

# The potential use of poly(methacrylic acid) hydrogels for oral administration of drugs and vaccines to ruminants

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## Abstract

Poly(methacrylic acid) hydrogels were investigated for the delivery of a model antigen to the lower gastrointestinal tract of sheep. Hydrogels were tested by incorporating a radiopaque material, administering them orally to a sheep, and then radiographing the sheep. The potential for loading high molecular weight proteins into hydrogels was determined by absorbing hydrogels with culture supernatants of the bacterium *Pasteurella haemolytica*. The hydrogels were dried, hydrated, and the culture supernatants eluted. The eluents were assayed for the presence of the 102 kDa proteinaceous exotoxin. The hydrogels readily bypassed the first stomach and swelled releasing a radiopaque dye into the lower gastrointestinal tract. Chromium-loaded hydrogels were then administered to a sheep and intestinal contents were collected for 5 days. Chromium was detected in the intestinal contents of the sheep for 96 h with peak levels detected at 12–15 h after administration. Eluents of the hydrogels loaded with culture supernatants contained readily detectable amounts of the proteinaceous exotoxin. PMA hydrogels were then absorbed with a vaccine consisting of culture supernatants of a pulmonary bacterium *P. haemolytica*. Hydrogels containing vaccine were administered orally to calves. Calves were challenged by an intrabronchial dose of bacteria. The length of time each calf survived was noted. All surviving calves were killed 3 days post-challenge. A post-mortem examination was performed to evaluate the severity of the pneumonic lesions. Vaccinated calves had less pneumonia and lived longer than control calves. Results of this study indicate that poly(methacrylic acid) hydrogels could be used to administer drugs and proteinaceous vaccines orally to ruminants.

**Keywords:** Hydrogel; Poly(methacrylic acid); Oral delivery; Antigen; Vaccine; Vaccine delivery; *Pasteurella haemolytica*

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## 1. Introduction

The oral medication of ruminants is much more challenging than other species because these animals have a complex upper gastrointestinal system composed of four stomachs: rumen, reticulum, omasum, and abo-

masum. The first stomach or rumen has several components: liquid, several solid fractions of feed particles of different sizes, and microbes [1]. Rumen microbes aid digestion by breaking down cellulose and some proteins into smaller units. Thus, orally administered drugs must be either stable in the rumen, or delivered in a way to bypass the rumen to avoid the potential for microbial breakdown. Sustained delivery of low

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molecular weight compounds has been accomplished using devices that take advantage of a density (specific gravity) greater than 2.5, and a size that prohibits regurgitation or transport through the stomachs [2,3]. These smaller molecular weight therapeutic agents may be released by diffusion from the device or by pumping activity into the reticulo-rumen [3,4]. These devices have revolutionized treatment of cattle for the administration of antibiotics, anthelmintics, and trace minerals. A one time treatment reduces subsequent handling of individual animals. This reduces labor costs to the owner, and stress to the animals induced by the need for corraling and restraining each animal in a head catch in order to administer the therapeutic agent. For the administration of essential amino acids, small coated pellets have been produced which can be administered in the feed, totally eliminating the need for individual administration of the components [5]. Although these delivery systems have been very useful, they still have some limitations. Many of the present delivery systems for ruminants require the use of organic solvents during manufacturing to incorporate the therapeutic agent into the device. Most therapeutic agents delivered are of low molecular mass, usually less than 1000 Da in size. The delivery of very high molecular weight components, or agents that are susceptible to organic solvents or microbial degradation cannot be administered using the present delivery systems. An example of a material that has not been administered orally to ruminants to date for these reasons is a (proteinaceous) vaccine.

Oral vaccines have not been used in cattle because unprotected proteins are likely to be degraded by bacteria and protozoa in the rumen (first stomach), as well as by the low pH of the abomasum (fourth stomach). Fermentative digestion by bacteria and protozoa in the rumen breaks down cellulose and other complex nutrients [6]. Ingesta is either absorbed, passed on to subsequent stomachs, or is regurgitated and subjected to further mastication. Therefore, an oral vaccine for cattle must bypass the rumen in order to deliver antigens to the gut-associated lymphoid tissue (GALT) in the lower gastrointestinal tract (GIT). The uptake of antigens by GALT can then result in an immune response at mucosal sites as well as systemically [7,8]. A delivery method is needed to protect antigens in oral vaccines from degradation in the rumen. In this study we propose that orally administered poly(methacrylic acid) hydrogels can bypass the rumen and deliver anti-

gens in a sustained release manner to stimulate mucosal immunity in the lungs of calves.

