UNIVERSITI PUTRA MALAYSIA

SEROPREVALENCE OF MELIOIDOSIS AMONG CATTLE IN FOSTER FARM PROGRAMME OF THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA.

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In partial fulfilment of the requirement for the
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It is hereby certified that we have read this project paper entitled "Seroprevalence of Melioidosis among Cattle in Foster Farm Programme of the Faculty of Veterinary Medicine, Universiti Putra Malaysia" by Muhamad Syazrin bin Abd Kadir and in our opinion it is satisfactory in term of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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DEDICATION

I would like to dedicate this project to:

ABD KADIR & NORHAYATI

(ABAH & IBU)

And all people that I love…

ACKNOWLEDGMENTS

In the name of Allah, the most Merciful and the Most Compassionate

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SEROPREVALENS MELIOIDOSIS ANTARA LEMBU DI DALAM PROGRAM LADANG ANGKAT FPV, UPM.

Oleh

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2017

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Dalam usaha untuk memajukan Malaysia sebagai hab halal haiwan ternakan, Malaysia hendaklah bebas daripada penyakit berjangkit. 'Melioidosis' adalah endemik di Asia Tenggara dan utara Australia. Penyakit ini juga memberi impak besar terhadap kesihatan haiwan serta mengurangkan produktiviti haiwan dan kehilangan protein. Kajian sebelum ini mengenai seroprevalens terhadap 'Melioidosis' kepada haiwan ternakan pada 2000-2009 telah dijalankan oleh Jabatan Perkhidmatan Veterinar Malaysia serta menunjukkan bukti daripada 100.262 haiwan yang diuji, sejumlah 5,729 (5.7%) adalah positif untuk 'Melioidosis'. Sejak itu tidak ada kajian susulan.

Kajian ini memberi tumpuan kepada seroprevalens terhadap ‘Melioidosis’ dalam Program Ladang Angkat FPV, UPM. Lima puluh lembu dengan campuran umur dan jantina telah dipilih secara rawak untuk pengumpulan sampel darah daripada Program Ladang Angkat FPV, UPM. Uji Fiksasi Komplemen (CFT) telah digunakan untuk mengesan antibodi terhadap Melioidosis. Hasil dari CFT menunjukkan negatif untuk semua sampel diperoleh daripada Program Ladang Angkat FPV, UPM. Kekurangan kes positif mungkin disebabkan oleh bilangan sampel yang kecil dan juga yang berkaitan dengan amalan pengurusan di mana lembu kebanyakannya disimpan di bawah sistem separa intensif di mana mereka kurang terdedah dengan tanah dan oleh itu risiko yang lebih rendah untuk dijangkiti organisma. Ini menunjukkan terdapat seroprevalens sifar dalam Program Ladang Angkat FPV, UPM, yang bebas daripada ‘Melioidosis’.

Kata kunci: Melioidosis, Seroprevalence, Uji Fiksasi Komplemen, Ladamg Angkat UPM

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine (FPV) in partial fulfilment of the course VPD 4999 - Project.

SEROPREVALENCE OF MELIOIDOSIS AMONG CATTLE IN FOSTER FARM PROGRAMME OF FPV, UPM.

By

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2017

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In the endeavour to advance Malaysia into a 'halal' hub the livestock animals should be free from infectious disease. Melioidosis is endemic in South-East Asia and northern Australia. The disease is also a significant animal health that reduces the productivity of animals and loss of valuable animal protein. A previous study on the seroprevalence of Melioidosis in livestock animals in 2000-2009 was carried out by the Department of Veterinary Services Malaysia indicate evidence of 100,262 animals tested, a total of 5,729 (5.7%) were positive for Melioidosis. Since then there was no follow-up study. This study focuses on seroprevalence of Melioidosis in Foster Farm Programme of FPV, UPM. Fifty cattle with mix-age and gender were selected randomly for blood sample collection from the Foster Farm Programme of FPV, UPM.

