Oral Vaccination and Population Management Focused on Juvenile Golden Jackals Halts a Rabies Epizootic in Israel

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ABSTRACT

Wildlife rabies has been well controlled in Israel due to regular oral rabies vaccine (ORV) campaigns targeting the primary rabies reservoirs, red foxes (*Vulpes vulpes*) and golden jackals (*Canis aureus*). During 2017, a rabies outbreak was detected in golden jackals in the Jezreel and Hama'ayanot Valleys covering approximately 500 km^2 area in Israel's Northern District. From October 2017 to March 2018, 68 of 93 (73%) reported rabies cases were golden jackals and the majority were juveniles of less than 1 year of age. Unusually high jackal population densities in the region (>80 animals/km^2), with a large proportion of juveniles born after the autumn ORV campaign, fueled the outbreak to a peak of 19 reported cases during January 2018. Two high-density ORV campaigns (one during October 2017 and another during March 2018) targeted jackal habitat with bait densities up to 200 baits/km^2. In addition to routine November and January baiting cycles, additional ORV baits were deployed during the summer months (July-October) to increase bait uptake by the unvaccinated juveniles. Due to an abundance of aquaculture in the area, bait acceptance studies were performed to confirm sufficient vaccine uptake. As high ambient temperatures conditions are common in the outbreak area, thermostability studies were conducted prior to these campaigns to determine whether the vaccinia-vectored recombinant oral vaccine was suitable for use under extreme weather conditions. Geographically and demographically targeted ORV distribution, paired with population control through focused culling, followed by enhanced rabies surveillance and rapid laboratory testing of suspect wildlife cases, contributed to rapid outbreak control by late March 2018.

Keywords: Rabies; *Canis aureus*; Oral Rabies Vaccination; Bait Acceptance; Thermostability, Vaccine Efficacy; Field Effectiveness; Focused Culling.

Abbreviations

ORV – Oral rabies vaccine/vaccination
RFITT – Rapid Fluorescent Focus Inhibition Test
DFAT – Direct Fluorescent Antibody Testing
$TCID_{50}$ – Tissue Culture Infectious Dose 50%
GPS – Global Positioning System

INTRODUCTION

The epidemiology of rabies in Israel is complex and involves a range of interacting animal species that differ in their behavior, ecology, and potential for contact with humans (1,2). Domestic dogs and wild carnivores – primarily red foxes (*Vulpes vulpes*) and, to a lesser extent, golden jackals (*Canis*

Figure 1. Number of domestic dog and wild carnivore rabies cases reported by five-year periods – Israel, 1960-2019

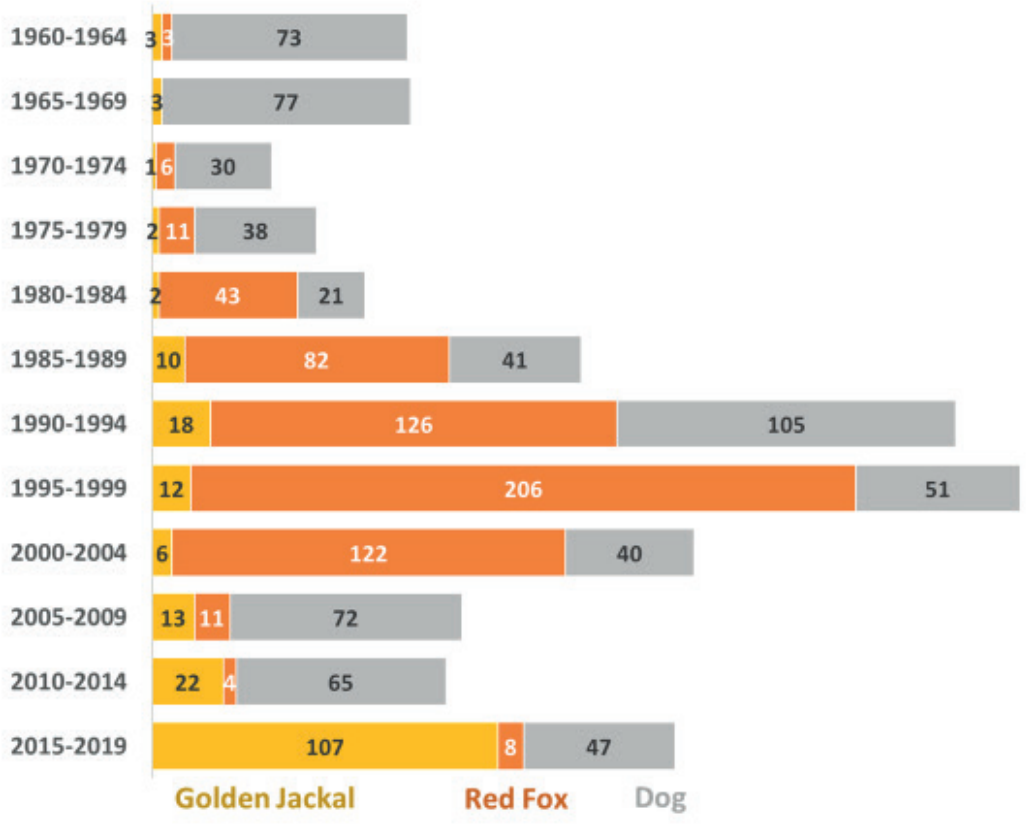

Figure 1 shows the number of rabid domestic dogs and wild carnivores (golden jackals and red foxes) reported in Israel from 1960-2019, summarized by five-year periods. During recent years (1990-2019), these three species comprised the majority of confirmed animal rabies cases in Israel (median annual percentage of cases attributed to dogs, foxes, or jackals = 78%, range 47%-100%; see also Figure 2).

aureus) –have historically comprised the majority of annual animal rabies cases reported in Israel (from 1990-2019, median of 78% of reported cases annually, with a range of 47%-100%). From 1960 to the early 1990's, red foxes were the most reported wildlife rabies cases (Figure 1) (2). During this time, golden jackals comprised a median of 3% (range 0-12%) of annual rabid animal reports (Figure 2).

In response to increasing rabies case counts and three human rabies deaths, Israel instigated an oral rabies vaccination (ORV) program during 1996 using a recombinant poxvirus oral rabies vaccine (RABORAL V-RG®, Boehringer-Ingelheim, Duluth, Georgia, USA). The immunization of wildlife by oral rabies vaccination (ORV) baiting was based on the success of preliminary bait acceptance trials in the field (3) and preliminary safety and efficacy studies in target and non-target wildlife species (4-10). Oral rabies vaccine efficacy and safety studies in captive foxes and non-target species were conducted before launching a large-scale ORV program with a baiting strategy designed to target red fox and golden jackal lifecycles and ecology (4, 11, 12, 13).

