MONTANA STATE UNIVERSITY

Infectivity and immunogenic capability of Dictyocaulus species from elk and cattle in experimentally infected bovine calves

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Abstract:
The level of protection induced in bovine calves by exposure to Dictyocaulus species from elk and cattle was investigated by subsequent challenge with homologous lungworm larvae. The criteria used for evaluation of protection at challenge were as follows % l) presence or absence of infection; 2) length of prepatent period; 3) duration of patent period; 4) larval output during patent period; 5) changes in respiration; 6) complement-fixing antibody response and 7) histopathology in lung tissues.

Four Holstein calves were inoculated initially with lungworm larvae isolated from elk. Two of these animals, which were immunized when 3.0-months old, were almost completely refractory to challenge with 46,000 homologous larvae 19 months later. The other two Holstein calves, which were immunized when 1.5-months old, developed light patent infections following challenge with 21,000 larvae 5 months later. Compared with the response of the challenge control calf, infections in the immunized calves had a longer prepatent period of 27 vs. 23 days, a shorter average patent period of 30 vs. 43 days, reduced larval production, and showed only transient respiratory distress.

Results of complement fixation tests indicated that calves immunized with elk lungworm demonstrated a secondary response following homologous challenge, suggesting that these animals were primed by this previous exposure. Low antibody titers resulting from immunization were detected when fat-extracted, whole mature lungworm antigen collected from elk was used. Positive results were not achieved when the cattle lungworm antigen was used.

Eosinophilia, lymphiod hyperplasia and interstitial emphysema were observed in lung tissues from the immunized animals at necropsy 2 months post-challenge. Three Holstein calves, which were inoculated with 5,000, 6,000 and 20,000 homologous infective larvae, respectively, developed patent infections that lasted 64, 95 and 55 days. Maximum outputs were 5.0, 9.8 and 45.5 larvae per gram of feces, respectively. Marked respiratory distress was observed during these infections.

One of these calves developed a patent reinfection following homologous challenge with 57,000 larvae 38 days after the termination of the initial infection. The other two animals did not develop patent infections and showed only transient clinical reactions to challenge with 35,000 and 51,000 larvae up to 6.5 months following the end of their initial infections.

In a reciprocal cross infection a captive elk calf, which was inoculated with 24,000 cattle lungworm larvae, developed a patent infection that lasted for 24 days. The maximum larval output was 6.6 larvae per gram of feces.

Results from these preliminary studies indicate that at least partial protection is obtained in cattle against homologous lungworm infections from the use of elk lungworms as an immunizing agent.

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INFECTIVITY AND IMMUNOGENIC CAPABILITY OF DICTYOCAULUS SPECIES FROM ELK AND CATTLE IN EXPERIMENTALLY INFECTED BOVINE CALVES

by

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ABSTRACT

The level of protection induced in bovine calves by exposure to Dictyocaulus species from elk and cattle was investigated by subsequent challenge with homologous lungworm larvae. The criteria used for evaluation of protection at challenge were as follows: 1) presence or absence of infection; 2) length of prepatent period; 3) duration of patent period; 4) larval output during patent period; 5) changes in respiration; 6) complement-fixing antibody response and 7) histopathology in lung tissues.

Four Holstein calves were inoculated initially with lungworm larvae isolated from elk. Two of these animals, which were immunized when 3.0-months old, were almost completely refractory to challenge with 46,000 homologous larvae 19 months later. The other two Holstein calves, which were immunized when 1.5-months old, developed light patent infections following challenge with 21,000 larvae 5 months later. Compared with the response of the challenge control calf, infections in the immunized calves had a longer prepatent period of 27 vs. 23 days, a shorter average patent period of 30 vs. 43 days, reduced larval production, and showed only transient respiratory distress.

Results of complement fixation tests indicated that calves immunized with elk lungworm demonstrated a secondary response following homologous challenge, suggesting that these animals were primed by this previous exposure. Low antibody titers resulting from immunization were detected when fat-extracted, whole mature lungworm antigen collected from elk was used. Positive results were not achieved when the cattle lungworm antigen was used.

Eosinophilia, lymphoid hyperplasia and interstitial emphysema were observed in lung tissues from the immunized animals at necropsy 2 months post-challenge.

Three Holstein calves, which were inoculated with 5,000, 6,000 and 20,000 homologous infective larvae, respectively, developed patent infections that lasted 64, 95 and 55 days. Maximum outputs were 5.0, 9.8 and 45.5 larvae per gram of feces, respectively. Marked respiratory distress was observed during these infections.

