

RELATIONSHIP BETWEEN LOAD OF MAREK'S DISEASE VACCINE DNA IN PERIPHERAL BLOOD AND LEVEL OF PROTECTION

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Introduction

- Marek's disease (MD) has been successfully controlled by vaccination since 1968 but sporadic outbreaks still are of concern for the poultry industry
- There is not an appropriate methods to monitor MD vaccine efficacy
 - Level vaccine viremia studies (Thorton et al 1975, Cho et al 1976)
 - Load of vaccine DNA in feather pulp (Baigent et al 2005)
 - Load of challenge virus DNA in blood (Gimeno and Silva 2006)

Objectives

- To determine if the load of DNA vaccine virus in blood (whole blood and buffy coats) measured by real time PCR is correlated with the level of protection
- To compare load of DNA vaccine virus and load of DNA challenge virus in blood as criteria to predict development of tumors

Experimental Design

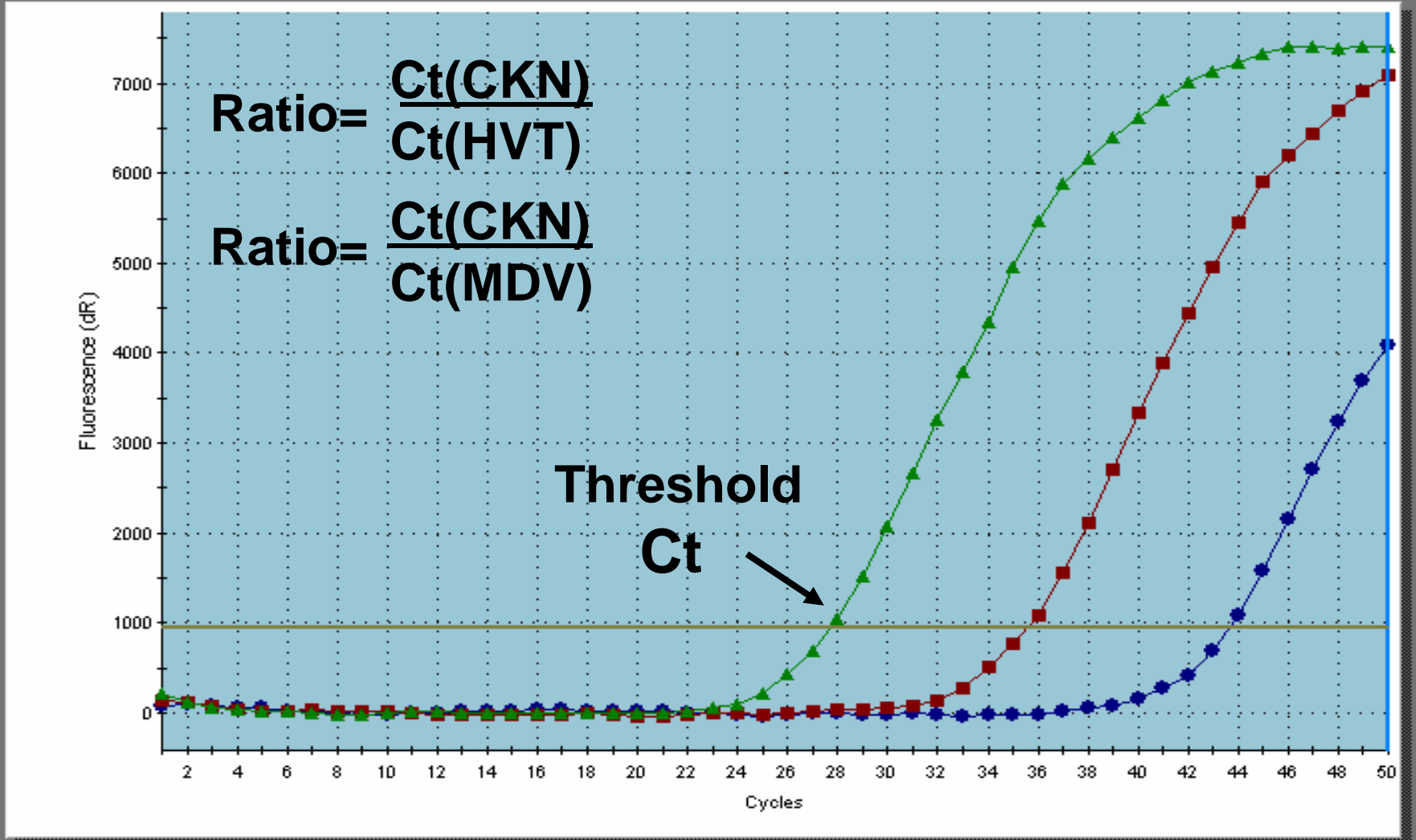
15x7Ab+		Vaccine			Challenge			Expected %tumors
Lot	#ck	Strain	Doses PFU	Age	Strain	Doses PFU	Age	
1	17	HVT	2000	1d	GA v	500	6d	< 5%
2	17	HVT	2000	1d	Md5vv	500	6d	> 50%
3	17	HVT	2000	1d	648Avv+	500	6d	> 90%
4	17	HVT+SB1	1000+1000	1d	Md5vv	500	6d	< 25%
5	17	HVT+SB1	1000+1000	1d	648Avv+	500	6d	> 50%
6	17	_____			_____			0%

