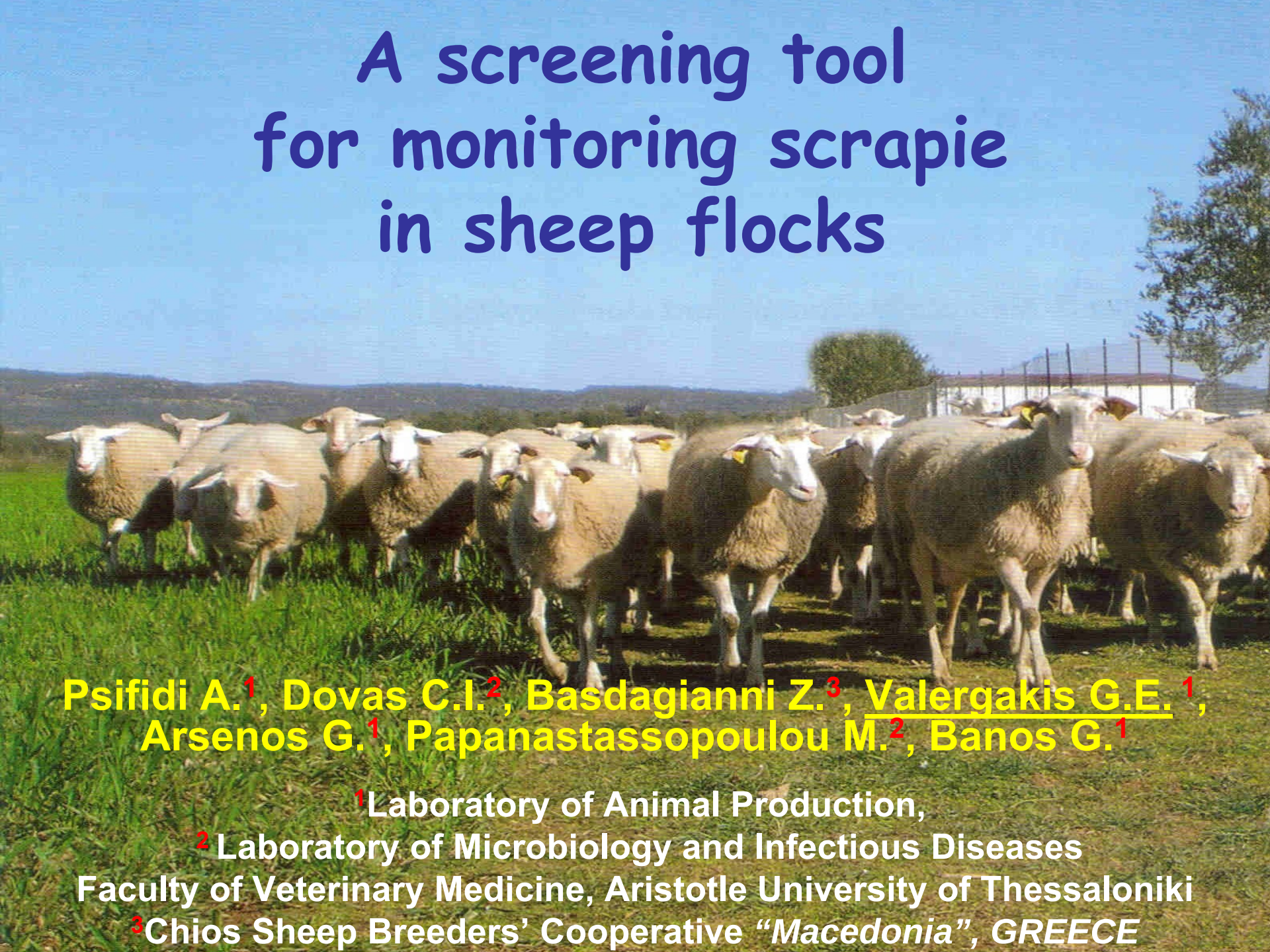
A screening tool for monitoring scrapie in sheep flocks



