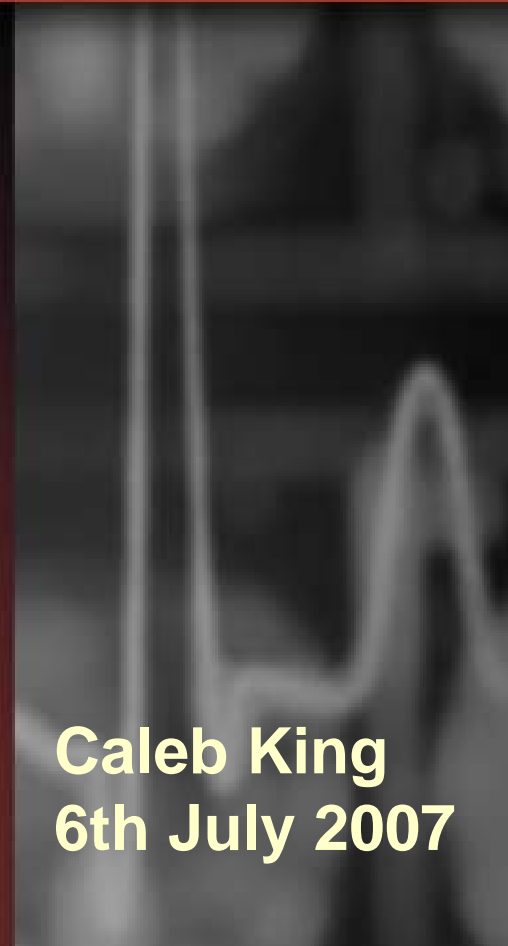
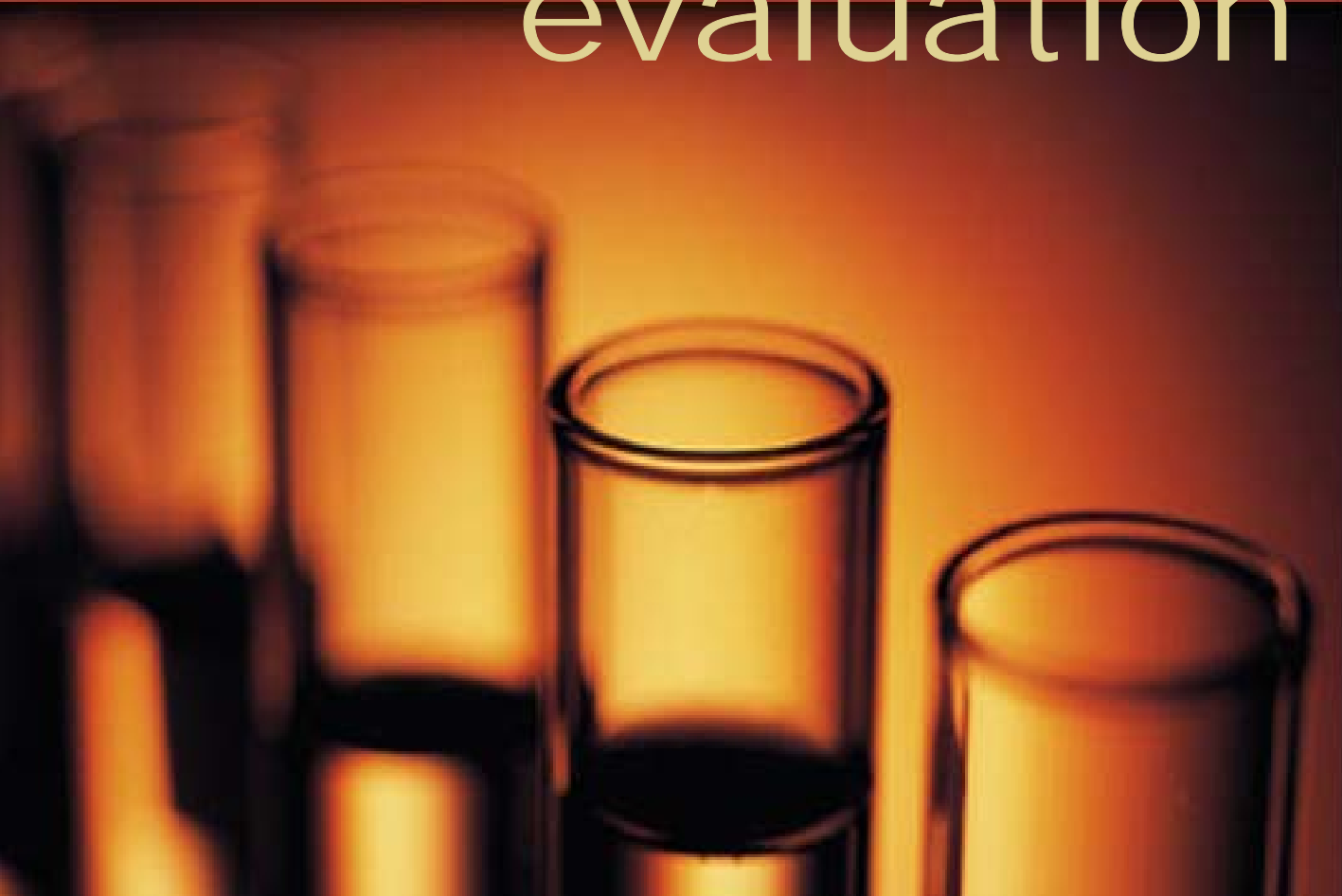


Diagnostic test evaluation



Caleb King
6th July 2007

TB tests, deer in NZ

- The current testing for deer involves the skin tests, the ELISA TB test (ETB) and the blood TB test (BTB)
- Skin tests have moderate sensitivity and specificity, but are cheap, the ancillary tests are more accurate, but cost more.

Problem - testing

Mycobacterium bovis (*M bovis*),
Mycobacterium intracellulare
and *Mycobacterium avium* have similar antigens,



any can cause a positive reaction in the host's skin, to the intradermal skin test.

The purified protein derivative (PPD) used as the antigen in the skin test for bovine tuberculosis contains antigens that can react to all of these bacteria, lowering the specificity of the primary test.