Materials and Methods

- **Sampling:** blood 3, 5, 15 wpc (whole blood, buffy coats)
- **Evaluation of tumors development**
- **Real time PCR:**
 - SYBR® green
- **Primers:**
 - MDV serotype 1 (gB)
 - HVT gene (spanning internal repeat regions)
 - Chicken genome (GAPDH)
- **Statistical analysis:** T student, $p < 0.05$

Real time PCR

Amplification Plots



Materials and Methods

- **At individual level** (regardless of the treatment group)
 - Lesions
 - No lesions
- **At group level**
 - Protected (HVT/GA, HVT+SB1/Md5)
 - Non protected (HVT/Md5, HVT/648A, HVT+SB1/648A)

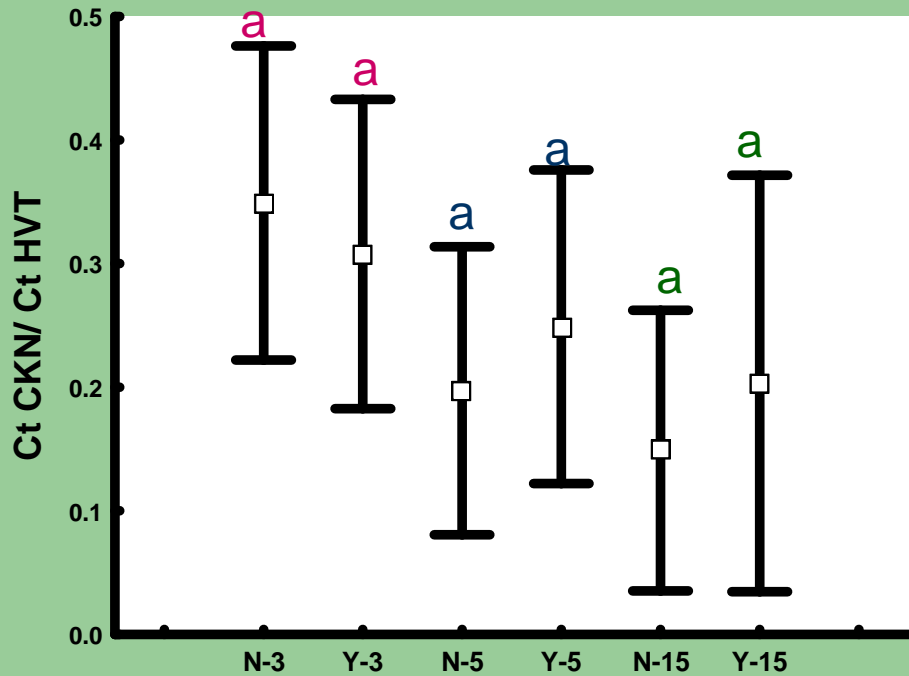
Results

Percentage of tumors per group

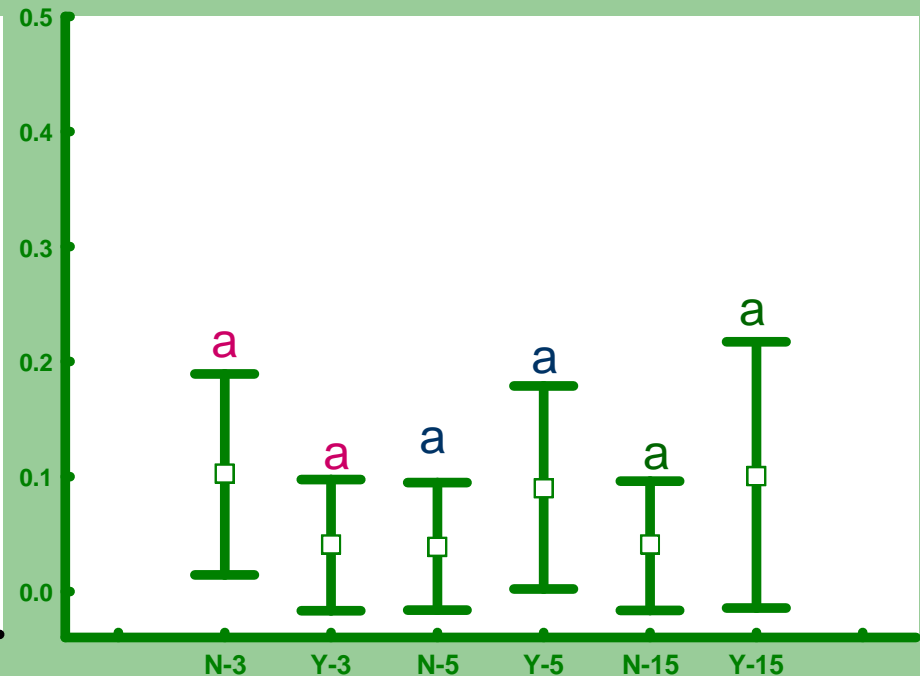
Lot	Level of Protection	Vaccine	Challenge	Expected %tumors	Real %Tumors
1	Protected	HVT	GA v	< 5%	5.8%
2	Non protected	HVT	Md5vv	> 50%	76.5%
3	Non protected	HVT	648Avv+	> 90%	86.7%
4	Protected	HVT+SB1	Md5vv	< 25%	18.7%
5	Non protected	HVT+SB1	648Avv+	> 50%	53.3%
6	NA	Control	—	0%	0%

Lesions vs. non lesions (HVT load)

