Veterinary and Comparative Clinical Pathology

Announcement & Third Call for Delegates, Presenters, Exhibitors, & Sponsors for the

13th Conference of the ESVCP / ECVCP

Jointly with the 9th Conference of the AECCP

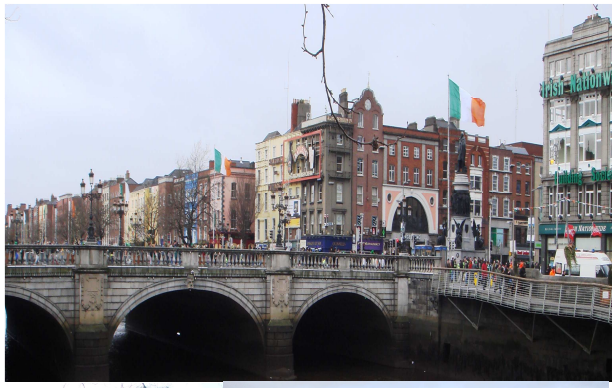
and the 12th Conference of the ACCP

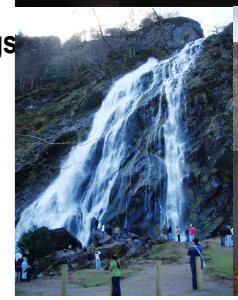
In Collaboration with the ASVCP

(ie Eu Soc & College of Vet Clin Path; Assoc of Eu Comparative Clin Pathologists, Amer Soc of Vet Clin Pathologists, Assoc for Comparative Clin Path)

Aug 31st to Sept 3rd 2011 at Trinity College, Dublin City Centre, Ireland

- **Lectures and free oral and poster communication streams**, including Keynote Speakers, in
  - a) Clinical Biochemistry – potent biomarkers, optimization of use, endocrinology
  - b) General Clinical Pathology - pathophysiologic basis of clinical pathology change
  - c) Haematology – infectious diseases of blood, novel parameters of modern haematology analysers, cytochemical and immunocytochemical diagnostics, bone marrow
  - d) Cytology – evidence-based approach, urinary sediment, lymphoma
  - e) Laboratory Management – quality assurance
- **Toxicologic Clinical Pathology stream**
- **Clinical Pathology Technology Exhibition**
- **Remarkable Cases session**
- **Resident Training - Coaching Session**
- **Breakfast round-table sessions with the experts**
- **Irish-interest sessions:**
  "Clinical Pathology of Canine / Equine Athletes"
  "High Content Imaging"
- **Concurrent ESVCP, ECVCP, AECCP, ACCP annual meetings**
- **Irish social half-day and Irish Banquet**





Registration: a) 100€ for ECVCP / ASVCP Resident, or ESVCP Student or or internal invited speaker (with accepted abstract for poster / oral presentation submitted before Augst); **b)** 200€ for ESVCP members or Veterinary Ireland members registered and paid up before Aug 1st; **c)** 300€ for late registrants or non-members; **d)** 150 € for one day attendance

Optional costs: a) Breakfast Round-table workshops with the experts (includes full Irish Breakfast) - 50€ each (25€ for group a) above); b) Conference banquet - 50 €; c) Social half-day - Trinity, Dublin & surrounds tours - 50 €; d) 4-day wireless access - 20 €

Exhibition booth / table: 500 € (for 2 days, Note: all attendees must register for conference)

Sponsors: Silver sponsorship - 500 €; Gold - 1000 €; Platinum -2000 €

Accomodation on the historic campus - per diem rate, includes continental breakfast and taxes: 1) single room at Goldsmith Hall adjacent to campus - 54.50 €; 2) on-campus, ensuite, single room or newly-renovated, apartment, single room - 66.50 €; c) on-campus, ensuite, twin room - 90.00 €. For further information and to make reservations, see www.tcd.ie/accommodation/visitors. **Note: You must use the promotion code: ESVCP to avail of the above discounted rates.**

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Invited Speakers and Chairpersons

External Invited Speakers
(registration, travel and accommodations paid courtesy of the sponsoring society or company)

Kirstin Beckhardt (USA, ASVCP)
JP Braun (France, ISACP)
Guillermo Couto (USA, ESVCP)
Anthony Davies (Ireland, ADL)
David Eckersall (Scotland, AECCP)
Francois Ballet (France, AECCP)
Arno Lindner (Germany, ESVCP)
Paula Lyons (Ireland, UCD)
Sam Maher (Ireland, ADL)
Houria Mechiche (UK, AECCP)
Rose Rasken (USA, ESVCP)
Allan Rositer (Ireland, ADL)
Alaa Saad (Sweden, AEECP)
Leslie Sharkey (USA, ESVCP)
Sue Shaw (UK, ESVCP)
Harold Tveden (Sweden, ACCP)
Bill Vernau (USA, ESVCP)
Bo Wiinberg (Denmark, ESVCP)

Internal Invited Speakers
(registration and roundtables discounted to rate of residents)

Joy Archer (UK)
Natalie Bauer (Germany)
Anne Beamonte (France)
Jean-Sylvain Beaufils (France)
Barbara von Beust (Germany)
Mary Christopher (USA)
Stefano Comazzi (Italy)
Guillaume Counotte (Netherlands)
Maria Elena Gelain (Italy)
Kathy Freeman (UK)
Peter Graham (UK)
Alessia Giordano (Italy)
Abigail Guya (Austria)
Mads Kjelgard (Denmark)
Martina Klinkon-Ogrinec (Slovenia)
David Ledieu (Switzerland)
Ernst Leidinger (Austria)
Reinhard Mischke (Germany)
Peter O'Brien (Ireland)
Saverio Paltrineiri (Italy)
Kam Seehra (UK)
Joze Staric (Slovenia)
Stratos Papakonstantinou (Ireland)
Mark Pinches (UK)
Zoe Polizopoulou (Greece)
Eve Ramery (Belgium)
Ilse Schwendenwein
Ivana Uhríková (Czeck Republic)

Chairpersons

Joy Archer (UK)
Natalie Bauer (Germany)
Barbara von Beust (Germany)
JP Braun (France)
Mary Christopher (USA)
Stefano Comazzi (Italy)
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Leslie Sharkey (USA)
Harold Tvedten (Sweden)
Bill Vernau (USA)
Bo Wiinberg (Denmark)

Free Communications Accepted for Presentation (1st author of paper identified below)

Allegret, Virginie (Canada)
Amato, Concetta (France)
Amininajafi, Fatemeh (Iran)
Athanasiou, Labrini (Greece)
Barbosa, Tatiana (Spain)
Bonfanti, Ugo (Italy)
Castejón-Riber, Cristina (Spain)
Christensen, Michelle (Denmark)
Defontis, Myriam (Germany)
DeNicola, Dennis (USA)
Ferkau, Annika (Germany)
Forlani, Annalisa (Italy)
Formisano, Principia (Scotland)
Fritz, Dennis (France)
Gavazza, Alessandra (Italy)
Ghisleni, Gabriele (Italy)
Giordano, Alessia (Italy)
Giori, Luca (Italy)
Goldman, Fee (Germany)
Granat, Fanny (France)
Haddadzadeh, Hamidreza (Iran)
Hammond J, (USA)
Hillstrom, Anna (Sweden)
Hooijberg, Emma (Austria)
Jaillardon, Laetitia (France)
Jalali, Missagh (Iran)
Jezek, J (Slovenia)
Kampfmann, Iris (Germany)
Khaki, Z (Iran)
Khazraiinia, Parvaneh (Iran)
Koutinos, Christos (Greece)
Laursen, S (Denmark)
Lilliehöök, Inger (Sweden)
Lynch, Ailish (Ireland)
Metzger, J (USA)
Mischke, Reinhard (Germany)
Muñoz, A (Spain)
Papakonstantinou, Stratos (Ireland)
Pinto da Cunha, Nazare (Portugal)
Poggi, Alessia (Italy)
Polizopoulou, Zoe (Greece)
Rattray, Jayne (Netherlands)
Riondato, Fulvio (Italy)
Rodriquez, R (Spain)
Rossi, Gabriele (Italy)
Rutgen, Barbara (Austria)
Satué, Katiuska (Spain)
Schmidt, E (Brasil)
Simčič, M (Slovenia)
Siska, Bill (USA)
Smollnaars, A (Netherlands)
Strage, Emma (Sweden)
Turinelli, Vanessa (Italy)
Tvarijonaviciute, A (Spain)
Tvedten, Harold (Sweden)
Wiinberg, BO (Denmark)
Woods, Anita (Ireland)
Yasini, Parastoo (Iran)

Sponsors

Platinum Sponsors

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Gold Sponsors

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Sysmex Europe - http://www.sysmex-europe.com/

Merck Millipore - **www.merckmillipore.com**

Autoscribe - http://www.autoscribe.co.uk/

Sebia – http://sebia.com

13th ESVCP, 9th AECCP, 12th ACCP Conference Programme

(Note: titles of talks are abbreviated, for full title see abstract)

Wed, Aug 31st			
8:30 - 9:15	Registration		
9:15 – 9:30	Welcome and "House-keeping" Details		
9:30 – 5 pm	**GENERAL CLINPATH**	**EDUCATION & EXAM**	**TOX CLIN PATH 1**
9:30 - 11 am	**1) PATHOPHYSIOLOGY OF CLIN PATH CHANGE** Chairs – **Bo Wiinberg, Mads Kjelgaard-Hansen**	Examiner Training: How do I know what you know? Chair - **Alessia Giordano**	**STATE-OF-THE-ART TECHNOLOGY: 1) Haematology**
9:30 - 10 am	Hemostasis – **Bo Wiinberg** (Denmark)	**(exam training programme under construction)**	The modern automated haematology analyser - **Sysmex** (speaker sponsored by Sysmex)
10 - 10:30 am	Acute phase reaction – **Mads Kjelgaard-Hansen** (Denmark)		The modern automated haematology analyser – **Siemens technology**
10:30 - 11 am	Enzymemia – **JP Braun** (France) (speaker sponsored by ISACP)		**Keynote Lecture**: Bone marrow analysis by flow cytometry & Sysmex XT - **Houria Mechiche** (speaker sponsored by AECCP)
11 - 12:30 pm	**2) POTENT BIOMARKERS IN CLIN BIOCHEM** Chairs – **Zoe Polizopoulou, Mark Pinches**		**2) Electrophoresis** Chairs – **Saverio Paltrinieri, Guillaume Counotte** (session sponsored by Sebia)
11 - 11:30 am	Renal biomarkers – **Mark Pinches** (UK)		Electrophoresis - **Saverio Paltrinieri** (Italy)
11:30 - 12 pm	Cardiac biomarkers – **Zoe Polizopoulou** (Greece)		Analytical evaluation of proteinogram using Capillarys 2 (Sebia) – **Anne Beamonte** (France)
12 - 12:30 pm	Pancreatic biomarkers – **Joy Archer** (UK)		Capillary electrophoresis in multiple species - **Guillaume Counotte** (Netherlands)
12:30 - 2 pm	**Go out for Lunch at the Local Irish Pub (Kennedy's, Lombard, Pavillion)**		
2 - 3:30 pm	**CASE PRESENTATIONS** Chairs: **Harold Tvedten, Zoe Polizopoulou**	How to write exam questions? Chair - **Alessia Giordano**	**3) Molecular Diagnostics** Chairs –
2-2:30 pm			Quantitative PCR of feline infectious disease in France – **Denis Fritz** (France)
			Genetic analysis of haemophilic Havanese dogs – **Reinhard Mischke**
2:30 - 3 pm			Antigen receptor rearrangement PCR in lymphoma diagnosis – **Barbara Rutgen** (Austria)
3 - 3:30 pm			
3:30 – 5:30 pm	**Education of Residents** Chairs - **Barbara von Beust, Mary Christopher**		**4) High Content Imaging** Chairs – **Anthony Davies, Stratos Papakonstantinou** (session / speakers sponsored by ADL)
3:30 - 4 pm	1. ECVCP Summer School 2012 (General ClinPath) **Barbara von Beust** (Switzerland)		**Keynote Lecture**: Overview of High Content Imaging –**Anthony Davies** (Ireland)
4 - 4:30 pm	2. Policies & Procedures of EBVS & ECVCP Brochure - **Barbara von Beust** (Switzerland)		HCI for predictive cytotoxicity - **Sam Maher** (Ireland)
	3. Residency Training Guidelines - **Ilse Schwendenwein** (Austria), **Mary Christopher**		HCI of macrophage response to organic dust - **Eve Ramery** (Belgium)
4:30 - 5 pm	4. Advice to Residents - **Ilse Schwendenwein** (Austria), **Mary Christopher** (USA)		HCI for diagnosis of lymphoma - **Stratos Papakonstantinou** (Ireland)
			HCI for *in vivo* monitoring of anticancer drug toxicity - **Paula Lyons** (Ireland)
5:00 - 5:30 pm	5. Experiences of Former Residents - **Abigail Guija** (Austria), **Maria Elena Gelain** (Italy)		
6:30 - 7 pm	**Relax and enjoy nature photography – TBD** (large lecture hall)		
7 - 8 pm	**Residents' reception** Chair: **Ilse Schwendenwein**	**ECVCP AGM** Chair: **Saverio Paltrinieri**	**AECCP AGM** Chair **Jean-Sylvain Beaufils**
8 - 9 pm			**ACCP AGM**

			Chair **Peter Cotton**
Thurs, Sept 1st			
7:30 – 9 am	**Breakfast Round-tables in the Dining Hall**		
8:30 - 9:30 Registration	**POSTERS AND EXHIBITS 1**		
9:30 - 12:30 pm	**LAB MANAGEMENT** Chair **Kathy Freeman**	**CLIN PATH OF CANINE & EQUINE ATHLETES** Chairs: **Arno Lindner, Guillermo Couto**	**Tox Clin Path 2**
9:30 - 10:30 am	**Keynote Lecture:** The science of quality assurance of vet lab analysis – **Kathy Freeman** (UK)	**Keynote Lecture:** Clin Biochem for safe, effective conditioning of horses - **Arno Lindner** (Germany)	**Keynote Lecture**: Practical tools to explore haematotoxicity in preclinical drug development - **Alaa Saad** (Sweden)
10:30 - 11:30 am	Biological variation: **Mads Kjelgaard-Hansen** (Denmark)	**Keynote Lecture:** Unique clinpath of greyhounds – **Guillermo Couto** (USA)	**Keynote Lecture:** Toxicologic clinical pathology of the non-human primate – **Kirstin Burkhardt** (USA) (speaker sponsored by ASVCP)
	Biological variation of equine haemostatic parameters – **Bo Wiinberg** (Denmark)		
11:30 - 12:00 pm		Broncho-alveolar lavage analysis in underperforming athletes - **Kathy Freeman** (UK)	Platelet function, evaluation & risk assessment in preclinical studies - **David Ledieu** (Switzerland)
12:00 - 12:30 pm	Use of an error management system to identify pre-analytical error – **Emma Hooijberg** (Austria)	Clinpath changes in chronic overtraining of horses – **Concetta Amato** (France)	Evaluation of CRP assays for non-human primates - **Virginie Allegret** (Canada)
	ELISA evaluation for canine IGF-1 – **Emma Strage** (Sweden)	Vitamin E anti-lipoperoxidation during exercising horses - **Tatiana de Sousa Barbosa** (Brasil)	Whole blood aggregation in beagles and rats. **Myriam Defontis** (Germany)
		Clinpath change in equine endurance events - **Cristina Castejón-Riber** (Spain)	Urinary prostate biomarkers - **Kam Seehra** (UK)
12:30 - 2 pm - Lunch	**POSTERS AND EXHIBITS 2**		
1:30 – 1:55 pm	**Relax and enjoy nature photography – Reinhardt Mischke** (large lecture hall)		
2 - 6 pm	**HAEMATOLOGY**: Chairs **Natali Bauer, Rose Raskin** (session sponsored by Sysmex)	**FREE COMMUNICATIONS 1**	**Tox Clin Path 3**
2 – 2:30 pm	**Keynote Lecture:** Diagnostic cytochemistry of haematopoietic neoplasia – **Rose Raskin** (USA)	Hemostasis in the greyhound -- **Guillermo Couto** (USA)	**Keynote Lecture:** Using acute phase proteins in animals - **David Eckersall** (Scotland) (speaker sponsored by AECCP)
2:30 - 3 pm		Leukocyte distribution and gene expression in exercise – **Eve Ramery** (Belgium)	
3 - 3:45 pm	Evaluating reticulocyte response with modern haematology analysers – **Natali Bauer** (Germany)	Monitoring of heparin treatment – **Reinhard Mischke** (Germany)	Kidney injury molecule: **Mark Pinches** (UK)
		Novel parameters of the modern haematology analyser - **TBA**	
		Diagnostic value of conventional reticulocyte parameters – **Gabriele Rossi** (Italy)	
3:45 - 4:15 pm Break	**POSTERS AND EXHIBITS 3**		
4:15 – 4:50	Platelet pathology – **Reinhardt Mischke** (Germany)	Seasonal variations in erythrogram of pregnant mares – **Katy Satue** (Spain)	
4:50 – 5:25	**Keynote Speaker** - Emerging infectious diseases of the blood in EU - **Susan Shaw** (UK)		
5:25 – 6 pm	Haemostasis – TEG – **Bo Wiinberg** (Denmark)		
6 – 8 pm	Dinner break		
8 - 10 pm	**ESVCP AGM** – Chair **Stefano Comazzi**		
8 - 9 pm	**Keynote address:** Vet Clin Path – Where are we & where are we going? **Kirstin Burkhart**, **Stefano Comazzi**, **Saverio Paltrinieri** – Presidents ASVCP, ESVCP,		

	ECVCP		
9 - 10 pm	**ESVCP Business agenda**		
Fri, Sept 2nd			
7:30 - 9:00 am	**Breakfast Round-tables in the Dining Hall**		
8:30 - 9:30 Registration	**POSTERS AND EXHIBITS 4**		
9:30 - 12:30	**CYTOLOGY:** Chairs: **Ernst Leidinger, Leslie Sharkey**	**FREE COMMUNICATIONS 2**	**Tox Clin Path 4**
9:30 - 10:30	**Keynote Lecture:** Diagnostic cytology – an evidence-based approach - **Leslie Sharkey** (USA)		**Keynote Lecture:** Managing drug induced liver injury during drug development: example of translation toxicology. **François Ballet** (France) (speaker sponsored by AECCP)
10:30 - 11 am - Break	**POSTERS AND EXHIBITS 5**		
11 - 11:45 am	**Keynote Lecture**: Cytology of the skin – **Harold Tvedten** (Sweden)	Diagnostic value of cytology of oral cavity tumours – **Ugo Bonganti** (Italy)	
		Novel feline effusion classification scheme – **Alessandra Gavazza** (Italy)	
		Immunocytochemistry in effusion cytology - **N Pinto da Cunha** (Portugal)	
11:45 - 12:30 pm	**Keynote Lecture:** Urinary sediment analysis – an evidence-based approach" – **Ernst Leidinger** (Austria)	Feline conjuctival cytology in chlamidiosis - **Anna Hillstrom** (Sweden)	
12:30 - 2 pm - Lunch	**POSTERS AND EXHIBITS 6**		
2 - 8 pm	**Social Afternoon**		
8 - 10 pm	**Conference Banquet**		
Sat, Sept 3			
9 - 12 pm	**CLIN BIOCHEMISTRY** Chairs: **JP Braun, Joy Archer**	**FREE COMMUNICATIONS 3**	
9 – 10 am	**Keynote Lecture** – Reference intervals for small populations – **JP Braun** (France) (speaker sponsored by ISACP)	CK isoenzymes in septic and asphyctic foals – **Luca Giori** (Italy)	
		Leptin secretion in hypothyroidism – **Laetitia Jaillardon** (France)	
		Automated multispecies SAA assay evaluation – **Michelle Christensen** (Denmark)	
		Evaluation of an automated canine SAA assay – **Michelle Christensen** (Denmark)	
		cTnI correlation with clinical staging in canine mitral valve disease – **Zoe Polizopoulou** (Greece)	
10 – 10:40 am	Early biomarkers for renal disease in dogs and cats – **Joy Archer** (UK)	cTnI in canine monocytic ehrlichiosis – **Christos Koutinas** (Greece)	
		Acute phase proteins in bovine fascioliasis and strongyloidiasis – **Elizabeth Schmidt** (Brasil)	
		Prognostic value of HMGB1 cytokine in gastric dilatation and volvulus - **Ivana Uhrikova** (Czeck Republic)	
		ELISA versus chemistry analyser - IgG measurement - **Martina Klinkon** (Slovenia)	
10:40 – 11:20 am	Lab diagnosis of thyroid and adrenal endocrinopathy – **Peter Graham** (UK)	Pregnancy and age effects on equine clin biochem -**Katy Satue** (Spain)	
		Bovine bone biomarkers – **Joze Staric** (Slovenia)	
		Urinary adiponectin as a renal injury	

		biomarker – **Asta Tvarijonaviciute** (Spain)	
11:20 – 12:00 pm	Optimisation of the use of diagnostic clinical biochemistry – **Leslie Sharkey** (USA)		
12:00 - 1 pm	**Lunch**		
1 - 4 pm	**LYMPHOMA:** Chairs: **Stefano Comazzi, Bill Vernau**	**Open Forum**	
1 - 2 pm	**Keynote Lecture**: Cytology and adjunctive diagnostics of WHO lymphoma types in dogs – **Bill Vernau** (USA)	What does today's vet practitioner really need from the vet clinpath lab? – **Alan Rossiter** (Ireland)	
2 – 2:40 pm	European Network – is dog model valid for human lymphoma? **Stefano Comazzi** (Italy)	What does today's vet clinical pathologist need from the technology vendor? TBD	
2:40 – 3:20 pm	Apoptosis in canine lymphoma and leukemia – **Alessia Poggi** (Italy)		
	Cytomorphometry in diagnosis of canine lymphoma – **Ailish Lynch** (Ireland)		
3:20 – 4:00 pm	Cytology of bone marrow in haematopoietic neoplasia – **Rose Raskin** (USA)		

Abstract Guidelines and Submission Form for ESVCP 2011 Conference

Fill in one submission form for each abstract

NOTE: 1) Absolute deadline for receipt of abstracts for publication in the journal "Veterinary Clinical Pathology": Jun 15th, 2011

2) **ECVCP / ASVCP Resident; and ESVCP students: Deadline** for receipt of abstract for poster / oral presentation (or for case report) for eligibility for two-thirds registration reduction: **June 15th 2011.**

Presenter of abstract:

Email address of presenter:

Indicate whether poster or oral presentation is preferred:	POSTER	ORAL
Confirm that all authors have agreed on the content and submission of this abstract.	YES	NO

ABSTRACT FOR SUBMISSION FOR THE ESVCP 2011 CONFERENCE
A.J. Smith[1], C.D Jones[2], H. Chen2, [1]Department of Clinical Pathology, University of Hastings, Devon, UK and [2]Vet Labs, Paignton, Devon.

Please read carefully and follow these ten points. 1) Abstract submission provides assurance: a) of adherence to rules, and scientific validity of presentation. b) that reported investigations involving animals have been conducted in compliance with guidelines for experimental procedures as set forth in the NIH "Guidelines for the Care and Use of Animals" or equivalent, as applicable. c) That this work will not be presented in any form at a national or international meeting prior to this meeting. 2) Abstracts should be informative, containing: a) **Background:** b) The study's specific **Objectives:** unless given by the title. c) Brief statement of **Methods:** if pertinent. d) Summary of **Results:** obtained. e) Statement of **Conclusions**. Subsections should be bolded and followed by a colon. It is NOT SATISFACTORY to state "results will be discussed." 4) An abstract may be rejected for publication if submission instructions are not followed. If accepted, your author-prepared abstract will be published exactly as it appears here. ESVCP is not responsible for author errors. 5) Fill in all requested information in the abstract submission form. 6) Abstracts will be peer-reviewed. 7) All abstracts must be in plain text, in English with no embedded symbols or formatting characters. Submissions may not include charts, graphs, pictures, tables or references. Please use words to spell out symbols. 8) Abstracts may not exceed 300 words (including title, authors and body). 9) Type abstract title in all capital letters. Include a listing of all authors (use initials for first and middle name and spell out the last name, do not include degrees) and their corresponding departments and affiliation/institution. 10) Indent the first line of each paragraph.

REGISTRATION FORM for ESVCP Conference – Aug 31st to Sept 3rd, 2011

(Trinity College, Dublin, Ireland)

Please complete one form per delegate. (BLOCK CAPITALS please)

NAME: .....

INSTITUTION: .....

ADDRESS .....

.....

EMAIL: .....

PHONE: .....