Complement Fixation Test (CFT) was used to detect antibody against melioidosis. The result from CFT showed negative for all samples obtained from Foster Farm Programme FPV, UPM. The lack of positive case probably due to small sample number and also related to the management practices where the cattle mostly kept under a semi-intensive system in which they had less contact with soil and therefore at a lower risk of contracting the organism. This indicates there is zero seroprevalence in Foster Farm Programme FPV, UPM, which is free of Melioidosis.

Keywords: Melioidosis, Seroprevalence, Complement Fixation Test. Foster Farm UPM

1.0 INTRODUCTION

Melioidosis is a saprozoonosis caused by soil saprophytic bacterium *Burkholderia pseudomallei*. The disease is endemic in Southeast Asia and northern Australia (Puthuchery et.al 1995). It is a significant public health problem because of its propensity to affect poor rural populations, immune-suppressed individuals and the death in facilities for its accurate diagnosis in the affected regions (Inglis and Sousa, 2009). The disease is also a significant animal health problem leading to chronic debility that reduces the productivity of animals and loss of valuable animal protein due to the condemnation of carcases at the abattoir (Ketterer et.al 1986). Moreover, it is an emerging infectious disease with serious public health implications in most countries (Center for Food Security and Public Health, 2003). The disease is mostly transmitted through ingestion and inhalation of contaminated water and/or soil. Transmission occurred when infected animal body fluids such as urine, milk or nasal secretion or blood came into direct contact with susceptible hosts depending on the site of infection or through contact with infected soil or water (Bettey et al, 2002; Center for Food Security and Public Health, 2003); as well infection appears to be the inhalation and aspiration of contaminated dust particles (Thomas et al, 1988).

Subclinical diseases are regular in the animal, and asymptomatic abscesses might be found at slaughter. Symptomatic melioidosis might be acute, subacute or chronic, and mild or severe. The lungs, spleen, liver and related lymph nodes are regularly involved in the animal, yet any organ can be influenced. Melioidosis has rarely been described in cattle. Most cases in adult cattle have been chronic. Fever, dyspnea, continuous profuse salivation and neurologic signs were reported in one

animal. (The Center for Food Security & Public Health, 2016). At necropsy, the major findings are multiple abscesses containing thick, caseous, greenish-yellow or off-white material. The Center for Food Security & Public Health of Iowa State University also stated that the abscesses are generally not calcified and the regional lymph nodes, lungs, spleen, liver and subcutaneous tissues are most often involved, but abscesses can occur in most organs.

Data from Department of Veterinary Services Malaysia stated that from the year 2000 to the year 2009 the seroprevalence rate in animals was 7.6, 48.2, 2.6, 13.6 and 3.6% in cattle, buffaloes, goats, sheep and pigs respectively. The seroprevalence of the disease varies in different states of the federation. For all species, the seroprevalence varies between 2.6% and 48.2%. The seroprevalence over the years increased from 4.2% in 2000 to 12.0% in 2003 after which it varies between the period 2004 - 2007 and apparently declined between 2007 and 2009. In veterinary diagnosis, serology has always been used for detection *Burkholderia pseudomallei* whereby anti-Burkholderia antibodies are detected in cattle (Malaysian Journal of Veterinary Research, 2012). From the international conference on One Health and 24th VAM Congress 2012, there is a retrospective study which was carried out to collect data on total number livestock were recorded positive for Melioidosis in Malaysia on 2007 to 2011. The study was based on documented confirmed cases of Melioidosis that occurred in Malaysia in the 5 years from January 2007 until December 2011. All data were extracted from Laboratory Information and Management System, VRI (LIMS).

The complement fixation test is one of the major conventional tests for the demonstration of the presence of specific antigens or antibodies (Acharya, 2016). In

the positive test, the available complement is fixed by antigen-antibodies complex and no haemolysis of red blood cell (RBC)s occurs. Therefore the test is positive for the presence of antibodies. In the negative test, there is no antigen-antibodies reaction occurs and the complement is free. The free complement binds to the complex of RBC and its antibody causes haemolysis and the development of pink colour (Giri, 2015)

There were no screening up to date have been conducted on Melioidosis status among cattle under the Foster Farm Programme of The Faculty of Veterinary Medicine (FPV) Universiti Putra Malaysia (UPM). Thus, this study was designed to obtain the current status of seroprevalence of Melioidosis among cattle under the Foster Farm Programme FPV, UPM. This study also will identify the risk factor and its association towards the seroprevalence of the Melioidosis in the selected cattle farms in Foster Farm Programme FPV, UPM.

