Progress Towards the Replacement of *In Vivo* Leptospirosis Vaccine Potency Testing in the United States

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Abstract

Vaccines are available to prevent leptospirosis—a potentially lethal illness—in cows, pigs, and dogs. To meet regulatory requirements, manufacturers must test the potency of each batch of vaccine to ensure its relative strength.[1] Historically, this has been measured with a lethal test in hamsters. In the U.S, the number of hamsters used in this test each year is reported by vaccine manufacturers to the U.S. Department of Agriculture (USDA). In this public reporting, hamsters used in lethal potency tests are counted in Category E, indicating that they undergo painful procedures in which pain is not relieved.

A 2012 workshop addressed the continued use of hamsters to test the potency of veterinary leptospirosis vaccines given the availability of non-animal methods that replace the *in vivo* method.[2] We assessed the impact of the workshop's recommendations to reduce hamster use and found that manufacturers have achieved an approximately 55 percent decrease in the number of Category E hamsters used since 2014, the earliest year for which records are available.

We propose steps to accelerate broader use of *the in vitro* methods to further reduce, and ultimately replace, hamster use.

Background

Leptospirosis is a zoonotic disease caused by *Leptospira* bacteria. There are no human leptospirosis vaccines in the U.S., though veterinary vaccines can help avoid the spread of the disease to humans in addition to preventing illness among vaccinated animal populations.[3] In the U.S., leptospirosis vaccines are available for the following leptospirosis serovars: *Leptospira interrogans* serogroups *pomona*, *canicola*, and *icterohaemorrhagiae*; *Leptospira kirschneri* serogroup *grippotyphosa*; and *Leptospira interrogans* serogroup *hardjo* and/or *Leptospira borgpetersenii* serogroup *hardjo*.[1,3,4]

Regulatory guidelines require that leptospirosis vaccines are evaluated for purity, potency, safety, and efficacy. The USDA Center for Veterinary Biologics (CVB) regulates the batch potency testing of leptospirosis vaccines through standard requirements that stipulate the *in vivo* test in hamsters.[1]

However, an *in vitro* ELISA test was published for four leptospirosis serovars more than 20 years ago as Supplemental Assay Methods (SAMs) and are accepted as alternatives to the *in vivo* test.[5-8] CVB notes that each of these *in vitro* methods is a "reproducible, sensitive, specific, and inexpensive alternative" to the *in vivo* test.[3] Vaccine manufacturers may also propose modifications to the SAM protocols for their specific products.[9]

Methodology & Results

Data was collected from public USDA records documenting vaccine manufacturers' animal use between 2014 and 2020, showing that the use of hamsters declined during this period: there was a 40% decrease in overall hamster use and a 55% decrease in hamsters used in Category E procedures.

Since the leptospirosis vaccine potency test is the only mandated use of hamsters in the standard requirements for these products, the number of Category E hamsters is used as an approximation of the number of hamsters used each year in U.S. leptospirosis vaccine potency tests.

Annual Hamster Use in the U.S.		
Year	**Category E**	**Overall**
2014	34,170	79,946
2015	29,048	68,857
2016	30,234	74,336
2017	27,354	65,426
2018	18,167	56,321
2019	17,525	51,724
2020	15,439	49,016

Table 1. The number of overall and Category E hamsters used in the U.S. from 2014 to 2020.

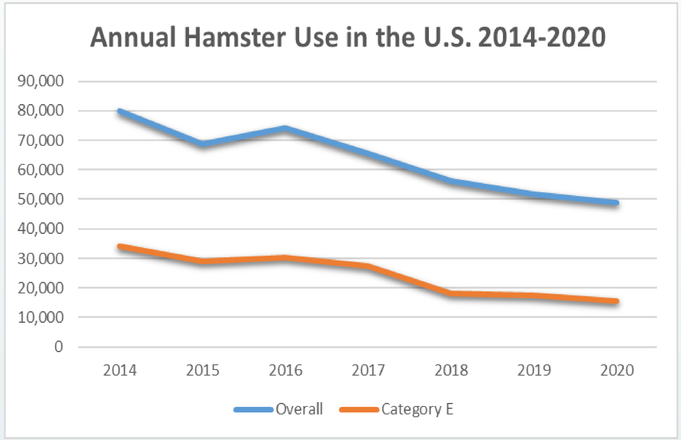

Figure 1. Trend lines of overall and Category E hamster use in the U.S. from 2014 to 2020.

Category E Only: Annual Hamster Use in the U.S. by Company or Agency											
	Company A	**B**	**C**	**D**	**E1**	**E2**	**F**	**G**	**H**	**USDA CVB**	**Total**
2014	2,565	18,633	2,899	0	4,001	0	187	3,669	1,413	803	**34,170**
2015	1,728	14,007	1,237	1,894	3,900	0	302	3,759	1,194	1,027	**29,048**
2016	1,796	15,016	0	3,028	1,189	2,999	74	3,977	1,157	998	**30,234**
2017	1,870	3,318	0	12,450	0	4,110	76	3,448	1,382	700	**27,354**
2018	1,770	2,762	0	7,563	0	4,444	3	688	804	133	**18,167**
2019	635	6,231	0	4,831	0	4,502	56	549	696	25	**17,525**
2020	0	6,118	0	3,461	0	3,803	253	828	966	10	**15,439**

Table 2. The number of Category E hamsters used by each company with an active leptospirosis vaccine license in one or more of the above years. Some companies are associated or have since merged and are noted with the same letter.

2012 Workshop

Alternatives to *in vivo* leptospirosis vaccine potency testing were discussed at a 2012 workshop that established a consensus on the value of manufacturers achieving product-specific validation of the *in vitro* ELISA and CVB evaluating the need for back-titration testing requirements for the *in vivo* potency test, among other targets.[2] The workshop was co-organized by USDA CVB, National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), International Alliance for Biological Standardization (IABS), and other partners.

In Vivo & *In Vitro* Methods

In vivo Hamster Test:

- Incorporated into requirements in 1974, developed in the 1960s[4]
- Can take months and costs about $1,000[2]
- Is a challenge test and uses at least 20 hamsters[1]

In vitro ELISA Test:

- Published as SAMs from the USDA by 2000[5-8]
- Can be completed in days and costs about $500[2]
- Is an ELISA test and uses animal-derived antibodies but no live animals[5-8]

Discussion

- While effective steps to reduce hamster use have already been made, opportunities to move toward the complete replacement of animals for assessing leptospirosis vaccine potency remain.
- USDA published waivers in 2015-2016 that effectively reduced the amount of hamsters needed for the *in vivo* test by half.
- The *in vitro* potency tests have the potential to replace most of the routine use of hamsters for leptospirosis vaccine potency testing.
  - Further uptake of the *in vitro* methods could continue to reduce the number of hamsters used annually.
  - There are numerous advantages of the *in vitro* methods, including reduced cost and time besides sparing animals.
  - As the *in vitro* assay uses animal-derived antibodies as critical reagents, results may be impacted by problems associated with animal-derived antibodies—namely, uncertain monospecificity and variability between batches.
  - We suggest that transitioning to recombinant antibodies could allow for more consistent assessment of vaccine potency over time.[10]
- Communication between stakeholders is needed to better understand the impact of the back-titration waivers and the *in vitro* ELISA methods in reducing hamster use, and to identify the remaining hurdles preventing adoption of the *in vitro* methods by all vaccine manufacturers internationally.
- An expert meeting or targeted workshop would facilitate this discussion and highlight developments and challenges that have emerged since the 2012 workshop. We are contacting vaccine manufacturers as a next step.
- A detailed summary of this research has been published.[11]

References

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