Within five years of implementing ORV for wildlife rabies control, wildlife case reports had declined substantially. From 2012 to 2016, small numbers of wildlife and domestic animal rabies cases were detected annually (Figure 2). Most of these cases were detected near borders and were attributed to incursion of infected animals from neighboring countries, as evidenced by geographic location and molecular genetic analysis of viral isolates (14-17).

Despite continuous and sustained ORV program effort, during 2017-2018 a large-scale rabies outbreak in golden jackals was detected in the Northern District (74 cases over 6

Figure 2. Number of golden jackal, red fox, dog, and other animals (domestic and wildlife) rabies cases reported by year – Israel, 1990-2019

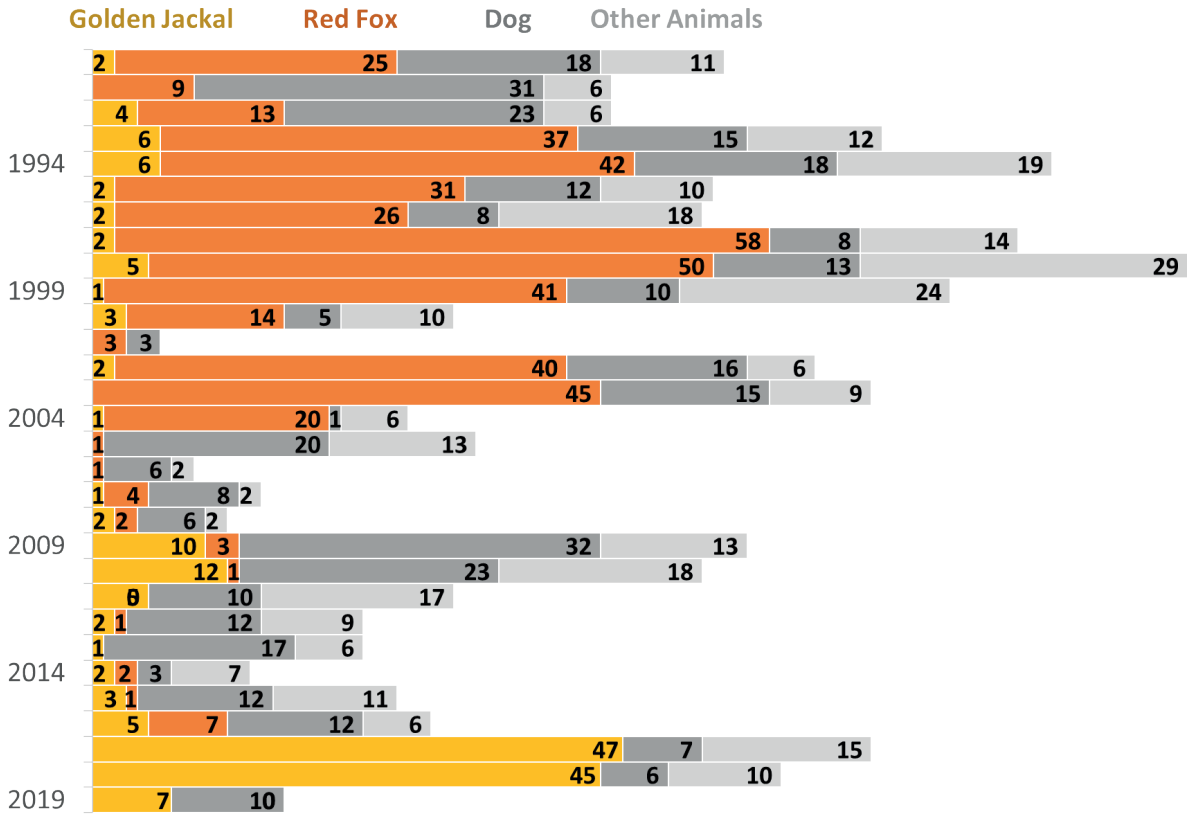

Figure 2 shows the annual number of rabid jackals and other rabid animals (domestic and wildlife species) reported in Israel from 2000-2019. During 2017-2018, rabid jackal case reports increased dramatically due to a rabies virus incursion near the border with Jordan in the Israel's Northern District.

months in an area of 850 km^2 originating in a valley adjacent to the Jordanian border).

This paper describes the epidemiology of this rabies incursion into the jackal population of this region of the Jordanian border and documents a successful outbreak response that included intensive, targeted ORV campaigns supplemented by significant focused culling.

MATERIALS AND METHODS

Study Site and Routine Rabies Surveillance

The Emek Hama'ayanot, Hagilboa and Jezreel Valley regional authorities are situated in Northern Israel and comprise roughly 850 sq. km in size of inland area known for its agricultural potential, mostly cultivated with field crops, orchards and fishponds, the latter of which jackals often use as a food source. The Lower Galilee region borders the area from the north, the Mount Carmel range from the west, the Samarian highlands from the south and the Jordan Valley to the east. The regional authority of Emek Hama'ayanot encompasses 24 settlements, including 16 kibbutzim and 6 moshavim settlements and 2 community villages. The regional authority of Hagilboa encompasses 33 settlements including 14 moshavim, 8 kibbutzim, 6 settlements and 5 Arab villages. The regional authority of Jezreel Valley encompasses 38 settlements in the Jezreel Valley, including 15 kibbutzim, 15 moshavim, and 6 community settlements, and 2 Bedouin villages. The city of Beit She'an lies in the center of the territory is an independent municipality. The total population of the three councils is approximately 88,000 people (268 people/sq. mi, or 104 people/sq.km).

The area has approximately 680 ruminant holdings containing approximately 65,000 cattle and 45,000 sheep and

goats. Most of the holdings do not have fencing to exclude wild carnivores. Most ruminant owners do not vaccinate their herds against rabies, as this vaccine is not mandatory. Approximately 12,000 microchipped dogs that were vaccinated against rabies have been registered in this region. Approximately 70% of these owned dogs have been spayed (82%) or castrated (55%). A relatively large number of unvaccinated feral animals, mostly cats and dogs are also present in the region.