One of these calves developed a patent reinfection following homologous challenge with 57,000 larvae 38 days after the termination of the initial infection. The other two animals did not develop patent infections and showed only transient clinical reactions to challenge with 35,000 and 51,000 larvae up to 6.5 months following the end of their initial infections.

In a reciprocal cross infection a captive elk calf, which was inoculated with 24,000 cattle lungworm larvae, developed a patent infection that lasted for 24 days. The maximum larval output was 6.6 larvae per gram of feces.

Results from these preliminary studies indicate that at least partial protection is obtained in cattle against homologous lungworm infections from the use of elk lungworms as an immunizing agent.

INTRODUCTION

The lungworm, Dictyocaulus viviparus, is the causal agent for parasitic bronchitis or "husk" in cattle. This parasite has caused serious economic loss in many parts of Eurasia, the British Isles and in North America. Poor weight-gaining ability and mortality in calves are important consequences of this disease (Brown and Spedding, 1958; O'Donoghue, 1958; Djafar et al., 1960). Lower milk yields have been attributed to lungworm infections in dairy cows (Hudson, 1951; Campbell and Wetherill, 1957). Several investigations on the epizootiology of parasitic bronchitis began about 1950. The emphasis of this research was placed on control, treatment and prophylactic measures.

Improved herd management resulted from studies on factors affecting survival and transmission of D. viviparus larvae on pasture. Jarrett et al. (1955b) reported that susceptible calves acquired infections on pastures contaminated with larvae 6 and 13 months earlier. Świetlikowski (1959) found that infective larvae could overwinter in Poland, but in southern England and Alabama larvae did not survive more than 6 to 8 weeks (Soliman, 1952; Porter, 1942). Seasonal variations in rainfall and temperature had a direct effect on the rate of development and survival of infective larvae (Rose, 1960). Spedding and Michel (1957) showed that gang-mowing of pastures in spring and fall was conducive to the dissemination of larvae onto the pasture. Soliman (op. cit.) suggested that a rotational grazing program would prevent the acquisition of infections. In a controlled experiment, Grégoire et al. (1960) showed this method worked effectively when calves were moved to different pastures every 4 days with a

32-day interval before the animals returned to a particular area. It was suggested that the level of herbage infestation could be reduced by allowing resistant cows to graze on pastures contaminated with lungworm larvae (Baxter et al., 1959). Michel (1957) recommended that all calves graze pastures in early spring to ensure an initial exposure to low levels of herbage contamination, followed by continued access to these pastures to maintain high levels of resistance.

Treatment and evaluation of lungworm anthelmintics improved considerably during this period of research on parasitic bronchitis. Previously, infected calves were treated intratracheally with a variety of chemicals. Good results were reportedly achieved with such solution as 2% picric acid (Cremona and Monaco, 1933); "GH 25", a mixture of menthol, thymol, turpentine and phenol (Middeldorf, 1932); 15 to 20 cc of a mixture of turpentine, olive oil, creosote and chloroform (Kennedy, 1934); or three injections of an emulsion of one part carbon tetrachloride and four parts olive oil (Niverd, 1947). Birkett (1942) suggested that the violent coughing caused by intratracheal injection of turpentine and carbolic acid effected expulsion of the lungworms. The improved clinical condition of treated animals may have lead to erroneous conclusions about the efficiency of these chemicals, since D. viviparus infections tend to be short-lived and self-limiting (Colglazier and Enzie, 1961), and because untreated infected cattle were not used. Taylor (1942) pleaded for controlled experimentation in assessing the results of treatment and Audureau (1954) outlined principles to use in drug testing. The critical test, using tracheotomy tubes

(Walley, 1957) or total tracheotomy (Vodrážka, 1959; 1960a), was an additional method developed to evaluate lungworm anthelmintics. Worms expelled after treatment were recovered in gauze bags attached to the trachea by a plastic tube and this count was compared with the number of worms found in the lungs at necropsy.

Intratracheal injections of aqueous iodine or iodine preparations such as Lugol's solution or "Iodinol" were highly effective in controlled tests (Popova, 1950; Olteanu and Fromunda, 1961; Evdokimov, 1963; Litoshko, 1963). A colloidal iodine preparation, "Merkojod," and "GH 25" were shown to have little effect in laboratory and field trials (Enigk and Düwel, 1963).

