HERD HEALTH AND PRODUCTION MEDICINE

Comparative Evaluation of Three Tests for Failure of Passive Transfer and Association with BRD and NCD Pathogen-Specific Antibodies in Neonatal Calves

Egon Thesing[1]; Bart Sustronck[2]; Geert Vertenten[3]

INTRODUCTION

- Testing Failure of Passive Transfer (FPT) involves assessing immunoglobulins (IgG) concentration in the calf's blood serum.
- A serum IgG concentration of less than 10 mg/mL indicates FPT.
- The most common diagnostic method is measuring BRIX% using a refractometer, which indirectly estimates IgG levels.
- For a definitive diagnosis, directly measuring IgG concentration using quantitative enzyme-linked immunosorbent assay (ELISA) or radial immunodiffusion (RID) test is ideal.

OBJECTIVE

To assess the correlation between three different tests used to evaluate FPT and the serological status of specific antibodies against various pathogens in neonatal calves.

MATERIALS AND METHODS

- This field study was performed on five different German dairy farms that are not vaccinating against Bovine Respiratory Disease (BRD) and Neonatal Calf Diarrhea (NCD) and with at least 100 lactating cows.
- Neonatal calves (n=106) aged 2-7 days were enrolled **(Fig.1).** All calves had received at least 2L of colostrum in the first 24 h of life.
- The IgG concentration in the serum was evaluated with three different methods: see **Fig. 1.**
- Furthermore, antibody levels were determined (Center for Diagnostic solutions, Boxmeer) against different pathogens as shown on **Fig. 2.**
- The 3 diagnostic methods were compared using a Bayesian latent class analysis to evaluate the FPT of the 3 tests. Mixed ordinal logistic regressions were used to evaluate the association between the IgG serum concentration and the pathogen specific antibodies in serum (p= 0.05).

IgG ELISA-Munich and IgG ELISA-BioX methods have comparable performance in detecting FPT, while the Brix method showed lower accuracy.

There is a strong association between passive immunity transfer and pathogen-specific antibodies which highlights the importance of adequate passive transfer for calf health and immunity.

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FIGURE 1. Study protocol including 3 compared diagnostic tests

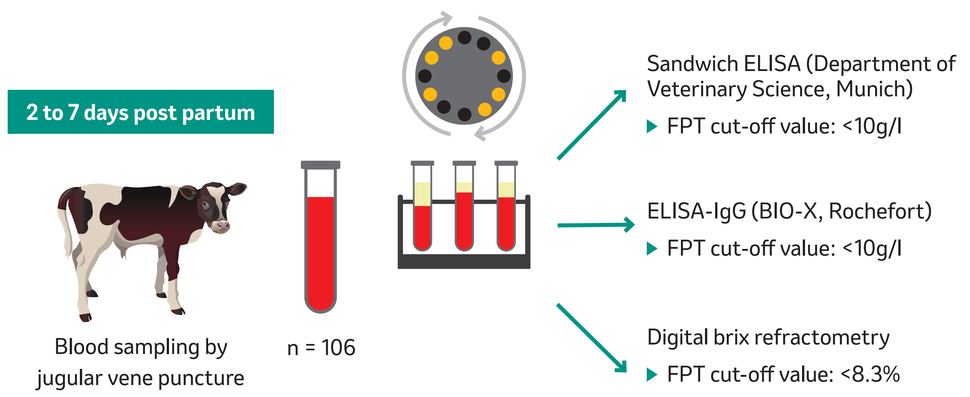
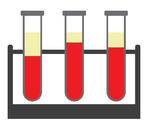


FIGURE 2. Determination of antibody levels against BRD and NCD pathogens



Parameter	Unit
ELISA-BRSV (Boxmeer)	Titer (2Log)
ELISA-PI3 (IDEXX)	Semiquantitative result (0,+,++,+++)
ELISA-*Mycoplasma bovis* (Bio-X)	Semiquantitative result (0, +)
ELISA-*Mannheimia haemolytica* (Boxmeer)	Titer (2Log)
ELISA-*Pasteurella multocida* (Boxmeer)	Titer (2Log)
ELISA-Bovine Coronavirus (BIO-X)	Value (% Inhibition)
ELISA-Bovine Rotavirus (BIO-X)	Value (% Inhibition)

RESULTS

The three tests have comparable sensitivity (±75%) **(Fig.3).**

The specificity of the two ELISA's is high (96%), however the specificity of the Brix method is lower (67%) resulting in a low positive predictive value **(Fig. 3).**

For the practitioner, the negative predictive value (NPV) of a FPT test is the most important. This one is shown in the last column of **(Fig. 3).**

The overall accuracy of the two ELISA tests is comparable (92%). In this study the overall accuracy of the Brix method is considerably lower (69%) **(Fig. 4).**

There is a strong association between passive immunity transfer and pathogen-specific antibodies **(Fig. 5).**

FIGURE 3. Comparison of the three tests

	Se Median (95%CI[1])	Sp Median (95%CI)	PPV Median (95%CI)	NPV Median (95%CI)
Sandwich-ELISA[2]	0.76 (0.50-0.96)	0.96 (0.89-0.99)	0.83 (0.54-0.99)	0.94 (0.83-0.99)
ELISA-IgG	0.76 (0.49-0.97)	0.97 (0.90-0.99)	0.88 (0.59-0.99)	0.94 (0.83-0.99)
Brix Refractometry[3]	0.78 (0.56-0.93)	0.67 (0.56-0.78)	0.38 (0.21-0.58)	0.90 (0.82-0.97)

FIGURE 4. Test characteristics of the three tests

	Accuracy Median (95%CI[1])
Sandwich-ELISA[2]	0.91 (0.83-0.97)
ELISA-IgG	0.91 (0.83-0.97)
Brix Refractometry[3]	0.69 (0.59-0.79)

Se: sensitivity; **Sp:** specificity; **PPV:** positive predictive value; **NPV:** negative predictive value;[1]**CI:** Credibility interval [2]**Sandwich ELISA:** cut-off value 10g/L serum immunoglobulins [3]**Brix refractometry:** cut-off value used 8.3%.

FIGURE 5. Association between the IgG serum concentration and the pathogen specific antibodies

Mixed ordinal logistic regression outcome with different IgG tests as explanatory variables

	ELISA-BRSV (in-house) OR	p-value	ELISA-PI3 (IDEXX) OR	p-value	ELISA-MB (Bio-X) OR	p-value	ELISA-MANH (in-house) OR	p-value	ELISA-PM (in-house) OR	p-value	ELISA-BCV (BIO-X) OR	p-value	ELISA-BRV (BIO-X) OR	p-value
Brix	**2,97**	<0,001	**1,52**	0,080*	**3,17**	<0,001	**3,22**	<0,001	**2,37**	<0,001	**3,76**	<0,001	**3,04**	<0,001
IgG M	**1,14**	<0,001	**1,05**	<0,001	**1,15**	<0,001	**1,14**	<0,001	**		**1,18**	<0,001	**1,15**	<0,001
IgG B	**1,25**	<0,001	**1,10**	0,080*	**1,20**	0,006	**1,31**	<0,001	**1,11**	<0,001	**1,19**	<0,001	**1,20**	<0,001

Brix 1 unit = 1 %
ELISA 1 unit = 1 g/l IgG
→ Significant association
*except the yellow marked cells
**for *Pasteurella multocida* the model with IgG- Munich did not converge

AUTHORS' AFFILIATION

1. Intervet Deutschland GmbH
2. MSD Animal Health, Brussels.
3. MSD Animal Health, Boxmeer.

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MSD Animal Health