Routine rabies surveillance in Israel is performed in the Rabies Diagnostic Laboratory at the Kimron Veterinary Institute in Bet Dagan, Israel, which is recognized as a OIE reference laboratory. Israeli Nature and Parks Authority rangers or municipal and state veterinary office staff collect clinically suspected or dead wild animal reported by citizens for passive surveillance. Brains submitted for routine rabies diagnosis were tested by Direct Fluorescent Antibody Test (DFAT; World Organization for Animal Health, 2018) (18). Each positive sample was then confirmed by virus isolation and PCR analysis (World Organization for Animal Health, 2018). Viral sequences were routinely compared to sequences of rabies viruses present in Israel and neighboring countries to infer the likely origin of the virus detected (15). Wildlife collected for active surveillance were also tested for consumption of the ORV baits by analysis of bone for the presence of tetracycline biomarker (3). When possible, serum was also collected for rabies antibody testing using Rapid Fluorescent Focus Inhibition Test RFFIT (18).

Target Species

Golden jackals are mid-sized (8-12kg), coyote-like canids that have a broad geographic distribution across North and East Africa, Southeastern Europe, and South Asia extending to Burma (Ivory, 1999) (19). The geographic distribution of golden jackals in Europe has expanded to some central European countries as well. Dispersing animals have been observed further to the North and West (e.g., Denmark, Germany, Poland, Switzerland, The Netherlands; as reviewed in Fenton *et al.*, 2021, (20), according to Magory Cohen *et al.*, (2013) (21). In Israel, golden jackals are found throughout Israel and have relatively recently expanded into previously uninhabited territories in both the north and south (21).

Golden jackals mate in monogamous pairs and in Israel typically give birth to 3-8 pups during February-March. Juveniles typically wean at 50-90 days of age and disperse from their birth territories during the fall. Based on research by Blasco *et al.*, 2001 (22), red foxes demonstrated loss of maternal rabies immunity after weaning (22). Therefore it is likely that dispersing juvenile golden jackals also lack protection against rabies derived from maternal vaccination by ORV. Golden jackals are considered juveniles until sexual maturity at approximately 11 months old. They form breeding pairs during the early spring of their second year. Golden jackals have a life expectancy of 8-9 years in the wild (19).

Golden jackals are omnivores, have a diet that can include human and agricultural refuse, which has allowed for dramatic population growth and expansion into human-dominated landscapes in some areas of the Middle East and Europe. Access to these anthropogenic resources has released jackal populations from natural constraints on carrying capacity (23, 24). The resulting high-density populations of jackals living near agriculture and urban centers has resulted in human-wildlife conflict, particularly because of the economic impacts of livestock predation (23, 24, 25).

Vaccine Efficacy

As background information for this report, during 1997-1999, a captive rabies challenge study and a pilot field trial were conducted to compare the efficacy and field effectiveness of two commercially available ORV in jackals at Kimron Veterinary Institute, Bet Dagan, Israel. The caged study evaluated the efficacy of a vaccinia-vectored recombinant oral rabies vaccine (RABORAL V-RG®, $10^{8.0}$ $TCID_{50}$/dose manufactured by Merial, now owned by, Boehringer Ingelheim, Athens, GA, USA) to (SAG2, RABIGEN®, $>10^8$ $CCID_{50}$/dose, Virbac, Carros, France) in golden jackals. While some findings of this study have been mentioned in the literature previously (9), the full details of the captive RABORAL V-RG vaccine efficacy trial have not been published to date. We include details of the RABORAL V-RG challenge trial in this report to document Israel's previous experience with the use of this product in golden jackals in order to add information for future research or experimental use. RABIGEN results are available by request from the corresponding author.

Challenge virus dose study.

A pilot study was performed prior to the challenge study to determine the proper challenge dose of a dog rabies virus isolated during 1997 from the local jackal population.

The virus was identified and typed using both monoclonal anti-rabies antibodies and sequencing. The challenge virus was produced in suckling Swiss mice in 50% rabbit sera and was stored at -70° C, until use (26). The initial challenge virus suspension titer of 10^4 median tissue culture infectious dose (TCID) per microliter (ml) was determined by serial 10- fold dilutions in mice according to the Reed & Muench method (Brown, 1964) (27).

The rabies virus titration challenge used three groups of four wild-caught jackals each and one group of controls (n=4). Free-ranging adult golden jackals (n=15) were trapped during February-March 1998 in central and northern Israel using leg hold traps as jackals avoid box traps. The animals were sedated and transported to quarantine, where they were held in individual cages. The animals were fed a mixture of commercial canned and dry dog food and water was provided *ad libitum*. After seven days of acclimation, animals were sedated using ketamine hydrochloride (10mg/kg; Ketaset, Zoetis, Kalamazoo, MI, USA) and xylazine hydrochloride (1mg/kg; Rompun, Bayer Healthcare, Animal Health, Shawnee Division, Shawnee Mission, KS, USA) administered intramuscularly by pole syringe. Anesthetized jackals received a complete veterinary examination, cephalic blood was sampled for baseline bloodwork, and vaccination against canine distemper, parvovirus, canine hepatitis, parainfluenza, and leptospirosis.

Each jackal received 1.0 ml volume of virus inoculated into the temporal muscle of 630 $TCID_{50}$/ml (4 jackals), 2000 $TCID_{50}$/ml (4 jackals and 4 foxes), and 6300 $TCID_{50}$/ml (4 jackals). The four control jackals and one control fox were inoculated with non-infected mouse brain suspension. Back titration of the challenge virus was also performed in mice. The inoculated jackals were observed clinically each day from Day 1-14 and twice a day from Day 15 until death. From Day 10 after inoculation, saliva was collected from each animal by cotton swab and individually frozen for subsequent testing for rabies virus, by virus isolation in tissue culture and reverse-transcription PCR (RT-PCR).

Vaccine efficacy study.

Twenty-one golden jackals were captured during the fall of 1998 to test RABORAL VR-G efficacy in this species. Jackals were acclimated to captivity and screened for medical concerns as described previously. After acclimation, eleven jackals received a RABORAL V-RG and 10 jackals were included as unvaccinated controls. Consumption of vaccine bait was documented by video recording and the vaccine capsules were collected afterward to measure the amount of fluid left. Nine jackals were given oral baits for voluntary consumption and two received a direct squirt of oral installation of vaccine. Two animals died during the study from a non-specific infection that did not appear to be related to the study, therefore, total samples sizes varied through the follow up period (10 jackals at 30 days after vaccination and 9 jackals at 150 days). At 160 days post vaccination, all jackals were challenged with a 1.0 ml injection in the temporal muscle of the same jackal-origin rabies virus suspension (2000 $TCID_{50}$/ml). The procedure was carried out under sedation using the previously described medication protocol. The animals were then monitored daily over a 180-day period to detect development of signs of rabies.