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Genetics of Scrapie

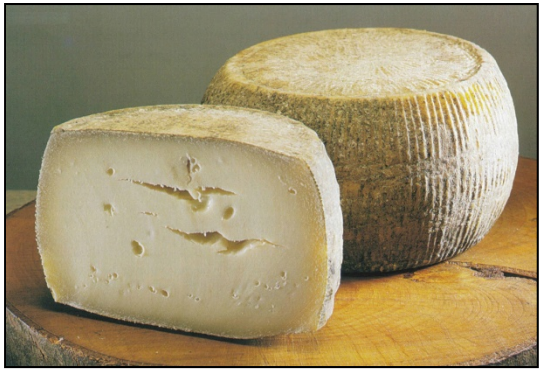
- *PRNP* gene locus
- Three codons
  - 136, 154, 171
- ARR (resistant)
- VRQ (susceptible)

Genetic selection for Scrapie

In the EU

- **Increase ARR (resistant)**
- **Eradicate VRQ (susceptible)**

- **DAIRY PRODUCTS FROM "VRQ-free" FLOCKS!**

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Genetic selection for Scrapie

In the EU

- **Increase ARR (resistant)**
- **Eradicate VRQ (susceptible)**

- **Conventionally, individual PRNP genotyping using blood samples**
- **We have developed an individual PRNP genotyping method using milk somatic cells**

Aim of our project

- Development of a practical **method** for assessing the presence of **VRQ** allele at **flock** level using **bulk milk** samples

Materials and methods

- **The Chios dairy sheep breed**

Previous research

23 flocks, 4600 ewes

⬇

Sample of 1013 ewes

⬇

VRQ frequency: 0.4%
(only in 3 flocks)

