

«Cross-stitch» vertebrae in *A. salmon*

Pathology and the possible link to vaccines

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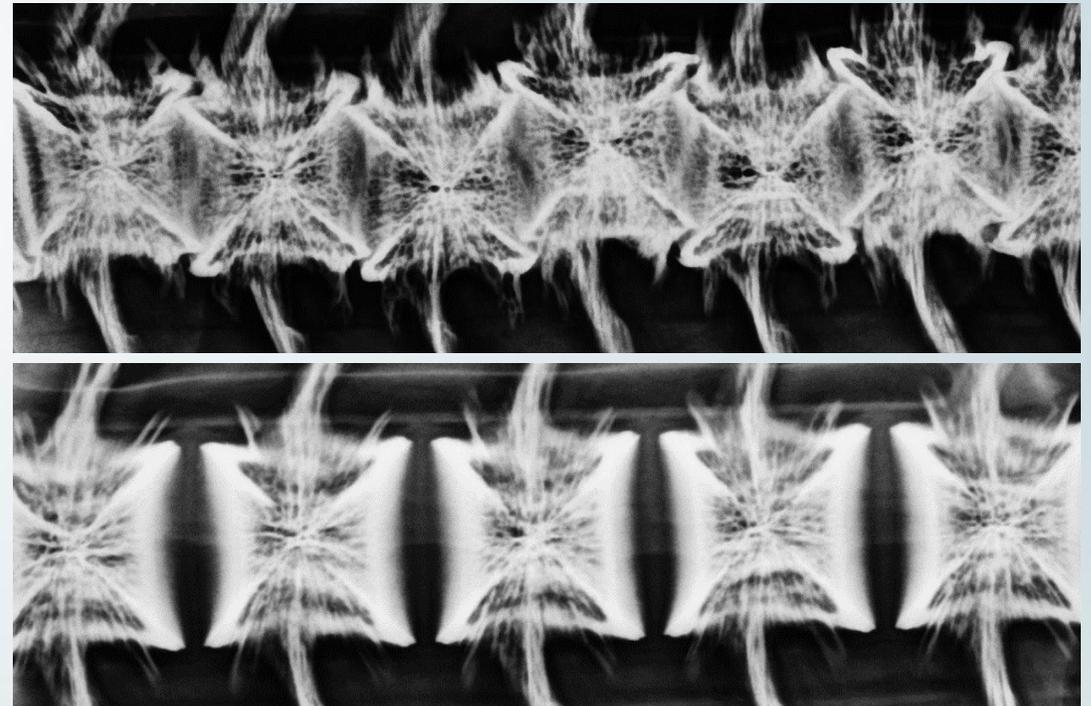
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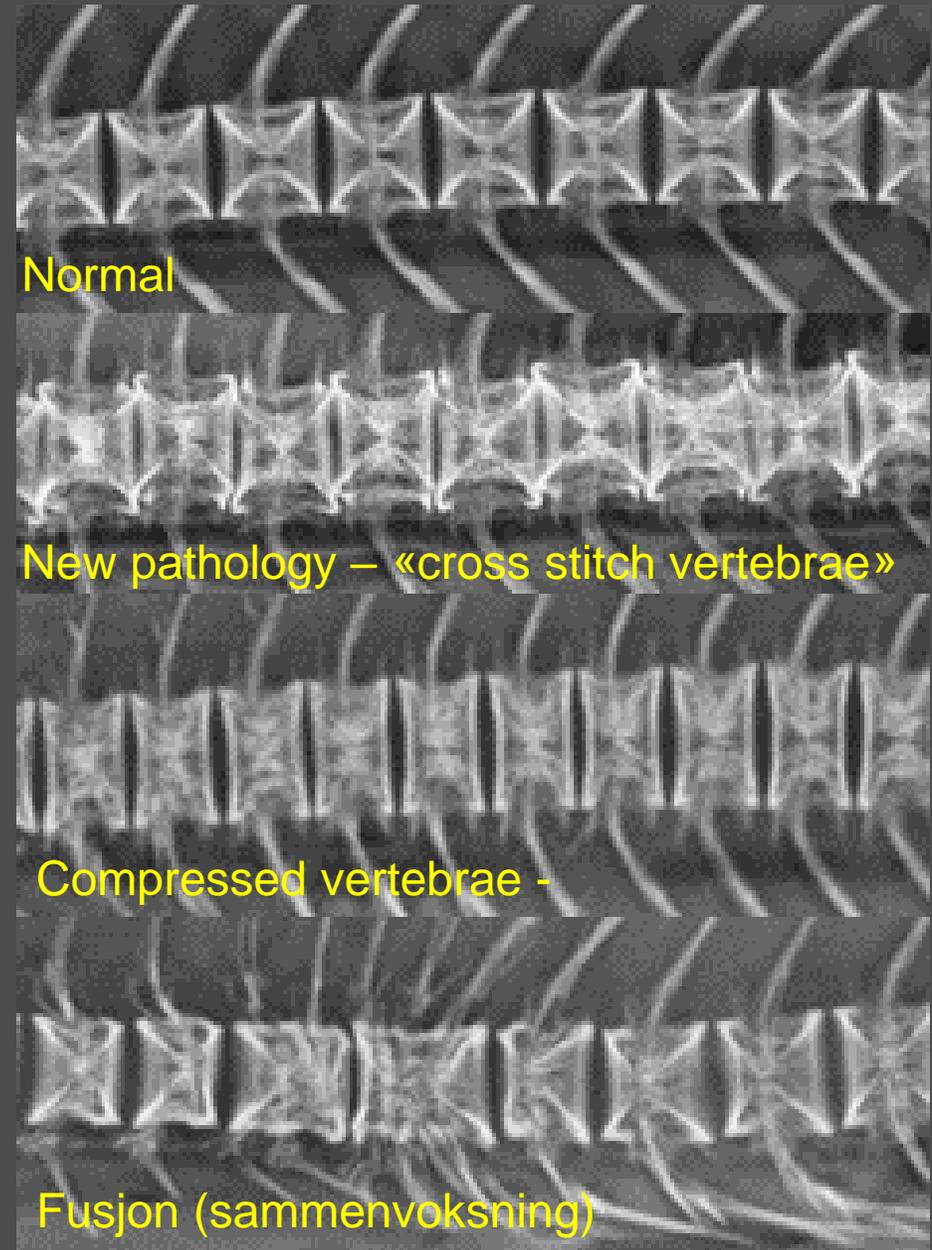
Skeletal deformities in farmed salmon

