PEER REVIEWED

# A Case-Control Study: *Comparing the Effects on Calf Health After Use of A Commercially Available Mycoplasma bovis Vaccine in Dairy Herds in Scotland*

**Background**
*Mycoplasma bovis* is a common cause of bovine pneumonia in calves. Treatment and control of the disease is challenging. Treatment can be ineffective and control is hampered by a lack of a licensed vaccine in Europe.

**Results**
This retrospective observational case-control study looked at evaluating growth rates, mortality and antimicrobial usage for 932 calves born into 4 herds over 2 time periods – before and after using a 3-strain *M. bovis* vaccine (Myco-B One Dose, American Animal Health, Grand Prairie, Texas, USA) programme in commercial herds in the UK which demonstrated previous prevalence of *M. bovis*. The vaccinated groups demonstrated a significant reduction ($P < 0.05$) in post-weaning mortality in calves aged 70-200 days old. No significant changes in pre-weaning or total mortality were observed. A significant reduction in antimicrobial usage post-vaccination was observed when compared to 2 control farms with qualifying data.

**Conclusions**
Due to study design weakness and data gathering insufficiencies in a commercial setting, the significant reductions in post-weaning mortality and antimicrobial usage can only be broadly suggestive of an effect of the vaccine in this preliminary case study. The study does outline the proof of concept for the vaccine's commercial use in the UK. Further work on assessing the effect of this vaccine in UK cattle herds infected with *M. bovis* respiratory disease is warranted.

**Keywords**
Mycoplasma bovis, vaccine, dairy calves, mortality, antibiotic reduction

## Introduction

*Mycoplasma bovis (M. bovis)* is a significant bacterial pathogen of cattle with rising significance in the UK (APHA 2020, Burr 2018). It has recently been cited as the most commonly identified pathogen in bovine respiratory disease diagnoses (APHA 2020, SRUC 2019). Morbidity and mortality rates from pneumonia outbreaks in calves have been reported up to 74% and 30% respectively (Maunsell 2009, Mahmood and others 2017).

*M. bovis* infection can cause acute disease and create chronic carrier animals. Clinical signs are varied: pneumonia, septic arthritis, otitis media, mastitis and less commonly meningitis, caesar wound seromas, keratoconjunctivitis and subfertility (Calcutt and others 2018, Maunsell and others 2011, Nicholas 2011, Nicholas and others 2006). Dudek and others (2013) found *M. bovis* infection facilitated systemic distribution and the immune system became ineffective in clearing *M. bovis* from the host. *M. bovis* spread in an endemically infected herd can be via aerosol, colostrum or milk (Dudek 2020). Calves can be infected from a young age: in one study nasal swabs from calves and vaginal swabs from their dams found 40% were positive for *M. bovis* at 4 days of age, suggesting possible pre or peri-partum infection (Stipkovits and others 2001).

Treatment of *M. bovis* is unreliable, treatment failures are high and increasing antimicrobial resistance patterns have been documented over the last 30 years (Gautier-Bouchardon and others 2014). *M. bovis* has no cell wall rendering penicillins and cephalosporins ineffective and *M. bovis* also has natural resistance to other antibiotics including trimethoprim-sulphonamides (Lysnyansky and Ayling 2016). Confirmation of the ability of *M. bovis* to produce a biofilm helps explain the lack of response to antibiotics and the hosts' immune system (McAuliffe and other 2006).

Bovine mycoplasmosis is a challenging disease to firstly identify and then control. Bacterial culture can be unreliable, time-consuming and be affected by faster growing polymicrobial infections resulting in poor bacterial recovery rates (Calcutt and others 2018).

Control methods of infection include extended antibiotic courses metaphylactically (Williams 2010), segregating and culling infected animals (Nicholas and others 2016) and pasteurising colostrum (Gille and others 2020). Herd biosecurity to prevent disease incursion for *M. bovis* negative herds has been recommended – either a closed herd policy (Calcutt and others 2018) or screening added replacements (Nicholas and others 2016).

