

Granuloma disease in flocks of productive layers caused by *Tetratrichomonas gallinarum*

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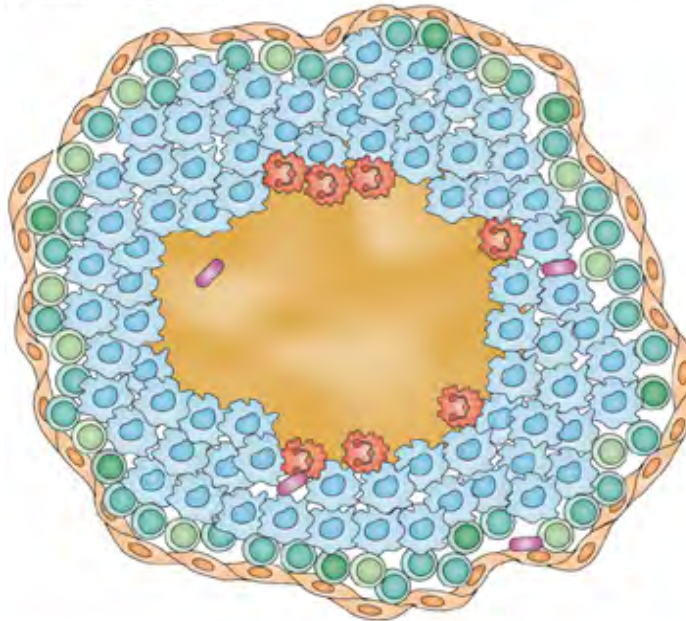
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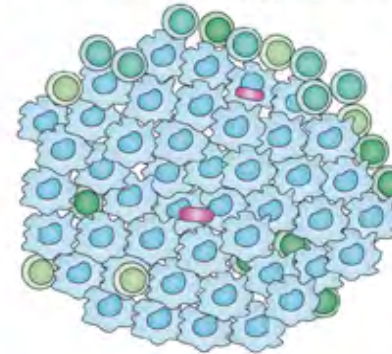
- 1. Outbreak in 7 layer flocks**
- 2. Case report**

Granuloma

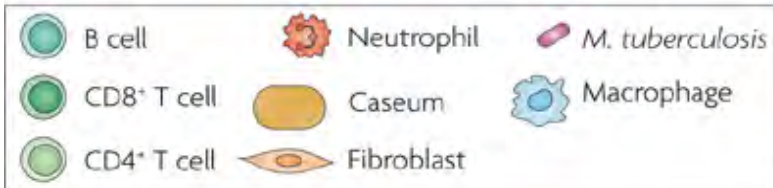
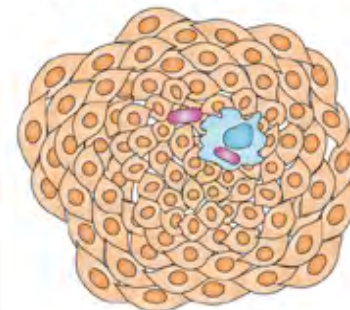
a Caseous granuloma



b Non-necrotizing granuloma



c Fibrotic granuloma





Escherichia coli



Histomonas meleagridis



Mycobacterium avium



M. avium



Salmonella Pullorum



Aspergillus fumigatus

Case history & clinical signs

- 7 Flocks
- White & (1) brown layers
- Flock sizes 8100-71,700
- Start increase mortality 21-35 (87) weeks
- 3.7 to 11.0% above norm
- Duration 9 to 48 weeks
- Maximum % egg drop 0-10.1
- Directly or indirectly linked to one rearing farm



Post-mortem & sampling

13 to 134 hens per flock (pm, histology, bacteriology, culture & PCR)



Apparently healthy $n = 310$



Dead non-autolytic $n = 98$



Gross post-mortem examination

Hens submitted	<i>n</i>	Number of hens with granulomas in			Number of hens with			
		liver	ceca	other organs ^a	productive ovary (%)	sepsis	peritonitis	other lesions ^b
Dead	98	23	92	3	59 (60)	10	2	2
Alive	310	46	248	2	251 (81)	4	0	4

n = 13-134 per flock

Granulomas 353/408 = 87% (50%-100%)

