THE VOLUNTARY IOWA BVDV SCREENING PROJECT

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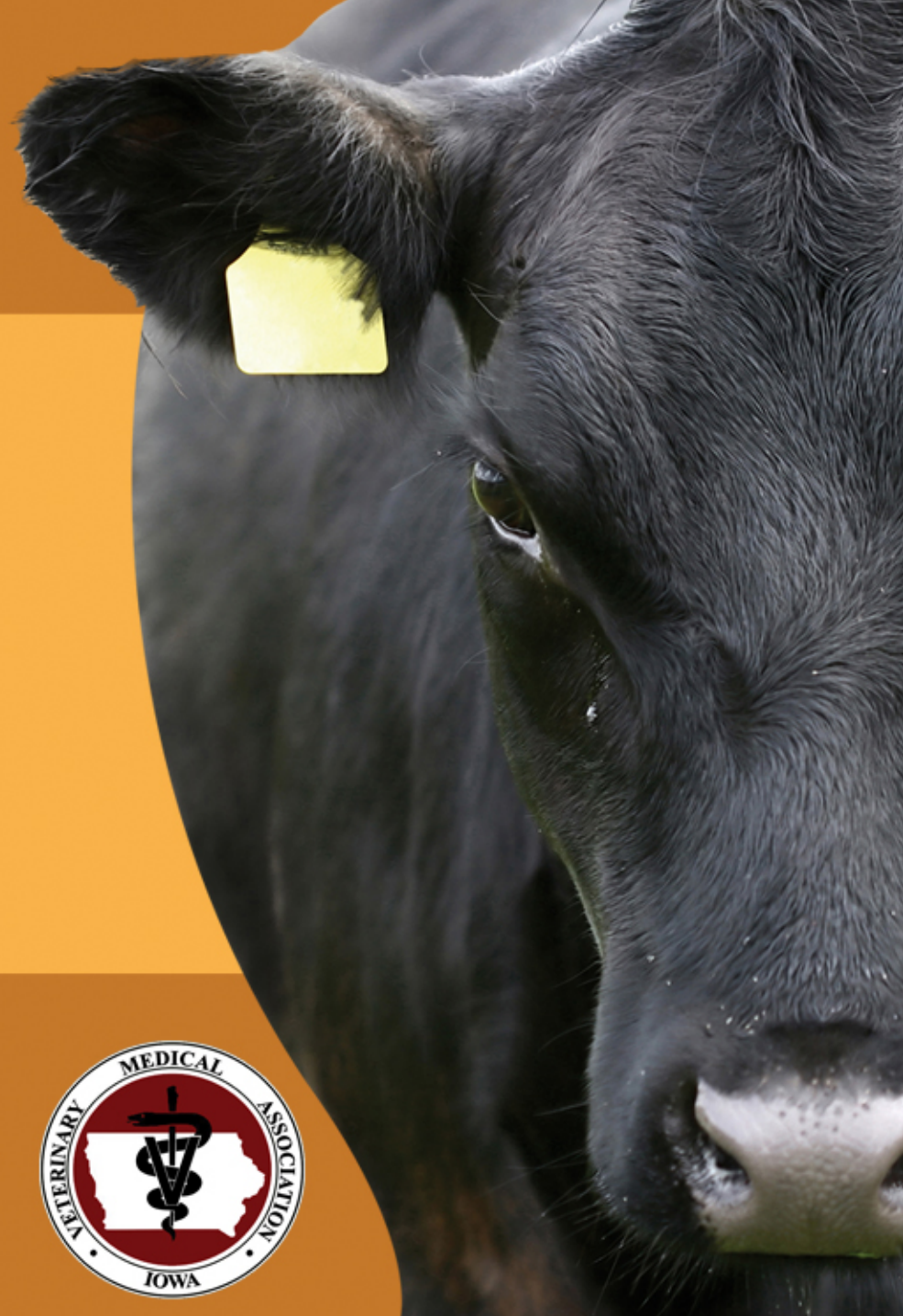

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THE VOLUNTARY IOWA BVDV SCREENING PROJECT

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THE VOLUNTARY IOWA BVDV SCREENING PROJECT

- VIBSP
  - –Recruiting 400 herds or 40000 calves for testing for BVDV in Spring 2006
  - –From Iowa based beef cow-calf producers

THE VOLUNTARY IOWA BVDV SCREENING PROJECT

- What are the aims?
  - Evaluate pooled testing as a screening tool
  - Estimate prevalence of BVDV in Iowa
  - Estimate risk factors for BVDV in Iowa

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- What it's not?
  - –Not a certification program
  - –Not state sponsored

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Motivation

"..it is the resolve of the Academy of the Veterinary Consultants that the beef and dairy industries adopt measures to control and target eventual eradication of BVDV from North America"

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Immediate impact

- –Provide beef breeders with cheap and effective methods for screening for BVDV
- –Sensitivity and specificity of the pooled RT-PCR screening program

- Screening tests
  - –Cheap
  - –Associated with false positive’s
  - –Positive’s further securitized
    - PCR on Bulk milk
    - Serology of sentinel heifers

Diagnostic tests

- Confirm or classify disease
- Animals are abnormal and the challenge is to identify the disease
- Individual animal: IHC, AC-ELISA

Long-range impact:

- A cost-benefit assessment of BVDV eradication program
- Prevalence of BVDV-PI positive herds
- Impact of eradication on performance

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- Enrollment phase: Oct 2005-Mar 2006
  - –Recruiting veterinarians
  - –Recruiting producers

- Ensure veterinarians are comfortable with BVDV control programs
  - Available for veterinary meetings, extension, ICA, CVM

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- Recruiting veterinarians
  - –Explain the goals, enrolling, sample collection, submission, receiving results
  - –Explain BVDV to vets
  - –NCBA BVD and AVC BVD materials