ACCOMODATIONS: I will arrange my own. I will stay on campus

Please make your own on-line reservation: per diem rate, includes continental breakfast and taxes: 1) single room at Goldsmith Hall adjacent to campus - 54.50 €; 2) on-campus, ensuite, single room or newly-renovated, apartment, single room - 66.50 €; c) on-campus, ensuite, twin room - 90.00 €. For further information and to make reservations, see **www.tcd.ie/accommodation/visitors**. **Note: You must use the promotion code: ESVCP to avail of the above discounted rates.**

Days Attending: Aug 31st Sept 1st Sept 2nd Sept 3rd

REGISTRATION COSTS

Category of Registrant	Registration Deadline	Registration Cost
ECVCP / ASVCP Resident or Student or internal invited speaker (with accepted abstract for poster / oral presentation submitted before July 15th)	Aug 1st	100 €
ESVCP member or Veterinary Ireland member registered	Aug 1st	200 €
Non-members or late registration		300 €
One-day registration	Aug 1st	150 €

OPTIONAL COSTS

Option	If book before Aug 1	Late booking
1st Breakfast round-table workshop with the experts (full Irish Breakfast) Student / resident ESVCP members or invited speaker	50 € 25 €	100 €
2nd Breakfast round-table workshop with the experts (full Irish Breakfast) Student / resident ESVCP members or invited speaker	50 € 25 €	100 €
ESVCP Banquet	50 €	100 €
ESVCP Social afternoon - Trinity, Dublin & surrounds tours	50 €	100 €
Exhibition booth / table	500 €	750 €
4-day wireless access	20 €	30 €

Breakfast Round Tables (These focus on supplementary material to the lectures and allow participants to meet up with and ask questions of the experts)

Check 1st four choices

1) Diagnostic cytochemistry - **Rose Raskin**
2) Lymphoma diagnostics - **Bill Vernau**
3) Clinpath labs quality issues - **Kathy Freeman**
4) Bone marrow analysis - **Rose Raskin**
5) Urine sediment analysis - **Ernst Leidinger**
6) Greyhound clinpath - **Guillermo Couto**
7) Clinpath in conditioning horses - **Arno Lindner**
8) Infectious diseases of the blood - **Sue Shaw**
9) Cytology of the skin – **Harold Tvedten**
10) Diagnostic cytology – **Leslie Sharkey**
11) Thromboelastography - **Bo Wiinberg**
12) Teaching quality management - **Kathy Freeman**
13) Clinpath testing in toxicology - TBA
14) What is possible with the XT-iV? - **Sysmex**
15) Acute phase proteins - **David Eckersall**

Conference Sponsorships: Silver sponsorship - 500 €; Gold - 1000 €; Platinum - 2000 €

Please make payment by **cheque or bank draft** in €, payable to the **ESVCP**.

Or pay by PayPal to treasurer.esvcp@googlemail.com

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Please indicate if you require a receipt **Yes** **No**

Please complete registration form and return to me at the address below, ASAP.

Peter O'Brien, Postal Address: Room 013, Veterinary Sciences Centre, University College Dublin, Dublin 4, Ireland
Email: Peter.James.OBrien@ucd.ie. Telephone number +353 - 1716 6048 Fax number + 353 - 1716 6157

Abstracts of Poster and Oral Presentations for the ESCVP – AECCP – ACCP Conference in Dublin, Ireland

NOTE: 1) Titles in red are oral presentations for publication in VCP; titles in **green are posters for publication in VCP**. **VCP does not publish abstracts of cases nor of invited presentations (blue titles)**. Instead, these will be published on-line on the ESVCP website and also in the proceedings along with all the others.

2) Abstracts received up to Aug 1st will not be published on-line with VCP, but will be collated for submission for publication in the Dec 2011 issue of VCP, as well as in the proceedings.

3) Most but not yet all abstracts have been scheduled, please contact the conference organiser if there are any mistakes in your abstract or its scheduling.

4) Where appropriate abstracts have been edited and reformatted according to the requirements of Veterinary Clinical Pathology

Abstracts are in alphabetical order according to the first author.

EVALUATION OF TWO IMMUNOASSAYS FOR THE MEASUREMENT OF C-REACTIVE PROTEIN CONCENTRATION IN NON-HUMAN PRIMATES. **V. Allegret, L. Huard, S. Lavallée, S. Legris, R. Falvo, L. LeSauteur, J. McCartney, F. Poitout,** Charles River Preclinical Services Montreal, Canada. **Background**: C-reactive protein (CRP) is an acute phase protein present in low concentration in normal sera that increases rapidly with acute inflammation in non-human primates (NHP). **Objectives**: We validated and compared immunoassays for the measurement of CRP in NHP: a quantitative immunoturbidimetric assay using a monoclonal human CRP antibody (Roche Diagnostic®) and a quantitative ELISA assay using monkey specific reagents (Life Diagnostics, Inc®). **Methods:** These assays were validated using healthy *Cynomolgus* monkey serum and compared using serum from two *Cynomolgus* monkeys administered Lipopolysaccharide or anti-CD3 monoclonal antibody. The immunoturbidimetric assay was adapted on an automated chemistry analyzer (Modular *Analytics*, Hitachi®). The Elisa assay was performed manually. **Results:** Limit of detection using the immunoturbidimetric assay was 0.1 mg/L. Measurements followed a linear manner until concentration of 300 mg/L. Intra-assay and inter- assay coefficients of variation were 0.9% and 1.5%, respectively. Reference range was: 1.2 – 10.2 mg/L. Storage at 2-8°C and -20°C for 3 and 28 days respectively, and three freeze-thaw cycles had no effect on CRP concentration. Limits of quantitation using the ELISA assay were 2.34 and 200 ng/mL. Acceptable dilution factors ranged from 12800 to 102400. Intra-assay and inter-assay coefficients of variation were 8.37% and 8.73%, respectively. Storage at room temperature and 4°C for 6 and 24 hours, respectively, and four freeze-thaw cycles had no effect on CRP concentration. **Conclusions:** *Cynomolgus* monkeys administered Lipopolysaccharide or antiCD3 showed similar post-stimulation increases in CRP with both assays, but higher concentrations of CRP were detected post-dose with the ELISA assay (11 to 1368 mg/L) compared to the Immunoturbidimetric assay (2.5 to 399 mg/L).

VARIATIONS OF BIOLOGICAL PARAMETERS IN EXERCISING HORSES DURING A WORK SEASON. **C. Amato, L. Jaillardon, L. Martin, H. Dumon, P. Nguyen, B. Siliart**. ONIRIS, Department of Biology, Pathology and Food Sciences, Nantes, France.
Background: In athletes, chronic fatigue due to workload increase leads to a decrease in performance called "overtraining syndrome". This syndrome is complex and difficult to assess. We aimed to investigate the variations in several routine biological parameters in show horses. **Methods**: Sixteen Iberian horses (11 geldings and 5 stallions, 7 to 16 years, mean body weight (BW)=477±40 kg) were included in the study. All the horses were weighed twice a month and received individually a suitably ration. Blood samples were collected monthly at rest for hematocrit, albumin, creatinine, urea, total proteins (TP), CK, LDH, AST, ALT, ALP, GGT, glucose, cholesterol, HDL-cholesterol, triglycerides, NEFA, lactate, sodium, potassium, magnesium, chloride, calcium and phosphates assays. Workload was determined according to heart rate. **Results**: The workload increased gradually from 3.3 to 7.6 shows/week. During the work season, BW significantly decreased ($p<0.0001$) despite an increase in energy intake adjusted according to workload ($p<0.0001$). All electrolytes and minerals significantly decreased ($p<0.0001$) with concentrations remaining in the reference ranges. Albumin, LDH, AST, ALT, TP, NEFA and triglycerides were not affected by the workload. The monthly workload was positively correlated to glucose ($r=0.45$, $p=0.004$) and NEFA ($r=0.32$, $p=0.042$) and negatively to creatinine ($r=-0.36$, $p=0.024$), urea ($r=-0.33$, $p=0.039$), CK ($r=-0.38$, $p=0.014$) and glucose after exercise ($r=-0.32$, $p=0.047$). The cumulative workload was positively correlated to albumin ($r=0.49$, $p=0.001$), CK ($r=0.44$, $p=0.004$) and negatively to creatinine ($r=-0.44$, $p=0.004$), Na ($r=-0.42$, $p=0.008$), Mg ($r=-0.43$, $p=0.005$) and Cl ($r=-0.47$, $p=0.002$). **Conclusions**: The observed BW loss could be related to fatigue. The observed decrease in electrolytes correlated with cumulative workload and might, therefore be a marker of exercise intolerance.

THE ROLE OF OPIOID SYSTEM AND ITS INTERACTION WITH SYMPATHETIC NERVOUS SYSTEM IN THE PROCESSING OF POLYCYSTIC OVARY SYNDROME MODELING IN RAT. **F. Aminiinajafi[1], F.Z. Zangeneh[2], A. Tavasoly[1], A. Mohammadi.[3], Sh. Ejtemaeimehr[4], M.M. Naghizadeh[5].**[1]Dept of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran Iran; [2]Vali-e-Asr, Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran; [3]Medicine Faculty, Azad University, Tehran, Iran; [4]Medical Faculty, Pharmacology Department, Tehran University of Medical Sciences Iran; [5]Central Biostatistics, Fasa University of Medical Sciences, Fasa, Iran.
Background: Polycystic ovary syndrome (PCOS) is a heterogeneous and an important metabolic disorder. Early evidence identified the importance of abnormal gonadotropin secretory dynamics. For example, PCOS cases are characterized by increased luteinizing hormone (LH). An increased frequency of gonadotropin-releasing hormone (GnRH) secretion secondary to decreased sensitivity of the GnRH pulse generator to the negative feedback effects of estradiol and progesterone may be a contributing mechanism. **Materials and Method**: Ninety adult female rats were treated with stradiol valerat (EV) for 60 days in order to induce follicular cysts (PCO modeling). Clonidine and yohimbine were used as sympathetic agonist and antagonist, respectively, and nalterxone was used for opioid system inhibition. Interactions of two systems were studied. Blood samples were collected from the heart. Serum LH, follicle-stimulating hormone (FSH), and estradiol levels were determined by ELISA. **Result**: Our results indicate that both systems and interaction of both systems have effectiveness in processing of modeling of PCOS in rat. In interaction of two systems, these drugs decreased estradiol ($P<0.05$). Qualitative analysis showed that the bulk of cysts and corpus lutea and dominant follicles were increased in PCO rats in comparison to control group. **Conclusion**: The present study addressed the question of whether the opioid system has direct effect on PCO rat ovary or any interaction with its sympathetic nervous system.

CORRELATION BETWEEN AQUEOUS HUMOR AND SERUM BIOCHEMISTRY IN SHEEP. **L.V. Athanasiou[1], G. Christodoulopoulos[1], Z.S. Polizopoulou[2], E. Kalaitzakis[1], S.M. Papadakis[1]**. [1]Clinic of Medicine, Faculty of Veterinary Medicine, University of Thessaly; and [2]Diagnostic Laboratory, School of Veterinary Medicine, Aristotle University of Thessaloniki.
Background: Aqueous humor is easily accessible and relatively isolated after death while blood undergoes contamination and autolysis. Since there is difficulty in establishing reference intervals in aqueous humor, the determination of the possible correlation of the values of various biochemical parameters and blood will enable the diagnosis based on the value of each biochemical parameter in the ocular fluid and the calculation of the corresponding value in blood, where reference intervals are well established. **Objectives:** This study was undertaken to evaluate the usefulness of the concentration of total solids, albumins, creatinine, calcium, magnesium and the activity of GGT in aqueous humor in estimating the antemortem levels of the same biochemical constituents in sheep blood. **Methods:** A blood sample was taken ante-mortem and aqueous humor from both eyes was collected twelve hours post-mortem from 126 sheep. Most of the biochemical parameters were determined employing photometric methodology, calcium and magnesium using atomic absorption techniques and proteins (total solids) using a refractometer. The relationships between chemical values of ocular fluids and serum were determined using simple linear regression. **Results:** A significant correlation was found for creatinine and magnesium concentration and GGT activity in aqueous humor and serum. There was a poor correlation between ocular fluid and serum values for all other biochemical constituents while the concentration of total solids in aqueous humor was below the limit of quantitation. **Conclusions:** Aqueous humor analysis of creatinine and magnesium concentration and GGT activity can be used for the estimation of the corresponding concentration in blood, while albumin and calcium levels are of limited value.

DETECTION OF DRUG-INDUCED LIVER INJURY (DILI) IN DRUG DEVELOPMENT: AN EXAMPLE OF TRANSLATIONAL TOXICOLOGY. **F.A. Ballet**. Medicen, Paris, France.

It is estimated that 45% of compounds where drug-induced liver injury (DILI) was identified during clinical development were negative in animal toxicology studies. There have been many attempts to improve detection of hepatotoxic potential of new molecules at a preclinical stage. *In vitro* detection of reactive metabolites in human liver microsomes and/or cytotoxicity in human hepatocytes is associated with a high incidence of false positives and/or false negatives. Also it has been suggested that use of new liver biomarkers or -omics approaches in animal toxicology studies could improve preclinical detection of DILI. However, despite significant progress in this area, these approaches have important limitations and cannot be used routinely. DILI is usually a rare event with less than a few percent of patients showing ALT elevations in clinical trials. Accordingly, a drug showing remarkable activity may be stopped because of "idiosyncratic" toxicity occurring in a small number of patients especially when there are cases of Hy's law, i.e. a combined elevation of ALT and total bilirubin. Idiosyncrasy is often considered a result of interplay of genetic and non-genetic factors specific to humans that cannot be detected in animals. However, there is evidence that disease or "sensitized" rat models could represent useful tools to detect idiosyncratic DILI. Also recently, an inbred panel of mouse strains was used to identify genetic polymorphisms associated with susceptibility to acetaminophen-induced liver injury. This suggests pre-clinical "pharmacogenetic" studies could be a valuable tool to detect genetic biomarkers used to prescreen human populations.These recent developments clearly show that a more "translational" approach in drug safety from clinical safety to toxicology and vice versa will be a key driver to improve the detection and management of DILI.

EFFECT OF VITAMIN E SUPPLEMENTATION ON LIPID PEROXIDATION OF HORSES SUBMITTED TO EXERCISE ON HIGH-SPEED TREADMILL. **T.S. Barbosa, L.A. Yonezawa, C.L. Marinho, J.L. Knaut, M.J. Watanabe and A. Kohayagawa**. School of Veterinary and Animal Science, Sao Paulo State University (Unesp), Botucatu-SP, Brazil.
Background: Exercise induces changes of the oxidant/antioxidant balance. When antioxidant systems are insufficient, oxidative processes may damage lipids and contribute to degenerative changes. Lipids are directly protected by membrane α-tocopherol and by other antioxidants. Antioxidant supplements appear to be satisfactory in preventing organ dysfunction, however only a few studies have evaluated whether administration, mainly orally in horses, improved antioxidant status and some clinical parameters. **Objectives**: This study aimed to investigate the efficiency of vitamin E supplementation on lipid peroxidation of horses submitted to exercise on high-speed treadmill. **Methods**: Ten untrained horses were used, five Arabian and five Crioulo horses, and they performed first, low intensity and long duration test (TLD1), with 60 minutes exercise protocol at 35% VO2max on a high-speed treadmill with +6% slope. Plasma malondialdehyde (MDA) was determined by HPLC at rest before exercise, during 30 min of exercise, immediately after exercise, 15 min, 30 min, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h after test end. The animals were supplemented with dl-α-tocopherol at a dose of 1.000 UI/day during 59 days and then they performed the second test (TLD2) with the same protocol of TLD1. Results: Vitamin E supplementation significantly ($P<0.05$) decreased plasma MDA concentration, 1.461±0.345µmol/L to 0.788±0.215µmol/L, in all analyzed moments. **Conclusions**: The antioxidant supplement in these conditions decreased the production of MDA, thus suggesting that the presence of vitamin E in adequate amounts might provide defense against peroxidation in this exercise protocol. Financial support: Sao Paulo State Research Foundation (FAPESP), Brazil.

ANALYTICAL EVALUATION OF THE PROTEINOGRAM USING CAPILLARYS®2 IN LABORATORY ANIMALS. **A. Beamonte**. Clinical Pathology Department, Servier R & D, Gidy, France.
Background: Automated capillary electrophoresis is increasingly gaining use in clinical pathology to replace electrophoresis on agarose gel for proteinogram. **Objective**: The aim of this study was to evaluate, in compliance with Good Laboratory Principles, the performance of a multicapillary instrument (Capillarys®2, Sebia) for serum protein analysis in laboratory animals. **Method**: Method accuracy was evaluated on 2 levels of Quality Control (QC) in conditions of repeatability and intermediate precision (5 days, 2 technicians). Bias, intra- and inter-assay plus inter-capillary precision were calculated for all fractions and albumin to globulin (A:G) ratio. Carryover between samples was assessed as well as the effect of storage conditions (room temperature, +4 and –20°C) and of haemolysis on the protein profile. Reference data were estimated on at least 13/sex control Wistar rats (11-13 weeks), beagle dogs and monkeys (Macaca Fascicularis). **Results**: On QC samples, the maximal value for the relative bias, intra- and inter-assay relative standard deviations were respectively –0.8, 1.0 and 1.3% for albumin and 4.2, 5.0 and 5.2% for globulins. Thus, the maximal relative total error for albumin and globulins, respectively, reached 2.0 and 8.5% compared to the 12 and 20% required for this assay. No carryover between samples was evidenced. The tested serum could be stored for 24h at room temperature, 7 days at +4°C or one month at –20°C. Haemolysed samples (at least ++) increased significantly beta1-globulins (dog, monkey) or alpha2-globulins (rat). When compared to standard agarose electrophoresis, control data obtained on each species by capillary electrophoresis showed a difference in the alpha-globulin and albumin fractions and thus, in the A:G ratio. **Conclusion**: Results showed that Capillarys®2 is an accurate and rapid instrument that can replace standard proteinogram in laboratory animals.

EVALUATION OF CELL-FREE mRNAs IN BLOOD FROM RATS AS NOVELS BIOMARKERS OF NEPHROTOXICITY. **J.S. Beaufils[1], F Gimie[1], S.Lezmi[2]**, [1] Department of drug safety evaluation, Porcheville, sanofi aventis, France; [2]Toxicology department, Covance laboratory SAS, Porcheville-Paris, France. During drug development, many projects are limited by drug induced toxicity. Our study evaluated organ specific mRNAs blood detection by RT-PCR as a new approach in biomarkers development. Only 14h after the administration of acetaminophen at 2000 mg/kg, we detected albumin mRNA associated with minimal hepatocellular necrosis and minimally increased AST and ALT values. mRNAs of nine kidney proteins were detected in rats treated with gentamicin at 25 or 75 mg/kg/d during 3, 5 or 10 days. Three days after the beginning of the treatment, α-gst mRNA was detected, but the specificity remains to be established; after ten days, kim-1, timp-1 and clusterin mRNA were modified and associated with kidney toxicity.These preliminary results permitted us to validate the measurement of blood mRNA as biomarker of tissue injury.

HEMATOLOGIC ABNORMALITIES IN VAV-JAK2^{V617F} (C57Bl/6J N2) TRANSGENIC MICE. **U. Bonfanti[1], G. Texido[2], W. Veronelli[2], E. Pesenti[2], P. Gnocchi[2], A.M. Giusti[1], M. Germani[1]**. [1]Accelera S.r.l., Nerviano, Italy and [2]Pharmacology and Cell Biology Dept. Oncology, Nerviano Medical Sciences, Nerviano, Italy
Background. Janus kinase 2 (*JAK2*) gene abnormalities occur in human myeloid neoplasms. An activating mutation (V617F) in *JAK2* is identified in some patients with myeloproliferative neoplasms (MPNs), mainly polycythemia vera (PV), essential thrombocytemia (ET) and primary myelofibrosis (PM). Vav-Jak2^{V617F} transgenic mice (TM) develop myeloproliferative diseases such as PV, ET and possibly PM. **Objectives:** to describe hematological abnormalities in Vav-Jak2^{V617F} TM, and evaluate disease progression over time in TM compared to non-TM littermates. **Methods.** EDTA blood was drawn from 17 Vav-Jak2^{V617F} TM and 22 non-transgenic controls. Samples were analyzed (ADVIA 120 hematology analyzer) at four different sampling times within 28 weeks for RBC, Hgb, HCT, MCV, MCH, MCHC, RDW, HDW, Platelets, MPV, PCT, PDW, total and differential WBC, MPC, PCDW, MPM, PMDW, large platelets, clumps count, RBC fragments and ghosts. In each group, data were collected within 5 age groups. *P* values are for comparisons of transgenic and non transgenic groups. **Results.** RBC, RDW, HDW, MCV, MPM, PMDW and large platelets of TM (both sexes) were different from controls in every age group, with RBC, RDW, HDW, MPM, PMDW, large platelets higher, and MCV lower than controls. Hgb, HCT, PLT and PCT were higher in every age group in TM females. WBC and subpopulations differed only occasionally in a few age groups. **Conclusions.** Transgenic expression of Vav-Jak2^{V617F} in mice causes hematologically-abnormal phenotypes resembling human PV and ET: increased number of RBC, PLT, and large platelets - which are described in humans with this disease. TM model of PV and ET is stable in the timeframe examined in this study for most relevant parameters.

DIAGNOSTIC VALUE OF CYTOLOGIC EVALUATION OF TUMORS AND TUMOR-LIKE CONDITIONS OF THE ORAL CAVITY IN DOGS AND CATS: A PROSPECTIVE CASE STUDY ON 114 CASES. **U. Bonfanti[1], W. Bertazzolo[2], M. Gracis[3], G. Romanelli[4], P. Roccabianca[5], G. Avallone[5], E. Zini[6]**. [1]Accelera S.r.l., Nerviano, Italy; [2]Clinica Veterinaria Tibaldi, Milan, Italy; [3]Clinica Veterinaria San Siro, Milan, Italy; [4]Clinica Veterinaria Nerviano, Italy; [5]DIPAV, Faculty of Veterinary Medicine, Milan, Italy; [6]Istituto Veterinario di Novara, Novara, Italy
Background. Diverse benign and malignant tumors, as well as non-neoplastic tumor-like conditions, frequently arise in the canine and feline oral cavity. Also, cytologic assessment is complicated by hemorrhage or inflammation. **Objectives:** to prospectively compare cytology results from FNA or impression smears of canine and feline oral cavity lesions with histologic results. **Methods.** Biopsy and cytology specimens from oral cavity lesions from 85 dogs and 29 cats of different genders and breeds were collected. Diagnostic potential of cytologic methods was evaluated: accuracy, sensitivity, specificity, positive and negative predictive values (PPV, NPV), and *k*-agreement between cytological and histological results. **Results.** 16 cytological specimens were unsatisfactory and excluded (retrieval rate: 85.7%). 60 of 67 (89.6%) dog lesions and 21 of 29 (72.4%) cat lesions were neoplastic. Accuracy in discriminating inflammation from neoplasia was 97% (93/96); discrepancy arose from 3 false negatives. In dogs, sensitivity, specificity, PPV and NPV for neoplasia diagnosis were 0.97, 1, 1 and 0.78, respectively, and in cats 0.95, 1, 1 and 0.89. After grouping both species, and considering as partial agreements three false negatives and partial correlations (e.g., agreement on neoplasia but not cell type), *k* value was 0.82 (CI: 0.75-0.88), 0.80 in dogs (CI: 0.73-0.87), and 0.88 in cats (CI: 0.83-0.94). **Conclusions.** High sensitivity and specificity, with good accuracy, suggest oral cavity lesion cytology is a valid procedure in dogs and cats comparable to definitive histological diagnosis.

FACTORS DETERMINING THE PLASMA CATALYTIC ACTIVITY OF ENZYMES. **J.P. Braun[1], C. Médaille[2], A. Geffré[1], C. Trumel[1].** [1]Dept Clinical Sciences, Veterinary School, Toulouse; and [2]Vébiotel, Arcueil, France.
Plasma activity of intracellular enzymes results from the equilibrium created between release from cells, distribution and entry into the vascular compartment and finally, clearance from plasma. Most plasma activity changes are interpreted as modifications of release by cells, the main causes being cell damage and/or synthesis induction. Leakage of intracellular enzymes through intact cell membrane is the main cause for the input of enzymes into plasma of healthy animals. This leakage is normally exceedingly low, estimated for instance to be between 0.01 and 0.1 % of the amount of trypsinogen synthesized by pancreatic cells. It is dramatically increased in case of cell damage. Diffusion towards plasma depends on cell location and molecular weight of the enzyme. Hepatocyte release of enzymes occurs in an extracellular compartment in direct contact with plasma. Thus enzymes reach plasma immediately and totally. High MW enzymes, such as CK, are released by muscle cells into the interstitial compartment and come into the plasma through the lymph. They are thus delayed and sometimes partially inactivated. Clearance occurs via renal excretion (for low MW enzymes) or by inactivation/catabolism in the plasma or after internalization by cells, often liver macrophages. The latter can be activated or inactivated by drugs and/or infections, thus modifying clearance of enzyme activities from plasma.