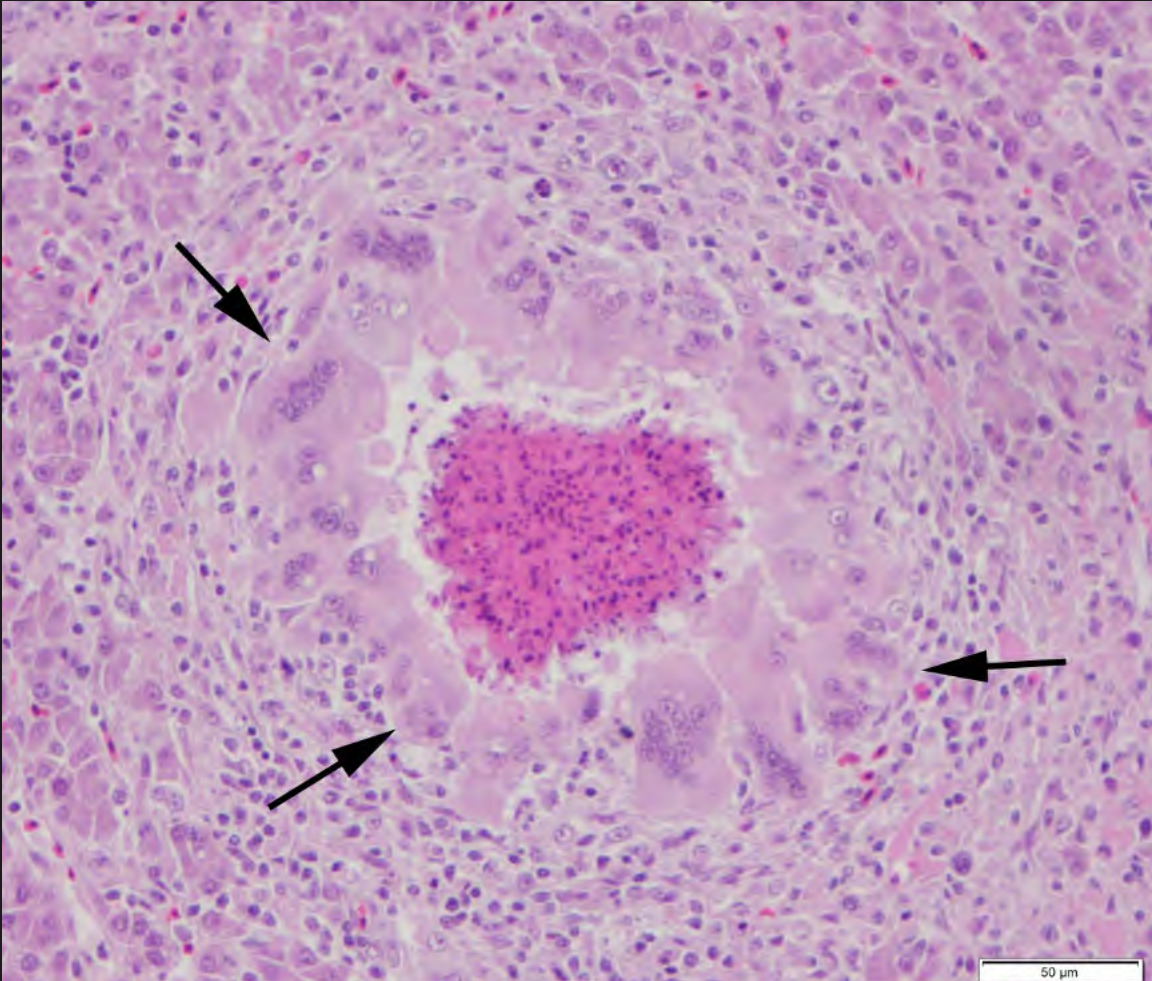
Ceca 83%

Liver 17%

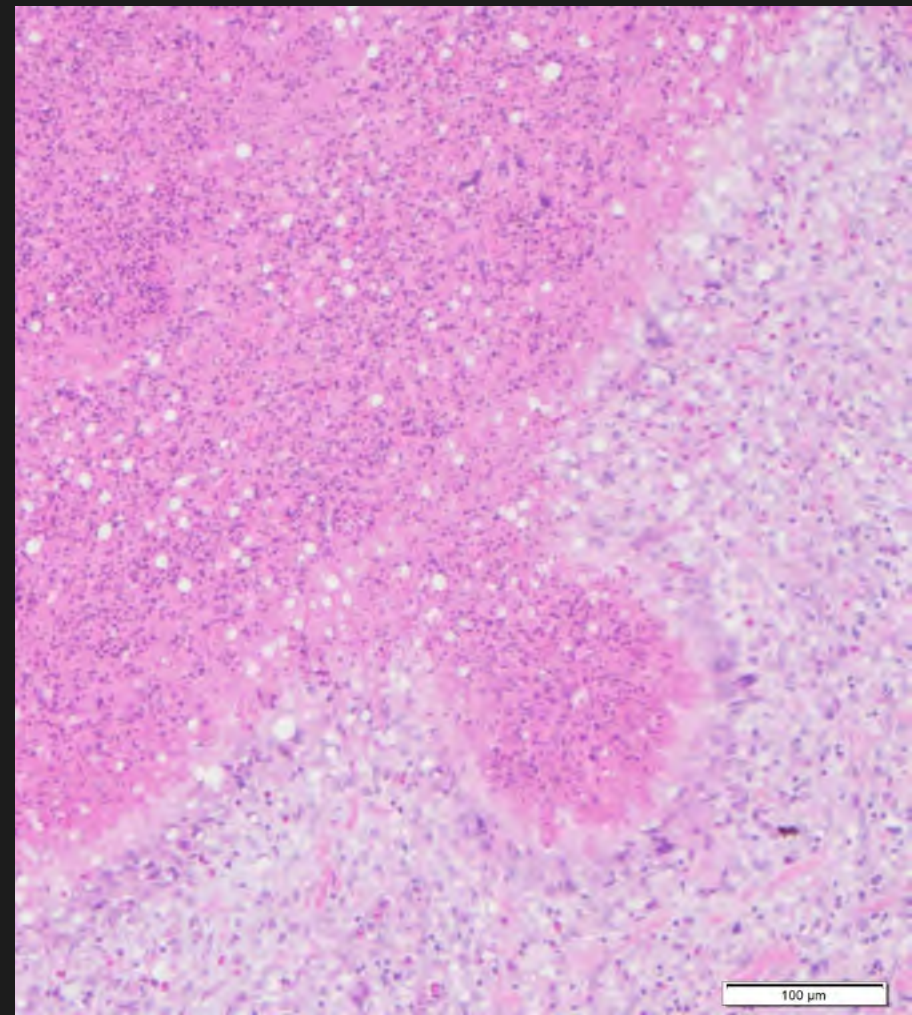
Ceca flock G used as inoculum

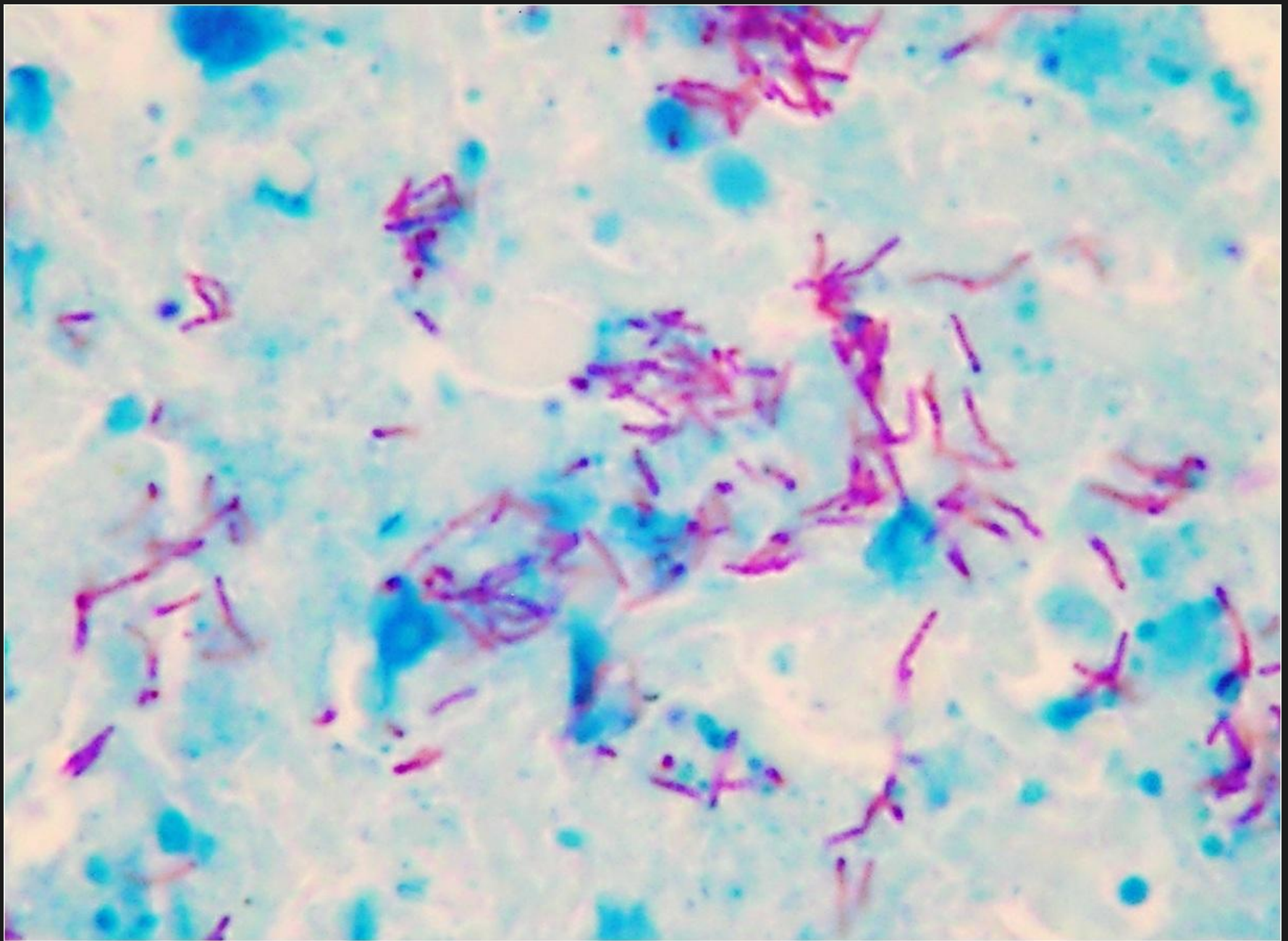


T. gallinarum