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- Vet contacts
  - –3 mailings : brochures and publications
  - –Phone call for each vet
  - –Follow up letters for enrolled vets
  - –Producer meetings for enrolled vets

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- Recruiting producers
  - –IVMA vets
  - –Iowa Cattleman's meetings and magazine
  - –Iowa State University Extension officers

After enrollment phase

- Determine if there is sufficient interest to warrant continuation
- Ensure veterinarians are comfortable with BVDV control options

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- Study phase runs from Jan-July 2006
  - –Sample collection
  - –Testing and interpretation
  - –Analysis

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- Enrolled producers will receive
  - –sample collection tubes
  - –sample submission boxes
  - –shipping labels
  - –sample submission record sheet

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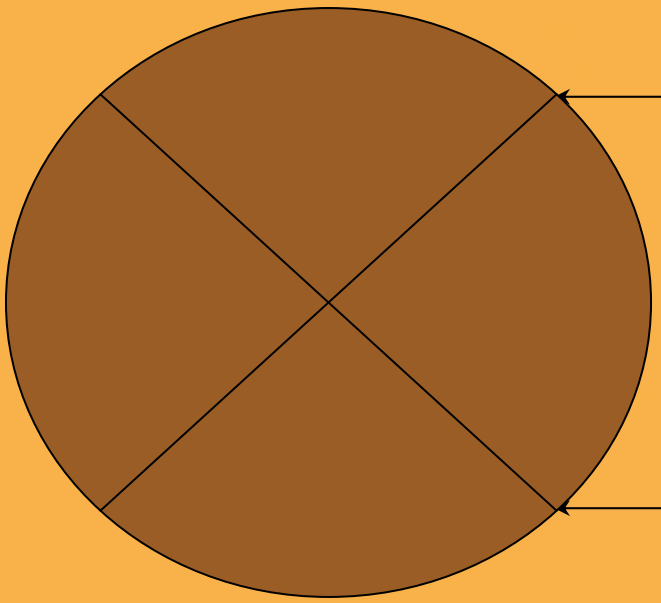

Calving

Breeding

Window of opportunity for testing

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- Sample collection either at
  - –Birth when calves are ID
  - –Spring processing pinkeye/blackleg vaccintion

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- Study phase
  - –Producers notch calves
  - –Freeze notches in tubes
  - –Submit frozen notches to our lab within 1 week of collection

- At the lab
  - –Process and freeze samples
  - –When collection complete then thaw and pool
  - –Submit to pooled sample to VDL for PCR

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- Study phase
  - –Pooled RT-PCR on PBS soaked ear notches
  - –Follow-up of ACE on positive pools
  - –Notify veterinarians for follow up sample

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Testing and interpretation

- –Available through veterinarian
- **–Testing paid for by grant**
- –Vet and client decide next step
- –Follow-up outcome

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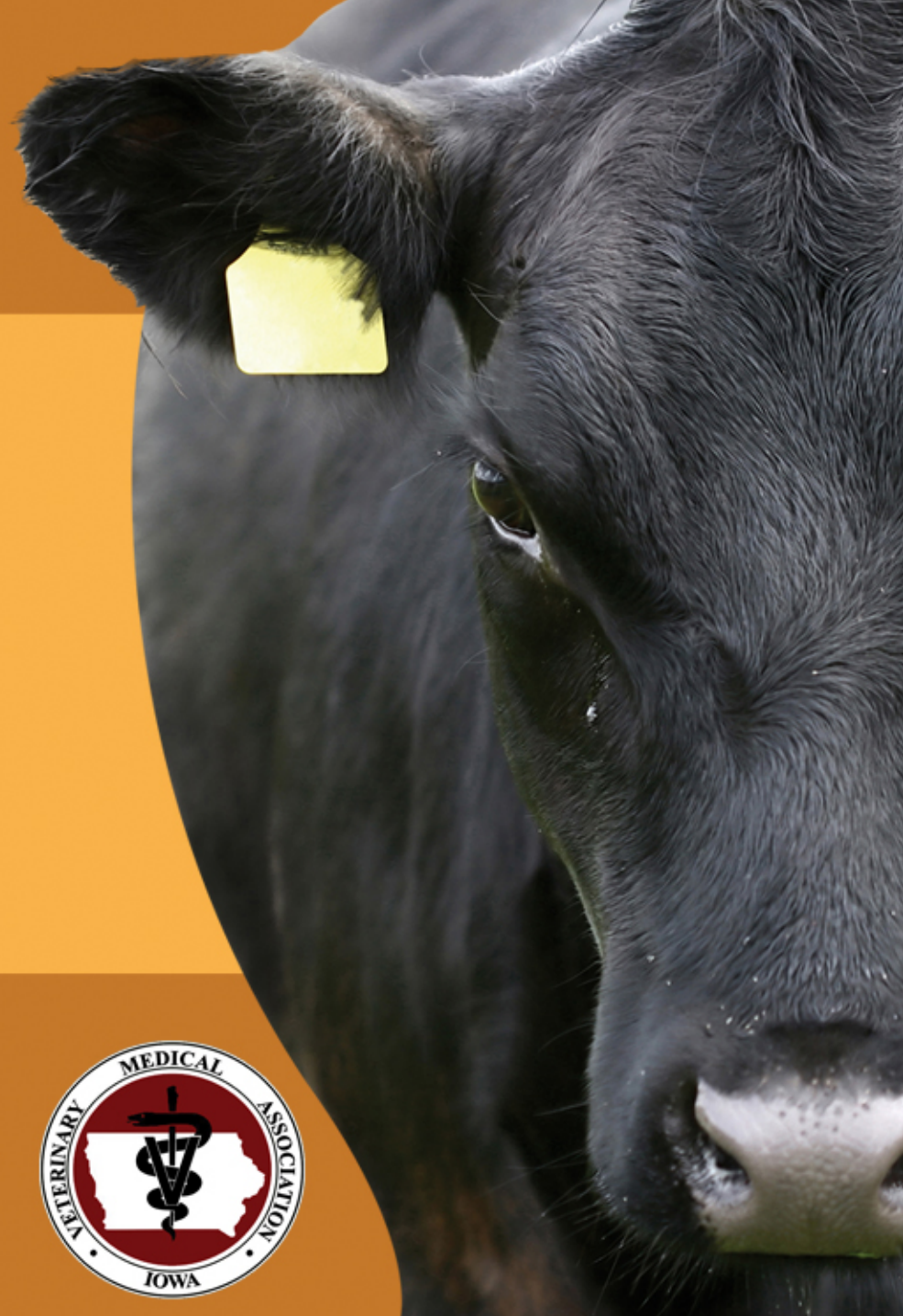