REFERENCE VALUES IN SMALL REFERENCE SAMPLE GROUPS. **J.P. Braun, A. Geffré, D. Concordet, C. Trumel**. Dept Clinical Sciences, Veterinary School, Toulouse, France.
In veterinary clinical pathology, the number of healthy reference subjects available for determination of reference intervals is frequently much lower than the minimum 120 recommended by the IFCC-CLSI. In these cases, nonparametric determination of reference limits can still be used if the number of healthy subjects is ≥40, but other methods, such as the robust method, may also be used. These methods are based on a parametric estimation of the reference limits. When the number of reference animals decreases, imprecision of determination of the reference limits increases: the width of their 90% confidence interval (CI) greatly increases, being frequently larger than 20% of the width of the reference interval (limit suggested by the IFCC-CLSI). Very small reference sample groups are frequently found in wild and zoo animals, for which larger numbers are not available. In such cases, the number of reference individuals may be even lower than 20. Testing distribution can be hazardous and probably no more accurate than a simple visual inspection. Even after making the distributions more symmetrical using transformations, the results yielded by parametric methods yield results must still be considered with much caution. At the best (i.e. a Gaussian distribution) the percentage of the 90% CI of the limits as regards to the width of the reference interval is ~ 31%, 35%, 42% and 55% for n = 25, 20, 15, and 10 respectively. For these reasons, it is probably better to report all reference values as well as the median of small reference sample groups, and not forget to describe fully the demographics and pre-analytical and analytical conditions.

HEMATOLOGY, PLASMA PROTEINS AND FIBRINOGEN IN ENDURANCE HORSES COMPLETING 500 Km IN 6 DAYS. C**. Castejón-Riber[1], A. Muñoz[1,2], J. Roldán[2], P. Trigo[1], M. Gómez-Díez[1], C. Riber[1,2].** [1] Equine Sport Medicine Centre; [2] Department of Animal Medicine and Surgery, Córdoba University, Spain.
Background: Prolonged exercise in horses leads to significant losses of water and electrolytes because of sweating, with hemoconcentration, neutrophilia, and, in very stressful situations, lymphopenia. In endurance events of two days, it has been demonstrated that water deficit persists overnight. Therefore, horses can start the second day of competition dehydrated. This fact could limit their performance, promoting metabolic disturbances. **Objectives:** This research assesses the changes in hematological parameters and plasma proteins as indicators of hydration status, and fibrinogen (FIB) as marker of inflammation in endurance horses that completed an endurance competition of 500 Km in 6 days. **Methods:** Venous blood samples were taken from 27 horses, before and immediately after exercise, during the 6 days of competition. RBC and WBC parameters, total plasma proteins (TPP) and FIB were measured. **Results:** Daily competitions led to an increase in WBC, neutrophils, microhematocrit and TPP and a reduction in lymphocytes. In comparison with the values obtained in the first day, before exercise, horses had lower hemoglobin concentration (days 3, 4, 5 and 6), microhematocrit (days 4, 5, and 6), TPP (days 4 and 6), and, lymphocytes (day 3). Thus, mean pre-exercise microhematocrits during the six days were: 40.20, 38.27, 35.92, 34.03, 33.46 and 32.38%. FIB concentrations were within the reference values (< 400 mg/dl) until the end of the 6th day, reaching a mean of 690,0±114,5 mg/dl. **Conclusions:** Endurance horses that were able to cover 500 Km in 6 days maintain their hydration status, with a mild and progressive overhydration. Laboratorial signs of inflammation appeared at the end of competition.

EVALUATION OF AN AUTOMATED CANINE SERUM AMYLOID A ASSAY BASED ON MONOCLONAL HETEROLOGOUS ANTIBODIES. **M. Christensen[1], T. Ichiyanagi[2], S. Jacobsen[3], M. Kjelgaard-Hansen[1].** [1]Department of Small Animal Clinical Sciences; and [3]Department of Large Animal Sciences University of Copenhagen, Denmark, [2]EIKEN Chemical, Tokyo, Japan.
Background: Major acute phase proteins (APP) have proven diagnostic useful as routine inflammatory markers in dogs. However, the heterologous and polyclonal nature of immunoglobulins in available automated assays makes long-term and inter-batch performance potentially highly variable. Monoclonal-based assays would reduce this variability. **Objective**: The objective was to evaluate the analytical performance of an automated latex agglutination turbidimetric immunoassay based on monoclonal heterologous antibodies for measurements of canine serum amyloid A (SAA). Material: 216 serum samples from client-owned dogs obtained for diagnostic purposes were used in the study. **Method**: The analyses were performed using an automated clinical chemistry analyser (ADVIA 1800, Siemens). Intra- and interassay coefficient of variation (CV) was determined based on replicate determinations (n=8) of serum pools containing intermediate and high SAA concentrations. Inaccuracy was investigated by linearity under dilution of pooled serum with high SAA concentration (linear regression). Detection limit (DL) was determined from replicate determinations (n=8) of distilled water. Parallel measurement of C-reactive protein (CRP) another major APP (n=216) was performed to evaluate diagnostic agreement (Kappa statistics). **Results**: Intraassay CV was 1.9% and 4.1% and interassay CV was 9.7% and 12.5% for high and intermediate concentrations, respectively. Acceptable linearity was observed under dilution with a slope of 1.0[0.95;1.05] and y-intercept of 4.03[-23.0;31.0]. DL was 1.06mg/L. Excellent agreement (Kappa=0.78) on clinical classification was observed between SAA and CRP measurements (cut-off of 75mg/L and 35mg/L, respectively). **Conclusion**: The assay demonstrated acceptable analytical performance and excellent diagnostic agreement with CRP. The monoclonal nature of the assay is expected to reduce batch-to-batch variation facilitating long-term routine diagnostic use.

EVALUATION OF AN AUTOMATED MULTI-SPECIES ASSAY FOR MEASUREMENTS OF SERUM AMYLOID A IN DOGS, CATS AND HORSES. **M. Christensen[1], S. Jacobsen[2], T. Ichiyanagi[3], M. Kjelgaard-Hansen[1].** [1]Department of Small Animal Clinical Sciences; and [2]Department of Large Animal Sciences, University of Copenhagen, Denmark; [3]EIKEN Chemical, Tokyo, Japan.
Background: Major acute phase proteins (APP) have proven diagnostic useful in dogs, cats and horses, when used routinely. However, dissemination of routine diagnostic use is hampered by available automated assays not being applicable across all major companion animal species. An automated multispecies assay for a major APP would facilitate dissemination of routine use. **Objective**: The objective was to evaluate analytical performance of an automated latex agglutination turbidimetric immunoassay (LAT) for measurements of serum amyloid A (SAA) across dogs, cats and horses. **Material**: Serum samples from 216 dogs, 40 cats and 40 horses obtained for diagnostic purposes were included in the study. Method: The analyses were performed using an automated clinical chemistry analyser. Intra- and interassay coefficient of variation (CV) was determined based on replicate determinations of serum pools (n=8) containing intermediate and high SAA concentrations. Inaccuracy was investigated by linearity under dilution of serum pools with high SAA contents (linear regression). Detection limit (DL) was determined from replicate determinations (n=8) of distilled water. Equine and feline measurements were compared to parallel SAA measurements using a previously validated assay. **Results**: Intraassay CVs ranged between [1.9%;4.0%] and [4.1%;4.6%] and interassay CVs [3.0%;14.5%] and [6.7%;12.5%] for high and intermediate concentrations, respectively. Acceptable linearity was observed with slopes and y-intercepts not differing from one and zero, respectively. DL was 1.06mg/L. Method comparison revealed acceptable agreement of the two SAA assays. **Conclusion**: The new LAT performed acceptable measuring SAA in equine, feline and canine serum and thus poses a relevant multispecies assay for a major APP in companion animal species.

CAPILLARY (ZONE) ELECTROPHORESIS IN MULTIPLE SPECIES. **G. Counotte.** Animal Health Service, Deventer, The Netherlands.
Background: Separation of serum proteins is a widely used technique in veterinary laboratories. Analysis began with cellulose acetate, continued with agarose gel electrophoresis and now capillary (zone) electrophoresis (CZE) has started to enter the laboratories. Due to the higher different resolution levels, patterns of the serum proteins can be different variable when using these techniques. Therefore, technicians have to learn how to interpret electropherogram and reference values have to be re-established. **Objectives**: To establish a robust working protocol including many animal species with many different protein patterns using a CZE-machine (Sebia Capillarys). **Methods**: Serum from different species (bovine, equine, canine, feline, caprine, ovine) (healthy and diseased) were used and the electropherograms were compared to those obtained with other electrophoretic techniques. Influence of fat and other possible interactions were studied. **Results**: After optimizing the settings like spectral resolution, it appeared that in several

species the albumin peak was no longer a single peak. Using anti-albumin, it was revealed that these variable peaks were indeed albumin. Because of the higher resolution and the better protein separation, many more proteins can be seen. Comparison with the Paragon-system of Beckman showed for most species and proteins-fractions good correlation. Not only serum proteins, but also for example, IgG in milk can be analyzed with CZE. **Conclusion**: Capillary (zone) electrophoresis is a robust technique useful for veterinary laboratories. Due to the principle of CZE, patterns of serum proteins change and new reference values have to be calculated. Establishing an atlas of all different kind of animal species electropherograms can be helpful for new users of CZE or users analyzing other animal species than they routinely do.

CORRELATION OF SERUM CARDIAC TROPONIN I (cTnI) WITH CLINICAL STAGING IN 56 DOGS WITH MITRAL VALVE DISEASE FOLLOWED UP FOR SIX MONTHS. **A. Dasopoulou[1], Z.S. Polizopoulou[1], C.K. Koutinas[2], M.N. Patsikas[2], M. York[3], I. Roman[3], M. Gandhi[3], S. Patel[3], A.F. Koutinas[2], P.J. O'Brien[4].** [1]Diagnostic Laboratory and [2]Clinic of Companion Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece; [3]Clinical Pathology, Safety Assessment, GlaxoSmithKline, Ware, Hertfordshire, UK; [4]Clinical Pathology Laboratory, University Veterinary Hospital, University College Dublin, Ireland.
Background: Cardiac troponin I (cTnI) is a highly specific and sensitive marker of myocardial injury in many mammalian species, rising rapidly after cardiomyocyte damage regardless of the original insult. **Objectives**: The purpose of this study was to evaluate cTnI changes and to correlate them with the clinical staging in dogs with mitral valve degeneration (MVD). **Materials and methods**: A total of 56 dogs, diagnosed with MVD were included in the study. For their allocation into three groups, notably dogs with asymptomatic disease (n=22) for group I, with mild to moderate congestive heart failure (CHF) (n=18) for group II, and with advanced CHF (n=16) for group III, a modified classification scheme, based on the guidelines of the International Small Animal Cardiac Health Council, was applied. During the 6-month follow-up period of each of the dogs, serum samples for biochemical testing were obtained every 15 days. Clinical progression and response to treatment were evaluated every two months, at which time thoracic radiographs and echocardiograms were also performed. **Results**: A positive correlation was demonstrated in all groups between the magnitude of changes in serum cTnI and the severity of clinical signs. **Conclusion**: In dogs with MVD the measurement of cTnI could be a useful diagnostic tool for assessing clinical stage and long term monitoring of patients with chronic heart failure.

EVALUATION OF THE AUTION STICKS 10PA FOR PROTEINURIA AND 10EA STRIPS IN THE DOG AND CAT. **M. Defontis, N. Bauer, A. Moritz**. Department of Veterinary Clinical Sciences, Justus-Liebig University Giessen, Germany.
Objectives: Prospective evaluation of the dipsticks AUTION 10PA for proteinuria and AUTION 10EA (Arkray, Axon Lab) in canine and feline urine samples. Semiquantitative results were compared to microscopy, refractometry and quantitative analysis of glucose, protein, creatinine, and UPC ratio. Agreement between visual reading of two blinded observers and between automated and visual reading was assessed. **Methods**: Urine specimens from 101 dogs and 46 cats were evaluated. Spearman correlation coefficients (rs), inter-observer agreement (κ), sensitivity and specificity were calculated. **Results**: Good correlation between dipsticks' automated reading and quantitative measurement of protein (dog: rs=0.87, cat: rs=0.84) and creatinine (dog: rs=0.85; cat: rs=0.82) was found. Sensitivity and specificity of the AUTION 10PA dipstick to detect an increased UPC ratio by automated reading were 75% and 100% in the cat (cut-off:0.2) and 73%, 98% in the dog (cut-off:0.5). Despite a good correlation between automated SG reading (10EA) and refractometry (dog: rs-=0.78, cat: rs=0.83), a high 95% confidence interval was seen. There was a poor correlation for leucocyte results between dipstick and microscopy. Agreement between two observers was good to very good except for ketone and creatinine values in the dog (10PA). Agreement between visual and automated reading was very good to moderate for both dipsticks except for leucocyte count (κ=0.26), UPC (κ=0.24), and creatinine (κ=0.22) in the dog. **Conclusions**: Using the cut-offs 0.2 (cat) and 0.5 (dog), the AUTION 10PA dipstick can be used as a highly specific screening test for detection of proteinuria. Considering higher agreement with reference methods, automated reading of the UPC ratio is recommended. However, dipsticks cannot be recommended for detection of pyuria and measurement of the specific gravity.

WHOLE BLOOD PLATELET AGGREGATION USING THE MULTIPLATE® ANALYZER IN THE BEAGLE DOG AND WISTAR RAT. **M. Defontis[1], M. Stirn[2], S. Coté[2], D. Ledieu[2]**. [1]Department of Veterinary Clinical Sciences, Giessen University, Germany; [2]Preclinical safety, Novartis, Basel, Switzerland.
Objectives: Standardization of Multiplate® whole blood platelet aggregometry in the Beagle dog and Wistar rat after blood incubation in 0.1% DMSO as a vehicle for further ex vivo testing of drugs' toxicity. **Methods**: First, three anticoagulants (hirudin, citrate and heparin) were compared (dogs: n=12, rats: n=10) and the effect of recalcification on spontaneous aggregation in citrated blood (dogs: n=12, rats: n=5) was evaluated. Then, platelet response to four aggregating agents (ADP, collagen, arachidonic acid and Par-4 agonist) in hirudin blood (dogs: n=12, rats: n=15) was assessed and agonists' EC50 were determined. **Results**: Significant spontaneous aggregation was observed in recalcified citrate samples compared to hirudin and heparin ($p<0.05$). After ADP stimulation, there was no difference between hirudin and heparin anticoagulated samples and for the dog AUC values in citrate samples were lower than in hirudin ($p<0.05$) or heparin ($p<0.01$) samples. After collagen stimulation, AUC values were higher in canine hirudin samples than in heparin ($p<0.05$) and citrate ($p<0.001$) samples whereas for the rat there was no difference between hirudin and heparin. Although no effect of recalcification was found on spontaneous aggregation in canine citrate blood, a significant effect was observed in the rat ($p=0.004$). Agonists' EC50 calculated in hirudin samples in the rat are: 2.7µM ADP, 0.85µg/ml collagen, 0.03mM arachidonic acid, 165.7µM Par-4 agonist and in the dog: 0.95µM ADP, 0.23µg/ml collagen and 0.05mM arachidonic acid. Par-4 agonist didn't induce aggregation in the dog. **Conclusions**: Whereas anticoagulation with recalcified citrate cannot be recommended, use of hirudin should be preferred for Multiplate® whole blood platelet aggregometry. Par-4 agonist cannot induce platelet aggregation in the Beagle Dog.

AUTOMATED RETICULOCYTE COUNTS FROM ANEMIC AND NONANEMIC DOGS ON THE IDEXX PROCYTE DX HEMATOLOGY ANALYZER. **D.B. DeNicola, J. Russell, S. Burger, J.M. Hammond**, IDEXX Laboratories, Inc., Westbrook, ME
Background: Several in-house hematology analyzers now provide reticulocyte counts on all dog and cat complete blood counts (CBC). Increased reticulocyte counts are occasionally seen in non-anemic animals, which can be confusing to the veterinary diagnostician. Some of the proposed scenarios resulting in reticulocytosis without anemia include transient physiologic reticulocytosis associated with splenic contraction, recovery phase from anemia until baseline hematocrit (HCT) values are reached, conditions of partially or fully compensated hemolytic disease, as well as primary and both absolute appropriate and inappropriate erythrocytosis. If reticulocyte counts are only evaluated on anemic patients, potential early developing disease processes can be overlooked; veterinarians should be aware of and alerted to any of these conditions. **Objectives:** The purpose of this study was to document the frequency of reticulocytosis in nonanemic dogs. **Methods:** Canine CBC data from IDEXX ProCyte Dx® Hematology Analyzers (PDx) in the field were collected through IDEXX's SmartService™ internet connection during the first ten months after the launch of the PDx. The canine reference interval (RI) determined for the PDx is 6-110 $x10^3/\mu L$ for reticulocyte counts and 37.3-61.7% for HCT. **Results:** A total of 163,736 CBC results were available for evaluation. A total of 8,354 nonanemic dogs (5.1%) had reticulocyte counts above the upper RI limit. During PDx development, the percent of nonanemic dogs with reticulocytosis was between 10 and 15% for several universities; however, a higher percentage of non-healthy animals are typically evaluated at the university compared to the average veterinary practice. **Conclusion:** Although not a dramatically high incidence of this occurrence, reticulocytosis in nonanemic dogs is still significant and the veterinarians in the field need to be aware of this possibility.

A DYNAMIC FLOW CHAMBER BASED ADHESION ASSAY TO ASSESS CANINE PLATELET MATRIX INTERACTIONS EX VIVO. **A. Ferkau[1,2], S. Ecklebe[1], S. Calmer[1], F. Echtermeyer[1], R. Mischke[2], G. Theilmeier[1]**. [1]Department of Anesthesiology and Intensive Care Medicine, Hannover Medical School, Hannover; and [2]Small Animal Clinic, University of Veterinary Medicine, Hannover, Germany.
Background and objectives: In humans platelet function, disorders and drug effects on platelet function can be examined using dynamic adhesion assays like parallel plate flow chambers. We here establish this method in healthy dogs to develop a potential tool for diagnosis of platelet dysfunction in diseased dogs.
Method: $DiOC_6$-stained whole blood of 10 healthy dogs of differing background was perfused through the flow chamber for 180 seconds across biochips coated with canine or bovine skin collagen (Cellix, VenaEC™). Shear rates ranged from 14 to 60 dynes/cm^2. For activation canine protease-activated-receptor-4-agonist (TRAP-PAR4) was used. After perfusion attached platelets were recorded in ten high-power fields. Percent area covered and average size of thrombi were measured using ImageJ. **Results:** Bovine skin collagen did not support platelet adhesion. Platelet adhesion and thrombus formation was best supported by 200µg/ml canine collagen. With up to 1000µg/ml no further increase in adhesion of platelets could be detected. At lower concentrations (30µg/ml) no platelet adhesion was supported. Most consistent results were obtained at 14 dynes/cm^2, whereas higher shear rates only increased variability but not thrombus formation. Under these conditions activation of platelets with TRAP-PAR4, but not TRAP-PAR1 increased adhesion (2800±1450 vs. 448±110 platelet covered area in μm^2, TRAP-PAR4 vs control; n=8; $p<0,05$) and average thrombussize (16.5±4.9 vs 7.9±1.8 μm^2, TRAP-PAR4 vs control; n=8; $p<0,05$). **Conclusion:** We confirm published differences in platelet function between dog and man and established a novel method to determine changes in platelet-matrix interactions in dogs that could be useful to assess platelet dysfunction.

INJECTION SITE REACTION (PSEUDOLYMPHOMA) MIMICKING A PRIMARY CUTANEOUS LYMPHOMA IN A CAT. **A. Forlani, G. Ghisleni, A. Sacchet, M. Caniatti, P. Roccabianca.** DIPAV, University of Milan, Italy.
A 4-years old, spayed female, domestic shorthaired cat was presented for a slow growing, non painful, not ulcerated, 1 cm, subcutaneous mass in the dorso-lateral thorax in the site of a previous drug injection (Ranitidine). The cat was in a good body condition, CBC and serum biochemistry were unremarkable. Fine-needle aspirate cytological samples were interpreted by the referring veterinarian as cutaneous lymphoma. Stained and unstained samples were sent for consult. Cytology was characterized by a prevalence of medium sized lymphocytes with round to indented nuclei, finely granular to diffuse chromatin (centrocytes) admixed with small mature lymphocytes, occasional centroblasts and macrophages. Findings were consistent with chronic lymphohistiocytic dermatitis. To definitely rule out a cutaneous lymphoma the lesion was surgically excised. Histopathology revealed a subcutaneous oval, well demarcated, unencapsulated mass consisting of a mixed inflammatory and reparative reaction containing plump reactive fibroblasts, plasmacells, histiocytes, eosinophils and, occasional mast cells associated with small mature and medium sized reactive lymphocytes either infiltrating the nodule or organized at the periphery in small pseudofollicular and perivascular aggregates.
The lesion resembled the so-called vaccine induced cutaneous pseudolymphoma described in man that is characterized by a reactive B cell proliferation associated with abundant histiocytes often in clusters. Pseudolymphoma is a term applied to a group of inflammatory to proliferative lesions that may be misdiagnosed as lymphoma as was in this case by cytology. In cats, arthropod bites and vaccination are probably the main triggers of pseudolymphoma. These lesions are mostly polyclonal (B or T) benign, self-limiting reactions induced by persistent antigenic stimulation. However, in some cases pseudolymphoma may evolve into cutaneous lymphoma.

CHARACTERISATION OF EQUINE MESENCHYMAL STEM CELLS. **P. Formisano[1], K. McDonald[3], S. Duthie[3], X. Donadeu[2], E. Milne[1].** [1]Division of Veterinary Clinical Sciences, University of Edinburgh, Scotland, United Kingdom; [2]The Roslin Institute - University of Edinburgh, Scotland and [3]Biobest Laboratories, Edinburgh, Scotland.
Background: Mesenchymal stem cells (MSCs) are multipotent cells able to proliferate in vitro and to differentiate into multiple adult mesodermal cells including tenocytes and fibroblasts. They are easily isolated from many tissues and are particularly useful in mesenchymal tissue injury repair. In veterinary medicine, the therapeutic use of MSCs has centred on the repair of tendon injuries in horses. Tendon injuries are a major cause of equine morbidity and a common reason for euthanasia, particularly in performance horses. It is not possible to differentiate tenocytes from fibroblasts on the basis of their morphology alone, and at present there are no specific equine markers able to characterize these cells, although recent studies have identified genes that may be useful as markers because they are differentially expressed in MSCs and then in tenocytes. In order to demonstrate regeneration, markers are required to identify tenogenic potential in the MSCs. **Objectives and methods:** The aim is to characterize equine MSCs at different stages of multiplication using a combination of methods (gene expression with PCR, cell surface molecules and proteins produced, with immunocytochemistry and flow cytometry) and to identify a reliable panel of tenogenic markers. **Results:** Standard growth conditions have been developed. The MSCs are positive for the stem cell markers (Oct4, Sox2, SSEA1, Nanog), positive for adherence markers (CD90 and CD 105) and negative for haematopoietic markers (CD45). **Conclusions:** These markers appear to predictably identify MSCs and will provide a reliable panel for further classification.

BRONCHOALVEOLAR LAVAGE ANALYSIS. **K.P. Freeman**. IDEXX Laboratories, Grange House, Sandbeck Industrial Estate, Wetherby, West Yorkshire, United Kingdom.
Bronchoalveolar lavage (BAL) has been long used in evaluation of equine respiratory tract, BAL use in dogs and cats has increased over the last decade. It is used to evaluate respiratory disease and investigate poor athletic performance. Clinical and technical factors, as well as those of evaluation and interpretation need be considered. Clinical factors include patient selection, collection instrument availability, and sample collection competency and experience. Clinical factors also are important in choosing BAL versus transtracheal or tracheal washing, and whether a blind or guided technique is used. Technical considerations apply after sample collection and before microscopic evaluation and may involve the clinician and laboratory staff. These include decisions on submission of pooled or separate samples, microbiologic testing, fixing samples, and anticoagulant use. Sample processing includes decisions on use of direct or concentrated smears versus cytospin or filter preparations, on use of total and differential cell counts or semi-quantitative estimates. Stain choice may influence information availability. Issues of evaluation include specimen quality, use of differential or semi-quantitative estimates of cell types, and establishment of reference intervals. Interpretation factors include knowledge of the species of interest and conditions occurring in them, as well as correlation with results of ancillary testing. BAL use in investigation of poor performance in athletes requires special attention to ancillary testing and synthesis of data from multiple sources as patients may have no relevant clinical signs. Serial collections may be of benefit for monitoring of response to treatment and prediction of relapsing respiratory disease following treatment. Although there are limited number and types of responses, BAL may provide valuable information in determining whether respiratory disease is implicated in poor performance and in determining the most likely underlying cause.