## 2. Materials and methods

### 2.1. Hydrogel preparation

Poly(methacrylic acid) (PMA) hydrogels were produced by cross-linking a 40% solution of methacrylic acid (Aldrich Chemical Company, Milwaukee, WI) with a 0.8% solution of *N,N'*-bisacrylamide (Biorad Laboratories, Richmond, CA). Ammonium persulfate (Polysciences, Inc., Warrington, PA) was used as the initiator and sodium bisulfite (J.T. Baker Inc., Phillipsburg, NJ) as the co-initiator. The solutions were degassed by applying a vacuum to the vessel containing the reactants, and then purged with nitrogen. The monomer solutions were subsequently placed into 1 ml syringe barrels where polymerization was carried out at 60°C for 18 h under nitrogen. The gels were removed from the syringes and cut into 5 mm in diameter by 3 mm long discs. The discs were washed repeatedly in distilled deionized water, and then dried at 37°C for 1 week or until the discs attained a hard and glassy state.

### 2.2. Rumen bypass studies

The location of the hydrogels within the gastrointestinal tract was studied by administering hydrogels loaded with a radiopaque material, diatrizoate meglumine/sodium diatrizoate (Gastrografin, E.R. Squibb and Sons, Inc., Princeton, NJ), to a sheep. PMA hydrogels were loaded with Gastrografin as previously described [9]. Briefly, hydrogels were allowed to swell in a 45% (v/v) solution of Gastrografin for 32 h at 37°C. The hydrogels were then air dried for 1 week and oven dried at 37°C for another week. A dose of 300 Gastrografin-loaded hydrogels was administered to a sheep at one time using a balling gun. Sheep were chosen as a model for cattle since they are ruminants that are smaller and therefore easier to radiograph. Movement of the hydrogels through the upper GIT and release of the Gastrografin from the hydrogels was monitored over time by radiography. Radiographs were taken from pre-administration to 3.5 h after adminis-

tration at which time the hydrogels were no longer visible.

### 2.3. Antigen release studies

The use of hydrogels as a vaccine delivery system was studied using chromium chloride (Sigma Chemical Co., St. Louis, MO) bound to EDTA (Sigma Chemical Co., St. Louis, MO) (Cr-EDTA) as a model antigen [10]. Chromium-EDTA was chosen for this study because it is virtually unabsorbed by the ruminant gastrointestinal tract and can therefore be readily detected in the intestinal fluid. Chromium-EDTA was prepared according to a method previously described [10,11]. Dried PMA hydrogels were loaded with Cr-EDTA by swelling them in a 10% (w/v) Cr-EDTA solution for 48 h at 37°C. Each hydrogel was loaded with an average of 14 mg of chromium. After loading, the hydrogels were dried at 37°C for 1 week. A total of 300 Cr-EDTA loaded hydrogels were placed in two 7.5 ml gelatin capsules and administered to a sheep by balling gun. At the end of the study the sheep was humanely killed by an overdose of pentobarbital (Succomb, Butler Co., Columbus, OH).

Samples of ileal contents were collected over 96 h through a cannula placed in the ileum 6 cm proximal to the ileal-cecal junction. The cannula was placed by transecting the ileum, suturing the cut ends and inserting a T-shaped cannula in the proximal and distal ends [11]. The ends of each cannula were passed through the flank by blunt dissection, clamped securely to the skin, and the two ends connected so that all intestinal contents passed through the cannula. All intestinal contents were collected for 120 h for one study to look for hydrogels. Ileal contents were collected, poured through a 60 mesh sieve, examined for hydrogels or fragments of hydrogels, and the liquid portion injected into the caudal aspect of the cannula. A 50 ml sample was collected, centrifuged to remove large debris, and stored at -20°C until assayed for Cr-EDTA content. All feces were collected for 1 week, broken apart and passed over a 60 mesh sieve and examined for the presence of hydrogels.