A more efficient method of administering drugs into the respiratory tree was described by Enigk (1953). He used an apparatus which formed aerosols from the anthelmintics and these were inhaled by the infected animals. Enigk (1957) reported cure or clinical improvement in 78% of 5,760 cattle using an ascaridol-5% santonin mixture. Twelve calves that were treated for periods of 8 to 30 minutes were free from infection 5 days later (Langeler, 1959). Ascaridol aerosol killed more than 80% of mature and some immature worms when experimentally infected calves were treated at two different intervals (Enigk and Düwel, 1961). A 3-minute treatment with iodine vapor of various concentrations cured 80 to 100% of infected cattle (Smirnov, 1963).

Cyanacethydrazide, an anthelmintic which acts only against lungworms, was first tested by Walley (1957a,b) in both critical and controlled experiments. He found that 60 to 100% of the worms were expelled, although the

drug had no effect on migrating larvae (Walley, 1960). Three daily subcutaneous injections were ineffective at 14 days post-inoculation (p.i.), 72.9% efficient at day 20 p.i., and removed 86.1% of mature worms (Rubin, 1959; Rubin and Tillotson, 1960). In critical tests, Vodrážka (1960b) reported that three consecutive daily doses administered subcutaneously resulted in expulsion of 67.8% of the worms. The piperazine salt of cyanacethydrazide eliminated 70% of the lungworms, although mature worms were only paralyzed and immature forms were only slightly affected (Enigk and Düwel, 1961). Little or no effect on clinical condition or lung pathology was reported following treatment in other controlled experiments (Swanson et al., 1959; Colglazier and Enzie, 1961). Conflicting results have been reported from field experiments using cyanacethydrazide. The response to treatment was poor, especially when the animals were heavily infected (Enigk et al., 1958; Langeler, 1959; Rosenberger and Heeschen, 1959; Larsen, 1960). Swanson et al. (op. cit.) reported that the controls gained 27 lb/head more than the treated animals by 87 days post-treatment. Improved condition after treatment was observed by O'Donoghue (1958) and Sirigu (1960).

Diethylcarbamazine, developed originally for the treatment of human filariasis, was highly effective against 2 week-old D. viviparus infections (Parker, 1957; Jarrett et al., 1962; Rubin and Tillotson, 1962). Treatment before day 10 p.i. was without action, and elimination of the worms during the second week interfered with the development of acquired resistance (Parker and Roberts, 1958; Parker, 1963; Kendall, 1965). The drug

was less effective against mature worms (Parker, 1957; Vodrážka, 1960b; Enigk and Düwel, 1961; Jarrett et al., 1962). Neither cyanacethydrazide nor diethylcarbamazine is considered completely satisfactory for treatment since they do not work efficiently against all stages of lungworm infection (Gibson, 1961).

Phenothiazine, methyridine, thiabendazole and tetramisole are broad-spectrum anthelmintics which have activity against lungworms as well as gastrointestinal nematodes. Daily doses of phenothiazine in salt killed first-stage larvae in the feces and reduced the incidence of infection (Enigk and Düwel, 1962; Paskalskaya and Zaitseva, 1963; Pivkov, 1963). Walley (1963) reported that methyridine was 86 to 99% effective against mature lungworms and removed over 60% of the immature worms in critical tests. A 95% reduction in worm burdens was reported by Ross (1966) in a controlled test. Oral administration of thiabendazole had little effect on cattle lungworms, but intratracheal injections were moderately efficient against both immature and mature worms (Ross, op. cit.; Rubin and Ames, 1966). Three intramuscular injections were 100% effective against larvae and removed 99.5% of mature worms. However, some residue was found at the site of injection (Ross, op. cit.). The newest broad-spectrum anthelmintic, tetramisole, eliminated 93.8 and 100% of the lungworms from two calves in a critical test (Nilsson and Sorelius, 1967). Forsyth (1966) and McCulloch (1968) reported high efficiency against D. viviparus with clinical improvement following treatment.