It is important to know the current status of Melioidosis in Foster Farm Programme FPV, UPM for biosecurity, planning of herd health programme and safety of students, staff and farm workers as Melioidosis in zoonotic and importance. Prior to planning detailed HHP, all the importance disease status such as Melioidosis is needed.

2.0 LITERATURE REVIEW

2.1 Aetiology Agent and Epidemiology

Burkholderia pseudomallei is a Gram-negative bacillus that can be naturally found as an environmental saprophyte in soil or stagnant water (Currie, 2003). In the laboratory, *B. pseudomallei* cultivates aerobically on most agar media and produces clearly observable colonies within 24-48 hours at 37°C (White, 2003).

The organism is universal throughout Southeast Asia, northern Australia, and the South Pacific. *B. pseudomallei* that had been introduced to new environments with the export of animals and shipments of contaminated soil and water could potentially produce the same results (Choy, 2006).

Ouadah, Zahedi and Perumal (2006) stated that Animal Disease Research Centre (ADRC) in Kota Kinabalu, Sabah records showed the most common disease was pasteurellosis followed by Melioidosis during the period between 1994 and 2003. Their epidemiology study showed the data on animal Melioidosis was obtained from ADRC records for the period 1994 to 2003 that 341cases of Melioidosis were identified from 12139 cases of post-mortem.

As reported by Quadah, Zahedi, and Perumal (2006) large ruminant such as cattle and buffalo were rarely seen infected with Melioidosis. Perhaps, the inactive behaviours of these animals on pasture lessen the contact with the disease agents. In conjunction with, the animal may have developed some tolerance or resistance to the bacteria, e.g buffaloes are semi-aquatic spending a large part of their time either in

water or swamp wallows, therefore protecting skin injuries or abrasion from infections (Quadah, Zahedi, & Perumal, 2006).

2.2 Transmission

B. pseudomallei is the etiologic agent of Melioidosis, a serious infection acquired by ingestion, inhalation or inoculation (Cheng & Currie, 2005). Infection is thought to be opportunistic and primarily a result of transmission from the environment (eg, contaminated soil and surface waters) rather than from animal to animal (Choy, 2006).

In addition, infection in humans and animals is believed to occur by inoculation, ingestion or inhalation of environmental organisms (Leelarasamee & Bovornkitti, 1989). Animal-to-human transmission has rarely been documented but can result in mortalities (Yap et al., 1995; Idris et al., 1998; Choy et al., 2000) as *B. pseudomallei* has a tremendously wide-ranging host range.

2.3 Clinical Signs

Melioidosis is most commonly seen in sheep, goats, and pigs, other affected species include cattle, buffalo, horses, mules, deer, camels, alpacas, dogs, cats, dolphins, wallabies, koala, primates, birds, tropical fish, reptiles, and people (Choy, 2006).

Bovine Melioidosis is very rare and has a tendency to run a chronic course in mature animals (Nicholls, 1930; Laws & Hall, 1963; Egerton, 1964; Laws & Mahoney, 1964; Ketterer et al., 1975). Nicholls (1930) determined that susceptibility

to *B. pseudomallei* in cattle is low but abscess formation may occur from infection. Egerton (1964) reported a case of bovine Melioidosis which was diagnosed post-mortem. Ketterer et al. (1975) reported two cases of Melioidosis in south-eastern Queensland after a severe flood. The clinical signs observed in one case were fever, aggressive behaviour, rapid, panting respiration, continuous profuse salivation and staggering gait. The additional case developed acute arthritis after a deep cut developed into a chronic granulating sinus. One case of acute Melioidosis in a calf has been described. Some authors guessed that cattle and water buffalo may be immune to *B. pseudomallei* (Strauss et al., 1969). In cattle, abscesses and nodules can be found in the lung and spleen (Nicholls, 1930; Egerton, 1964). Experimental inoculation leads to local abscess formation and resolution (Nicholls, 1930; Laws & Hall, 1963). Adrenal abscess, meningoencephalitis and meningitis have been reported by Laws and Hall (1963).

