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Review

Comprehensive Evaluation of Direct Methods for Failure of Passive Transfer Diagnosis in Neonatal Calves

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Abstract: Failure of Passive Transfer (FPT) is a critical concern in neonatal calves, marked by inadequate maternal immunoglobulin transmission due to the synepitheliochorial placenta. This leads to heightened disease prevalence, significantly impacting calf health and imposing substantial economic burdens. The prevalence of FPT varies globally, with studies reporting incidence rates ranging from 13% to 41%, one of the explanations for such high variability being the use of different methods and non-adapted thresholds. Efficient diagnostic methodologies are essential to address this challenge, with ongoing exploration of alternative approaches beyond the established gold standard of radial immunodiffusion (RID). This review critically evaluates the available direct methods for FPT assessment gathering the most recent data on both established methods such as enzyme-linked immunosorbent assay (ELISA) and innovative methodologies such as immunoturbidimetry, split trehalase IgG assay (STIGA), ionization techniques (IT), mass spectrometry (MS), proteomics, and infrared spectroscopy (IS) with attenuated infrared spectroscopy (ATR). Exploring beyond conventional practices is vital for enhancing diagnostic accuracy and addressing the complexities of FPT in calf health management.

Keywords: failure of passive transfer; radial immunodiffusion; calf management; immunoglobulins, total serum protein

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1. Introduction

The synepitheliochorial placenta in cows, with its capacity to separate maternal and fetal blood supplies, effectively inhibits the *in-utero* transmission of protective immunoglobulins [1]. Consequently, calves are born with insufficient immunity, making them highly dependent on colostrum intake [2].

The colostrum incorporates blood serum components [3], such as immunoglobulins, mainly IgG [4]. The secretion is transitory, and the antibody levels gradually decline over time, reaching very low levels 24 hours after birth [5]. Antibodies from the colostrum are absorbed by epithelial cells in the small intestine, particularly in the jejunum and ileum, via pinocytosis [1]. Afterward, immunoglobulins are transferred to the main lymphatic tissue components, entering systemic circulation via the thoracic duct [1]. Unfortunately, the pinocytotic mechanism fully ceases the transfer of large molecules after the first 24 hours [2]. Beyond

this point, additional intake of colostral immunoglobulins can offer local intestinal protection but does not contribute to the calf's overall systemic protection [2].

The neonatal calf's inability to absorb sufficient colostral immunoglobulins during the initial hours of life leads to a failure in passive transfer (FPT) [6]. FPT was associated with high economic losses [6] and increased disease prevalence [7, 8]. Dairy calves experiencing FPT were twice as likely to develop diarrhea and necessitate antibiotic therapy before weaning [9-12]. Various thresholds for serum IgG were contemplated based on the animal category. In the case of the mentioned dairy calves, the designated cut-off value for FPT was established at 10 g/L, with lower levels correlating to elevated rates of mortality and morbidity [2, 13]. The situation appears to be more alarming in beef cattle, with the established threshold set at 24 g/L [14]. Beef calves with serum IgG levels below 24 g/L may be 1.6 times more susceptible to death compared to those with higher IgG values [14]. Other studies report even more concerning findings, considering a mortality rate 2.7 times higher before weaning, under the same circumstances [15]. An alternative approach is represented by the measurement of serum total protein concentrations (STPC), considering a threshold of 52 g/dL as equivalent to IgG levels inferior to 10 g/L [16]. These results motivated a series of researchers to orient their work to different treatment and prophylactic alternatives, such as the use of hyperimmune plasma [17], along with non-enriched blood products [18].

The prevalence of FPT was intensively studied in the last decade different prevalence rates being recorded, ranging from 13% [19] to 33% [20] and even 46.5% [21].

However, these results may be method-dependent, and several direct or indirect techniques for FPT assessment being scrutinized such as: Brix refractometry (BR), gamma-glutamyl transferase activity, zinc sulfate turbidity test, enzyme-linked immunosorbent assay (ELISA), radial immunodiffusion (RID), electrophoresis, capillary electrophoresis (CE), turbidimetric immunoassays, Split trehalase IgG assay (STIGA), Ionization techniques (IT) and mass spectrometry (MS), Infrared spectroscopy (IS) with Transmitted & Attenuated Infrared Spectroscopy (ATR) and proteomics. The suitability of various methods is still a topic of discussion, with some techniques proving more fitting for on-site calf applications but potentially lacking in sensitivity. The aim of this paper is to gather the most recent information on the direct methods available for FPT evaluation, as indirect methods have been recently reviewed [22].