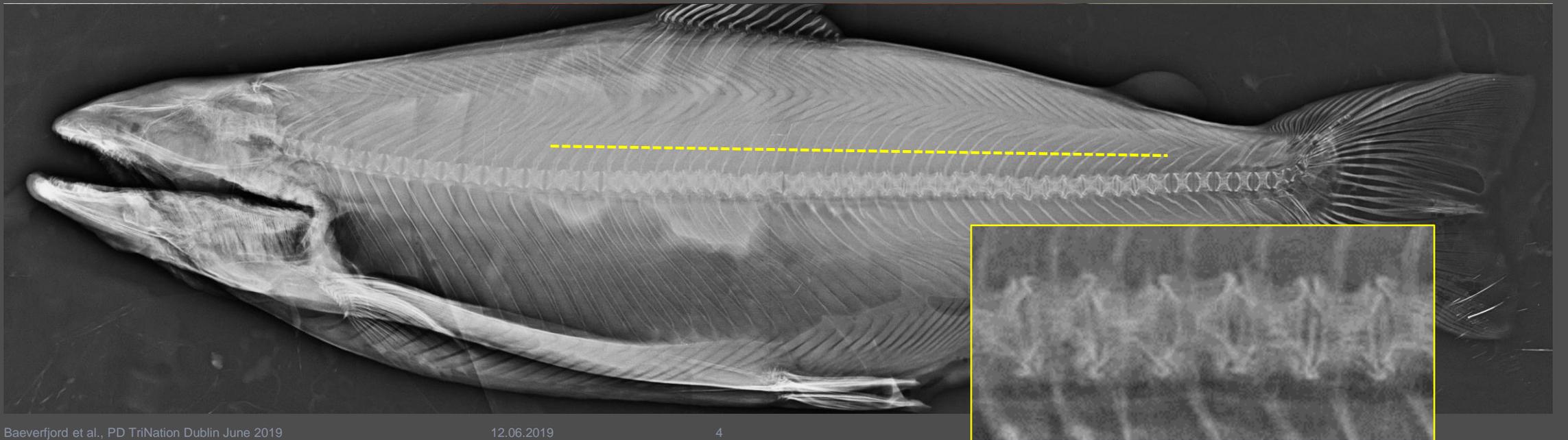
- First recognized as a problem 1995-96
- Systematic research gave results: Deformities gradually disappeared from the radar during the 2000s
- Most cases were explained by two factors: High temperatures and reduced mineralization, or a combination of the two
- Some cases remained unexplained
 - Documented effects of certain vaccine products during a defined time period
- By 2010-12, it was quiet

