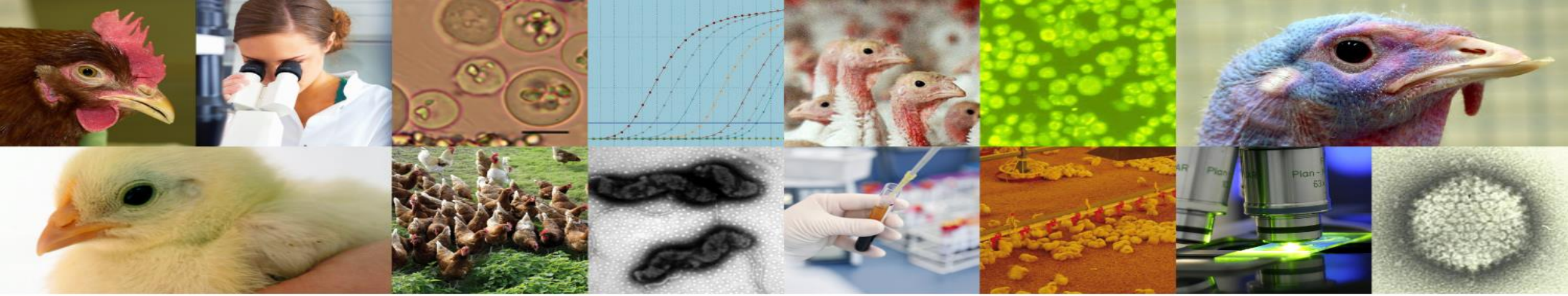

Histomonosis – new approaches on disease prevention

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Topics of the lecture

Histomonosis – current situation in poultry flocks and latest experimental data on the disease

Recent investigations to prevent the disease

Histomonosis

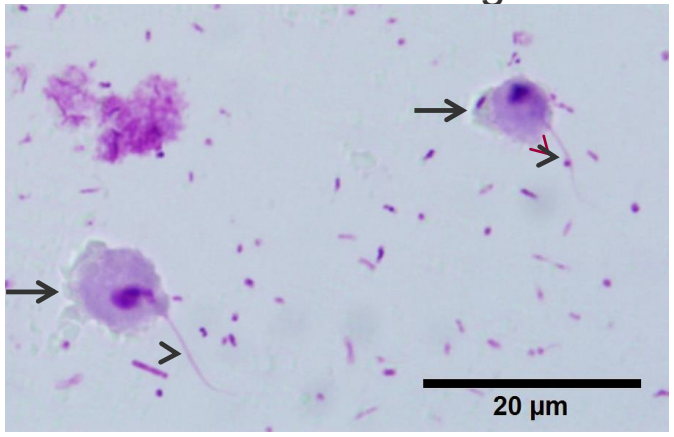
- synonyms: blackhead disease, enzootic typhlohepatitis
- turkeys and chickens are the mainly affected birds
- transmission: exclusively horizontal, directly and via the vector *Heterakis gallinarum*
- clinical signs: drowsiness, dropping of wings, sulfur-colored diarrhea
- course of disease is variable - low morbidity to high mortality
- common pathological findings
  - inflammation of caecum and/or liver

Lesions in the liver and the caeca of a turkey suffering from histomonosis

Histomonas meleagridis

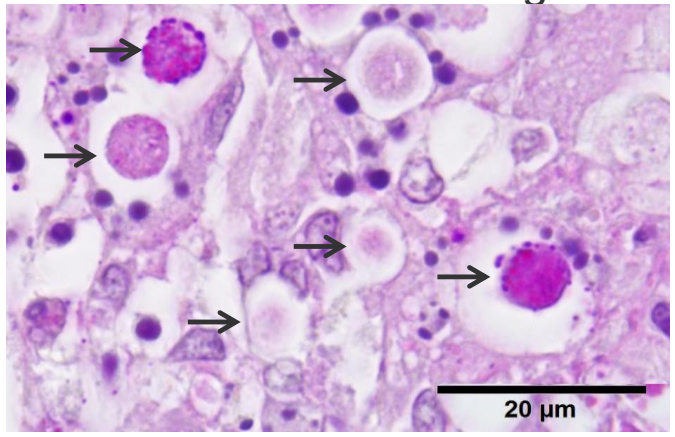
- flagellate
- spherical to amoeboid shape, about 10 µm in diameter
- morphology:

caecal lumen form: flagellated



smears of histomonads from culture (Giemsa stain)

tissue form: without flagella

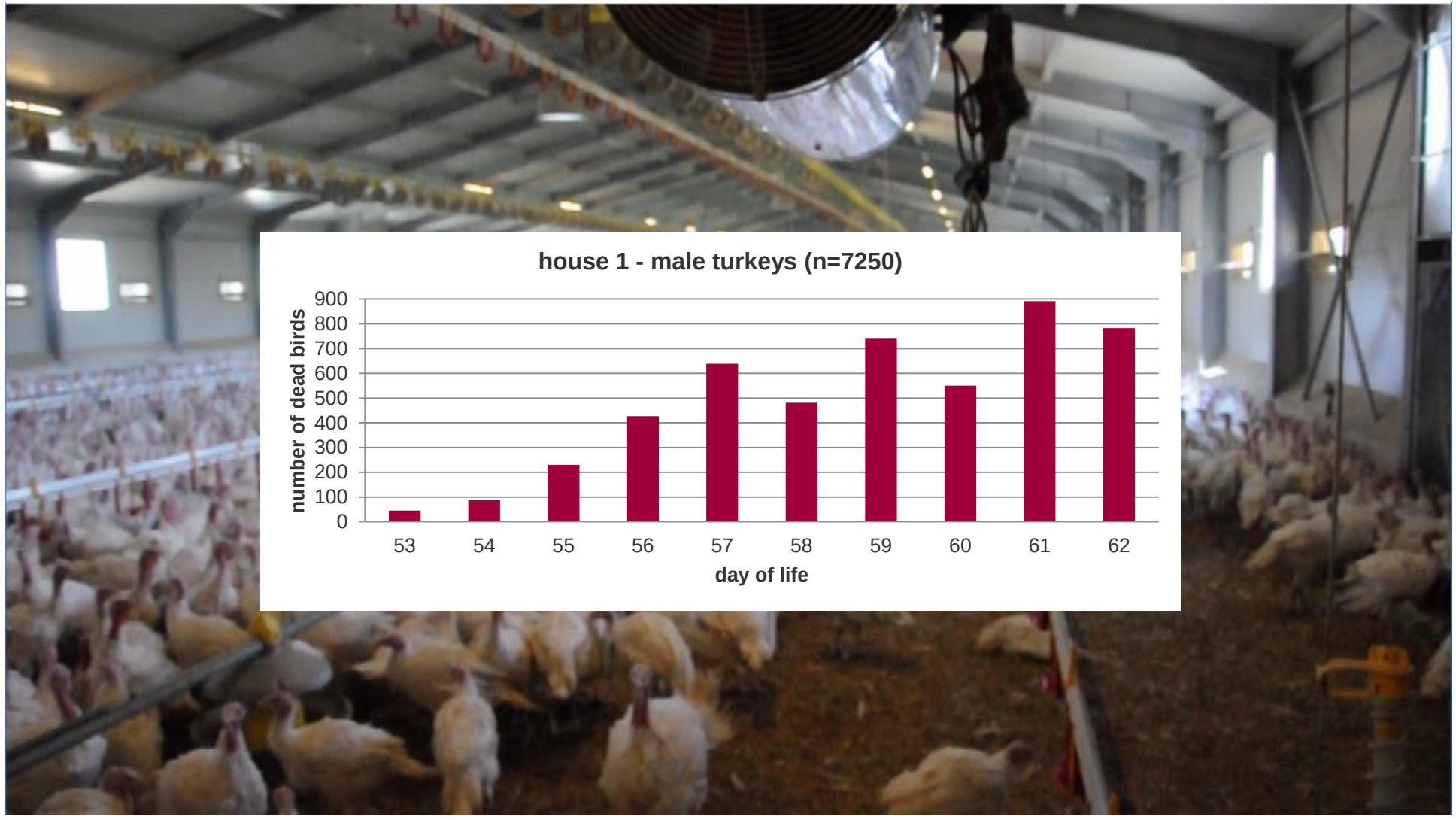


histomonads in the liver of a chicken (PAS stain)