THE SCIENCE OF QUALITY ASSURANCE OF VETERINARY LABORATORY ANALYSIS. **K.P. Freeman**, IDEXX Laboratories, Grange House, Sandbeck Industrial Estate, Wetherby, West Yorkshire, United Kingdom.
'Planned and systematic activities to provide adequate confidence that quality requirements will be met' are quality assurance (QA). QA science lies in determining objective quality standards to judge analytical performance. Biologic variation information in various species determines quality requirements, but information about a variety of analytes in various species is generally lacking. Determining quality requirements uses results interpretation method, most commonly expressed as total allowable error (TAE) at a particular medical decision level. Results varying more than acceptable for medical interpretation make tests unsuitable. Desirable TAE is compared to calculated total error (Bias% + 2CV) to see if analytic performance meets user need. It's used with mean, standard deviation, coefficient of variation and bias, to evaluate analytical performance using a Method Decision Chart for analysis validation. Ongoing stable performance for analysis is achieved by QC validation to determine QC rules and number of controls needed for high probability of error detection and low probability of false rejection: >90% error detection ensures unacceptable variation is detected; < 5% false rejection reduces delays in patient result production and expenses from investigating 'failed QC' when a problem is not present. Performance indices and desirable TAE also are used to determine sigma metric for a test, a unitless way to express quality of performance: 'world class' is >6; < 3 is unstable and unsuitable. QA strategies for various analytes may also be determined using sigma metric: if < 6 quality goal index can be calculated to see if low performance is from imprecision, inaccuracy or both. Findings may identify solutions for improved performance. QA science helps decision making on quality management less arbitrary and provides an evidence-based approach for QA.

STUDY OF TICK BORNE AND FLEA RELATED INFECTIONS BY QUANTITATIVE PCR IN CATS IN FRANCE: PRELIMARY RESULTS FROM 100 CASES OUT OF 150. **D. Fritz[1], B. Carcy[2], T. Schetters[3]**. [1]Companion Animal Laboratory, Troyes, France; [2]Laboratoire de Biologie Cellulaire et Moléculaire, UMI, Montpellier cedex 5, France; [3]Intervet/Schering–Plough Animal Health, Boxmeer, The Netherlands.
The aim of the study was to investigate tick-borne and flea-related infection in cats from France, to identify blood-borne pathogens, and determine the main clinical signs associated with different infections. The inclusion criterion was a sufficient amount of EDTA-K3 blood to perform analyses in blood samples submitted to a commercial laboratory for diagnostic assays by referring veterinarians. Pathogen-specific primers were based on sequences that were retrieved from Genbank. The following positive PCR results were obtained among the first hundred cases: FIV (23%) and FeLV (20%), *Erhlichia* sp.(14%), *Cytauxzoon felis* (12%), *Anaplasma* sp.(9%) and *Rickettsia* / *Neorickettsia* sp.(9%), *Babesia felis* (8%), *Mycoplasma haemofelis* or *candidatus, Mycoplasma haemominutum* (7%), *Theileria annae* (7%), *Bartonella* sp.(2%) and *Babesia rossi*.(1%). Analysis of the PCR product by two laboratories confirmed that the sequence was similar to that of *B. rossi*. To confirm the possible présence of *B. rossi*, or a closely related strain, a second PCR using specific BrEMA1-derived primers according to Matjila et al. (2009) was performed. No PCR-product could be detected(2). Thus, the presence of B. rossi sensu stricto or of B. rossi-like in cat remains to be determined. Sequencing was also used to confirm the detection of Cytauxzoon *felis* (1,2) and to identify *Ehrlichia canis* (1) and *Anaplasma cytophagophilum* (1) in one sample of *Erhlichia* sp. and *Anaplasma* sp., respectively. Interestingly, FeLV and FIV were not associated with other systemic infections. Clinical signs were not specific and hematologic abnormalities were not always associated with infection.

CLASSIFICATION OF 396 FELINE EFFUSIONS ACCORDING TO NEW PROPOSED SCHEME. **A. Gavazza[1],V. Turinelli[2], G. Lubas[1], S. Thuere[2].** [1]Dept. Veterinary Clinic, University of Pisa, Italy; [2]Idexx Laboratories, Ludwigsburg, Germany.
Effusion is the abnormal accumulation of fluid within a body cavity and it is a common sign of several disorders in the cat. A new etiological classification has been recently proposed (Dempsey & Ewing, 2011). It divides effusions into four main groups: 1. Transudates, subtyped into protein-poor (<1.5 g/dL) and protein-rich (≥ 2 g/dL); 2. Exudates, subtyped into septic and non-septic (eosinophilic, neutrophilic and FIP) (≥ 2 g/dL); 3. Effusions resulting from vessels or viscous disruption, subtyped into hemorrhagic, lymphorragic, uroperitoneum and bile peritonitis (≥ 2 g/dL); and 4. Effusions resulting from cell exfoliation, subtyped into neoplastic (carcinoma and lymphoma) and reactive

mesothelial proliferation (≥ 2 g/dL). This retrospective study was conducted between September 2006 and June 2010. Three hundred and ninety-six thoracic and abdominal effusions were classified using both cytological criteria and refractometrical estimates of total protein concentration. Samples were collected from cats referred for effusion investigation affected by several disorders. Results were: transudates 21.2%, exudates 28.8%, disruption of vessels or viscous effusions in 26.3% and cell exfoliation effusions 23.7%. Results obtained in each type of effusion were: protein-poor transudates 5.6%, protein-rich transudates 15.6%; septic exudates 8.6%, non-septic exudates 20.2% (eosinophilic 0.8%, neutrophilic 9.8%, FIP 9.6%); hemorrhagic 2.8%, lymphorragic 23.2%, uroperitoneum not found, bile peritonitis 0.3%; carcinoma 14.4%, lymphoma 8.8% and reactive mesothelial proliferation 0.5%. This study outlines the major incidence of lymphorragic effusions and the rarity of bile peritonitis, mesothelial hyperplasia and eosinophilic exudates in feline species. Moreover, this new etiological classification is interesting because it seems to be more clinical useful problem oriented and can provide further information to perform additional diagnostic.

INTRA-ABDOMINAL FLUID ASPIRATE FROM A DOG. **G. Ghisleni, A. Forlani, G. Avallone, M. Caniatti.** DIPAV, University of Milan, Italy.
CASE PRESENTATION: A 12-year-old, female, Siberian Husky, was presented with a 6-months history of progressive abdominal distension, and weight loss. The dog appeared normal on physical examination except for marked abdominal distension. A fluid wave was balloted, strongly suggesting an abdominal effusion. Ultrasound examination confirmed this clinical finding. Results of the CBC included mild non regenerative anemia. No abnormalities in serum chemistry were detected. An abdominal centesis gave three liters of serosanguineous, and mildly turbid abdominal fluid. The fluid had a protein concentration of 3g/dL, and a total nucleated cell count of 2,500/µl. Several direct smears and centrifuged samples were May-Grünwald-Giemsa-stained. The background consisted of an amorphous, lilac proteinaceous material. A predominant population of cells, round to polygonal, single and in small clusters was found. These cells had moderate bluish cytoplasm with variably distinct margins. Nuclei were round to oval, central to paracentral. Moderate number of activated macrophages containing hemosiderin were also present. Few lymphocytes, non degenerated neutrophils, and cholesterol crystals were present. The fluid was cytologically interpreted as a modified transudate. Gradually increasing abdominal discomfort developed, and a laparotomy was performed and revealed a large (40 cm in diameter) solitary unilocular cyst with a thin wall, without septation and containing serous fluid. The cyst occupied the whole abdominal cavity. The histologic diagnosis was cyst of subsurface epithelial structures. **COMMENT**: Cyst that occur adjacent to the ovary can derive from a variety of structures, including the mesonephric duct and tubule, paramesonephric duct, uterine tube, and mesosalpinx. These are fluid filled cysts that are located adjacent to the ovary or uterine tube. The presence of cholesterol crystals is a very helpful cytologic finding to differentiated epithelial origin of the lesion from mesothelioma.

FREQUENCY OF HEMATOLOGICAL AND ELECTROPHORETICAL ABNORMALITIES IN DAIRY COWS AFTER CALVING: THE ProZoo PERSPECTIVE
A. Giordano[1,2], P. Moretti[2], N. Morandi[3], E. Scanziani[1,2], S.Paltrinieri[1,2] [1]Department of Veterinary Pathology, Hygiene and Public Health – Milan, Italy; [2]Central laboratory, Large Animal Hospital –Milan, Italy; [3]Parco Tecnologico Padano - Lodi, Italy
Background – Metabolic and inflammatory diseases are common in dairy cows after parturition. Frequently, these diseases have a subclinical course but are characterized by laboratory abnormalities. **Objective** – To determine the frequency of hematological and electrophoretical abnormalities potentially consistent with subclinical diseases in dairy cows after calving. The early identification of sublicnical changes is important within the ProZoo project, which is aimed to investigate the association between genetic traits and resistance/susceptibility to diseases. Methods – 109 dairy cows from 3 herds (n=35, 36 and 34) were sampled on day 3±1 and 30±3 after parturition. Hematology with a laser counter followed by microscopical differential leukocyte counts and agarose gel serum protein electrophoresis (SPE) were performed. In both samplings, values from the 3 groups were statistically compared to each other. The number of values outside the reference intervals at each sampling was calculated in each herd. **Results** – For many hematological and electrophoretical parameters, significant differences between the herds were found in both samplings, likely due to a different management or to environmental factors. Abnormal values were found in all the groups with variable frequency (0 to 64% of samples). The most frequent changes were found for hemoglobin concentrations neutrophils and monocytes. Other hematological parameters and globulin fractions were occasionally altered. **Conclusions** – Routine hematology and SPE can detect abnormalities potentially consistent with subclinical diseases, thus allowing a preliminary selection of animals to be further investigated on a clinical standpoint and/or to be characterized genotypically within the ProZoo project. However, laboratory results can be influenced by the management of the herd.

CREATINE KINASE ISOENZYMES IN HEALTHY NEWBORN FOALS AND PRELIMINARY EVALUATION IN SEPTIC AND IN ASPHYCTIC ANIMALS. **L. Giori[1], S. Panzani[1], E. Tagliabue[1], A. Giordano[1], C. Castagnetti[2], M.C. Veronesi[1], S. Paltrinieri[1].** [1]University of Milan, Italy; [2]University of Bologne, Italy.
Background: no information about the possible role of the serum activity of Creatine Kinase (CK) isoenzymes as a biomarker for septicemia and asphyxia in newborn foals are available. **Objectives**: to evaluate the activity of CK isoenzymes in healthy newborns and in septicemic or asphyctic foals. **Methods**: electrophoretic separation of CK isoenzymes (CK-MM, CK-MB, CK-BB) and macroenzymes (Macro-CKI and Macro-CK2) was performed on sera from healthy foals sampled 30 minutes after birth, at 3, 12, 24 hours and daily until day 7. Sera from 9 septicemic and 5 asphyctic foals were also examined at admission and during the follow up. The results were compared with those of age-matched controls. For both healthy and sick foals, differences among the sequential time samplings were also assessed. **Results**: in healthy foals, a total CK activity significantly increased in the first day then decreased on day 2. CK-BB is the main isoenzyme at birth and up to day 7, except at 3 and 12 hours, when CK-MM is the prevalent isoenzyme. CK-MB and macroenzymes are negligible. Compared with age-matched controls, asphyctic foals have increased CK-MM activity, while electrophoretic changes in septicemic foals are variable and generally mild. During the follow up, isoenzyme activities normalized in both groups of sick foals. **Conclusions**: the proportion and activity of the different isoenzymes vary with age, with CK-BB being the main serum isoenzyme in most samplings. Thus it is important to compare the results of sick foals with those of age-matched controls. This comparison reveals severe CK-MM increases in asphyctic foals and variable changes in septicemic foals.

COMPARISON OF THE PROCYTE DX ANALYZER TO THE ADVIA 2120 AND MANUAL DIFFERENTIAL FOR VALIDATION OF EQUINE AND BOVINE HEMOGRAMS. **F. Goldmann, N. Bauer, A. Moritz.** Department of Veterinary Clinical Sciences, Clinical Pathophysiology and Clinical Pathology, Justus-Liebig University Giessen.
Background: ProCyte Dx® (IDEXX) was introduced as in-house hematology analyzer with laser flow cytometry, optical fluorescent and laminar flow impedance technologies. It performs a complete hemogram for five different species including horses and cattle in two minutes. **Objective** of this study was to evaluate the performance, precision, linearity and accuracy of ProCyte Dx for bovine and equine blood samples. **Methods**: EDTA-anticoagulated blood samples from clinically healthy or ill horses (n=175) and cattle (n=115) were analyzed within 6 hours. Routine hemogram parameter results including a five-part leukocyte differential from ProCyte Dx were compared to ADVIA 2120 (Siemens Medical Solutions GmbH) results and a 200-cell manual leukocyte differential. Statistical analysis was performed on all results and as well as after selected exclusion following scattergram/dot plot validation. Correlation coefficient (R), intercept, slope, with 95% CI and mean bias of instrument-to-instrument comparison are reported. **Results**: Handling of the ProCyte Dx was user friendly and no technical faults occurred. Coefficient of variation results were <3% (CBC) and <7% (differential count) except for eosinophils and monocytes (cattle) and PLTs (horse). Linearity was excellent except for platelets. Spearman´s rank correlation coefficients (rs) revealed good to excellent (rs=0.99-0.80) agreement between both analyzers for all variables except for MCHC (rs=0.33-0.65) and equine impedance based PLTs (PLT-I) (rs=0.79). Agreement between PLT-I and laser-based platelets (PLT-O) was good (rs=0.88). Biases were close to 0 except for MCHC, HGB and PLTs in both species. **Conclusion**: Data indicate ProCyte Dx results - especially with addition of visual data report to numeric data - are comparable to information provided by reference laboratory analyzers.

FELINE HEMATOLOGY REFERENCE INTERVALS WITH THE PROCYTE DX® ANALYZER. **F. Granat, M.-L. Théron, N. Bourgès-Abella**, **A. Geffré, R. Froment, C. Trumel**. Laboratory of Clinical Pathology, Veterinary School of Toulouse, France
Background & objective: To our knowledge hematology reference intervals have not been yet determined in cats with the Procyte DX® (Idexx Laboratories) analyzer. The aim of this study was thus to determine *de novo* feline hematology reference intervals according to CLSI C28-P3. **Material and methods:** 0.5-mL K3-EDTA blood samples were obtained from 95 clinically healthy cats of various breed, both sexes, and 0.5-14 years of age. FeLV and FIV status was tested before vaccination. Blood samples were analyzed on the Procyte DX®. Distributions were tested for normality and reference intervals (2.5-97.5 limits) with their 90% confidence intervals were determined by the nonparametric method with the Reference Value Advisor freeware (http://biostat.envt.fr/spip/spip.php?article 63). **Results:** Only 85 specimens were included because of macroscopic clots, FeLV or FIV positive status or incompletely filled tubes. Cats were mainly European (80/85) between 0.5 and 10 years old, male (47/85) and female (38/85). The reference intervals were [6.5-11.3].10^{12}/L for Red Blood Cells, [95-161]g/L for Haemoglobin, [0.297-0.535]L/L for Hematocrit, [34.5-52.1]fL for Mean Corpuscular Volume, [11.9-15.8]pg for Mean Corpuscular Haemoglobin, [29.3-35.3]g/dL for Mean Corpuscular Haemoglobin Concentration, [20.3-30.6].% for Red blood cell Distribution Width, [3.5-49.4].10^9/L for Reticulocytes, [3.9-21.4].10^9/L for White Blood Cell, [1.4-9.2].10^9/L for Neutrophils x10^9/L, [1.3-10.4].10^9/L for Lymphocytes, [0.1-1.0].10^9/L for Monocytes, [0.1-1.8].10^9/L for

Eosinophils, and [42-505].10^9/L for Platelets. **Discussion & Conclusion:** Reference intervals obtained in this study were close to previously reported data except for leukocytes and neutrophils (wider range). They have been determined according to international recommendations, thus can be used in clinical cases and in interlaboratory comparisons.

IDENTIFICATION OF HYALOMMAANATOLICUMANATOLICUM USING NUCLEOTIDE SEQUENCE ANALYSIS OF SECOND INTERNAL TRANSCRIBED SPACER (ITS2). **H. Haddadzadeh[1], M. Ganjali[1], P. Shayan[2].** [1]Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Iranian Research Center for Tick and Tick-born Disease (IRCTTD).
Background: Ticks are considered as important vectors for transmission of many viral, bacterial, rickettsial and parasitical pathogens. Although many identification keys are available for different species of ticks as well as Hyalommaanatolicum(koch 1844, Hoogstral and Kaiser 1959) but morphological identification keys are always time consuming and need expert peoples. There are permanent problem for tick diagnosis at the species and subspecies levels specially for identification of Hyalomma. excavatum complex members which are prevalent ticks in Iran. Hyalommaanatolicumanatolicum is closely similar to Hyalommaanatolicumexcavatum and this subject has caused some confusion in their identification. **Objective:** To dissolve this kind of difficulties, We decided to use polymerase chain reaction(PCR) for the identification and phylogenetic studies of Hyalommaanatolicumanatolicum. **Method:** In this study the gene sequence of second Internal Transcribed Spacer (ITS2) of Hyalommaanatolicumanatolicum was detected. **Result:** According our finding, nucleotide sequence of ITS2 region of Hyalommaanatolicumanatolicum in Iran has 93% hemology to the other in GenBank registered ITS2 sequence of this subspecies (Accession no FJ593700.1). The length of the ITS2 sequence of Hyalommaanatolicumanatolicum were 963bp in our study. The complete sequence of ITS2 region of rRNA gene from Hyalommaanatolicumanatolicum of Iran registered under accession no HQ123320 by GenBank, respectively. **Conclusion:** The result of this study showed that nucleotide sequence analysis of ITS2 gene can provide a useful diagnostic tool for identification and differentiation of closely related ticks.

PATIENT-BASED FEEDBACK CONTROL FOR ERYTHROID PARAMETERS FOR IN-HOUSE HEMATOLOGY DIAGNOSTIC AUTOMATED ANALYZERS
J.M. Hammond, W.C. Lee, D.B. DeNicola, J. Roche, IDEXX Laboratories, Inc., Westbrook ME
Background: Automated in-house diagnostic analyzers, most commonly used for hematology and chemistry measurement, are generally calibrated and then confirmed with control materials. One potential deficiency with using control materials is that there is no direct knowledge of system performance between control runs, though there is indirect knowledge that the system is performing correctly, such as consistent MCHC. **Objectives**: The purpose of this paper is to describe a method to assess automated analyzer performance using patient samples as they are analyzed and apply correction factors to mitigate instrument-driven biases if they develop. **Methods**: Data analysis and associated algorithm development will be presented to describe a means to collect data, group data into weighted moving average batches, analyze system performance with control chart rules, and apply fuzzy logic when systems are out of control to improve system performance by applying optimized adjustments. **Results**: A total of 102 hematology analyzers that have developed biases in RBC, HCT, HGB, MCV, MCH, and MCHC were evaluated. Correction factors were derived from the patient results utilizing weighted moving averages and optimization logic. Adjustment validations were made by comparison of fresh sample PCV measurements to original and adjusted HCT values. Fundamental assumptions must be met for the approach to assure proper functionality. Repeated samples within a batch and large populations of samples with common abnormalities were excluded. **Conclusions**: The proposed system provides feedback control to minimize system bias in RBC, HCT, HGB, MCV, MCH, and MCHC.

CONJUNCTIVAL CYTOLOGY IN CATS WITH CHLAMODOPHILA FELIS. **A. Hillström[1,2], H. Tvedten[1,2], M. Källberg[2], S. Hanås[2], A. Lindhe[3], B. Ström Holst[1,3].** [1]Swedish University of Agricultural Sciences, Uppsala; [2]Strömsholm Animal Hospital, Strömsholm, Sweden; [3]National Veterinary Institute, Uppsala, Sweden.
Background: When typical epithelial inclusions are detected in cytological samples from cats with conjunctivitis, a presumptive diagnosis of chlamydiosis can be made. **Objectives**: The effectiveness of conjunctival cytology for diagnosing C. felis in cats with conjunctivitis was determined. The duration of clinical signs in cats with cytologic diagnosis of chlamydiosis was evaluated. **Methods**: Conjunctival smears from eighty-eight cats with conjunctivitis were collected and stained with Hemacolor, a quick Romanowsky-type stain. Information about duration of clinical signs was obtained from owners. Cytological examinations were performed blinded by one of the authors (AH). PCR analysis for C. felis was performed on all samples. **Results**: Eight cats were PCR positive for C. felis and all these had inclusions interpreted as chlamydiae in the conjunctival smears. Five of the 8 cats had cytoplasmic inclusions interpreted as chlamydiae with high certainty. The duration of clinical signs was 1-2 weeks in 4 of the cats, whereas 1 cat had a duration of clinical conjunctivitis of 5 weeks. Three cats with longer duration, 4-6 weeks, were suspected to be infected with chlamydiae based on the cytological examination. The suspicion was rather weak because inclusions were few and/or atypical. Eighty cats were PCR negative for C. felis. In 3 of the smears from PCR negative cats, few inclusions suspected to be chlamydiae were identified. **Conclusions**: Cytology was effective in diagnosing chlamydiosis in cats with distinct inclusions and a duration of clinical signs shorter than 2 weeks. Cytologic diagnosis of chlamydiosis in C. felis PCR positive cats with disease duration longer than 2 weeks was less certain.

MONITORING OF PRE-ANALYTICAL ERRORS USING A LIS-INTEGRATED ERROR MANAGEMENT SYSTEM IN AN ISO-CERTIFIED VETERINARY DIAGNOSTIC LAB. **E. Hooijberg, E. Leidinger.** InVitro Labor, Vienna, Austria.
Background: Various quality management systems, including the standards ISO 9001 and ISO 15189 (medical laboratories), require that laboratories have processes in place for identifying and controlling non-conformities and errors as part of a quality management system. Our laboratory has an error-reporting and management system integrated into the laboratory information software which has been in use for the past 8 years. Errors are recorded according to type, with 15 categories covering pre-, intra- and post-analytical errors defined. Pre-analytical errors have been shown to comprise 46-68% of all errors in studies in human medical laboratories. **Objectives**: An evaluation of the amount, types and frequencies of pre-analytical error recorded from 2003 to 2010 was performed. **Methods**: The total pre-analytical error, pre-analytical error as a percentage of total error and the percentage of each sub-type of pre-analytical error was calculated for each year from 2003 to 2010 using data recorded in the error management program. Results were displayed graphically and annual percentages compared. **Results**: The mean number of recorded errors per year was 374. Annual pre-analytical error ranged from 52-87% (median: 71%) of total errors recorded. Most pre-analytical errors arose from mistakes during data entry (21-51%), followed by sample unpacking and labeling (15-47%) and in-house courier service errors (7-27%). **Conclusions**: The percentage of pre-analytical errors recorded in our laboratory is higher than that reported in human medical literature. Reasons are unclear; comparison of results with those from other veterinary laboratories could provide further information as to whether this is a phenomenon specific to this laboratory or a finding associated with veterinary laboratories in general. The majority of recorded pre-analytical errors arise during sample handling and data entry.