Problem definition- farmer

- **The number of lesion positive reactors for deer is very low, leading to farmers not trusting the ancillary tests**
- **More “reactors” lowers return for the meat value of deer.**

Problem – annual cost

- Approx 7000 deer in herds clear of tuberculosis for more than 5 years
- Average reactor rate is 55 kg
- Difference in schedule price \$1.80
- Number of reactors approx 700
- A 10% improvement in specificity can save around \$7000

Problem - Strategy

- Reduced stakeholder faith in the ancillary tests increases the likelihood of non-compliance and increases the risk of spread of TB back in to areas where it has been previously reduced
- Increased length of time to completion, and associated inc cost

Proposed solution

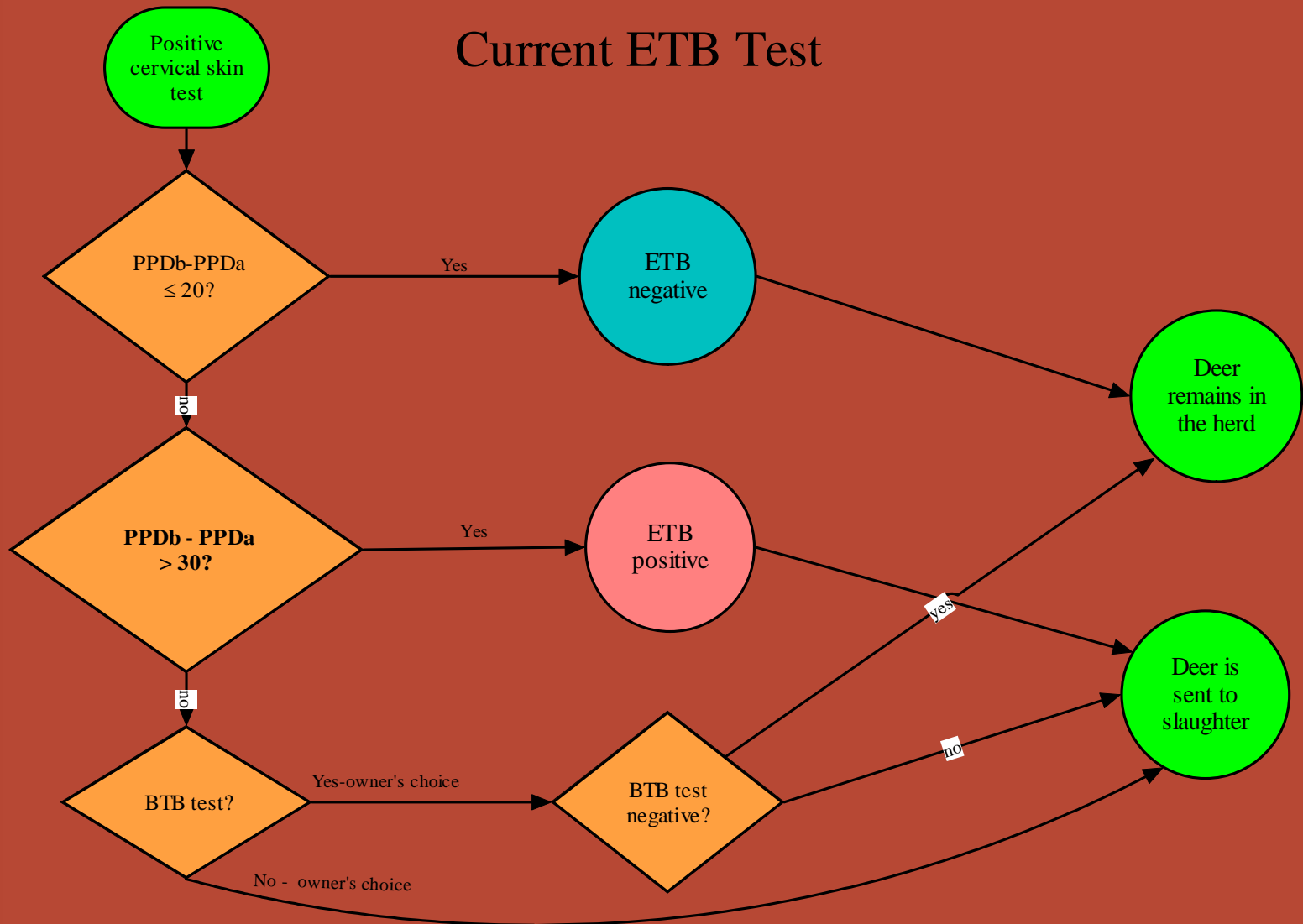
- Improve the current ETB test by adding further ELISA's to differentiate TB from Johnes better
- Start using the new modified ETB test in herds with a low prior probability of TB to reduce the risk of sensitivity losses affecting program

ELISA test units

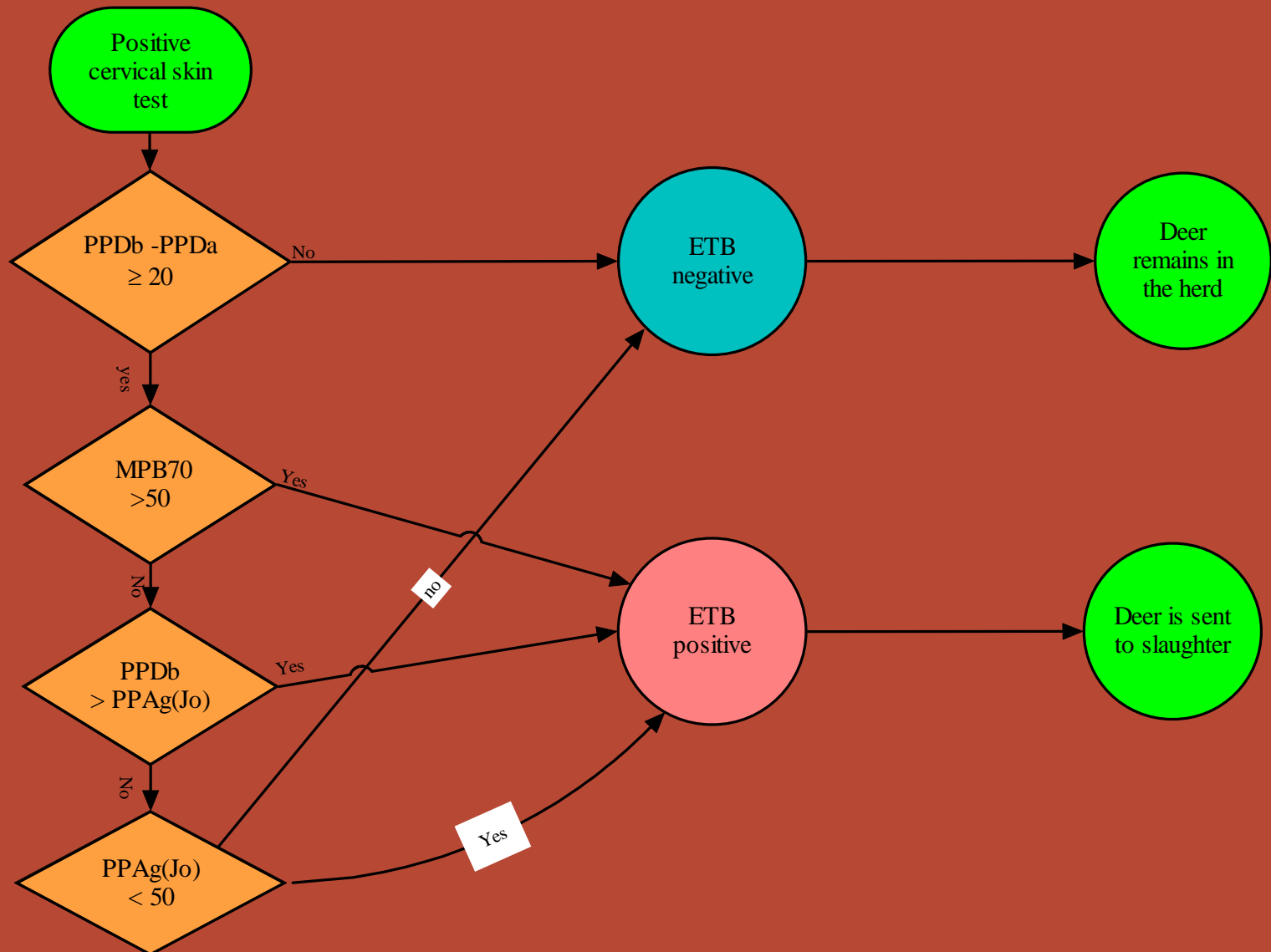
- The raw output of each ELISA is the observed optical density (OD) value at 490nm wavelength of light. Positive and negative control sera are used to establish the validity of each assay. The observed OD value of negative reference sera is subtracted from the OD value of test sera and the resultant difference is multiplied by 100 to convert it to an ELISA test unit (EU). The ELISA test unit is therefore a continuous variable. A cutoff value is used to dichotomize the continuous variable into a test status of positive or negative.