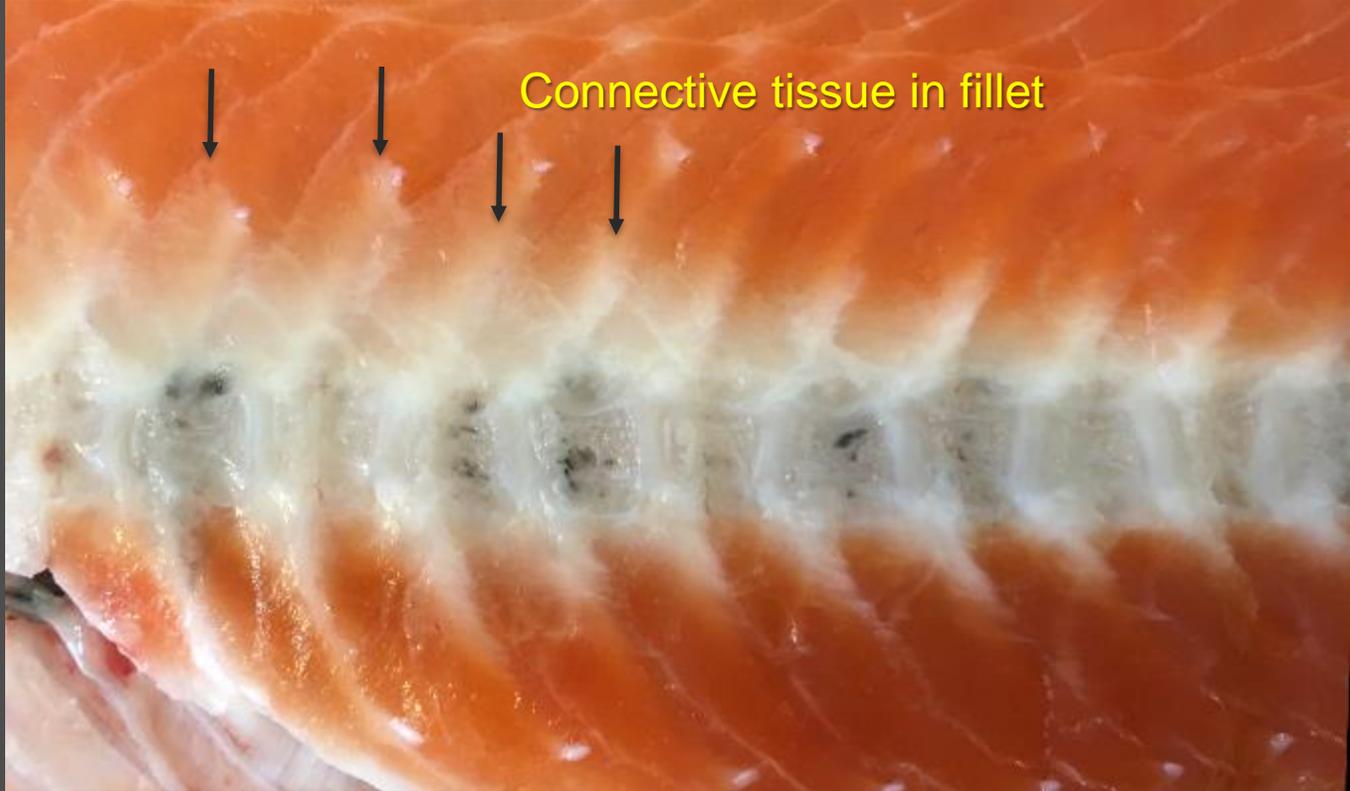


Vertebral deformities winter 2016-2017

- A flood wave of new cases
- Harvest size fish, fish groups with no previous history of deformities
- 0+ smolts, 2015
- X-ray observation: «New» pathology
- West coast of Norway







Field observations, reports from producers

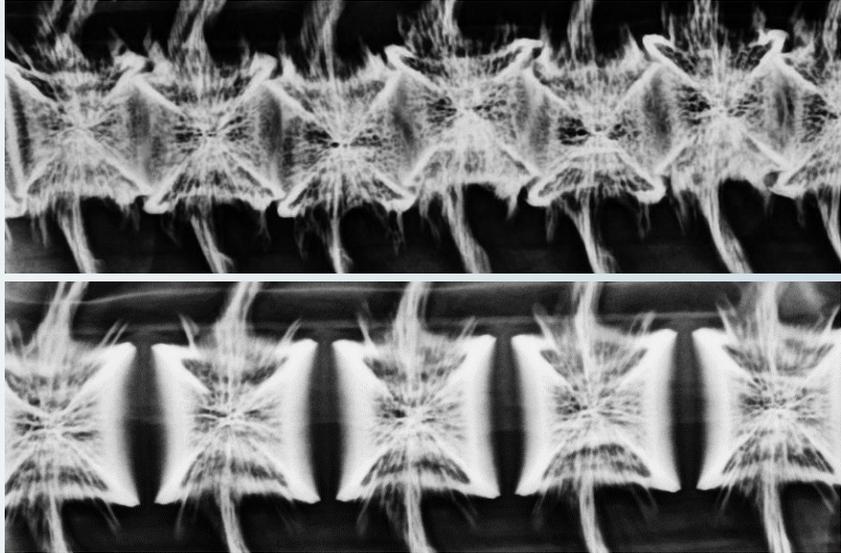
- Linked to vaccination with PD-vaccine
- Worse in 0+ than 1+ groups
- Fish producers reported that fish fed «health feeds» were more severely affected than those fed diets without active ingredients

- Unexplained variation in severity
- Worst groups 30-40% fish affected at harvest

The 2013-2015 study (Nofima and Pharmaq)

- Nofima did a long-term study in collaboration with Pharmaq
- Initiative based on observations of severe skeletal deformities in field trial
- Comparison of effects: Known risk factors for vertebral deformities (freshwater temperature and reduced P-supply) and various vaccines and –products.
- From 17g to harvest at 4,5kg
- X-ray (live) at seawater transfer, at 700g and at harvest (4,5kg)

The 2013-2015 study, key findings

- A specific type of pathology was identified, later provisionally named «cross-stitch» vertebrae
 - The X-ray pathology differed from changes associated with high temperature and reduced dietary P
 - The changes were particularly severe
- 
- The reaction required adequate P supply during initial immunization
 - The reaction was not influenced by temperature during early immunization
 - The prevalence of cross-stitch pathology varied between vaccine groups
 - The reaction was enhanced by feeding a commercial health feed 7 months in sea
- X-ray was only reliable diagnosis