- anaerobic metabolism
- low tenacity and viability outside the hosts
- intermediate host *Heterakis gallinarum* bear histomonads for years

Outbreak of histomonosis in a turkey flock

Outbreak of histomonosis in a turkey flock



Post mortem

- turkeys – case PA14/21403

Post mortem

- chickens – case PA09/12408

Prophylaxis and therapy

- first reports on effective drugs against histomonosis
  - arsenicals (Tyzzer, 1923, J Exp Med. 37:851-873)
    - not used in Europe
  - nitroheterocyclic compounds (Waletzky *et al*., 1950, Science 111:720-721)

- previously used drugs against histomonosis

	prevention	therapy
	nitrofuran	nitroimidazole
mode of action	undergoes reduction and eventually creates oxygen radicals such as superoxide and hydrogen peroxide which have a toxic effect (Maya *et al*., 2003, Biochem Pharmacol. 65:999-1006)	inhibition of nucleic acid synthesis by disrupting the DNA of anaerobic microorganism (Müller *et al*., 1976, in Biochemistry of Parasites and Host-Parasite Relationships, 537–544)

Ban of chemicals for the use in poultry

- food safety regulations were implemented in the last years in the EU and other industrial countries
  - ban of nitroimidazoles[1] and nitrofurans[2] in food producing animals

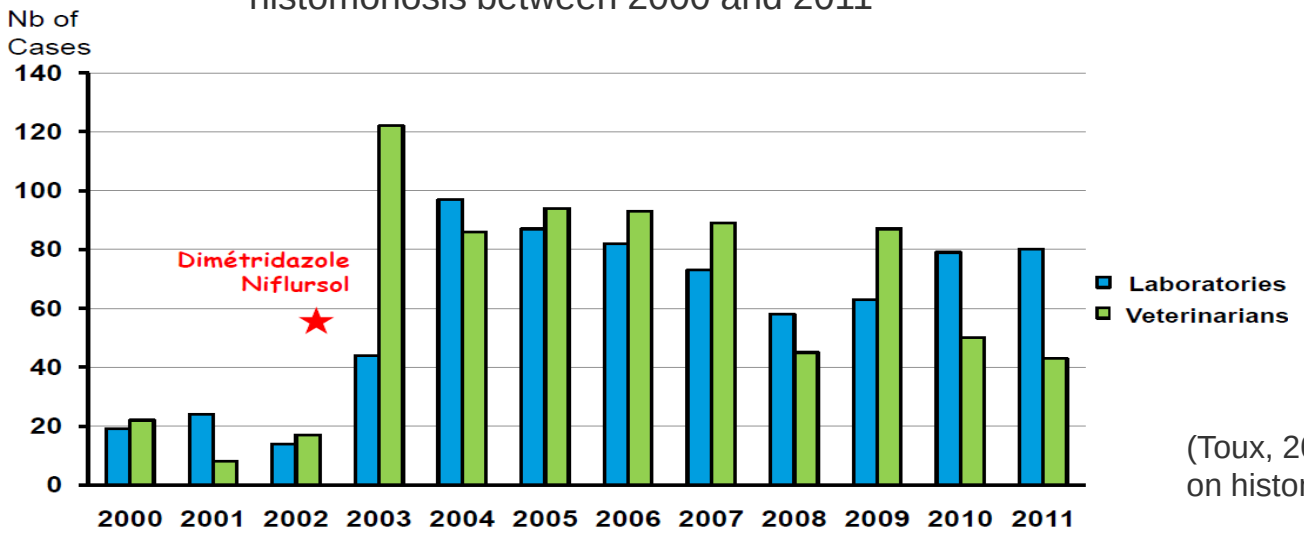
➡ prophylaxis and therapy of histomonosis not possible

[1]Commission Regulation (EC) No 1798/95, 1995, O.J. L174:20–21;
[2]Council Regulation (EC) No 1756/2002, 2002, O.J. L265:1–2;

Consequence: re-emergence of *H. meleagridis*

- France

histomonosis between 2000 and 2011



(Toux, 2013, Technical meeting on histomonosis in turkeys, EFSA)

- Germany

Year	Chicken	Turkey	Peacock	Species not known	Total
2004	1	9	1	1	12
2005	4	9	0	0	13
2006	4	5	0	1	10
2007	10	6	0	0	16
2008	7	6	1	0	14
Total	26	35	2	2	65

(Hauck and Hafez, 2010, Avian Dis. 54:1021-5)

- Poland

Case	Date of diagnosis (month, year)	Type of production	Age of birds (weeks) at which clinical signs were noticed	Farm location (voivodeship)	Clinical & postmortem diagnosis (Yes/No)	Confirmation by PCR (Yes/No)	Undertaken therapy (Yes/No)
1	11.2002	BB	40	Mazovian	Yes	No	No
2	01.2010	BB	16	Mazovian	Yes	Yes	Nd
3	01.2010	BB	16	Lubusz	Yes	Yes	Yes
4	02.2010	BB	44	Mazovian	Yes	No	Nd
5	12.2010	BB	44	Warmian-Masurian	Yes	No	Yes
6	03.2011	BB	40	Mazovian	Yes	No	Yes
7	03.2011	CL	25	Greater Poland	Yes	Yes	Nd
8	03.2011	CL	51	Mazovian	Yes	Yes	Yes
9	08.2012	BB	19	Warmian-Masurian	Yes	No	Yes
10	02.2014	BB	42	Mazovian	Yes	Yes	Nd

(Dolka *et al*., 2015, Vet Res Commun., in press.)