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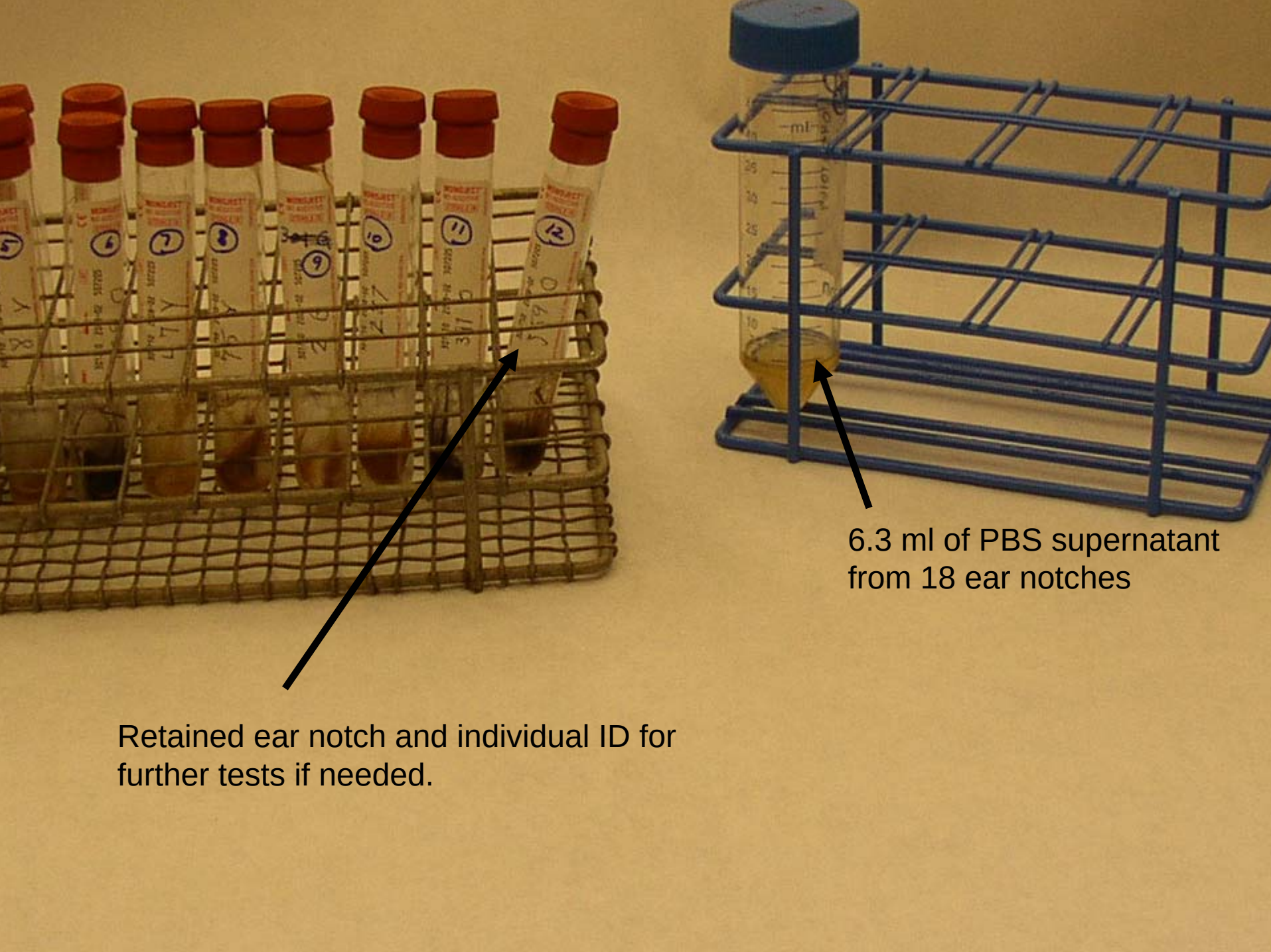
6.3 ml of PBS supernatant
from 18 ear notches
Retained ear notch and individual ID for
further tests if needed.