Repeated X-ray of same individuals

Cross-stitch

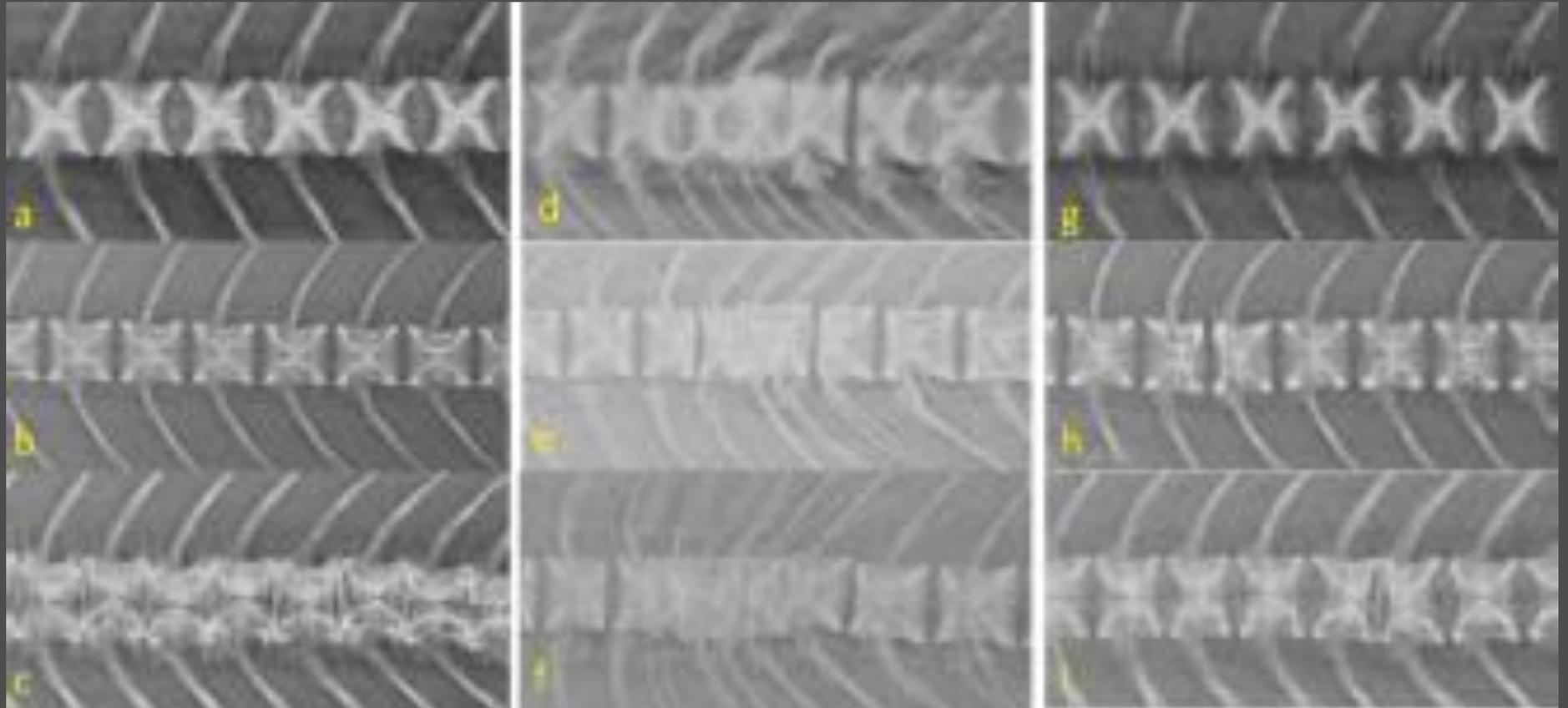
Fusion

Compressed

Seawater transfer

7 months post transfer
(ca 700g)

Harvest
(ca 4,5 kg)



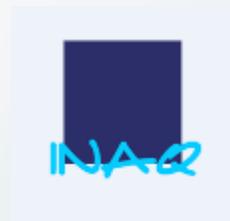
No early signs of cross stitch pathology, differs from the two other main types of lesions

FHF – Norwegian Seafood Research Fund

Project # 901430:

Prevention of cross-stitch vertebrae in farmed salmon, 2017-2020

Collaboration between:



The 2017-2020 project

1. Analysis of field data, with harvest of samples for initial analyses
2. Controlled experiment
 - Vaccines, feeds, temperature post transfer
 - Fish are currently ca. 500g
3. Pathogenesis and early markers
4. Predictive models
 - Cell culture studies

Analysis of field data, to identify risk factors

Classification of fish groups (harvest) based on:

- X-ray
- Screening at harvest
- Quality reports

Production data:

- CV for the fish group
- Selected information from freshwater production

For analysis:

- 201 fish groups/harvest groups/cages tested (2015G and 2016G)
- Categorical variable: Affected by cross stitch **yes/no**
- Tested against vaccine type: 118 groups A, 83 groups B
- No vaccines without PD-component in material

Main result: Prevalence differed between vaccines



N	DF	-LogLike	RSquare (U)
201	1	28,701471	0,2060

Test	ChiSquare	Prob> ChiSq
Likelihood Ratio	57,403	<,0001*
Pearson	54,249	<,0001*

118 vs. 83 fish groups

Fisher's Exact Test	Prob	Alternative Hypothesis
Left	<,0001*	Prob(Ryggradsdeformasjonsproblem (0/1)= 1) is greater for Vaksine navn= [] than []
Right	1,0000	Prob(Ryggradsdeformasjonsproblem (0/1)= 1) is greater for Vaksine navn= [] than []
2-Tail	<,0001*	Prob(Ryggradsdeformasjonsproblem (0/1)= 1) is different across Vaksine navn

Analysis of field data, additional risk factors

Test of interactions between

- Vaccine
- Breed
- Feed product type (health feed or not)

Best model:

- Effect of vaccine type ($p < 0,001$)
- Interaction between breed and vaccine ($p < 0,02$)
- No effect of feed product type was identified
 - Incomplete information and complex feeding histories
 - No chance

Experimental study 2018-2020

- Four vaccine groups
 - Unvaccinated control, multicomponent vaccines without (1) and with PD component (2)
- Freshwater diets
 - high and low P in freshwater
 - with or without betaglucan in freshwater
- Seawater
 - Tanks with different temperatures post transfer (terminated February 2019)
 - Sea cages, commercial feeds with or without active ingredients
- The PD vaccines induced a significant growth depression in the weeks following vaccination
 - No differences 3-4 months post transfer
- No signs of X-ray pathology so far (350g)



