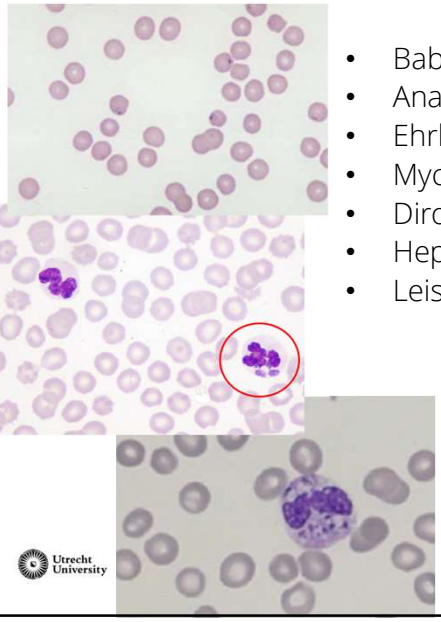
Utrecht University

Identification of parameters and formulation of a statistical and machine learning model to identify Babesia canis infections in dogs using available ADVIA hematology data
&
CHr in the diagnosis of iron deficiency

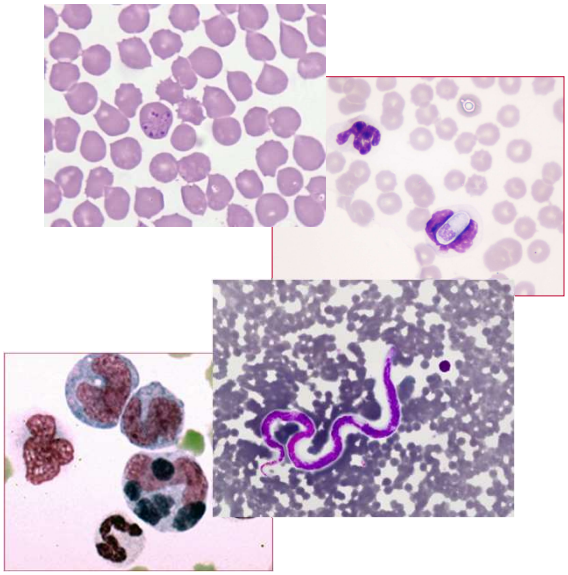
Erik Teske

Dept Clin Scie, Veterinary Faculty
Utrecht University

Some common canine hematologic infections



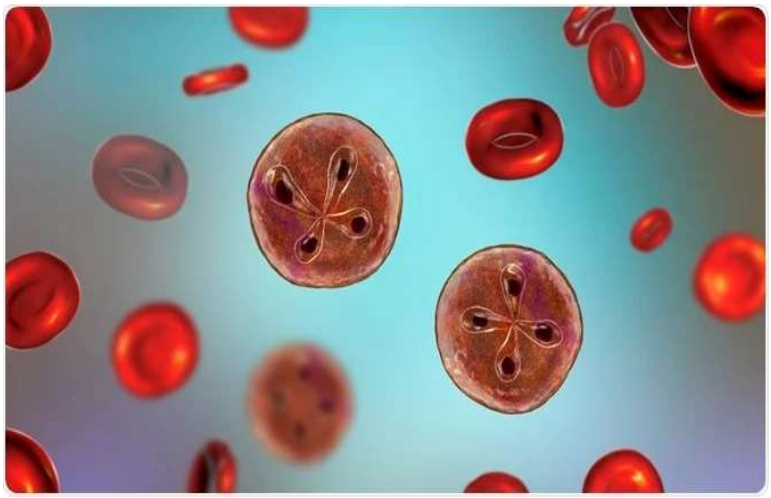
- Babesia spp
- Anaplasma spp
- Ehrlichia
- Mycoplasmata
- Dirofilaria
- Hepatozoon
- Leishmania

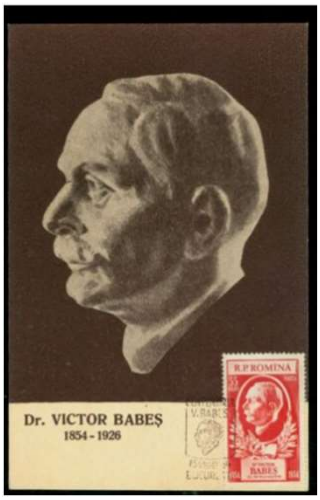


Utrecht University

2

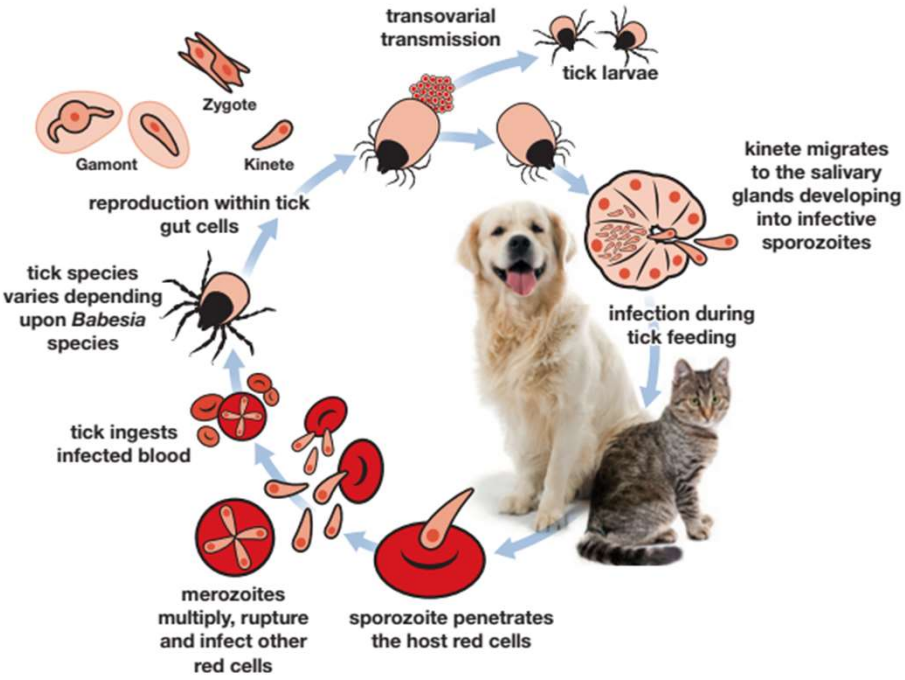
Canine Babesiosis



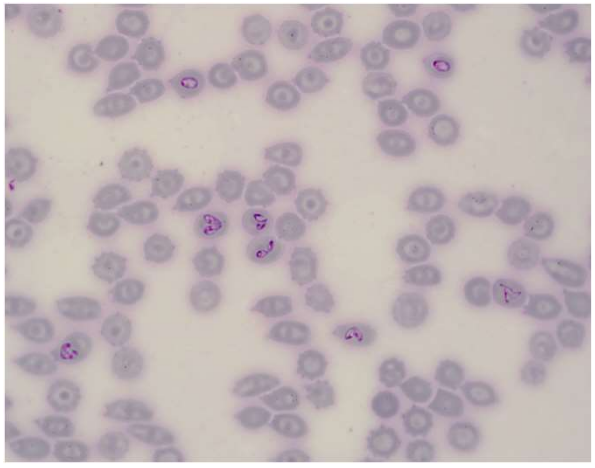


Utrecht University

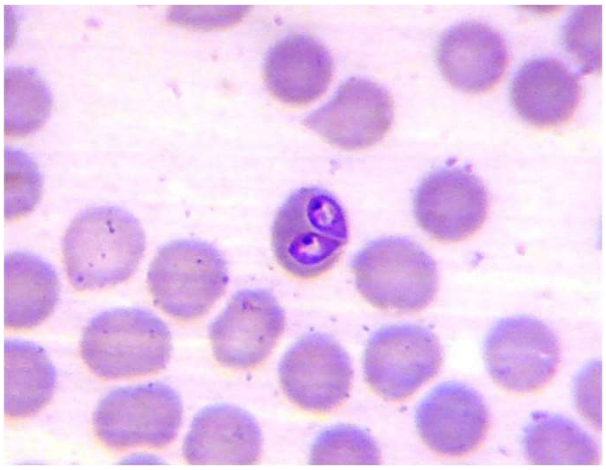
3



Large Babesia species found in Europe



Babesia canis

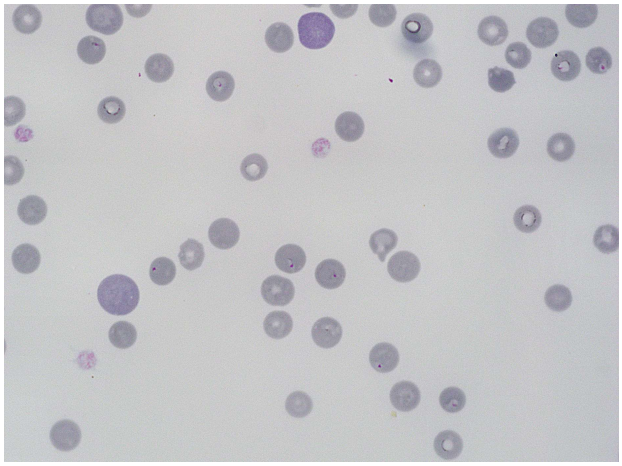


Babesia vogeli

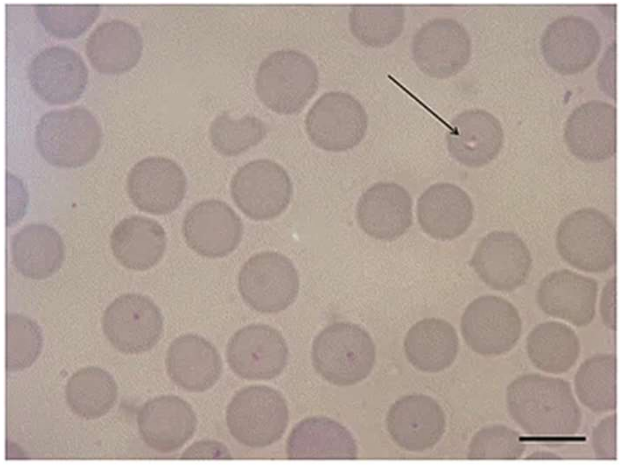
Utrecht University

5

Small Babesia species found in Europe



Babesia gibsoni
in a 1.5 year old Pit Bull terrier



Babesia microti (B vulpes)
Solano-Gallego et al. Parasites & Vectors (2016) 9:336