2.4 Pathogenesis

The virulence of *B pseudomallei* appears to fluctuate among isolates, but these virulence factors are not well understood. The incubation period ranges from a few days to months or even years. *B pseudomallei* is a facultative intracellular pathogen that can remain inactive for many years before emerging as an active infection (Choy, 2006).

B. pseudomallei like many soil bacteria is a difficult organism to eradicate. It can last in triple distilled water for years (Wuthiekanun, Smith, & White, 1995) and yet it has the capability to transcend the environmental saprophytic state to become a

pathogen of humans and animals (Puthucheary, 2009). Melioidosis is an interesting infection in terms of pathogenesis. The result of the host-pathogen interaction ranges from asymptomatic seroconversion (the time period during which a specific antibody develops and becomes detectable in the blood) to rapidly lethal and sepsis (Puthucheary, 2009). Amongst these extremes, the infection may run a chronic or relapsing course, or remain latent for many years before recurrence into an active infection. This consequence will depend on the relationship of several factors such as the size of the inoculum, the virulence of the infecting strain and the susceptibility of the host as well as possible as yet unknown genetic factors (Puthucheary & Vadivelu, 2002).

2.5 Diagnostic Tests

Culturing *B. pseudomallei* is currently considered to be the gold standard test for Melioidosis in both animals and people (Center for Food Security & Public Health, 2016). It will grow on most media including blood agar. Selective media such as Ashdown's medium are often used in endemic regions.

The diagnostic value of all serological tests is debatable in endemic areas, as healthy individuals may display persistent IgG levels. In non-endemic areas, the tests might be convenient for detection of chronic infections (Jesudason et al., 2001; Wongratanacheewi et al., 2001; O'Brien et al., 2003). It can be presumed that all tests described so far cross-react with antibodies raised against *B. psuedomallei*. Regardless of all these limitations, serology has constantly been used in veterinary medicine for the identification of anti-Burkholderia antibodies, such as in horses, goats and dairy

cows (Thomas et al., 1988; Srikitjakarn et al., 2002). In a study evaluating indirect haemagglutination (IHA), complement fixation (CF) and microtitre agglutination, Thomas et al. (1988) established that screening with the IHA and confirmation using the CF test is sensitive and specific in caprine melioidosis (Thomas et al., 1988)

Serologic tests such as complement fixation and indirect hemagglutination are effective herd surveillance tools (Choy, 2006). Thus, CFT was used to study the seroprevalence of Melioidosis among cattle in foster farm programme of FPV, UPM.

2.6 Treatment and Prevention

Appropriate antibiotics are needed for treatment, thus it should be based on culture and sensitivity results. Treatment may be expensive, prolonged, and possibly unsuccessful, with the risk of recurrence once treatment is discontinued. The possibility of underlying immunosuppressive conditions should be investigated in less susceptible species (Choy, 2006).

Melioidosis is a fatal disease and treatment is rarely attempted in animals as it requires long-term antibiotic treatment. Due to the risk to human health, affected animals should be euthanised (Fitzpatrick, 2008).

An ounce of prevention is worth a pound of cure. Preventive measures are more practical and economical in intensive farming environments and involve raising the animals off the soil, especially avoiding exposing animals to muddy or water swamped regions and providing clean drinking water via chlorination and filtration (Choy, 2006). Furthermore, Fitzpatrick (2006) stated measures that may reduce disease occurrence consist of restrictive access of animals to high-risk areas and/or

provide drainage to prevent surface water accumulation. Thus, minimization of environmental contamination by diseased animals is also an important control measure.

3.0 METHODOLOGY

3.1 Blood Sample Collection

Four cattle farms under Foster Farm Programme of Faculty Veterinary Medicine, UPM were selected including university farm in 'Taman Pertanian' Universiti, UPM. 50 cattle with mix-age and gender were selected via random-convenient sampling in the total population of all the farms. Blood collection was performed via jugular venipuncture using 18G, 1inch, vacutainer into 2/3 of a plain red tube and stored in the ice box during collection on the farm.