Current ETB test

Current ETB Test



Proposed modified ETB



Test components

PPDb - bovine purified protein derivative

PPDa – avian purified protein derivative

MPB70 – secreted mycobacterial protein unique to *M bovis* and some *M tuberculosis* strains, not always present

PPAgJo – protoplasmic antigen of *M avium paratuberculosis*

Values are ELISA units

Review of proposal

- The parameters of the b-a step is known from the current ETB [Diagnosis of tuberculosis due to Mycobacterium bovis in New Zealand red deer \(Cervus elaphus\) using a composite blood test and antibody assays.pdf](#)
- The parameters of the PPAgJo has recently been published.
- The B>J step is a complete unknown

Development of a model

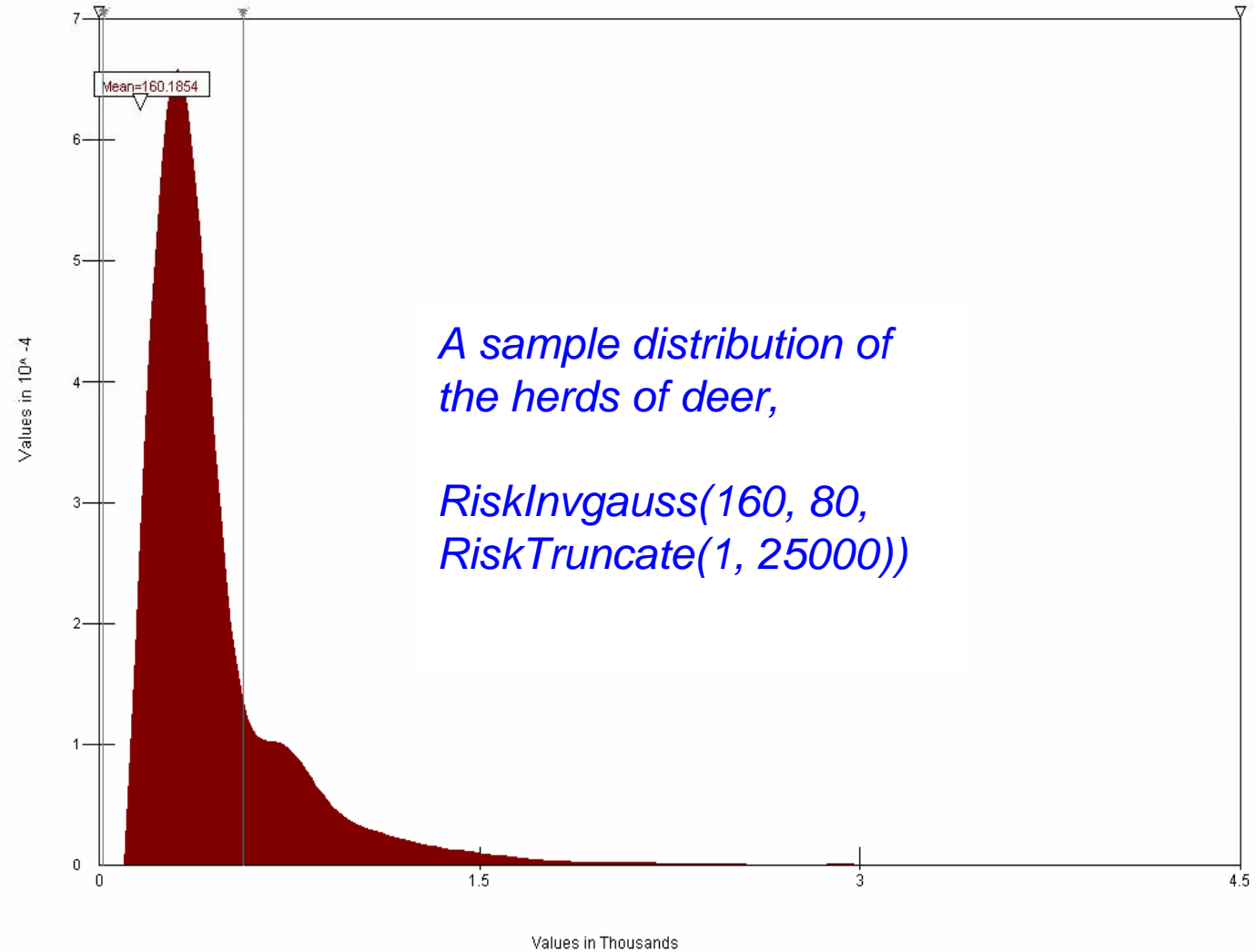
Using At risk can take the known values of sensitivity and specificity, simulate herds and prevalence of both M bovis and MAP (Johnes)

Scenario 2 vary b-a\Copy of
Simulate sensitivity and specificity
effects_9 Basic.xls

Model inputs

- Deer herds
- Prevalence of M bovis
- Prevalence of Johnes
- Proportion of Johnes ST +ves
- Sensitivity of B>J step
- Known Se & Sp values for ELISA components

Distribution for Herd size of deer / Model/G3 (Sim#5)



