



Refinement of the Canine Leptospira Vaccine Hamster Potency Test

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The EP challenge potency test

- Inject 1/40 dog dose into 5 hamsters (for each serovar)
- 15-20 days pi, challenge 5 vaccinated hamsters and 5 unvaccinated controls
- At least 4/5 control hamsters should die of leptospirosis within 14 days
- At least 4/5 vaccinates should remain healthy
- Clearly a severe test



The 3 Rs options

- **Replacement** – two options are available in the EP, but alternatives are difficult to develop
- **Reduction** – numbers are specified and probably the minimum
- **Refinement** – humane end-points



Measure we have taken

- More frequent observation
- Training and awareness
- Clearly defined clinical end-points
- Temperature monitoring to predict end-points
- Reverse lighting
- Standardisation and familiarity with the challenge model



Frequent Observation

- Immediately after inoculation
- Daily by the animal care workers
- More frequently by the Study Director after challenge
- Critical phase is 4-7 days after challenge
- Training and involvement of animal care workers

Hamster



- Healthy hamster and sick companion with head pressed into the corner

Hamster



- Healthy hamster with sick companion showing hunched posture and dull sunken eyes

Hamster



- Infected hamster bleeding from the nostrils



Clinical end-points

Clinical Signs	Score	Actions
Normal behaviour	0	None
Arched back/slightly rough coat	1	Observe again in 2 hours. If symptoms persist, observe again in 2 hours
Dull sunken eyes, moderately rough coat, unstable on feet, subdued but responds on stimulus, disinterested in surroundings	2	Observe as for score 1. If a 2 is observed at the end of the day – euthanase
Prostrate, nasal bleeding, blood in urine, permanently closed eyes	3	Euthanase



Temperature Monitoring

- Could a drop in temperature predict sickness (end-point)?
- Did not really work
- Hand held devices, so hamsters were handled
- May hold promise for the future?



Reverse Lighting

- Hamster are nocturnal
- So more active in the dark
- The lights are on during night-time, but off during the day
- Helps identify sick animals – less active



Standardisation of the challenge model

- Good control of the challenge material
- Source of the hamsters
- Regular testing is helpful
 - Share controls
 - Critical phase



Does it work?

- It is difficult to measure success, ie to prove it
- But we “feel” we have improved animal welfare, or at least doing all we can



Change in clinical profile

	<i>L. canicola</i>			<i>L. ictero.</i>		
	KS	KM	FD	KS	KM	FD
1999 to 2001 (18 tests)	16	19	34	20	33	30
%	23	28	49	24	40	36
2001 to 2005 (29 tests)	193	3	55	134	6	35
%	77	1	22	77	3	20

KS = killed sick, KM = killed moribund, FD = found dead



Conclusions

- Death as an end-point is not necessary
- We have introduced measures to improve welfare and use a clinical end-point
- These measures have reduced the severity of the test and improved animal welfare



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