All jackals were tested for the presence of rabies serum neutralizing antibodies prior to vaccination, one and six months after vaccination, and two months after the challenge, by RFFIT (28-29). Animals that did not die from rabies were humanely euthanized at the end of the study and all subjects were necropsied. Blood was collected from the heart cavity for serology, brain and spinal cord samples were collected for virus identification by DFAT, RT-PCR, and histopathologic and immunohistochemical testing. Samples of femur bone were collected from each animal and cut into 50-75 µm slides for examination using a fluorescent microscope, to assess oral uptake of the tetracycline biomarker included in the vaccine bait matrix (Johnston *et al.*, 1987) (30).

Field Effectiveness

In parallel to the rabies challenge study to demonstrate ORV safety and efficacy in jackals, an ORV program was initiated in Israel during the fall of 1998 to control rabies in both red foxes and jackals. While multiple ORV products are labelled for use in fox species, there was currently no oral vaccine licensed specifically for immunizing jackals. Israel's ORV program primarily used RABORAL V-RG and also used a modified live attenuate rabies virus vaccine (RABIGEN SAG-2), both applied off-label when vaccination efforts included jackal populations. Safety, efficacy and field effectiveness of oral rabies vaccination has been demonstrated in jackal species for both vaccines used in Israel (4,5,6,7,8, 9, 10, 31).

Israel's ORV bait distribution parameters were devel-

Figure 3. Number of golden jackal rabies cases reported in the Northern District by month – Israel, 2017-2019

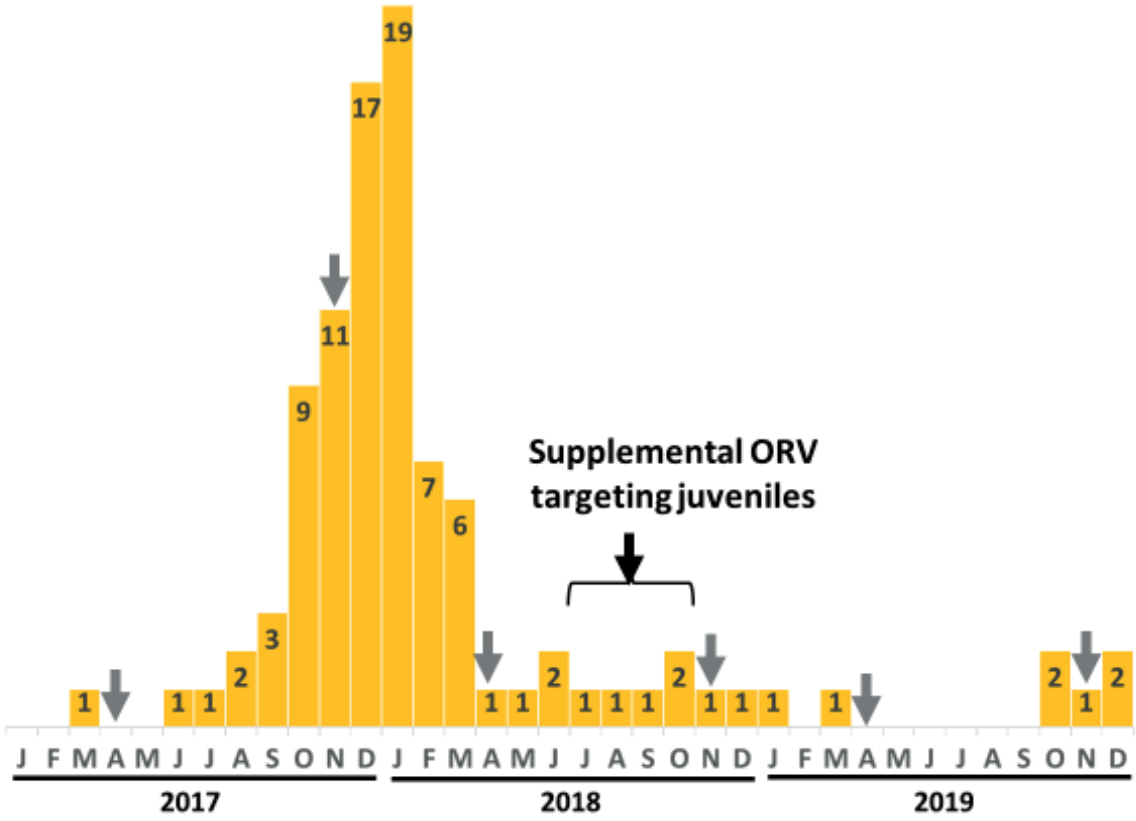

Figure 3 shows the number of rabid jackals reported in the Northern District of Israel during 2017-2019. Arrows indicate timing of oral rabies vaccination (ORV) campaigns. Grey arrows indicate the timing of routine ORV distribution, and the black arrow indicates an intensive targeted ORV distribution to prevent resurgence of rabies in juvenile jackals.

oped to match both red fox and golden jackal lifecycles and ecology in the region. Distribution began in the northern part of the country and moved south over time to encompass 21,000 km^2, almost all the inhabited areas in Israel and Judea and Sumeria (4, 12, 13, 32). Oral rabies vaccine baits were distributed primarily by airplanes or helicopters, supplemented by ground distribution by hand, either twice a year (during November and April from 1998-2004) or once a year (during November from 2005-2017). Distributions were scheduled to prioritize immunization of juvenile pups soon after dispersal from their natal dens. Bait distribution was reduced to one campaign per year during 2005 due to substantial reductions in wildlife rabies case counts that persisted until the outbreak described herein. Since 2011, the Israeli ORV project had included ORV bait distribution along the border with Jordan, a primary site for incursion of rabid wildlife and dogs (15). Aerial distribution over uninhabited areas in a density of 14-19 baits/km^2 (36-50 baits/mile^2), using planes or helicopters that fly in parallel lines approximately 300 meters apart as directed by global position system guidance from predetermined polygons. In high-risk border areas and in hot spots of high jackal population density, a higher baiting density of up to 50 baits/km^2 (130 baits/mile^2) was used. Approximately 5% of total baits were distributed by hand near urban areas. Tetracycline deposition in bones as a biomarker for bait consumption was checked as described previously by Linhart, *et al,* (3).

Outbreak Response

A considerable ORV distribution, using bait densities up to 300 baits/km^2, was implemented in response to the severe

outbreak of rabies in jackals during 2017-2018. Baits were distributed during October 2017, and again during April 2018, November 2018 and April 2019 (Figure 3).

Mathematical modeling of rabies control approaches conducted in Southern Israel suggested higher effectiveness of ORV campaigns when matching bait distribution density to target reservoir density in desert environments (33). Baiting densities ranged from 29-80 baits/sq. km (75-207 baits/sq. mi) and were matched to estimated jackal population densities in the outbreak area, with the highest bait densities in the valleys where jackal densities and transit were highest, including a belt along the Israel-Jordanian border.