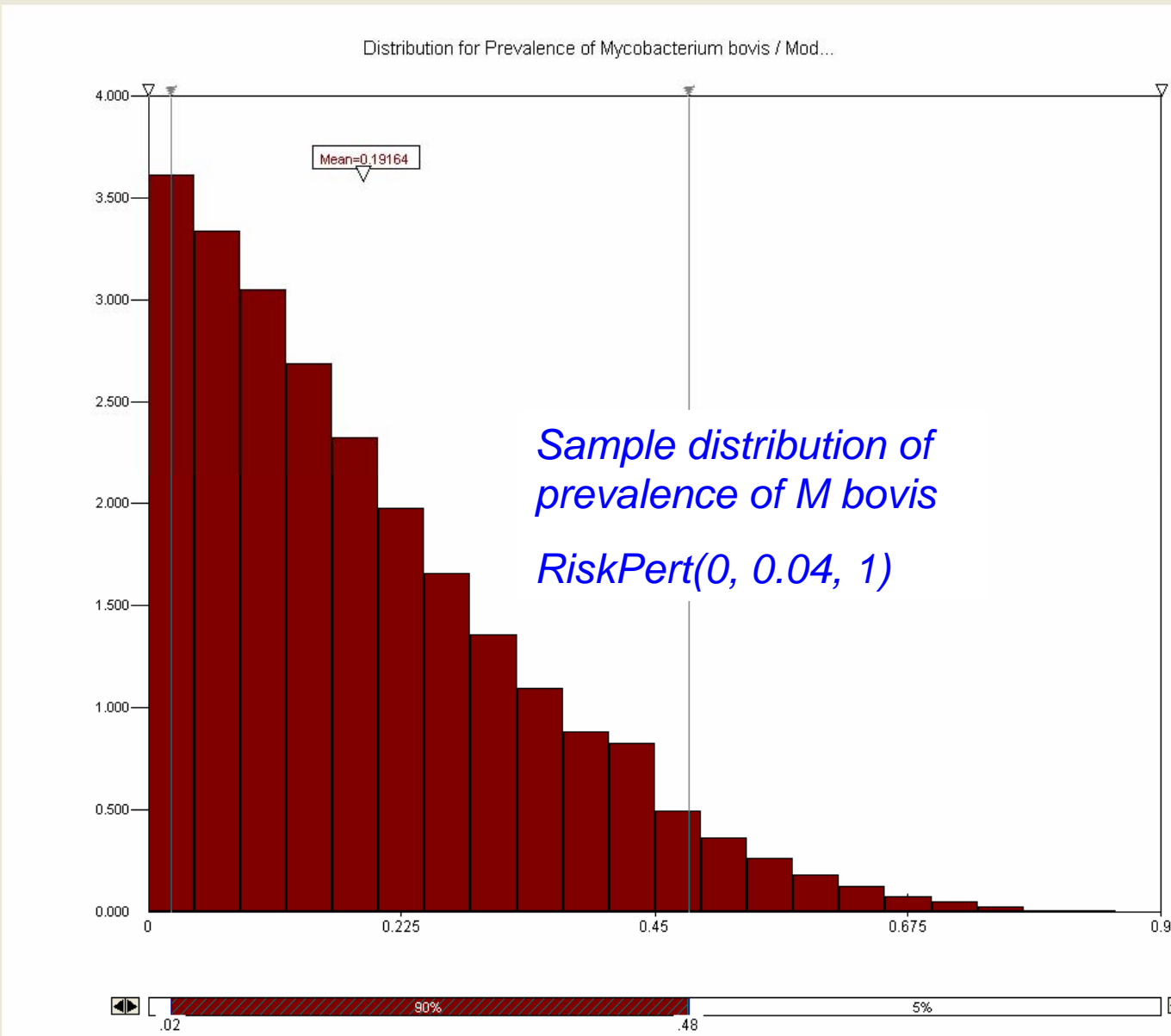
*A sample distribution of
the herds of deer,

RiskInvgauss(160, 80,
RiskTruncate(1, 25000))*

Name	Herd size of
Cell	33 Output (Sim#5)
Minimum	4
Mean	160.1854
Maximum	4215
Std Dev	229.0402
Variance	52459.39
Skewness	4.623451
Kurtosis	42.08083
Mode	24
Left X	17
Left P	5%
Right X	567
Right P	95%
Diff. X	550
Diff. P	90%
5th Perc.	17
95th Perc.	567
#Errors	0
Filter Min	
Filter Max	
#Filtered	0

- Outputs
 - G3- Herd siz
 - G4- Prevale
 - G5- Number
 - G6- Prevale
 - G7- Percent
 - Q8- ETB Se
 - Q9- ETB Sp
 - Q10- ETB F
 - Q11- ETB N
 - L24- Youde
 - Q25- Overa
 - Q26- Overa
 - Q29- Sensit
 - Q30- Specif
 - Q31- Youde
 - Q32- Youde
 - Q33- Improv
 - Q34- Improv
 - Q35- Youde
 - Q54- INTEF
 - G97- Numb
 - G98- Numb
- Inputs
 - F3- Herd siz
 - F4- Prevale
 - F6- Prevale
 - F7- Percent
 - G66- Sensit