2. Direct methods for FPT evaluation

2.1 Radial immunodiffusion (**RID) – golden standard for FPT assessment**

Currently, the reference test for IgG determination is RID but even this method has several drawbacks being impractical for clinical use. This belief is backed up by the relatively high cost, the need for skilled operators, and a specifically equipped laboratory [23]. In addition, this method implies working on low-volume assays [23], the reagents and required antibodies having limited durability [24]. Results are usually obtained in 18 or 24 hours after admitting the samples to work, the measurements applied to the precipitation zones being time-consuming [25, 26]. In addition, if the coefficient of variation between the samples exceeds 10%, carrying out the test at least twice may be recommended [27, 28].

However, the method described by Mancini et al. (1965) with its variations has been extensively applied for different purposes in immunoglobulin quantitative studies [29, 30].

In contemporary literature, the use of RID is acknowledged as a valuable approach for assessing IgG levels in bovines. Findings indicate that, in certain instances, results obtained through RID may diverge from those obtained through alternative methods such as ELISA or electrophoresis [29] [31-33]. Gathering four different analytical methods of assessing FPT (ELISA, electrophoresis, and refractometry), a German research group used RID as gold standard, with results from the other procedures being referred to the latter [32]. Even if the same authors considered that the results from the other techniques are highly correlated to the standard, they stated that a true quantitative comparison between RID and ELISA or electrophoresis is inadequate. Moreover, the demand to adapt the thresholds for every assay depending on the sample type, serum or plasma, has been pinpointed.

Beyond several studies comparing other methods to the RID IgG results in order to assess the applicability of different techniques [31-34], there is also data available regarding the comparison between commercial RID kits [35]. Thus, it has been ratified that even different available kits may be subject to variability. Accordingly, it was considered that a three internal standard kit formulation may reveal with higher precision IgG levels compared to a single RID kit [35].

2.2. Immunoturbidimetry

Turbidimetric immunoassays have been recently developed and proposed for different applications such as acute phase protein determination in swine [36] and IgG serum evaluation in bovines [37]. Published research has indicated the potential for rapid analysis, with absorbance readings through spectrophotometry yielding results within 10 minutes [37]. This became achievable after adapting the original method [38] to the use of a portable laboratory instrument for in-farm applications [37]. The suitability of the method was backed up by analytical survey. Based on regression analysis, the automated immunoassay results closely paralleled those obtained by RID, with a correlation coefficient of 0.988,and a coefficient of determination value between immunoturbidimetry and RID of 0.98 [37].

Point-of-care assays based on turbidimetry have proven good sensitivity and specificity [39]. Based on these premises other authors compared bovine specific turbidimetric assays to several techniques for FPT evaluation [40]. However, the turbidimetric immunoassays were deemed to be only fairly correlated to RID, and a large bias was determined between the two [40].

Further studies have analyzed the use of turbidimetry-based calf-side tests [41, 42]. The high efficiency of the above-mentioned for FPT diagnosis in calves is considered relatively sensitive and suitable for initial screening [42]. Moreover, to reduce processing time requirements, further validation of whole blood tests, through semiquantitative IgG determinations were proposed [41]. Unfortunately, the method did not prove to be sufficiently accurate for immune status assessments, exhibiting a 77% sensitivity and 44% specificity compared to RID [41].

2.3. Split trehalase IgG assay (STIGA)

A novel detection platform based on a glycolytic enzyme, trehalase respectively, was recently proposed [43].

Further research has proven the possibilities of measuring IgG levels in more than one species, through the use of three bacterial immunoglobulin binding proteins (protein G, protein A, and

protein L) [44]. Thus, the use of this method has been ratified in a large number of species, with activation being detected in almost all tested situations, except for birds [44].