Concurrent with improvements in treatment against parasitic bronchitis

were attempts to find a prophylactic means of controlling this disease. Field observations (Wetzel, 1948; Taylor, 1951; Jarrett et al., 1955b) and experimental infection studies (Porter and Cauthen, 1942; Michel, 1955; Rubin and Lucker, 1956a; Weber, 1958a; Jarrett et al., 1959a) showed that calves demonstrated strong resistance to later exposure with lungworm larvae after recovery from initial lungworm infections. Partial protection was conferred to susceptible calves by the passive transfer of immune serum. The immunized group harbored fewer worms than the controls at necropsy (Jarrett et al., 1955a). Calves given 5 ml gamma globulin/lb body weight survived the inoculum of 50,000 larvae, but developed patent infections after a relatively longer prepatent period of 31 days (Rubin and Weber, 1955). Active immunization experiments using injections of antigens prepared from dead larvae or adult worm material did not induce a protective immunity in calves (Jarrett et al., 1960a; Wade et al., 1962).

In summarizing research on the development of immunity to helminths, Soulsby (1958; 1961) concluded that the presence of the living worm was necessary to achieve protective immunity. Metabolic products, particularly exsheathing and molting fluids, were considered to be important functional antigens. When infective ova of Ascaris lumbricoides were injected subcutaneously into guinea pigs, few larvae hatched and their development was incomplete. Good protection was demonstrated when these animals were challenged later (Soulsby, 1957). This approach was attempted with fourth-stage D. viviparus larvae that were administered

intraperitoneally. A 70% reduction in worm burden was achieved at challenge; however, three of the four vaccinated calves developed light patent infections following immunization (Cornwell, 1962a). Wade and Swanson (1958) reported the establishment of patent infections in calves when large numbers of first-stage or infective larvae were injected subcutaneously.

Protection resulting from immunization with small numbers of D. viviparus larvae given per os has also been investigated. Five of six calves given 500 or 1,500 infective larvae demonstrated strong resistance to challenge with 25,000 larvae. Although patent reinfections were not prevented, fewer larvae were recovered from the feces and the patent period was much shorter for the immunized calves than for their controls. Light patent infections resulted from the immunizing doses and moderate respiratory distress occurred 3 to 8 weeks p.i. (Weber and Lucker, 1959). Very few worms were recovered from the lungs of four calves 30 days after challenge with 15,000 larvae. These animals were immunized 5 months previously with 25 doses of 300 larvae inoculated over a 62-day period (Jarrett et al., 1959a).

Jarrett and his coworkers (1960b) studied the biological effects of X-irradiation on D. viviparus larvae using techniques similar to those of Gould et al. (1955) for artificially attenuating the pathogenicity of Trichinella spiralis larvae. After exposure to 40,000 roentgens, the infective larvae underwent only partial development when given per os to calves, but the vaccinated animals exhibited strong immunity when challenged subsequently. In field trials, they found that a single dose of

irradiated larvae did not completely protect calves when they were exposed to heavy pasture contamination (Jarrett et al., 1958). The vaccinated animals developed patent infections and 20% of them died. A higher degree of protection was obtained if two doses of 1,000 X-irradiated larvae were given 6 weeks apart (Jarrett et al., 1959b). The lungworm vaccine went into commercial production in 1959 and a high degree of protection resulted from vaccination of susceptible calves (Eck et al., 1960; Engelbrecht, 1961; Nelson et al., 1961; Pierre et al., 1961; Edds et al., 1963; Nelson, 1964; Downey, 1965; 1968). Inadequate protection was obtained from a single dose of irradiated larvae when calves were challenged with 15,000 normal larvae 18 weeks after vaccination (Lucker and Vegors, 1960; 1964). Düwel (1963) reported that 67% of the vaccinated calves developed patent infections when exposed to larvae on contaminated pastures. These vaccinated calves may then serve as carriers of infection to susceptible animals (Cornwell, 1959; Cornwell and Berry, 1960).

An alternate procedure for immunizing against helminth infections was proposed by Stoll (1961). He suggested using naturally occurring species or strains of helminths of low pathogenicity to immunize against similar, more pathogenic strains. This principle was applied by Allen and Samson (1961) to Haemonchus contortus infections in sheep. They found significant resistance to challenge with homologous infective larvae following exposure to a relatively non-pathogenic strain of H. contortus larvae isolated from pronghorn antelope. Protection against homologous lungworm larvae was obtained in calves given Dictyocaulus filaria larvae

from sheep (Hildebrandt, 1962; Parfitt and Sinclair, 1967). Lucker et al. (1964) found that older calves tolerated these larvae well but in calves 1 to 4 months old, D. filaria larvae were excessively pathogenic. Parfitt and Sinclair (op. cit.) killed three of six calves using large doses of these larvae, and Parfitt (1963) reported the establishment of a patent infection in a calf with 30,000 larvae. Therefore, D. filaria do not appear to be the most suitable agent for immunizing against cattle lungworms.