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RT-PCR Pools/ AC-ELISA	+ RT-PCR Pool	-RT-PCR Pool
One or more Positive AC-ELISA	39*	0
No Positive AC-ELISA	0	33

*Includes 36 samples spiked with a single known positive sample

THE VOLUNTARY IOWA BVDV SCREENING PROJECT

POOL	Samples	BVD RT-PCR Results	AC-ELISA & IHC results by Sample #
1	1-100	not detected	No Positives
2	101-200	not detected	No Positives
3	201-300	not detected	No Positives
4	301-400	not detected	No Positives
5	401-500	not detected	No Positives
6	501-600	not detected	No Positives
7	601-700	not detected	No Positives
8	701-800	not detected	No Positives
9	801-900	not detected	No Positives
10	901-1000	not detected	No Positives
11	1001-1100	not detected	No Positives
12	1101-1200	not detected	No Positives
13	1201-1300	not detected	No Positives
14	1301-1400	not detected	No Positives
15	1401-1500	Positive	1433 & 1442 positive all others negative
16	1501-1600	not detected	No Positives
17	1601-1700	not detected	No Positives
18	1701-1800	Positive	1718 positive all others negative
19	1801-1900	Positive	1899 positive all others negative
20	1901-2000	not detected	No Positives
21	2001-2100	not detected	No Positives
22	2101-2200	not detected	No Positives
23	2201-2300	not detected	No Positives
24	2301-2400	not detected	No Positives
25	2401-2500	not detected	No Positives
26	2501-2600	not detected	No Positives
27	2601-2700	not detected	No Positives
28	2701-2800	not detected	No Positives
29	2801-2900	not detected	No Positives
30	2901-3000	not detected	No Positives
31	3001-3100	not detected	No Positives*
32	3101-3200	not detected	No Positives*
33	3201-3300	not detected	No Positives*
34	3301-3400	not detected	No Positives*
35	3401-3500	not detected	No Positives*
36	3501-3599	not detected	No Positives*

*AC-ELISA correlated with IHC on first 3016 samples afterward IHC was discontinued

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RT-PCR pools/ AC-ELISA	RT-PCR BVD +	RT-PCR BVD -
Pool contained positive AC-ELISA (1 or more)	49	0
Pool did not contain positive AC-ELISA	2 *(one pool contained 50 samples* the other contained 38 samples**)*	38

*Samples from herd where a PI was found: **testing not completed for herd

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- 400 + herds
- 40 PI-BVDV positive herds

THE VOLUNTARY IOWA BVDV SCREENING PROJECT

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- Identifying PI's early allows them to be removed from the herd before the next breeding season;
- Otherwise, PI suckling calves may infect cows carrying early-gestation fetuses and create more PIs in next year's calf crop.
- Only need to test dams of + calves (dams are rarely positive, but must test).

Finding BVDV PI Calves

- Options for identifying BVDV PI neonates:
  - **–BVDV isolation (VI) from serum or buffy coat:**
    - False negatives due to interference by maternal Ab.
  - **–BVDV antigen detection in serum with Ag-capture ELISA:**
    - False negatives due to interference by maternal Ab.

- Options for identifying BVDV PI neonates:
  - **RT-PCR on serum (individual or pools):**
    - Need blood samples, $$ unless pool.
  - **Ear notch tests**

Three tests for BVDV can be performed on ear notches:

1. Immunohistochemistry (IHC):

   Fix notch in formalin, embed in paraffin, apply antibody to section to detect antigen.

- 2. Ag-capture ELISA:
  - –Soak notch in saline, (freeze), run ELISA on fluid to detect antigen.
- 3. PCR:
  - –Soak notch in saline, run RT-PCR on fluid to detect RNA. Can pool fluid of 100 notches.

1. Immunohistochemistry (IHC):

   Sensitivity = ~100%, but a small % of acutely infected calves are positive.

2. Ag-capture ELISA:

   Sensitivity = ~100%, but a small % of acutely infected calves are positive

3. PCR:

   Sensitivity = ~100% on individuals and pools. May also detect acute infections

THE VOLUNTARY IOWA BVDV SCREENING PROJECT

- Producers notch calves at ear-tagging.
- Place notches in numbered tubes with saline and freeze.
- After all calves have been notched, send frozen notches to VDL.
- At VDL, ear notch fluids for each herd pooled into 1 or more pools of ≤100.
- RT-PCR for BVDV RNA on each pool.

VIBSP Testing Strategy

- If pool is PCR-negative, no PIs present.
- If pool is PCR-positive
  - –Ag-capture ELISA will be done on fluid from each calf in pool.
  - –Ag-capture ELISA positive calves should be confirmed PI by re-testing (buffy coat PCR) >30 days after notches obtained.

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- Aim:
  - –Estimate the prevalence of BVDV PI in Iowa beef breeding herds
  - –Evaluate the efficacy of pooled testing to cheaply screen herds for BVDV

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Enrollment phase: Winter 2005

Study phase: Spring and Summer 2006

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THE VOLUNTARY IOWA BVDV SCREENING PROJECT

- Enrollments to date
  - –Calves -15000
  - –Producers-102 average herd size ~150
  - –Vets-51
  - –Clinic-40

THE VOLUNTARY IOWA BVDV SCREENING PROJECT

- Issues to date
  - –What to do with fall and spring calving herds
  - –What to do with spread out calving seasons
  - –Concern about ears appearance after notching

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- Do you produce seedstock? 36/71
- Do you background or feedlot calves? 49/71
- Do you purchase replacements, bulls or cows?70/71
- Do you exhibit etc? 38/71*

THE VOLUNTARY IOWA BVDV SCREENING PROJECT

- Do you have nose to nose contact?53/71
- Do other animals have contact with cattle feed etc ? 71/71
- Conception > 90%? 60/71
- Weaning rate> 90%?62/71
- BVDV vaccination yearly? 64/71

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- 81% of non BVDV herds vaccinate?
- 90% of herds diagnosed with BVDV vaccinate?