Utrecht University

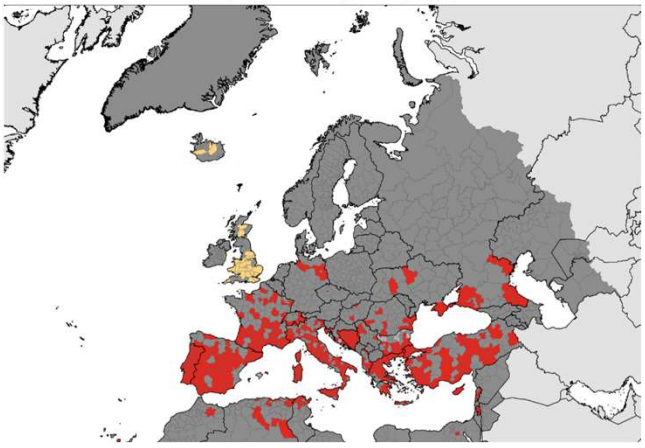
6

Utrecht University

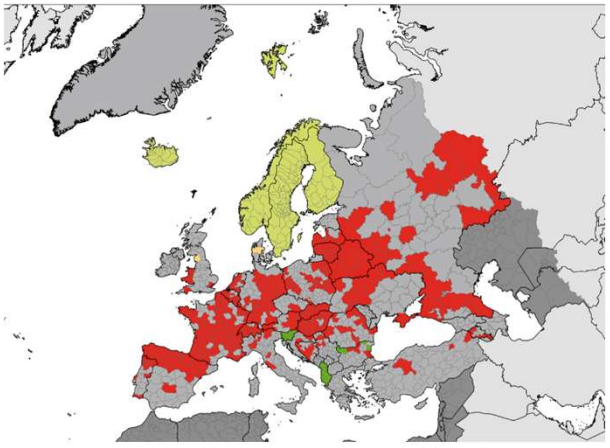
Ticks

Babesia canis	*Dermacentor reticulatus*
B. vogeli	*Rhipicephalus sanguineus*
B. gibsoni and *B. gibsoni*- like	*Haemaphysalis* spp., *Dermacentor* spp.
Babesia microti-like/*Babesia vulpes*	*Ixodes hexagonus*[2]

Rhipicephalus sanguineus group, March 2021



Dermacentor reticulatus, March 2021



Legend

- Present
- Introduced
- Antic.Absent
- Obs. Absent
- No data
- Unknown
- Outside scope

Utrecht University

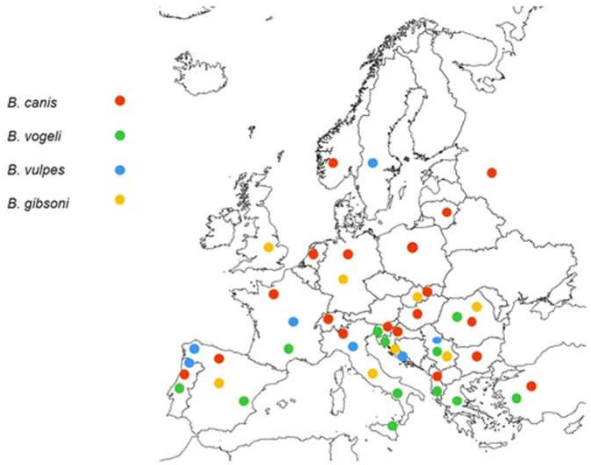
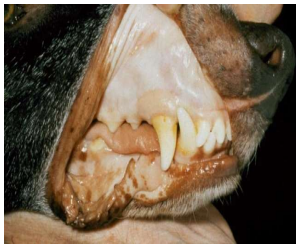


Table 9: Currently recognised distribution of canine *Babesia* spp. in Europe

Babesia spp. in dogs	Distribution
B. canis	Endemic in northern Spain, Portugal, France, The Netherlands, Italy, focally in central and eastern Europe up to the Baltic region associated with the distribution of *Dermacentor* spp. At least one endemic focus in the UK.
B. vogeli	Southern Europe, associated with the distribution of *Rhipicephalus sanguineus*.
B. gibsoni or *B. gibsoni*-like spp.	Sporadic and rare in Europe, imported from Asia. Associated with distribution of *R sanguineus*
B. microti-like (*B. vulpes*)	Northwest Spain and Portugal (in foxes found in Croatia, Italy, Germany). Recently found in ticks from UK dogs. Associated with distribution of *Ixodes*

Babesiosis (symptoms)



Clinical Findings in Dogs with Babesiosis[138]

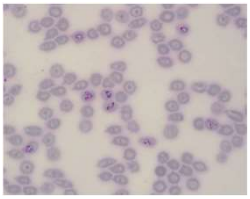
SPECTRUM	DURATION
Nonspecific Signs	**Hyperacute Symptoms**
Anorexia	Hypothermia
Lethargy	Shock
Weakness	Coma
Pyrexia	Disseminated intravascular coagulation
Weight loss	Metabolic acidosis
Atypical Signs	Death
Ascites	**Acute Symptoms**
Edema	Hemolytic anemia
Constipation	Icterus
Diarrhea	Splenomegaly
Ulcerative stomatitis	Lymphadenopathy
Hemorrhage	Vomiting
Congested mucous membranes	**Chronic Symptoms**
Polycythemia	Intermittent pyrexia
Ocular and nasal discharge	Partial anorexia
Respiratory distress	Loss of body condition
Masticatory myositis	Lymphadenopathy
Temporomandibular joint pain	
Back pain	
CNS signs	
Seizures	
Ataxia	
Paresis	

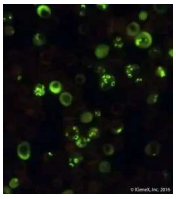
Utrecht University

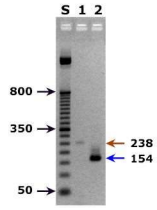
10

Babesiosis Diagnosis

- Blood smear (peripheral capillary blood, buffy coat)
  - Clinical babesiosis: often positive
  - Chronic infections/carrier dogs: low and often intermittent parasitemia
- Serology (IFT/ELISA)
  - After two weeks: not suitable for acute babesiosis
  - In endemic areas only proof of contact with parasite
- PCR
  - Sensitivity higher than blood smear
  - Also for identification subspecies







All methods not suitable for screening

Utrecht University

Utrecht University

Identification of parameters and formulation of a statistical and machine learning model to identify Babesia canis infections in dogs using available ADVIA hematology data

Tera Pijnacker
Erik Teske

Dept Clin Scie, Veterinary Faculty
Utrecht University

Rationale study

- Especially in chronic babesiosis clinical signs are not always very specific
- In a non-endemic regio Babesiosis is not always on the top of the differential list
- A large number of samples are analyzed daily in a (commercial) laboratory. Most hematology samples are solely analyzed on a machine.
- A warning system to identify cases with increased chance of finding parasites on manual blood smear analysis would offer advantages

[Results published in: Parasites and Vectors, 2022, Jan 29;15(1)]

Utrecht University

Utrecht University

Formulating a conventional statistical model to identify Babesia canis infections in dogs using ADVIA hematology data

Erik Teske

Materials & Methods

- Model building dataset:
  - All dogs with confirmed parasitemia in period 2002-2013 (n=87)
  - Control dogs (n=1144): all canine blood samples send to hematology lab in period Nov 2010-Jan 2011
- Validation dataset:
  - 13 dogs with confirmed *B. canis* in period Jan 2017-June 2020
  - Control dogs (n=5649, with 5540 unique dogs): all blood samples send to hematology lab period Jan 2017-Sept 2018

Utrecht University

Materials & Methods

- All blood samples were analyzed on ADVIA-120 in period 2002-2013 and on ADVIA-2120i in period 2017-2020
- In both datasets 214 different parameters related to erythrocytes, platelets and leukocytes were recorded
- Parameters were exported to Excel and analyzed in SPSS 27.0 and MedCalc 20.0

Utrecht University

Results I Model Building dataset

- After calculating means and 1SD and 2SD for each of the 214 different parameters related to erythrocytes, reticulocytes, platelets and leukocytes, in the modelling dataset, those parameters of which >30% of the values of the babesia dogs were outside 1SD of the mean of control dogs were identified (Table 1) =>