Autogenous vaccines have been used to prevent *M. bovis* infection, some successfully in finishing cattle (Nicholas and others 2019), whilst others have demonstrated higher rates of morbidity than the control groups when used in young calves (Maunsell and others 2009). In the UK autogenous vaccines may only be manufactured with recovered isolates epidemiologically linked to the site where the vaccine is to be used (Ridgeway Biologicals 2020). Isolation of *M. bovis* can take several weeks in some cases (Nicholas 2011). Autogenous vaccine manufacture takes 8-10 weeks followed by an on-farm safety test at the herd of origin for every batch manufactured (Ridgeway Biological 2020). Good results can be achieved when vaccines are used correctly and other BRD pathogens are either well controlled or not present (Nicholas and others 2019).

The author is unaware of any published data on the efficacy of US manufactured commercial *M. bovis* vaccines, none of which are licensed for use in very young calves (Nicholas and others 2006), (Maunsell and others 2009). Although they have been licensed for some time in the US, they have not been available in the UK, until now. In April 2019 the author acquired authorisation from the Veterinary Medicines Directorate to allow importation of Myco-B One-Dose (American Animal Health, Texas, USA), a 3 strain *M. bovis* bacterin vaccine into the UK. The first use of the vaccine in the UK was in this study. Therefore the objectives of this study were to evaluate this commercial vaccine under commercial UK field conditions in terms of: safety, ease of incorporation into pre-existing vaccine protocols on commercial UK dairy farms (i.e. 'proof-of-concept'), reducing mortality and reducing antimicrobial use in calves up to 200 days old.

## Materials and Methods

### 2.1 Herd Selection and Vaccine Acquisition

The selection criteria for enrolment included: a previous

herd history of calf pneumonia in dairy calves confirmed or clinically suspected of *M. bovis* and a willingness to participate in the observational study. Only herds with uniformly positive serological findings from 5 home bred calves over 5 months of age (tested positive by *M. bovis* serology, SRUC, Aberdeen, UK) or confirmed *M. bovis* infection in calves via bacterial culture or PCR were selected. Maternal antibody is likely to make a significant contribution to the titres in animals aged less than 4 months and may inhibit seroconversion (Geraghty, personal communication).

Three dairy herds from North East Scotland were enrolled in the study following positive calf serology screening results: DH, TY, and TB. A fourth dairy farm, CU, was enrolled 2 months later after *M. bovis* was confirmed in a clinical outbreak by PCR (*M. bovis* PCR SRUC, Aberdeen, UK) in pre-weaned calves. Most pre-vaccination calves (time period 1) in this study were born in the 'winter' (October/November to April). Most post-vaccination calves (time period 2) were born in the 'summer' (June to Oct). To offset this confounding time-point difference control farms datasets were included in the study. Four farms of comparable management practices and size were selected. Two tested positive for *M. bovis* during the course of the study (PCR and serology – PG and MK respectively), two were not tested for *M. bovis* presence (CS and CG). Pre-vaccination absolute mortality risk data (time period 1) analysis showed no significant difference (P = 0.3703) to reduce any herd selection bias effect. All 8 farms were located within the same region to account for differing geography and seasonal weather variations.

### 2.2 Study Populations

**Vaccinating Farms**

All 8 farms made no material changes to their respective health plans during the 2 time periods.

| Farm | Milking Herd size | Cow Management | Cow Dry Off vax | Calf Rearing | RSV/PI3 vax @ 10d | M. haem + H. somni vax @ 4wks |
|---|---|---|---|---|---|---|
| **TB** | 170 | Summer grazed | Yes | Buckets | Yes | No |
| **DH** | 280 | Housed | Yes | Machine | Yes | Yes |
| **TY** | 140 | Summer Grazed | Yes | Buckets | Yes | Yes |
| **CU** | 240 | Housed | Yes* | Machine | Yes | Yes |
| **MK*** | 260 | Housed | Yes | Machine | Yes | No |
| **CG*** | 180 | Housed | No | Machine | No | No |
| **CS*** | 240 | Housed | No | Machine | No | No |
| **PG*** | 170 | Summer Grazed | Yes | Machine | Yes | Yes |

*Table 1: Farm Management Summary (* - control farms)*

**Housed** – all-year round housed milking/dry cows, summer grazing from May till September, otherwise housed.
**Dry cow vaccination** – Rotavec Corona vaccination (MSD Animal Health UK Ltd, Milton Keynes, UK) given at time off dry off, 8 weeks pre-calving. * CU gave dry cow vaccinations at 4 weeks pre-calving.
**Calf-Rearing** – buckets – paired calves reared on twice daily milk feeding via buckets with teats before weaning into larger groups. Machine – individually reared for up to 10 days before added to a larger rearing group fed by milk machine with an identification collar for each calf.
**RSV/PI3 vaccination** – Rispoval Intranasal (Zoetis UK, Leatherhead, UK) used routinely from 10 days of age in all calves.
*M. haem + H. somni* – Hipra Bovis Somni Lkt (Hipra UK Ltd, Nottingham, UK) at one month old.