Due to the timing of the outbreak and the swift increase in rabid jackal incidence, an immediate rabies control response was implemented. In addition to ORV distribution twice annually at higher bait densities in the outbreak area, a focal ORV campaign was performed during the summer of 2018 to ensure rabies transmission did not re-emerge in juveniles missed by the spring campaign. Baits were distributed also in the evenings during August through October of 2019, to ensure that juveniles born during the spring had sufficient bait uptake opportunities to prevent resurgence of rabies virus transmission. Aerial and manual distribution of the bait was carried out in the late afternoon to minimize bait exposure to direct sun light and high temperatures. Due to high numbers of wild carnivores especially jackals in targeted areas, up to 85% of the baits were consumed each night following distribution as estimated by manual bait placement and monitoring over an 18-hour period in the distribution areas. Motion-triggered cameras were used to monitor bait uptake. A total of 620,000 additional vaccine baits were distributed by air and 30,000 by local municipality veterinarians on the ground in areas near urban centers.

Oral vaccination was paired with a focused population reduction strategy. A total of more than 6,000 jackals were culled by hunting and a subset of these animals were sampled 3-4 weeks after bait dispersal to monitor sufficiency of bait distribution to immunize the susceptible population of jackals by measuring bone tetracycline biomarker detection and rabies antibody serology. Lower mandibles from 461 jackals and 23 foxes were tested for the presence of tetracycline hydrochloride-specific fluorescence using a standard protocol (34). Blood samples were drawn by intra-cardiac puncture of 2 of the red foxes and 103 jackals sampled for biomarker surveillance from the vaccination target areas. Sera were stored at -20°C, until tested by rapid fluorescent focus inhibition test (RFFIT) (18).

In addition to the ORV campaigns and the focused culling to reduce rabies virus spread in wildlife, public outreach campaigns educated the public about rabies in residential and farming areas and about control and prevention measures, with an emphasis on post-exposure treatment to prevent human deaths. Communication was carried out through local and national TV, radio and internet. Information was also distributed using community internet resources. Communication messages raised awareness about rabies and provided guidance to avoid contact with wildlife or domestic animals that were behaving abnormally, to treat wounds appropriately immediately after a suspected exposure, to seek medical attention including post-exposure prophylaxis if contact with such animals occurred and to keep pets updated on vaccinations. The Ministry of Agriculture and Rural Development provided special support to farmers for livestock vaccination and to municipalities to identify previously unvaccinated pets to microchip, register and vaccinate against rabies. Free booster vaccinations were provided to all dogs that were vaccinated only once or were more than one year beyond receipt of their last rabies vaccination.

Thermostability

Thermal and mechanical stability of ORV vaccines and baits are important criteria for wildlife vaccination programs. Prior to implementation of wildlife ORV programs in Israel, thermostability of various wildlife rabies vaccines were tested across a relatively wide range of storage and field conditions (7, 12,35, 36), but prior test conditions did not include the environmental temperature extremes potentially encountered during this outbreak response. As part of ORV distribution targeting juvenile jackals to address this outbreak, a thermostability experiment was performed to assess the effect of bait distribution in hot habitats during the summer months on vaccine titer.

Thirty baits each of three ORV products (RABORAL V-RG and two modified-live attenuated rabies oral vaccines) were evaluated for vaccine titer and bait integrity. The doses were placed inside a wire cage to prevent disturbance by animals. The experiment was conducted for 6 days during May 2018 at Bet Dagan. Vaccines were in place before sunset and samples were collected for vaccine titer measurements

beginning at 7:00 am the following day (12 hours after placement) and subsequently approximately every 24 hours over 3 additional days. At each sampling, five baits were removed from the cage and stored frozen for analysis. Temperatures of the air, soil, and baits were measured over the course of the experiment using thermologgers. One thermologger was destroyed by presumed rodent damage, resulting in a gap in the temperature monitoring due to erroneous data point removal.

Vaccine titers were measured using standard titration methodology (Reed and Muench, 1938) (37). Briefly, a mouse neuroblastoma cell line suspension containing $5x10^4$ cells/ml was prepared in Eagle's minimal essential medium (EMEM-10) containing 10% fetal calf serum supplemented with antibiotic (Pen-Strep Amphotericin Solution, Biological industries 03-033-1B) at 1% concentration. Cell concentration was determined using a Neubauer counting chamber with a calibrated grid using the manufacturer's instructions (Saaringia, Germany). Two hundred microliter aliquots of the cell suspension were placed in each well of an 8 well chamber (LabTek, Nunc) microtiter plates for vaccine virus titration and plates were incubated at 37˚C (±0.5˚C) in an atmosphere of 0.5% CO_2 for 5 days.

For each vaccine sample, a ten-fold serial dilution was created by mixing bait contents with Dulbecco's modified Eagle medium without fetal calf serum to create a range of dilutions from 10^{-1} to 10^{-12}. Original baits stored under refrigeration condition served as control. One hundred microliters of each dilution were distributed per well with six replicates per dilution. Six wells were left empty of vaccine dilution to serve as negative controls. Cells with vaccine dilutions were incubated for five days at 35˚C (±2˚C) in an atmosphere of 5% CO_2.

After incubation, wells were observed, and a qualitative reading was recorded; wells showing lysis of the cell layer were recorded as positive and cells showing no lysis were recorded as negative. Log_{10} median tissue culture infectious dose ($TCID_{50}$) were then calculated using CombiStats software (38, 39) using the Spearman Kärber formula by means of the neoprobit graphic method (38).

Bait acceptance

Due to the presence of aquaculture ponds in the outbreak area and common knowledge that jackals use these ponds as a primary food source, studies were conducted to evaluate fishmeal bait uptake by jackals. Baits were distributed along five transect lines of 1-5 km in length and having 9-14 checkpoints. Ten trail cameras were placed, 2 per transect line to allow identification of species consuming the baits.

Each checkpoint was marked with a tape, and precise bait positions were recorded using the Global Positioning System (GPS). The distances between the points in lines 1 & 2 were 50 meters, and between lines 3-5, about 100 meters. Bait acceptance was observed each morning. Other observations included puncture of the vaccine container. Bait uptake studies were also performed in a remote area for comparison.

Data analysis and visualization

Historical rabies case count data were obtained from Kimron Veterinary Institute from B. Yakobson. Contemporary data are available on the Israel Ministry of Agriculture and Rural Development website (39) and from the World Organization for Animal Health's World Animal Health Information System database (40). Data were compiled and graphed using Microsoft Excel. Basic statistics, including Fisher's exact test to compare proportions and Pearson's correlation to assess association between variables, were performed in R (version 3.6.1) statistical language (41).