Table 1 Parameters of which >30% of the values of babesia patients were outside 1SD of mean of control dogs were identified

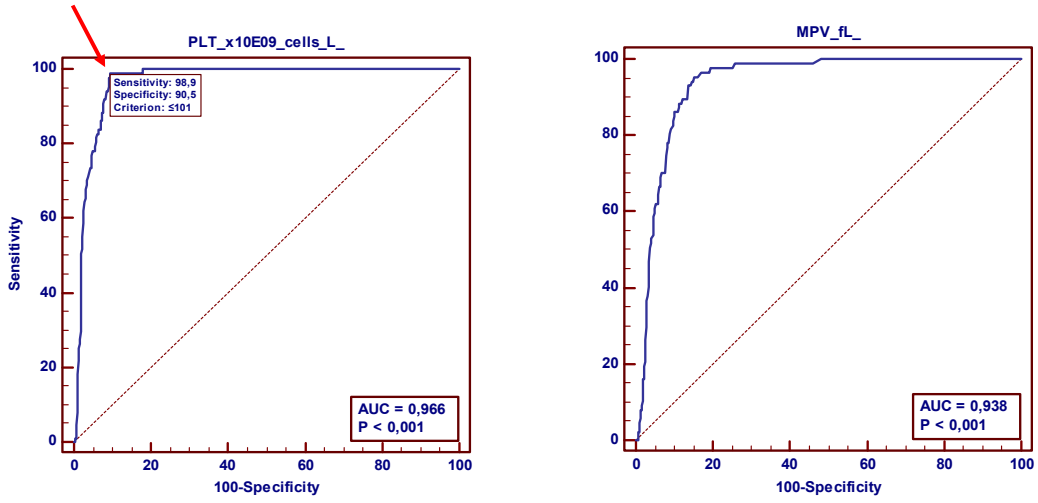
TEST	%		< or >	n
	1 SD	2 SD		
|RBC(x10E12 cells/L)|	43,7	5,8	<	87
HGB(m mol/L)	34,5	3,5	<	87
|HCT(L/L)|	42,5	5,8	<	87
|%LUC(%)|	60,9	33,3	>	87
MN_y_peak([No Units])	80,5	50,6	<	87
lob_Index([No Units])	79,3	3,5	>	87
pcnt_low_retics(%)	39,7	0,0	>	63
pcnt_med_retics(%)	33,3	0,0	<	63
retics_cells_tresh([No Units])	44,4	0,0	>	63
med_retic_tresh([No Units])	82,5	0,0	>	63
high_retic_thresh([No Units]	100,0	0,0	>	63
retic_MCV(fL)	34,9	0,0	<	63
retic_HDW(m mol/L)	36,5	12,7	>	63
retic_H_mean(fmol	31,8	1,6	<	63
% abnormal_cells([No Units])	42,9	18,4	>	87
pcnt_high_px(%)	36,8	4,6	<	87
Lymph_noise_valley	32,2	11,5	>	87

TEST	%		< or >	n
	1 SD	2 SD		
|IRF-M+H(%)|	39,7	0,0	<	63
|MCV_rm_delta(fL)|	41,3	3,2	<	63
HDW_rm_delta(m mol/L)	36,5	7,9	>	63
|CH_rm_delta(fmol)|	41,3	12,7	<	63
CHDW_rm_delta(fmol)	48,4	12,9	>	63
|%macro_r([No Units])|	49,2	0,0	<	63
%lowCH_m([No Units])	28,6	7,9	>	63
|%highCH_r([No Units])|	41,3	0,0	<	63
|RBC_2-D_count(x10E12 cells/L)|	43,7	5,8	<	87
PLT(x10E09 cells/L)	98,9	0,0	<	87
MPV(fL	89,7	59,8	>	87
|MPC(g/L)|	74,7	40,2	<	87
PCDW(g/L)	41,4	1,2	>	87
MPM(pg)	58,6	18,4	>	87
|PMDW(pg)|	89,7	63,2	>	87
RBC_Ghosts(x10E12 cells/L)	30,3	18,2	<	65
BaroxNRBCCount([No Units])	31,0	0,0	>	87
endCurveMu([No Units])	28,7	8,1	>	87

Results I Model Building dataset

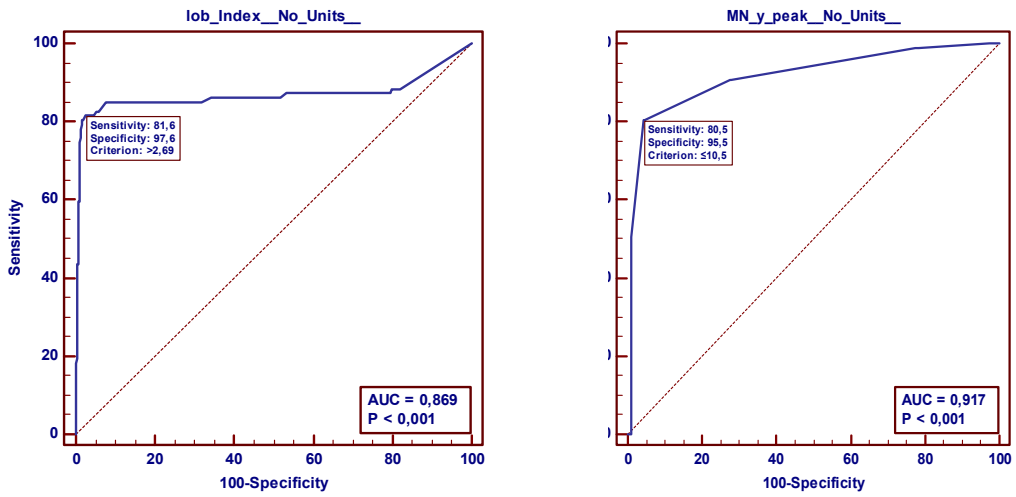
- After calculating means and 1SD and 2SD for each of the 214 different parameters related to erythrocytes, reticulocytes, platelets and leukocytes, in the modelling dataset, those parameters of which >30% of the values were outside 1SD were identified (Table 1).
- For these parameters ROC curves were drawn and parameters with a high AUC were selected, cut-off values were chosen, and sensitivity, specificity and LR+ were calculated (Table 2) =>

Utrecht University



ROC curves for selecting cut-off point

Utrecht University



ROC curves for selecting cut-off point

Utrecht University

21

Table 2 Selected parameters based on ROC curves

	N=	AUC	Sensitivity (%)	Specificity (%)	LR+
PLT (≤101 x10E09 cells/L)	1231	0.966	98.85	90.47	10.37
MPV (>14 fl)	1231	0.938	95.40	84.70	6.24
(>2.69) %Luc (>1.8)	1231	0.929	89.66	88.72	7.95
MN-y-peak (≤10.5)	770	0.917	80.46	95.54	18.04
High_retic_tresh (>70)	1231	0.708	100	58.42	2.41
PMDW (>1.09 pg)	1231	0.939	97.70	75.87	4.05
Lob_Index (>2.69)	1231	0.869	81.61	97.50	32.64
MPC (≤ 200 g/l)	1231	0.890	82.76	81.56	4.49

Utrecht University

22

Results I Model Building dataset

- After calculating means and 1SD and 2SD for each of the 214 different parameters related to erythrocytes, reticulocytes, platelets and leukocytes, in the modelling dataset, those parameters of which >30% of the values were outside 1SD were identified (Table 1).
- For these parameters ROC curves were drawn and parameters with a high AUC were selected and sensitivity, specificity and LR+ were calculated (Table 2).
- To increase the diagnostic accuracy several combinations of parameters were selected (Table 3) =>

Utrecht University

Table 3 Combinations of parameters to increase diagnostic accuracy in modelling dataset.

	Sensitivity	Specificity	LR+
PLT (≤101 x10E09 cells/L)	98.9	90.5	10.37
PLT< 102 and PMDW >1.09	96.6	93.0	13.80
PLT<102 and MPV>14	94.3	94.3	16.54
PLT<102 and %Luc >1.8	89.7	97.7	39.00
PLT<102 and PMDW>1.09 and MPV>14	93.1	94.8	17.90
PLT<102 and PMDW>1.09 and %Luc>1.8	88.5	98.1	46.58
PLT<102 and MPV>14 and %Luc>1.8	87.4	98.6	62.43

Utrecht University

Results II Model evaluation dataset

- Parameters identified in the modelling dataset as having a high AUC (Table 2) were used in the validation set.
- The known prevalence for *Babesia canis* in this set was 0.23%.
- Using this prevalence, apart from the sensitivity and specificity, positive predictive values (PV+) were calculated for each of these parameters (Table 4) =>

Utrecht University

Table 4 Selected parameters evaluated in validation dataset with prevalence of 0.23%

N=5663	Sensitivity (%)	Specificity (%)	LR+	PV+
PLT (≤101 x10E09 cells/L)	100%	89.4%	9.43	2.1%
MPV (>14 fl)	84.6%	78.4%	3.92	0.9%
Lob_Index (>2.69)	76.9%	33.6%	1.16	0.3%
MN-y-peak (≤10.5)	100%	1.5%	1.02	0.2%
High_retic_tresh (>70)	61.5%	58.1%	1.47	0.3%
PMDW (>1.09 pg)	92.3%	77.2%	4.05	0.9%
%Luc (>1.8)	84.6%	93.9%	13.87	3.1%
MPC (≤ 200 g/l)	61.5%	69.0%	1.98	0.5%