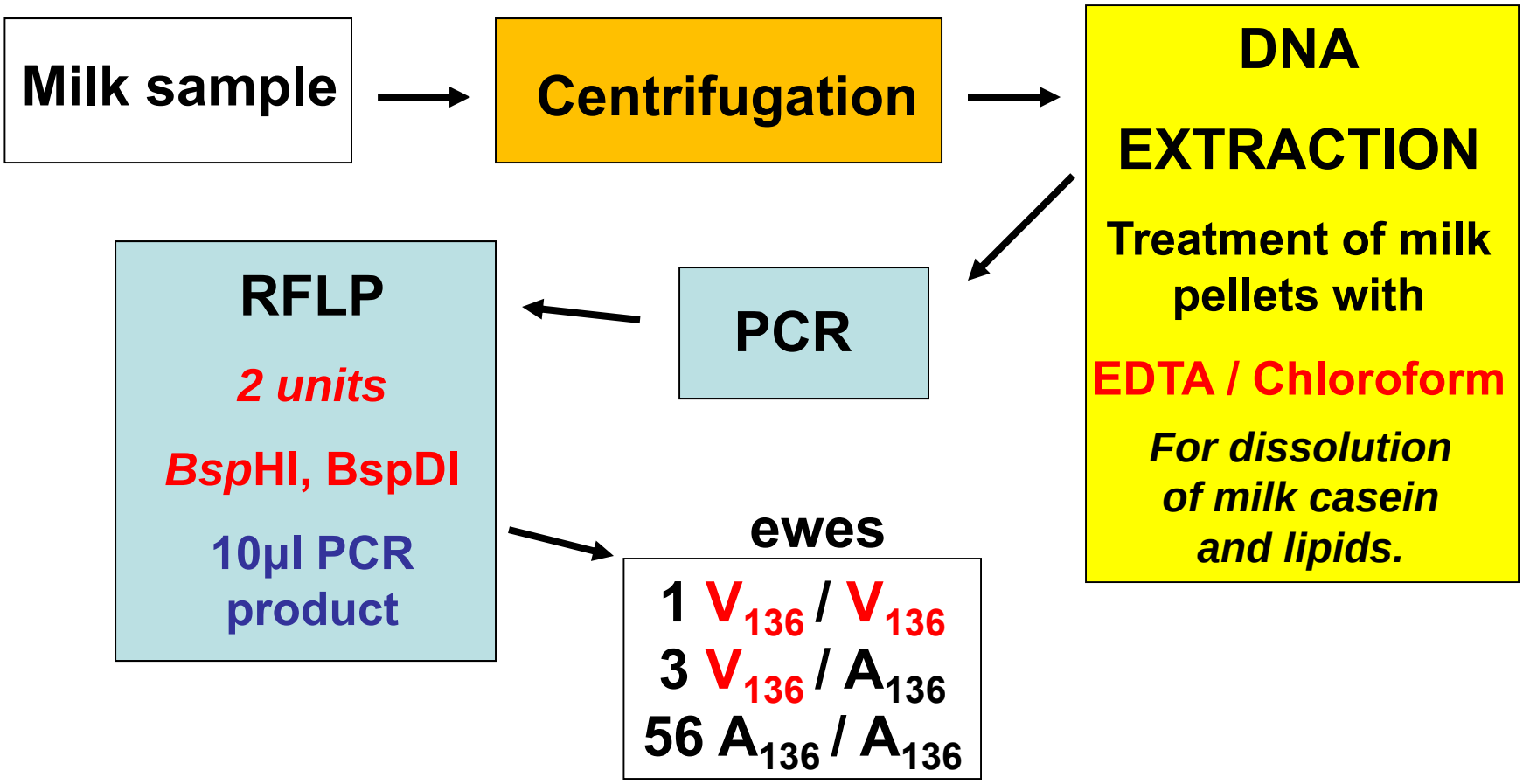
Materials and methods

- One "VRQ" flock selected
- 60 individual milk samples (50ml) collected

Materials and methods

Treatment of samples

Modified Nucleospin Blood (Mackerey -Nagel®)



Materials and methods

Artificial bulk milk samples

- **Mixing different individual milk samples**
- **Different V_{136} frequencies**
  - **64%, 32%,16%, 8%, 4%, 2%, 1%, 0.5%, 0.25%**
  - **2 different sets**

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Plasmid DNA standard pools

- **VRQ clone / ARQ clone**

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Both in triplicates
20µl PCR product
10 units of BspHI and BspDI

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Detection limit
Clear detection
to all 3 digested samples