### 2.3 Study Design and Reactive Changes

All cows at drying off (or 4 weeks pre-calving – farm CU only) received vaccination with 2mls of Myco-B One-dose injected subcutaneously in the neck area, alongside the herds' routine Rotavec Corona vaccination (MSD Animal Health UK Ltd, Milton Keynes, UK). Calves born to these dams were fed dam's colostrum as per each farms' usual management system. These calves then received a Myco-B booster vaccination at approximately 60 days of age as per datasheet recommendation, alongside the calf vaccine plan described in table 1.

Calves at farm DH began showing mild clinical signs suspicious of M. bovis at 6–7 weeks of age (unilateral ear droop, nasal discharge, reduced milk intakes and inconsistent responses to treatment) in the first batch of calves born to Myco-B vaccinated dams. In response to this the age at first booster for Myco-B at all vaccinating farms was immediately reduced to 4 weeks.

No other changes were made in routine herd management on either vaccinating or control farms.

### 2.4 Study Data Collection

Mortality data was collected for pre-vaccination calves born on all farms between October to April (time period 1) and for post-vaccination calves born (time period 2) between June to October. Calves were observed until 200 days old. Farm CU was enrolled in July. Farm CU's post-vaccination data was collected from July to October.

Farm records supplied for individual calf treatment records and mortality due to suspected pneumonia could not be used due to substantial inconsistencies. Therefore, all mortality records for calves born in each cohort observation period and followed up to 200 days old for each calf, were taken from official farm movement records supplied by Scot EID, Huntly, UK. The control farms were recorded in the same way.

Antimicrobial sales recorded by the author's practice to both vaccinating and control farms were analysed. The four vaccinating farms and two of the control farms had specific calf pneumonia treatments that were not used on other classes of stock and were sold at regular intervals onto farms making them suitable for recording and analysis. Volumes of these direct sales of antimicrobials during each of the two time periods were recorded and converted to a population correction unit/100kg body weight, representing the approximate average liveweight of calves throughout the observation periods (PCU= mg of antimicrobial/100kg bodyweight). Two farms, CG and CS had unusual medicine bulk-purchasing activities rendering this method of data analysis unusable and were judged as non-qualifying.

On at least one occasion during the study, on all vaccinating farms only, up to 6 calves aged 2–7 days old were blood sampled for total protein levels. This was to assess the level of passive immunity, in line with their usual monitoring practises, outlined in their existing health plans. All farms had at least 1 calf measured at below the recommended level of 5.6g/dl TP (MacFarlane and others 2015).

### 2.5 Statistical Analysis

Mortality data was assessed on relative risk of mortality before and after vaccine use (time period 1 – pre-vaccination,

time period 2 – post-vaccination). A Kruskal-Wallis Rank Sum Analysis was performed on pre-weaning, post-weaning and overall relative mortality risks.

Antibiotic usage was analysed as an absolute usage change with a Student's Independent T-Test two-sample assuming unequal variances.

A Post-Hoc Dichotomous Endpoint, Two Independent Sample Study Power Calculation yielded a power of 0.937 for calf mortality during time period 2, with $p<0.05$.

**Results**

1. Safety
No adverse reactions were recorded when using Myco-B One-Shot on any of the vaccinating farms in either cows or calves.

2. Incorporating Myco-B One-Shot into existing Vaccine Plans
During the time each farm remained enrolled in the study all farms successfully completed the vaccine protocols routinely and timeously in both cows and calves. Farmers continued to use the vaccine after the observation period of the study had been concluded, possibly confirming Myco-B's ease of use.