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- Project issues of the outcomes
  - Sensitivity of Pooled PCR
  - Risk factor analysis
  - Prevalence estimates

- Sensitivity
  - –The PCR test on the calf subset
  - –The calf subset as a herd test

- Causes of imperfect sensitivity in the calf subset
  - –Excessive dilution
  - –Inhibition

THE VOLUNTARY IOWA BVDV SCREENING PROJECT

- 100 of 400 samples
- Prevalence of false-ve pools- 1%
- ACE=95% sensitive & 100% specific

- If all samples test negative, there is 95% confidence that the true prevalence is false negative is < 2.76%
  - –1- false negative prevalence=sensitivity
  - –95% confident the sensitivity of pooled PCR is greater than or equal to 97.24%

- 100 of 400 samples; prevalence of false -ve pools- 1%
  - –100% sensitivity & 100% specificity of ACE
  - –If all samples test negative, there is 95% confidence that the true prevalence is < 2.1%

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		BVDV+	BVDV -	
Test result	+	39	4	PVP=39/43=91%
	–	1	356	PVN=356/357=97%
		40 (sen=97%)	360 (spec-99%)	

THE VOLUNTARY IOWA BVDV SCREENING PROJECT

		BVDV+	BVDV-	
Test result	+	39	0	PVP=39/39=100%
	–	1	360	PVN=360/361=99.7%
		40 (sen=97%)	360 (spec-100%)	

- 99% sensitivity & 100% specificity of ACE
  - From an infinite population testing 300
  - If all samples test negative, there is 95% confidence that the true prevalence is < 0.01.
- Prevalence estimates from: http://www.ausvet.com.au/epitools/content.php?page=Freedom

- Sensitivity of the calf subset as a herd level diagnosis
  - –Follow-up of herds that decide to herd test after finding a calf PI
  - –Next funding phase

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- Project issues of the outcomes
  - Risk factor analysis
  - Prevalence estimates

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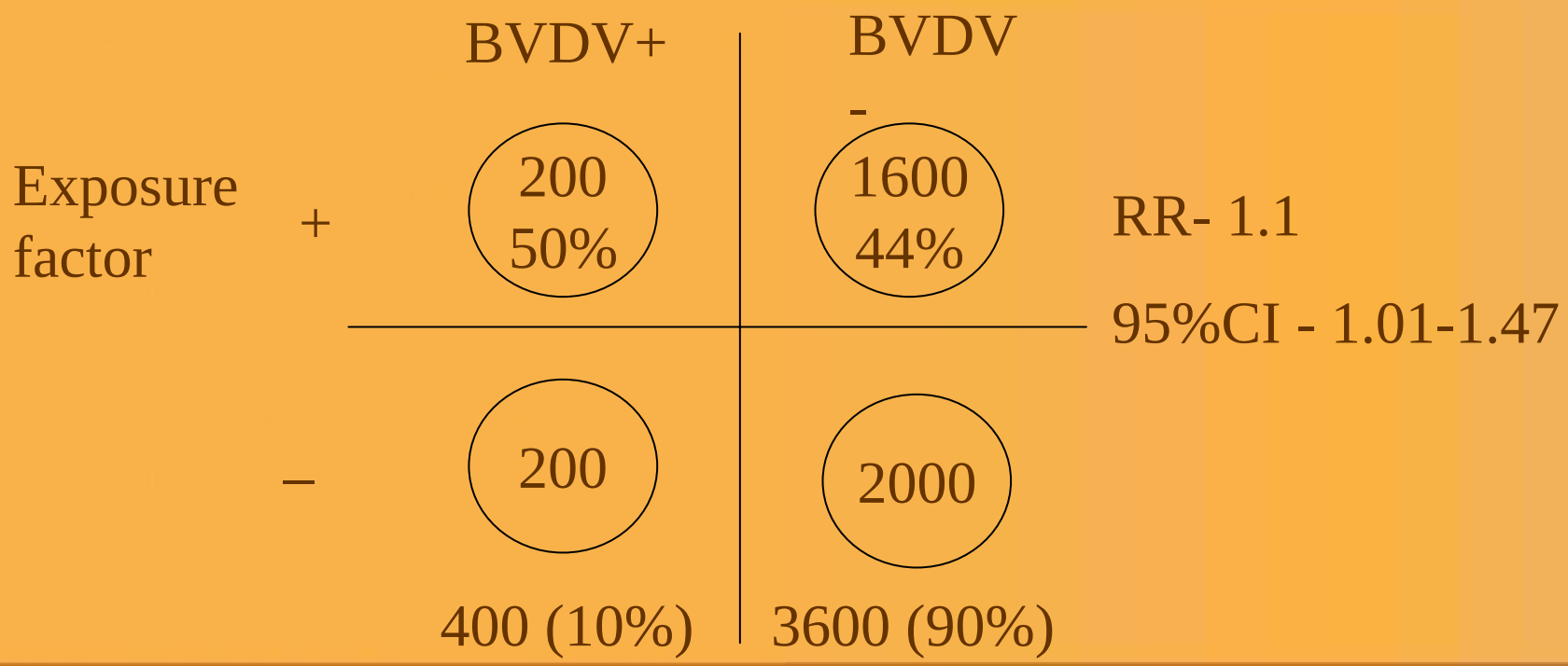

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Exposure factor	BVDV+	BVDV-
+	20 50%	160 44%
–	20	200
	40 (10%)	360 (90%)