In bovines, the results are spectacular, at an absorbance optical density of 0.2 sensitivity rate reaching 77.8% and specificity 98.2% [27]. Moreover, a blinded trial has been carried out, with high correlation values being observed when compared to RID for both colostrum and serum [27]. In this manner, the authors managed to obtain 100% sensitivity and 94.7% specificity, for FPT diagnosis when using serum samples [27].

The advantages of the method have been further depicted, highlighting its high efficiency in the direct detection of IgG levels through a single-step analysis [27]. In addition, STIGA provides swift results, requiring only 90 minutes as opposed to longer time intervals needed for RID (up to 48 h) [27, 45]. From an economic perspective, STIGA is less expensive due to the lower production costs of the reagents compared to RID, less laborious, and suitable for automatization [27]. The latter statement was backed up by the user-friendly format that could be optimized for field testing, STIGA$^{FIELD\ ®}$, which rendered the same high correlation between its results and the ones obtained by RID [27].

2.4. Enzyme-linked immunosorbent assay (ELISA)

ELISA is broadly used for different means regarding veterinary practice. Among its diverse applications, this technique has been utilized extensively to determine IgG levels in colostrum, plasma, and sera [31, 33] [46-49].

Additionally, as a direct, laboratory-based method [49], ELISA proved to be a good alternative to RID in terms of costs [33]. Comparative studies have shown that the level of agreement between the two stated methods is considered to be high (94%), this assay being suitable for both screening and diagnosis of FPT. Another study gathering 206 Holstein-Friesian calves, compared ELISA to other methods while using different sample types. The Pearson correlation coefficients between the latter and RID remained at a high level (0.9) when serum was analyzed [32]. Also, a high correlation has been established between CE and ELISA (0.89), with the same authors proposing method-dependent cut-off values (5.4 mg IgG/mL for ELISA and 6.9 mg IgG/mL for CE) [32]. In contrast to these results, other authors were able to display only a relatively weak correlation between ELISA and RID ($r= 0.59$, $P < 0.01$), when using plasma samples [33], their results being closer to the ones obtained through immunoturbidimetry, also consistently lower than the RID values [50]. However, not only one statement rendering the comparison between such methods (RID vs ELISA) as being inadvisable, has been formulated [31, 33]. The main reason for the hindmost statement refers to the poor agreement between the assays [31].

A rapid test based on a competitive immunoassay was also compared to ELISA using 277 samples obtained from calves housed in 16 farms [51]. The quick method and ELISA were highly significantly correlated but the strength of the correlation was only moderate ($r_s = –0.36$) [51]. Moreover, the fast diagnosis kit revealed questionable sensitivity and specificity rates, reaching 61.1% and 58.7%, respectively [51].

Even though ELISA is a time-consuming method, it is still broadly used when laboratory infrastructure is available. However, current data analysis does not consider it to have high potential for point-of-care or calf-side applications.

2.5. Ionization techniques (IT) and mass spectrometry (MS)

IT such as solid or liquid matrix-assisted laser desorption/ionization (MALDI) are considered core techniques for molecular analysis through MS [52].

Solid MALDI MS profiling has been applied in many areas [53]. Thus, these attempts were carried out with variable success rates because of many reasons, including the limited analytical power [53]. Generally, liquid MALDI has additional advantages compared to the solid alternative such as greater sample homogeneity, greater ion beam stability, and lower sample consumption [54]. This kind of advanced diagnosis has been used for the detection of dairy cow mastitis, proving 98.5% accuracy and 100% specificity [53].

Liquid MALDI MS is relatively affordable and highly efficient in terms of operating times as it has been previously shown [55] while dealing with sheep and goat milk protein and lipid profiling. Therefore, the latter study has highlighted a new potential alternative for colostrum discrimination via IgG detection. MALDI MS was compared to the presently employed methods, ELISA and BR, and was deemed globally superior [55].

Even if current literature does not state the use of these techniques for FPT evaluation in calves, it has been previously hypothesized that these advanced methods would be at a higher value in comparison with current alternatives [23]. Thus, research activity concerning these applications should be considered.

2.6. Proteomics

Fluid and tissue sample characterization through proteomic studies have been recently discussed, along with their implications in veterinary practice [56-59].