Recent studies with elk in the northern Yellowstone National Park herd have shown that both calves and mature animals have a high incidence of lungworm infection (McBee et al., 1964). This lungworm was originally named Dictyocaulus hadweni, but was later synonymized with D. viviparus (Dikmans, 1936; Dougherty, 1946). In this manuscript, elk lungworms will be considered as the elk strain and those from cattle as the cattle strain of D. viviparus. Serious morbidity has not been associated with these infections in elk, which suggests the occurrence of a well-adapted strain of lungworms. This may have resulted from a long association of the elk with this parasite. Preliminary observations in five bovine calves inoculated with 4,000 to 43,000 elk strain larvae have shown that the infections were self-limiting and practically asymptomatic (Barrett and Worley, 1965). The possibility of using this elk strain of lungworm larvae as an immunizing agent in cattle was investigated in experimentally infected calves.

MATERIALS AND METHODS

All but one of the nine Holstein calves used in this experiment were purchased when less than 7-days old and fed milk replacer for a period of 6 weeks. Alfalfa hay was fed ad libitum. The exception was a challenge control calf (no. 17) that was raised for 7 months under conditions precluding exposure to lungworm larvae. The four principal animals in the study (no. 8, 9, 12 and 13) were raised indoors in stalls with concrete floors from the time of purchase until the date of immunization, and for the interval until challenge. Source animals (no. 10, 14 and 15) and challenge control calves (no. 17 and 11) were housed individually in sheltered stalls during the prepatent and patent periods of their infections.

The elk used were from the northern Yellowstone National Park herd. In late March of 1966 and again in 1967, two pregnant cow elk were brought to this laboratory and housed in a specially modified stall. The calves born in captivity were also used in the study.

First-stage elk strain larvae were obtained from the feces of one of the naturally infected cows in 1966 and from eggs of mature female lungworms recovered from elk in 1967. These eggs were hatched by placing them in containers with a thin film of distilled water with an animal charcoal substrate. They were aerated continuously for 2 to 3 days at room temperature using an aquarium air pump. These first-stage larvae and those recovered by Baermannization from the feces were then cultured to infective stage at 19 to 26°C for 9 to 12 days by the method described by Rubin and Lucker (1956b).

Cattle strain larvae were obtained from source calf 15 which was fitted with a fecal collecting bag. First-stage larvae were recovered from the feces in the following manner: the daily collection of feces was separated into approximately 200 g samples, placed in cheesecloth and set up in 12 to 30 250 ml plastic funnels filled with lukewarm water. These funnels were equipped with an 80-mesh screen 6 cm in diameter placed 4 cm from the top, and a short rubber hose with a pinch clamp attached at the bottom. After 17 to 24 hours, about 25 ml of fluid were tapped from the bottom of each funnel; larvae and associated debris were concentrated by centrifugation and washed several times with distilled water. These larvae were cultured to infective stage as described previously for the elk strain larvae.

Infective larvae were separated from the animal charcoal by washing the contents of the culture through a 100-mesh screen and concentrated by centrifuging the mixture that passed through the screen. The larvae were stored in water at 4°C for less than 2 weeks before being used.

The number of viable larvae was determined by dilution count immediately prior to the time of inoculation. Larvae that were considered viable were either active, tightly coiled, or nearly straight in shape. Animals were inoculated *per os* by means of a stomach tube or a 4 oz drenching syringe with an 18 in Whitlock nozzle according to the schedule outlined in Table I. Of the four animals immunized with elk strain larvae, calves 12 and 13 were challenged with cattle strain larvae 5 months later and calves 8 and 9 after 19 months. All cattle strain larvae used to challenge immunized animals and control calf 11 were collected from source

Table I. Schedule of Inoculations with Elk and Cattle Strain Dictyocaulus viviparus in Experimental Animals.

