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ASSISTANCE IN THE PRODUCTION OF VETERINARY DRUGS IN SADCC COUNTRIES

DP/RAF/86/012

ZAMBIA

Technical report: The supply of veterinary drugs and vaccines in Zambia*

Prepared for the Government of the Republic of Zambia
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

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Vienna

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INTRODUCTION

Zambia is a landlocked country of some 743,000 sq.km, between 8° and 17° South of the Equator, bordered by Tanzania, Angola, Namibia, Botswana, Zimbabwe, Mozambique and Malawi.

The human population was estimated at 5.8millions in 1980 growing at at least 3.1% p.a.

About 24% of the total land area is suitable for arable agriculture, which provides livelihoodfor about 60% of the population, but only constitutes about 17% of G.N.P. The economy is totally dominated by copper production which is now in serious decline.

Livestock production is concentrated mainly in the South, South East and North of the country.

1 LIVESTOCK POPULATION AND PRODUCTION TRENDS

Livestock numbers were derived from annual reports of census by the Ministry of Agriculture and Water Development (Annex 1 and 2). These census excluded poultry, and commercial cattle were not always included. Even when they were, no distinction was made between beef and dairy cattle. Cattle population between 1964 and 1979 (Annex 1) increased by 68.7 over the 16 years, representing an average increase of 4.2% p.a. However, between 1979 and 1985 (Annex3) the average increase dropped to 2.5% p.a. The increase in the numbers of commercial cattle, over the same six years was even lower at 2% p.a.

Thus the increase of the cattle population as a whole over the six years prior to 1986 averaged 2.2% p.a. (Annex 3).

The reasons for the decline in recent years include draught, between 1980-1983, and the larger increase of cost of cattle production compared to smaller livestock and poultry. Although there is new emphasis on the importance of agriculture and livestock industry in the economic life of the country, it is thought unlikely that the rate of increase of cattle in the traditional sector would exceed 3% p.a., and that in the commercial sector, 2-2.5%.

The number of sheep in the country is very small, just over 30,000 in 1985 showing an average decrease of 1.1% p.a. over the last six years. Their number is not likely to increase in the next few years. However, with more commercial involvement in sheep breeding it is hoped that during the last decade yearly increases may average 2% leading to ca. 36,000 animals in the country.

The number of goats increased by an average of 2.9% p.a. over the last six years, reaching almost 400,000 in all. They are likely to increase by the same rate during the next 15 years increasing to ca. 0.57million by the year 2000.

The number of commercial pigs increased steadily during the last six years averaging 4.6% p.a. However, continued increase even at that rate is somewhat prejudiced by the shortage of dependable and competitively priced compounded feed. Should an average increase of 4% p.a. be achieved, just under 0.25million pigs should represent the commercial sector by the end of the century. There are, in addition, some 150,000 swine (estimated number) in the country kept around small holdings as scavengers, but these seldom receive any medication at all.

The number of poultry between 1970-1976 increased very rapidly indeed (Annex 4) averaging 35% p.a. This was followed by a sharp decline in the ensuing three years averaging -5%, due to a combination of factors, including lack of good quality compounded feed (shortage of feed supplements and protein) and a decline in purchasing power of potential consumers. The decline in the poultry sector affected the commercial producer primarily because of its heavy reliance on compounded feed. At the present time (1986) it is estimated that there are 0.8-1.0million layers and about 2.0million broilers in commercial poultry farms. It is difficult to predict what is going to happen over the next 15 years or so in a lifestock sector which is capable of dramatic changes in either direction over a very short period of time. It is anticipated that there would be a modest increase of 3% p.a. in the next few years, increasing perhaps to 5% p.a. during the last decade of the century.

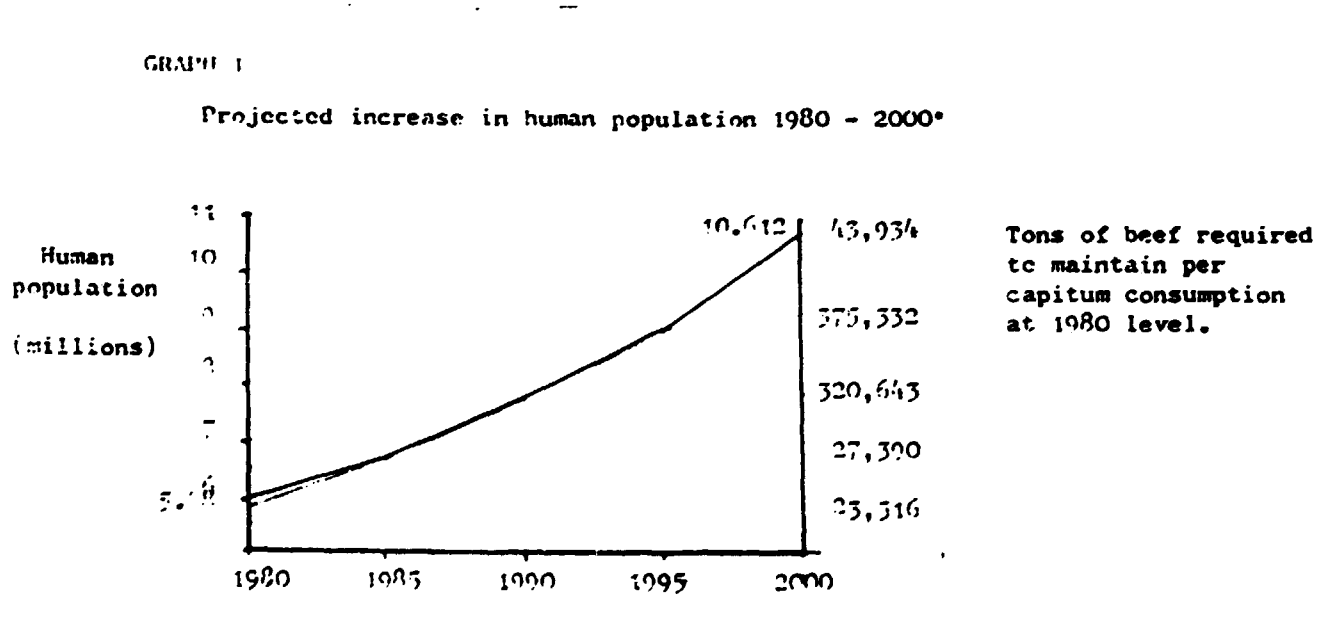
This would increase the number of poultry, including all but day-old chickens, to 14.7 million and 22.0million by 1990 and 2000 respectively. These forecasts, however, must be viewed with caution since there is no assurance at the present time that the feed supply would improve significantly.

From the point of view of vaccine usage the output of the large hatcheries is of the greatest importance. These hatcheries also export many millions of chickens to neighbouring countries. Although their fortunes have also undergone quite dramatic changes in the 1970's, they recovered in more recent years. It is estimated that they produce some 16-17.0million day-old chicks at the present time, increasing to over 18.0million by 1990 and almost 24.0million by year 2000.

Reference to Graphs 1 and 2 show that in order to maintain even the 1980 level of beef consumption per capita, over 7000 tons of beef needs to be imported or beef production increased by over 20 per cent.

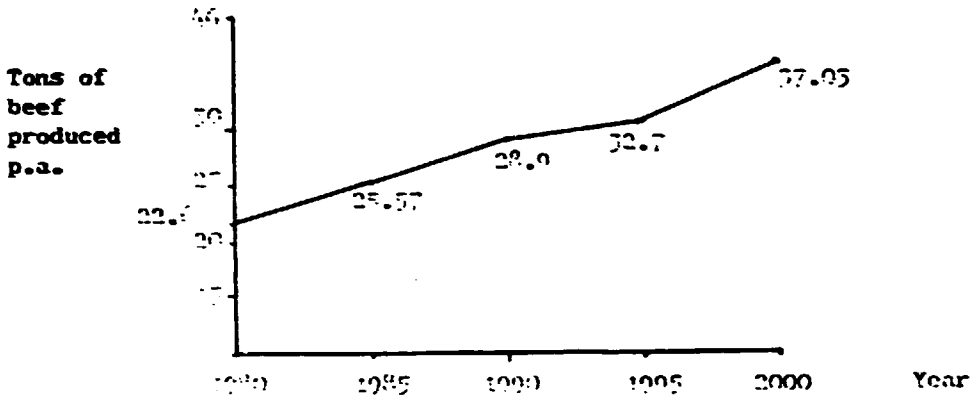
GRAPH 1

Projected increase in human population 1980 - 2000*



GRAPH 2

Beef production in Zambia if 1970-1979 increase continues (2.5% p.a.)