RESULTS

Vaccine efficacy

Challenge Virus Dose Study. One of four experimental foxes used to determine the appropriate dose of challenge virus suspension titer for challenge dosing died five days after virus inoculation from a non-specific illness. No rabies virus was detected in the brain by DFAT or PCR. The median incubation period of the other three foxes challenged with rabies was 27 days (range: 22-31 days) and death occurring at 28-39 days (median 31 days) after virus inoculation.

As for the titration of the challenge virus inoculated, jackals died from rabies within a median incubation period of 42 days (range: 24-58 days), median duration of illness of 4 days (range: 2-11 days), and median number of days to death of 47 days (range: 26-66 days). Length of incubation period was dose dependent with a median incubation period of 50 (range: 31-58), 47 (range: 24-58), 38 (range: 27-49) days corresponding to 630, 2000, 6300 TCID/ml. Jackals in the highest dose group had a slightly lower median time to death, 42 days (range: 37-56) compared to

52 days (ranges: 37-61 days for the lowest dose and 26-66 days for the middle dose). The length of clinical disease once signs were noted did not differ substantially based on inoculum titer. The control group of one fox and three jackals remained healthy throughout the observation period. All animals showed similar DFAT fluorescence intensity and distribution viral inclusions in brain smears regardless of the inoculation titer. Virus isolated from animals that died during the experiment was molecularly identified as the inoculation virus. Virus was also identified by PCR from all salivary glands. All jackals were negative for rabies virus neutralizing antibodies before the challenge. Antibodies were detected in five of 12 inoculated jackals upon post-mortem examination.

Vaccine Efficacy Study.

Rabies virus neutralizing antibodies were detected at a dilution of 1:5 or higher at 30 days after vaccination in a portion of vaccinated jackals (30.0% of 10 jackals). Antibody detection was slightly higher at 150 days after vaccination (44.4% of 9 jackals).

All jackals in the unvaccinated control group died within 17-45 days of rabies virus challenge (median: 27 days). Two of the nine jackals that received RABORAL V-RG died from rabies on day 26 and day 27 after challenge (both were individuals offered vaccine baits for voluntary consumption). At 30 days after rabies virus challenge, rabies virus neutralizing antibodies were detected in almost all vaccinated jackals tested (7/8 jackals, one sampled at time of death on day 26; the second jackal that died of rabies was not sampled) and in some unvaccinated controls (4/8). Rabies virus identical in sequence to the challenge virus was isolated from all dead animals.

Field Effectiveness

Since the onset of oral vaccination activities in Israel beginning in 1998, annual bait acceptance in the vaccination zones has been evaluated by tetracycline biomarker detection in about 52% of bone samples of target animals (primarily jackals and foxes) submitted for testing. Vaccine bait consumption and induction of immunity in animals collected from the vaccination zones were also reflected by an average annual seroconversion percentage of less than 20% and substantial declines in wildlife rabies reports after ORV distribution began during 1997 (Figure 1).

During 2017, the proportion of jackals that consumed baits as detected by biomarker was lower (43% positive animals from 98 samples). During 2018, field samples collection performed in response to the outbreak-detected biomarker in 61% of 461 animals sampled. Blood samples for seroconversion detection, were taken from 103 of the jackals, and 16% were positive.

Outbreak response

During 2017, one rabid dog (May 17$^{th.}$) was diagnosed in proximity to the Jordanian border. On June, 8, one rabid jackal was found adjacent to the Jordanian border and then again on October 8, furthermore, one additional rabid jackal was detected in the same settlement (Hamadiya) and another in a close settlement. These early cases marked the beginning of the intensive outbreak as described previously. Molecular analysis of the isolates showed 100% similarity among them, and a marked difference from the other isolates that were found along the Lebanese border in the north, belonging to a different subtype. Within two weeks, on October 22, spillover into cattle had already been observed.

During October 2017 to March 2018, 68 of 93 (73%) reported rabies cases were golden jackals from an area of about 850 km^2 in the previously described area in the Northern District of Israel. Cases rose rapidly from October 2017 peaking at 19 rabid jackals reported during January of 2018 before declining through the spring after two ORV distributions (Figure 3). The majority (68/93 or 73%) of rabies cases reported during the first six months of this outbreak (October 2017 to March 2018) were jackals from a 500 km^2 area of the Hamaynot and Jezreel Valleys. Most of which were juveniles <1 year of age that may not have been immunized during the spring ORV distribution and were found negative for tetracycline on bone examination. Due to the timing, maternal immunity would not be expected to be a protective factor either. Rabies cases occurred in an area that was overpopulated by jackals, estimated roughly at about 50 jackals/sq. km.

In total, 91 jackals were diagnosed positive during 2017-2018 with 68 identified in the approximately 850 sq. km area of the outbreak in both areas and 23 identified outside of the outbreak zone near the Lebanese and Syrian borders. Cases identified within the outbreak area differed on molecular analysis from those identified along the northern border area. During the same period, 16 cattle, 4 dogs, 2 cats

Figure 4. Thermostability of a recombinant poxvirus rabies vaccine (V-RG) exposed to ambient temperatures during May in Northern Israel

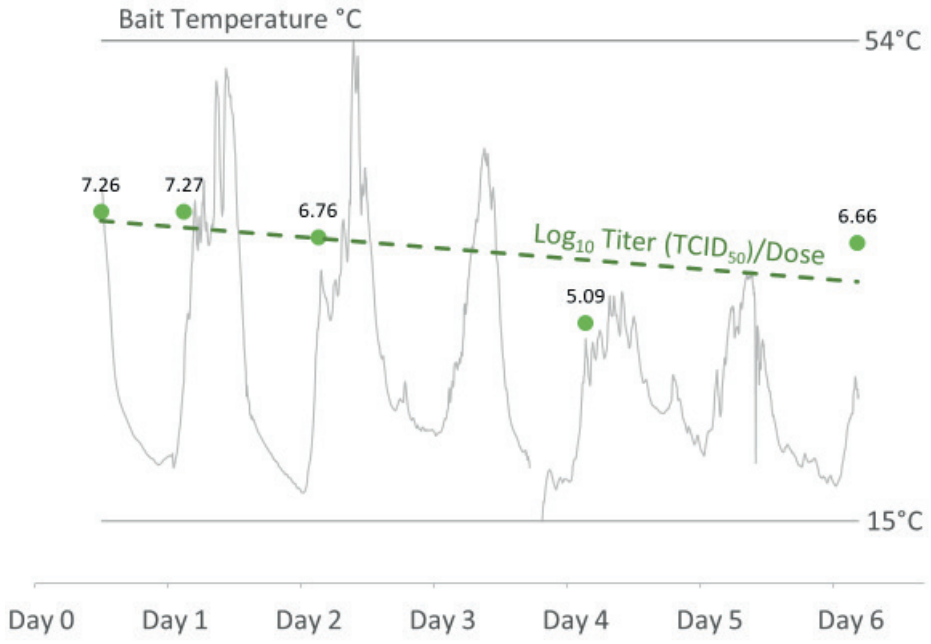

Figure 4. Shows decline in RABORAL V-RG® vaccine titer (green dots) compared to daily temperature variation (light grey) over a six-day period of vaccine bait exposure in the field during summer in the Northern District of Israel.