Recent outbreaks of histomonosis in turkeys

13 cases 2014-2016
(Sulejmanovic *et al*., 2017,
Wien Tierarztl Monatsschr 104:277-287)

101 cases in 2016
(Clark & Kimminau, 2017, Avian Dis 61:281-288)

12 cases in 2014
8 cases in 2015

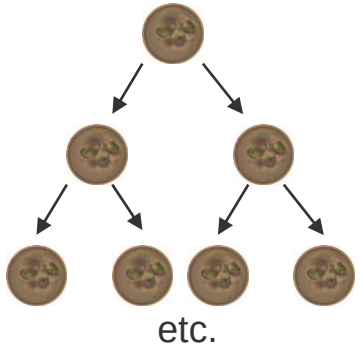
40 cases in 2015/2016

10 cases in 2014
25 cases in 2015

32 out of 199 turkey flocks positive
from 2012-2014

Experimental histomonosis as infection model

- clonal culture of *H. meleagridis*
  - *H. m.*/Turkey/Austria/2922-C6/04 passage(P) 21
  - infection with histomonads via cloaca
  - group of birds kept in pens on deep litter
  - host age 14 days of life

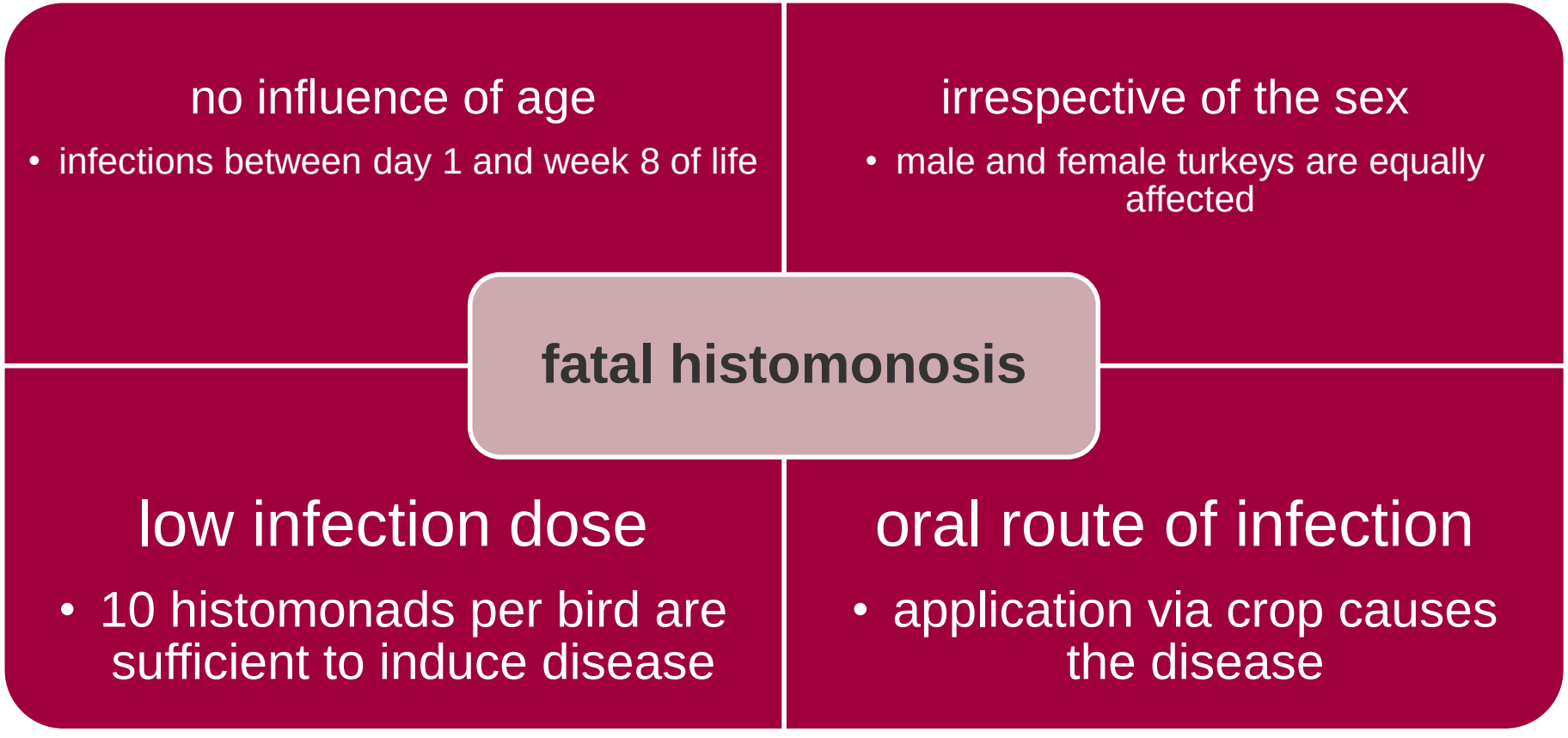


- turkeys
  - severe clinical signs of histomonosis
  - causes up to 100% morbidity and mortality of infected and in-contact birds

- chickens
  - absence of clinical signs, no mortality
- rapid spread of the parasite from infected to in-contact birds

(Hess *et al*., 2006, Avian Pathol. 35:280-5)

Infection studies in turkeys

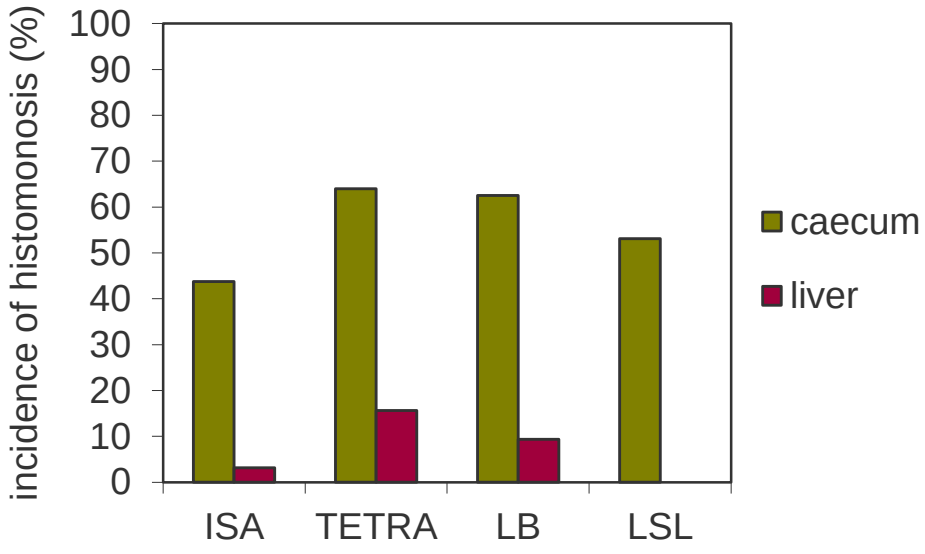


(Liebhart *et al*., 2008, Avian Dis. 52:168-72,
Liebhart and Hess, 2009, Avian Pathol. 38:223-227)