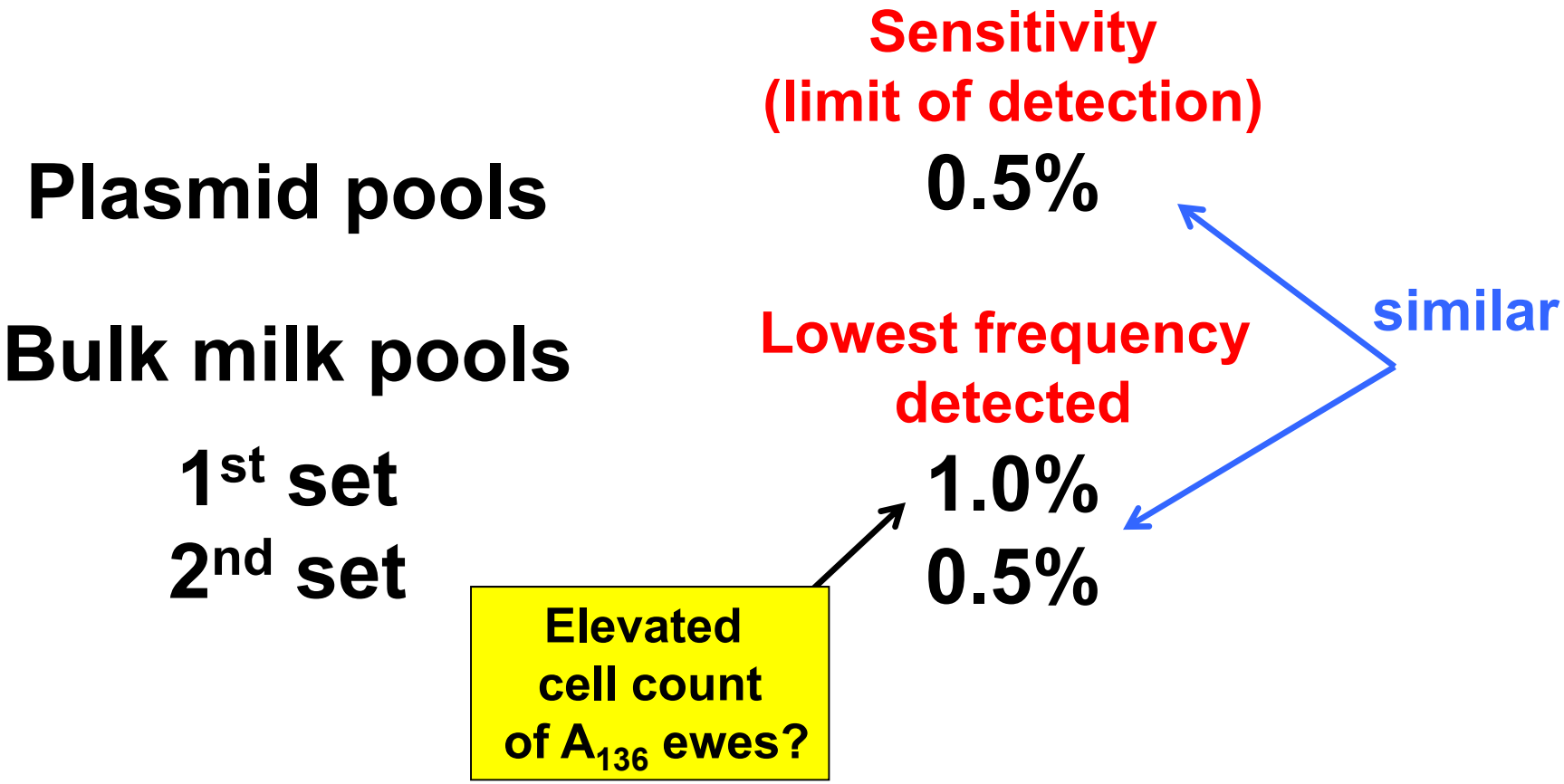
Results

	Sensitivity (limit of detection)
Plasmid pools	0.5%

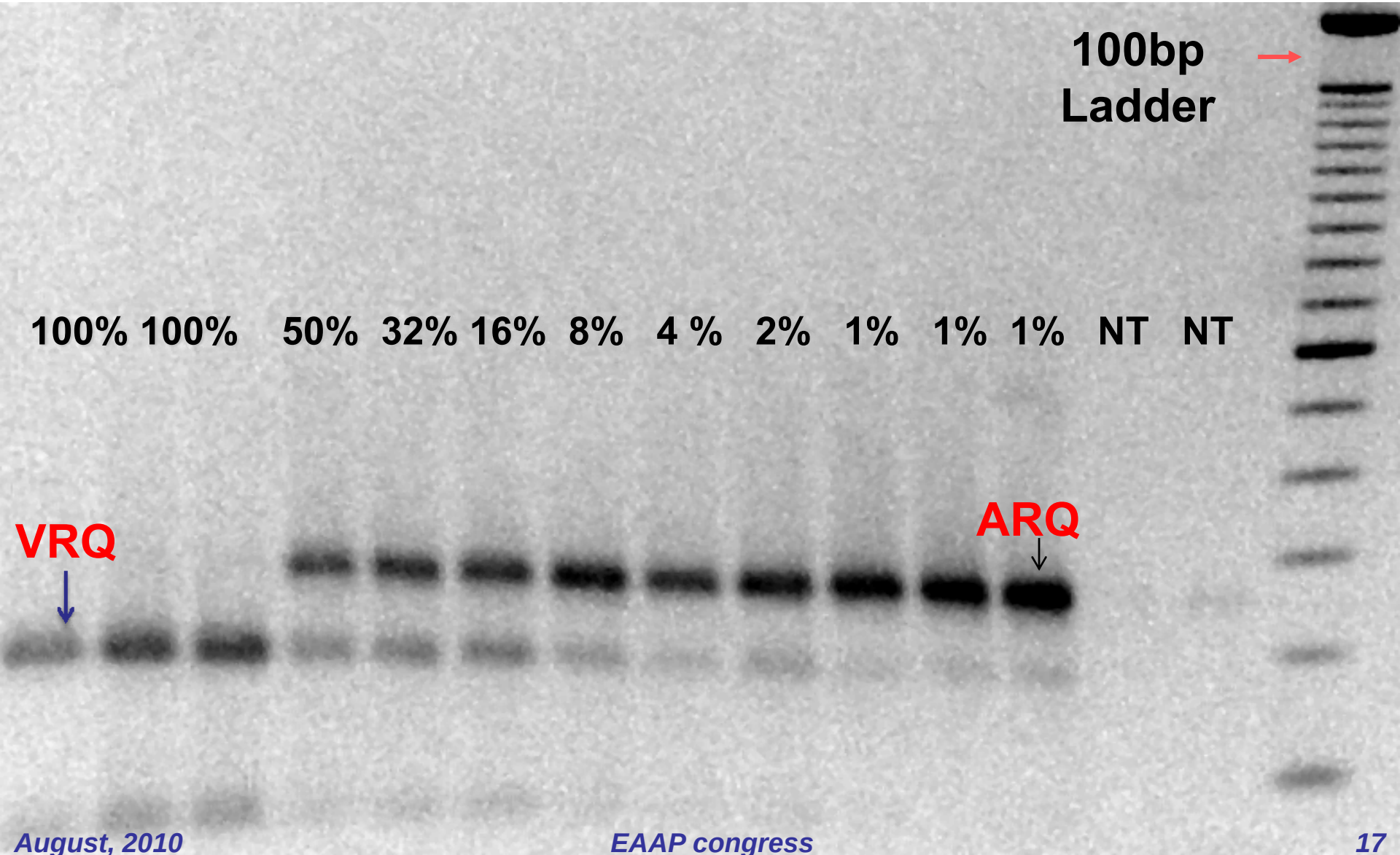
Results

	Sensitivity (limit of detection)
Plasmid pools	0.5%
Bulk milk pools	Lowest frequency detected
1st set	1.0%
2nd set	0.5%