**Calf Mortality**

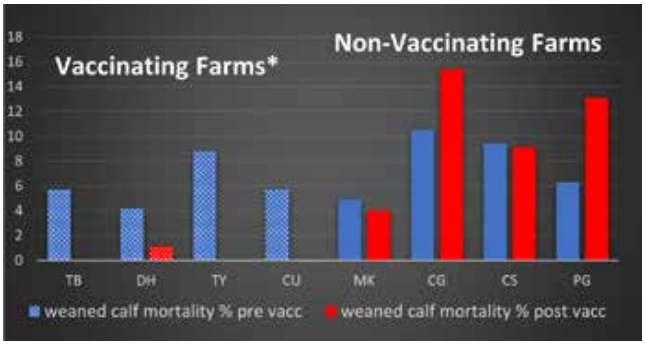


*Figure 1: 4 vaccinating farms on the left, control farms on the right*

Figure 1 shows individual weaned calf mortality as % of calves born during each recording period pre and post vaccination. All vaccinating farms had reduced post-weaning mortality; all controls had similar or increased mortality.

Table 1. Kruskal Rank Sum Test Analysis of mortality risk across all farms during both time periods (TP). TP1 describes time period 1, before any vaccine used. TP2 describes time period 2, when vaccinating farms began using the vaccine. When the relative risk of mortality is equal to 1 then no difference in risk has been observed pre and post vaccination. If the relative risk is below 1 then a reduction in mortality has been observed.

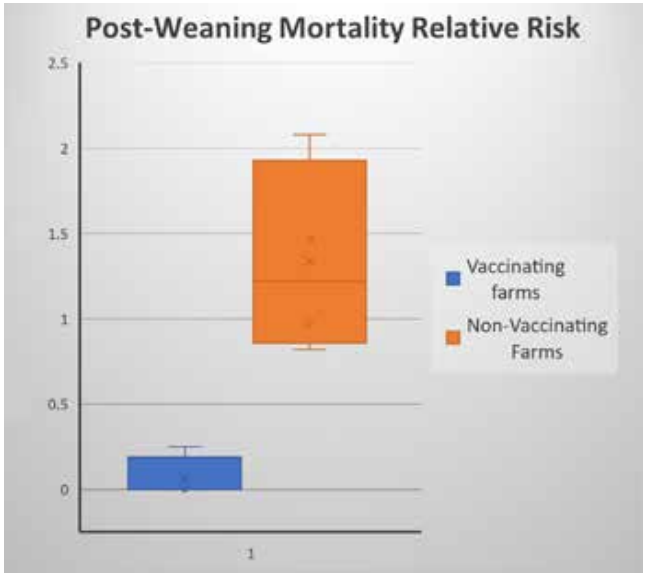


*Figure 2: Box and Whiskers of Post-weaning Mortality significant reduction on post-weaning mortality on vaccinating farms p <0.02*

Post-weaning mortality risk showed a significant reduction after vaccination. There were no other significant changes in risk of mortality after vaccination.

| Farm | Mortality Stage | Vacc | Calves TP1 | TP1 Mort | Risk TP1 | Calves TP2 | TP2 Mort | Risk TP2 | Relative Risk |
|---|---|---|---|---|---|---|---|---|---|
| **TB** | Prewean | 1 | 106 | 1 | 0.009 | 82 | 2 | 0.024 | 2.67 |
| **DH** | Prewean | 1 | 192 | 4 | 0.021 | 176 | 8 | 0.045 | 2 |
| **TY** | Prewean | 1 | 113 | 4 | 0.035 | 89 | 8 | 0.09 | 2.57 |
| **CU** | Prewean | 1 | 123 | 6 | 0.049 | 61 | 3 | 0.049 | 1 |
| **MK** | Prewean | 0 | 122 | 4 | 0.033 | 101 | 2 | 0.02 | 0.61 |
| **CG** | Prewean | 0 | 86 | 3 | 0.035 | 65 | 2 | 0.031 | 0.89 |
| **CS** | Prewean | 0 | 96 | 2 | 0.021 | 55 | 8 | 0.145 | 6.9 |
| **PG** | Prewean | 0 | 64 | 4 | 0.063 | 61 | 0 | 0 | 0 |
| **TB** | Postwean | 1 | 106 | 6 | 0.057 | 82 | 0 | 0 | 0 |
| **DH** | Postwean | 1 | 192 | 8 | 0.042 | 176 | 2 | 0.01 | 0.25 |
| **TY** | Postwean | 1 | 113 | 10 | 0.088 | 89 | 0 | 0 | 0 |
| **CU** | Postwean | 1 | 123 | 7 | 0.057 | 61 | 0 | 0 | 0 |
| **MK** | Postwean | 0 | 122 | 6 | 0.049 | 101 | 4 | 0.04 | 0.82 |
| **CG** | Postwean | 0 | 86 | 9 | 0.105 | 65 | 10 | 0.154 | 1.47 |
| **CS** | Postwean | 0 | 96 | 9 | 0.094 | 55 | 5 | 0.091 | 0.97 |
| **PG** | Postwean | 0 | 64 | 4 | 0.063 | 61 | 8 | 0.131 | 2.08 |