2.NATIONAL DISEASE CONTROL STRATEGIES

2.1 Typanosomiasis, a scheduled disease

Approximately 40% of the area of Zambia is tsetse infested, putting at risk an estimated 60% of the cattle to Trypanosomiasis, and causing an estimated 30% of cattle deaths in the traditional sector.

During recent years as a result of the difficulty in maintaining previous policies of tsetse control - aerial and ground insecticide spraying, habitat destruction, erection of game fences etc., the disease has actually been on the increase.

However, the EEC has established a co-ordinated campaign in Zimbabwe, Zambia and Mozambique with the intention of controlling tsetse by the use of fly traps (attractants, acetone and octonal, insecticide Deltamethrin) and the use of trypanocides in the cattle population at risk to Trypanosomiasis.

Under this campaign widespread use of trypanocides have been applied.

In Zambia the cattle most at risk to Trypanosomiasis are in the following provinces:

Province	No. Cattle	% at risk	No. at risk
Southern	1.05 million	15%	150,000
Southern	0.47 million	20%	94,000
Western	0.29 million	15%	43,500
Eastern	0.263 million	100%	263,000
	TOTAL		550,000

i.e., 23% of the National Herd

However, this strategy has yet to be made fully operational.

2.2 Tick Borne Diseases

Included in this category are ECF and Corridor Disease (Theileria parva and lawrencii respectively) heartwater, anaplasmosis and babesiosis. Together they are second only to Trypanosomiasis in economic importance.

All are controlled by routine dipping under a nation-wide official Government policy.

However the government policy to charge livestock owners for dipping their animals has meant that very little of the traditional herd is regularly treated, so regular weekly dipping is carried out mainly by the commercial farmers.

The purchase of dips in the commercial sector is via licenced traders, Shell, Hoechst, Coopers, Chemico and some others; an import licence is necessary. Only Coopers and Shell formulate locally.

The requirement for dip. The market can be divided into two sectors:

1. Commercial sector. Commercial farmers are aware of the danger of tick-borne disease and buy acaracide and apply it.
2. The traditional sector. A total of 321 dip baths are available, being located in the Southern (132) Eastern (78) Northern (29) Central (816) and Lusaka Provinces.

In the recent past it is only when International Agencies have donated funds for the purchase of Acaracide e.g. EEC, FAO, Dutch Aid etc., that the Veterinary department has had supplies. This lack of availability, combined with the policy of charging livestock owners for dipping have resulted in a very low proportion of animals being regularly dipped.

The actual usage 1.5-2million immersions/sprays for the traditional sector appears low (about 2% of the theoretical requirement for mass weekly dipping). Even allowing for the seasonal variation in tick threat, the relative frequency of ECF and other tick-borne diseases and the wish to preserve some innate resistance to protozoan disease, the ectoparasite usage is too low as evidenced by the increasing losses from ECF, Corridor Disease and other protozoan diseases. Given the appropriate extension services and infrastructure, it is likely that an increased usage of at least 10-fold would prove cost effective.

2.3 Helminthiasis

Helminthiasis is widespread but largely untreated, particularly in the Traditional Sector. The great majority of the anthelmintic usage is in the commercial sector (probably over 99%) and the choice is left to the individual farmer.

2.4 Bacterial Disease

As a result of the increasing role of the diagnostic services in the country, an assessment of the relative significance of various diseases, including those caused by bacterial and viral infections, is improving. Although all the significant infectious diseases are "scheduled" (notifiable) it must be accepted that at the present time varying proportions of these never get reported, hence the picture emerging from the records of the diagnostic services should be regarded as indicative of trends rather then reflecting the true extent of these diseases.

In order of their perceived potential importance, the various bacterial and viral infections may be enumerated as follows:

Rinderpest is a notifiable disease but has not been diagnosed in the country for decades. However, the recent outbreak in Tanzania is a warning of the constant

danger of the disease, especially in the Northern province. Here, vaccination of animals constituting a buffer zone, started in April of 1984 and finished in 1985. 62136 cattle were vaccinated representing 68% of the population of ca. 90,000 animals in the region. The aim has been to vaccinate all cattle in the buffer zone twice in consecutive years. This is followed by calf vaccination for three years. This policy is adequate and together with the natural barrier between cattle in the buffer zone and those nearest in the SW should serve well in preventing the disease to break into Zambia.

Contageous Bovine Pleuro Pneumonia (CBPP) is a disease of cattle caused by a mycoplasma. Apart from the semi-arid zones of the Southern borders of the Sahara, it occurs in South-West Africa, including Angola. For that reason a buffer zone is maintained along the Angolan border, where serological surveillance of this notifiable disease is carried out. Positive reactors are slaughtered and a stand still order comes into operation until all in-contact animals are cleared by serological testing. Cattle can only move out of the zone to the slaughter house after 90 days in quarantine and two negative serological tests 30-60 days apart. In 1984 31,375 cattle were vaccinated and 8729 were tested. In 1985 28,509 were vaccinated and 3140 tested. These measures are adequate but it must be emphasised that constant monitoring of the disease must be continued for as long as the disease is present in Angola together with all other control measures.

Foot and Mouth Disease is another notifiable disease occurring, in some years, sporadically in the border region with Tanzania, and in the Kafue and Zanbezi zones. Characteristic of its potential importance was the 1981-82 outbreak, causing an estimated loss of just under 12 million Kwacha. As a result of the outbreak the price of beef increased from 5 to 15 Kwacha per kg. The control strategy for the prevention of the disease is the formation of buffer zones in endangered border regions. Thus along the Tanzanian border vaccination started in December 1984 concurrently with Rinderpest vaccination. Similarly, two other vaccination campaigns started in the Zambezi zone. In case of an outbreak the region comes under a standstill order, concurrently ring vaccination is carried out around the outbreak. To maintain the buffer zones in the Northern, Southern and Western provinces ca. 140,000 doses of the vaccine was used in 1985. Maintenance of buffer zones by vaccinating cattle in endangered border areas, together with movement control of animals and prohibition of imports of potentially contaminated goods and livestock should be adequate for the prevention of major outbreaks. For greater security twice yearly vaccination in the buffer zones is recommended.

Blackquarter disease is said to be widespread in the country especially in the Western and Southern provinces where well over half of the cattle in the country are kept. It is regarded as the most important bacterial disease and vaccination against it is widespread. From Government importations 0.25 - 0.6million doses were available in recent years together with 0.20 - 0.38 million doses imported by the private sector. There is also local production of this vaccine providing ca. 0.2million doses p.a. Although that dosage would be sufficient for the vaccination of 25-35% of cattle in the country yet the disease is allegedly "widespread". One of the reasons for this is that vaccine is not always available when it is required and is used for the control of an outbreak rather than for its prevention. The other is that many of the reported cases are not confirmed.

It is suggested that the economic significance of this disease should be the subject of a study, based upon adequate diagnosis, and control measures adopted accordingly.

Anthrax is another of the scheduled diseases which occurs sporadically in some years in various parts of the country, especially on the flood plains. Prophylactic

vaccination using Blackquarter/Anthrax vaccine is practiced where it is known to occur and the vaccine is applied before the advent of the rainy season. Since the current measures have kept this disease under reasonable control it appears to be adequate.

Rabies, a notifiable diseases attains its importance by being transmissable to man. Its control is attempted by vaccination of dogs and destruction and safe disposal of infected animals. Compared to requirements, very little vaccine is available, hardly any in 1985. Local production of some 10,000 doses is grossly inadequate.