LEPTIN SECRETION BEFORE AND AFTER TREATMENT OF CANINE PRIMARY HYPOTHYROIDISM. **L. Jaillardon, M. Klett, S.B. Oniris**. Department of Biology, Pathology and Food Sciences, Nantes, France.
Background: Leptin is closely related to body weight (Sagawa et al., 2001) and increases in canine primary hypothyroidism (Mazaki-Tovi, 2010). The effect of primary hypothyroidism treatment on leptin values has not been studied to date. **Methods**: 55 hypothyroid dogs (median 5 years), including 29 females (17 neutered) and 26 males (8 castrated), treated for 3 months with thyroxin (µg/kg/day). Treatment efficiency was scored with a 30% decrease in c-TSH value and satisfactory clinical improvement. Inclusion criteria: Clinical and biological signs of primary hypothyroidism i.e. lethargy, body weight (BW) gain, dermatologic signs, high cholesterol (>6.5 mmol/L), high c-TSH (>0.5 ng/mL) and low fT4 values (≤12 pmol/L). **Assays**: Radioimmunoanalysis (fT4: Immunotech kit1363, leptin: Millipore, Canine Leptin ELISA kit) and chemiluminescence (c-TSH: Siemens Immulite CanineTSH). **Results**: Results are expressed as the median[range]. 40 dogs exhibited a good therapeutic response (group A, dose=16.2[7.4-37.2], pre-therapy BW=38[6-69], fT4=10[0-12], c-TSH=1.5[0.6-9.1] vs. post-therapy BW=38[7-70], fT4=22[13-38], c-TSH=0.4[0.03-4.8]; p<0.001) and 15 dogs were insufficiently-treated (group B, dose=10[6.2-13.3], pre-therapy BW=40.5[20.5-54.0], fT4=10[7-12], c-TSH=1.0[0.6-4.3] vs. post-therapy BW=38[26-51], fT4=16[13-28], c-TSH=1.0[0.5-4.2]; p<0.05 except for c-TSH, NS). Before treatment, leptin (ng/mL) significantly increased in group A compared to B (12.9[0.6-107] vs. 7.2[2.2-26.3], p=0.007), was significantly negatively correlated with fT4 (p=0.038, r=-0.34) and significantly decreased after treatment only in group A (12.9[6-107] vs. 6.9[0-31.8], p<0.0001). No correlation was found between BW and leptin or between delta BW and delta leptin, whatever the group. **Conclusions**: Leptin secretion significantly decreased 3 months after treatment of canine primary hypothyroidism only in dogs exhibiting a good therapeutic response, without a significant correlation with BW change. This study highlights that the relationship between leptin secretion and BW might change when the thyroid state is altered.

MAMMARY ADENOCARCINOMA IN A 14 YEARS OLD DOMESTIC SHORT HAIR FEMALE CAT. **M. Jalali, N. Atyabi, P. Yasini, R. Shafiee.** Department of Clinical Pathology, College of Veterinary Medicine, University of Tehran, Iran.
Background: Mammary tumors have not been commonly reported in cats in Iran. If presented, the majority of them usually are malignant. A 14 years old domestic short hair queen with mammary tumor was presented to the small animal hospital, college of veterinary medicine, university of Tehran. Cytology and histopathology were conducted to

diagnose and classify mammary tumor. **Methods**: The tumor mass was seen in cranial abdominal mammary gland. It was 8 cm in diameter. The mass was surgically excised and impression smears was prepared and stained with Giemsa for cytologic study. The rest of the tissue was subjected to histopathologic evaluation. **Results**: Cytologic examination revealed high cellularity, composed predominately of neoplastic secretory and ductular mammary epithelial cells. The cells were characterized by increased nuclear-to-cytoplasmic ratio; marked anisocytosis and anisokaryosis; nuclear molding; coarse nuclear chromatin; binucleation and large, prominent, multiple nucleoli. Punctate cytoplasmic vacuoles were observed in a number of secretory cells. Acinar formation was also present. Background was containing marked amounts of red blood cells, neutrophils, basophilic proteinaceous material, and foam cells. **Conclusion**: The mammary adenocarcinoma was diagnosed based on Cytologic and histopathologic evaluation.

MEASURING THE IMMUNOGLOBULIN G CONCENTRATION IN CALVES SERUM WITH ELISA AND BIOCHEMICAL ANALYSER – COMPARISON OF METHODS. **J. Ježek, M. Nemec, J. Starič, M. Klinkon**. Clinic for ruminants, Veterinary faculty, University of Ljubljana, Slovenia.
Background: It is well known that it is crucial for calves to absorb colostral immunoglobulins shortly after birth in order to provide immunity until their own immune system sufficiently develops to provide protection. **Objective** was to compare two different methods for measuring the immunoglobulin G (IgG) concentration in calves' blood serum; measuring with ELISA (enzyme linked immunosorbent assay) and with a biochemical analyser. **Methods:** Measurements were performed on 166 samples of calves' blood serum. Concentration of IgG was measured with ELISA Quantitation Kit (Bethyl Laboratories, Great Britain) according to manufacturer's instructions. All measurements were performed the same day. Results were compared with measurements carried out on biochemical analyser Cobas Mira (Hoffman La Roche). IgG concentration was measured with reagents from producer Midland Bioproducts made for measuring IgG concentration in bovine serum and their software (application) for Cobas Mira. For comparison of results of both methods the correlation coefficient was calculated with SPSS (version 15). For calculation of agreement the method by Bland and Altman (1986) was used. **Results:** The mean concentration of IgG measured with ELISA (23.06 ± 14.02 g/L) was higher than IgG concentration measured with biochemical analyser (11.91 ± 6.80 g/L). The correlation coefficient was 0.611. With calculation of agreement it was established that ELISA gave on average 11.15 ± 11.24 g/L higher results than the biochemical analyser. **Conclusions:** Higher concentrations measured with ELISA were most likely due to influence of standard which is producer specific producer. Method differences should be considered by interpretation of results. Advantages of biochemical analyser methods are their automation and appropriateness for single samples.

BONE MARROW ANALYSIS WITH THE SYSMEX XT-2000iV HEMATOLOGY ANALYZER IN DOGS AND COMPARISON WITH MICROSCOPICAL EVALUATION. **I. Kampfmann[1], N. Bauer[2], S. Johannes[1], M. Ginder[3], T.Sakata[4], A. Moritz[2]**. [1]Institute of Toxicology, Merck Serono GmbH, Darmstadt, Germany; [2]Department of Veterinary Clinical Sciences, Clinical Pathology and Pathophysiology, Justus-Liebig University, Gießen, Germany; [3]Sysmex Europe GmbH, Norderstedt, Germany; [4]Sysmex Corporation, Kobe, Japan.
Background: Bone marrow is routinely assessed by microscopic evaluation. But this method is time consuming and results depend and vary on the experience of the investigator. Therefore, a standardized automated measurement of bone marrow samples might be useful. **Objectives**: The aim of this study was to compare bone marrow samples analyzed with the automated laser based hematology analyzer Sysmex XT-2000iV with standard microscopic evaluation. **Methods**: Sternal bone marrow were obtained from 90 beagle dogs during necropsy and measured with the Sysmex XT-2000iV hematology analyzer. Samples were assayed in the WBC/BASO channel and reanalyzed after creating a template for the XT-2000iV software. 10-run intra assay precision was calculated for the analyses with the Sysmex XT-2000iV. Resulting populations were total nucleated red blood cells, total neutrophils, poly-/orthochromatic red cells and mature neutrophils. 500-cell differential counts were prepared on May-Grunewald-Giemsa stained bone marrow smears. For both methods, the M:E ratio was calculated. **Results**: Intra-assays CVs were ranging between 0.95% and 2.03%. Good correlation was found for the M:E ratio (Spearman´s rho rs = 0.91). The correlations for the other compared cell population were fair (rs = 0.65 to 0.71). Bias was -0.11% for M:E ratio, 7.10% for total neutrophils, -7.71% for immature erythrocytes, 14.67% for mature neutrophils and 0.76% for total NRBCs (Bland-Altman analysis). **Conclusions**: Especially for the assessment of M:E ratio, the automated analysis provides a rapid reliable result and is a useful tool to get an immediate, objective overview of the marrow's cell status.

IDENTIFICATION OF SERUM BIOCHEMICAL PARAMETERS OF DAIRY CATTLE FED WITH HERBAL ADDITIVES WHICH KNOWN AS MILK PRODUCTION INCREASER. **Z. Khaki, M. Jalali, F. Gharagozloo and M.Vojgani.** Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Background: Fennel (Foeniculum vulgare) and Anise (Pimpinella anisum) are plant species that was suggested to act as phytoestrogens and have potentially beneficial effects on milk production. **Objectives**: The aim of this experiment was to investigate the effects of the herbal galactagogue on milk production and some serum biochemical parameters in dairy cows. **Methods**: 102 healthy cows were completely randomized to treatment and control groups (51 heads in each group). All animals were fed from fibers and concentrates diet but in treatment groups from day 20 after parturition enriched with 180 g/day galactogogue and continued for a period of 45 days. In both groups each cow's milk production was recorded, and Milk and blood samples were taken every 15 days. Serum biochemical parameters including urea, creatinine, uric acid, triglyceride, cholesterol, HDL, LDL, VLDL, total protein, albumin, amylase, GGT and AST were assessed. The data obtained was analyzed statistically using SPSS software and P value <0.05 was considered significant. **Results**: In treatment group serum cholesterol concentration on the day 60 of experiment and serum uric acid on day 45 were significantly lower than control group. On the other hand, serum total protein concentration on the day 45 and 60 and serum amylase activity on the day 60 were significantly higher in treatment group. There was no significant difference in milk production rate between two groups. **Conclusion:** We concluded that using of herbal galactogogue has considerable effects on serum biochemical markers. It may need further investigations to evaluate the efficacy of herbal drug on lactational performance by using different amounts of it and/or prolonged duration of consumption.

A SURVEY ON IMHA IN ANEMIC CATTLE. **P. Khazraiinia[1], S.M. Nassiri[1], S. Darvish[1], H. R. haddadzade[2], S. Sattari[1]**[1]. [1]Department of clinical pathology, Faculty of veterinary medicine, University of Tehran, Tehran, Iran; [2]Department of parasitology, Faculty of veterinary medicine, University of Tehran, Tehran, Iran.
Background: Immune mediate hemolyticanemia is a primarily or secondary to other disease, disorder that leads to red blood cells hemolysis. **Objective:** There is low incidence of reported cases of Immune Mediate HemolyticAnemia (IMHA) in cattle. **Methods:** In this study 42 anemic cattle with common clinical signs of anemia: pale mucosa membrane, lethargy, weakness, anorexia and PCV<27.5 were investigated for IMHA. These cattle were referred to the large animal hospital of veterinary faculty, university of Tehran during 10 months period. The blood samples of anemic cattle were subjected to direct Combs test. **Results:** 13 anemic cases of 42 cows were positive in Combs test (30%). The mean age of the animals (11 males and 31 females) was 3.5 year. They were evaluated for complete blood count and osmotic fragility test. Combs positive cattle's showed lower PCV compare to combs negatives (16±1.7% versus 21±0.8%). The hematological and biochemical findings in cattle with IMHA included: anisocytosis (in 2 cattle), reticulocytosis (in 2 cattle), spherocytosis (in 2 cattle), hyperfibrinogenemia (in 5 cattle), and left-shifted neutrophilia. Four osmotically different populations of erythrocytes were detected in cattle with IMHA whereas the populations of erythrocytes in other types of anemia were the same in fragility. **Conclusion:** A significant proportion of anemia in cattle had immune-mediated mechanism. Certain infectious diseases and administration of certain drugs were identified in association with IMHA in this study.

CHARACTERISATION OF THE ACUTE PHASE REACTION. **M. Kjelgaard-Hansen**. Department of Small Animal Clinical Sciences, University of Copenhagen, Denmark.
When systemic inflammatory activity is suspected, not only the detection, but also a characterisation of the possible acute phase reaction (APR) is important. Studies on critically ill animals have found that measuring the mere presence and degree of pro-inflammatory activity without concurrent assessment of the balance and character may be an inadequate measure of APR. The PIRO model for staging sepsis, which was presented by the '2001 International Sepsis Definitions Conference', is a very relevant skeleton for organizing such a characterisation. The PIRO model is meant as a tool for generating hypothesis and for stratifying septic patients in clinical intervention trials. The concept of PIRO, is to address the pathophysiological process/state in four categories; Premorbid factors, Insult, Response and Organ dysfunction. Although PIRO was designed to stage septic processes, using it in the wider context of APR is just a matter of including other types of Insults than infections. Traditionally, most veterinary clinical pathology APR markers are within the Response category with an obvious focus on pro-inflammatory factors, such as quantification of cytokines, cytokine-induced changes of e.g. hepatic protein synthesis (the acute phase proteins) and bone-marrow leukopoiesis. The use of biomarkers to characterize Organ dysfunction as a sequel to the illness is well established for most important organs. However, when studying critical illnesses in animals, PIRO can be a useful tool for identifying markers that complement the present routine APR markers and thus facilitate a more complete characterisation of the APR in such animals. This includes, routine Response markers for anti-inflammatory activity, markers stratifying Insult into processes such as aseptic/septic and perhaps a more detailed focus on markers of Premorbid factors.

GENERATION, INTERPRETATION AND APPLICATION OF DATA ON BIOLOGICAL VARIATION. **M. Kjelgaard-Hansen**. Department of Small Animal Clinical Sciences, University of Copenhagen, Denmark.
Measurements of biomarkers for diagnostic and toxicology purposes in animals are subjected to both biological (i.e. within-animal and between-animal) and analytical variations. Detailed knowledge on these different levels of variability is beneficial in multiple aspects of clinical pathology, e.g. assay development, validation, quality control and diagnostic interpretation. The theoretical background assessing the various components is that fluctuations observed in repetitive measurements of the same sample (imprecision) is randomly and normally distributed around a mean (the true concentration), like the fluctuations across repetitive observations are in the same healthy individual (intra-individual variation) fluctuating around a mean (homeostatic set-point) and finally like variations observed across individuals (intra-individual variation) are around a population mean. Thus, a nested study design with repetitive measurements of repetitive samples of multiple healthy individuals in a population will facilitate assessment of the three levels of variability. Population-based reference intervals are also generated for diagnostic purposes by means of verified healthy individuals, however based on single point measurements. Data on biological variation must be viewed as a very valuable supplement, as the mere relevance of these reference intervals depend on the ratio between intra- and interindividual variation, as this determines the diagnostic capacity of a -based reference interval. Data on biological variation are widely used in human laboratory medicine to establish critical differences for diagnostic interpretation, to assess the utility of population-based reference intervals and to set objective analytical performance standards for laboratory tests, and it has been advised to routinely estimate and analyze data on biological variation of quantities considered implemented for routine application.

CARDIAC TROPONIN I CONCENTRATION IN NATURALLY-OCCURRING CANINE MONOCYTIC EHRLICHIOSIS. **C. Koutinas[1], M. Mylonakis[1], P. O'Brien[2], L. Leontides[3], V. Siarkou[4], E. Breitschwerdt[5], A. Koutinas[1].** [1]Companion Animal Clinic, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece; [2]Veterinary Science Centre, College of Life Sciences, School of Agriculture, Food Science; and Veterinary Medicine, Ireland; [3]Laboratory of Epidemiology, Biostatistics and Animal Health Economics, School of Veterinary Medicine, University of Thessaly, Greece; [4]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki; and [5]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, USA.
Background: Clinical and subclinical myocardial injury has been implicated in canine monocytic ehrlichiosis (CME, Ehrlichia canis). **OBJECTIVES**: To investigate whether serum cardiac troponin I (cTnI) concentration, measured on admission, was associated with the clinical phase of CME, or was predictive of the clinical outcome (survival or death). **Methods**: cTnI concentration was compared among 22 dogs with nonmyelosuppressive CME (group A), 22 dogs with myelosuppressive CME (group B) and 10 healthy dogs (group C). Diagnosis of CME was based on clinical and clinicopathological findings, E. canis seropositivity, PCR amplification of E. canis DNA and/or the microscopic observation of Ehrlichia sp. morulae. **Results**: A total of 10/22 group A, 13/22 group B and none group C dogs had increased serum cTnI concentration (cut-off value: ≤0.052 μg/L). There was no difference in the frequency or mean cTnI concentrations between groups A and B. Mean cTnI concentration was significantly lower in healthy dogs, as compared with groups A or B. Using a linear regression model, there was no association between cTnI concentration and clinical outcome. **Conclusions**: Increased cTnI concentration signifies myocardial damage in dogs naturally infected by E. canis. However, cTnI on admission is not a useful indicator of the clinical severity or a predictor of clinical outcome.

BIOLOGICAL VARIATION OF HEMOSTATIC PARAMETERS IN HORSES. **S.H. Laursen[1], P.H. Andersen[1], M. Kjelgaard-Hansen[2], B. Wiinberg[2],** [1]Department of Large Animal Sciences; and [2]Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark.
Background: The result of the coagulation analyses aPTT, PT and fibrinogen in horses are traditionally evaluated by comparing to a normal reference range, however no studies to date have evaluated the suitability for applying such reference ranges in horses. Studies on biological variation provide detailed information on the components of variation, evaluate performance standard and determine the suitability of population-based reference intervals for clinical purposes. **Objectives:** To set objective analytical performance standards for aPTT, PT and fibrinogen in healthy horses and to assess applicability of population-based reference limits for those parameters**. Methods:** Eight healthy adult mares were sampled for five consecutive weeks on the same weekday and time. Plasma samples were frozen at -80°C and analyzed in one batch on an ACL Top 500 using the standard reagents supplied by the manufacturer: aPTT (aPTT SynthAFax, Instrumentation Laboratory), PT and Fib (PT-Fibrinogen, Instrumentation Laboratory). The components of biological variation were estimated by a nested variance analysis as described by Fraser and Harris (1989). **Results:** The observed CV_A; CVmax for each parameter were: aPTT = 0.9%;1.0%, PT = 2.2%;0.7% and Fibrinogen = 2.3%;3.6%. Both aPTT and PT showed intermediate individuality ($1,4>R_{II}>0,6$), whereas fibrinogen was the only parameter with high individuality ($R_{II}<0,6$) in this study. **Conclusions:** Overall aPTT, PT and fibrinogen showed a low analytical variation. Nevertheless, PT did not live up to the objective performance standards. Both aPTT and PT showed intermediate individuality, indicating population-based reference intervals can be considered acceptable clinical interpretation criterion for these parameters, whereas fibrinogen showed high individuality in this study indicating that critical difference may be a more suitable interpretation criterion.

URINE SEDIMENT: AN EVIDENCE-BASED APPROACH. **E. Leidinger, J. Leidinger**. Invitro Diagnostic Lab, Vienna, Austria.
The evaluation of urine sediment is incontrovertibly an indispensable method for the evaluation of the urinary tract. However, surprisingly few publications are available on this topic in veterinary medicine. **STABILITY OF URINE SAMPLES** Many textbooks recommend that urine sediment should be analyzed within 6-8 hrs, although interestingly the new ASVCP QC guidelines state that urine can be examined within 24 h without loss of clinical information in many cases. This correlates with our experience; however some structures like casts are more unstable. **COMPONENTS OF URINE SEDIMENT** RBC: according to our data detection of blood (dipstick) and RBC (sediment) correlate well in dogs and cats even in samples >24 hrs old. There is no evidence that the discrimination between eumorphic and dysmorphic RBC is useful in veterinary medicine. **LEUKOCYTES**: leukocytes degenerate rapidly in urine and may resemble small epithelial cells and lyse in hyposthenuric or alkaline urine. The lysis of leukocytes does not cause measurable proteinuria. BACTERIA: more than 10,000 rods/μl (100,000 cocci) must be present in order to be detected in urine sediment. A quantitative urine culture is recommended in all cases of pyuria and/or bacteriuria, Isolation of more than 2 bacteria species frequently indicates contamination. **EPITHELIAL CELLS**: may originate from the kidney, the urinary tract collection system and/or the genital tract. A transitional epithelial carcinoma may be suspected from a sediment wet-prep but has to be confirmed by a sediment smear stained with a cytology stain. CASTS: casts are the most fragile elements in urine; they always originate from the renal tubuli and the rare RBC and leukocyte casts are always significant. CRYSTALS: there is little evidence associating the occurrence of urine crystals with calculi. Calculi can be present without finding crystals in urine.

ADVIA 2120 PLATELETCRIT FOR ASSESSING PLATELET STATUS IN CAVALIER KING CHARLES SPANIELS. **I. Lilliehöök[1], H. Tvedten[2], J. Öberg[1], I. Ljungvall[2], J. Häggström[2]**. [1]University Animal Hospital and [2]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.
Background: The plateletcrit method of the IDEXX VetAutoread hematology analyzer (QBC) is the best way to evaluate platelet status in dogs with very large platelets such as Cavalier King Charles Spaniels (CKCS) with macrothrombocytosis. It is unknown if Adviaplateletcritis accurate in CKCS. **Objective**: To test if the plateletcrit (A-PCT) of the Advia 2120 (Siemens Healthcare Diagnostics) could be used to evaluate platelet status in CKCS with and without macrothrombocytosis. **Methods**: Fresh EDTA blood samples from 43 CKCS in a study of myxomatous mitral valve disease were analyzed with Advia 2120 and compared with the QBC plateletcrit, which is reported as a platelet count (QBC PLT). **Results**: The number of CKCS considered thrombocytopenic varied with method. Advia PLT counts were under 150 x 10^9/L in 23 CKCS (53%), A-PCT was under preliminary reference values of 0.18-0.44 in 9 (21%) CKCS, while no CKCS had a QBC PLT count under 150 x 10^9/L. A-PCT agreed quite well with QBC (correlation r = 0.84), but appeared to underestimate PCT in CKCS with pronounced macrothrombocytosis. **Conclusions**: Adviaplateletcrit should reflect the platelet status of dogs with large platelets fairly well but seem to underestimated plateletcritin CKCS with pronounced macrothrombocytosis and thus overestimate how many CKCS that have decreased platelet volume. The Advia PCT is, however, much better than the platelet count to evaluate platelet status in dogs with macrothrombocytosis.

QUANTITATIVE CYTOMORPHOMETRY OF ROMANOSKY-STAINED SMEARS FOR THE DIAGNOSIS OF CANINE LYMPHOMA. **A. Lynch, S. Papakonstantinou, P.J. O'Brien**. Veterinary Clinical Pathology Laboratory, and Advanced Diagnostics Laboratory, UCD, Ireland.
Background: Fine needle aspirates are used for cytological diagnosis of canine lymphoma. Non-neoplastic lymph nodes are mainly composed of small lymphocytes (nuclear diameter 1-1.5 RBCs) and <15% cells are medium or large (nuclear diameter >2-2.5 RBCs). >50% medium to large cells is a reliable threshold for cytological diagnosis of lymphoma. **Objective:** to evaluate use of quantitative cytomorphometry for diagnosis of large cell canine lymphoma. **Methods:** Romanowsky-stained smears from normal or reactive lymph nodes (n=5) and large cell lymphomas (n=6) were examined using 50x and 100x objectives and images were analyzed using imaging software (Cell B, Olympus). Cell and nuclear length (largest diameter across cell or nucleus) and width (largest diameter perpendicular to length) were measured. Red blood cell and neutrophil diameters were used as calibrators**.** Statistical analysis was performed using GraphPad Prism 5 (La Jolla, USA). **Results:** 73.6±34.6 (mean±SD), well-preserved, well-stained cells were analyzed in each sample. For normal or reactive lymph nodes, nuclear length was 7.83±0.7 μm, nuclear width 6.9±6 μm, cell length 8.8±0.8 μm and cell width 7.3±0.7 μm.

Neutrophil diameter was 11.4±1.0 µm (n=38 cells) and red blood cell diameter was 7.3±0.6 µm (n= 251 cells). For lymphoma samples, nuclear length was 9.6±1.0 µm, width 8.4±1.0 µm, cell length 11.45±1.7 µm and width 9.5±1.1 µm. Bonferroni mutliple comparison test showed statistically significant differences ($\alpha<0.05$) for all parameters between normal and cancerous lymphocytes ($P<0.0001$). Normal or reactive lymph nodes had ≤17.3% lymphocytes with diameter > 1.5 RBCs, whereas all the lymphoma cases had ≥50% (maximum of 96.3%). **Conclusion**: Quantitative cytomorphometry was determined to be an efficient and reliable technique that can be readily applied for diagnosing large cell canine lymphoma.

CLINICAL BIOCHEMISTRY FOR SAFE, EFFECTIVE CONDITIONING OF HORSES. **A. Lindner.** Arbeitsgruppe Pferd, Juelich**,** Germany.
Blood lactate (LA) measurements provide a means to guide the exercise intensity to condition horses. Several studies have been published on the effect of different LA guided exercise conditions. So far the most effective have been 45 min at v2 3 times a week and 2 x 5 min at v10 2 times a week (v2, v10 = speed at which under defined conditions the blood lactate concentration (LA) reaches 2 or 10 mmol/l. The experimental training studies as well as the training in practice needs to stress the horses sufficiently to prepare them for competition without harm. Therefore biochemical variables have been and are being sought to control for excessive stress that could lead to overtraining and health impairment. The technical side of the analysis of biochemical variables is carefully studied in most cases, but not the value of the measurements for the practice. This is because studies on the best timing for collecting the samples in relation to exercise for optimizing diagnosis are seldom done.