□ Mean I ± 0.95 Conf. Interval

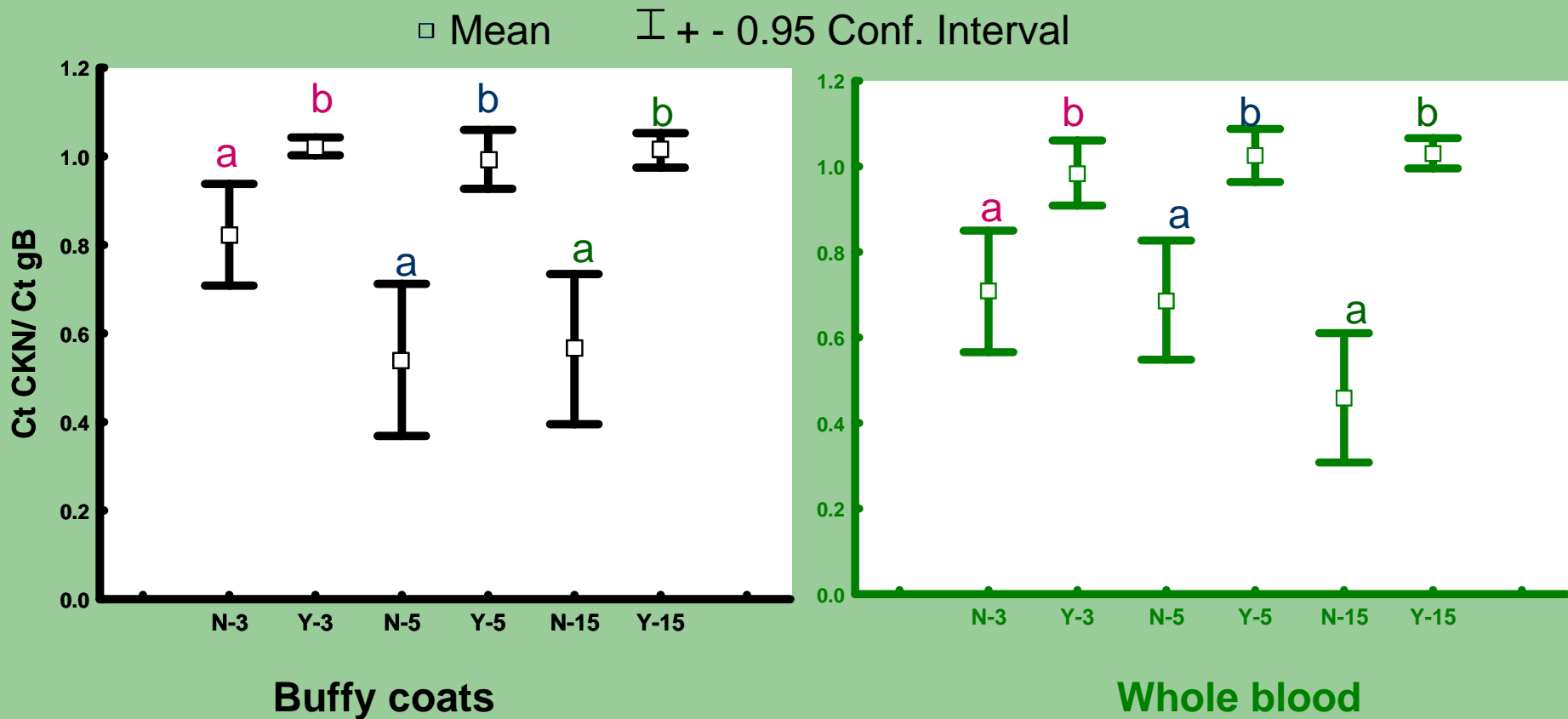


Buffy coats

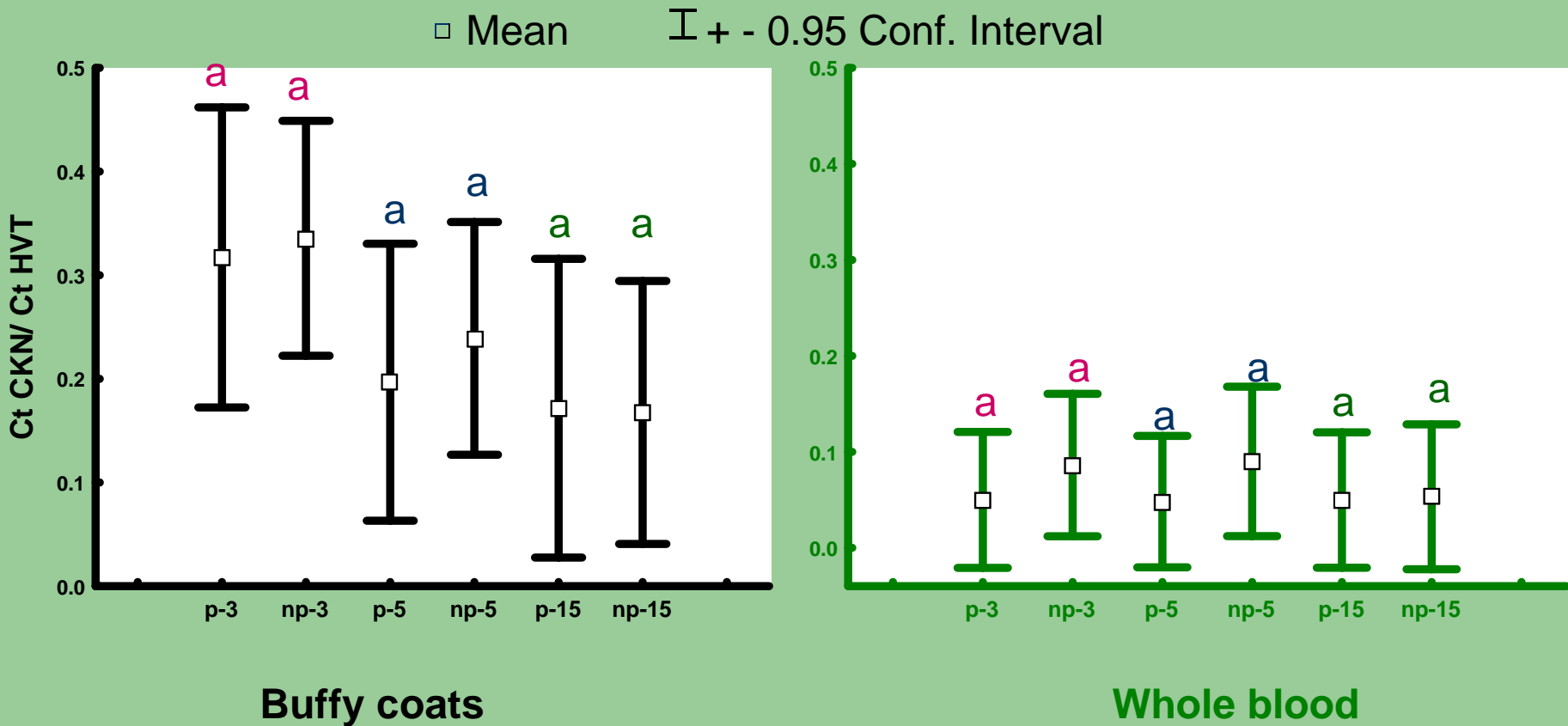


Whole blood

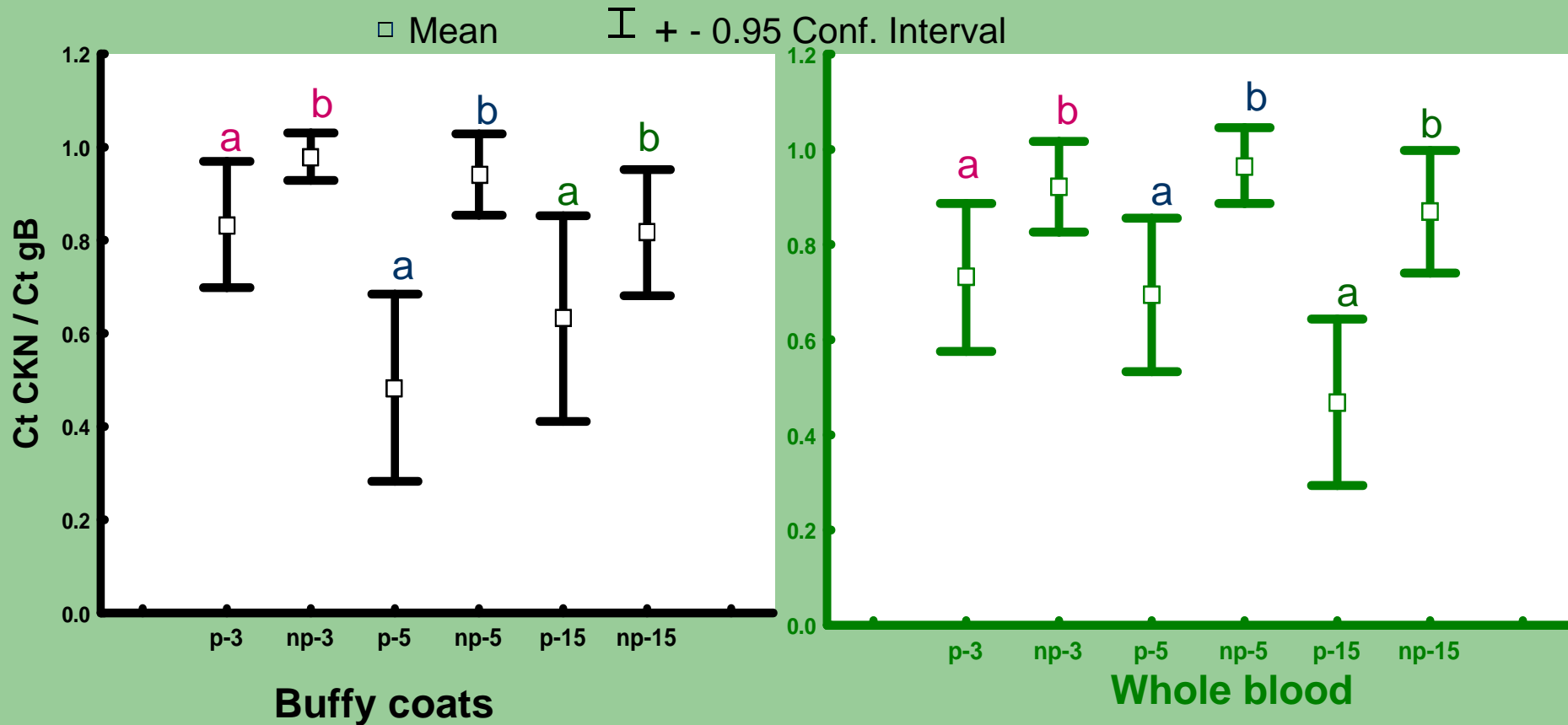
Lesions vs. non lesions (MDV load)



Protected vs. non protected (HVT load)



Protected vs. non protected (MDV load)



Discussion

- In the challenged model used in this work, protection against MD was not correlated with load of DNA vaccine virus but did correlate with load of challenge MDV DNA in blood
- Measuring the load of DNA vaccine virus from buffy coats is more sensitive than using whole blood
- Load of challenge MDV can be evaluated in both buffy coat and whole blood

Further studies

Conclusion: Load of challenge MDV DNA in blood can be used to monitor protection against MD in laboratory conditions

- Could this criterion be useful if using feather pulp as sample?
- Is this technique going to work under field conditions?

Thanks!!!