Haemorrhagic septicaemia is mainly confined to three districts Namwala, Monze and Mazabuka where it is endemic and when it occurs causes 7-10% mortality on the flood plains. Outbreaks tend to be seasonal and control of the disease is by prophylactic vaccination in the regions where it is known to occur, prior to the onset of the rainy season. For example, in 1984, 254,602 doses were used in the Western province supplied by FAO and paid for by the Dutch Government. The last major outbreak was during the rainy season of 1977/78 when out of 200,000 animals at risk some 10,000 deaths were reported causing an estimated loss of approximately 6.25million Kwacha in retail value. Intensive vaccination campaigns, using locally produced vaccine, in recent years prevented further spread of the disease and much reduced its frequency in affected areas. This experience is conducive of maintaining this policy. Yet, an in depth study, carried out by FAO expert in recent years, would suggest that the cost of vaccine and its administration is not commensurate with the losses caused. A full report on the subject should be extremely valuable in formulating future control measures.

Tuberculosis is most prevalent in the South, followed by the Eastern province as seen in cattle on slaughter house inspections. However, a lack of tuberculin makes it impossible to assess its real significance. Apart from its potential economic significance by the low productivity of infected animals it represent a real health hazard for man. Positive diagnosis followed by compulsory slaughter has not been introduced.

Bovine Brucellosis is endemic in the whole of the country causing an economic problem through abortions and infertility and a real danger to the health of man. Its control is attempted by vaccination. A single injection of female calves with the live S19 vaccine, between 4 to 8 months of age confers life-long immunity on the recipient without causing difficulties in diagnosis. At the present time, however, only dairy calves and those in grade beef herds get vaccinated and very few in traditional herds. Same vaccination policy practiced in commercial herds should be gradually extended to cover traditional herds, thus increasing the demand for this vaccine very markedly.

In poultry New Castle disease is by far the most important, and it does occur everywhere in the country. Since the vaccine became available at the hatcheries, which can be purchased together with the day-old chicks, control of the disease in the commercial sector greatly improved. However, for the traditional sector, vaccine is scarcely available. A large proportion of the day-old chicks leaving the hatcheries are vaccinated. These vaccinations should be followed up by one more application for broilers and two more for layers and stock birds. Although supply of this vaccine improved in the last few years by importation by the private sector, meeting the demands of the commercial sector alone would require very much more vaccine.

Fowl Pox is of lesser significance compared to New Castle disease but it does occur sporadically in the country and there is a sizable demand for the vaccine. Layers

and stock birds should be vaccinated once at 10 weeks of age. There is very little and erratic importation of poultry vaccines by Government departments. The private sector imported, in recent years, between 0.5 -1.0 million doses of Fowl Pox vaccine, very much less than would be required even in the commercial sector.

Marek's disease The importance of this disease in the country is rather obscure. Neither the state nor the private sector imported Marek's vaccine in recent years.

Sheep (ca. 30,000 conventional and similar number in commercial flocks) are relatively unimportant nationally as are pigs (ca. 150,000). Commercial sheep may be vaccinated against clostridial diseases (Covexin-8), Anthrax, Blue tongue, Rift Valley Fever and Enzootic abortion. Traditionally kept sheep and goats very seldom get vaccinated just as traditionally kept pigs which account for the major proportion of swine kept in the country.

2.5 Deficiency Diseases, productive disorders
Growth promoters, vitamins, minerals are used especially in the poultry sector. These amount to some 20% by value of the total animal health market, but the sector is subject to considerable fluctuation depending principally on foreign aid available, particularly with the relative decline in the poultry industry.

3. ORGANISATION OF VETERINARY SERVICES

3.1 The organisation follows the typical Veterinary Department pattern with Administration, Field, Diagnostic, Research and Vaccine Production Departments.

3.2 The two over-riding national campaigns are Tsetse and Trypanosomiasis control and Tick and Tick-borne disease control.

3.3 Specific preventive control campaigns are effectively mounted against Foot and Mouth Disease, Rinderpest and Contagious Bovine Pleuro-pneumonia.

4. THE VETERINARY DRUG AND VACCINE MARKET (1985/86) AND ESTIMATED FUTURE REQUIREMENTS (1990-2000)

4.1 Production, Importation and Marketing of Veterinary Drugs

Registration. There is no official registration of Veterinary drugs in Zambia, although traditional Animal Health Companies do submit drugs for trials and evaluation by the Veterinary Authorities.

Import Licences. Import Licences may be obtained from the Veterinary Authorities on payment of a Registration Fee.

Foreign Exchange Control. The method of Foreign Exchange Control changed on 4.10.85 from an application for foreign currency to a weekly "Auction" of foreign exchange. This has had a very disruptive effect on the Animal Health Market and an adverse effect on local Veterinary Pharmaceutical Producers.

Control of Importation. There is no central control on the type and value of Veterinary Medicines imported.

Marketing and Distribution. This is carried out through the usual commercial channels of importer/manufacturer wholesaler to retailer/customer, the total

margins being between 15-40% depending on quantity, complexity and number of links in the chain.

Official Control of Drug Usage. There is no official control of drug usage even when they form a component of an official campaign (e.g. ectoparasite control).

Local Manufacture

Manufacturing Standards. The reputable multinational companies (Coopers, Shell and Hoechst) institute their own internal control in line with International Standards.

Final Production Control. These standards too are monitored by the reputable companies.

4.2 Current Use and Estimated Requirements for Drugs

Aid Donations to Zambia in 1986

Aid donations in the Veterinary sector in 1986 include the following:

Ectoparasiticides	740,500
Antiprotozoan drugs	137,500
Antibacterials	174,000
Miscellaneous, steroids aerosols, syringes etc.	108,000
	$1.160,000

Although recent foreign exchange control policies and Aid Donations have caused wild swings in the nature and value of the Zambian Veterinary Market recently. the approximate breakdown is:

Product	Units	Value US$000's
Ectoparasiticides	Litres	
Amidine	16,000	1,100
Organo Phosphorus	90,000	
Antiprotozoons	Doses	
Samorin	680,000	340
Berenyl	320,000	160
Antibiotics	Doses	
Tetracyclines	500,000	650
Others	150,000	
Anthelmintics	Litres 6,000	500
Minerals, vitamins etc.		450
Total		3,200

Future requirements (Table 1) can only be realised, especially in the traditional sector, if the Government, Aid Agencies and the livestock industry co-ordinate their efforts to control major economic diseases, such as trypanosomiasis, theileriosis, etc., and gear the provision of veterinary drugs to them.

4.3 Current Use and Estimated Optimal Requirement for Vaccines

Veterinary vaccines in Zambia until 1986 originated from three major sources, (i) vaccines imported by the Government and paid for, in the main, by different aids, (ii) vaccines produced by the Central Veterinary Research Institute (CVRI) and (iii) imported by two major private companies Coopers (Zambia) Ltd and Hoechst (Zambia) Ltd.

The range and quantities of vaccines from the first source have been determined more by donations and the availability of foreign exchange for vaccine purchases than the real need for them. Vaccines from this source were mainly directed towards the traditional sector of the livestock industry. Vaccines supplied by CVRI have gained in importance during the last few years, supplying a substantial part of vaccines against the most important bacterial diseases. These vaccines too were supplied mainly to the traditional sector of livestock industry.

Vaccines from the private sector on the other hand were almost exclusively purchased by the commercial sector for dairy and grade beef cattle and by the large commercial hatcheries.

There is no registration of vaccines in the country. Although the Department of Veterinary and Tsetse Control Services issue a permit for the importation of vaccines, this does not necessarily mean that the quantities applied for are imported. Therefore to assess the sales of vaccines, information must be obtained from the two major companies. Because of the various constraints limiting vaccine usage especially in the traditional sector, past use should not be taken as an indication of the real requirements for them but rather indicating their importance relatively to one another, both in dosage and value.

Quantities of the three bacterial and one virus vaccine produced by the CVRI since it started issuing vaccines in 1980 are shown in Annex 5 together with current retail values for vaccines produced in 1985. What detracts from their potential use to livestock was that they were not always available at the time when required. For example, in 1985 vaccine production was suspended for two months due to shortages of local funds and foreign exchange for equipment, chemical and reagents. Thus to increase the significance of this valuable asset to the livestock industry greater harmonisation of supply and demand is called for.