Animal No.	Age at Inoculation (months)	No. of Elk Strain Larvae	No. of Cattle Strain Larvae	Interval to Challenge (months)	Age at Challenge (months)	No. of Cattle Strain Larvae
Immunized Animals						
8	1.5	10,000	-	19.0	20.5	46,000
9	1.5	9,500	-	19.0	20.5	46,000
12	3.0	6,500	-	5.0	8.0	21,000
13	3.0	6,500	-	5.0	8.0	21,000
Challenge Controls						
17		-	-		7.0	21,000[a]
11	0.5	-	2,000[b]	7.0	8.0	24,000
Elk Calf	6.5	-	24,000			-
Source Animals						
10	6.5	-	6,000	6.5[c]	19.0	51,000
14	1.5	-	5,000	1.5[c]	5.5	57,000
15	2.0	-	20,000	0.0[d]	4.5	35,000

[a]Larvae obtained from the Beltsville Parasitological Laboratory, Beltsville, Maryland

[b]Larvae stored in water at 4°C for 2 months before use

[c]Interval from the termination of the patent period of the initial infection

[d]Challenge was 9 days before the patent period ended

calf 15. Infective larvae used to inoculate challenge control 17 were obtained from Dr. F. W. Douvres at the Beltsville Parasitological Laboratory.

Calves 8, 9, 12 and 13 were observed several times a week for changes in respiration following both immunization and challenge. Fecal samples were collected from all inoculated animals beginning about day 18 p.i. Samples were collected daily for the first 3 weeks and at least twice weekly throughout the duration of the patent period. Determinations of the total numbers of larvae recovered by Baermannization and larvae per gram of feces (l.p.g.) were made using fecal samples weighing 50 to 150 g.

Blood samples were collected from the immunized animals prior to, and for 6 weekly intervals following challenge. Calves 12 and 13 were sampled for 4 weeks following immunization with the elk strain larvae. Complement-fixing antibody titers were determined for these blood samples following the techniques described by Lennette and Schmidt (1964) and Weber (1958b). Two full units of complement were used, and test tubes were kept in a 4°C cooler overnight before the hemolytic system was added. The highest serum dilution at which a four plus reaction occurred was the end point used for demonstrating changes in titer. Fat-extracted, whole mature worm antigens were prepared for both the cattle and elk strains according to the method described by Kent (1963). Antigen concentrations used for the tests were 1.4 mg/ml for the cattle strain and 3.6 mg/ml for the elk strain.

Immunized calves 12 and 13 were slaughtered 4 weeks following the termination of patency, and yearling 9 was killed on the same date. The

lungs and trachea were removed and examined grossly for lesions and for worms in the air passages. Representative tissues were fixed in 10% buffered formalin. These were sectioned, stained with azure-eosinate and examined for pathological changes. Portions of the lungs were cut up and Baermannized to recover any immature worms overlooked on gross examination.

The criteria used for evaluation of the protection demonstrated following challenge with homologous strain larvae were as follows: 1) presence or absence of infection; 2) length of the prepatent period; 3) duration of the patent period; 4) larval output during the patent period; 5) changes in respiration; 6) complement-fixing antibody response and 7) histopathology in lung tissues.

An additional experiment conducted during this study was the reciprocal cross infection of an elk calf with 24,000 cattle strain larvae. Also, the course of initial infections with cattle strain larvae was followed in source calves 10, 14 and 15 (Table I). Fecal samples were collected frequently throughout the patent periods and changes in respiration were noted following initial inoculations. The three source animals were challenged with homologous strain larvae at different intervals following or during the patent period. Similar observations were made on respiratory changes and larval production following these inoculations. Larvae for challenge and those used to inoculate the elk calf were obtained from source calf 15.

RESULTS

The four calves did not develop patent infections following immunization with elk strain larvae (Tables I and II). None of the animals exhibited coughing or prolonged changes in respiration.

Yearlings 8 and 9 (Table II) were almost completely refractory to challenge with 46,000 cattle strain larvae 19 months after immunization. A patent infection did not develop in yearling 8 and only two larvae were recovered from fecal samples collected from yearling 9. Some coughing was observed between days 14 and 35 post-challenge (p.c.), but no obvious respiratory distress occurred.

Immunized calves 12 and 13 (Tables I and II) developed light patent infections from homologous challenge when 8-months old. Compared with the response of challenge control calf 17, the immunized calves demonstrated a relatively longer prepatent period of 27 vs. 23 days, a shorter average patent period of 30 vs. 43 days, and reduced larval output. In Figure 1, larval output data are presented graphically for calves 12, 13 and 17. Values are averaged for the two immunized calves because these determinations were very similar.

The clinical manifestations associated with these infections differed markedly. Immunized calves coughed only a few times between days 12 and 19 p.c. Respiration was somewhat labored during this period, but there was no marked change in the respiratory rate. In contrast, the control calf 17 developed a light cough by day 14 p.i. which increased in severity and frequency until day 27, when this animal stood with head lowered, neck