RR-1.1

95%CI - 0.6 - 2.1

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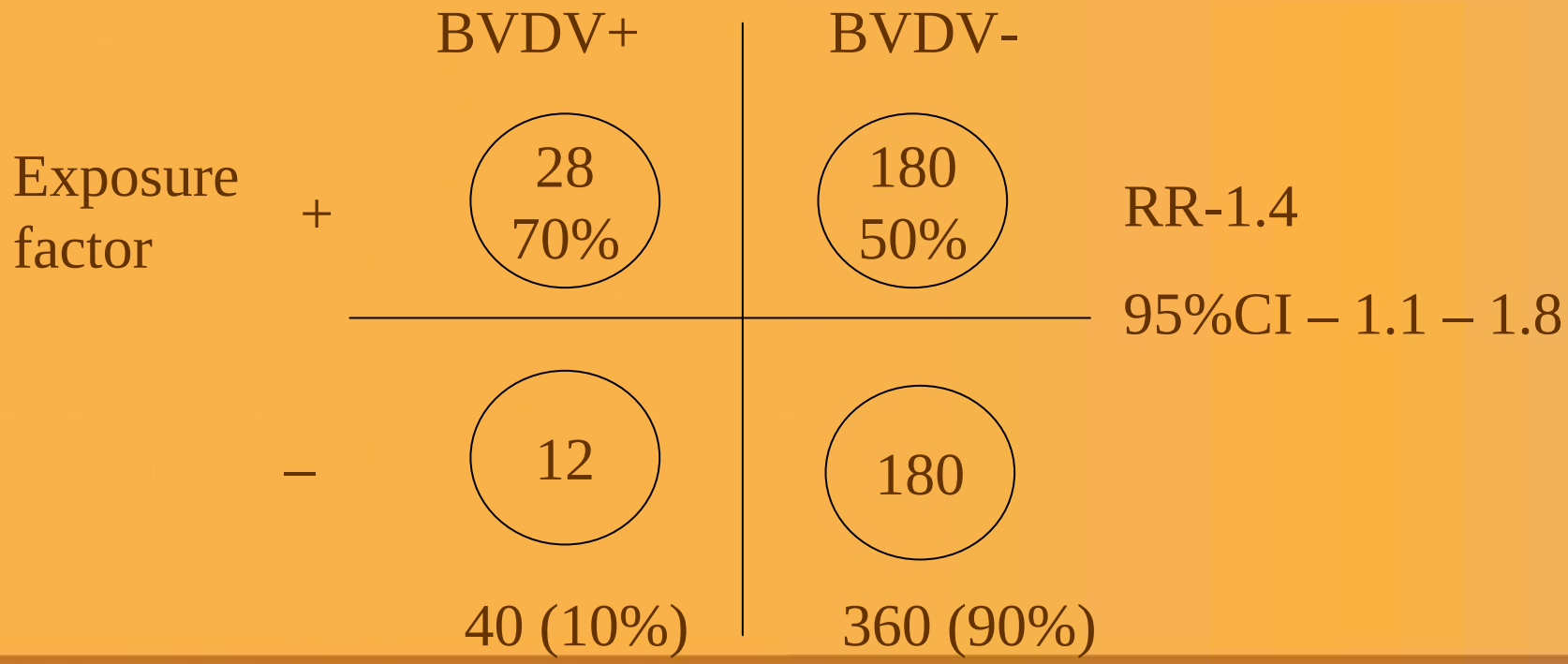

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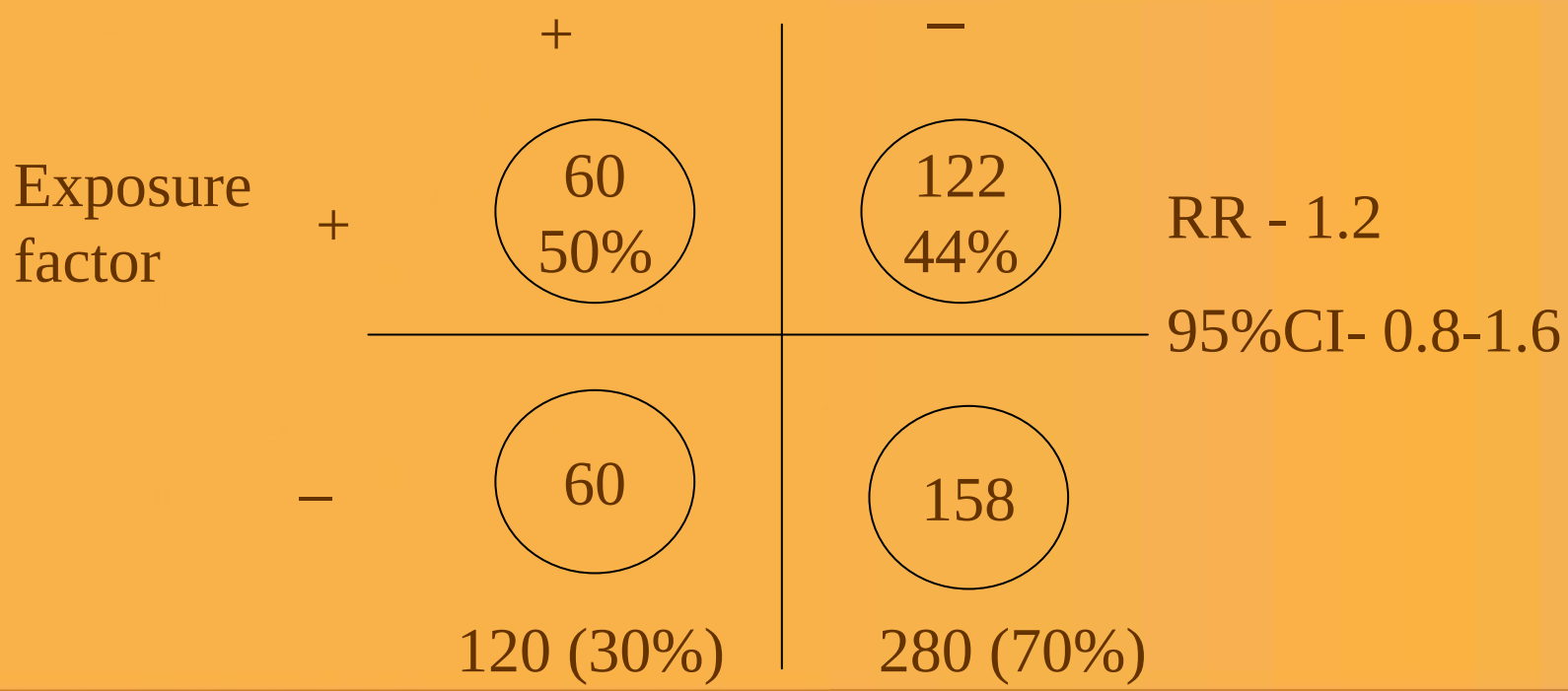