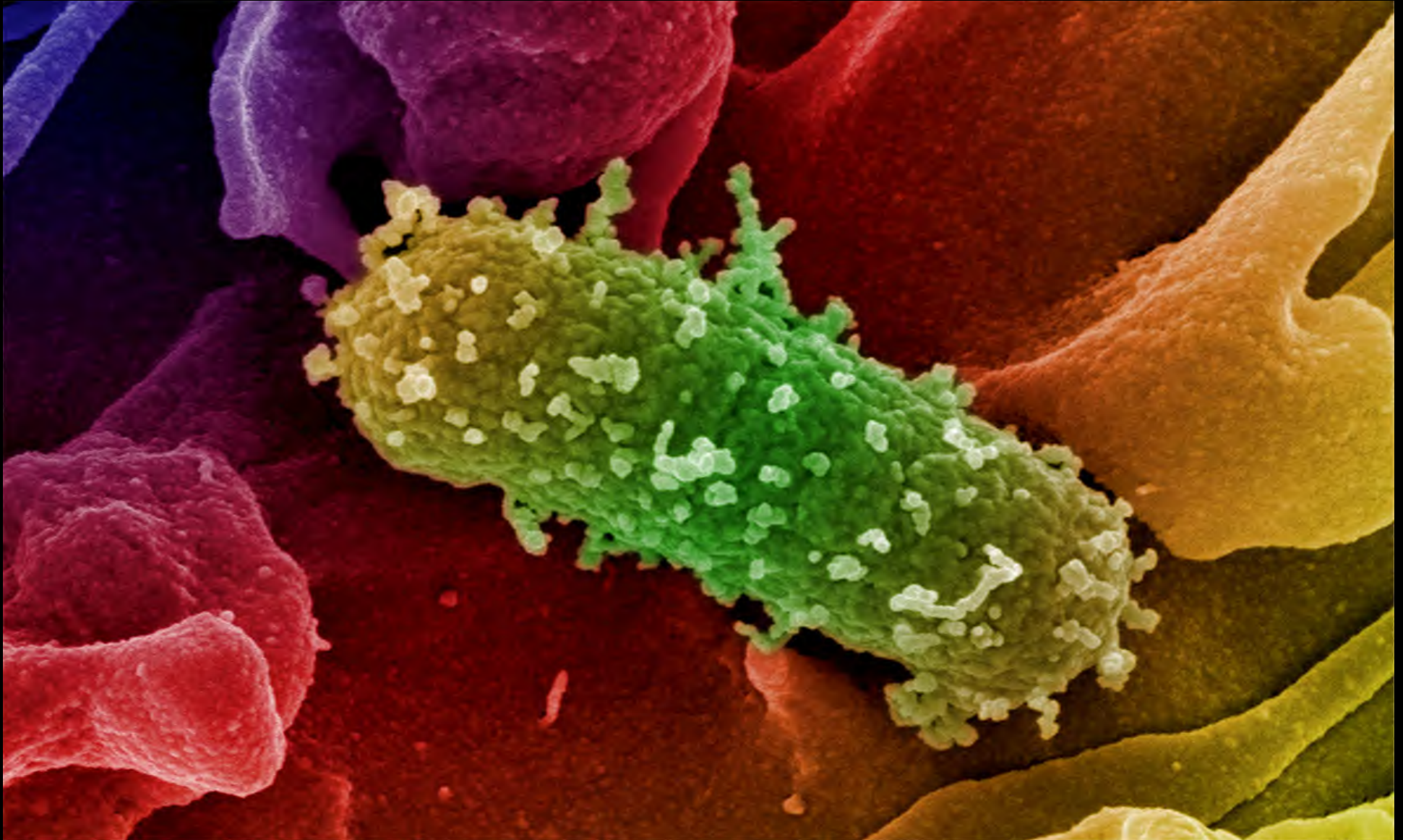


$n = 184$ livers & ceca





Ziehl Neelsen $n = 22$ livers & ceca

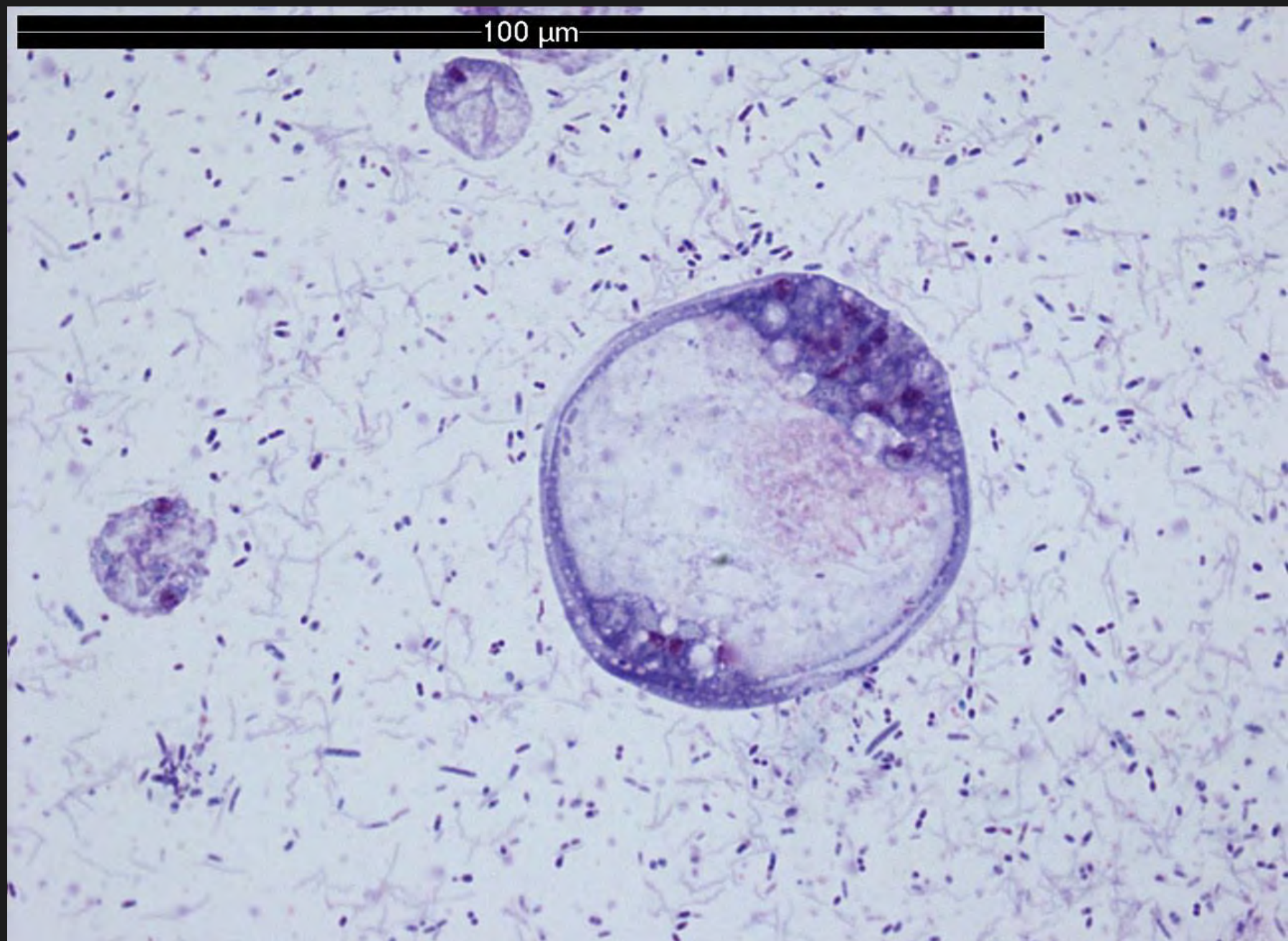


S.E.M. *E. coli* on macrophage

100 μ m

Culture, PCR, sequencing ITS1-5.8S rRNA-ITS2

100 μ m



PCR products 100% identical



Tg

GenBank Accession number

LK031731

(Liebhart *et al.*, 2014)



Liver *Alectoris rufa*



Assesment infectiousness



- 10 SPF WL
- Contact period 6 wks
- Shared feed, water & bedding (fence)
- 2/6 small cecal granulomas
 - 0.5 cm

Animal experiment (I)

Group	Inoculum	<i>n</i>	Dose per hen ^a	Inoculation route ^b
1	negative control	20	-	-
2	<i>E. coli</i>	20	10 ^{6.7} cfu	iv
3	<i>E. coli</i>	20	10 ^{9.0} cfu	it
4	<i>Blastocystis</i> spp. & <i>E. coli</i>	20	10 ^{6.6} cells 10 ^{8.2} cfu	ic
5	Tg	20	10 ^{5.1} cells	ic
6	Tg	20	10 ^{4.8} cells	iv
7	homogenized ceca	20	25% v/v	ic