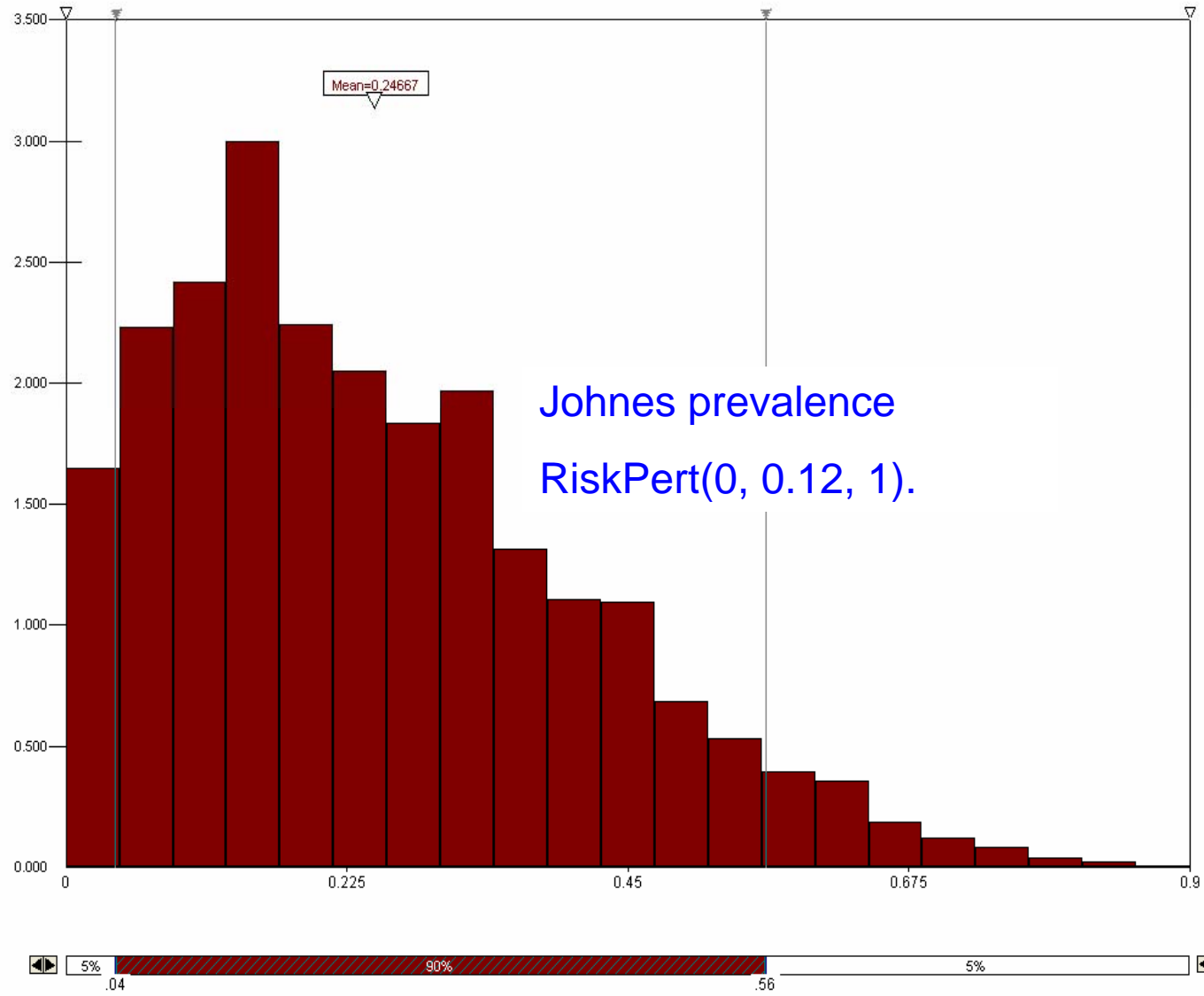
Distribution Tornado Range Summary



Stats	Data	Sen
Name	Prevalen	
Cell	G4	
Minimum	0	
Mean	0.19164	
Maximum	0.86	
Std Dev	0.1478575	
Variance	2.186184E-	
Skewness	1.023379	
Kurtosis	3.72897	
Mode	0.04	
Left X	0.02	
Left P	5%	
Right X	0.48	
Right P	95%	
Diff. X	0.46	
Diff. P	90%	
5th Perc.	0.02	
95th Perc.	0.48	
#Errors	0	
Filter Min		
Filter Max		
#Filtered	0	

Name	Prevalence
Cell	G6
Minimum	0
Mean	0.24667
Maximum	0.9
Std Dev	0.1629795
Variance	2.656232E-02
Skewness	0.7774876
Kurtosis	3.14098
Mode	0.13
Left X	0.04
Left P	5%
Right X	0.56
Right P	95%
Diff. X	0.52
Diff. P	90%
5th Perc.	0.04
95th Perc.	0.56
#Errors	0
Filter Min	
Filter Max	
#Filtered	0

Distribution for Prevalence of Johnes disease / Model/G6



Skin positive deer

- Percent of Johnes deer that are skin test positive, RiskTriang(0, 0.5, 1), i.e half of the deer with Johnes disease are skin test positive
- B>J step – most uncertain input
- RiskPert(0, 0.5, 1, RiskTruncate(0.05, 0.95))

Model outputs

- For skin test, current ETB and modified ETB calculate for each herd
- Net Sensitivity and Specificity
- Positive and Negative predictive values
- Youden's J
- Change in J over skin test, current ETB
- Change in sensitivity and specificity
- Number of animals with *M. bovis* remaining in herd at the end of testing

Name	Cell	Minimum	Mean	Maximum
Herd size of deer / Model	G3	5.00	160.00	3675.00
Prevalence of Mycobacterium bovis / Model	G4	0.00	0.18	0.85
Number of deer infected with M bovis	G5	0.00	28.72	929.00
Prevalence of Johnes disease / Model	G6	0.00	0.25	0.90
Percent of Johnes deer that are skin test positive / Model	G7	0.01	0.50	1.00
ETB Sensitivity	Q8	0.67	0.88	1.00
ETB Specificity	Q9	0.94	0.98	1.00
ETB Positive predictive value	Q10	0.00	0.94	1.00
ETB Negative predictive value	Q11	0.00	0.87	1.00
Youden's J of the skin test	L24	-1.51	0.67	1.00
Overall Sensitivity	Q25	0.50	0.77	1.00
Overall Specificity	Q26	0.91	1.00	1.00
Sensitivity lost with ETB	Q29	0.00	0.10	0.33
Specificity gained with ETB	Q30	0.00	0.20	2.27
Sensitivity loss from current ETB	Q33	0.00	0.08	0.33
Specificity gained from current ETB	Q34	0.00	0.01	0.18
Youden's J of the modified ETB test	Q35	0.60	0.86	1.00
Youden's J of the current ETB test	Q36	0.74	0.89	1.00
Difference in Youden's J from current ETB	Q37	-0.33	-0.03	0.17
Difference in Youden's J over the skin test	Q38	-0.32	0.20	2.33
Youden's J overall	Q39	0.48	0.77	1.00
INTERNAL VALIDITY	Q54	0.00	0.00	0.00
Number of animals remaining with M bovis	G97	0.00	2.23	97.00
Number of animals ETB positive following PPAgJo stepI	G98	0.00	2.30	98.00
Sensitivity of B-J per model	G66	0.05	0.50	0.95

Second model

- First model used vendor's estimates of $Se = 0.95$ and $Sp = 0.9$ for the b-a step (current ETB) however the trial flock of deer this was based on did not have Johnes disease so these estimates may be inaccurate.
- range of sensitivity reported from trial = $0.9 - 0.95$, and lowered Sp by an arbitrary 10%, to give =RiskOutput("Specificity b-a / Model") + RiskPert(0.7, 0.8, 0.9)

	Name	Cell	Minimum	Mean	Maximum
ETB Sensitivity		Q8	0.67	0.86	1.00
ETB Specificity		Q9	0.83	0.94	1.00
ETB Positive predictive value		Q10	0.00	0.86	1.00
ETB Negative predictive value		Q11	0.00	0.86	1.00
Youden's J of the skin test		L24	-0.86	0.67	1.00
Overall Sensitivity		Q25	0.50	0.76	1.00
Overall Specificity		Q26	0.84	0.99	1.00
Sensitivity lost with ETB		Q29	0.00	0.12	0.33
Specificity gained with ETB		Q30	0.00	0.19	1.59
Sensitivity b-a / Model		G32	0.90	0.93	0.95
Specificity b-a / Model		G33	0.70	0.80	0.90
Sensitivity loss from current ETB		Q33	0.00	0.07	0.33
Specificity gained from current ETB		Q34	0.00	0.03	0.20
Youden's J of the modified ETB test		Q35	0.50	0.80	1.00
Youden's J of the current ETB test		Q36	0.46	0.79	1.00
Difference in Youden's J from current ETB		Q37	-0.45	0.01	0.35
Difference in Youden's J over the skin test		Q38	-0.39	0.13	1.65
Youden's J overall		Q39	0.42	0.74	1.00
INTERNAL VALIDITY		Q63	0.00	0.00	0.00
Number of animals remaining with M bovis		G97	0.00	2.05	103.00
Number of animals ETB positive following PPAgJo		G98	0.00	2.27	106.00
Herd size of deer / AtRisk		F3	4.20	159.97	3707.70
Prevalence of Mycobacterium bovis / AtRisk		F4	0.00	0.17	0.85
Prevalence of Johnes disease / AtRisk		F6	0.0004171	0.2466656	0.9143127