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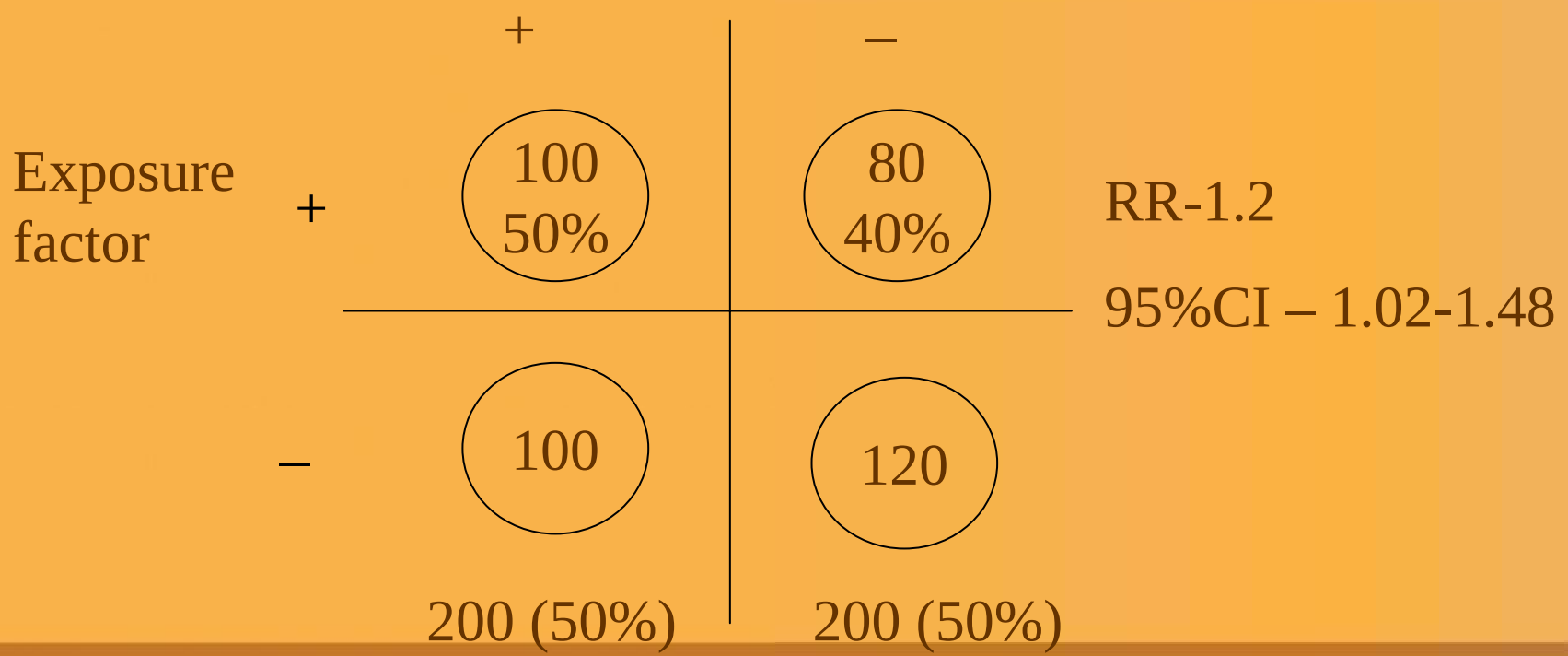

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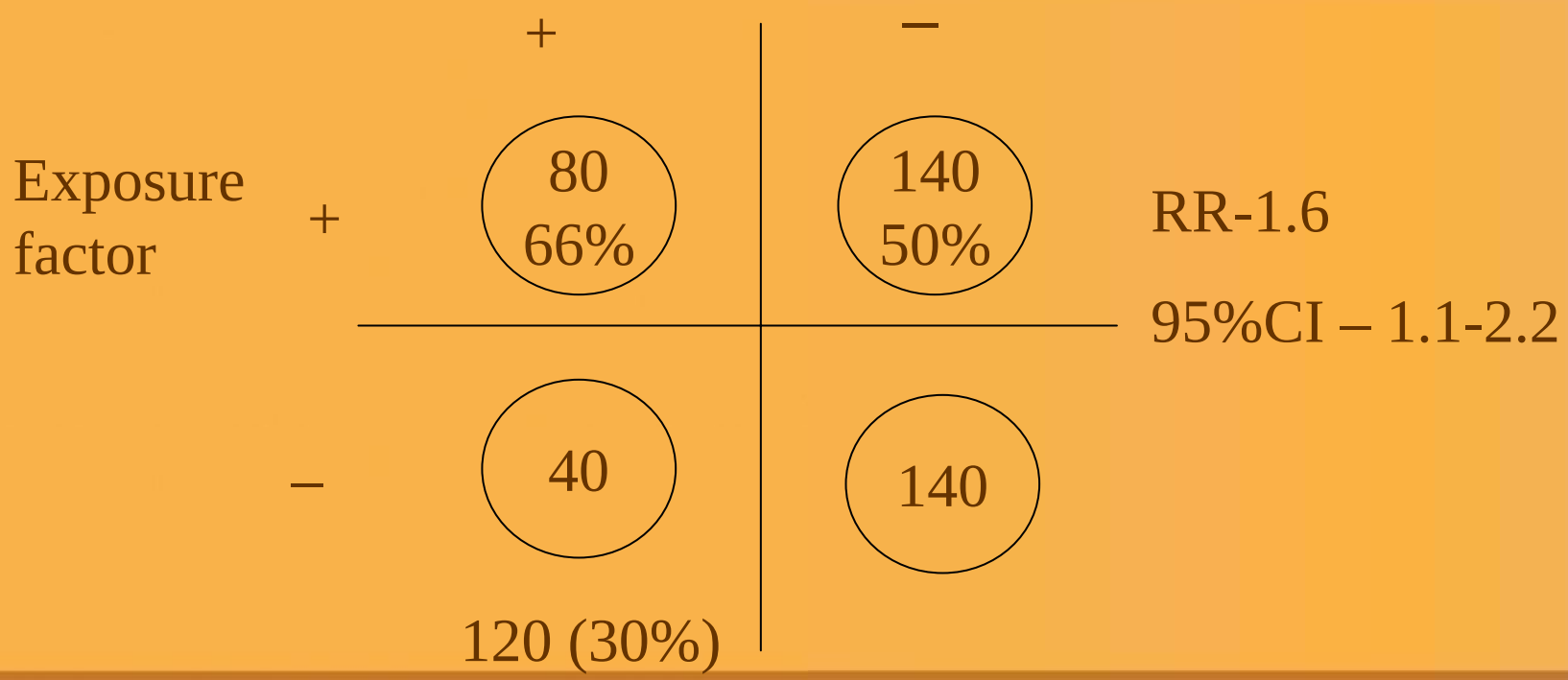