Utrecht University

Results II Model evaluation dataset

- Parameters identified in the modelling dataset as having a high AUC (Table 2) were used in the validation set.
- The known prevalence for *Babesia canis* in this set was 0.23%.
- Using this prevalence, the sensitivity and specificity, positive predictive values (PV+) were calculated for each of these parameters (Table 4). The single parameter with highest PV+ was %LUC>1.8 (PV+=3.1%).
- This was repeated for the combination of parameters found to have the highest diagnostic accuracy in the modelling dataset. (Table 5). Combining with a third parameter did not significantly increased accuracy =>

Utrecht University

Table 5 Selected combinations of parameters evaluated in validation dataset with prevalence of 0.23%

N=5663	Sensitivity	Specificity	LR+	PV+
PLT (≤101 x10E09 cells/L)	100%	89.4%	9.43	2.1%
PLT< 102 and PMDW >1.09	92.3%	91.3%	10.61	2.4%
PLT<102 and MPV>14	84.6%	92.0%	10.58	2.4%
PLT<102 and %Luc >1.8	84.6%	97.7 %	36.78	7.7%
PLT<102 and Lob_Index >2.69	76.9%	93.6%	12.02	2.7%
PLT<102 and MPC (≤ 200 g/l)	61.5%	93.8%	9.92	2.2%
PLT<102 and MN_y_Peak (≤10.5)	100%	89.6%	9.62	2.8%
PLT< 102 and PMDW >1.09 and MPV>14	84.6%	92.5%	11.28	2.5%
PLT< 102 and PMDW >1.09 and %Luc >1.8	76.9%	97.9%	36.62	7.9%
PLT<102 and %Luc >1.8 and MPV>14	69.2%	98.0%	34.60	7.4%

Utrecht University

Conclusion

- The combination of PLT<102 and %LUC>1.8 had one of the highest sensitivities and PV+ (7.7%). Combining with a third parameter did not significantly increased accuracy.
- All blood smears that were indicated false positive by the combination PLT<102 and %LUC>1.8 were re-evaluated microscopically and an additional 6 *Babesia canis* and 7 *Anaplasma phagocytophilum* cases were identified. Including these Babesia cases the PV+ would increase to 12.0% in a population with a prevalence of 0.23%.

Utrecht University

Utrecht University

Formulating a machine learning model to identify acute Babesia canis infections in dogs using ADVIA hematology data

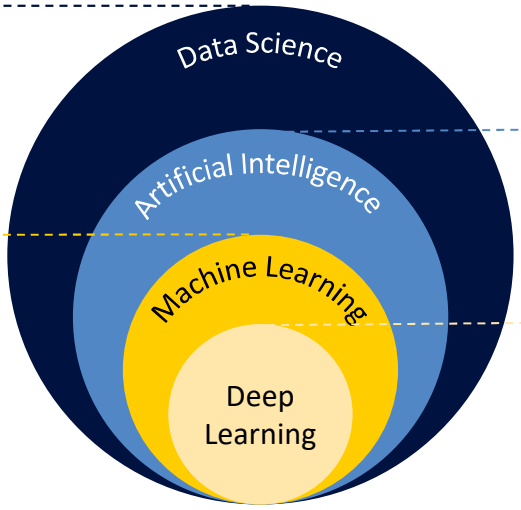
Tera Pijnacker (Dip ECVIM-CA)

Internal medicine, Utrecht University

What is machine learning

Data Science
- Collection, preparation and analysis of data
- Leverages AI/ML, statistics and domain knowledge to make decisions

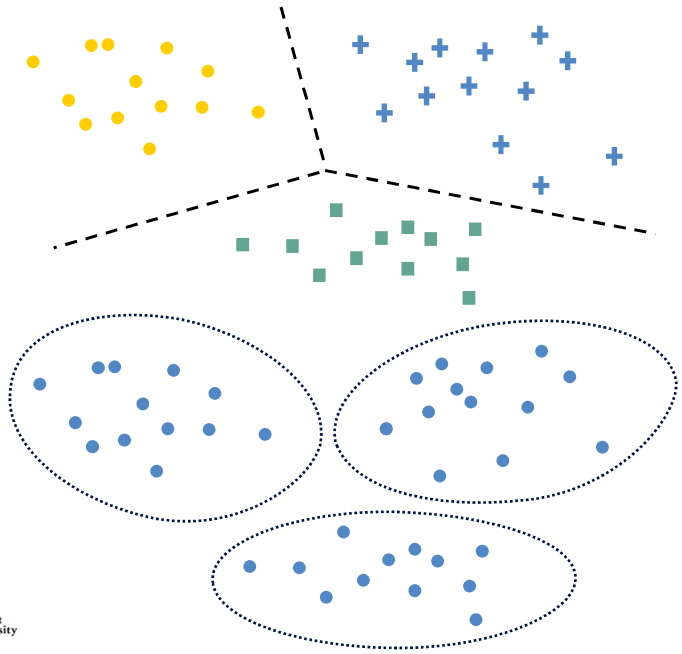
Machine Learning (ML)
- Algorithms that use (big) data to improve automatically by supervised, unsupervised and reinforcement learning



Artificial Intelligence (AI)
- Technology for machines to interpret, learn, and make 'intelligent' decisions

Deep Learning
- Subset of ML using deep neural networks

Utrecht University



What is machine learning

Supervised Learning

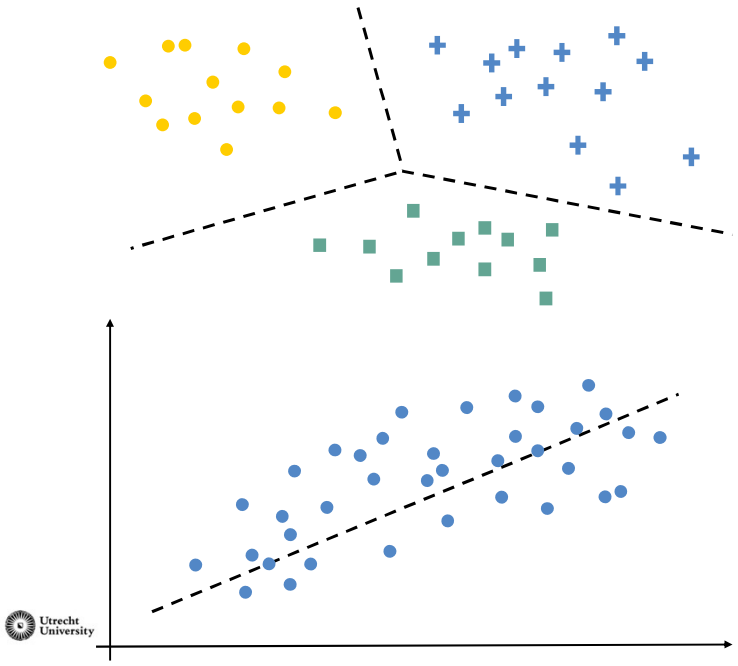
$$f(x) \rightarrow y$$

computer learns what is the best model f

Unsupervised Learning

algorithm groups cases without guidance about possible target groups, i.e. labels

Utrecht University



Supervised learning

$f(x) \rightarrow y$

Classification

Predict discrete classes:
e.g. has patient babesiosis

Regression

Predict continuous outcomes:
e.g. milk production based on food, age, breed etc

Utrecht University

33

How is machine learning used for medical purposes

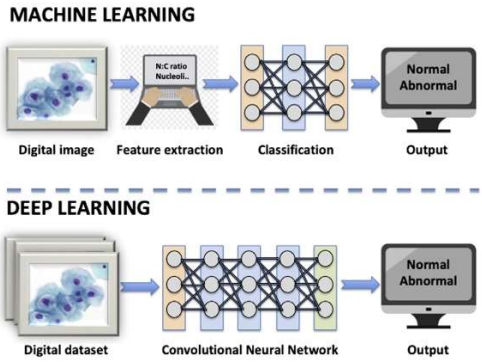
Diagnosis

- Image analysis (radiograph analysis, cytology, histology)
- Predicting disease from lab results, vital parameters, etc
- Immunophenotyping
- Etc.

Utrecht University

34

How is machine learning used for medical purposes



Cytology

Feature-based neural network (learning vector quantization) that used metrics based on nuclear size, shape, and texture.	Distinguished benign from malignant thyroid follicular cells with sensitivity of 91.5% and specificity of 92.4%.	Varlatzidou et al. 2011
Feature-based neural network (back propagation) using 5 morphometric features with histologic and/or clinical follow-up.	Distinguished benign from urothelial carcinoma in all cases, and also distinguished almost all high-grade urothelial carcinoma from low-grade urothelial carcinoma.	Muralidaran et al. 2015

Utrecht University

How has ML been used in veterinary medicine

- Machine learning algorithm as a diagnostic tool for hypoadrenocorticism in dogs (Reagan et al, 2020)
- Machine-learning based prediction of Cushing's syndrome in dogs attending UK primary-care veterinary practice (Schofield et al, 2021)
- Predicting early risk of chronic kidney disease in cats using routine clinical laboratory tests and machine learning (Bradley et al, 2019)
- An artificial neural network-based model to predict chronic kidney disease in aged cats (Biourge et al, 2020)
- Computerized assisted evaluation system for canine cardiomegaly via key points detection with deep learning (Zhang et al, 2021)
- Etc..