*Table 2: mortality data before and after vaccination on all farms*
*Relative risk = <1 = reduction in risk of mortality in second time period >1 = increase in risk of mortality in second time period, TP = Time Period*

**Antimicrobial Usage**
Calf Pneumonia Antibiotic Use*

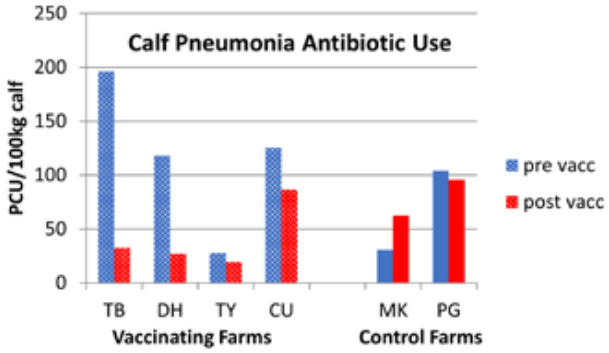


*Figure 3: Calf pneumonia antibiotic usage as population correction unit/100kg liveweight (approximated average weight throughout observation periods) Significant reduction in post-vaccination antibiotic use on the vaccinating farms (p<0.05) compared to non-vaccinating farms with qualifying data.*

Reduction in antibiotics after vaccination compared to 2 control farms using an Independent T-Test two-sample assuming unequal variances saw a significant reduction (P <0.05).

All vaccinating farms showed a reduction in the calf pneumonia antibiotic use PCU/100kg of calf pneumonia specific antimicrobials purchased by farm per live calf born during observation period.

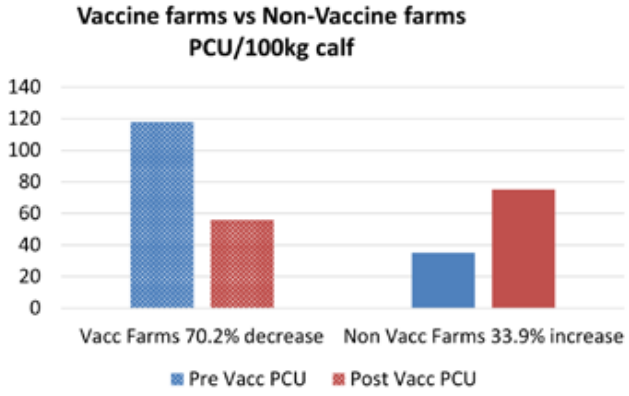


*Figure 4: Antibiotic usage on vaccinating and non-vaccinating control farms*

The four vaccinating farms had a combined antibiotic usage reduction of 70.2% against a 33.9% rise in antibiotic usage on the combined non-vaccinating control farms with qualifying data.

**Overall Results**
The commercial 3-strain *M. bovis* bacterin vaccine assessed in this study caused no adverse reactions, was easily incorporated into different pre-existing vaccination protocols and was effective in reducing post-weaning mortality as well as antimicrobial usage on the vaccinating farms.