Numerous proteins have been individualized through proteomics, during colostrum or milk analysis. One group identified 212 compounds, 208 being also quantified [59]. The same study highlighted the link between the decrease in protease inhibitors and the subsequent drop in immunoglobulins, suggesting that certain enzymes may have the ability to protect IgG from proteolytic degradation [59]. Recently, IgG identification was reported in a proteomic study regarding colostrum compounds time-related variability [60]. Thus, using a peptide-centric LC–MS/MS, 212 individual proteic sequences have been isolated, >50 % of the colostrum protein content being represented by IgG [60].

Due to still absent information regarding serum compounds analysis in calves for FPT diagnosis, proteomics is still not established for this purpose. However, the results previously reported, particularly those concerning milk or colostrum examination, provide a foundation for a potential new tool that warrants further investigation.

2.7. Infrared spectroscopy (IS) and transmitted & attenuated infrared spectroscopy (ATR)

By courtesy of modern analytical chemistry, another tool has been proposed for different qualitative and quantitative measurements. IS has proved various applications being able to carry out multianalyte analysis [61, 62]. For IgG levels assessment IS has been trialed in humans [63], equines [64], and bovines [65, 66].

One group tested ATR and IR for the measurement of bovine serum IgG concentration, thus FPT diagnosis [67]. The authors have stated that both methods are suitable for the proposed

applications, based on the sensitivity, specificity, and predictive values obtained. Moreover, the use of different sample types was appraised, with optimal cut-off values leading to high levels of agreement (88.1%) between results derived from testing serum and plasma by transmitted IR spectroscopy [21].

Transferability into field conditions was also scrutinized, and a portable ATR-IR spectrometer rendered suitable for use in alpacas [68]. Furthermore, the other rapid methods described [42] in correlation with the previously sketched portable ATR spectrometers [61, 62] have suggested their potential for calf-side applications.

2.8. Electrophoresis and capillary electrophoresis (CE)

Electrophoretic methods can be used to measure immunoglobulin concentrations in serum thanks to the final fraction migration [69]. The latter statement refers to IgG which is the least negatively charged molecule that will determine a slow migration through the electric field [23]. Therefore, it is considered that IgG electrophoretic analysis can be carried out with higher accuracy (89%) [29]. Moreover, a study regarding serum protein profiling has highlighted the utility of electrophoretic findings in general practice situations such as neonatal calf diarrhea [70]. Nevertheless, the studies on ruminants have identified variables that could have affected the outcomes, including the animal's age and physiological condition as well as sample pre-analytical preparations in the lab [71-73].

For FPT assessment in calves, electrophoretic techniques have been applied and current data suggests adequate accuracy when compared to the gold standard (RID) [74].

Electrophoresis was successfully applied for IgG level determination in serum samples after colostrum supplementation in calves [75]. More data is available in equines, and a good agreement between RID and electrophoresis when determining IgG levels in foals being highlighted [76, 77].

Thus, laboratory-based, electrophoresis is an interesting alternative to RID being a faster method, which still relies on specific cut-off values [32].

CE is a superior technique that has been used in human medicine for serum protein recognition [78, 79]. Additionally, CE is more suitable for monoclonal components detection clinical implications of these features being already depicted in related scientific works [79].

Fully automated [79] and cost-efficient [80], CE has been also used for veterinary research purposes regarding FPT. The method was applied in lambs, being considered reliable for serum IgG determination, due to its accordance with the gold standard RID [81]. CE was applied to samples from 216 clinically healthy Holstein Friesian calves, the results proving the high reliability of the method for FPT diagnosis when a cut-off value of 6.9 mg IgG/mL is considered [32].

3. Conclusions

The heightened awareness of a significantly high prevalence of FPT in neonatal calves accentuates the imperative for reliable diagnostic methodologies. This underscores the need for accurate FPT assessment, given the economic implications and increased disease susceptibility that further emphasize the urgency of precise diagnostic tools. While RID serves as the established gold standard, persistent practical limitations necessitate the exploration of alternative approaches. Emerging technologies like immunoturbidimetry, and innovative methodologies such as the STIGA, MS,

and proteomics show promise but require thorough validation for the establishment of practical, efficient modalities to ensure optimal calf health and productivity.

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