Infection studies in chickens at 14 days of age

- layer chicks of different breeds

severe lesions of a commercial juvenile chicken in the caecum and the liver

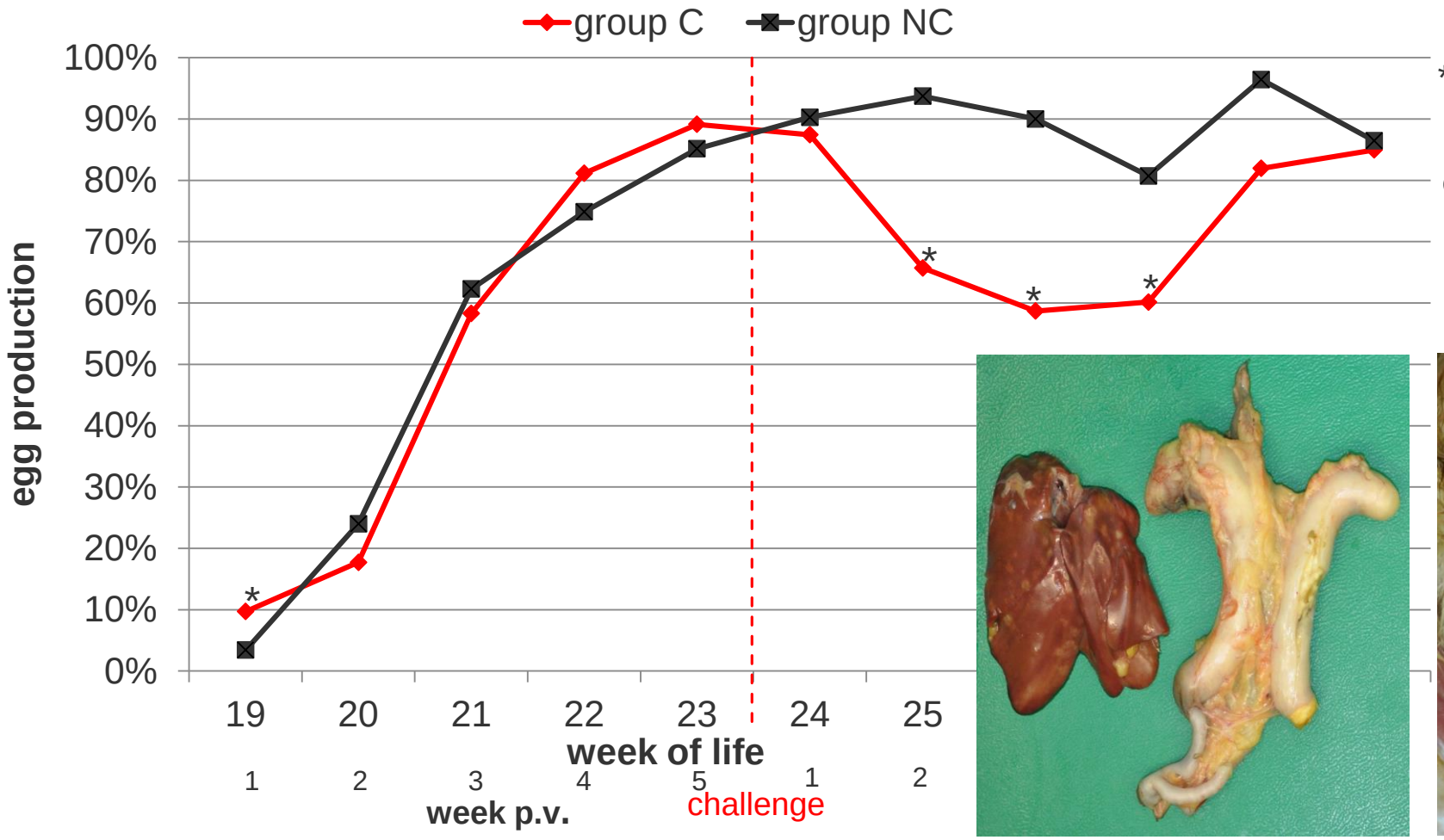


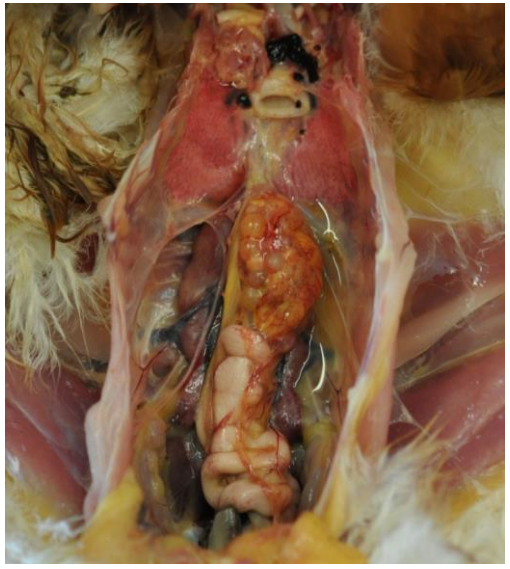
commercial strains of chickens

(Zahoor *et al*., 2011, Avian Dis. 55:29-34.)

Experimental infection of layers

- effect of histomonosis on performance





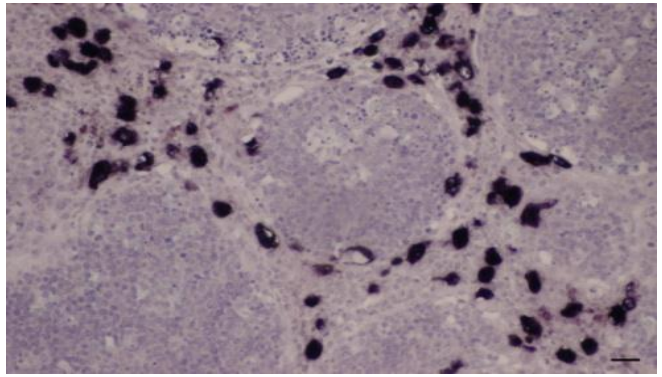
(Liebhart *et al*., 2013, Avian Pathol. 42:79-84.)

Recent improvements in diagnosis in laboratory diagnosis

- PCR
  - several protocols were developed
    (Huber *et al*., 2005. Vet Parasitol. 131, 311–316; Hafez *et al*., 2005. Avian Dis. 49, 366–370; Grabensteiner and Hess, 2006. Vet Parasitol. 142, 223–230; Bleyen *et al*., 2007. Vet Parasitol. 143, 206–213, Landman *et al*., Avian Pathol., 2015, in press; Hussain *et al*., Vet Parasitol., accepted for publication)

- histological detection tools
  - *in situ* hybridisation (ISH)
    - using oligonucleotide probes

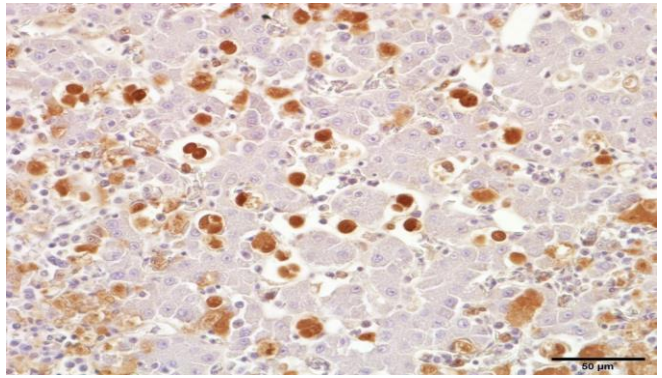
    (Liebhart *et al*., 2006. J Comp Pathol. 35, 237–242)



histomonads in the bursa of Fabricius of a turkey

  - immunohistochemistry
    - using polyclonal antibodies

    (Singh *et al*., 2008. Exp Parasitol.118, 505–513)



histomonads in the liver of chicken

Current demand to combat histomonosis

- in countries that observe outbreaks of histomonosis

- urgent need to prevent histomonosis in poultry flocks
  - high severity of the disease
    - suffering of infected birds - animal welfare considerations
    - losses in turkey and chicken flocks
    - decrease of egg production in layers

Topics of the lecture

Histomonosis – current situation in poultry flocks and latest experimental data on the disease

Recent investigations to prevent the disease

Focus on prevention

- pharmaceuticals
- plant derived compounds
- vaccination

Pharmaceuticals

- recently tested drugs

	paromomycin	nifurtimox
medicinal category	aminoglycosid antibiotic	nitrofuran
effectivity	reduction of mortality of turkeys[1]	reduction of liver lesions and mortality of infected turkeys[2]
application	prophylactic	prophylactic
adverse effects / license	prophylactic use causes antibiotic resistance of intestinal bacteria[3]	antimicrobials of this group are banned in many countries