### 2.4. Analysis of samples for chromium

The intestinal samples were assayed for chromium content by atomic absorption spectrophotometry as

previously described [10,12]. Samples of ileal contents were centrifuged at 500 × g for 30 min to separate the fibrous matter from the liquid fraction. A 0.8 ml aliquot of the supernatant was added to 15 ml of 1 N HNO<sub>3</sub> and heated for 6 h at 60°C to precipitate any soluble proteins. The samples were then filtered through a 0.2 μm pore size filter. Chromium levels were detected by an atomic absorption spectrophotometer (Model 2300, Perkin-Elmer Corp., Norwalk, CT) at a wavelength of 357.9 nm and a slit setting of 0.7 nm. Samples containing 5 and 15 μg/ml of chromium in 1 N HNO<sub>3</sub> were used as standards.

### 2.5. In vitro antigen release studies

Culture supernatants (CS) of the respiratory bacterial pathogen *Pasteurella haemolytica* were used to determine whether high molecular weight antigens can be absorbed into PMA hydrogels. The primary component of CS is a 102 kDa protein exotoxin. This is an important immunogen in the stimulation of immunity to *P. haemolytica*. Culture supernatants were produced in the following manner. Colonies from an overnight growth on a blood agar plate were used to inoculate tryptic soy broth which was incubated at 37°C in a shaking water bath for 4.5 h. A 10% inoculum of this broth was used to inoculate fresh tryptic soy broth. The secondary culture was incubated for 1 h at 37°C with 5% CO<sub>2</sub> bubbled through it. The broth was agitated to the maximum possible without foaming. The bacteria were removed from the culture following a 1 h incubation by ultrafiltration (Pellicon, Millipore, Inc., Bedford, MA). CS free of bacteria were lyophilized and stored desiccated at -20°C until used to load hydrogels. Loading the hydrogels was accomplished by reconstituting the lyophilized CS to 22% w/v in sterile distilled deionized water. The premade hydrogels were placed in the reconstituted CS and allowed to fully swell for 2 days. Once all the fluid was absorbed, the hydrogels were dried to a hard glassy state by placing them in an incubator at 37°C for 2 days.

Hydrogel discs absorbed with CS were placed individually in phosphate-buffered saline and allowed to fully hydrate for 2 days to determine whether the proteinaceous 102 kDa exotoxin could be eluted. Eluent was then removed from the hydrogels (three were tested each time) daily for 3 days. An equal volume of fresh buffered saline was placed on each hydrogel after

removing the eluent. The eluents were tested for the presence of leukotoxin using an enzyme-linked immunosorbent assay (ELISA). The ELISA was performed by placing 50  $\mu$ l of each sample of eluent in triplicate into a well on a 96-well polystyrene plate (Immulon 2, Dynatech Laboratories, Chantilly, NY) and allowing the material to bind overnight at 4°C. A polyvalent rabbit antibody made in our laboratory to the 102 kDa leukotoxin of *P. haemolytica* was used to detect the presence of leukotoxin in eluents by incubation at 37°C for 3 h. A secondary anti-rabbit IgG conjugated to horseradish peroxidase (Bethyl Laboratories, Montgomery, TX) was placed into each well and the plate incubated at 37°C for 2 h. A solution containing the substrate orthophenyldiamine (Sigma Chemical Co., St. Louis, MO) was added to each well and the plate incubated in the dark at room temperature for 30 min. The reaction was stopped by adding sulfuric acid to each well and the plate was read at 490 nm using an EIA spectrophotometer (Kinetics VMAX, Molecular Devices Co., Menlo Park, CA)