Escherichia coli
peritonitis
syndrome
(55%-90%)



Animal experiment (I)

Group & treatment	Mortality ^c	Number of hens with							Histology	Protozoal culture ^d	
		productive ovary ^e	degen. ovary ^e	inactive ovary ^e	Macroscopic granulomas						
					cecum	liver	spleen	Total	granuloma lesions	liver	cecum
1 NC	0	20	0	0	0	0	0	0	neg	neg	neg
5 Tg ic	0	19	0	1	18	4	1	18	pos	neg	Tg
6 Tg iv	20	6	3	11	0	20	9	20	pos	neg	Tg
7 Hom. caeca ic	2	18	1	1	9	3	0	10	pos	neg	Tg, B ^f

PCR & sequencing ITS1-5.8S rRNA-ITS2 showed *T. gallinarum* in groups 5, 6 & 7

Case report

- Flock 33088 layers
- Keep indoors until 39 weeks of age
- Mortality
 - 0.85% till 55 weeks of age (norm 3.5%)
 - 20.3% 55 - 84 weeks of age (norm 3.9%)
- Egg production above norm
- Egg weight 0.5-3 g under norm



Post-mortem examination 61-week-old white layers with granuloma disease

		# hens					
		Granulomas in liver	Granulomas in cecum	Productive ovary	Degenerated ovary	Inactive ovary	Reduced condition
12 dead hens		12 (+++*)	10 (++ to +++)	1	7	4	7
12 live hens	5 clinically depressed hens	1 (+++)	2 (+ and +++)	2	1	2	3
	7 clinically healthy hens	2 (++)	1 (+)	7	0	0	0

*+ = one granuloma per hen, ++ = some granulomas per hen and +++ = many granulomas per hen