Histology of cross-stitch vertebrae

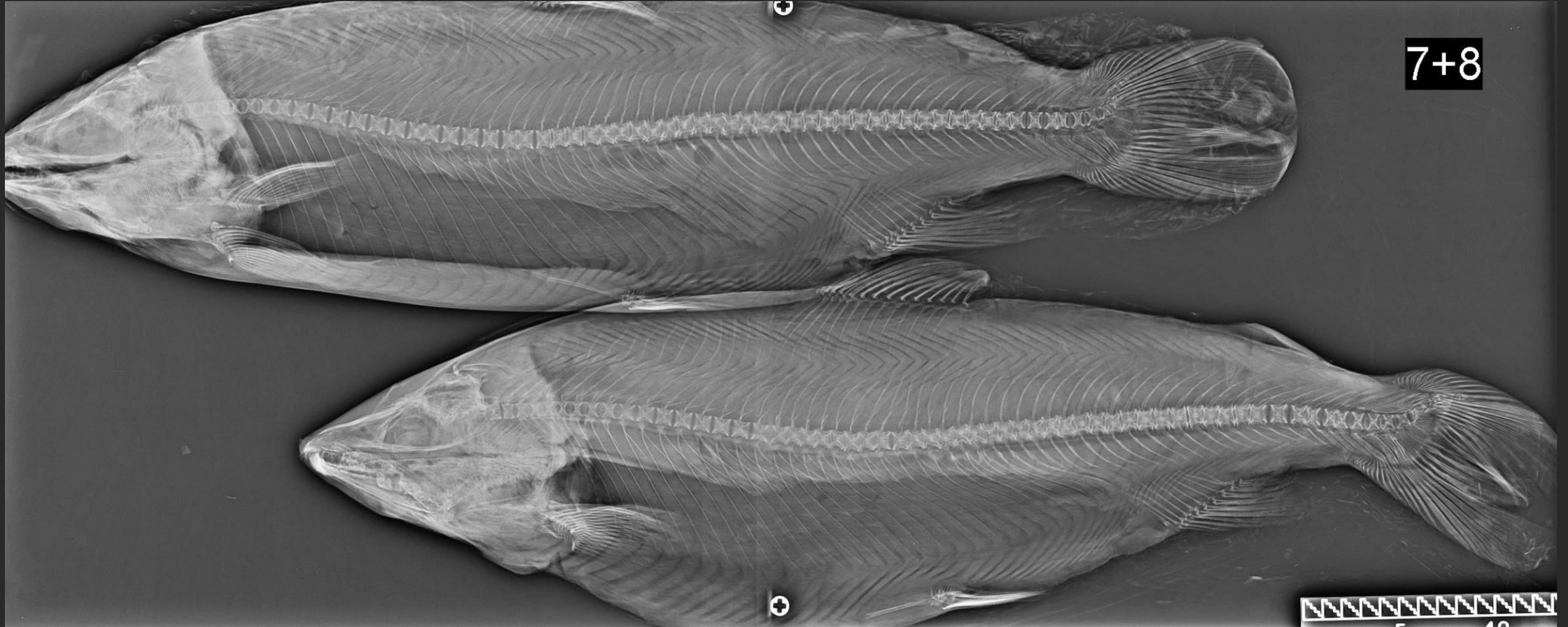
- Symmetrical defects in vertebral endplates
- Corresponds approximately to position of the tip of the vertebra at time of vaccination
- On scanning electron microscopy, it was observed that the defect covered the whole circumference of the vertebrae, like a ring