3.2 Serum Collection

Plain tubes were placed in the centrifuge machine. Then, plain tubes were centrifuged at 3000 revolutions per minute (rpm) for 5 minutes. The serum was transferred with a pipette to the Eppendorf. Serum should be clear and free from all red cells. At least 1000 µl serum on each plain tube was collected, then separate them into two Eppendorf each 500 µl which stored at a different temperature. The serum sample used for the complement fixation test were stored at -20°C while backup serum was maintained at -80°C.

3.3 Complement Fixation Test (CFT)

To conduct the test, the materials compulsory to run the test are test serum, one known negative serum and one known positive serum, diluent glucose normal saline

(GNS), diluent and standard solutions; Melioidosis antigen, Melioidosis complement, and hemolytic system.

Serum was inactivated at 58°C in water bath for 50 minutes. Then, 25 µl serum was transferred into the well H. 25 µl diluent was added into wells number A (anti-complementary control well to replace the Melioidosis antigen), C, D, E, F, and G. Titration was performed from well H to G, F, E, D, and C. Then, 25 µl of inactivated serum from well H was transferred into the well A (control well). Standardised 25 µl of Melioidosis antigen was added into wells C, D, E, F, and G. 25 µl of the Melioidosis complement was added to well A, C, D, E, F, and G. Then, incubate the plates at 37°C for first 15 minutes. Next, 25 µl of the haemolytic system was added to wells A (control well), C, D, E, F, and G. All the content in the plates were mixed using a shaker and incubated at 37°C for another 15 minutes. All the plates were shaken and left to settle at room temperature for 2-3 hours before reading the result.

Table 1: Dilution of serum in each well

Well No	Dilution
A	1/64
B	(empty)
C	1/32
D	1/16
E	1/8
F	1/4
G	1/2
H	Inactivated serum

3.4 Interpretation of Result

Row A that contain no antigen would not give fixation of complement, hence complete lysis shall occur. Under other conditions, the serum can be considered as Anti-Complementary (A/C) if there is fixation occurred. In the positive well, there will be button formation which is a fixation of complement. Whereas in the negative well, the result shows complete lysis or no button formation meaning there is no fixation of complement.

RESULTS

50 serum samples from our cattle farms under Foster Farm Programme of FPV, UPM including cattle farm in TPU, UPM were tested to detect the presence of an antibody the test system is formed by the patient's serum and a known antigen.

Table 2: Complement Fixation Test result.

FARM	SAMPLE COLLECTED	POSTIVE	NEGATIVE	ANTI-COMPLEMENTARY
Farm 1	12	0	8	4
Farm 2	13	0	13	0
Farm 3	14	0	14	0
Farm 4	11	0	11	0
TOTAL	50		46	4

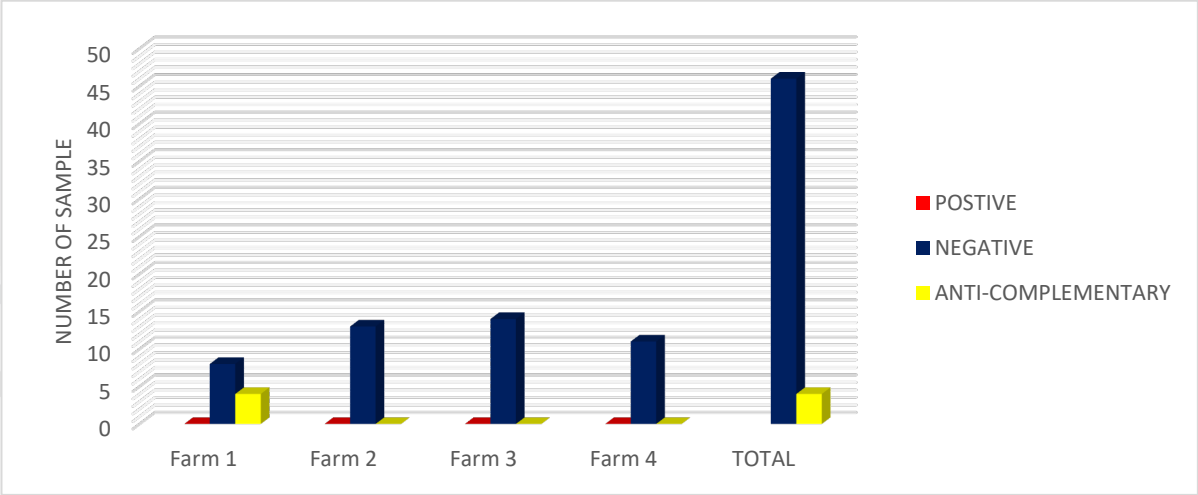

Figure 1 Complement Fixation Test result.