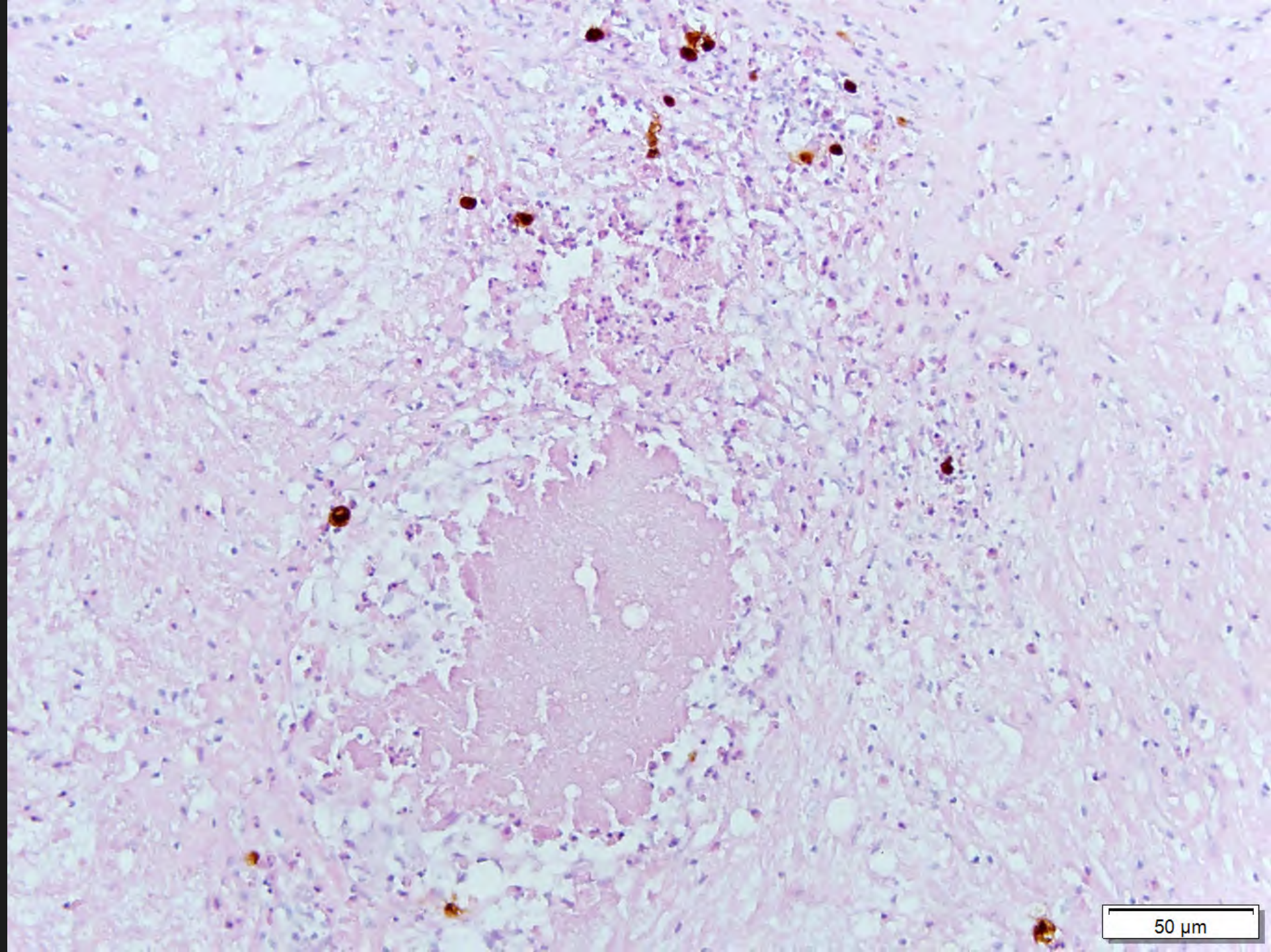




Further diagnostics 9 hens

- *In situ* hybridization
 - 9 x positive
- Nested PCR & sequencing
 - Organs
 - 3 x *T. gallinarum*
 - 4 x *Simplicimonas* spp.
 - Culture
 - 2 x *T. gallinarum*
 - 1 x *Simplicimonas* spp.

T. gallinarum strains
genetically identical to
2013 outbreak strain
(strain 13/16632)



50 μm

Google Maps



Afbeeldingen ©2018 Google, Kaartgegevens ©2018 Google 500 m

Afstand meten

Totale afstand: 3,81 km (2,37 mijl)





Afbeeldingen ©2018 Google, Kaartgegevens ©2018 Google 50 m

No trichomonadal DNA found
1/5 wild ducks *Simplicimonas* spp.

Persistence in wetland area?

- In agreement with Trylich et al., 1977
 - Canadian turkey farm
 - Severe granulomatosis
 - Two years in successive flocks
 - After source pool was fenced off, granulomatosis outbreaks stopped

Undersökningar över en med specifika granulom förlöpande hönssjukdom orsakad av mukoida kolibakterier (Koli-granulom).

AV

A. HJÄRRE och G. WRAMBY

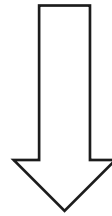
SÄRTRYCK UR

SKANDINAVISK VETERINÄRTIDSKRIFT FÖR BAKTERIOLOGI, PATOLOGI ETC. 1945

Coligranuloma (Hjärre & Wramby's disease)

- 1941-1945
- $30/8000 = 0.4\%$
- Granulomas
 - Caeca 63%
 - Liver 50%
 - Bone marrow 3%
- Reproduction only by artificial routes
 - Intramuscular, intravenous
 - Mucoid *E. coli*
- Little practical relevance
 - Ddx tuberculosis (localization, microscopy, etiology)

If severe granuloma disease is observed



T. gallinarum should rank high in differential diagnoses



Thank you for your attention