## 2.6. Oral inoculation of calves

An oral vaccine was prepared by absorbing CS into PMA hydrogels. This vaccine was used to orally vaccinate calves. The calves were challenged later with viable bacteria to determine if the vaccine was able to stimulate protective immunity in their lungs. Culture supernatants of *P. haemolytica* were suspended to a 22% (w/v) solution in deionized water. PMA hydrogel discs were placed into this solution and allowed to absorb the CS for 48 h. The hydrogels were then dried in a 37°C incubator for 48 h. These hydrogels were used to vaccinate calves orally for *P. haemolytica*. An equivalent number of plain hydrogels were also prepared. Twelve Holstein-Friesian 12-week-old male calves were purchased from the same local dairy and assigned to either a vaccinated or control group. Vaccinated calves were given a dose containing 300 hydrogels per day for 5 days. Control calves were given a dose of plain hydrogels. All dosages of hydrogels were administered in a bolus once daily using a balling gun. Each calf was challenged by an intrabronchial dose of 25 ml of  $10^9$ – $10^{10}$  CFU of viable *P. haemolytica*. The experiment was divided into two trials with three calves per group for each trial. Calves were killed (Succomb, Butler Co., Columbus, OH) 72 h after challenge. The

time of death of calves prior to the end of the study was noted. Any calf that was suffering was killed before this time.

At post-mortem, the lungs were evaluated for the severity of pneumonic lesions and scored for both gross and histopathological lesions. Lesions were scored from 0–3 with 3 the most severe and 0 no lesions noted. A total score of 24 was possible for gross lesions. Parameters evaluated included the presence of fibrin in interlobular septa, on pulmonary pleura, and in bronchiole-alveoli, the presence of interlobular edema, lobular congestion and exudate in bronchiole-alveoli, pleural effusion, and survival time. The maximum histopathological lesion score was 42 and included the parameters of hemorrhage or edema, fibrin, necrosis, and cellular infiltrates in each of three sites: bronchioles and bronchi, interlobular areas, and alveoli; pleuritis, and the presence of Gram negative bacilli in sections. Three sections of lung were examined from each of the right anterior, middle, and caudal lung lobes. Each section was cut from the same location (caudal anterior part) of each lobe in each calf. The mean score of all the sections was used as the histopathological lesion score for each calf. The surface area of lung affected was diagrammed on a chart and the percentage pneumonic lung measured using a computer planimetry program (Kontron Image Analysis, Zeiss Co., New York, NY). The determination of the area of pneumonia and assignment of lesion scores was performed by a pathologist who had no prior knowledge of the group assignment of each calf. A pneumonic index was computed by multiplying the percentage pneumonic lung by the gross lesion score times the histopathological lesion score. The calves were ranked by time of survival and pneumonic lesion score and analyzed using the Wilcoxon rank sum statistical test.

## 3. Results

### 3.1. Rumen bypass studies

A significant number of PMA hydrogels entered the reticulum by 15 min after administration (Fig. 1). As shown in the radiograph in Fig. 2, approximately 60% of the gels were in the reticulum within 45 min after administration. As the gels began to swell, Gastrografin was released due to its pH-dependent solubility [9]

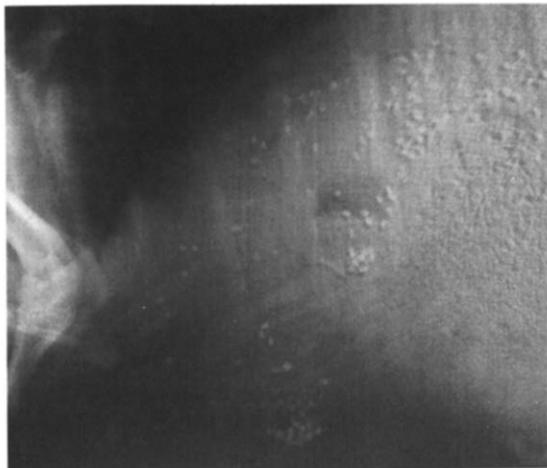


Fig. 1. Lateral radiograph of the abdomen of a sheep made 15 min after the oral administration of poly(methacrylic acid) hydrogels containing Gastrografin. Most of the hydrogels have already entered the reticulum.