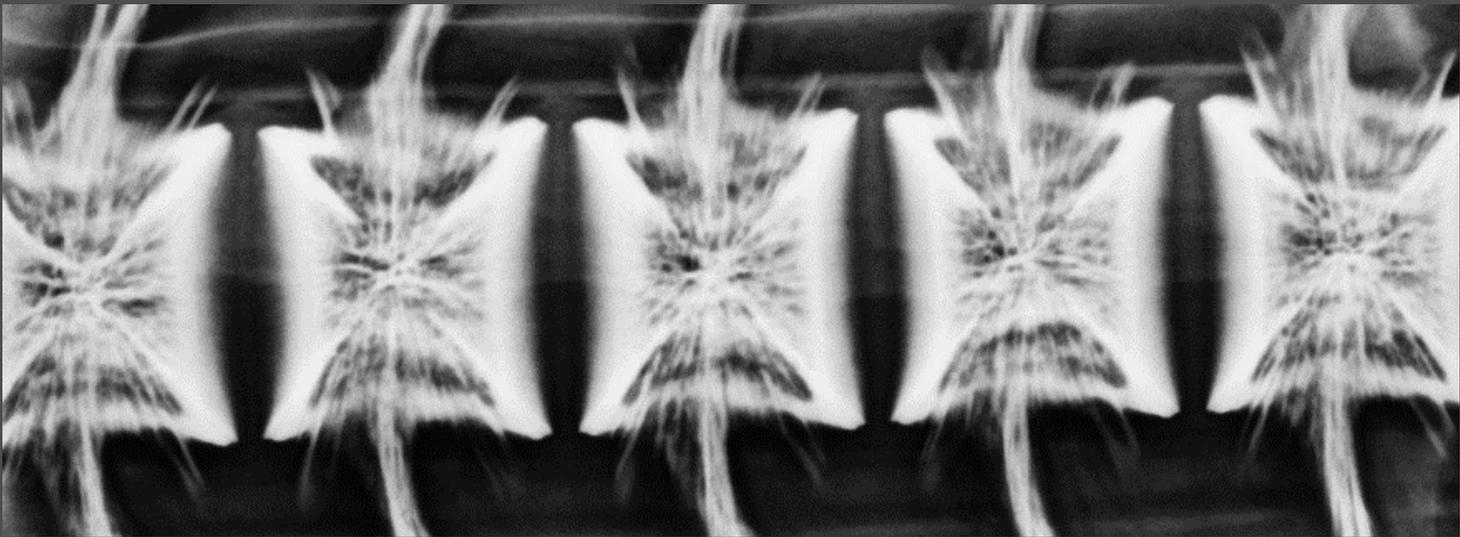
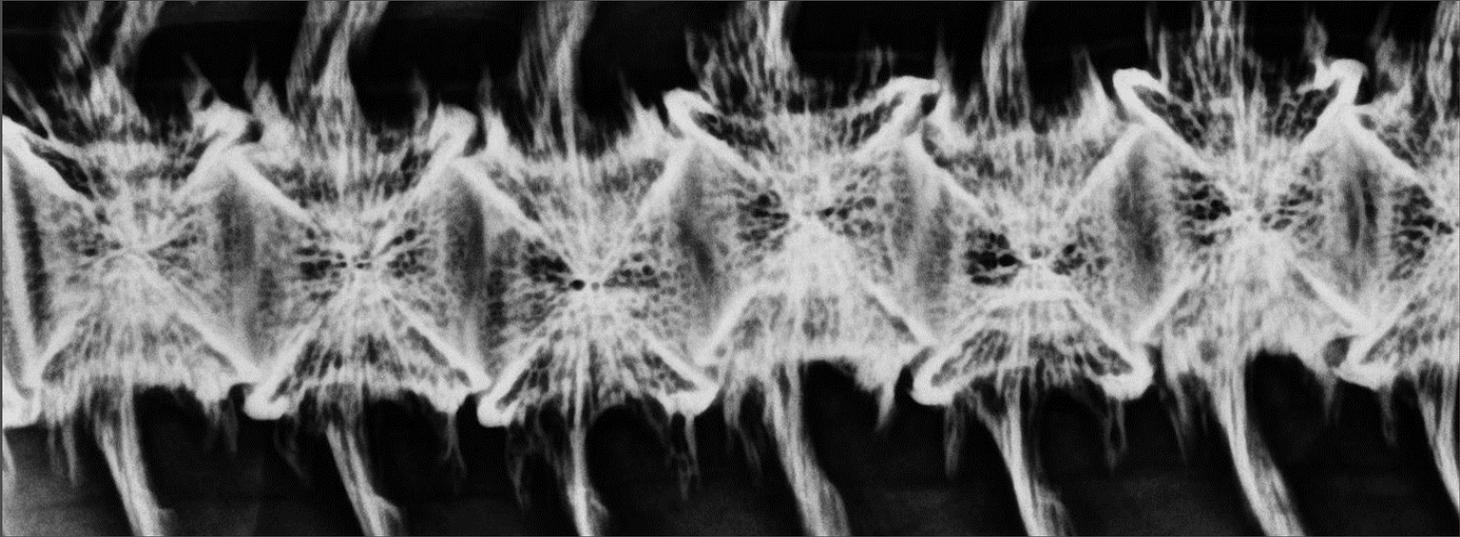
Is this a «new» pathology?

Is it exclusive for the new generation of PD-vaccines?

- Seen in fish from the Trøndelag coast in 2006, from one specific company, then disappeared
- Any similarity to the vaccine induced deformities in the early 2000s remains an open question
 - Published X-rays from the relevant period show mainly compressed vertebrae and fusions
 - A paper showing cross-stitch vertebrae (Gil-Martens et al. 2003) refers to them as compressed (platyspondylia)
- Cross-stitch pathology seen in low numbers (1%) in fish group vaccinated with multivalent vaccine with no PD component (CtrlAqua Benchmark experiment, 450 fish examined)

Vinter 2017-18 (H16)





Thank you for
the attention