HIGH CONTENT ANALYSIS OF INTRACELLULAR BIOCHEMISTRY OF LYMPHOID NEOPLASIA FOR DIAGNOSTIC AND PROGNOSTIC CLINICAL PATHOLOGY BIOMARKERS. **P. Lyons, P.J. O'Brien**. UCD Veterinary Science Centre, Dublin, Ireland.
Objectives: 1) To establish whether high content analysis cytotoxic biomarkers are effective *in vivo* using non-adherent, peripheral blood lymphocytes from lymphomatous dogs, while being treated with chemotherapeutic drugs. 2) To determine a reference range for these biomarkers. 3) To determine whether drug-induced cytotoxicity of the lymphocytes could be detected *in vivo* both during and immediately prior to chemotherapy. 4) To determine whether a biphasic (hormetic) response occurs in lymphocytes *in vivo*. **Methods**: Four fluorescent dyes were used to demonstrate these biomarkers. They included Hoechst for DNA, Fluo-4 for calcium, tetramethylrhodamine methyl ether for mitochondrial membrane permeability (MMP) and TOTO3 for plasma membrane permeability. 12 controls, 8 2 week post-chemo and 3 24 hr post chemo dogs were analysed. **Results**: Statistically significant changes were seen in MMP total area and intensity, and nuclear area and intensity. A decrease of 34.8% in MMP total area was seen in dogs treated with chemotherapy 24 hours prior. In contrast, there was then an increase of 11.4% in dogs treated two weeks prior. The same pattern was seen for MMP total intensity, but with a decrease of 30% followed by an increase of 11.4%. A large decrease of 43.5% in nuclear intensity was seen in dogs treated with chemo 24 hours prior. In contrast, there was then a large increase of 56.6% in dogs treated two weeks prior. **Conclusion**: This study demonstrated. 1) reference range for these biomarkers. 2) High content analysis can be applied to non-adherent live peripheral lymphocytes. 3) This in vivo model was at least as sensitive as an in vitro model. 4) Hormesis was seen in the lymphocytes with response to chemotherapy.

CANINE RETICULOCYTE DYNAMICS SURROUNDING ANESTHETIC EVENTS. **F. Metzger[1], J.A. Christian[2], D.B. DeNicola[2],** [1]Metzger Animal Hospital, State College, PA, [2]Purdue University School of Veterinary Medicine, West Lafayette, IN, [3]IDEXX Laboratories, Inc, Westbrook, ME
Background: Reticulocytosis is occasionally observed in nonanemic animals using in-house hematology analyzers that provide reticulocyte counts (RC) on all dog complete blood counts (CBC). A role for the spleen in retaining and conditioning reticulocytes has been suggested for many years and physiologic transient release during splenic contraction and retention/pooling during splenic congestion may be contributing to changing RC. **Objective:** The purpose of this study was to indirectly and non-invasively investigate the potential role of the spleen on reticulocyte counts. **Methods:** CBCs from 12 nonanemic dogs presented for various reasons (4 spay/neuter surgeries, 4 prophylaxis dentals, 2 orthopedic surgeries, 1 mass removal and 1 myelogram) were used. For each dog a CBC was performed before, during and after the associated anesthetic event. Animals with relatively high initial RC (72.3-173.4 $x10^3$/ µL; reference interval, 6-110 $x10^3$/µL) were selected for evaluation. CBCs including RC were performed on the IDEXX ProCyte Dx® Hematology Analyzer. Changes in RC were compared to respective RBC counts. **Results:** Before, during and after anesthesia, respectively, average (range) RC were 102.0 (72.3-173.4), 35.4 (15.1-63.2) and 50.1 (10.2-109.1) $x10^3$/µL and average (range) RBC counts were 6.74 (4.23-9.27), 5.12 (3.24-6.88) and 5.96 (3.83-7.67) $x10^6$/µL. Anesthesia period RC decreased to 36.6% of baseline preanesthetic values; RBC counts decreased to only 76.4% of baseline. Recovery period RC returned to only 53.2% of baseline while RBC counts returned to 88.5% of baseline.
Conclusion: Dramatic changes in RC can be observed surrounding anesthetic events in the dog. Excitement-associated splenic contraction and anesthesia-associated splenic blood pooling may be associated with these dynamics and may prove helpful in explaining certain physiologic causes of reticulocytosis.

MONITORING OF HEPARIN TREATMENT. **R. Mischke.** Small Animal Clinic, University of Veterinary Medicine, Hannover, Germany.
Unfractionated (UFH) and low molecular weight heparins (LMWH) are important standard short acting anticoagulants in small animal medicine. Adequate laboratory monitoring is a challenge and there is an ongoing debate on indications and methods in human and in veterinary medicine. Subcutaneous administration of UFH in dogs and cats is associated with a great individual variation of anticoagulant effects. Therefore, frequent laboratory testing is necessary especially in the initial phase of treatment with high dosages of UFH. Activated partial thromboplastin time (APTT) is non-specific to heparin, but may better reflect the in vivo anticoagulatory effect than the chromogenic substrate assays, which represent only specific aspects (anti- factor [F] IIa or –ant-FXa activity) of the anticoagulatory potential (target range in humans: 0.3–0.7 IU/ml). Heparin sensitivity differs significantly between different APTT reagents, which supports the necessity to calibrate the specific test. Measurement of anti-factor Xa activity has been established as the gold standard for LMWH monitoring. However, laboratory monitoring of established, body weight adjusted LMWH treatment protocols is not routinely performed in humans and current guidelines in human medicine advise determination of anti-Xa in specific conditions with markedly altered LMWH metabolism (e.g. pregnancy, extreme body weights, and renal insufficiency). The reasons therefore are: (1) high bioavailability of subcutaneously (SC) administered LMWH due to reduced binding to proteins and cells results in predictable heparin plasma activities, and (2) limited correlation between peak plasma activities and antithrombotic effect, patient`s clinical outcome and bleeding risk indicate anticoagulatory effects apart from the anti-Xa activities. Methods aiming to determine a broader spectrum of LMWH depending anticoagulant activity (e.g. viscoelastic testing, thrombin generation) are supplementary tools for LMWH monitoring.

PLATELET PATHOLOGY. **R. Mischke.** Small Animal Clinic, University of Veterinary Medicine Hannover, Hannover, Germany.
This review focuses on pathogenesis and diagnosis of quantitative and qualitative platelet disorders in small animals. Thrombocytopenia is clinically more important and one of the most important causes of haemorrhagic diathesis. Possible pathomechanisms include reduced production due to bone marrow diseases (e.g. hypoplasia, leukaemia, myelodysplasia) or increased turnover (loss, distribution abnormalities [splenomegaly, septicaemia] or consumption [immune-mediated, DIC, vasculitis]). Various laboratory tests help to verify the underlying pathomechanism and disease. Bone marrow examination does not only assess thrombopoietic activity, but may also deliver further hints on the underlying disease. Increased mean platelet volume can indicate increased thrombopoietic activity, but may be low in primary immune-mediated thrombocytopenia despite high thrombopoietic activity. Reticulated platelets are an alternative parameter of thrombopoietic activity. Flow cytometric detection of platelet-associated antibodies is helpful, but this test does not differentiate between primary and the more frequently occurring secondary immune-mediated thrombocytopenias. Whereas primary functional abnormalities of platelets (e.g. Glanzmann`s thrombastenia; signalling defects, storage pool deficiency, and Chediak-Higashi syndrome) are extremely rare in animals and humans, acquired platelet dysfunctions are more frequent and occur associated with many disorders (e.g. hepatopathy, monoclonal gammopathy) and after application of anti-platelet agents (aspirin, clopidogrel) and other drugs (NSAIDs, dextran, antibiotics). A wide variety of platelet function tests is available to characterize functional platelet disorders which, however, often lack standardisation. Capillary bleeding time assesses overall capacity of primary haemostasis. The clinical use of the platelet function analyser PFA 100 is limited, because the result depends significantly on the haematocrit. When compared to the turbidimetric method, the impedance platelet aggregometry is easier and faster to measure, requires smaller sample volumes and may also better reflect in vivo platelet functionality. Additional platelet function tests include flow cytometry, thrombelastometry and mean platelet component.

GENETIC ANALYSIS OF HAEMOPHILIC HAVANESE DOGS. **R. Mischke[1], P. Kühnlein[2], A. Kehl[2],M. Jahn[2], R. Ertl[3], D. Klein[3], A. Cecil, T. Dandekar, E. Müller[2].**
[1]Small Animal Clinic, University of Veterinary Medicine Hannover, Hannover, Germany; [2]LABOKLIN, Labor für Klinische Diagnostik GmbH, Bad Kissingen, Germany; [3]VetCore Facility for Research, University of Veterinary Medicine Vienna, Vienna, Austria; and [4]Department of Bioinformatics, Theodor-Boveri-Institute, University of Würzburg, Würzburg, Germany.

Objective: to define the underlying genetic defect of a mild form of haemophilia A in Havaneses. Genetic studies were performed on two phenotypically affected male dogs (factor VIII: 10 and 15%), three carriers with subnormal factor VIII activity and different controls (29 normal Havaneses, 100 dogs of different breeds). **Results:** Comparison of the entire coding region of the canine factor VIII DNA sequences and exon-intron junctions from affected dogs with the wild type canine factor VIII DNA revealed a tRNA-derived SINE (*s*hort interspersed *e*lement) insert of 220 bp in exon 14 of the canine factor VIII gene. The mutation occurs at nucleotide 2675 of the factor VIII cDNA and at nucleotide 580 of exon 14, respectively. The insert was flanked by a 16-bp target site duplication. The open reading frame of the duplication and insert contains 58 amino acids before the first of three stop codons. All affected dogs were hemizygous for this mutation and carriers were heterozygous, whereas none of the other dogs showed the mutation. No further alterations in the sequences of affected dogs could be observed. In the samples of an affected dog less exon 14 mRNA was produced and the whole amount of exon 14 mRNA contained the insert. Protein modelling of the translated factor VIII molecule indicated some residual functional activity. **Conclusions:** The described SINE exonic insertion in the factor VIII gene is responsible for haemophilia A in Havaneses.

CLINICAL SIGNS, HEMATOLOGY AND COAGULATION PROFILE IN HORSES IN RESPONSE TO VACCINATION. **A. Muñoz[1,2], C. Castejón-Riber[2], S. Agüera[2], A. Galán[1], I. Mesa[1], C. Riber[1,2].** [1]Medicine and Surgery Dpt.; [2]Equine Sport Medicine Centre, Córdoba University, Spain.
Background: A link between inflammation and coagulation was suggested in dogs, but it has not been investigated in horses. Vaccination in humans is used as a standardized mild inflammatory stimulus for experimental studies. **Objectives:** This research aims to assess the inflammation caused by vaccination (tetanus and influenza) in 8 adult horses and its relationships with coagulation profile. **Methods:** Rectal temperature (RT), heart rate (HR), respiratory rate (RR), pain, nodule o heat in the site of the injection were recorded and venous blood samples were taken before vaccination, and at 4, 8, 12, 24, 28, 32, 26, 48, 60, 72, 84, 96 and 108 hrs after vaccination. In blood with EDTA, total WBC and subsets, hemoglobin, microhematocrit and platelets were measured. Fibrinogen (FIB), prothrombin time (PT) and activated partial thromboplastin time (aPTT) were determined in citrated plasma. A horse developed fever and leukocytosis (18,83 10^3/µl) at 12 hrs and he was excluded from the study. **Results:** Statistically significant changes elicited by vaccination were: increased heart rate (at 28 hrs), respiratory rate (at 12 and 36 hrs), total WBC (at 12 hrs), monocytes (at 12, 48, 60, 108 hrs), neutrophils (at 12 and 24 hrs), FIB (at 84, 96 and 108 hrs) and PT (at 4, 24, 28 and 84 hrs). Hemoglobin decreased at 60 hrs. aTPP was not modified by vaccination. PT was positively correlated with HR, RR, RT, WBC and neutrophils, and aTPP with HR, RR and neutrophils. **Conclusion:** Vaccination in horses leads to an inflammation with significant but mild correlations with coagulation profile and additionally, PT is increased in response to inflammation.

RAPID AND USER-FRIENDLY IMMUNOPHENOTYPING OF CANINE LYMPHOCYTES IN NORMAL BLOOD AND LYMPHOMA USING A PERSONAL FLOW CYTOMETER. **S. Papakonstantinou, I. Berzina, A. Lawlor, E.J. O'Neill, P.J. O'Brien.** School of Agriculture, Food Sciences and Veterinary Medicine, College of Life Sciences, University College Dublin (UCD), Ireland.
Background: Widespread use of flow cytometry in clinical veterinary medicine is limited by cost and requirement for considerable laboratory space, staff time, and expertise. The Guava EasyCyte Plus (Guava, Guava Technologies, Hayward, CA, US) is a personal, bench-top flow cytometer designed to address some of these limitations. **Objective:** To assess the Guava performance for immunophenotyping T and B canine lymphocytes using monoclonal antibodies (CD3, CD21), compared with two, conventional, flow cytometers (Cyan Dako, FACSCalibur). **Methods:** Lymphocytes from 18 peripheral blood samples of healthy (n=18), leukemic (n=2), and from lymph nodes of lymphomatous (n=8) were stained with monoclonal antibodies for T and B cells: CD3 conjugated with fluorescein isothiocyanate (FITC) and CD21 conjugated with phycoerythrin (PE), respectively. **Results:** a) Peripheral blood samples: for (CD3+) T-lymphocytes Spearman's correlation coefficient was 0.79 (p (two-tailed) <0.001, n=20) and for (CD21+) B-lymphocytes it was 0.99 (p (two-tailed) <0.001, n=20). The Bland-Altman analysis gave a mean positive bias of 1.3% for both (CD3+) T-lymphocytes and (CD21+) lymphocytes (SD of bias = 7.9, n=40). b) Lymph node aspirates: Spearman's coefficient of correlation for (CD3+) T-lymphocytes was r=0.89 (p (two-tailed) =0.005, n=8) and for (CD21+) B-lymphocytes it was 0.93 (p (two-tailed) =0.002, n=8). The Bland-Altman analysis gave a mean positive bias of 0.28% for both (CD3+) T-lymphocytes and (CD21+) B-lymphocytes (SD of bias = 74.4, n=16). **Conclusions**: T and B lymphocyte percentages determined by Guava were indistinguishable from those measured using conventional analyzers. This personal flow cytometer may be effectively used for immunophenotyping canine lymphoma.

IMMUNOCYTOCHEMISTRY IN EFFUSION CYTOLOGY. **N. Pinto da Cunha[1,2], G. Ghisleni[2], G. Avallone[2], M. Caniatti[2]**. [1]CEDIVET, Porto, Portugal; [2]DIPAV, University of Milan, Italy.
Background: A prompt accurate diagnosis on effusion cytology has both prognostic and therapeutic significance. However, cell morphology alone is not always sufficient to formulate such a diagnosis. Immunocytochemistry could provide a reliable help on various diagnostic dilemmas. **Objective**: To estimate the value of a panel of antibodies in identifying cells in canine and feline effusions. **Methods**: Western-blot analysis was used to check antibody cross-reactivity on human, feline and canine cells. Forty-four cytospined or smeared effusion specimens from dogs and cats with a cytological diagnosis of reactive effusion or non-hematopoietic malignancy were stained with a standard panel of markers. Vimentin, Cytokeratin (CK)AE1/AE3, CK5/6 and HBME-1 were used as mesothelial cell markers; desmin as mesothelial cell malignancy marker; coordinate expression of CK7/CK20 as a marker of epithelial metastasis. Malignancy was confirmed by histologic evaluation; non-malignant conditions were confirmed by follow-up. Sensitivities, specificities and predictive values were calculated. **Results**: No significant differences were found between canine and feline results. Vimentin/CK coexpression had 79% sensitivity and 92% specificity, HBME-1 had 89% sensitivity and 23% specificity, and CK5/6 had 26% sensitivity and 100% specificity for mesothelial cells. Desmin had only 20% specificity for benign mesothelial cells, while CK7-/CK20+ had a specificity of 79% and a sensitivity of 30% for metastatic cells on effusions. **Conclusions**: Immunocytochemistry can be applied in effusion samples, and valuable results can be obtained. The marker with the highest overall accuracy for the identification of mesothelial cells is the Vim/CK coexpression, being CK5/6 the more specific and HBME-1 the more sensitive antibody. Desmin is not useful for discriminating between benign and malignant mesothelial cells. The CK7-/CK20+ expression does not seem to be promising on the identification of metastatic cells on effusions.

APOPTOSIS IN CANINE LYMPHOMAS AND LEUKEMIAS: PRELIMINARY RESULTS. **A. Poggi[1], B. Miniscalco[1], E. Morello[1], S. Comazzi[2], M.E. Gelain[2], L. Aresu[3], F. Riondato[1].** [1]DPA, University of Turin; [2]DIPAV, University of Milan; and [3]DSPPCIV, University of Padua -Italy.
Background: Alteration of apoptosis contributes to carcinogenesis, tumor progression and treatment resistance. Apoptotic activity might be determined as percentage of apoptotic cells (PAC) by flow cytometry. **Objective**: Compare apoptosis in different canine lymphomas (LSA) and leukemias. **Methods**: PAC was detected in 48 LSAs (lymph node aspirates), 15 leukemias and 9 LSA/leukemias (EDTA blood) by flow cytometry using AnnexinV-FITC/PI staining. Samples were both fresh and shipped by courier. **Results**: Higher PAC was detected in B- respect to T-LSAs and in shipped related to fresh samples. Shipped B-LSAs showed higher PAC than fresh B-LSAs; no differences were detected among T-LSAs. No differences between fresh and shipped blood samples were recorded. B and T lymphoid leukemias presented similar values. Too few cases were available to explore differences among and between ALL and CLL and to confirm statistically the following descriptive results. Decreasing PACs were recorded in AML vs. AUL vs. ALL and CLL. LSA/leukemias showed higher PAC than leukemias. The only LSA/ALL case showed a clearly higher value than ALLs. LSA/CLLs reported higher medium PAC than CLLs but similar median values. **Conclusions**: B-LSAs show higher apoptotic activity than T-LSAs but only in shipped samples. 24h-storage seems to influence PAC in lymph nodes but not in blood samples. Detection of apoptosis could help in characterizing different leukemias. Comparison with blood from stage-V LSAs will help to evaluate usefulness in differentiating blood pictures. A larger series will allow to define the effect of B/T lineage respect to time on apoptosis and to explore its role in identifying subgroups with potentially prognostic significance among the main types of LSAs and leukemias.

EVALUATION OF ORGANIC DUST COMPONENTS CYTOTOXICITY ON THP1 MONOCYTES-DERIVED-MACROPHAGES USING HIGH CONTENT ANALYSIS.
E. Ramery, P. J. O'Brien. School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, 4 Dublin, Ireland.
Background: Organic dust contains pathogen associated molecular patterns (PAMPs) which can induce, following chronic exposure, significant airway diseases. Mononuclear phagocytes are key protecting cells of respiratory tract. Several studies have investigated PAMPs effects, and mainly endotoxins, on cytokine production. However sub-lethal cytotoxicity of organic dust components on macrophages has not been tested yet. The novel technology of high content analysis (HCA) is already used to assess subclinical drug-induced toxicity. It combines capabilities of flow cytometry, intracellular fluorescence probes, and image analysis and enables performing rapid multiple analysis in large numbers of samples. **Objectives** of the present study were, by using HCA, to investigate cytotoxicity of 3 major PAMPs contained in organic dust, ie. endotoxin (LPS), peptidoglycan (PGN) and β-glucans (zymosan) on THP-1 monocyte-derived macrophages. **Methods**: LPS was used at concentrations of 0.005, 0.01, 0.02, 0.05, 0.1 and 1 µg/ml; PGN and zymosan were used at concentrations of 1, 5, 10, 50, 100 and 500 µg/ml. Cells were exposed to PAMPs for 24 hours. In addition, oxidative burst and phagocytic capabilities of cells were tested. **Results**: An overlap between PGN intrinsic fluorescence and red/far-red fluorescent dyes occurred, rendering evaluation of some parameters

impossible for PGN. LPS induced sub-lethal cytotoxicity at lowest dose (from 10 ng/ml). However, most spectacular changes occurred with zymosan. In addition, zymosan, but not LPS, induced phagosome maturation and oxidative burst. **Conclusions**: Given that β-glucans can be up to 100 fold more concentrated in organic dust than LPS, these results suggest that β-glucans could play a major role in macrophages impairment following heavy dust exposure.

EXERCISE AND EPINEPHRINE AFFECT EQUINE LEUKOCYTES DISTRIBUTION AND GENE EXPRESSION IN A DISTINCT BUT CLOSELY RELATED PATTERN. **E. Ramery, J. Schoenecker, T. Art, P. Lekeux.** Department for Functional Sciences, Faculty of Veterinary Medicine, University of Liege, Liege, Belgium.
Background: In horses-athletes, exercise has been shown to be an important regulator of immune cells and their functions, and catecholamines to be essential components of the physiological response to exercise. **Objectives**: The purpose of the present study was to compare immunological reactions and molecular events during exercise and epinephrine infusion. **Methods**: Horses were submitted to exercise or epinephrine infusion for 10 min. Blood samples were collected at T0, T5, TI0, T20 and T40 min for biochemistry and haematology at T0 and T20 min for RNA extraction and microarray analysis. **Results**: In both protocols, packed-cell-volume and neutrophils peaked at T5 and T10, and returned to baseline at T40. Lymphocytes increased at T5 with exercise and at T20 with epinephrine and then remained high until protocol end. Between T0 and T20, 55 probes, coding for 25 genes, and 19 probes, coding for 11 genes, were differentially regulated with epinephrine and exercise respectively (P<0.001). Ten of genes over-expressed with epinephrine were related to inflammatory response and leukocytes trafficking and activation. Among genes with highest significant fold changes following exercise were FOS, DUSP-1 and CXCL1. FOS was over-expressed in both conditions. Among genes differentially regulated, Ingenuity software identified 3 overlapping functional networks with epinephrine and 1 functional network with exercise that overlapped the 3 epinephrine networks. **Conclusions**: Circulating leukocytes distribution and gene expression are rapidly affected following exercise and epinephrine infusion. Distinct but closely-related sets of gene activities were shown to be altered with exercise and epinephrine, with lesser changes in immunological pattern with physical stress than epinephrine alone, arguing for compensatory mechanisms in exercise.

HEXAGRAM MODEL OF HORSE NATURAL RESISTANCE. **J.E. Rattray, G. Counotte.** Department of Diagnostics, Research and Epidemiology, Animal Health Service, Deventer, The Netherlands.
Background: Use of antibiotics is being scaled down due to increasing bacterial resistance. It is therefore important to quantify natural immune defense responses so that problems can be rapidly identified and animal health improved. **Objectives**: to create a model at animal and company level that can quantify horse natural resistance. **Methods**: Full biochemistry and white blood profile was created from 120 samples of horse blood sent for routine health screening. A hexagram model was then applied to the data. Our hexagram model is based on 6 cornerstones: 1. Primary defense mechanisms (digestive system, beta-globulin as biomarker); 2. ROS reactive oxygen species (vitamin E, GSH as biomarkers); 3. Cellular immunity; 4. Acute phase proteins (haptoglobin and albumin as biomarkers); 5. B & T lymphocytes 6.WBC white blood cells, neutrophil leukocytes (bacterial infections) as biomarker Each cornerstone was sorted into five classes ranging from poor to good health (score 1-5). Data was corrected for sample frequency distribution and a hexagram model created for each animal, giving an indication of general health. The VI (vitality index, the average score of the cornerstones) was then correlated with trace element (zinc and magnesium) and alkaline phosphatase concentrations to give an indication of poor health and loss of appetite. **Results**: The VI positively correlated with increases in zinc (n=28) and magnesium (n=82) concentrations and negatively correlated with concentrations of alkaline phosphatase (n=83). High VI score appears to indicate good health and vice versa. **Conclusion**: The hexagram model and VI are useful for an overview of general animal health and facilitate decision making. The model is being further developed to include aspects of cellular immunity and to validate the procedure for use in daily practice.