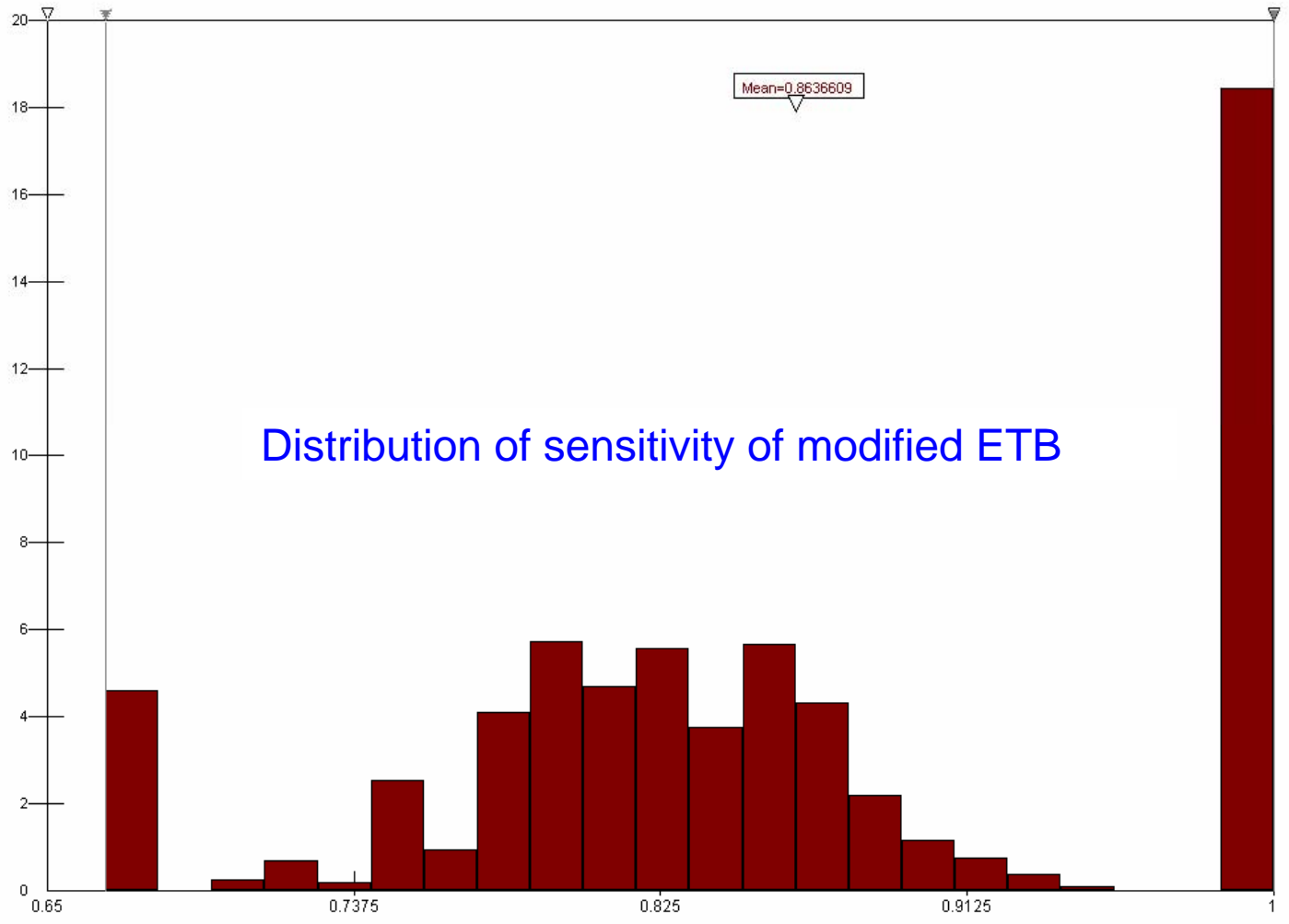
Scenario of intended use

- Higher prevalence of Johnes, 50%
- Lower prevalence of M bovis
- All other parameters the same
- Simulating the effect of using the modified ETB test on herds with a clear five year status, but still having Johnes disease on their farms. Scenario 4,

Clear 5 herds with endemic Johnes\Copy of Simulate sensitivity and specificity effects 10
C5+J.xls

Name	Cell	Minimum	Mean	Maximum
ETB Sensitivity	Q8	0.67	0.86	1.00
ETB Specificity	Q9	0.83	0.92	1.00
ETB Positive predictive value	Q10	0.00	0.75	1.00
ETB Negative predictive value	Q11	0.00	0.91	1.00
Youden's J of the skin test	L24	-1.55	0.51	1.00
Overall Sensitivity	Q25	0.50	0.76	1.00
Overall Specificity	Q26	0.79	0.97	1.00
Sensitivity lost with ETB	Q29	0.00	0.12	0.33
Specificity gained with ETB	Q30	0.00	0.33	2.20
Sensitivity b-a / Model	G32	0.90	0.93	0.95
Specificity b-a / Model	G33	0.70	0.80	0.90
Sensitivity loss from current ETB	Q33	0.00	0.07	0.33
Specificity gained from current ETB	Q34	0.00	0.04	0.30
Youden's J of the modified ETB test	Q35	0.50	0.78	1.00
Youden's J of the current ETB test	Q36	0.29	0.76	1.00
Difference in Youden's J from current ETB	Q37	-0.44	0.03	0.55
Difference in Youden's J over the skin test	Q38	-0.37	0.27	2.39
Youden's J overall	Q39	0.41	0.73	1.00
INTERNAL VALIDITY	Q63	0.00	0.00	0.00
Number of animals remaining with M bovis	G97	0.00	2.04	150.00
Number of animals ETB positive following PPAgJo	G98	0.00	2.50	162.00
Herd size of deer / AtRisk	F3	3.00	159.95	3402.17
Prevalence of Mycobacterium bovis / AtRisk	F4	0.00	0.17	0.89
Prevalence of Johnes disease / AtRisk	F6	0.01	0.50	0.99
Percent of Johnes deer that are skin test positive / AtRisk	F7	0.00	0.50	1.00