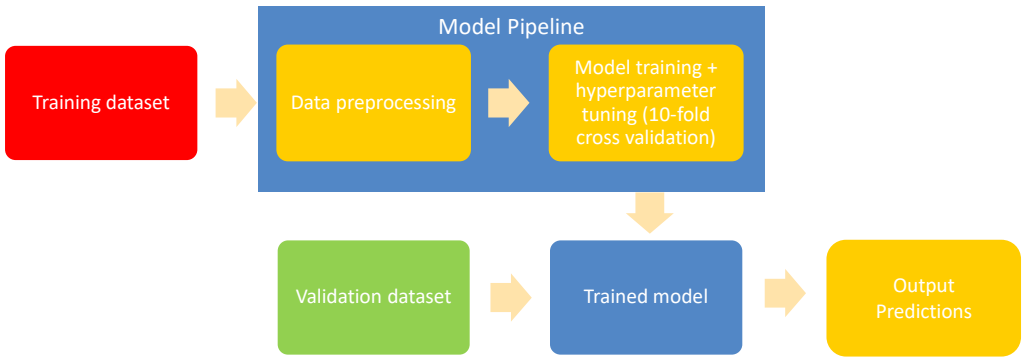
Utrecht University

36

Utrecht University

Study

Building a ML model to detect *Babesia canis* parasitemia



Materials & Methods

Identical to conventional statistics

- Model building (training) dataset:
  - All dogs with confirmed parasitemia period 2002-2013 (n=87)
  - Control dogs (n=1144): all canine blood samples send to hematology lab in period Nov 2010-Jan 2011
- Validation dataset:
  - 13 dogs with confirmed *B. canis* in period 2017-June 2020
  - Control dogs (n=5649, with 5540 unique dogs): all blood samples send to hematology lab period Jan 2017-Sept 2018

Utrecht University

39

Materials & Methods

Identical to conventional statistics

- All blood samples were analyzed on ADVIA 120 in period 2002-2013 and on ADVIA 2120i in period 2017-2020
- In both datasets 214 different parameters related to erythrocytes, platelets and leukocytes were recorded
- Parameters were exported to Excel

Utrecht University

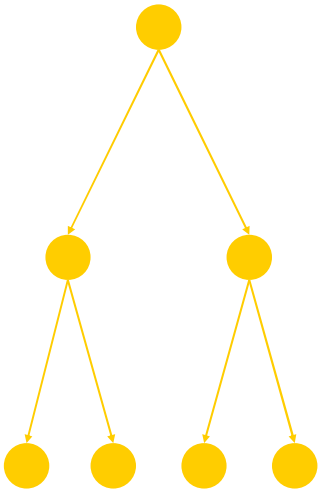
40

Materials & Methods

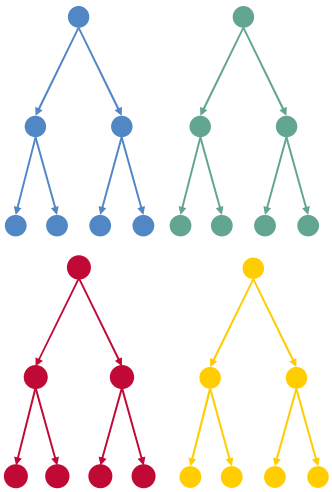
- 4 classification models (logistic regression, decision tree, random forest, XGBoost)
- Model training and hyperparameter tuning (HyperOpt) using 10-fold cross validation (to prevent overfitting).
  - Best model selected based on AUC
- Best trained model applied to validation dataset

Utrecht University

Decision Tree



Random Forest



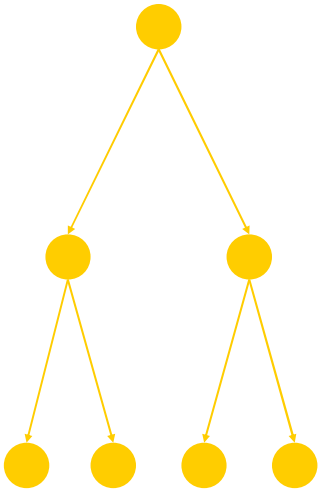
Tree methods

Decision Tree
- Single decision tree
- Trained on all samples and all parameters

Random Forest
- Multiple decision trees
- Each trained on random subset of samples and parameters
- Final classification by majority vote

Utrecht University

Decision Tree



Random Forest

Tree methods

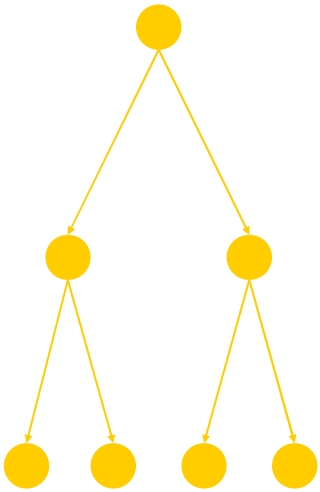
Decision Tree
- Single decision tree
- Trained on all samples and all parameters

Random Forest
- Multiple decision trees
- Each trained on random subset of samples and parameters
- Final classification by majority vote

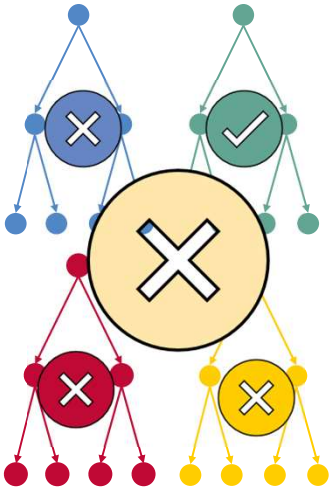
Utrecht University

43

Decision Tree



Random Forest



Tree methods

Decision Tree
- Single decision tree
- Trained on all samples and all parameters

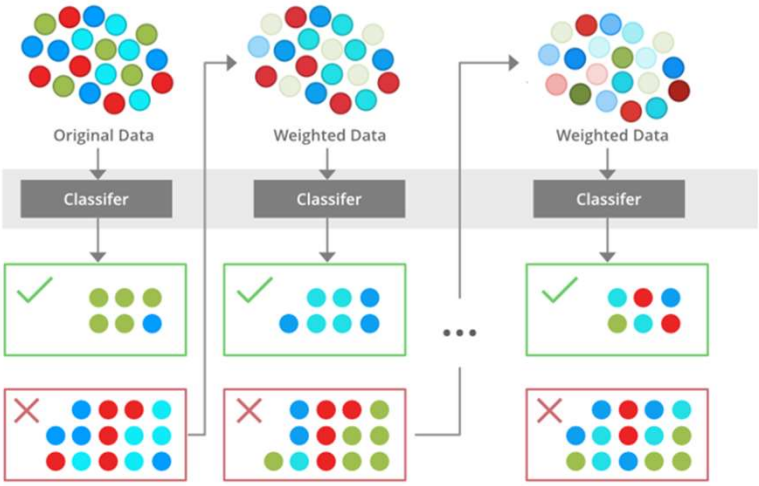
Random Forest
- Multiple decision trees
- Each trained on random subset of samples and parameters
- Final classification by majority vote

Utrecht University

44

Boosting

Building a model by using weak models in series. Firstly, a model is built from the training data. Then the second model is built which tries to correct the errors present in the first model. This procedure is continued and models are added until either the complete training data set is predicted correctly

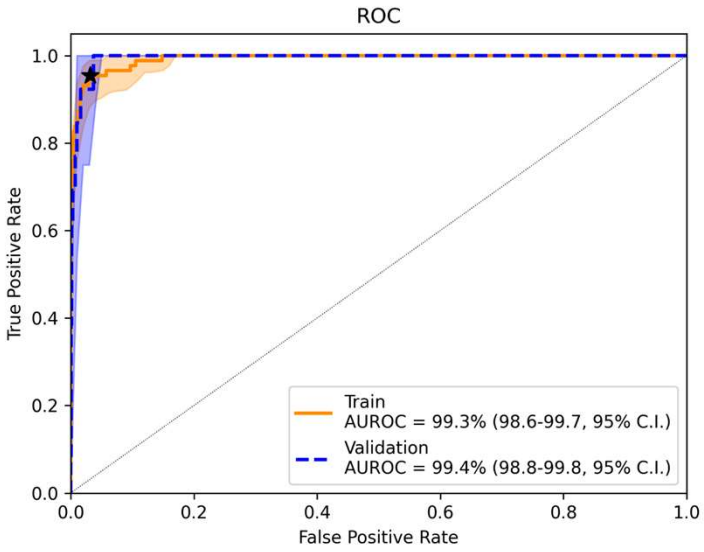


Utrecht University

Results

Model	Train			Validation		
	AUC (%)	Sensitivity (%)	Specificity (%)	AUC (%)	Sensitivity (%)	Specificity (%)
Decision Tree	97.0	95.4	89.1	98.0	100	87.0
Random Forest	99.3	95.4	96.9	99.4	100	95.7
XGBoost	99.3	95.4	96.8	99.4	100	93.7