PERFORMANCE OF A URINE DIPSTICK TEST IN DETECTING PROTEINURIC DOGS. **F. Riondato, R. Zanatta, S. Falco, B. Miniscalco.** Department of Animal Pathology, University of Turin, Italy.
Background: in the clinical practice the detection of proteinuria in dogs relies on protein detrermination by urine dipstick tests. However, to confirm results and to quantify proteinuria, urine must be sent to external laboratories for protein:creatinine ratio (UPC) assessment.**Objective:** assess the ability of CliniteckMicroalbumin 9 reagent strips (CM9) (Siemens) to detectproteinuric dogs. **Methods:** 61 samples were tested. In addition to proteins, CM9 provides semi-quantitative concentration of albumin (10, 30, 80, 150 mg/L),albumin/creatinineratio (UAC – negative, 30-300, >300mg/g) and UPC (negative, 300, 1500, 3000, ≥5000mg/g). UPC was determined also by quantitative reference method and 0,2 was used as cutoff to identify proteinuric dogs. Ability to detect proteinuric dogs was evaluated for the following CM9 parameters: protein (protUSG),UPC, UAC. Samples were intended as proteinuricwhen protein≥2+ or protein≥1+ and USG≤1012 (Zatelli et al. 2010), UPC≥300mg/g and UAC≥30mg/g. **Results:** protUSG, UPC and UAC yielded respectively: accuracy 80,3%, 75,4% and 85,2%; sensitivity79,5%, 89,7% and 94,9%;specificity 81,8%, 50% and 68,2%; positive (and negative) predictive value 88,6% (69,2%), 76,1% (73,3%) and 84,1% (88,2%);diagnostic odds ratio 15;8,75 and 39,64. Agreement with reference UPC was 0,56 (protUSG), 0,427 (UPC) and 0,663 (UAC).Negative UAC result was reliable (correctly excluding proteinuria) when USG >1012. Positive UAC result was reliable (correctly identifying proteinuria) when UAC>300 or UAC=30-300 with albumin=150 and/or UPC≥1500 and/or USG≥1030.**Conclusions**: UAC provided the highest performances with exception of specificity (higher for protUSG).UAC results from CliniteckMicroalbumin 9 reagent strips can improve correct identification of proteinuric dogs in the clinical practice. Exact quantitative determination of UPC is still mandatory in some cases of UAC<30 and UAC=30-300.

SEPARATION OF PERIPHERAL LYMPHOCYTES BY MAGNETIC CELL SORTING IN THE DOG. **F. Riondato**, **A. Poggi, B. Miniscalco** DPA, University of Turin, Italy.
Background: studying pure lymphocyte populations is often frustrated because of difficulties in sorting cells. Usually we rely on gradient PBMC separation, which requires large quantities of blood and does not yield specific subsets. **Objective**: sorting lymphocytes using small quantity of whole blood. **Methods**: MACS separation system (Myltenyi) was used. 300-500ul of EDTA blood was used directly and after RBC lysis. Positive selection (CD3+) and granulocytes+monocytes (CD11b+) depletion were tested. After staining with monoclonal antibodies (MAbs), cells were magnetically labeled with anti-FITC and anti-IgG1 microbeads and passed through MS columns placed in the magnetic field (MACS separator), thus collecting the CD3- or CD11b-negative fraction. Removing the column from the separator, the magnetically retained (CD3+ and CD11b+) fraction was eluted in a separate tube. Percentages of lymphocytes and granulocytes+monocytes (scatter properties and/or CD3 and CD11b expression) and vitality (PI staining) were recorded by flow cytometry. **Results**: whole blood yielded poor results. After RBC lysis, purity of lymphocyte population using anti-FITC microbeads was 85% (recovery 95%) and 21% (recovery 99%) for positive selection and depletion method, respectively. Better results were obtained for positive selection increasing beads/MAb ratio. Anti-IgG1 microbeads returned purity of 92% (recovery 90%) and 87% (recovery 99%) in CD3+ enrichment and CD11b+ depletion, respectively. CD11b+ depletion using blood stored 24h at 4°C yielded lower purity with equal recovery. PI-positive cells were <1% in all cases. Lymphocytes were successfully cultured in all cases. **Conclusions**: MACS separation system can be used to separate lymphocytes or lymphocyte subpopulations from small quantity of canine whole blood. Previous RBC lysis is required. Determination of the optimal concentration of MAb respect to microbeads is crucial. The lymphocytes collected are vital and can be cultured.

CLINICAL SIGNS AND LABORATORIAL ABNORMALITIES IN HORSES NATURALLY INFECTED BY THEILERIA EQUI. **R.G.M. Rodríguez[1], A. Muñoz[1,2], C. Riber[1,2], K. Satué[3], F. Castejón[1].** [1]Equine Sport Medicine Centre; [2] Animal Medicine and Surgery., Córdoba University; [3]Animal Medicine and Surgery Cardenal Herrera-CEU University. Valencia, Spain.
Background: Piroplasmosis, produced by Babesia caballi and Theileria equi, is one of the most important tick-transmitted hemoprotozoan disease in horses. T. equi leads to erythrocytolysi, fever, anemia, icterus, hemoglobinuria, and petechial hemorrhages. Severity of the clinical response is variable. **Objectives:** This study describes the laboratorial and clinical findings in horses positive to T. equi (polymerase chain reaction) in an endemic area. **Methods:** Twenty-three Andalusian horses of two groups were studied: group A (n=16; broodmares, 50% time stabled. 50% time at pasture) and B (n=7; stabled males used for dressage). A clinical examination was made and venous blood samples were withdrawn before treatment. **Results:** Clinical signs were more evident in group A (anorexia, 94%; pale mucous membranes, 81%; depression, 50%; icterus, 50%, edema, 38% and loss of weight, 25%). Other signs in this group were: chemosis, abortion and difficulty for movement. Horses of group B presented loss of performance during training or show, with a delayed recovery after exercise (57%) and depression (57%). Any of the studied horses had fever at the moment of sampling. Group A presented significantly lower lymphocyte numbers (3,115 vs. 5,024 10^3/µl), hematocrit (35,45 vs. 40,20%) and albumin concentrations (3.069 vs 3.343 mg/dl) and higher mean corpuscular volume (51.11 vs. 47.84 fl), mean corpuscular hemoglobin (18.59 vs. 15.80%), platelet numbers (137.9 vs. 94.43 10^3/µl), total bilirubin (1.888 vs. 1.157 mg/dl), fibrinogen (543.8 vs. 285.7 mg/dl) and lactate concentrations (2.594 vs. 1.873 mmol/l). **Conclusion:** Broodmares spending 50% of the time at pasture had more evident clinical signs and laboratory abnormalities than stabled males at training.

EVIDENCE BASED EVALUATION OF THE DIAGNOSTIC RELEVANCE OF CONVENTIONAL RETICULOCYTE PARAMETERS IN DOGS WITH REGENERATIVE ANEMIA. **G. Rossi, M. Manca, L. Giori, S. Paltrinieri, A. Giordano**. DIPAV, University of Milan, Italy.
Background: Reticulocyte counts (Ret#), reticulocyte percentage (Ret%), and reticulocyte production index (RPI) are used to estimate bone marrow regeneration. No information on the actual diagnostic relevance of these parameters for canine regenerative anemia is available **Objectives**: To assess the diagnostic relevance of reticulocyte parameters for canine regenerative anemia through a retrospective analysis of our caseload and a systematic review of literature. Methods: Data recorded at our Institution since 1993 to 2009 were screened to select 148 cases classified as: 1) non anemic (n=57): 2) regenerative anemia (RA; n=37); 3) non-regenerative anemia (NRA; n=27); 4) "pre-regenerative" anemia (PRA; n=23). Values of Ret#, Ret%, and RPI were used to calculate sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively), and positive likelihood ratios (LR+). ROC curves were designed to assess how reticulocyte parameters identify dogs with RA and dogs with RA or PRA . A systematic review of the literature was also performed followed by the calculation of sensitivity, specificity, PPV, NPV and LR+ on published data. **Results**: in our caseload, dogs with RA had significantly higher Ret#, Ret%, and RPI than other groups. The area under the ROC curve to diagnose RA, as well as sensitivity, specificity, PPV, NPV, and LR+, were significantly higher for Ret% and RPI than for Ret#. When dogs with RA and PRA are merged in a single group reticulocyte parameters provided unsatisfactory diagnostic performances. The systematic review of literature confirms that Ret% and RPI identify RA better than Ret#. **Conclusions**: Ret% and RPI are more powerful than Ret# to identify RA in dogs. Reticulocytes parameters are not able to differentiate RA from PRA.

ANTIGEN RECEPTOR REARRANGEMENT PCR (PARR) AS AN ADJUNCT TOOL FOR DIAGNOSIS AND SUB-STAGING OF CANINE HAEMATOPOIETIC MALIGNANCIES. **B. C. Rütgen[1], A. Saalmüller[2], S. E. Hammer[2], I. Schwendenwein[1]**. [1]Central Laboratory, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria; and [2]Institute of Immunology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria.
Canine lymphoma is the most common spontaneously occurring haematopoietic neoplasia in dogs and has many similarities to non-Hodgkin's lymphoma (NHL) in humans. In recent years, molecular techniques have become available for the diagnosis of canine lymphoproliferative disorders. Polymerase chain reaction (PCR) for antigen receptor rearrangement (PARR) is a method used for the detection of clonal lymphocyte populations based on the assessment of diversity within the complementary determining region 3 (CDR3) of the immunoglobulin heavy chain variable region genes (IgH) and the T cell receptor gamma (TCRγ) genes. In this study, 18 samples of canine haematopoietic malignancies showing ambiguous results in flow cytometry analysis and 10 samples of non malignant lymph node material were screened with this method. For refinement of data additionally heteroduplex PCR method was used in comparison to "classical" PARR. Out of the 18 patients, 12 patients exhibited a clonal population for TCRγ, two displayed IgH-clonality, whereas four patients turned out to represent mixed clonal B and T cell populations. Heteroduplex PCR confirmed the results of the PARR method. The PARR method represents a sensitive (≤ 1,000 cells) and flexible PCR assay system for the analysis of various source materials e.g., PBMC, FNA, whole blood, bone marrow, CSF also allowing retrospective studies based on formalin fixed paraffin embedded material. In addition to the clinical, cytological, histological and immunophenotyping data of a single patient, PARR is a suitable method for the differential diagnosis of ambiguous cases of haematopoietic malignancy and for more precise sub-staging.

EFFECTS OF AGE ON PLASMA IRON, FERRITIN AND OTHER BIOCHEMICAL PARAMETERS IN HEALTHY PREGNANT SPANISH PUREBRED BROODMARES. **K. Satué, P. Montesinos, A. Calvo.** Department of Animal Medicine and Surgery, CEU-Cardenal Herrera University, Valencia, Spain.
Background: To perform comparisons between individuals and with reference data in a clinical diagnostic situation, it is necessary to consider normal variations due to breed and age within a specific reproductive condition, in order to increase diagnostic precision. **Objectives**: To evaluate the effect of age on plasma iron (Fe), ferritin (Ferr), total proteins (TPP), albumin (ALB), creatine kinase (CK), creatinine (CREAT), gamma glutamyl transferase (GGT), aspartate aminotransferase (AST) and blood urea nitrogen (BUN) in pregnant Spanish Purebred mares. **Methods**: This research was approved by the Animal Ethics Committee of the CEU-Cardenal Herrera University. Jugular venous blood samples were extracted from 30 Spanish Purebred mares. Mares were classified into three aged groups: I (5-9 years; N: 10), II (10-15 years; N: 10) and III (16-19 years; N: 10). Blood samples were collected in tubes with lithium heparin. Fe, Ferr, TPP, ALB, CK, CREAT, GGT, AST and BUN were analyzed by spectrophotometry (Metrolab 2300 Plus V3®) using specific reagents (RAL®; Barcelona, Spain). **Results**: Although pregnancy caused an increase in Fe, Ferr and TPP and a decrease in AST, CK and GGT ($p<0.05$), with no modifications in ALB, ALT, LDH or ALP, age alone induced significant reductions of the BUN (A: 29.32±6.21; B: 28.59±5.61; C: 26.38±5.89 ug/dl) and activity of AST (A: 170.58±35.02; B: 152.86±29.35; C: 139.29±34.63 U/L) ($p<0.05$) Conclusions: These results shows that the variations associated with age in mares occur regardless of the physiological state of the mare, although pregnancy may mask some effects.

AGE DECREASES SIZE OF PREOVULATORY FOLLICLE AND SERUM CONCENTRATIONS OF IGF-1 AND ESTRADIOL-17β IN CYCLING HEALTHY SPANISH PUREBRED MARES. **K. Satué, P. Montesinos, A. Calvo**. Department of Animal Medicine and Surgery, CEU-Cardenal Herrera University, Valencia, Spain.
Background: Although in fertile mares, increased follicular diameter (FD) is closely related to estradiol-17β (E2) and insulin-like growth factor-1 (IGF-1) at intrafollicular level, the relationship with plasma concentrations have not been considered in old mares. **Objectives**: To investigate the effect of aging on maximal follicular diameter and serum E2 and IGF-1 concentrations in 30 healthy Spanish Purebred mares. **Methods**: This research was approved by Animal Ethics Committee of the CEU-Cardenal Herrera University. To analyze the effect of age, mares were classified into three groups: A (5 to 9 years; N=10), B (10 to 15 years; N=10) and C (16 to 20 years; N=10). Blood samples were collected in tubes with activators of coagulation. Serum E2 and IGF-1 concentrations were analyzed by a competitive immunoassay and immunoassay (DRGÒ IGF-1 equine (EIA-3982), respectively. Follicle development was controlled ultrasonographically. **Results**: In the younger group, E2 and FD to ovulation (30.94±7.47 pg/ml; 4.58±0.65 cm) were greater ($p<0.05$) than in older mares (E2: 10.16±5.48 pg/ml; FD: 4.07±0.36 cm). IGF-1 levels were maximum in younger (75.42±17.98 ng/ml) than in older mares (4.07±0.36 ng/ml) ($p<0.05$). In young adult mares 24.63±4.00 pg/ml, 4.22±0.40 cm and 69.66±23.30 ng/ml were obtained to E2, FD and IGF-1, respectively. Significant positive correlations were found between E2 and FD. **Conclusions**: These results indicate that the follicular diameter and E2 and IGF-1 concentrations show a gradual decrease with advancing age in Spanish mares. This effect was more marked in older than in adult mares. These events may be related to the higher rate of ovulatory failure and decrease fertility in older mares.

INFLUENCE OF PLASMA PROTEIN LEVELS ON THE CONCENTRATIONS OF RENIN AND ALDOSTERONE IN SPANISH PUREBRED BROODMARES. **K. Satué, P. Montesinos.** Department of Animal Medicine and Surgery, CEU-Cardenal Herrera University, Valencia, Spain.
Background: Several hormonal alterations appear in relation to variations in dietary intake and plasma protein level, including changes in renin (REN) and aldosterone (ALD). Relationship between plasma concentrations of protein (TPP) and the components of renin angiotensin aldosterone system (RAAS) have not been described in pregnant mares. **Objectives**: To investigate the effect of TPP concentrations on components of RAAS in pregnant Spanish broodmares. **Methods**: This research was approved by the Animal Ethics Committee of the CEU-Cardenal Herrera University. Jugular venous blood samples were extracted from 31 Spanish Purebred mares aged between 4 and 17 years. Blood samples were collected into tubes with activators for coagulation and lithium-heparin to obtain serum and plasma. TPP were analyzed by spectrophotometry (Metrolab 2300 Plus V3®) using specific reagents (RAL®; Barcelona, Spain). Serum REN, ANG-II and ALD concentrations were measured by competitive immunoassay. **Results**: Mean values of REN increased progressively from the 6th month (3.26 pg/ml), achieving maximum mean values at 10th (7.20 pg/ml) and 11th months (7.23 pg/ml). ANG-II and TPP fluctuated without significant changes (ANG-II: range 0.05-7.66; mean: 1.28 ng/ml; TPP: range 6.0-8.5 g/dl; mean: 6.88 g/dl). Mean values of ALD increased progressively from the third month reaching in the fifth month the mean maximum level (795.19±71.22 pg/ml), subsequently decreasing ($p<0,05$). Non significant correlations between TPP and ALD ($r=0.20$), TPP and REN ($r=-0.10$) and TPP and ANG-II ($r=0.13$) were found. **Conclusions**: Unlike what happens in other species, changes in TPP does not modify the renin and aldosterone concentrations in Spanish Purebred mares during pregnancy.

SEASONAL VARIATIONS IN THE ERYTHROGRAM IN PREGNANT CARTHUSIAN MARES. **K. Satué[1], A. Muñoz[2], P. Montesinos.** [1]Department of Animal Medicine and Surgery, CEU-Cardenal Herrera University, Valencia; [2]Department of Animal Medicine and Surgery, University of Córdoba, Spain.
Background: An accurate examination of the scientific literature shows that many studies have been carried out on equine hematological parameters, but studies on seasonal periodicity are scarce, particularly concerning the influence of pregnancy. **Objectives**: To investigate whether pregnancy interacts with the season to induce hematological variations in pregnant Carthusian broodmares. **Methods**: This research was approved by the Animal Ethics Committee of the CEU-Cardenal Herrera University. The total study duration was 12 months. Jugular venous blood samples were extracted from 44 pregnant Carthusian mares and poured in tubes with EDTA. Red blood cell (RBC; 106/mm3), hemoglobin concentration (HB; g/dl), packed cell volume (PCV; %), mean corpuscular volume (MCV; fl), mean corpuscular hemoglobin (MCH; pg) and mean corpuscular hemoglobin concentration (MCHC; g/dl), and platelets (PLT; 103/mm3) were analyzed (Sysmex F-820®) and total plasma proteins (TPP; g/dl) was determined by spectrophotometry (Metrolab 2300 Plus V3®). **Results**: RBC, PCV, MCV and PLT during the summer were significantly higher than the values obtained in spring, autumn and

winter (p<0.05). However, HB in spring was significantly higher and MCV, MCH and TPP in spring and summer were significantly lower than other seasons (p<0.05). There were not significant seasonal variations in MCHC. **Conclusions**: The results of the present investigation suggest that season in an exogenous factor that could modulate the dynamic of blood components with pregnancy in Carthusian mares.

BLOOD LEUKOCYTE RESPONSE IN CARTHUSIAN MARES WITH ENDOMETRITIS AND ENDOMETRIOSIS. **K. Satué[1], J. Lacuesta[1], M. Pérez[1], A. Muñoz[2].** [1]Department of Animal Medicine and Surgery, CEU-Cardenal Herrera University, Valencia; [2]Department of Animal Medicine and Surgery, University of Córdoba, Spain.
Background: In several animal species, reproductive disorders such as retained placenta, pyometra and endometritis induce modifications in the leukogram. In mares it is unknown whether semen induces uterine leukocytosis and whether the reproductive localized inflammatory processes lead to a leukogram of inflammation.
Objectives: To compare the hemogram in mares with endometritis and endometriosis. **Methods:** Forteen venous blood samples were extracted from 10 Carthusian mares, aged between 6 and 12 years. The mares were classified into three groups: healthy (I: N = 4), mares with endometritis (II: N = 3) and with endometriosis (III: N = 3). Venous blood samples were taken in EDTA tubes. Red blood cell (RBC; 10^6/mm^3), hemoglobin concentration (HB; g/dl), packed cell volume (PCV; %), mean corpuscular volume (MCV; fl), white blood cell (WBC; cél/10^3), granulocytes and monocytes (GRAN+MON; cél/10^3), lymphocytes (LYMP; cél/10^3) and platelets (PLT; 10^3/mm^3) were analyzed (*Sysmex F-820®)* and total plasma proteins (TPP; g/dl) by spectrophotometry (*Metrolab 2300 Plus V3®).* **Results:** RBC, TPP and GRAND+MON were lower in mares with endometriosis. MCV and MCHC were higher in normal mares, while PCV and HB were higher in mares with endometritis. In healthy mares, WBC and LYMP were significantly higher than other groups (*p <0.05*). **Conclusions:** In mares with endometriosis, systemic leukocyte response is not a common hematologic sign as hematological parameters tend to stabilize over time, although the hemogram may help to diagnose acute or hyperacute stages. In chronic localized uterine inflammation, the use of other markers, such as acute phase proteins, would be more useful.

URINARY PROSTATE BIOMARKERS – ARE WE THERE YET? **K.K. Seehra**. H.M.Hill, Huntingdon Research Centre, UK.
Serum prostate specific antigen (PSA) is a primary screening tool for the diagnosis of prostate cancer (CaP/PCa), but is often flawed by its association with both false positives and false negatives although, once identified provides an excellent indicator of disease progression and/or response to therapy. The other major pathophysiological states of the prostate; prostatitis and benign prostatic hyperplasia (BPH) may also be associated with elevated levels of PSA. A major challenge is to differentiate between these diseases. The identification of novel and specific disease biomarkers that can achieve these goals without the aid of biopsies could become the gold standard. Non-invasive urinary biomarkers offer an attractive option for further analysis and evaluation in the diagnosis and differentiation of prostate disease. The accuracy, reliability and relevance of a biomarker requires characterisation for its measurement and application. In interpreting urinary results, limitations such as the presumed protein expression, abundance and localisation within the prostate need to be considered and understood particularly in the early onset of disease. The annexin A-3 (ANXA-3), early prostate cancer antigen-2 (EPCA-2), engrailed-2 (EN2), microseminoprotein-beta (MSMB) and prostate cancer (antigen) gene-3 (PCA3) are current biomarkers of interest and have been discussed in literature findings for clinical utility. The use of these emerging urine biomarkers will be reviewed and evaluated to determine whether we are nearing a biomarker panel strategy that enables prostate disease categorisation.

OPTIMISATION OF THE USE OF DIAGNOSTIC CLINICAL BIOCHEMISTRY. **L.C. Sharkey**. Veterinary Clinical Sciences Department, University of Minnesota, St Paul, MN .
Background: Serum biochemical analysis is a component of the minimum clinicopathologic database for veterinary patients, providing important diagnostic and prognostic information. Most academic and commercial diagnostic laboratories do not routinely provide interpretations due to lack of necessary contextual information, however consultation services are provided upon request. Familiarity with species idiosyncrasies and knowledge of the current veterinary medical literature is required. Utilization of the Standards for the Reporting of Diagnostic accuracy studies (STARD) is strongly encouraged for studies. **Objectives:** To suggest an approach for the evaluation of serum biochemical data that optimizes clinical diagnosis. **Results:** Serum biochemical data are ideally interpreted with a concurrently performed complete blood count with microscopy, urinalysis data, and knowledge of patient demographics and nutritional and hydration status. To avoid bias in interpretation, initial analysis of serum biochemical data should be blinded to history, physical examination findings, and working clinical diagnoses. Once a thorough mechanistic evaluation of the data is obtained, a secondary assessment with more comprehensive clinical information will allow refinement of the prioritization of mechanisms and differential diagnoses. While occasionally a definitive diagnosis can be achieved by integrating history, physical examination findings, and basic clinical pathology testing, in many cases, confirming a specific diagnosis will require additional testing. In these cases, the information gleaned from the clinical biochemistry data will facilitate risk stratification and appropriate selection of ancillary diagnostics, especially in resource limited cases. **Conclusions:** Clinical pathologists can be a valuable resource for clinicians in interpreting serum biochemistry data. Absence of clinical bias, a thorough mechanistic approach, and detailed familiarity with current literature can provide significant contributions to the assessment of patient data that positively impact patient care and maximize utilization of available resources.

DIAGNOSTIC CYTOLOGY: AN EVIDENCE-BASED APPROACH. **L.C. Sharkey**. Veterinary Clinical Sciences Department, University of Minnesota, St Paul, MN.
Background: Appropriate application of cytology as a diagnostic tool requires complete and accurate understanding of its performance in various medical scenarios. This is generally accomplished by comparison of the cytologic diagnosis with the reference standard of histopathology, however patient populations and the details of experimental design must be clearly reported and evaluated in a critical approach to this body of literature to ensure legitimate conclusions. **Objectives:** To highlight strengths and weaknesses of experimental design and to develop an approach for the evaluation of the published literature that evaluates cytology as a diagnostic tool in small animal practice. **Results:** Utilization of the Standards for the Reporting of Diagnostic accuracy studies (STARD) is strongly encouraged to facilitate proper experimental design and full disclosure of process and results. The use of statistical power calculations is preferred to determine appropriate samples sizes to address clinical hypotheses regarding diagnostic cytology. Inclusion and exclusion criteria based on the scientific question should be clarified prior to data collection even if retrospective data sets will be utilized. Careful definition of how agreement and disagreement between the cytologic and histologic diagnoses will be codified is required, as well as the limitations of histopathology as a reference standard in some applications. When evaluating the cytology literature, the sample size, characteristics of the patient population, definitions of diagnostic agreement, and the methods of sample collection and analysis must be considered. Variations in results are likely to be the result of differences in patient populations, lesion characteristics, or methodology. **Conclusions:** Determination of best practices for the application of diagnostic cytology requires appropriate experimental design and adherence to consistent standards for reporting of results, which will facilitate an evidence-based approach to evaluation of the data.