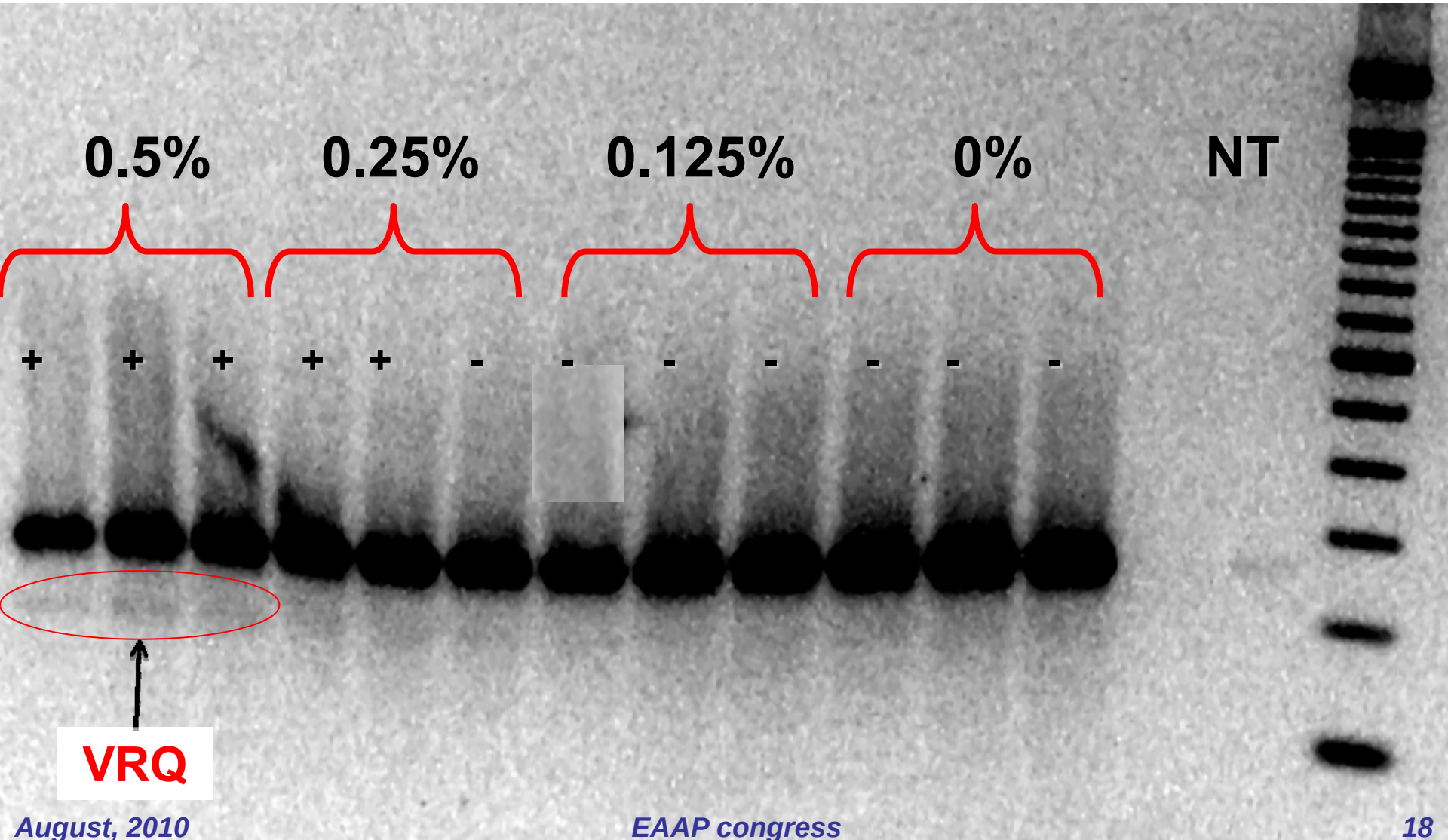
Results


Sensitivity
(limit of detection)
Plasmid pools
0.5%
Bulk milk pools
Lowest frequency
detected
similar
1st set
1.0%
2nd set
0.5%
Elevated
cell count
of A136 ewes?