[1]Lindquist, 1962, Am J Vet Res. 23:1053-1056; Bleyen *et al*., 2009, Vet Parasitol. 165: 248-255;
van der Heijden *et al*., 2011, Tijdschr Diergeneeskd. 136:410-416; Hafez *et al*., 2014, Arch Anim Nutr. 64:77–84;
[2]Hauck *et al*., 2010, Avian Dis. 54:28-32;
[3]Kempf *et al*., 2013, Vet J., 198:398-403;

Plant derived compounds

botanical / herbal product	minimal lethal concentration (MLC) *in vitro* (12 - 48h)	*in vivo* effect	supplementation	host species tested	reference
Cinnamomum aromaticum essential oil	0.5 µl/ml	not done (n.d.)	-	-	Zenner *et al*., 2003, Parasite 10:153–157.
Citrus limon pericarps essential oil	1 µl/ml	n.d.	-	-	
Allium sativum essential oil	1 µl/ml	n.d.	-	-	
Protophyt® oils from cinnamon, garlic, rosemary, lemon	-	30% reduction of mortality	0.2% via feed and 0.3% via drinking water	turkey	Hafez and Hauck, 2006, Arch Anim Nutr. 60:436–442.
cassia oil	0.60 µl/ml	n.d.	-	-	Grabensteiner *et al.,* 2007, Parasitol Res. 101:193–199.
carvacrol	0.5 µl/ml	n.d.	-	-	
thyme rosemary mixture essential oil	0.55 µl/ml	n.d.	-	-	
Quillaja saponaria saponin	0.90 µl/ml	n.d.	-	-	
RepaXol® (blend of double-coated oregano, cinnamon, thyme, citrus fruit extract, and capsicum essential oils)	1.25 µl/ml	n.d.	-	-	Hauck and Hafez, 2007, Avian Dis. 51:880–883.
blend of double-coated cinnamon, thyme, citrus fruit extract, and capsicum essential oils	1.25 µl/ml	n.d.	-	-	
coated grapefruit seed extracts	2,5 µl/ml	n.d.	-	-	
Thymus vulgaris ethanolic extract	5 mg/ml	100% mortality	1% via drinking water	turkey	Grabensteiner *et al.*, 2008, Parasitol Res. 103:1257–1264.
Serenoa repens ethanolic extract	5 mg/ml	100% mortality	1% via drinking water	turkey	
Vitis vinifera ethanolic extract	5 mg/ml	91.7% mortality	1% via drinking water	turkey	
*Cucurbita pepe*ethanolic extract	5 mg/ml	100% mortality	1% via drinking water	turkey	
Peganum harmala ethanolic extract	1.65 mg/ml	n.d.	-	-	Arshad, *et al.*, 2008, Phytother Res. 22:1533–1538.
Eucalyptus globulus	indeterminate	n.d.	-	-	
Aromabiotic™ derived from tropical plants	not available - no effect	n.d.	-	-	van der Heijden and Landman, 2008, Vet Parasitol. 154:1–7; van der Heijden and Landman, 2008, Avian Pathol. 37:45-50.
Enteroguard™ lyophilized garlic and cinnamon infusion with active compounds allicin and cinnemaldehyde and other thiosulfonates	not available - growth inhibition	100% mortality	500 ppm	turkey	
Protophyt SP™ oils from cinnamon, garlic, rosemary, lemon	not available - no effect	94-100% mortality	3000 ppm via feed	turkey	
Protophyt B™ oils from cinnamon, garlic, rosemary, lemon	not available - growth inhibition	100% mortality	0.2 % via drinking water	turkey	
Artemisia annua-derived materials (i.e. dichloromethane extracts of leaves and pure artemisinin	1.0 mg/ml or growth inhibition	turkeys: 85-100% mortality; chickens: no reduction of severe lesions in caecum and liver	0.1% via drinking water or 100 ppm via feed	chicken and turkey	Thofner *et al*., 2012, Avian Pathol. 41:487–496.

Vaccination against histomonosis

active vaccination

attenuated histomonads

protection

(Tyzzer, 1936, J. Comp. Pathol. Ther. 49:285–303; Lund, 1959, J. Protozool. 6:182-185; Lund, 1966, Exp. Parasitol.18:403-407; Hess *et al.,* 2008, Vaccine 26:4187-4193)

inactivated histomonads

(Hess *et al.,* 2008, Vaccine 26:4187-4193; Bleyen *et al*., 2009, Avian Pathol. 38:71-76)

passive vaccination

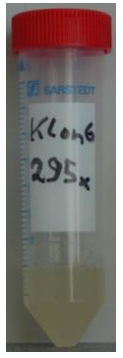
circulating antibodies

(Clarkson, 1963, Immunology 6:156-168; Bleyen *et al*., 2009, Avian Pathol. 38:71-76)

Vaccination – recent investigations

- experimental approaches to protect turkeys

  - vaccination with a clonal culture of *in vitro* attenuated histomonads
    (Hess *et al.,* 2008, Vaccine 26:4187-4193)



H. m./Turkey/ Austria/2922-C6/04

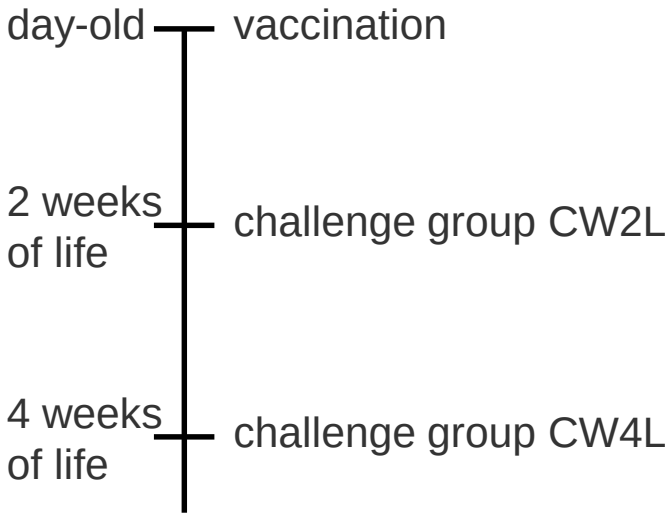
vaccine candidate challenge isolate

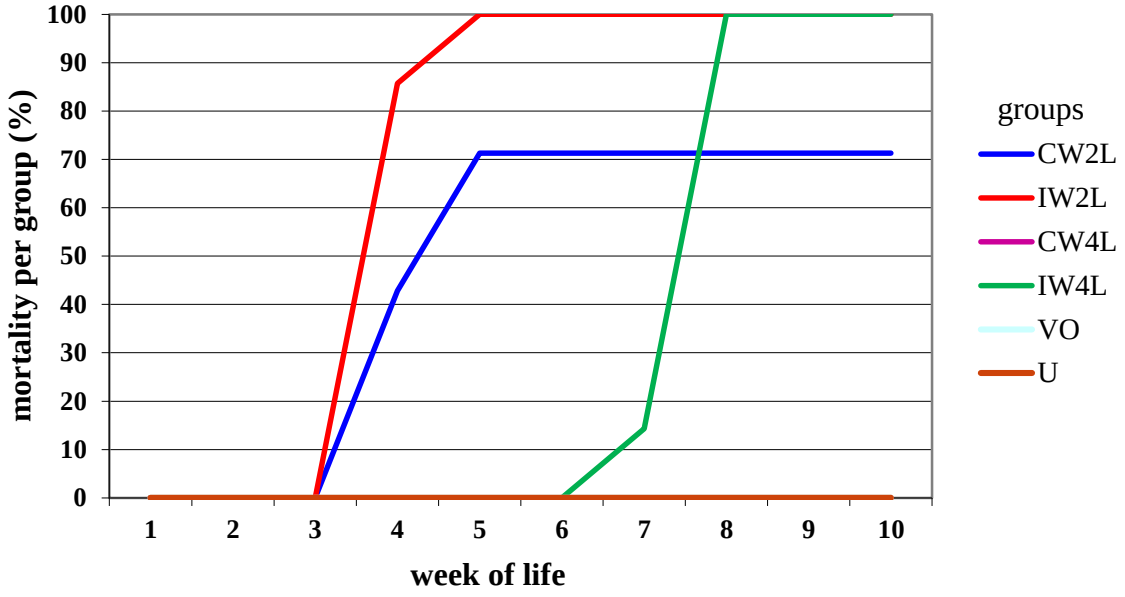
  - infection with virulent histomonads and treatment
    (Bleyen *et al*., 2009, Avian Pathol. 38:71-76)