Utrecht University

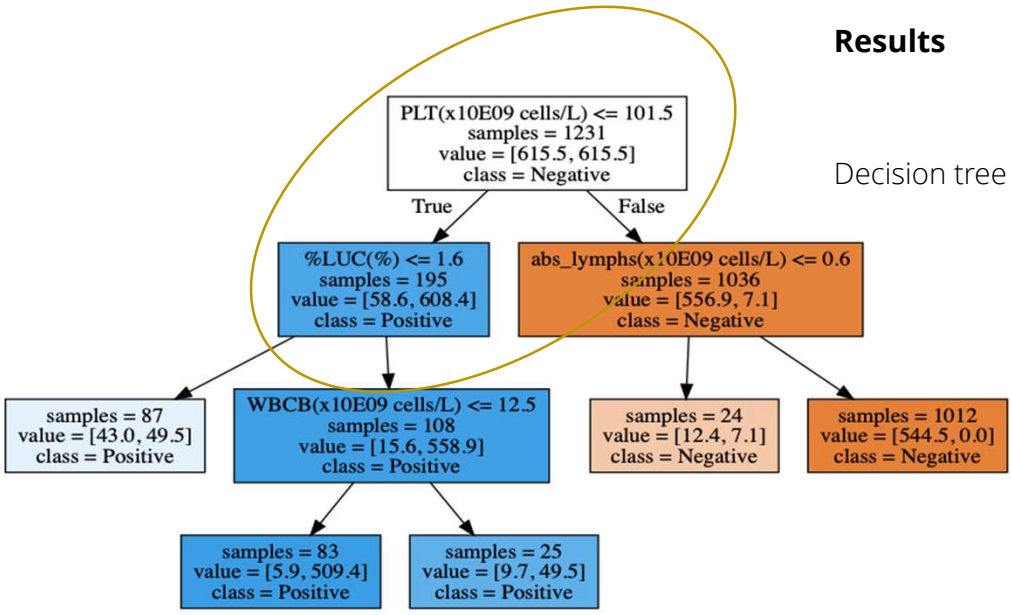


Results

ROC curves from the random-forest classifier for the training (orange) and validation (blue) sets. The star represents the model to whose performance is referred in the text (sensitivity of 95% on the training set).

Utrecht University

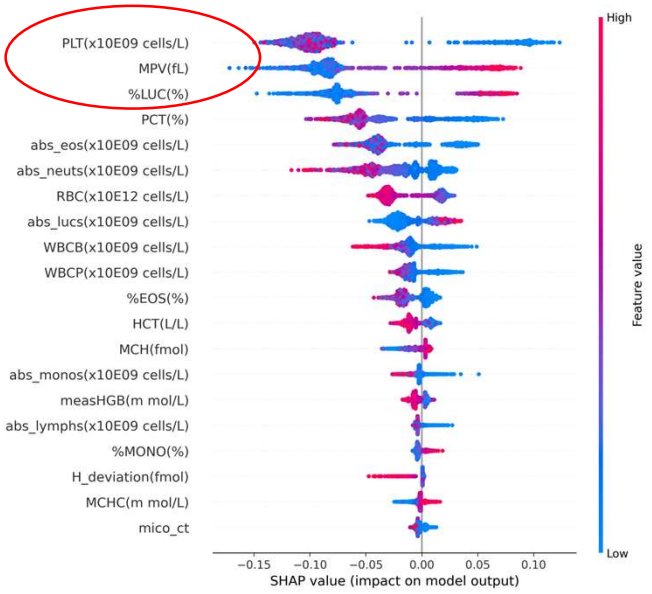
47



Results

Decision tree

48



SHAP plot (random forest)

The SHAP value indicates how much that feature contributes to the prediction of that data point, where large deviations from zero mean a larger contribution and positive values contribute towards a positive prediction of Babesia canis

Utrecht University

49

Machine learning compared to conventional statistics

Model	Train			Validation		
	AUC (%)	Sensitivity (%)	Specificity (%)	AUC (%)	Sensitivity (%)	Specificity (%)
Conventional statistics	93.7	89.7	97.7	91.1	84.6	97.7
Decision Tree	97.0	95.4	89.1	98.0	100	87.0
Random Forest	**99.3**	**95.4**	**96.9**	**99.4**	**100**	**95.7**

Utrecht University

50

Utrecht University

Overall conclusions

Comparing statistical method and machine learning method

- Logic behind decision tree similar to conventional statistics model (if / then).
- Performance both methods similar.
- Both methods identified the same important parameters (PLT, MPV, %LUC), while the random forest used additional parameters which were of lesser importance to the model
- Random forest and XGBoost perform slightly better, but more complex (black box).

Conclusions

- Screening for Babesia canis parasitemia on readily available CBC data from ADVIA made possible.
- Machine Learning offers a powerful complementary method to conventional statistics.
- Algorithms can easily be introduced in laboratories.
- Pos Likelihood Ratio of ~37.

Utrecht University

Questions?

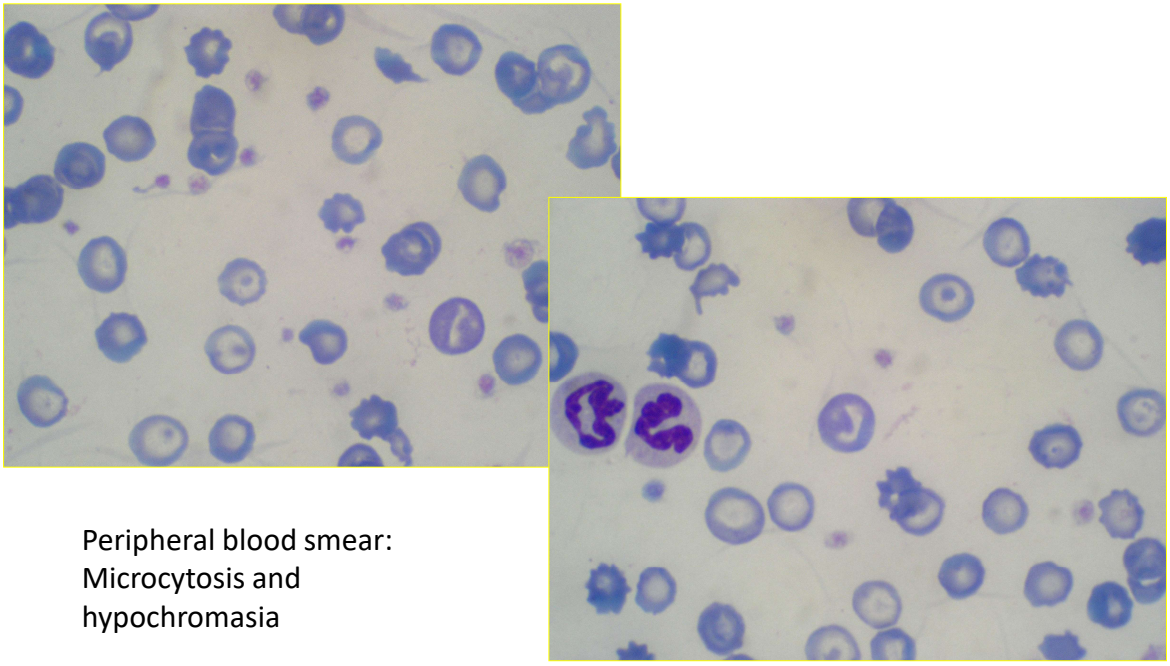
Utrecht University

Utrecht University

Use of reticulocyte hemoglobin content (CHr) for the diagnosis of Fe deficiency in dogs and cats

(Absolute) Iron Deficiency

- Microcytic, hypochromic anaemia (low MCV and MCH/MCHC)
- Often low reticulocyte count
- Low serum Fe and bone marrow iron
- Total Iron Binding Capacity increased (not in dogs?)
- Decreased transferrin saturation
- Often due to chronic blood loss (GI tract, urinary tract, massive parasite infestation)
- Less common in cats than in dogs



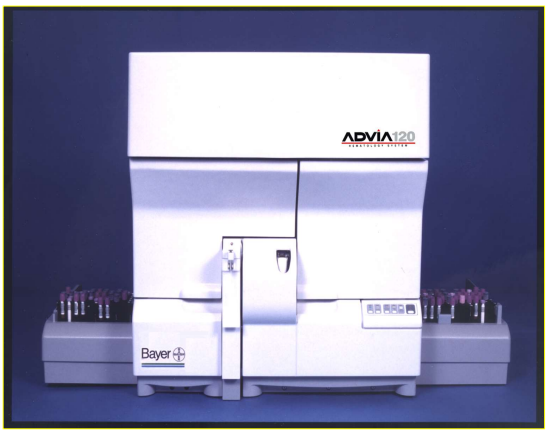
Peripheral blood smear:
Microcytosis and
hypochromasia

Disadvantages classic parameters

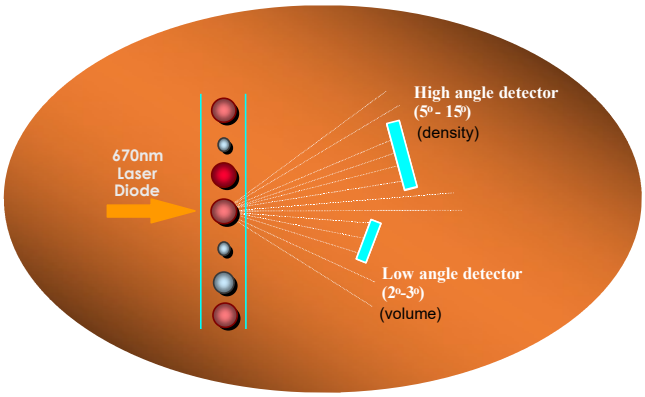
- Insensitive parameters
- Only abnormal in late Fe deficiency stage
- Respond to inflammatory diseases
- Require additional blood sampling or bone marrow biopsies
- Time consuming