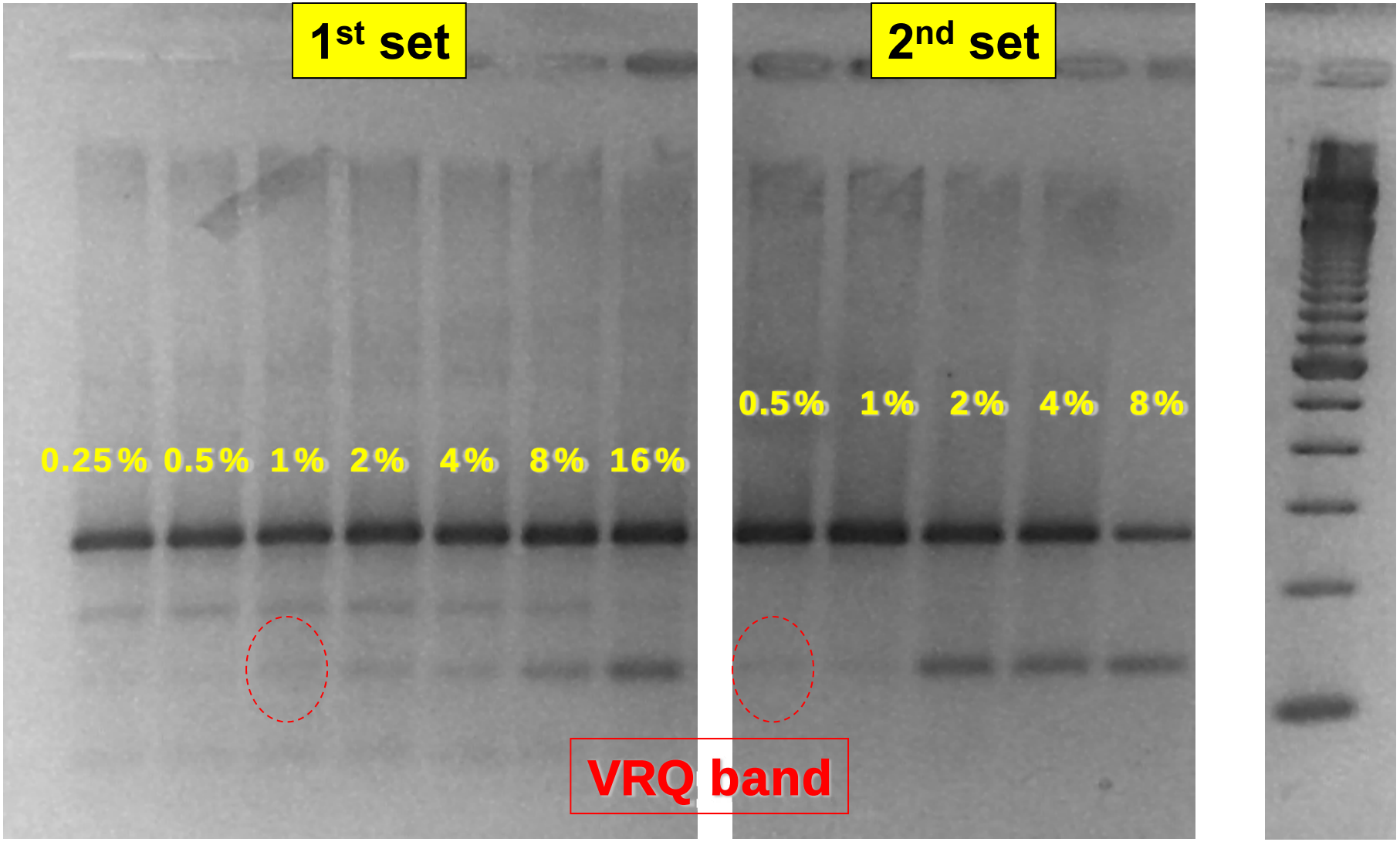
Results-plasmid pools



Results-plasmid pools



Results-bulk milk pools



Application

- **Bulk milk sampling from every 50 ewes**
- **2 monthly consecutive collections**

  statistical elimination of problems arising from fluctuating somatic cell counts
- **Milk recording samples can be used**

Application

Flock of 200 ewes

Genotyping from blood samples

200 samples

Application

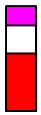
Flock of 200 ewes

Genotyping from milk samples
No "VRQ" animals

50 ewes	50 ewes	50 ewes	50 ewes
Negative	Negative	Negative	Negative

Genotyping from blood samples

200 samples



4 samples x 2 = 8 samples

Application

Flock of 200 ewes

Genotyping from blood samples

200 samples

Genotyping from milk samples
One "VRQ" animal

- 50 ewes — Negative
- 50 ewes — Positive
  - 25 ewes — Positive
    - 12 ewes — Negative
    - 13 ewes — Positive → 13 individual samples
  - 25 ewes — Negative
- 50 ewes — Negative
- 50 ewes — Negative

(8 + 13 samples) x 2 = 42 samples

CONCLUSIONS

- Individual and bulk milk genotyping
- High detection sensitivity
- Animal friendly
- No specialized personnel for sampling
- Fast and cost-effective
- Excellent tool for flock screening

Acknowledgement

RISKSCRA , E.C./COLL-CT-2006

Dairy products in Mediterranean sheep populations: quantification of scrapie risk


Thank you!
Any questions?