and one sheep were diagnosed with rabies in the outbreak zone and 5 cattle, 8 dogs, 1 sheep and 1 badger were diagnosed with rabies along the Lebanese and Syrian borders.

During 2019, after the outbreak response, only 17 rabid animals were reported throughout Israel, the majority (10/17 or 58.8%) of these being rabid dogs detected along the northern border (39). Seven rabid jackals were detected during 2019, sporadically distributed through the year and with no evidence of resurgence in sustained transmission (Figure 2, Figure 3). During 2020 and 2021, the number of rabies cases increased, but they were restricted to the proximity of the Lebanese border in the north. No new cases have been diagnosed associated with the previous outbreak as of June 4, 2022 (39).

Thermostability

Vaccine baits were exposed to a wide range of temperatures over six consecutive days in Bet Dagan. Baits and sachets were patent without any visible damage throughout the course of the experiment. Vaccine titers were measured prior to placement (Day 0) and then samples for titer measurement were collected after 14.92 (Day 1), 39.17 (Day 2), 87 (Day 4), and 136.42 (Day 6) hours of environmental exposure. Air, soil, and bait temperatures showed similar cycles throughout the experiment with bait and soil temperatures reflecting changes in air temperature. Mean ambient (air) temperature over the entire study period was 25.6˚C (range: 15.7˚C-49.2˚C). Mean soil and bait temperatures over the entire study period were slightly higher than air temperatures (soil: 27.8˚C, range 17.8˚C-48.3˚C and bait: 27.2˚C, range 15.1˚C-54.2˚C).

At the first field sampling time (15 hours after placement the previous evening), bait temperature had been at or above 25˚C for less than a quarter of the exposure period (3.58/14.92 hours) and above 40˚C for 3% of the exposure period (0.42/14.92 hours). This contrasted with subsequent samples that were collected after one or more full day-night cycles. At subsequent sampling times, vaccine temperature was at or above 25˚C for over 40% of the exposure time, between sampling periods and vaccine temperature was at or above 40˚C for nearly 10% of the exposure time.

During the first two days of exposure, RABORAL V-RG vaccine titer declined by 0.5 log (Figure 4; Table 1). The largest decline in vaccine titer was measured on the fourth day (2.17 log reduction). While longer environmental

Table 1: Average vaccine titers of a recombinant poxvirus rabies vaccine (RABORAL V-RG) and two other oral vaccine exposed to ambient temperatures during May in Northern Israel

Vaccine	Log Vaccine Titer ($TCID_{50}$)/Dose					Pearson's Correlation		
	Day 0	Day 1	Day 2	Day 4	Day 6	t	r2	P
Raboral V-RG*	7.26	7.27	6.76	5.09	6.66	-1.32	0.37	0.278
ORV 2	6.24	6.21	4.02	3.20	1.00	-6.71	0.94	0.007**
ORV 3	6.25	6.22	5.10	4.46	2.05	-5.69	0.92	0.011**

* The Day 6 value is a possible influential outlier by Cooks Distance. Pearson's correlation for Day 0-Day 4: t=-7.19, r^2=0.96, p=0.019)**
** Significant at alpha<0.05

exposure studies have used more complex models for assessing the decline of vaccine titers (for example, a Weibull distribution; Hermann *et al.,* 2011) (42), the short duration of this study fits within the initial linear period of decline typically observed in longer studies. A simple linear regression line fit to the change in vaccine titer over time was not significant when all sampling points were included (Pearson's Correlation: p=0.278, r^2=0.37), but was statistically significant when the possible outlier measurement was removed (Table 1; Pearson's Correlation: p=0.019, r^2=0.96). The slope of the latter estimated regression line was more like that observed for other vaccines studied when the day 5 sample was omitted (Figure 4). Based on this correlation, there was an imputed 0.97 log titer loss over the 6 days of exposure. The expectation for most ORV programs is for most vaccine doses to be consumed within three to five days post-distribution was the reason for ending the study on Day 6.

Bait Acceptance

The rate of bait consumption was found to be average of 97% monitored baits were consumed during the first night, and the rest were removed during the second night. We managed to find 31 ampoules afterwards, and four of them were not punctured (13%).

In the other area, that was not so rich with food sources, the removal rate was only 60%. The trail cameras showed that 67% were consumed by jackals and the rest were removed by rodents, mainly rats.

DISCUSSION

Rabies control in the Middle East can be challenging due to environmental and political factors that limit effective regional surveillance and control of rabies in wildlife and free-ranging dog populations. Transboundary migration of reservoirs incubating rabies virus is one of the main barriers to rabies control in Middle Eastern countries with established wildlife rabies control programs (43). In recent years, golden jackals have become an emerging rabies reservoir species in this region. Urbanization and agricultural development of rural areas followed by poor sanitation, had increased food resources in the region leading to higher carrying capacity for wild canids (10).

Since 1998, Israel has used ORV to control rabies in wildlife populations (4-10, 44). In recent years, migration of rabid animals, particularly dogs, from neighboring countries has been a primary threat to rabies control at the borders of Northern Israel (14, 15, 44, 45). Geographic features such as mountain ranges or rivers can deter or impede the movement of rabies reservoirs like golden jackals. Open valleys in Israel may permit long-distance animal migration (21, 46). Golden jackals may travel relatively long distances through habitat that is permissive to such migrations (46). Free-ranging dogs have also been reported to travel long distances, sometimes while infected with rabies virus, and thus acting as "super spreaders" by biting multiple targets while migrating (47). Dogs appear to be a major source of transboundary introduction of rabies virus into wildlife populations in northern Israel as well (14, 15, 39, 44).