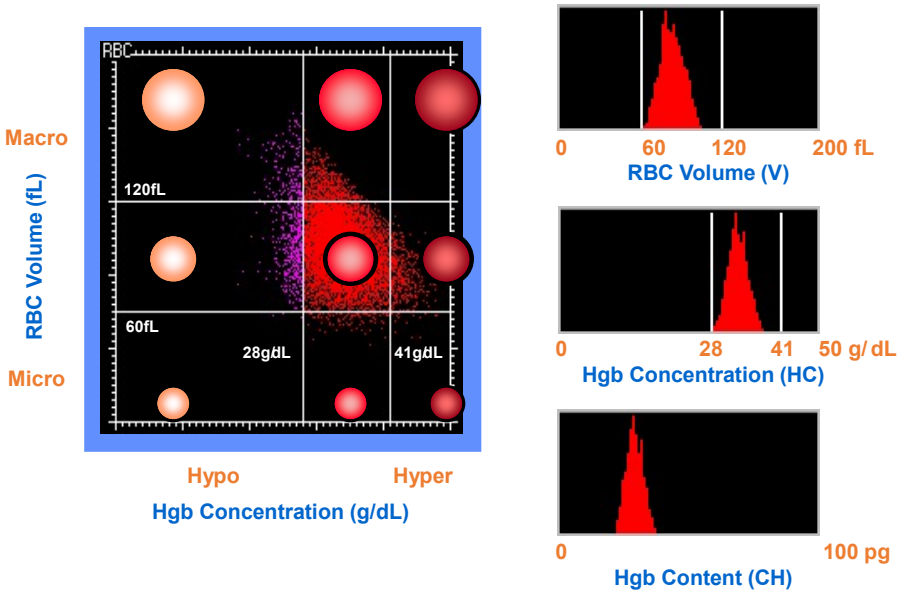
- **Hb content in reticulocytes better reflection?**

ADVIA®(2)120
Hematology System

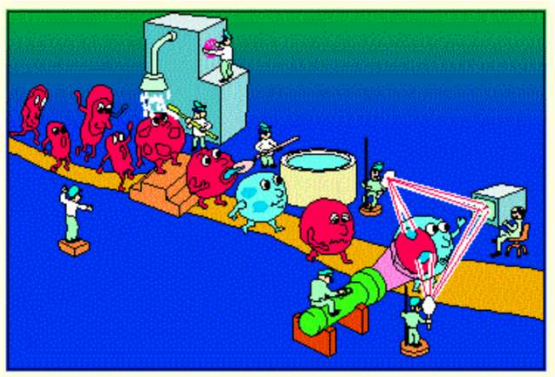


RBC Analysis

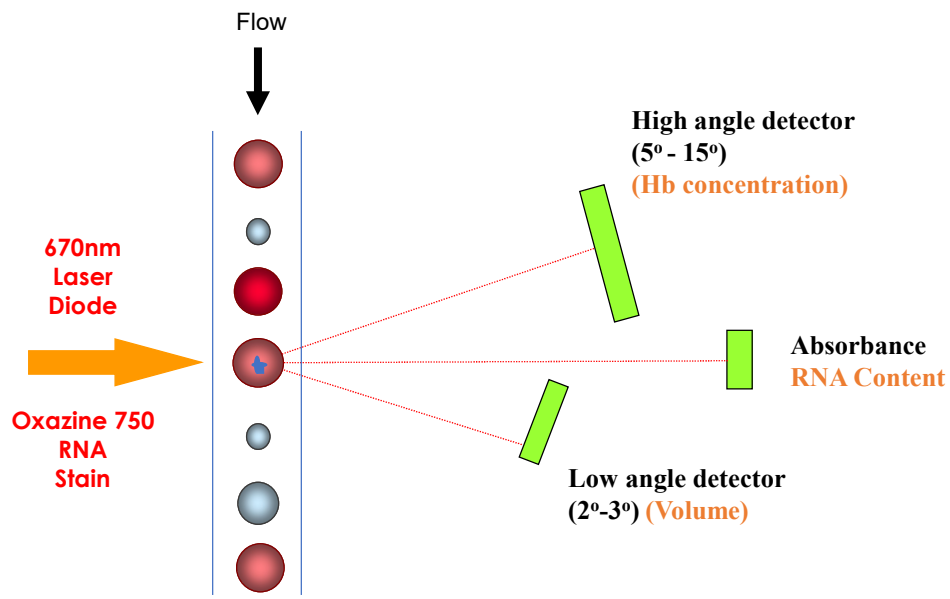




RETICULOCYTES ANALYSIS

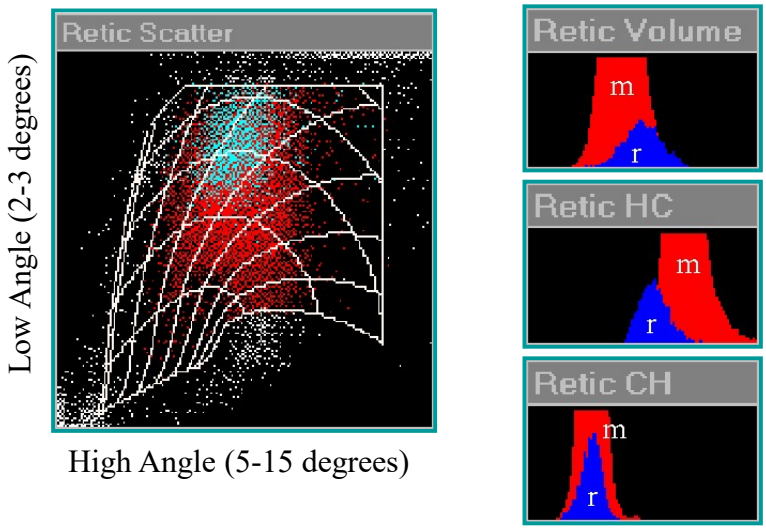


Oxazine 750 RNA Stain



Reticulocyte Analysis

Reticulocyte Parameters



Vet Clin Pathol 2005

Hematologic and biochemical abnormalities indicating iron deficiency are associated with decreased reticulocyte hemoglobin content (CHr) and reticulocyte volume (rMCV) in dogs

Jennifer D. Steinberg, Christine S. Olver

- Dogs with low CHr significantly lower mean values of HCT, MCV, serum Fe, and % sat values than did control dogs.
- Dogs with low CHr or low rMCV values had a higher frequency of microcytosis, anaemia, low serum Fe concentration, and low % sat than did control dogs.
- Low CHr was defined as below reference values

Veterinary Clinical Pathology ISSN 0275-6382 2015

ORIGINAL RESEARCH

Reticulocyte hemoglobin content does not differentiate true from functional iron deficiency in dogs

Lauren B. Radakovich, Kelly S. Santangelo, Christine S. Olver

Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

- Dogs with low CHr values often have evidence of inflammation, but low CHr did not reliably predict Fe deficiency.
- Fe deficiency due to:
  - Inadequate intake or excessive loss (Absolute Fe deficiency)
  - Functional Fe deficiency with anaemia of inflammation
- However, low CHr values were defined as all values below reference range

Veterinary Clinical Pathology ISSN 0275-6382 2015

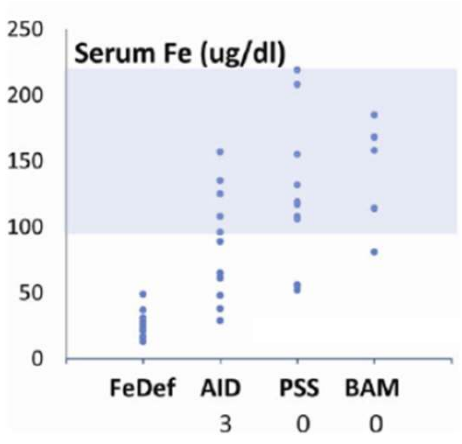
ORIGINAL RESEARCH

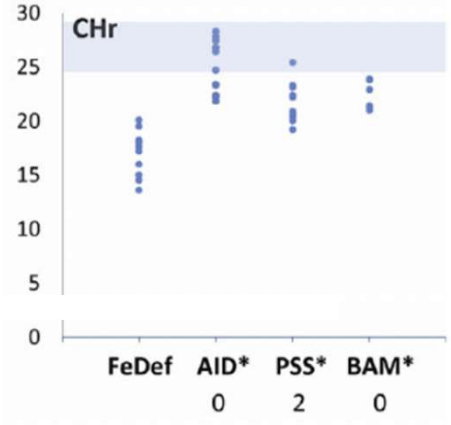
The utility of reticulocyte indices in distinguishing iron deficiency anemia from anemia of inflammatory disease, portosystemic shunting, and breed-associated microcytosis in dogs

Deanna M. W. Schaefer, Tracy Stokol

Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

- Reticulocyte indices were measured using the ADVIA 2120.
- Reference intervals were determined prospectively in 122 healthy dogs: 1.521-1.776 fmol
- Retrospectively compared between dogs with FeDef (n = 11), Anaemia of Inflammatory Disease (AID; n = 12), Porto-Systemic Shunt (PSS; n = 12), and Breed Associated Microcytosis (BAM; n = 7).





