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Using Off-the-Shelf Technologies to Mass Manufacture Oral Vaccine Baits for Wildlife

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ABSTRACT: Technology and infrastructure costs can limit access to oral vaccination tools for wildlife disease control. We describe vaccine bait mass manufacturing employing off-the-shelf technologies. Our approach has helped advance scaling-up of plague vaccination campaigns, but components of this production system could be translated into other wildlife vaccination applications.

Oral vaccination offers a prospective tool for controlling a variety of wildlife diseases (Cross et al. 2007; Artois et al. 2011). The most notable successes with wildlife vaccination have been the European and North American campaigns against rabies (Cross et al. 2007; Sterner et al. 2009; Artois et al. 2011). Mass oral vaccination of wildlife relies on availability of effective immunogens and suitable bait delivery technology (Cross et al. 2007; Artois et al. 2011). Unfortunately, the necessary investments in technology and infrastructure (Sterner et al. 2009) have largely limited access to oral vaccination for large-scale uses other than rabies control. Versatile and affordable approaches for wildlife vaccination could be beneficial.

Plague—a disease with human and wildlife health implications—occurs in wild rodent reservoirs worldwide (World Health Organization 2016). Since its introduction in the early 1900s, plague has disrupted grassland and shrub-steppe ecosystems throughout much of western North America, contributing to the near-extirpation of several native species, including prairie dogs (*Cynomys* spp.; Abbott et al. 2012). Consequently, work has been underway since the early 2000s to develop oral vaccination as a plague management tool (Abbott et al. 2012; Johnson et al. 2014; Rocke et al. 2014). Small-scale field trials have been undertaken, but landscape-scale application via mechanized delivery will be needed for biologically meaningful plague control (Johnson et al. 2014; Tripp et al. 2015; USFWS 2016). Here we describe key elements of a vaccine carrier bait mass-manufacturing approach employing off-the-shelf technologies. Our approach had immediate application in aiding the development of mechanical bait distribution for plague vaccination campaigns in western North America, but this production system could be adapted to other wildlife vaccination applications.

Baits carrying plague vaccine (the genetically-modified raccoonpox virus strain RCN-F1/V307; Rocke et al. 2014) consists mainly of distilled water, an attractant (peanut butter), and a patented, gelatin-based biopolymer matrix (Incortrix®, FoodSource Lures Corporation, Birmingham, Alabama, USA) used previously as a carrier for a recombinant vaccinia-Lyme disease vaccine (Table 1; Bhattacharya et al. 2011; FoodSource 2013). Three critical features of the biopolymer matrix are: 1) live vaccines can be incorporated into the mix at a temperature low enough to assure viability; 2) the vaccine bait mixture remains malleable at this lower temperature; and 3) the vaccine remains viable through drying (Bhattacharya et al. 2011; FoodSource 2013). Vaccine-laden baits made with this matrix can be shaped and sized according to target species because vaccine is distributed throughout the bait material.

Bench-top approaches for making vaccine baits suffice for laboratory studies and small-scale field trials, but cannot support landscape-level endeavors. As a step toward meeting larger-scale demands, we modified

TABLE 1. Ingredient amounts in a formulation for mass-produced baits carrying plague vaccine (RCN-F1/V307). We used 8-kg batches as a standard to accommodate processing through a semiautomated bait-making machine. See the Supplementary Material for additional details on vaccine bait making procedures using this system.

Ingredient	Weight (g)	Percentage	Change[a]
Distilled water	4,322	53.8	1.15×
Incortrix powder[b]	2,319	28.8	0.82×
Peanut butter[c]	1,159	14.4	0.92×
Blue dye powder	40	0.5	
Vaccine fluid	200	2.5	
Total	8,040		

[a] Multiplier for proportional difference from small-batch formulation provided by the US Geological Survey. Other manipulations explored as overviewed in the Supplementary Material (Table S1).

[b] FoodSource Lures Corporation, Birmingham, Alabama, USA.