SERUM PROTEIN CONCENTRATIONS OF CATTLE WITH NATURAL *FASCIOLA HEPATICA* AND STRONGYLIDS INFECTIONS. **E.M.S. Schmidt, C.M. Thomazini, L.F. Moraes, R.G.S. Dias, R.O. Reis, R.K. Takahira, R.S. Lopes, J.J. Fagliari, P.C. Silva**. School of Veterinary Medicine and Animal Science and School of Agrarian and Veterinary Sciences. Sao Paulo State University, Brazil.
Acute phase proteins (APPs) are considered a sensitive marker of inflammation especially in large animals. In this study was determined the profile of acute phase proteins in parasitic diseases using 22 Nellore cattle. They were naturally infected with *Fasciola hepatica* and strongylids which were 70% *Cooperia* sp., 22% *Oesophagostomum* sp., 7% *Haemonchus* sp. and 1% *Trichostrongylus* sp. by egg count per gram and coprocultures. Serum concentrations of acute phase proteins were determined by means of sodium dodecyl sulphate-polycrylamide gel electrophoresis. An increased were observed in concentrations of IgG 4,083±1,034mg/dL (maximum of 6,765 mg/dL), ceruloplasmin 78.9±22.7mg/dL (maximum of 119.7 mg/dL – 10 times fold), transferrin 361±114.4mg/dL, haptoglobin 22.8±14.8mg/dL (maximum of 66.7 mg/dL – 3 times fold), α1-acid glycoprotein 11.3±5.7mg/dL (maximum of 24.1 – 2 times fold). The liver enzymes AST and GGT were also increased in 132.2±33 UI/L (maximum of 199.5 UI/L) and 282.8±156.6 UI/L (maximum of 650 UI/L). Serum total protein, IgA, albumin, non-nominal identified protein of 23kD did not present significant increased (P>0.05). The elevated serum AST, GGT and the acute phase proteins (ceruloplasmin, haptoglobin, α1-acid glycoprotein and specially IgG) demonstrates an acute hepatic inflammation mainly caused by *Fasciola hepatica.* Otherwise, the other parasites may also contribute for the changes in the acute phase protein concentrations.

THE EFFECT OF BREEDING AREA AND AGE ON BLOOD PARAMETERS OF CIKA COWS. **M. Simčič[1], Z. Klinkon[2], M. Klinkon[3]**. [1]University of Ljubljana, Biotechnical faculty, Department of animal science, Domžale, Slovenia; [2]Veterina , Veterinary Clinic,Radomlje, Slovenia and [3]University of Ljubljana, Veterinary faculty, Ljubljana, Slovenia.
Background: Cika is the only autochthonous cattle breed in Slovenia. Today Cika population accounted around 2000 animals. **Objectives**: The aim of this study was to determine blood parameters of Cikacows regarding the effect of breeding area and cow's age. **Methods**: The study included 47Cikacowsfrom two breeding areas (23 from Bohinj and 24 from Kamnik). Farms with Cikacows were placed from 437-1100 m above the sea level with an average altitude of 833.7 m in Bohinj and 593.5 m in Kamnik area. The

average cows' age at sampling were 9.1 years, where the youngest cow had 3.0 years and the oldest 18.0 years. **Results**: The number of erythrocytes (RBC), leucocytes (WBC) and platelets (PLT) and values of haemoglobin (Hb), haematocrit (Ht), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were measured with haematological analyser ABC Vet. Data was analysed using GLM procedure (SAS/STAT) considering breeding area as fixed effect and cow's age as linear regression. Analysis of variance showed that the breeding area significantly affected only the number of PLT and the MCHC. The effect of age at blood sampling significantly affected the number of RBC and WBC as well as values of H band Ht and MCHC. **Conclusions**: Nevertheless, even the Cika is autochthonous breed blood parameters were within reference intervals despite the well-known difference in rearing technology of two included breeding areas of Cika in Slovenia.

VALIDATION OF THE IDEXX CATALYST DX CHEMISTRY ANALYZER FOR IN-HOUSE BIOCHEMICAL ANALYSIS. **W.D. Siska[1], N.K. Rosen[1], J.A. Christian[1], D.A. Taddeo[2], D.B. DeNicola[2]**, [1]Purdue University School of Veterinary Medicine, West Lafayette, IN, [2]IDEXX Laboratories, Inc., Westbrook, ME.
Background: The IDEXX Catalyst Dx® Chemistry Analyzer is a bench-top chemistry analyzer that uses dry slide technology to measure biochemical analytes. **Objective:** The purpose of this study was to validate the clinical results of the Catalyst Dx through comparison to a well established, dry slide technology chemistry analyzer, the Vitros ® 5,1 FS (Ortho Clinical Diagnostics). **Methods:** A minimum of 40 serum or plasma samples from healthy and ill dogs, cats, and horses were analyzed on both the Catalyst Dx and the Vitros 5,1 FS for each measured analyte, which included: glucose, blood urea nitrogen, creatinine, phosphorus, calcium, magnesium, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma glutamyltransferase, total bilirubin, cholesterol, triglycerides, amylase, lipase, and creatine kinase. Analyte results from both instruments were compared using regression analysis and bias plots with calculation of the R squared value, root mean square error, and mean bias. Ten replicates of a single sample were tested to calculate within-run analyte precision (mean, standard deviation, and coefficient of variation (CV)). **Results:** The Catalyst Dx showed a strong correlation (R squared greater than 0.9) with results from the Vitros 5,1 FS for all analytes except lipase, which had an R squared value of 0.85. For most analytes, the CV was less than 5 %, while all analytes had a CV that was acceptable within clinical decision limits. Mean biases were minimal for most analytes. **Conclusion:** Based on these results, the Catalyst Dx provides accurate and reliable biochemical analysis in domestic species, as compared to the Vitros 5,1 FS.

THROMBOCYTES IN CALVES ARE MORE STABLE THAN IN COW'S. **A. Smolenaars, C. Mathijssen-Brosens, G. Counotte**, Animal Health Service, The Netherlands.
Background: Bleeding calf syndrome (Bovine neonatal pancytopaenia BNP) is partly diagnosed by analyzing thrombocytes in calf blood. During previous validation studies, stability of the red and white blood cell count in cows (at two temperatures) was measured using the CellDyn 3700. Most red and white blood cell parameters were sufficiently stable to allow 24 hours transport and storage. Only the thrombocytes count was 40 % lower after 24 hours. Therefore, veterinarians were informed that diagnosis of pancytopaenia (in fact thrombocytopaenia) was not possible with blood older than 6 hours. **Objectives**: Validate the stability of thrombocytes in calves younger than 4 weeks of age. **Methods**: Blood samples (in K2EDTA treated vials) were taken from 5 calves and 5 cows on the same farm. Calves were under 4 weeks old. Blood was immediately transported to the laboratory and within 2 hours after collection the samples were analyzed on the CellDyn 3700, validated using cow thrombocytes. Samples were stored at 2 – 8 °C and at 20 – 25 °C and thrombocytes were analyzed after 6, 24, 48 and 72 hours. **Results**: The average count of cow's thrombocytes was the same as in the previous validation: at 6 hours blood stored at 20 – 25 °C was 30 % lower compared to the initial count. But the average count of calf thrombocytes remained almost the same during storage at both temperatures during 24 hours. **Conclusion**: Blood from calves can be stored for 48 hours at 20 – 25 °C or 2 – 8 °C before analysis of thrombocytopaenia. It is currently unknown why a difference has been found in the stability of thrombocytes in young compared to older animals.

BIOCHEMICAL MARKERS OF BONE METABOLISM – APPLICATION IN CATTLE. **J. Starič, M. Klinkon, J. Ježek, T. Zadnik.** Clinic for Ruminants, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia.
Background: Biochemical markers of bone metabolism in blood serum and/or urine are indicators of bone metabolic activity in real time. Biochemical bone resorption markers (for example C-terminal telopeptide (CTx)) indicate osteoclastic activity (catabolic) and bone synthesis markers (for example bone-alkaline phosphatase (bALP)) indicate osteoblastic (anabolic) activity. Bone metabolism is also closely connected to calcium (Ca) metabolism, which enables their utilisation as early indicators of disturbances in bone and Ca metabolism.. They can show us pathological trends in bone tissue metabolism before development of clinical disease. Disorders of bone and Ca metabolism represent a significant scope of health problems in all farm animals with intensive production. Because of the subclinical nature of many diseases involving disorders of bone tissue and Ca metabolism during the initial stages, early detection of these disorders is critical to prevent progression to clinical disease. **Objectives:** The evaluation of current use and our experiences with biochemical markers of bone metabolism in cattle. **Methods:** CTx and bALP were measured in blood serum of cattle (calves, primiparous cows, 4th or higher parity cows) in dry period and lactation, during summer when animals were on pasture and winter when they were housed. Animals with milk fever and animals that received high dose of vitamin D3 before calving were differentiated. **Results and Conclusions:** Variation in values of biochemical markers of bone tissue metabolism in different groups of animals in relation to age, different productive stages, physical activity and bone disease were observed by different researchers. Blood biochemical markers of bone metabolism can be implemented in monitoring of Ca metabolism in cattle (prevention and precalving risk assessment for milk fever).

EVALUATION OF AN IGFBP-BLOCKED ELISA FOR MEASURING IGF-I IN CANINE SERUM. **E. Strage[1], M. Lewitt[2], I. Lilliehöök[3], B. Ström-Holst[1,4], B. Jones[5], T. Fall[6]**. [1]Dept. of Clinical Sciences; and [3]University Animal Hospital, Swedish University of Agricultural Sciences; [2]School of Science, University of the West of Scotland; [4]National Veterinary Institute, Sweden; [5]Dept. of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden.
Background: Insulin-like growth factor I (IGF-I) measurements are used in veterinary medicine for diagnosing growth hormone disorders. These assays are subject to interference by the IGF-binding proteins (IGFBPs). One of the most commonly used methods for removing IGFBPs in veterinary medicine is the acid-ethanol extraction. However this may not remove IGFBPs completely. During the last decades, enzyme-linked immunosorbent assays (ELISA) have been developed, that measure total IGF-I without pretreatment of the samples. The interference from IGFBPs is instead blocked by the addition of excess IGF-II to bind IGFBPs. The aim of this study was to validate one of these ELISA assay for use with canine samples. **Methods**: IGF-I was analyzed with Mediagnost IGF-I E20 IGFBP-blocked ELISA (Mediagnost, Reutlingen, Germany). Serum samples with different IGF-I concentrations were measured in four runs for inter-assay CV and in 16-20 replicates for intra-assay CV. Interference of IGFBPs was evaluated by performing serial dilutions on serum with and without added IGFBPs. **Results**: The dilution curves of IGF-I standard and canine serum demonstrated parallelism and linearity upon dilution was 95-113% (mean 107%). Addition of three different IGFBPs at a total concentration of 8 mg/L did not show any interference in the assay. For samples with low, middle and high IGF-I concentrations the intra-assay and inter-assay CV was less than 10%. **Conclusions**: Mediagnost IGF-I ELISA E20 is a convenient assay, requiring no extraction step that can be used for measuring IGF-I in canine serum samples.

QUANTITATIVE AND QUALITATIVE ABNORMALITIES IN THE HEMOGRAM OF 2 IMMUNODEPRESSED PUPPIES: SUSPICION OF A FAMILIAL DISEASE. **V. Turinelli[1], A. Gavazza[2]**. [1]Idexx Laboratories, Ludwigsburg, Germany; [2]Dept. Veterinary Clinic University of Pisa, Italy.
In a litter of seven dachshund puppies one was born dead, four presented diffuses skin infections, consisting of dermatitis, abscess and ulcers. Two of them were referred for a clinical and pathological evaluation. The 4 months old, male presented fever, lethargy, anorexia, vomiting, diarrhea, dermatitis and conjunctivitis. The CBC showed: mild normocytic hypochromic non regenerative anemia, with presence of keratocytes, schistocytes, anulocytes; mild leukocytosis (neutrophilia) and severe signs of dysplasia in both neutrophils and monocytes, consisting of abnormal nuclear shape, atypical cytoplasmic granules; mild thrombocytopenia with dysplastic platelets resembling to blood parasites. His sister mate was presented 2 months later with the same symptoms. The CBC showed: normocytic, hypochromic, non regenerative anemia; moderate leukocytosis (monocytosis) and severe signs of dysplasia in both neutrophils and monocytes. Platelets count was normal but still mildly atypical. The male died 3 days after referral exams for septicemia; the female was euthanized at 8 months old because of the persisting and worsening of the clinical conditions. Necropsy and histopathological examinations were performed only in the male. The results were: neutrophilic lymphadenitis of the mesenteric lymph nodes, ulcerative enteritis of the small intestine; multifocal, necrotizing to suppurative hepatitis; extramedullary hematopoiesis in the spleen. Based on clinical, laboratory and histological findings a diagnosis of septicemia was made. Among the other puppies one died of peritonitis and suppurative mesenteric lymphadenitis, another one was euthanized and no information was given about the two other. Considering the clinical and hematological findings of two examined puppies and the history of the other three, a suspicion of familial immunodepression disorder secondary to neutrophilic and monocytic hypofunction was made.

ADIPONECTIN CONCENTRATION IN URINE OF DOGS WITH PROTEINURIA. A**. Tvarijonaviciute, J.J. Ceron, J.D. García-Martinez**. Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, University of Murcia, 30100 Espinardo, Murcia, Spain.

Background: Adiponectin is an adipose-derived cytokine of a small molecular weight, 30 kDa. Circulating adiponectin levels are decreased in obesity and during inflammation in dogs. Additionally, in human medicine increased excretion of this adipokine in patients with advanced nephropathy has been described. For this reason, it has been hypothesized that in humans increments of urinary adiponectin would indicate kidney damage. **Objectives:** to investigate whether in dogs urinary levels of adiponectin are changed in dogs with proteinuria using the canine leishmaniasis as a nature model of kidney disease. **Methods:** 59 dogs with diagnosed Leishmania (BCS 3/5, of different breeds, age, and sex) were used. These dogs were assigned to two different groups according urine protein: ceatinin ratio (UPCR): (1) group that was formed by dogs (number 16) that presented UPCR ≤ 0.5; and (2) group that was composed by dogs (number 43) that presented UPCR >0.5. **Results:** Urine adiponectin and adiponectin: creatinine ratio were significantly higher ($p<0.0001$ in both cases) in group of dogs with proteinuria in comparison with dogs that presented UPCR ≤ 0.5. **Conclusions:** Adiponectin in urine was elevated in dogs with proteinuria. This data indicate that, similarly as in humans, measurement of urinary adiponectin may emerge as a novel and easy-to-obtain method for the clinical assessment of renal injury.

IS SIMONSIELLA A SINGLE CELL ORGANISM? **H. Tvedten.** Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala Sweden.
All experienced cytologists have seen Simonsiella on stratified squamous epithelial cells from the mouth of dogs and other species. Nyby (1977) reported finding Simonsiella on cells from the palate of 66 of 67 dogs. Dogs, cats and people each have different types of Simonsiella in their oral cavity. Burkhard and Millward (Page 126, Chapter 5 of Raskin and Meyer's Canine and Feline Cytology 2010) defined Simonsiella as "large, rod-shaped bacteria that align in a row after division resulting in a distinctive pattern that resembles stacked coins." Most definitions describe bacteria as single cell organisms. Is Simonsiella a single-cell, rod-shaped organism in a chain? During this presentation Tvedten will attempt to redefine Simonsiella to veterinary cytologists as a flat, oval, multicellular organism. About 12-19 cells form subunits called trichromes. Individual cells become shorter (less wide) at the ends of the trichrome to give it a more oval shape. Simonsiella's trichrome is not a cylinder that resembles stacked coins but a flat oval structure with dorsal-ventral differentiation. Trichrome's ventral side has a fibrillar fringe by which Simonsiella binds to a mucosal cell's surface and allows one or more trichrome to glide along the surface. The dorsal side has somewhat of a capsule. Electron microscopy shows open connections between "individual" cells (Pangborn 1977), which may allow communication among cells. The trichrome looks and acts as a multicellular organism and not simple a chain of rods. It does not fit the routine definition of a bacterium.

INITIAL HMGB1 VALUES IN DOGS WITH GASTRIC DILATATION AND VOLVULUS-A PILOT STUDY. **I. Uhrikova[1], K. Rehakova[1], L. Rauserova-Lexmaulova[2], J. Doubek[1].** [1]Small animal clinical laboratory, University of Veterinary and Pharmaceutical Sciences Brno, Czeck Republic; [2]Department of Surgery and Orthopedic, Small Animal Clinic, University of Veterinary and Pharmaceutical Sciences Brno, Czeck Republic.
Background: Gastric dilatation and volvulus (GDV) is an acute life-threatening disease in dogs. Complicating factors include development of shock and gastric necrosis. Both states may increase high mobility group box 1 (HMGB1) as a result of his passive release during cell necrosis and active production as a late cytokine.
Objective: The aim of this study was to evaluate initial values of HMGB1 as a marker of disease severity in dogs with GDV. **Methods**: Blood was collected pre-operatively in 13 dogs with GDV. Routine biochemistry and hematology examination was done. Serum was stored and HMGB1 analysis was performed. Data related to the time since last feeding and degree of gastric rotation were recorded. Determination of HMGB1 was performed simultaneously in 7 healthy control dogs. **Results**: There was significant difference between HMGB1 concentrations in healthy dogs and dogs with GDV ($p<0.01$). Two dogs with GDV died, one due the hypovolemic shock, second dog with gastric necrosis and the highest HMGB1 level (16.3 ng/ml) died after surgery due the multiorgan failure. Concentrations of HMGB1 were moderately, but significantly correlated with initial lactate values ($r=0.72$; $p<0.01$). When compared HMGB1 levels with the degree of rotation, resp. time since last feeding none ($r=-0.09$), resp. weak ($r=0.49$) correlation was found. Multiplying the degree of rotation by time since last feeding resulted in moderately, but significant correlation with HMGB1 ($r=0.68$; $p=0.01$). **Conclusion**: Results indicate that HMGB1 may take place in the prognostic markers in dogs with GDV. More studies need to be done to confirm our results.

EVALUATION OF A NEW POINT OF CARE ANALYSER FOR MEASUREMENT OF APTT AND PT ON CITRATED WHOLE BLOOD IN DOGS. **B. Wiinberg and AT. Kristensen**. Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark.
Background: The prothrombin time (PT) and activated partial thromboplastin time (aPTT) are the most commonly ordered coagulation tests in veterinary medicine. The most common indications for ordering these tests include initial evaluation of haemorrhage, anticoagulant monitoring and, although not generally indicated, routine preoperative screening. **Objectives:** To evaluate aPTT and PT measured with a new whole blood point of care coagulation analyser, with the aim of determining 1) intra-assay analytical variation, 2) agreement with conventional plasma based assays and 3) positive (PPV) and negative predictive value (NPV) for detection of bleeding. **Methods:** Guideline reference intervals were based on duplicate blood samples from 25 healthy dogs. Clincal performance was based on blood samples collected from 114 diseased dogs. On blood samples was used for duplicate POC analyses and the other for reference PT and aPTT analyses on an ACL TOP 500 (Instrumentation Laboratories). **Results:** Using the POC, mean (SD) PT of citrated whole blood (CWB) from healthy dogs was 16.6 (0.6) seconds, whereas aPTT was 96.3 (6.7) seconds. Intra-assay variation for POC PT/aPTT was 1.2%/2.8% and whereas it was 1.6%/3.2%% for the reference method. Comparing POC with the reference method, Perason correlation (r^2) was 0.52 ($P<0.0001$) for PT and 0.87 ($P<0.0001$) for aPTT, respectively. Using the 99% CI as cut-off, PPV/NPV for detection of bleeding was 30%/87% for PT and 33%/91% for aPTT, respectively at a bleeding prevalence of 15%. **Conclusions:** The aPTT and PT POC assays perform well compared to the comparable ACL TOP assays under clinical conditions, with impressive low analytical variation and acceptable agreement to the ACL TOP system.

RET-Y IN CANINE ANAEMIA. **A. Woods, E. Niznikowska, P.J. O'Brien,** Advanced Diagnostics Laboratory (ADL), NovaUCD, University College, Dublin, Belfield, Dublin 4, Ireland
Background: Sysmex XT is an automated, fluorescence flow cytometer using a polymethine dye to stain cytoplasmic RNA and allow detection of reticulocytes. Forward light scatter measures a signal proportional to RBC and reticulocyte size and provides their respective mean values (RBC-Y and RET-Y) in arbitrary units (channel numbers). RET-Y is the mean value of forward-scattered light histogram within reticulocytes. It is equivalent to CHr on Advia 2120 analysers. These measure reticulocyte hemoglobinization. Both are used as gold standards for iron-deficient erythropoiesis in humans. **Objective:** Assess relevance of RET-Y in dogs. **Methods:** 130 dogs without haematological disease and 45 dogs with anaemia were studied. We measured a) RBC count, b) hemoglobin concentration, c) hematocrit as measures of anaemia severity, erythrocyte indices of d) mean cell volume (MCV) and e) mean cell hemoglobin concentration (MCHC), f) reticulocytes, g) red cell distribution width (RDW), h) RET-Y. Serum iron was measured in 42 normal and 9 anaemic dogs. **Results:** Reference range values are determined for RET-Y (1454 – 1660), reticulocytes, hemoglobin, RDW, MCV, and MCHC. Hemoglobin had no overlap between groups. One-quarter anaemic values fell outside reference range for MCHC, RDW, and reticulocytes, one-third MCV were outside reference range, whereas half anaemic RET-Y values were outside reference ranges. RET-Y correlates with MCH ($r = 0.4$) in all dogs, with MCHC ($r = 0.55$) in anaemic, but not non-anaemic dogs, with MCV ($r = 0.45$) in non-anaemic, but not anaemic dogs. RET-Y correlated with serum iron in anaemic ($r = 0.66$) and non-anaemic ($r = 0.4$) dogs. **Conclusion:** RET-Y identified significantly greater anaemic dogs (that were presumably iron deficient) than did MCV or MCHC, other parameters that are decreased in iron deficiency.

OCCURRENCE OF MAST CELL TUMOR IN A 5 YEARS OLD DOG IN IRAN. **P. Yasini, N. Atyabi, M. Jalali, R. Shafiee.** Department of clinical pathology, College of Veterinary Medicine, University of Tehran. Iran.
Background: Mast cell tumors are especially common in dog, and often arise in the skin but they may occur anywhere that mast cells are found. These tumors frequently appear on the trunk and limbs. Mast cell tumors are easily diagnosed cytologically, but histopathology is required to grade accurately for prognostic purposes. **Methods**: A 5 year old female terrier was presented to the small animal clinic at the college of Veterinary Medicine, university of Tehran, with the history of skin lesion on axillary region that was diagnosed as skin seborrhea. Ketokonazol was prescribed for treatment. After a month, the case was again referred to hospital, with a firm mass, 2.5 cm in diameter, on the proximal right front leg. There was no evidence of regional lymph node involvement. The mass was surgically resected and an impression smear of the tissue was prepared and stained with Giemsa. The rest of the tissue was subjected to histopathologic evaluation. **Results**: Cytological examination revealed mast cells with numerous metachromatic stained granules. Nuclei were varied in size and shape with high nuclear-to-cytoplasmic ratio, prominent nucleoli, marked atypia and mitotic figures. The background was filled with granules from ruptured cells and a few eosinophils were also present. **Conclusion**: A diagnosis of moderately differentiated solitary cutaneous mast cell tumor was made.

ANTIOXIDANT EFFECT OF DIFFERENT VITAMINS ON METHEMOGLOBIN PRODUCTION IN VITRO. **P. Yasini[1], N. Atyabi[1], M. Jalali[1], H. Shayegan[2].** [1]Department of Clinical Pathology, College of Veterinary Medicine, University of Tehran; [2]College of Veterinary Medicine, University of Garmsar, Iran.

Background: Nitrite intoxication occurs frequently in ruminants and equines. The most common treatment of this disorder is administration of 1% methylene blue, although the use of some antioxidant agents e.g. vitamins and complementary treatment may also be useful. **Objectives**: The aim of this study was to evaluate the antioxidative effects of particular vitamins on methemoglobinemia which is induced by sodium nitrite in vitro. Methods: For this purpose the blood sample of healthy dairy cattle was preincubated with three different concentrations (5, 10, 20 mMol/l) of each vitamin (E, C, B1 and A) as antioxidant agent at 4^{o} C for 24 hours. A control group with normal saline instead of vitamin was applied. Then, all samples were treated with sodium nitrite (10 mMol/l) as an oxidant agent for 10 minutes and the level of methemoglobin formation was measured spectrophotometrically. **Results**: The results revealed that the level of methemoglobin decreased significantly ($p<0.05$), when vitamin E (10 and 20 mMol/l) and vitamin C (5 and 10 mMol/l) was applied to the tests. Vitamin C at the concentration of 20 mMol/l not only was not effective but also increased methemoglobin formation significantly. Vitamin A and B1 were not seemed to be efficient in any concentration. **Conclusion**: It was concluded that vitamins especially vitamin C and E can reduce oxidative induced methemoglobin formation in vitro and can be used as an alternative medication.