The quantities of vaccines sold by Coopers (Zambia) Ltd and Hoechst (Zambia) Ltd between 1983 -85 and their retail values for the 1985 sales are shown in Appendices 6 and 7 respectively. Bearing in mind that vaccines sold by these two companies were almost entirely to the commercial sector it is worth pointing out that of the total sales of $173,600, $90,500 (52%) was used in ca. 350,000 commercial cattle, representing use of $0.26 worth of vaccine per head per annum (Annex 6 and 7). In contrast out of $284,000 worth of vaccines used in the traditional sector of the livestock industry (Annex 8) $218,000 worth (77%) was used in 2,075million traditional cattle representing use of $0.10 worth of vaccine per head per annum. It may be argued that the commercial sector, with its better understanding of the benefits of vaccination and its ability to pay for it, is a more reliable indicator of the real requirements for vaccine of the national herd than the total sales of vaccines in recent years to the whole of the livestock industry. It is also worth noting that of

Table 1

ESTIMATES OF VETERINARY DRUG REQUIREMENTS IN ZAMBIA

DRUG/BIOLOGICAL	1986		YEAR 1990		2000	
	UNITS	VALUE US$000's	UNITS	VALUE US$000's	UNITS	VALUE US$000's
Ectoparasiticides (Litres)						
Organo phosphorous	90,000	1,100	280,000	2,655	420,000	4,000
Amidine/Pyrethroid	16,000					
Anthelmintics (Litres)	6,000	500	12,000	1,000	1,800	1,500
Antibiotics (Doses)						
Tetracycline	500,000	650	1,000,000	1,000	1,500,000	1,500
Others	150,000					
Antiprotozoons (Doses)						
Samorin	680,000	340	1,000,000	500	1,000,000	500
Berenyl	320,000	160	500,000	250	500,000	250
Feed supplements etc.		450		600		800
Sub-total		3,200		6,005		7,050

the $460,866 worth of vaccine sold in 1985, 68% was used in cattle, 17% in poultry, 14% rabies and 1% in sheep, goats and swine.

Considering the requirements of the country for priority vaccines until the end of the century the use of these vaccines by the commercial and traditional sectors were taken into account as "moderators". It was assumed that availability of these vaccines was not a limiting factor and correct vaccination policies were employed for protection of animals perceived to be at risk during the next 15 years or so (Annex 9).

Anthrax vaccine In assessing the requirements for this vaccine the striking difference in the rate of use of this vaccine between the commercial and traditional sectors must be considered. Between 1982-1985 approximately 40% of the 350,000 commercial cattle was vaccinated with Anthrax vaccine but only 1-2% in the traditional sector (Annexes 6,7,8 and 3 for number of animals). This does not take into account the use of anthrax vaccine, which is incorporated in the Blanthax combination with Blackquoter vaccine, and also used for the commercial sector. Despite the difference in the rate of use between commercial and traditional sectors, the latter did not suffer unduly from the disease. The information about a more realistic need for this vaccine must come from a reliable appraisal of the significance of the disease. In the meanwhile a compromise between the commercial and traditional sectors about its use would seem reasonable. Assuming that ca. 20% of the national herd is at risk, this would call for 0.48million doses now increasing to 0.7million doses by the end of the century.

Blackquarter vaccine Between 1982 - 1985 ca. 80-90% of cattle in commercial sector was vaccinated with either monovalent Blackquarter or with bivalent Blackquarter-Anthrax vaccines (Annexes 6 and 7). During the same time in the traditional sector between 12-25% of cattle was inoculated with monovalent Blackquarter vaccine (Annex 8).Its overall rate of use in the national herd in 1985 was 35%. Until a better understanding of the significance of this disease is known, prophylactic vaccination of ca. 50% of the national herd may be regarded as a reasonable compromise requiring 1.2million doses in 1986 going up to 1.7million doses by year 2000.

Haemorrhagic septicaemia vaccine has not been used much by the commercial sector. In 1985 ca. 0.5million doses were manufactured locally. Since a recommendation for the control of this disease should be available soon, on the basis of the recent FAO study, maintaining the production of 0.25-0.50million doses p.a. is suggested.

Brucella S19 Vaccine Considering the need for this vaccine, demand forecast was made for the national herd. For the calculations half of the national herd was cnsidered as females, a calving rate of 50%, calf mortality at 20% and 50% of the calf crop as females requiring vaccination.

Thus the demand for vaccine in 1986 would increase from 0.24million doses to 0.316million doses by 2000.

Rinderpest In the Northern province some 90,000 animals are presently at risk increasing by ca. 40% to 126,000 by year 2000. Following double vaccination of all the cattle is the region only the new calf crop in each year requires a double injection. This required 40,000 doses in 1986 increasing to 58,000 by the end of the century.

FMDV Just to maintain twice yearly vaccination of cattle in the buffer zones ca. 0.35million doses of the vaccine is required in 1986 increasing to 0.47 million doses

by year 2000. For ring vaccination around outbreaks and for vaccination of cattle along trade routes the demand would be over and above the dosage indicated.

Rabies Vaccine The number of dogs in the country is not known but it is estimated to be around 0.20million. To cover these and the occasional demand for vaccine for cattle and cats 0.25million doses p.a. would meet the demand.

Contageous Bovine Pleuropneumonia (CBPP) Vaccine There is good evidence that this vaccine provides reasonably good protection for at least two years. Since there are ca. 32,000 animals at risk in the buffer zone this calls for 16,000 doses in 1986 increasing by 40% ie. to 22,000 doses by 2000.

Newcastle Vaccine Production of day-old chicks is big business in the country, large numbers are produced for the export market as well as for domestic sales. Currently some 16-17million birds are produced mostly by the large hatcheries. To meet the requirement of these, and to have some vaccine available for older birds and for ring vaccination, ca. 16million doses are required at the present time, increasing to ca. 24million doses by the end of this century.

Fowl Pox Vaccine There are some 13million birds in the country including a few million layers and stock birds. Adequate information was not available of their numbers nor of those in the traditional sector. For this reason assessment of demand for this vaccine, which is required for the 10-week old bird, can hardly be more than an inspired guess, aided by the sale of 0.5million doses in 1985 which was said to be much less than required. Perhaps provision of 1.5million doses now, increasing to 3.0million doses by year 2000 would not be unreasonable.

Marek's disease vaccine Although this vaccine has not been used regularly by the hatcheries in recent years it is probably "living on borrowed time". It is strongly suggested that at least those day-old chicks which become layers and stock birds, and those going for export, should be vaccinated requiring 8million doses now, increasing to 12million doses by 2000.

Fowl Typhoid Vaccine Another vaccine scarcely used in recent years mainly because the vaccine has not been available. It needs to be applied at the same time as the Fowl Pox vaccine and should be used for birds which become layers and stock birds. The estimated dosage is the same as for Fowl Pox 1.5million doses increasing to 3.0million doses by year 2000.

5. CONSTRAINTS ON DRUG AND VACCINE USE AND PROPOSALS FOR INCREASE USE

There are two main market sectors.

The Commercial Sector. This accounts for something like 80% of the whole market, but is subject to wild fluctuations as a result of Aid Donations.

The Traditional Sector. This market is poorly developed and unpredictable. As the government does not budget for drug purchase for the traditional market, the extent of it depends entirely on Aid donations. These fluctuate wildly both in the extent and nature of the donation made. (Table 2).

Aid Agencies. Within the general policies to control ticks, tick borne disease and tsetse and trypanosomiasis, the availability of drugs is dependent on aid donations. These come piecemeal and there is no guarantee that drugs essential for the continuation of a policy will be available the following year (e.g., trypanocides).