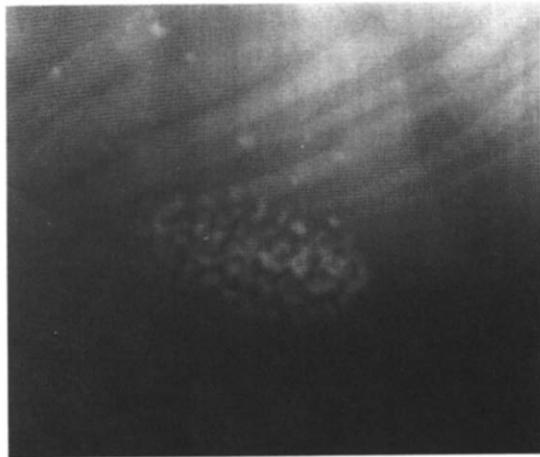


Fig. 2. Close-up lateral radiograph of the reticulum of a sheep made 45 min after the oral administration of hydrogels prepared as described in Fig. 1. Approximately 60% of the hydrogels were present in the reticulum by this time.

and the hydrogels appeared larger, less radiopaque, and with less distinct edges by 75 min after administration (Fig. 3). By 3 h after administration the outline of the omasum and abomasum could be seen (radiograph not shown). By 3.5 h the hydrogels could not be seen due to the release of Gastrografin. Hydrogels appeared to remain primarily in reticulum with the remaining ones in the rumen; no hydrogels were observed in either the omasum (third stomach), abomasum (fourth stomach), or intestinal tract during the study.

### 3.2. Chromium release studies

The release profile of the PMA hydrogels was studied using Cr-EDTA because it is not broken down by enzymes or changes in pH, it is not absorbed well by the ruminant gastrointestinal tract, and it is easily quantitated in intestinal contents using atomic spectrophotometry. Cr-EDTA was detected in the ileum from 3 to 96 h after administration with peak levels at 12–15 h (Fig. 4). In order for the peak levels of Cr-EDTA to occur at 15–18 h, Cr-EDTA had to be released a few hours before this in the upper GIT. This study showed that while retained in the upper GIT, hydrogels released a model antigen (Cr-EDTA) for 96 h into the ileum. Detection of Cr-EDTA in the intestinal contents was probably due to its release in the reticulum and not due to the hydrogels traversing the lower intestinal tract. This is supported by radiographs confirming the retention of hydrogels within the reticulum for some time, and the failure to find hydrogels in intestinal contents or feces during the studies. Moreover, the detection of Cr-EDTA 3 h after administration of hydrogels suggests that Cr-EDTA was released promptly from the

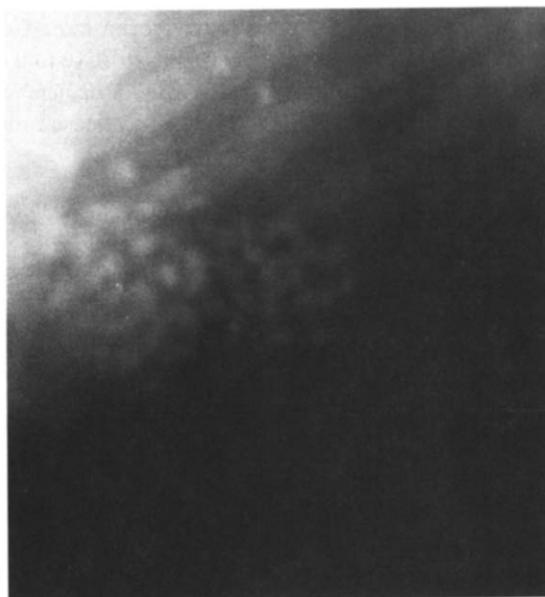


Fig. 3. Close-up lateral radiograph of the reticulum of a sheep made 75 min after the oral administration of hydrogels prepared as described in Fig. 1. The hydrogels appear less opaque, swollen in size and have less distinct edges due to swelling and the release of Gastrografin.

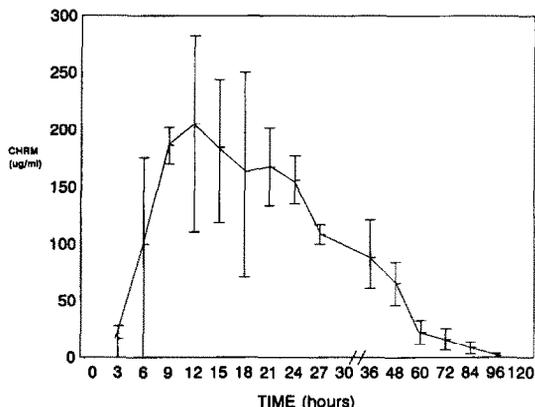


Fig. 4. The geometric mean and range (maximum and minimum) of chromium-EDTA detected in the ileal contents of a sheep over time. The mean and ranges represent the results of three separate studies.