  - application of intracloacally passaged low-virulent histomonads
    (Nguyen Pham *et al*., 2013, Vet Parasitol. 196:307-313)

Vaccination using *in vitro* attenuated histomonads

- oral vaccination of turkeys

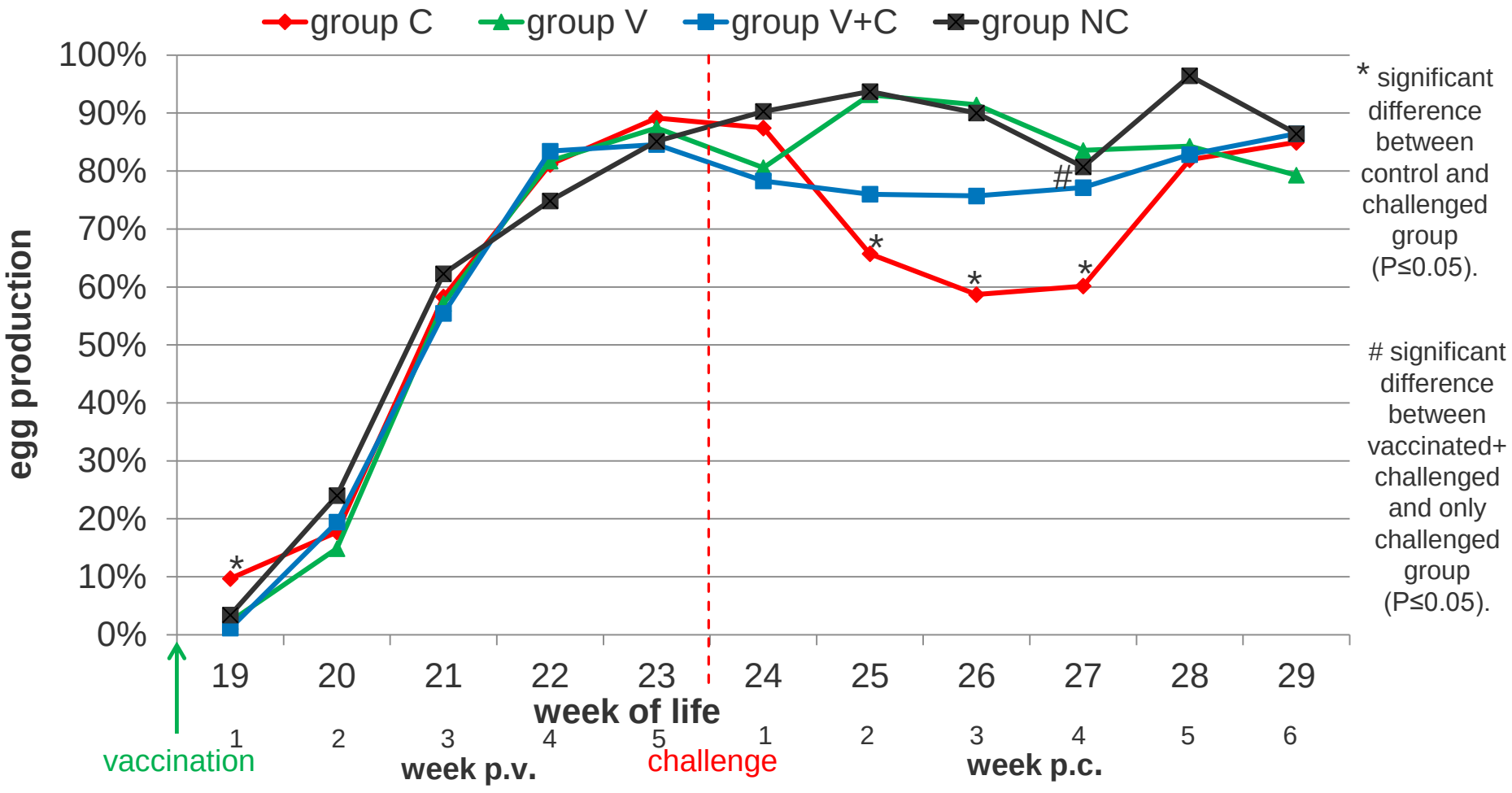




- partial protection 2 weeks post vaccination: 4 of 14 birds survived the challenge (group CW2L)
- full protection of orally vaccinated turkeys challenged after 4 week post vaccination (group CW4L)

(Liebhart *et al*., 2010, Avian Pathol. 39:399-403)

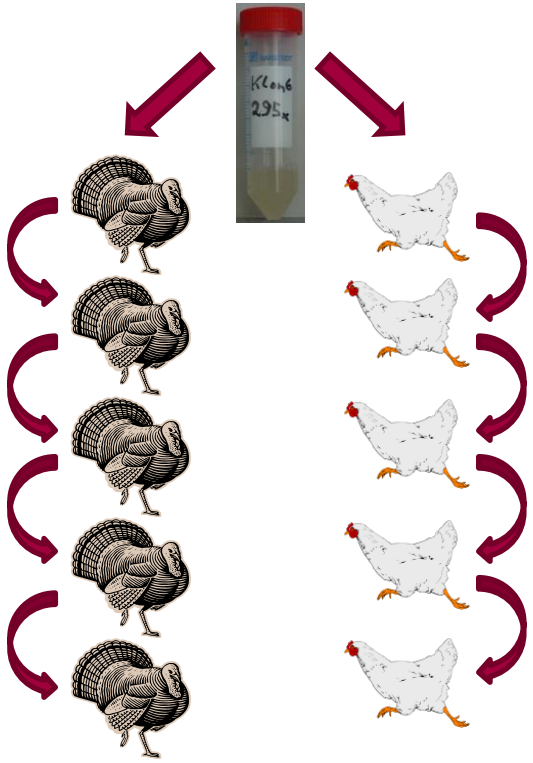
Protection of chickens following vaccination



(Liebhart *et al*., 2013, Avian Pathol. 42:79-84)