TABLE 2

EEC 1984/5 for 1985/6

Quantity	Description	Total Value DM
10,000 bottles	Oxytetracycline	43,000.00
10,000 "	Sulphadimidine	147,800.00
30,000 "	TMP/Sulphadiazine	3,900.00
1,250 "	TMP/Sulphadiazine	12,673.00
2,000 "	TMP/Sulphadiazine	20,280.00
10,000 "	Penicillin/Streptomycin	127,500.00
350 "	Parvaquone	4,056.50
	TOTAL	358,211.50
1,000 bottles	Imidocarb	7,240.00
3,000 tubes	Antibiotic Optholicreme	6,960.00
3,000	Wound Spray Aerosol	34,400.00
5,000 pcs	Intramammary Injectors (treatment)	5,800.00
1,500 bottles	Corticosteroids Injection	9,780.00
5,000 pcs	Intramammary Inj. (prophylaxis)	5,800.00
3 million dose	Newcastle Disease Vaccine	11,310.00
	TOTAL	81,290.00
1,000	Syringe 20 cc	13,540.00
1,000	Syringe 5 cc (Automatic)	31,000.00
6,000	Needles G16, 20mm	3,600.00
8,000	Needles G16, 35mm	3,600.00
4,000	Needles G16, 50mm	3,920.00
8,000	Needles G17, 20mm	3,600.00
8,000	Needles G17, 35mm	3,600.00
4,000	Needles G17, 50mm	3,760.00
500	Drenching Guns (Automatic)	16,605.00
500	Burdizzo	53,625.00
	TOTAL	136,390.00

These figures were extracted from the letter of contract between EEC and Meridian Breckwoldt. Unfortunately pack sizes are not stated and there seems to be a serious error in the price quoted for 30,000 bottles of TMP/Sulphadiazine.

ECTOPARASITICIDE DONATIONS

FEB 1985	UNDP	DELNAV	400 Litres	(UK)
*MAR 1985	COOPER Z Ltd	DELNAV	284x5L	(UK)
AUG 1985	FAO	DELNAV	82x5L	(UK)
MAR 1985	UNDP	TRIATIX	100x5L	
*APR 1985	COOPER Z Ltd	TRIATIX LS	780x5L	
APR 1985	ITALY	DIAZANON	417x4x6L	
APR 1985	ITALY	TACTIK	500x20L	
FEB 1986	FAO	CYPERMETHRIN	96x5L	

*Sending ?
Acaricide receipts by DVTCS

TABLE 2 (Cont'd)

Aid Donations to Zambia in 1986

Aid Donations in the Veterinary Sector in 1986 include the following:

	US$
Ectoparasiticides	740,500
Antiprotozoan drugs	137,500
Miscellaneous, steroids, aerosols, syringes etc.	108.000
TOTAL	1,160,000

Furthermore, donations from Aid Agencies may also compete directly with identical or equivalent drugs formulated locally or available through traditional importing companies (as in the case of the recent EEC tender). This has a catastrophic effect on traditional producers as they are unable to forecast meaningfully and have to compete against completely unpredictable (and sometimes cheap) importations.

Commercial Sector. The available evidence points to a reasonable balance between essential disease control and drug usage. Obviously better education at all levels and improved extension services would help, particularly in the case of productive diseases e.g., infertility and helminthiasis.

The short term goals should be improved diagnosis and monitoring of specific drug resistance by parasites, measuring dip wash concentrations and recommending specific disease control strategies.

5.1 The Traditional Sector
The scope for increased drug usage in this sector is much greater, but the problems are considerable.

Apart from the (often) fatalistic attitude of stock owners the situation is complex and is dependent on a number of inter-relating factors.

5.1.1 Quick accurate diagnosis.
5.1.3 Adequate extension services.
5.1.3 Adequate transport and distribution system for drugs and vaccines.
5.1.4 Education of the stock owners.
5.1.5 Guarantee of appropriate supply of medicaments.

The Government policy is for the Traditional stock-owners to pay for treatments. If this policy is to work satisfactorily it must be combined with proper attention to 1-5, above.

In this way the drug off-take in the traditional sector would rise many times and give a corresponding benefit in reduction in animal disease and greater productivity.

5.2 Co-ordination of Aid Agency Contributions
At least equal to the problems faced by Veterinary Pharmaceutical Producers in Zambia at the present time in respect of depressed markets, high interest rates, cut price competition (from entrepreneurs who import finished Veterinary Products for sale without incurring the overheads of companies which manufacture and service products) and exchange difficulties, is the question of unco-ordinated donations from Aid Agencies.

In the space of the last 12 months there have been 8 donations of ectoparasiticide, equivalent to a significant proportion of the normal market, comprised of four classes of acaricide, one of which was not the least suitable for use in Zambia!

Over the same time there have been donations of antibacterials, antiprotozoals and miscellaneous drugs equivalent to approximately half the normal annual usage of these products. (Table 2).

Such uncor-ordinated donations are most disruptive and can only have the long-term effect of making local manufacture an even more hazardous affair than it is at present.

Insofar as the long-term benefit to the livestock industry in Zambia is concerned there is no real benefit as there is no guarantee that appropriate donations will be made in subsequent years in the control (e.g.) of trypanosomiasis or ectoparasites.

5.3 Proposals for stabilising and improving the animal health market in Zambia.

The following suggestions are put forward for consideration:

5.3.1 General. Drugs for the control of animal disease of national importance should undergo formal registration and their use in the field should also be centrally controlled.

5.3.2 The monitoring of parasitic drug-resistance should be stepped-up as an adjunct to the formation of policies on drug usage.

5.3.3 Extension services and stock owner education should be improved in both the traditional and commercial sectors.

5.3.4 Preference for supply of drugs should be given to those Companies which formulate Veterinary Products in Zambia and provide technical services and support (e.g., dip-wash drug and pH testing).

The nature of preference could be:

5.3.4.1 Tarriff protection.
5.3.4.2 Concessions on taxes and duties.
5.3.4.3 Subsidies for local production.
5.3.4.4 Lower interest rates for capital tied up in raw material importation and processing.

5.3.5 Aid Agencies. The contributions from Aid Agencies should be co-ordinated both by the Agencies themselves and by the Zambian National Committee by Development Planning.

5.3.5.1 Donations from Aid Agencies should be channelled through bona fide Zambian Veterinary companies wherever they are in a position to supply the appropriate products at an acceptable price.

5.3.5.2 Donations from Aid Agencies which impinge on the control of scheduled diseases of National importance should relate to the National Disease Control Strategy and should provide for continuity over a period of at least several seasons.

6. CONSIDERATION FOR LOCAL MANUFACTURE

6.1 Pharmaceuticals

Three International companies are operating at a significant level, Coopers, Shell and Hoechst. Only Coopers and Shell are formulating locally and only Coopers has a major commitment to the Animal Health Field. Shell is essentially producing Agrochemicals plus two ectoparasiticides, Supona and Barricade (pyrethroid). (Annex 14)

Discussion

There is an urgent need to protect local acaricide production from the effects of the auction of foreign exchange and unco-ordinated Aid Donations. Tariff protection; registration of products and channeling aid through local producers (where appropriate) would be desirable.

6.2 Biologicals

Central Veterinary Research Institute

Production of veterinary biologicals is one of the major functions of the Central Veterinary Research Institute (CVRI) at Balmoral, some 25Km S.W. of Lusaka. CVRI was transferred to its present site from Mazabuka (Southern Province) in 1979. This coincided with the implementation of an FAO/UNDP Animal Disease Control Project (ZAM/77/002) which provided US $5.0million over a period of 8 years, to aid CVRI in attaining its various objectives including establishment of local production of veterinary vaccines.

The first vaccine, developed by CVRI, was against Haemorrhagic septicaemia. Its development was precipitated by the 1977/78 outbreak of the disease causing the death of many thousands of cattle in the Southern Province. In 1980 some 32,000 doses were produced increasing to over 3.45million doses by 1984.

CVRI produces three other bacterial vaccines (Blackquoter, Anthrax and Brucella S19) and one viral vaccine (Rabies). During 1984 vaccine production unit was aided by FAO Project (TCP/ZAM/23M) for the support of animal vaccine production as well as by Dutch Bilateral Aid, in anticipation of the implementation of large scale EEC assistance, aiming to establish a new vaccine production facility on the grounds of CVRI.

Very limited information was made available about this new vaccine production facility. What was made available may be summarised as follows. In 1981 a project proposal was put to the EEC for the establishment of a vaccine production facility. In 1983 EEC agreed and gave Zambia a soft loan of 2.0million US dollars and a grant of 1.0million US dollars for the project. The Zambian government also allocated the equivalent of ca. 0.79milion US dollars to the project. A tender (MF/W/3123) by the Central Supply and Tender Board was gazetted on July 28 1986 for the construction of a vaccine unit at CVRI, closing date 5.9 1986. The facility is expected to become operational in 1988. Once construction starts, a tender for the supply of laboratory equipment will be invited from EEC countries.

The New Vaccine Production Facility aim to produce the following vaccines.