[c] An organic, pure peanut butter product that does not contain palm kernel oil or other additives is recommended. See Supplementary Material Table S1.

bait formulation and manufacturing practices in order to facilitate adaptive plague vaccination campaigns in Colorado, US and elsewhere. We used a series of exploratory experiments (Supplementary Material Table S1) to increase malleability of the vaccine-bait slurry at room temperature. We ultimately effected the desired change by increasing relative water content and allowing ample time for matrix saturation (Table 1 and Supplementary Table S1). We also substituted FD&C Blue #1 food dye for the rhodamine B biomarker (Fernandez and Rocke 2011) to decrease bait size, maintain palatability, and enhance attractiveness to prairie dogs (Cain and Carlson 1968). This change sacrificed ability to biomark individuals consuming baits. Instead, we use observation of blue-stained feces to confirm vaccine uptake by target species.

These modifications allowed us to exploit an off-the-shelf mass production system for bait manufacture (Fig. 1 and Supplementary Material). We adapted a "carp bait" production machine (BoilieRoller Machine, Midland Carp and UAB Topas LT, Tauragė, Lithuania; Fig. 1A) to make vaccine baits. These machines produce round, uniform fishing baits or "boilies" of user-specified diameter. We elected to produce 14-mm-diameter baits (mass about 2.1 g wet, each). A video of vaccine bait manufacturing is given as part of the Supplementary Material.

We produced vaccine baits under Biosafety Level II conditions (detailed in the Supplementary Material). Each 8 kg batch yielded about 3,800 baits, a >10-fold improvement over bench-top production. Initial steps approximated those of Bhattacharya et al. (2011): we mixed all ingredients at 65–70 C except the plague vaccine (Yersinia Pestis Vaccine, Live Raccoon Poxvirus Vector, Code 11Y2.R0; Colorado Serum Company, Denver, Colorado, USA), then added the vaccine after cooling the bait slurry to 35–40 C. We then poured the complete vaccine-bait mixture—still as a liquid slurry—into an aluminum extrusion tube. We sealed the extrusion tubes with plastic wrap to reduce moisture loss and allowed the formulation to hydrate and solidify overnight (~12 h) at ~21 C. This essential time period allowed the matrix to fully hydrate, thereby ensuring cohesiveness during rolling. After hydration, the extrusion tube was connected to the manufacturing machine. Finished baits were dried for 48–96 h (relative humidity 40%), then weighed, bagged, and stored frozen until used. Finished baits weighed 0.9–1 g dry and remained generally uniform, but became somewhat less spherical during drying (Fig. 1B).

Extrusion under pressure 210,000–344,000 pascal (30–50 psi) did not appear to affect vaccine viability. Dried baits collected from the beginning and end of runs yielded comparable live RCN-F1/V307 virus counts (Supplementary Fig. S1). Virus counts from dry baits were lower than target doses (Supplementary Fig. S1) but approximated the ~10-fold discrepancies between target and measured RCN counts that we and others have encountered in bench-top-made vaccine baits (e.g., Mencher et al. 2004). These differences could reflect true losses in titer from the bait-making process or inefficiencies