Investigations on the vaccine

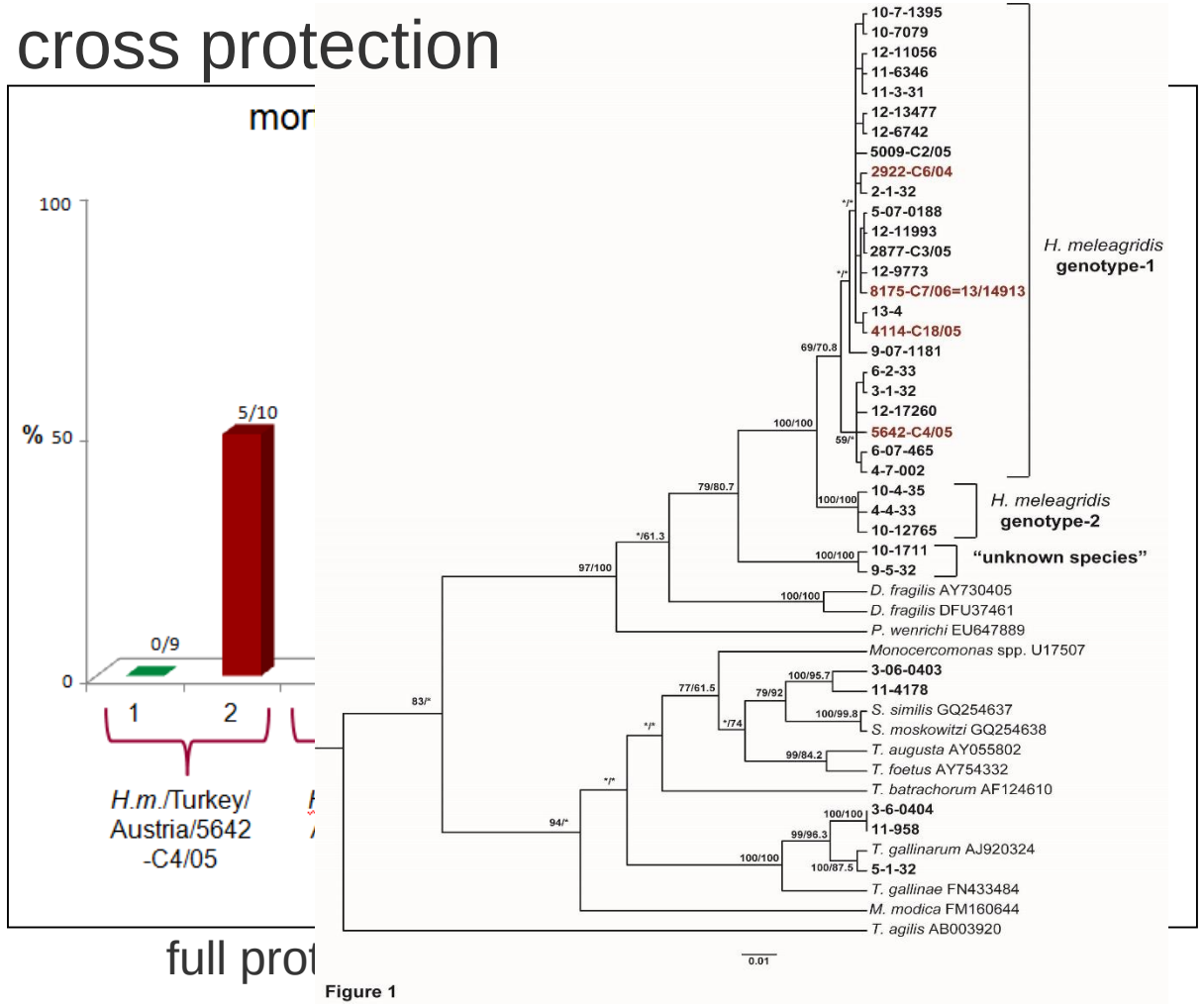
- safety of the live vaccine



no reversion to virulence following five consecutive passages *in vivo*

(Sulejmanovic *et al*., 2013, Vaccine 31:5443-5350)

- cross protection



full prot

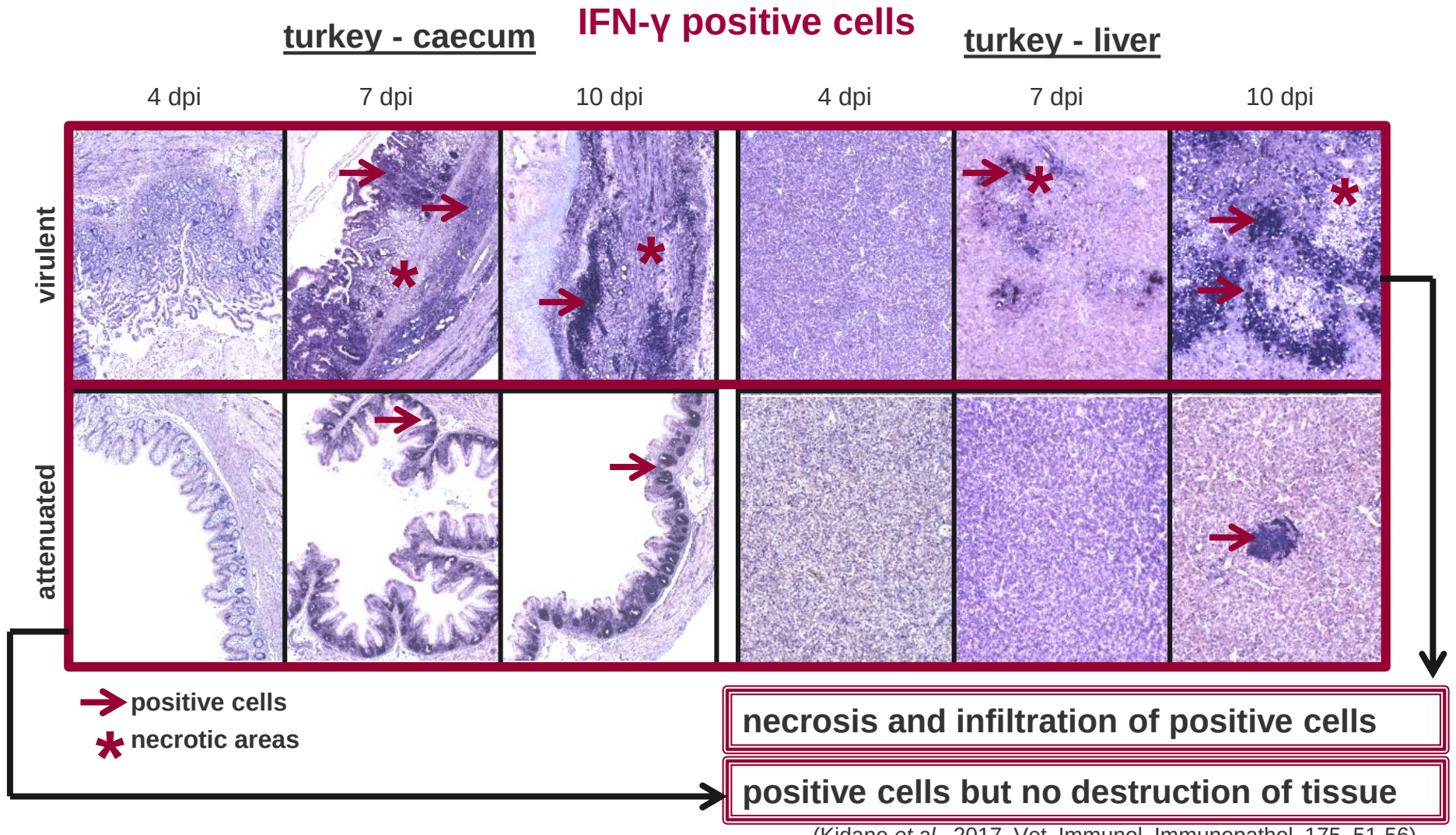
(Bilic *et al*., 2014, PLoS One 9:e92438; Bilic *et al*., 2016, Avian Pathol. 45:46-53)