Anthrax	0.5mill. doses
Blackquarter	0.5mill. doses
Haemorrhagic septicaemia	0.5mill. doses
Brucella S19	0.3mill. doses
Newcastle vaccine	3.7mill. doses
Rabies	0.5mill. doses
Contageous Bovine Pleuropneumoniae	0.2mill. doses.

These vaccines will be produced mainly for the home market although exports at a later date may also be considered. It is envisaged that as the new unit becomes operational the present vaccine production unit will be gradually phased out as its production is replaced by the new facility.

No information was made available about either the new buildings, equipment or production technologies.

However information was available about the present vaccine production unit. Since this unit shall be phased out in a matter of a few years, only the staff, production technologies and quality control measures shall be discussed here since these may well influence the work of the new unit.

Organisation, manpower and training at the Vaccine Production Unit (VPU) at CVRI

In terms of organisation Head of Vaccine Production Units reports directly to the Head of CVRI, who himself reports to the Director of Veterinary Service.

Head of VPU is an ex-patriot, vaccine expert from Ethiopia who is responsible for both production and quality control. Although it is fully appreciated that this is not a desirable practice there is no suitably qualified person, independent of production, who could take responsibility. There is a veterinary graduate trainee counterpart of the Head of VPU, a Zambian national.

For each of the 5 products (Anthrax. Blackquarter, Brucella S19, Haemorrhagic septicaemia and Rabies vaccines) there is a "product line manager", a senior technician with a diploma in medical laboratory technology (a 3 year course) who has trained "on the job". They are assisted by 6 junior technicians who, apart from school leaving certificates, also have a certificate in laboratory technology (2 year course) and training "on the job". There are also 6 laboratory attendants and 3 animals technicians. These are without formal qualifications.

For further training, graduate staff at CVRI are sent abroad, usually to do an MSc. course at an academic establishment. They may also have the opportunity of brief visits to places of vaccine manufacturing.

Outstandingly good technical staff may also be sent abroad for further training. Presently one is abroad and two more are to go to study lyophilization and media production.

Other deserving junior technicians, usually after a few years work, may be sent to the local medical Laboratory Technical College for a 3 years full time course leading to a diploma.

Undoubtedly one of the severe constraints on the development of vaccine production in the country is the shortage of suitably qualified staff. Technical training is medically orientated and therefore practical training for vaccine production is "on the job". Functioning of VPU at the present time is very heavily dependant on ex-patriot vaccine expert who does a most creditable job under the circumstances. However, to improve the present position it is important that more suitably qualified school leavers receive the basic technical training in microbiology and laboratory technology so that sufficient candidates are available to meet the needs of both the medical services as well as that of industry.

To acquire specialised training, required for vaccine production, is difficult both for technical as well as graduate staff.

For more senior staff in production and quality assurance this usually requires going abroad. However, to obtain the most appropriate training in vaccine manufacturing the candidates should go to the vaccine manufacturing industry itself. This is not readily possible because of the lack of incentives for industry. One of the ways of overcoming this is to purchase production technology complete with staff training.

For junior staff establishment of sandwich courses should be considered as a means of further education. Employment in a junior capacity combined with education on a day release basis has been very successful in many countries leading to graduates immediately productive unlike their counterparts who had recieved full-time education. Training for both junior as well as more senior technical staff should be offered on contractual basis which would prevent newly trained persons leaving for more attractive salaries.

Production technologies and quality control, vaccine production unit CVRI

The vaccine production unit is dealt with here only in such details which may help a fuller understanding of production technologies.

The building, which accommodates vaccines production, consists of a series of rooms on either side of a corridor. These are used either for media preparation (2), washing and sterilisation, chemical stores, changing room or were converted into a walk-in incubator or cold room. In addition three of these rooms were adopted as production suites. Each of them was sub-divided and the larger portion is used a general laboratory cum office. The smaller part, "the sterile room". which gains entrance from the general laboratory via a small changing room, serve for most of the activities involved in vaccine production. These include inoculation of seed and production cultures, sampling, harvesting and inactivation of cultures. It is also used for preparation of bulk vaccine as well as for filling and closing, both of these operations are entirely manual.

In between operations, a U.V. light is switched on and the floor and bench (the only permanent furnishing in the "sterile room") are wiped with 10% formalin solution. There is no control of air movement in any of the laboratories although production staff change gowns and wear cap, mask and gloves before entering the "sterile room".

Production of Cl.chauvoei (Blackquarter) and P.multocida (Haemorrhagic septicaemia) vaccines are carried out in devoted suites whereas production of anthrax and brucella S19 vaccines take place in the same suite on a time sharing basis.

Rabies vaccine is produced in a different building in a devoted suite.

As mentioned before, this vaccine production unit is to be phased out in a few years time and therefore no detailed comments on either the building or its use are offered here, other than to suggest that production of anthrax and S19 vaccines should be separated and carried out in separate suites. Prevention and recognition of cross contamination with these live vaccines is more difficult than with inactivated products. It would be a more sound practice to produce Blackquoter and Haemorrhagic septicaemia vaccines (both inactivated) in the same production suite on a time sharing basis. These changes would not require any extra expenditure only reorganisation of use of laboratories.

Preparation of Blackquarter vaccine

Cl.chauvoei is grown in 10L conical bottles containing 8L of medium. A batch of this vaccine consists of 35-45 bottles. Production cycle in 63 days between media production and issue of vaccine. Five to 6 batches p.a. are prepared yielding a total of ca. 500,000 doses.

Detailed description of production and quality control are appended. (Annex 10).

Briefly, the original seed (Master Culture) was obtained from Weybridge, U.K. from which a lyophilised Seed Lot (Master Seed Culture) was produced. This culture was adequately tested for identity and pathogenicity but not for purity, since extraneous yeasts and fungi may not show up readily with the test used. Sabouraud medium is recommended (B.P. Veterinary, 1985 App. XV1A, A132).

From the Seed Lot Culture, Seed Culture was prepared (stored at 4-8^{0}C as a liquid culture) to serve as the inoculum for Production Seed Culture which was used to inoculate the production culture.

The production culture is grown in a liver-meat extract medium and incubated at 37^{0}C. After 24hrs of incubation pH of the culture is adjusted to pH7.1-7.3 and after an additional 24hrs of incubation 0.6% formalin is added to inactivate the culture. This takes 4 days at 37^{0}C.

Following sterility and inocuity tests the culture is adjuvanted with potassium aluminium sulphate, and following sterility test on the bulk vaccine it is filled out in 20 dose packs, 5ml per dose.

The following comments and suggestions are offered:

(i) Yield of antigen is on the low side requiring over 4ml of culture per dose. It is possible to obtain several doses of vaccine from 1ml of culture, thus making production very much more economical in terms of media utilisation, labour and packaging.

(ii) Use of a fermenter for growing the culture would soon pay for the initial investment. A reappraisal of medium, production strain, pH control and feeding of glucose (in that order) should lead to significant improvement in yield of antigen.

Quality control and the tests employed were on the whole adequate. Introduction of a few changes, however would lead to improvements.

(i) Sterility test on Triptose blood agar plates is inadequate and use of Thioglycollate broth (for anaerobic and aerobic bacteria) and Soyabean Casein Digest Medium (for aerobic bacteria and fungi) should be adopted. Details of both of these media and their use is described in B.P. (Veterinary) 1985 App.XVIA, A126.

(ii) Potency test of bulk vaccine should replace that on the filling lot. Generally speaking potency test is advantageously carried out on liquid vaccines and especially on inactivated liquid vaccines at the bulk stage. Should it fail the test, it may be reprocessed and if improved it can be still used. This option is not open for failed filled vaccines, thus losing not only the vaccine but also the cost of filling.

Occasionally it may be advantageous to carry out potency assay on the antigen to determine its concentration required in the vaccine prior to blending of the bulk vaccine. In such cases to meet the statutory requirements of testing the potency of the vaccine, it may be better done on the filled vaccine.

(iii) Execution and interpretatio of the potency test should also be changed in that 5, rather than 3, non-vaccinated quinea-pigs should be challenged, and none of the vaccinates can die within 5 days of challenge (presently 80% survival is demanded) and only then pass the test.