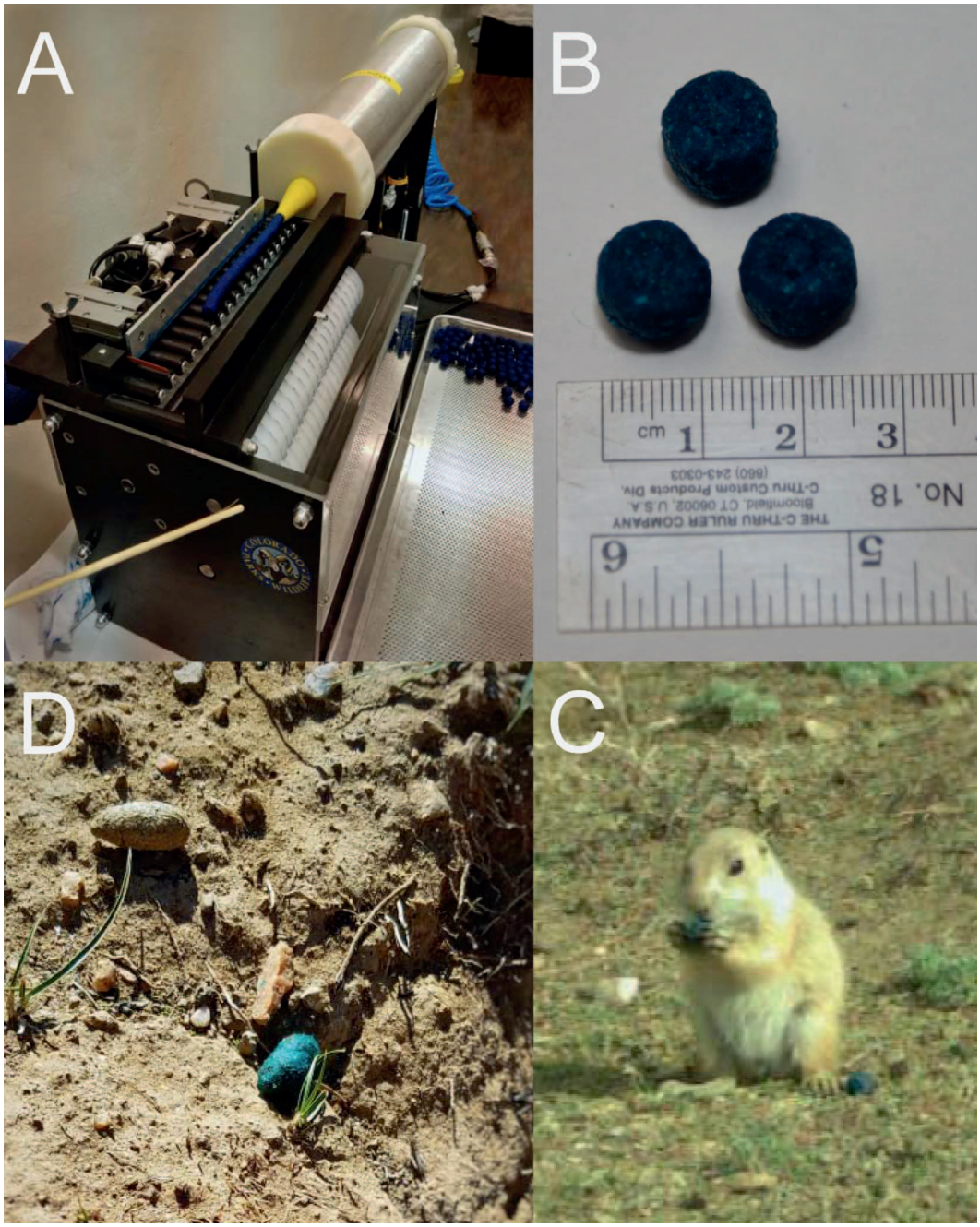

FIGURE 1. Plague vaccine baits were mass-produced using off-the-shelf technologies. Clockwise from upper left: We used a "boilie" machine (A) to manufacture relatively uniform bait balls (~14 mm diameter wet, ~10–12 mm dry) (B) that were readily consumed in the field by prairie dogs (*Cynomys* spp.; camera trap image) (C). Blue-stained prairie dog feces (D) deposited across treated field sites confirmed vaccine uptake by the target species. See the Supplemental Material for a video clip of vaccine bait manufacturing.

in extraction from the matrix (Mencher et al. 2004).

Manufacturing vaccine baits with boilie machines was efficient. We produced over 300,000 vaccine baits (~300 kg dry weight) in an initial 5-wk production run with two machines. Judging from efficiencies gained with experience, we anticipate that 100,000 baits/machine-week could be achieved. Labor savings and formulation modifications cut estimated per bait production costs by ~50%. Baits made as described were readily consumed by prairie dogs in the field (Fig. 1C, D) and already have been field tested in all-terrain vehicle-mounted delivery platforms and by the US Fish and Wildlife Service (USFWS) in an unmanned aerial distribution system (USFWS 2016). Mechanized distribution of baits in the field is likely to reduce costs further by replacing ground delivery on foot.

Palatable and homogeneous vaccine baits produced affordably in large quantities could be useful in combating wildlife diseases in a variety of species. More accessible vaccine bait formulation and manufacturing methods hopefully will facilitate broader access to oral vaccination for wildlife health management.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2017-01-013.

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