Previous investigations on the immune response against histomonosis

- Previous investigations
  - circulating antibodies do not protect against histomonosis
    (Clarkson, 1963, Immunology 6:156-168; Hess *et al*., 2008, Vaccine 26:4187-4193; Bleyen *et al.*, 2009, Avian Pathology 38:71-76)
  - intestinal antibodies (IgG, IgA and IgM) of chickens are increased following infection
    (Windisch and Hess, 2010, Parasite Immunology 32:29-35)
  - chickens have of a more effective innate immune response in the caecum than turkeys (higher expressions of IL-1β, CXCLi2 und IL-6 mRNA)

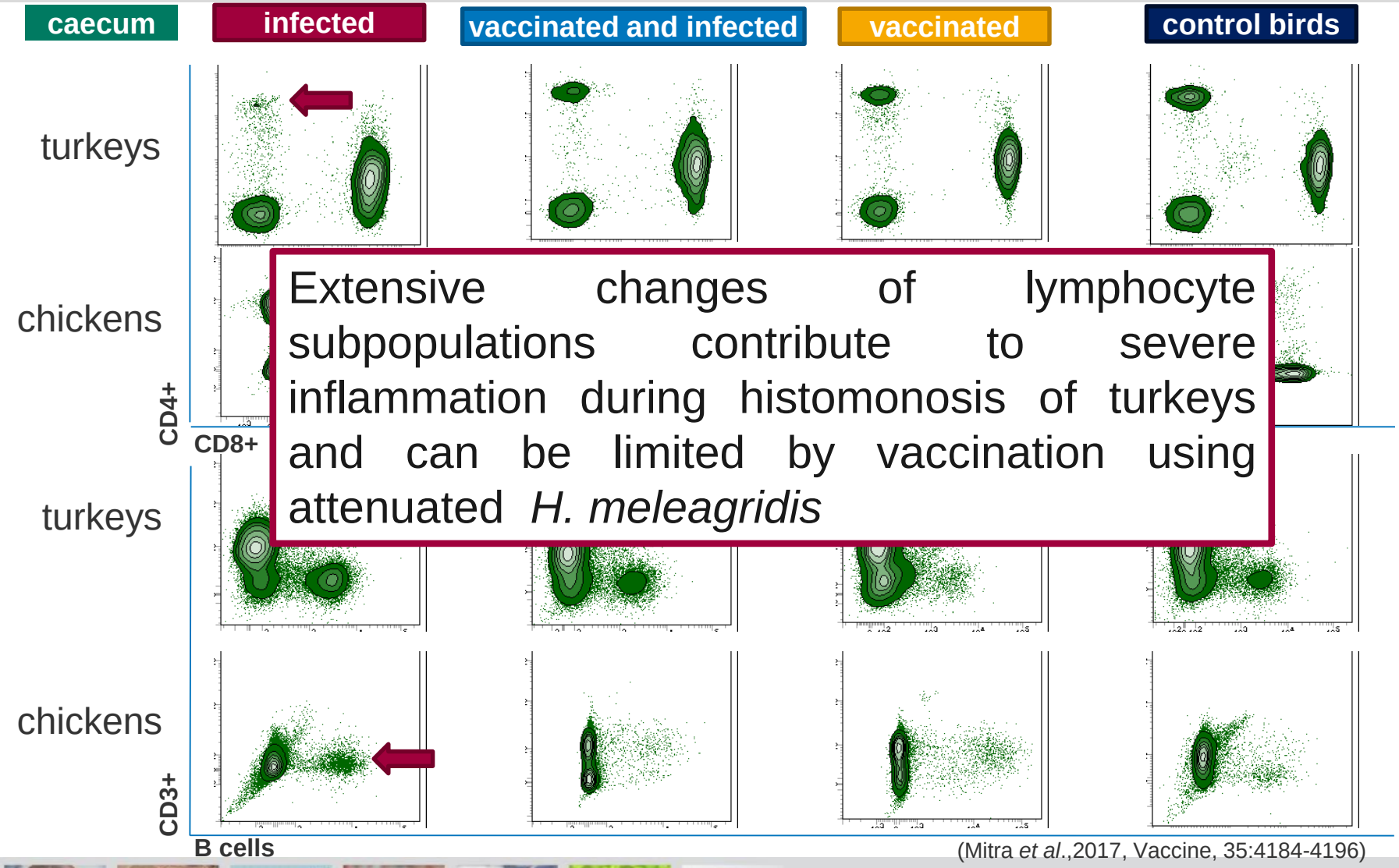
    and
  - specific immune cells ($CD4^+$, $CD8\alpha^+$, $CD28^+$ and $CD44^+$) and cytokines (IL-1β, CXCLi2, IFN-γ, IL-13, IL-4 und IL-10) are severely increased in infected livers of turkeys.
    (Powell *et al*., 2009, Parasite Immunology 31:312-327)

Studies on the effect of vaccination on the immune response



(Kidane *et al.*, 2017, Vet. Immunol. Immunopathol. 175, 51-56)

Effect of vaccination on immune cells

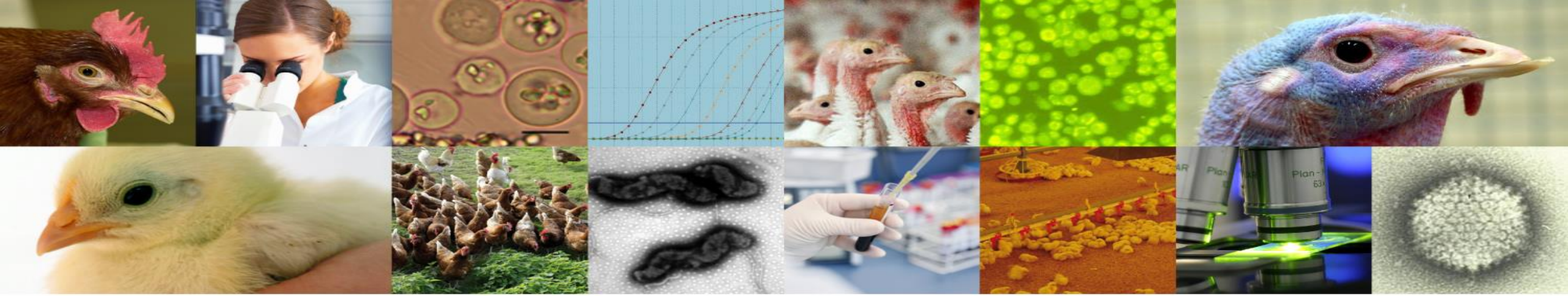


Extensive changes of lymphocyte subpopulations contribute to severe inflammation during histomonosis of turkeys and can be limited by vaccination using attenuated *H. meleagridis*

(Mitra *et al.*,2017, Vaccine, 35:4184-4196)

Summary and conclusion

- histomonosis is a re-emerging disease of poultry due to the ban of effective chemotherapeutics in Europe and the USA
- investigations were recently performed on pharmaceuticals, herbal products and vaccination
- experimental vaccination was shown to be a promising approach to protect turkeys and chickens from clinical histomonosis
- recent investigations on the immune reaction following vaccination and infection
  - low increase of different leukocytes following vaccination
  - measurable changes in T cell populations following infection with virulent histomonads
  - expression of cytokines of infected turkeys indicate activation of the type 2 immune pathway following infection with *H. meleagridis*



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Ivana Bilic
and the whole team
of the

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Department for Farm Animals and
Veterinary Public Health

Christian Doppler Laboratory
for Innovative Poultry Vaccines

FWF

Thank you for your attention!