Table and bar chart above showed the Complement Fixation Test (CFT) result from cattle farm in Foster Farm Programme. The result revealed negative result except 4 samples from first farm had anti-complementary activity.

This indicates zero serological prevalence of Melioidosis in cattle farms under Foster Farm Programme FPV, UPM and TPU UPM.

DISCUSSION

Serological test result reveals negative results except for 4 samples from first farm having anti-complementary activity. Anti-complementary activity means the characteristics of some serum to inhibit haemolysis to varying degrees (Lennette & Schmidt, 2003). To identify this property a serum control (test run without antigen) is included for each serum.

According to Lennette and Schmidt, the anti-complementary activity can be induced by immunoglobulin aggregation, rheumatoid factors or drugs and can repeatedly be found in haemolytic or contaminated serum and after repeated freezing and thawing. In order to make the serum available to be analysed in the CFT, serum should be pre-treating with undiluted complement which will absorb the anti-complementary activity.

For future use, we may perform pre-treatment; the serum will be pre-treated with undiluted complement. If persists in pre-treated serum, a new serum sample must be obtained. Patients whose serum repeatedly shows anti-complementary activity should be examined for pathological states such as autoimmune disease.

The lack of positive case probably due to small sample number and also related to the management practices where these animal species, mostly kept under a semi-intensive system in which they had less contact with soil and therefore at a lower risk of contracting the organism.

In terms of management, risk factor also was observed in the farms. Risk factors that being identified which are associated with the farm's management were,

the presence of other species (dogs, goat & sheep), bush clearing around the farms and flooding or waterlogging. Intended for risk factor bush clearing activities amplified the risk of exposure to *B. pseudomallei* as this activity typically comprises spreading of large amounts of dust particles into the atmosphere, thereby increasing chances of bacterial transmission to animals in the surrounding farms (Strauss et al 1969). This may have caused by bringing up *B. pseudomallei* located in the deeper layers of soil to the surface, thereby increasing chances of transmission to animals in the surrounding farms. *Burkholderia pseudomallei* usually favour deeper layers of soil with higher moisture content (Palasatienet al. 2008; Kaestli et al. 2009). Moreover, the presence of animals such as livestock, dogs and wallabies was found to be significantly associated with high *B. pseudomallei* in the environment (Kaestli et al. 2009). Lastly, the discovery of the effect of flood and/or waterlogging condition is consistent with the views of Munckhof et al. (2001) who stated that flooding enables an increase in infections of animals and humans with *B. pseudomallei.* The only risk factor showed on the farms is the presence of other species. However, not significant in this study due negative result obtained.

Furthermore, the number of cattle tested in the study is low and the outcome may not be truly representative of the situation in cattle farm in Malaysia. Therefore, larger sample size are required to obtain a more accurate result.

According to Kadam and Bhalerao (2010), we can estimate the effect size based on previously reported or preclinical studies. It is important to note that if the effect size is large between the study groups, then the sample size required for the study is less and if the effect size between the study groups is small, the sample size

required is large. In this study, the size was estimated by using prevalence reported by the Department of Veterinary Services which is seroprevalence of Melioidosis among livestock in Malaysia from 2000-2009.

As a recommendation for future study, more farms need to be included from all states of Peninsular Malaysia to obtained accurate seroprevalence of this disease.

CONCLUSION

Serological prevalence study revealed that there is zero seroprevalence among cattle in Foster Farm Programme of FPV, UPM. The lack of positive case probably due to small sample number and also related to the management practices. Good management practice is essential in order to prevent disease.

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