hydrogels and moved down the GIT in the liquid portion of the chyme. Radiographic studies showed that absorbed material is released rapidly as shown by the detectable loss of a radiopaque marker within 1.5 h after administration. Moderate amounts of Cr-EDTA were detected up to 36 h while levels declined rapidly from 36 to 96 h. This decline may be due to unequal loading of the hydrogels. Since the hydrogels were loaded by absorption, there may have been more Cr-EDTA in the outer part of the hydrogels relative to the most inner part resulting in less Cr-EDTA detectable as the inner most parts of the hydrogels released the load.

### 3.3. Elution of leukotoxin from hydrogels

The primary constituent of the culture supernatants of the bacterium *P. haemolytica* is a 102 kDa protein exotoxin [13]. This exotoxin was detected in each sample of eluents collected over 3 days (Fig. 5). The amount of exotoxin present was estimated from the absorbance values. The absorbance values of the eluents for days 1–3 were each significantly greater than the absorbance of the culture medium tryptic soy broth. The absorbance values of the eluents from days 1 and 3 were not significantly different from the CS positive control. The absorbance of the eluent from day 2 was significantly less than the CS control although it was greater than the tryptic soy broth. The absorbance values from eluents on days 1 and 2 were significantly less than for the eluent for day 3. The reason for this

variation is not certain but it is likely that there was some differences in size of the hydrogels since they were cut by hand. It is also likely that there may have been some slight differences in the total amount of CS loaded into each hydrogel. Overall, although there were day to day differences in the relative amounts detected, each eluent contained a substantial amount of exotoxin. Most importantly, the ELISA studies showed that it is possible to absorb such a large protein into the PMA hydrogels and that it can be eluted and not trapped within the hydrogel. The ELISA studies also demonstrated that the key epitopes of the exotoxin were not damaged by the PMA or the process used to absorb the exotoxin containing CS into the hydrogels. These results suggest that it would be possible to use the PMA

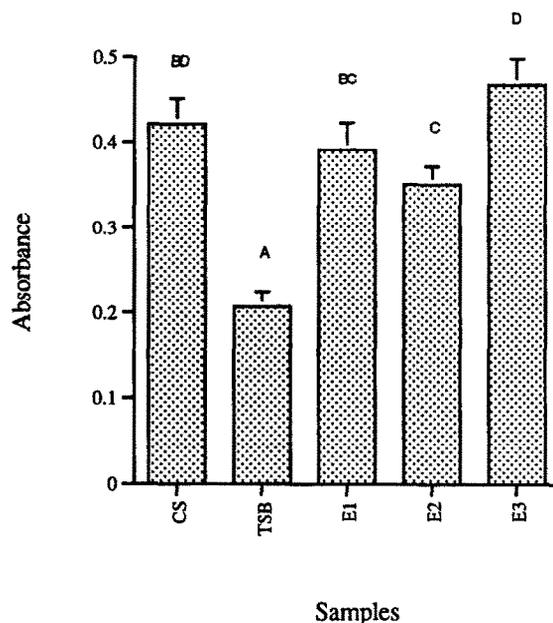


Fig. 5. Mean and standard error of the mean of absorbance values indicating the relative amounts of exotoxin in eluents from hydrogels absorbed with culture supernatants of *Pasteurella haemolytica*. Tryptic soy (T-soy) broth was used as a background control since it was the medium used to culture the bacteria. Culture supernatants (22% w/v) not previously absorbed into hydrogels were used as a positive control. CS = culture supernatant positive control, TSB = tryptic soy broth background control, E1 = eluent day 1, E2 = eluent day 2, and E3 = eluent day 3. Parameters with a different letter were significantly different from each other using the Waller-Duncan analysis of variance with the following *p* values: for A – BCD, *p* > 0.005; for B – C, *p* > 0.025; for B – D, *p* > 0.025; and for C – D, *p* > 0.005.