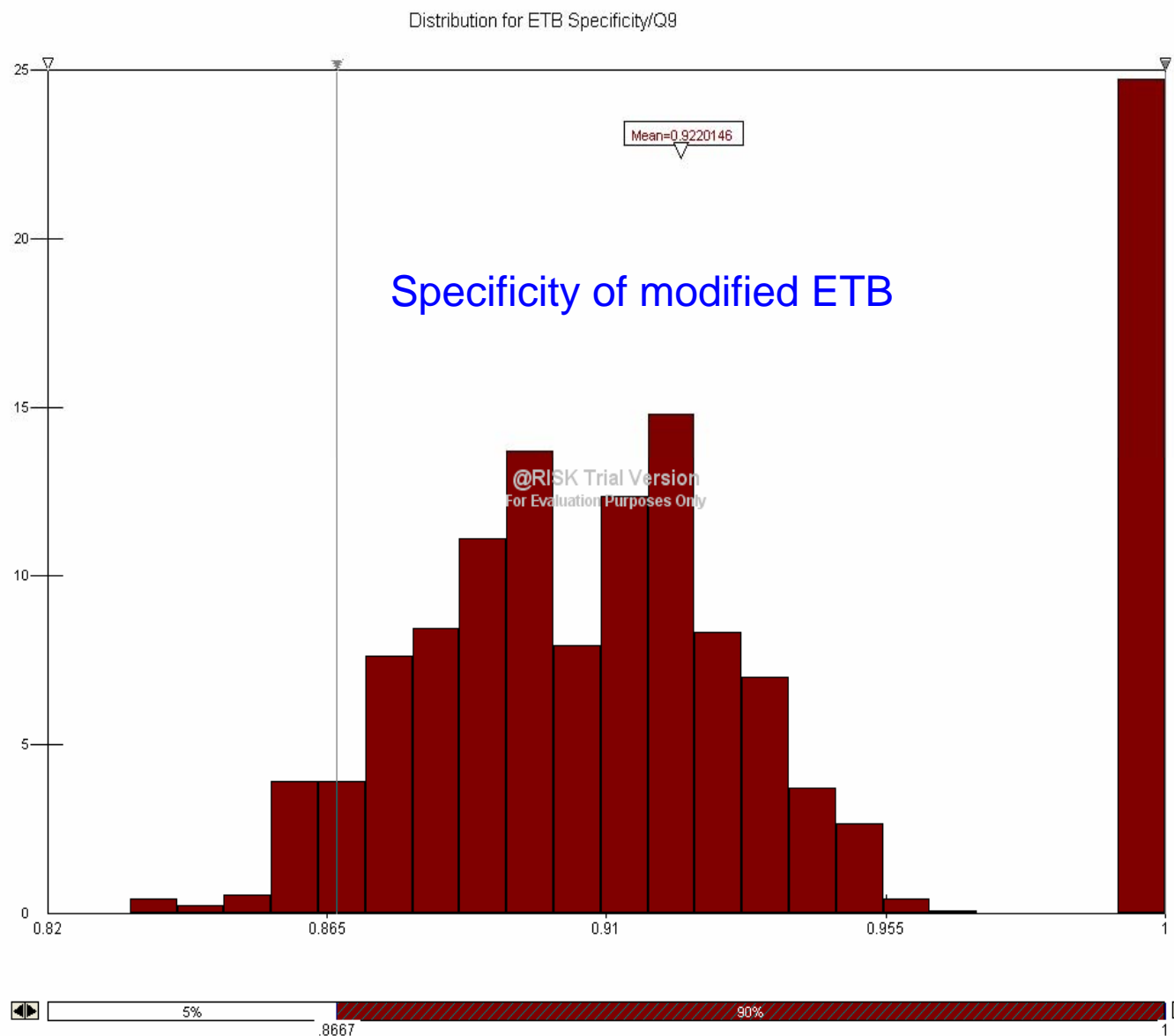
Distribution for ETB Sensitivity/Q8



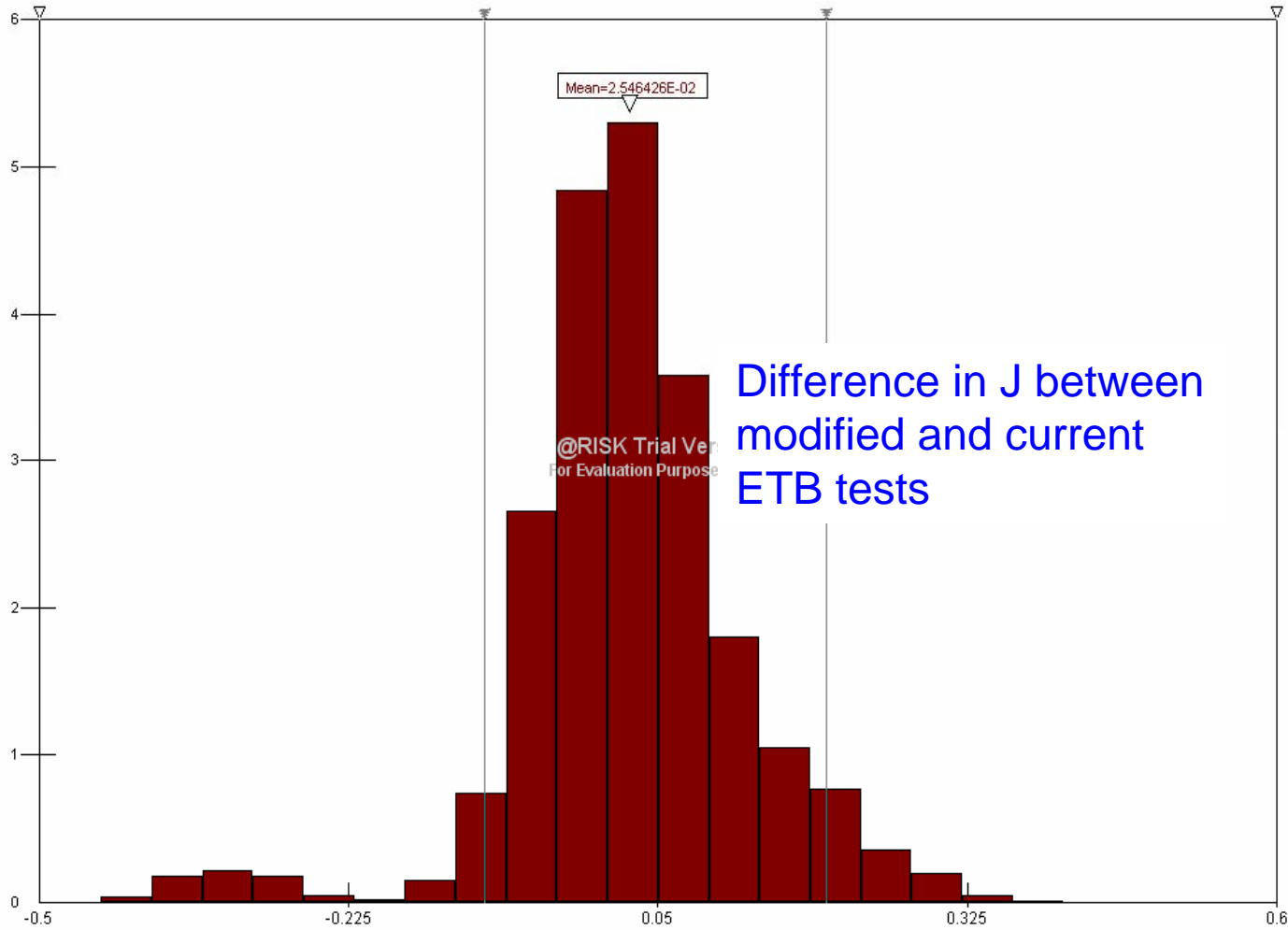
Stats	Data	Sens.
Name	ETB Sensitivity	
Cell	Q8	
Minimum	0.6666667	
Mean	0.8636609	
Maximum	1	
Std Dev	0.100707	
Variance	0.0101419	
Skewness	-1.861734E-02	
Kurtosis	2.173015	
Mode	1	
Left X	0.6666667	
Left P	5%	
Right X	1	
Right P	95%	
Diff. X	0.3333333	
Diff. P	90%	
5th Perc.	0.6666667	
95th Perc.	1	
#Errors	470	
Filter Min		
Filter Max		
#Filtered	0	