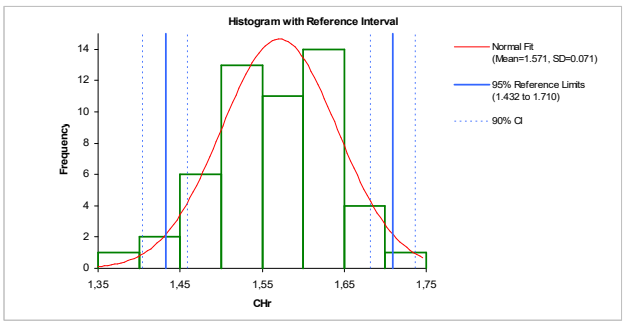
Conclusion:
Important to set low enough cutoff level!

CHr Research Project Utrecht

- Both in dogs and cats
- Reference values CHr
- Reproducibility
- Stability
- Determine optimal cut-off point
- Sensitivity/Specificity

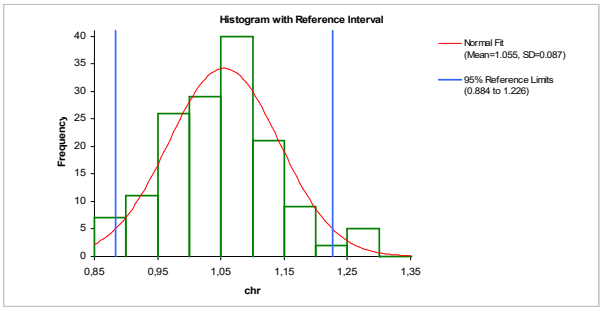
Reference values in dogs

- In 53 healthy dogs with normal Ht
- One outliner excluded
- Normal distribution (Shapiro-Wilk test)
- Reference values: 1.43 - 1.71 fmol



Reference values in cats

- In 150 cats with Ht 0.30-0.56 (median 0.37), Reticulocytes 0-1.6% (median 0.2%)
- Normal distribution (Shapiro-Wilk test)
- Reference values: 0.88 – 1.23 fmol



Reproducibility CHr

Coefficient of variation:

6 cats			3 dogs		
Ẋ (gem.)	n	CV (%)	Ẋ (gem)	n	CV(%)
0.79	10	1.63	1.36	10	0.54
0.82	6	1.34	1.53	10	0.64
0.85	10	1.74	1.86	10	0.62
0.96	8	2.10			
1.02	8	1.69			
1.11	10	3.79			

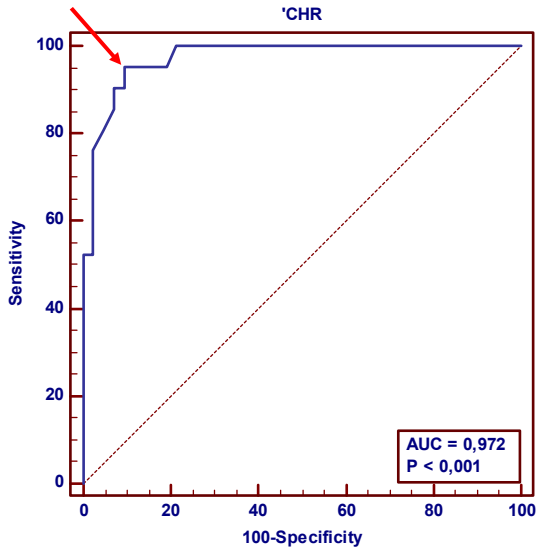
Influence of storage time on CHr

- 12 dogs: T_0 : mean CHr = 1.49 fmol

T_{16}	Δ = -0.007	(0.47%)	P=0.698
T_{24}	Δ = -0.022	(1.48%)	P=0.158
T_{40}	Δ = -0.043	(2.89%)	P=0.019
T_{48}	Δ = -0.084	(5.64%)	P<0.001
T_{64}	Δ = -0.093	(6.24%)	P<0.001
T_{72}	Δ = -0.107	(7.18%)	P<0.001

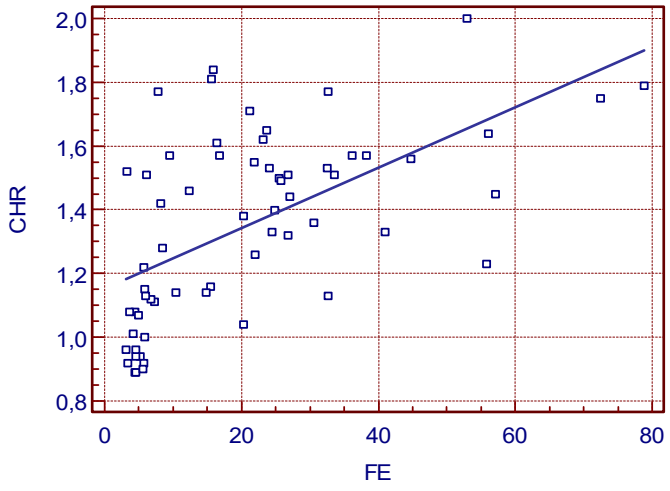
Accuracy to predict Fe def in dogs

- 63 dogs with different diseases
- Ht, Ret, MCV, MCH, MCHC, CHr, Platelets, serum Fe, Total Iron Binding Capacity
- 21/63 dogs classified as Fe deficiency based on patient's file
- Use of ROC curve to determine optimal cut-off point:

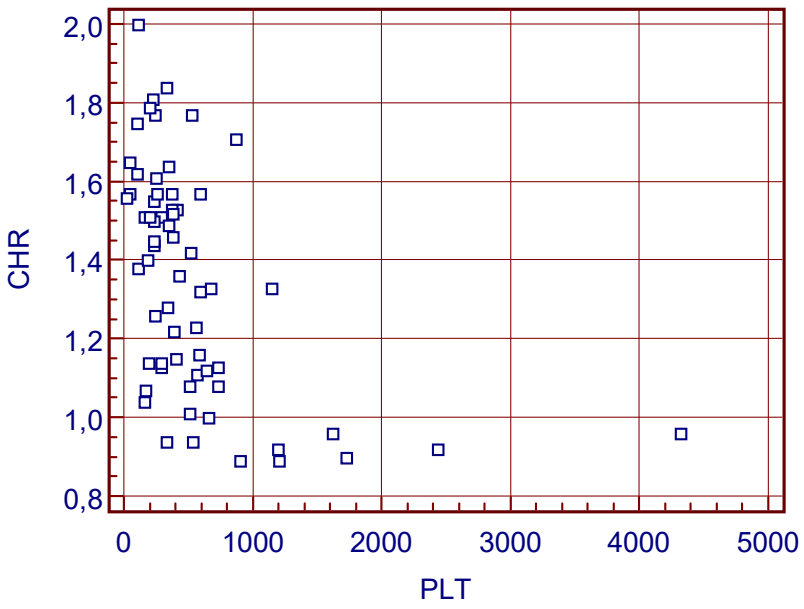


Cut-off point: 1.22 fmol

Sensitivity: 95.2% (95%CI: 76.7-99.9) Specificity: 90.5% (95%CI: 77.4-97.3)

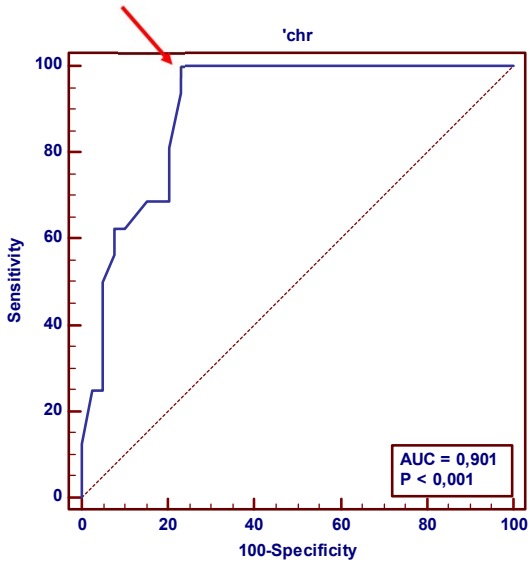


CHr = 1.1517 + 0.0095 Fe (R=0.58; P<0.0001)



Accuracy to predict Fe def in cats

- 55 cats with different diseases
- Ht, Ret, MCV, MCH, MCHC, CHr, Platelets
- 16/55 cats classified as Fe deficiency (based on patient's file
- Use of ROC curve to determine optimal cut-off point:



Cut-off point: 0.88 fmol

Sensitivity: 100% (95%CI: 79.2-100%) Specificity: 76.9% (95%CI: 60.7-88.8%)

Conclusions

- Fast, easy and reliable method to detect Fe deficiency in dogs and cats
- Its stability over time facilitates postage of blood samples to referral laboratories for measurement within 48 hours

Species	Reference range	Cut-off value	Sensitivity	Specificity
Dog	1.43 - 1.71 fmol	1.22 fmol	95.2%	90.5%
Cat	0.88 - 1.23 fmol	0.88 fmol	100%	76.9%