Preparation of Anthrax Spore Vaccine

CVRI aims to meet WHO standards for the preparation of this vaccine. The culture is grown on solid medium in Roux flasks, 30-50 flasks would represent a batch and 2 to 3 batches per annum may be produced. A production cycle takes 77 days. Each Roux flask may yield 2000-3000 doses and the yearly output is around 0.2million doses.

Detailed description of production and quality control are appended (Annex 11).

Briefly, Original Culture B.anthracis 34F2 was obtained from Weybridge, England from which a Seed Lot culture was prepared and lyophilised. From Seed Lot culture Production Seed Culture is prepared which is used as inoculum for Production Culture.

The production culture is grown on Bacto casitone yeast extract agar in Roux flasks. Each flask is inoculated with a few ml of Production Seed Culture and incubated 37^{0}. After 3 days of incubation sporulation of the culture is tested and when it exceeds 80% growth of each flask is harvested. Following sampling, vegetative cells in the harvest are inactivated by the addition of glycerol. Satisfactory spore concentrate is diluted in saline-glycerol mixture (containing 0.5% Saponin) to give 1 x 10^{7} spore count per 1ml dose. This bulk vaccine is filled out in 50 dose glass containers.

The following comments and suggestions are offered:

(i) Although the production of this vaccine in Roux flasks is wide spread, nevertheless it is very laborious and because it involves a lot of handling it runs a high risk of contamination. It would be very advantageously produced in an aerated fermentation vessel.

(ii) Yield of culturable spores is good but if anything less then maybe obtained using the same medium (without agar) in a fermenter.

(iii) The Original Seed and the Seed Lot are used in a manner compatible with the concept of the Seed Lot System in that the production culture is not more than three passages removed from the Seed Lot.

Quality Control and the tests employed were on the whole adequate. However, the following modifications would bring them in line with WHO requirements.

(i) Safety test as used in sheep and goats is appropriate for testing safety of the Seed Lot (5000million live spores) but too severe for testing routine batches where inoculation of twice the recommented dose (ie up to 10million spores) is required.

(ii) Safety test should be carried out on the bulk vaccine rather than on the spore concentrate.

(iii) Bulk vaccine should be tested for extraneous bacteria and fungi.

(iv) Potency test would be more advantageously carried out on the bulk rather than on the filled vaccine. Bulk vaccine may be re-processed in case of a failure and used, whilst filled vaccine cannot. Failing filled vaccine means not only the loss of the vaccine but also the cost of filling.

(v) Filling lot should be tested for the number of culturable spores.

(vi) A stability test for the filling lot should be adopted. Although there is no internationally accepted test for that end, repeat spore counts over the first three months of storage at 2-8^{0}C give a reasonable idea of the shelf-life of the batch.

(vi) Interpretation of potency test: 100% rather than 80% of the vaccinates must survive for the vaccine to pass the test.

Implementation of these changes in Quality Control test would bring the testing in line with WHO requirements.

Preparation of Brucella S19 Vaccine

CVRI aim to meet WHO standards for the preparation of this vaccine. Br.abortus S19 is grown on solid medium in Roux flasks. A typical batch may consist of 35 flasks and 3-4 batches per annum are produced. A production cycle takes 36 days. Each Roux flask may yield 1500-2000 doses (issue density is 1/10 of that recommended by B.P. (Veterinary) 1985 and about 150,000 doses are produced p.a.

Detailed description of production and quality control are appended (Annex 12)

Briefly, Original Culture is received from WHO Ref. Laboratory and is used for the preparation of Production Seed Culture which in turn is used for the inoculation of Production Culture.

Production Culture is grown on potato infusion agar in Roux flasks at 37^{0}C for 48hrs. Harvest of groups of 5 flasks are pooled and pending on tests these small

pools are pooled in turn to form bulk concentrate of cells. Suitable concentrate is diluted to give 3×10^9 viable cells per 2ml dose and this is dispensed into 25 dose glass bottles.

The following comments and suggestions are offered:

(i) This vaccine can be grown in a basic fermenter and superior yields may be obtained by the use of a more suitable medium. It is suggested that in order to improve medium utilisation and to save on labour and cost of quality control much larger batches of this vaccine should be produced in liquid medium in a fermenter.

(ii) Original Seed should be used more sparingly by the preparation and use of Seed Lot Culture. (Presently Original Seed Cultures are used in place of Seed Lot).

(iii) Use of 1/10 of viable cells recommended by WHO, B.P., Brit.Vet.Codex, per dose of vaccine is prejudicial to its efficacy especially when it is distributed in liquid form. It is recommended that the number of live cells per dose are increased to not less than 4×10^{10} live cells, per dose and that the vaccine is lyophilised.

Production Seed Culture and production culture are very adequately tested "in-process". However Quality Control procedures, with respect to final bulk and filled vaccine, require quite substantial changes as follows:

(i) Bulk vaccine must be tested for number of viable cells. This test, carried out on bulk antigen concentrate, is not and cannot be a substitute.

(ii) Bulk vaccine must also be tested for bacterial and mycotic contaminants. Similar tests on bulk harvest antigen cannot be a substitute.

(iii) Viability, safety and inocuity tests on filled vaccine are not enough and the following tests are needed:

Identity
Absence of extraneous organisms
Dissociation
Viability
Reactivity in guinea-pigs (Safety)*
Antigenicity and immunogenicity (Potency)*
Stability (on selected filling lots)

For details of these tests consult WHO Tech.Rep.Ser.No. 444 (1970). Annex.2, p66-67 and B.P. (Veterinary) 1985 p163

* These tests are optional.

Preparation of Haemorrhagic septicaemia Vaccine

This vaccine is produced from a locally isolated strain of Pasteurella multocida Carter's type E, cultured in liquid medium in 10 and 20L glass bottles and aerated by means of magnetic stirrer in the bottle. An average production batch consists of 4 bottles containing a total of 30L medium. Four of these batches, produced and inactivated within 25 days, are pooled to form bulk antigen (ca. 120L) for the preparation of one batch of bulk vaccine. Production cycle for a batch of vaccine is 58 days. Five production batches p.a. are prepared, ca. 0.5million doses.

Detailed description of production and quality control are appended (Annex 13).

Briefly, original isolate of P.multocida type E (identity verified by Carter) is passaged in calf and blood from the moribund animal is stored in liquid nitrogen

refrigerator. From this Production Seed Culture is prepared (via culturing the organisms on blood agar) and used for the inoculation of nutrient broth, enriched with 1% yeast extract, to grow the production culture at 37°C over 24hrs. The culture is inactivated by the addition of 0.5% formalin and adjuvanted with 1% potassium aluminium sulphate so as to contain 1×10^{10} cells per 2ml dose.

The following comments and suggestions are offered:

(i) Growing this organism in a fermenter would make aeration more efficient. This would lead to much shorter incubation time to reach the end of the log phase of growth and less endotoxin in the culture.

(ii) Adjusting antigen concentration of the vaccine on the basis of viable cell counts, taken after 24hrs of incubation, is likely to lead to the inclusion of substantially higher total number of cells than intended (1×10^{10} cells per dose).

It would pay to establish the relationship between optical density and viable counts of the culture during the log phase of growth. This information can be used to adjust cell concentration of an older culture (which contains a high proportion of dead cells) by optical means.

Quality control procedures and the tests employed were by and large good. However, the following changes would bring them in line with the standards of B.Vet.Codex.Supplement 1970.

(i) Tests carried out on filled vaccine (sterility, inocuity, safety and potency) should be applied to bulk vaccine and those applied to the bulk vaccine (sterility and inocuity) would be appropriate to the filling lot.

(ii) Potency assay is more elaborate than demanded by the B.Vet.Codex.Supplement 1970. It may be simplified to fall in line with the test in the Codex which specifies a single injection for immunisation of mice unlike the two injections currently used.

Preparation of Inactivated Rabies Vaccine for Canine

This vaccine is produced in the brain tissue of lambs. Ten batches of 10-12 lambs in each are produced per annum yielding ca. 100,000 doses of this inactivated alhydrogel adjuvanted vaccine.

Briefly, lyophilised canine rabies virus obtained from the WHO Ref.Laboratory, is passaged twice in the brain of infant mice and the second mouse-passaged virus in brain tissue (stored at -70°C) is used as Seed Lot culture for inoculation of lambs.