Name	ETB Specificity
Cell	Q9
Minimum	0.8333333
Mean	0.9220146
Maximum	1
Std Dev	4.305748E-02
Variance	1.853947E-03
Skewness	0.7295638
Kurtosis	2.515844
Mode	1
Left X	0.8666667
Left P	5%
Right X	1
Right P	95%
Diff. X	0.1333333
Diff. P	90%
5th Perc.	0.8666667
95th Perc.	1
#Errors	6
Filter Min	
Filter Max	
#Filtered	0



Distribution for Difference in Youden's J from current E...

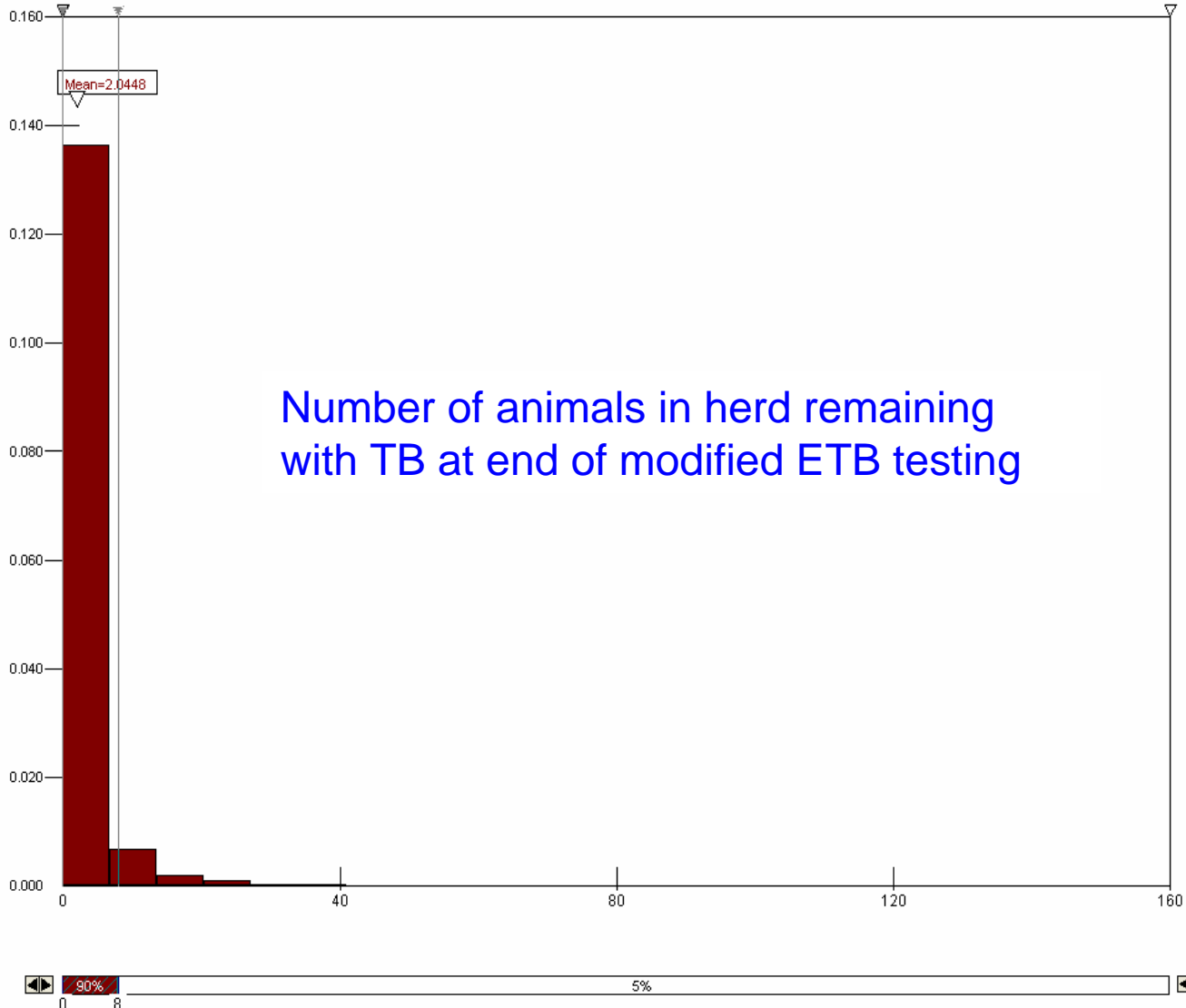


Stats Data Sens.

Name	Difference i
Cell	Q37
Minimum	-0.4449405
Mean	2.546426E-02
Maximum	0.5460373
Std Dev	0.1041998
Variance	0.0108576
Skewness	-0.6453912
Kurtosis	6.448966
Mode	0
Left X	-0.1043956
Left P	5%
Right X	0.2
Right P	95%
Diff. X	0.3043956
Diff. P	90%
5th Perc.	-0.1043956
95th Perc.	0.2
#Errors	475
Filter Min	
Filter Max	
#Filtered	0



Distribution for Number of animals remaining with M bovi...



Name	Number of
Cell	G97
Minimum	0
Mean	2.0448
Maximum	150
Std Dev	4.525422
Variance	20.47944
Skewness	9.182797
Kurtosis	177.9211
Mode	0
Left X	0
Left P	5%
Right X	8
Right P	95%
Diff. X	8
Diff. P	90%
5th Perc.	0
95th Perc.	8
#Errors	0
Filter Min	
Filter Max	
#Filtered	0

Factors to bear in mind

- Prior probability of TB on C5+ farms
- Prevalence of Johnes in deer
- Relative costs of false positives and negatives
- Improvement of understanding of B>J step with use
- Other ways of combining these tests?