While the timing of routine ORV campaigns in Israel takes both red foxes and jackal life cycles into consideration to maximize vaccine uptake, the significant increase in jackal population density together with the specific timing of this outbreak contributed to rapid spread of the disease, mainly among juveniles. In Northern Israel, irrigation, the rearing of fish and livestock, and the presence human settlements in areas that were previously rural habitats have provided many artificial food resources to red foxes and golden jackals, elevating the carrying capacity for them, enabling an almost

uncontrolled increase in the population especially in the vicinity of human settlements. Through their semi-natural dispersal, golden jackals have returned to habitats that they use to populate until massive poisoning in the 1960s and expanded also to the Negev areas and along the Dead Sea and the northern "Arava Valley". To survive in desert areas, opportunistic golden jackals have become dependent on anthropogenic sources of food and water (Mendelssohn & Yom-Tov, 1999) (48). Extermination programs in the 1960's justified by the accusation of the jackals as vectors of the rabies, sharply decreased their numbers while today irrigated farmland support increased populations of both red foxes and golden jackals (1, 21, 49).

The climate and topography of Northern Israel present unique challenges for ORV delivery. ORV campaigns are typically timed to accommodate both the target species' ecology and to distribute baits during moderate environmental conditions, typically during the fall or early spring, to preserve vaccine efficacy in the field setting. However, wild carnivore populations that whelp during the spring may experience declining population immunization, even with biannual ORV distribution, due to the addition of young, naïve animals to the population through the summer months as maternal antibodies decline (32, 50).

One of the key observations early on in this outbreak was the unusual number of juvenile golden jackals submitted for rabies testing. Prior to the outbreak, a warm winter followed by abundant spring rains led to high juvenile survival rates. After the fall dispersal, a "voles year" provided plentiful food resources and enhanced jackal pups survival. While adult animals already rendered immune by previous campaigns and would not contract rabies, seasonal influx of susceptible juveniles that were not immunized during the spring campaign resulted in higher infection rates and rapid amplification of the outbreak in this susceptible population. The possibility that because of the abundant fish sources, the baits are not attractive enough was excluded. The baits in this area were taken during the first night while the removal rate in the area without fishponds was only 60%, probably due to lower density of the jackals.

Due to the location and demographics of this outbreak, additional ORV distribution was required under suboptimal conditions for such operation. Oral rabies vaccines must maintain a relatively high vaccine titer under a variety of field conditions for several days to effectively vaccinate the animals that consume them. Oral rabies vaccine distribution during the spring and autumn optimizes the efficacy of the campaign because it exposes vaccines to more moderate environmental temperatures to reduce vaccine titer loss. However, using this distribution schedule in Israel leaves most of the juveniles which whelped during the spring unprotected through the summer months until the next ORV campaign in the fall. To enhance the chances for reduction of rabies virus transmission, ORV was deployed even in this warm habitat during more extreme summer conditions to ensure immunization of young-of-the-year. Evening temperatures were 33°C, and peak daytime temperatures exceeded 40°C in shadow. This approach exposed the vaccine to harsh conditions but also increased the chance of juvenile animals finding the baits and being vaccinated.

As part of this outbreak response, ORV thermostability under these extreme conditions was assessed. RABORAL V-RG and two other commercially available vaccines demonstrated a moderate decline in vaccine titer over the likely period for vaccine uptake. Under milder field conditions, ORV baits have relatively high thermostability across a variety of field conditions retaining adequate titers for effective immunization over days to weeks, depending on variability in maximum temperatures and degree of sun exposure (7, 12, 31, 45, 51, 52). For example, less than 1 log titer was lost over three weeks of field exposure when baits were placed in the shade and 2.2 log titer was lost when baits were placed in direct sun (35).

Evidence of retention of high vaccine titer over three days of exposure to extreme desert conditions, along with strong field evidence of bait uptake and seroconversion in juvenile jackals, suggests that ORV can be used successfully in desert habitat. Strategic distribution, including placing ORV in the evening to reduce exposure to high temperatures during the first day of bait uptake and distributing baits at high density in areas with the highest estimated populations may enhance effectiveness in these difficult habitats. Bait placement at dusk has also been recommended to increase uptake by carnivores over non-target species (Koeppel *et al.*, 2020) (53). Adequate titers for likely effective immunization were observed over the first three days after distribution even at high maximum daily temperatures. While the minimum protective dose has not been established for golden jackals, vaccine titers stayed near the known minimum protective dose for other species during the most likely period for bait

uptake. Previous bait uptake studies have demonstrated that red foxes and various jackal species consume most baits within 24-48 hours of placement (3, 10, 42, 53).

Even if baits are not immediately consumed due to caching behavior of the target species, temperature extremes in caches should be blunted compared to surface exposure, potentially extending the efficacy of vaccines under harsh conditions for weeks to months (51, 52).

Rapid modification of routine bait distribution to target the ecology and behavior of the emerging host, along with targeted efforts to reach juvenile jackals (summer timing, evening distribution to reduce heat damage, higher bait densities in higher animal density locations) to prevent re-emergence of rabies virus circulation effectively quelled this outbreak over a two-year period. To prevent similar future outbreaks, the routine November and January baiting ORV campaign cycles was updated by deploying an additional ORV cycle during the summer months (July-October), aimed to increase bait uptake by the unvaccinated juveniles, after losing maternal immunity.

CONCLUSIONS

A combination of intensive ORV distribution targeting juvenile animals, use of an effective, thermostable oral vaccine, focused population control, and enhanced surveillance and rapid testing and virus typing supported rapid control of an outbreak of rabies in juvenile golden jackals in Northern Israel.

Addressing wildlife rabies outbreaks in real time requires significant resources, expertise and adaptable field actions tailored to the geography and climate of the outbreak area. Due to the continued risk of rabies virus incursion across country borders, terrestrial rabies cannot be eliminated from wildlife populations in Israel without a strong partnerships with neighboring countries to support regional rabies control.

Golden jackals are found throughout the Middle East and jackal populations are expanding into parts of Europe (46, 54). These opportunistic carnivores adapt well to a variety of different environments, including recent adaptation to desert habitat (21). Urbanization and agricultural development of rural areas in Israel have increased food resources in the region leading to higher carrying capacity for wild canids (10, 55). Continued efforts to control rabies in the region will require not only continued investment in ORV and wildlife diseases surveillance, but also measures to control jackals' populations by reducing the area's carrying capacity through sanitation and elimination of artificial food sources.

Golden jackal populations have also experienced a substantial geographic range expansion in Eastern Europe (54, 56, 57), making them a potentially emerging rabies reservoir throughout the region. The approaches used here to quell an emerging outbreak in juvenile golden jackals may inform approaches to controlling rabies outbreaks in jackals species in similarly challenging landscapes and climates in other countries within this expanding range.

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