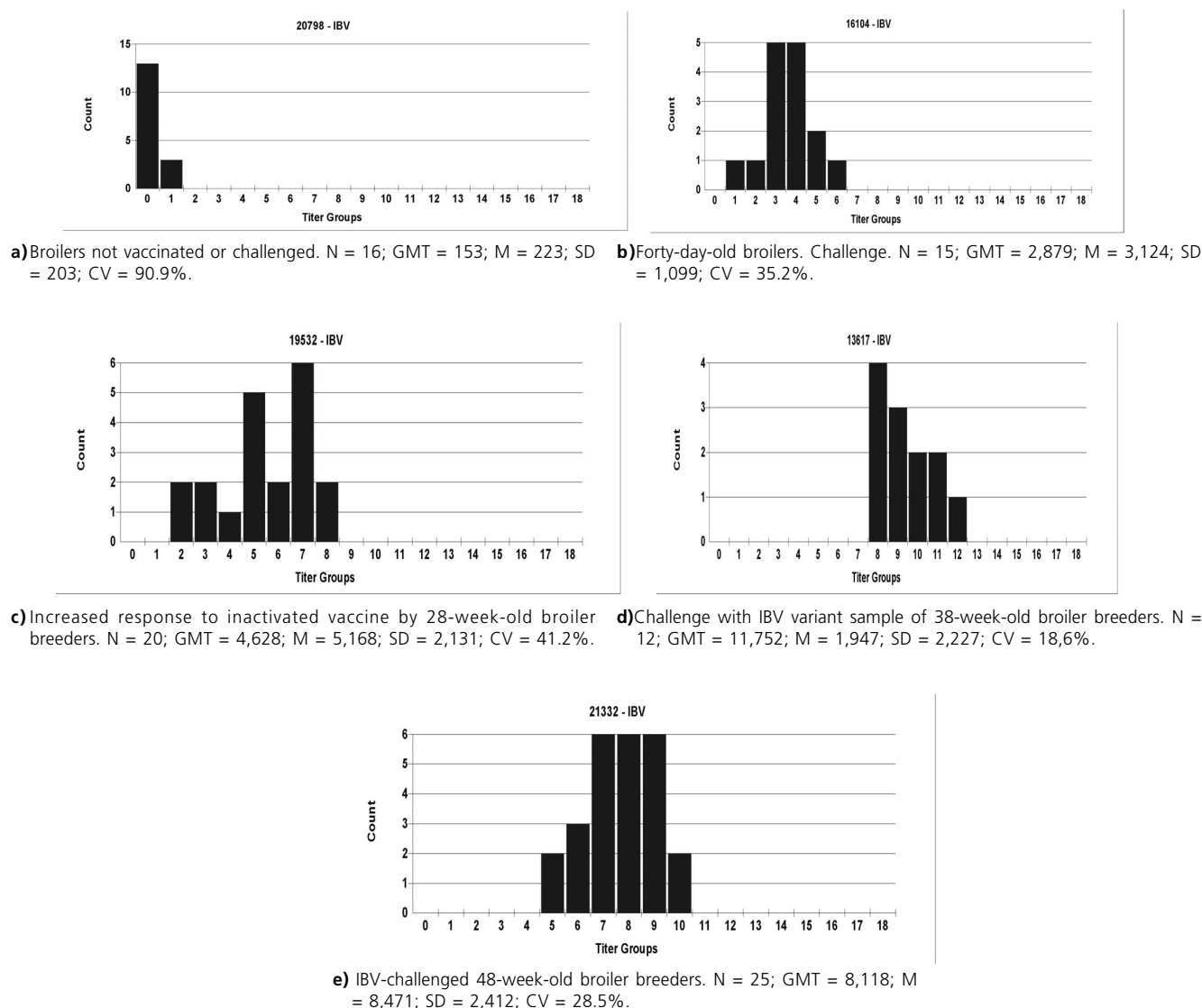


Figure 3 (a-e) - Results of IB serological monitoring using ELISA (Idexx). Source: Back, 2009). N = number of samples; GMT = geometric mean titer; M = mean titer; SD = standard deviation; CV = coefficient of variation.

that had been vaccinated against H120 at 1, 3, 9, and 14 weeks of age via coarse spray and drinking water, injected with inactivated vaccine at 20 weeks, and revaccinated every six weeks against H120 via drinking water, presented clinical IB after 30 weeks of age, with 3% egg production drop, and thin, white, and deformed eggshells, which resulted in 15% less settable eggs. In the lab, a GMT of 4,500 was detected using ELISA (Idexx), but the virus failed to be isolated.

FINAL REMARKS

The opinions and perceptions of field service people

are not unanimous. Back (2009) believes that the problems are being overestimated. He thinks respiratory disease currently observed in broilers are not beyond normal levels, as demonstrated by the usual levels of carcass condemnations due to airsacculitis (as an indication) in broiler processing plants. Seroconversion has been observed, but it is not common. Back admits that there are antigenic variants of IBV in the field, different from Mass, but he argues that this is not necessarily correlated with disease outbreaks. He points out that birds under good vaccination programs and proper serologic response can seroconvert without any clinical symptoms of the disease.



Finally, there is a general agreement that IBV has shown high variability in Brazil in terms of genotype, pathotype, and serotype. However, before developing a new vaccine, research should emphasize IBV phenotypic characteristics using birds as biological model. Every new IB vaccine must be submitted to standard protection test in birds.

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