Table 1  
Rank of calves per trial based on survival time and pneumonic index

Trial number	Treatment group	Survival time (h)	Pneumonic index
1	control	3.5	6349
1	control	3.5	4994
1	vaccinate	3.5	71
1	vaccinate	3.5	8
1	vaccinate	23	5105
1	control	72	1810
2	control	12	6830
2	control	12	3850
2	control	20	2500
2	vaccinate	72	526
2	vaccinate	72	487
2	vaccinate	72	211

Rank was based primarily on survival time with the shortest time ranked first within each trial. Pneumonic index was determined by multiplying the percentage pneumonic lung by the gross lesion score times the histopathological lesion score. Statistical rank sum analysis:  $p=0.040$ .

hydrogels to deliver high molecular weight proteinaceous antigens.

#### 3.4. Calf challenge studies

The pneumonic index and survival time in hours for each calf by group is shown in Table 1. The control calves had pneumonic indices that were greater than those for vaccinated calves with the exception of one calf. For most calves there was a 10–100-fold difference in the pneumonic index between vaccinated and control calves. The control calves also lived a shorter length of time than the vaccinated calves with one exception. The exceptions were in the first trial in which calves were challenged with 10-fold more bacteria than the calves in the second trial. This greater challenge dose of bacteria resulted in more severe disease in general for all calves and resulted in the early death of five of the six calves in the first trial, in which the longest surviving vaccinated calf was killed at 23 h. In the second trial where the challenge dose was decreased, all vaccinated calves survived the duration of the study whereas all control calves died within 24 h after challenge. Ranking of calves by survival time and pneumonic index as a secondary index within each trial demonstrated a statistically significant difference in

survival and pneumonic index between control and vaccinated calves ( $p=0.040$ ).

#### 4. Discussion

This study demonstrates that poly(methacrylic acid) hydrogels bypass the rumen and move rapidly into the reticulum. Rumen bypass is dependent on the size and density of particles. Hydrogels that were either too large and too light or too small and too light would be more likely to become suspended in the rumen and not enter the reticulum. This was found to be the case in our preliminary studies (data not shown) in which the delivery system tested tended to float in the rumen. Retention time in the rumen normally varies, lasting up to 54 h [14]. Retention of the hydrogels in the rumen for any length of time with the subsequent release of antigens would result in microbial degradation of the antigens. Particles with a specific gravity of 1.10–1.70 and less than 6 mm in diameter have a shorter retention time in the rumen. Such particles move rapidly into the abomasum (14–17). The PMA hydrogels used in this study entered the reticulum within 15 min after administration because they had an optimal size (5 mm in diameter by 3 mm in length) and density (specific gravity of 1.4) to bypass the rumen efficiently [18]. The hydrogels probably did not move into the abomasum because they swelled within the reticulum. The increase in size would have restricted further movement to the lower digestive tract.

Although it appears that gel retention and subsequent gel erosion may have occurred in the reticulum, further studies are needed to confirm this. The poly(methacrylic acid) hydrogels are very hard in the dry state. However, once hydrated they have a rubbery consistency. Hydrogel erosion would therefore not be unexpected due to the force of reticular contractions and the presence of fibrous matter in the diet. Both of these factors would facilitate mechanical breakdown [19]. One animal was used in this study to reduce the number of animals that had to undergo surgery and the extensive handling required in this project. Animal to animal variation may affect the transit time and release of material from the hydrogels. However, results of this study suggest that this delivery system was worth investigating for the delivery of oral vaccines to ruminants in challenge studies.

In the challenge studies there was a dramatic difference in the survival and severity of pulmonary lesions in orally vaccinated calves compared to control calves. This difference strongly supports the efficacy of orally administered antigens in stimulation of immunity at distant mucosal sites. In order for the oral vaccination to be effective the vaccine had to readily bypass the rumen to avoid degradation by enzymes and microbial digestion. The PMA hydrogels were able to protect the culture supernatants of *P. haemolytica* following administration. The protection included bypassing the rumen and entering the reticulum where the hydrogels swelled releasing the CS which then rapidly flowed through the omasum and abomasum into the lower GIT to stimulate GALT. Most hydrogels probably stayed in the reticulum for a substantial length of time. Feces of the calves were examined daily for 1 week after administration of the hydrogels. Of all the hydrogels administered, only five were found in the feces. At necropsy the entire GIT was examined and no hydrogels were found. The PMA hydrogels are not biodegradable by enzymes. However, when swollen the hydrogels are rubbery in consistency and the ingesta of the bovine is very coarse. There is also continuous contractions of the stomachs which would further enhance the interaction of the ingesta with the hydrogels. This probably resulted in the erosion of the hydrogels over time as seen also in the CR-EDTA studies in sheep. The erosion may actually have enhanced the elution of the CS from the hydrogels by increasing the surface area of the hydrogels in the GIT.