**Discussion**
The vaccination programme used in this study was 'off-label' from the supplied datasheet (calves mostly vaccinated at 30 days of age, instead of recommended 60 days). Dams were vaccinated during the dry period to potentially prevent *M. bovis* infection of the calf before birth (Stipkovits and others 2001) and reduce the shedding of *M. bovis* and potentially increase *M. bovis* specific antibodies in the colostrum (Gille and others 2020). Seasonal calving herds have seen a higher bulk tank PCR *M. bovis* presence 5-8 weeks after starting calving suggesting the post-calving period is a higher risk time for *M. bovis* shedding (Parker and others 2017) so potentially important to target in a vaccination programme. Further work with this vaccine on assessing the transfer of colostral *M. bovis* antibodies to neonatal calves and reduction in *M. bovis* shedding in colostrum is necessary. However, Maunsell and others (2009) showed no correlation with higher levels of *M. bovis* specific post-colostral antibodies and reduction in clinical signs associated with *M. bovis* infection. Pre- and post- calf vaccination *M. bovis*serum antibody levels would have been of interest especially given the 'off-label' use of the vaccine at 4 weeks of age complicated by the potential interference of maternally derived antibodies.

The study design and data gathering were sub-optimal because all calf mortalities recorded on official movement records had to be utilised, therefore non-respiratory causes of death were included. This was due to a lack of reliable cause-of-mortality data from all the farms involved. However, one study showed the most common cause of Holstein heifer mortality between 101 and 160 days was respiratory disease (Zhang and others 2019). This would suggest the reduction seen in this current trial in this comparable age group (time period 2, post-vaccination) was due to a reduction in respiratory disease. Better data gathering for farmer-recorded cause of mortality alongside on-farm post-mortems and pathogen identification would have been helpful to confirm this.

The significant reduction in antimicrobial use needs to be analysed in context. The author is unaware of any standardised PCU for rearing/weaned calves so the arbitrary weight of 100kg throughout the 2 times periods was used. The AMU measurements were antimicrobials prescribed by the author's practice to each farm during the 2 observed time periods, classed as calf-pneumonia specific as laid out in each farms' existing health plan. The precise time of use and which group received the medicines were not made available for the study. A period of overlap existed from May till October when either cohort could have received pneumonia treatments, potentially reducing the drop in AMU seen post vaccination.

Previous *M. bovis* vaccine studies have recorded individual calf records (Maunsell and others 2009, Nicholas and others 2019). However, these studies observed 373 and 141 calves respectively. This study observed 1581 in total, including 398 vaccinated calves. More accurate recording of calf medications and morbidity would have been beneficial in this study.

Previous vaccine studies have been based on single strain bacterin preparations (Maunsell and others 2009, Nicholas and others 2019). Using a multiple strain vaccine, as in this trial, has previously been suggested as a way of improving *M. bovis* vaccine efficacy (Calcutt and others 2018). It has been suggested that vaccine development should be based on conserved recombinant proteins, not bacterin-based vaccines (Perez-Casal and others 2017); trials using recombinant protein vaccines have been unsuccessful to date (Prysliak and others 2018).

This would suggest that currently bacterin-based vaccines, such as Myco-B One-Shot, provide the most promise for effective vaccination against *M. bovis*. The immediate availability of this commercial vaccine may prove useful in giving practising vets another control option

for *M. bovis* on commercial UK dairy farms when combatting rising calf mortality rates (Mee 2013) and increasing Dairy AMU in the UK (VMD 2019).

**Conclusion**

Due to study design weakness and data gathering insufficiencies in a commercial setting, the significant reductions in post-weaning mortality and antimicrobial usage can only be broadly suggestive of an effect of the vaccine in this preliminary case study. The study does outline the proof of concept and safety for the vaccine's commercial use in the UK. Further work on assessing the effect of this vaccine in UK cattle herds infected with *M. bovis* respiratory disease is warranted.

**Acknowledgements**

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**Graeme Fowlie**

Graeme Fowlie is a director of Meadows Vets Centre, a practice based in Aberdeenshire. Graeme lives in rural Aberdeenshire and in between work and looking after his young family has time for the odd game of cricket with the MCC (Methlick Cricket Club) at Lairds. Meadows is a very mixed practice with strong links to the local family farms of the area (which supply many of the current staff, Graeme included). Graeme has always had a strong interest in pneumonia prevention since helping 'dose beasts as a young loon' on the family farm before graduating from Glasgow Vet School. The mycoplasma vaccine project finally got off the ground when Graeme had an unexpected 'break' from clinical work due to a broken leg one winter. Utilising the practice's strong trading links with Kernfarm and with the assistance of the extremely helpful staff at the VMD the successful importation of a US-manufactured multi-strain mycoplasma bovis vaccine to the UK was finally achieved in April this year.

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