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- Where next?
  - –Funding for longitudinal animal health and enterprise analysis
  - –Collaboration and sample supply

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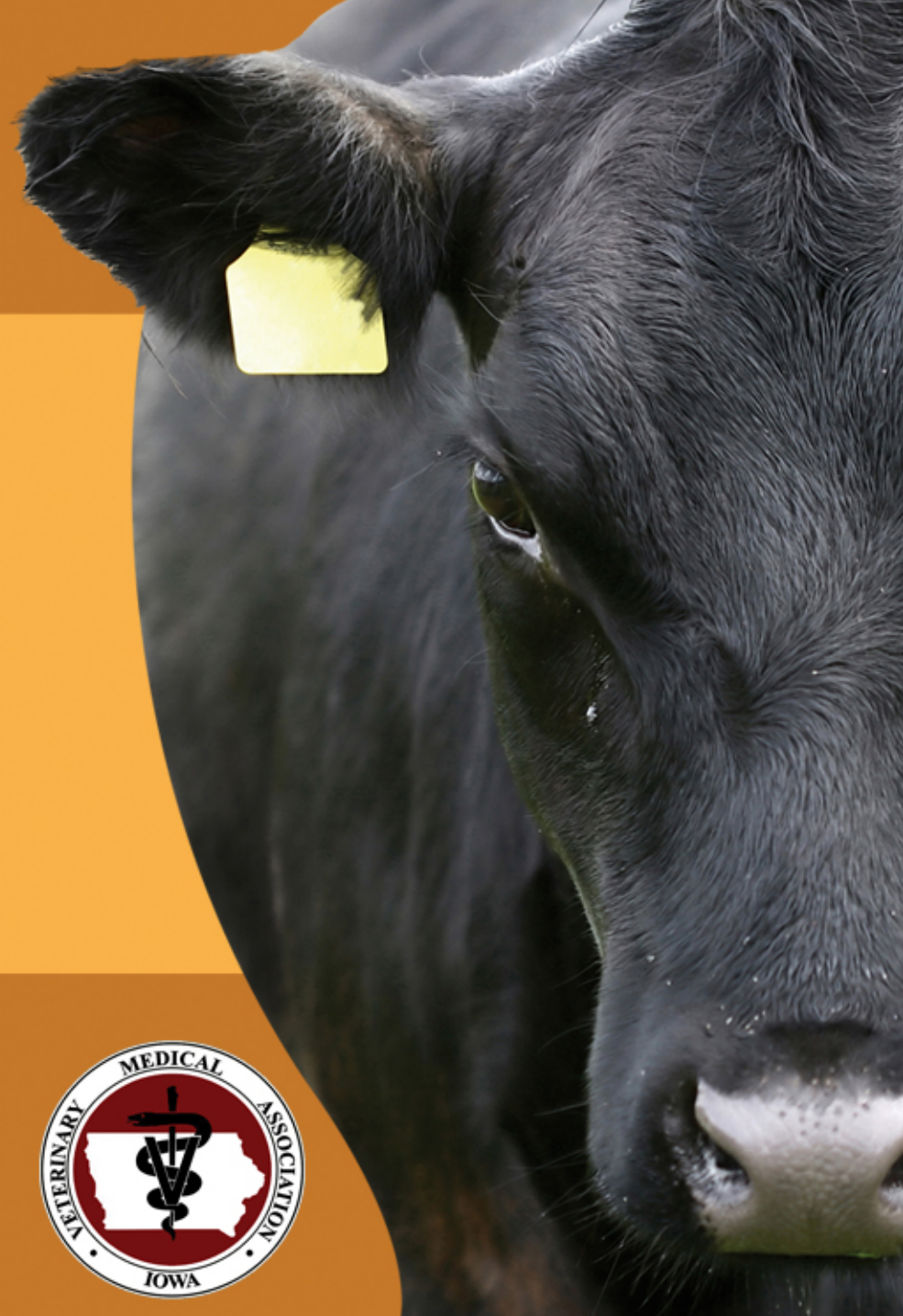

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