The results of the challenge studies are consistent with previous investigations using *P. haemolytica* administered intraduodenally to enhance a pulmonary immune response in calves [20]. The intraduodenal studies suggested that if intact antigens could be administered orally, an effective immune response is possible. The oral administration of vaccines to prevent respiratory disease has been shown to be effective in laboratory animals and humans [21,22]. These studies were all conducted in monogastric animals using liquid preparations of antigens in gastric gavage. This technique is possible in ruminants but not commercially practical. The use of a delivery system to administer oral vaccines to cattle is necessary to study the application of this method of inoculation. This is the first study in which the oral delivery of antigens in a non-degradable hydrogel demonstrated the effective stim-

ulation of pulmonary immunity to an infectious agent in cattle.

Hydrogels have been used to deliver a variety of drugs orally and are under extensive study for use in the delivery of peptide drugs [9,10,23,24]. Hydrogels are useful in the delivery of materials where controlled release is desirable. They can be biodegradable, non-reactive, and economical to produce. All these factors make them ideal for the delivery of therapeutic agents including vaccines. Hydrogels are excellent for the oral delivery of antigens in vaccines because they provide a means of protecting epitopes from degradation or alteration by proteases, pH, and other enzymes present in the gastrointestinal tract. Hydrogels have been used successfully to deliver vaccines to monogastric laboratory animals [25,26]. However, the preparations used in those studies would not be likely to traverse the complex stomachs of cattle. Yet, cattle are afflicted with many diseases of economic impact (such as respiratory disease complex) for which oral vaccines could be very useful [27].

Hydrogels must be able to be loaded with antigen and to release the antigen upon hydration within the body to be useful in the delivery of oral vaccines. It is very important that the antigen be incorporated within the hydrogel without being damaged. The PMA hydrogels used in this study were prepared using chemicals and temperatures that could harm antigens. However, the model antigen was protected by preparing the hydrogels first, washing them extensively, and then loading the Cr-EDTA within them. Cr-EDTA is much smaller than most antigens and less likely to be affected by adverse conditions used to incorporate larger proteinaceous antigens. PMA hydrogels loaded with bacterial antigens were eluted in vitro and proven to retain their antigenicity, further supporting the hypothesis that these hydrogels could be used to deliver oral vaccines to ruminants. Stimulation of an immune response using orally administered antigens that can protect animals from a challenge by a virulent pathogen such as *P. haemolytica* was an important test of the efficacy of PMA hydrogels for oral delivery of vaccines to ruminants.

## 5. Conclusions

Poly(methacrylic acid) hydrogels were evaluated for the delivery of an agent over time to the lower

intestinal tract of sheep. Radiographic studies showed the hydrogels rapidly entered the reticulum and stayed there where they swelled and released a radiopaque material. Release studies showed that a model drug was released and detected in the lower small intestine for 96 h. Culture supernatants containing a 102 kDa protein exotoxin of the bacterium *P. haemolytica* were absorbed into and eluted from PMA hydrogels in vitro. Analysis of the CS by ELISA demonstrated that epitopes of the exotoxin eluted from the hydrogels as well as from neat CS were recognized equally well by a polyclonal antibody. These studies suggest that PMA hydrogels can be loaded with large protein molecules without damaging their immunogenicity and could therefore be useful in the oral delivery of drugs or vaccines that would otherwise be damaged by ruminal conditions. A vaccine administered orally to cattle was effective in stimulating protection from a challenge of virulent *P. haemolytica*. The orally vaccinated calves lived longer and had less pulmonary damage than sham vaccinated control calves. These studies suggest that PMA hydrogels can be used to deliver oral products such as vaccines to ruminants.

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