In strictly specified position on the forehead a 2mm diameter, 15mm deep hole is drilled into the scull of each of 10-12 healthy one-month old lambs and inoculated intracerebrally with ca. $10^{6.2}$ MLD_{50} of second mouse-passaged virus. When complete paralysis sets in, head of animals is disinfected in 5% phenol, inserted into a plastic bag and decapitated. Brain tissue is harvested with aseptic precautions, homogenised with ice-cold sterile distilled water to form a 40% suspension. This is further diluted to 5% suspension and filtered through stainless steel mesh (0.425mm) and inactivated by the addition of betapropion lactone (final concentration of 0.025%). Alhydrogel (25%) is added to the suitably diluted homogenate and 0.25% phenol as a preservative. It is distributed as a liquid vaccine.

No detailed description or criticism of production or quality control of this method of vaccine production is offered here. Due to the singular cruelty involved in virus propagation and to the inherent instability of this vaccine, especially in liquid form,

it is most earnestly suggested that an alternative method, such as the use of embryonated eggs should be adopted in place of lambs.

Vaccine produced in embryonated eggs is no more complicated or expensive to produce and in lyophilised form offers a superior product.

Discussion

Determination in the country to become self sufficient in selected vaccines was most evident and plans for the establishment of a new vaccine production unit, with EEC assistance, have been in an advanced stage. Although information about the new unit and production technologies was very scarce nevertheless it is hoped that comments about current production of vaccines will be of help.

Generally speaking it may be stated that all production technologies were outmoded and very labour intensive, resulting in the production of small batches attracting proportionately higher cost of Quality Control. It also follows from the use of these technologies that yeilds on the whole were poor, as was batch to batch reproducibility, and an increased danger of contamination due to excessive handling.

There was an adequate appreciation of the role of Quality Control yet the actual tests have not always been employed at the right stage of downstream processing. The most consistent example of this was the application of tests on filling lots which would have been more appropriate for bulk vaccine. However, detailed criticism of production technology and quality control was advanced together with description of each product.

Animal testing facility was totally unsuitable for containment of highly pathogenic challenge strains and thus can be a source of infection of animals in the area.

The lack of provisions for continued education and training was another area where changes would be desirable.

With respect to the EEC-sponsored new vaccine production facility, planning of which is out with the terms of reference of this mission, nevertheless the following recommendations may be of some help.

Recommendations

1) Production technologies to be changed in favour of deep culture technology for all bacterial vaccines. This may be best attained in context of licencing agreements with established manufacturers coupling transfer of technology with training of key personnel in production and Quality Control.

2) Production of rabies vaccine in brain tissue of lambs should be abandoned. If home production of this vaccine is insisted upon, use of embryonated eggs should be adopted.

3) Production and Quality Control should be separated as soon as circumstances permit and not later than the commissioning of the new vaccine production unit.

4) Animal test facility must be capable of containing pathogenic organisms.

5) Provisions to be made for further education of staff not only by training abroad but also in-house. Assistance of outside teachers should be also sought.

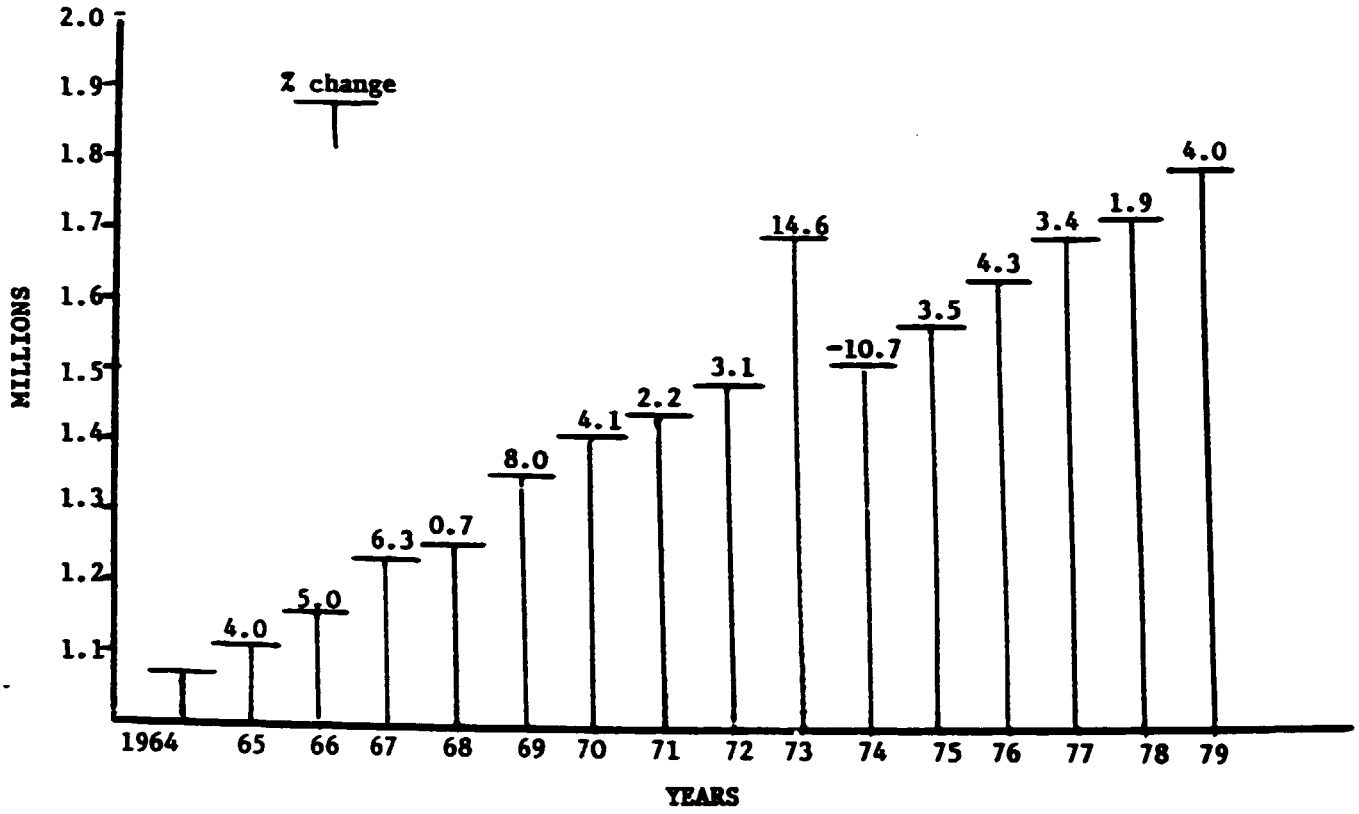
7. RECOMMENDATIONS

Acaricides. Steps be taken immediately to protect local acaricide production and build on this to expand the local production of veterinary pharmaceuticals, with possibility of exporting to neighbouring countries.

Biologicals. Should there be a desire to consider the facilities currently under construction as a regional supply source, the necessary steps should be taken to see that they meet international standards of manufacture, quality control and product specification. This is especially relevant to the production of CBPP vaccines where Zambia could be considered as the supply point of this vaccine for the SADCC countries.

ANNEX 1

Cattle Population Census, Zambia 1964 - 1979



Source: Department of Veterinary and Tsetse Control Services, Traditional Sector

ANNEX 2

Number of Livestock 1979 - 1985*

YEAR	COMMERCIAL CATTLE	TRADITIONAL CATTLE	SHEEP	GOATS	PIGS
1979	343,000	1,804,057	32,625	325,362	122,429
1980	N/AV	1,728,917	27,540	257,610	139,995
1982	N/AV	1,951,863	25,525	315,917	147,098
1983	324,884	2,048,160	30,624	349,593	168,588
1984	350,000	2,035,170	42,752	370,638	176,697
1985	N/AV	2,057,736	30,468	393,896	156,041

*Source: Census "Livestock Population Traditional Sector" 1979-1985. Ministry of Agriculture and Water Development Zambia

ANNEX 3

Number of Livestock* and Projected Increases 1979 - 2000

		Numbers(000) 1979	1985	%Animal Changes Past	Projected	Projected Incr.(000) 1990	2000
CATTLE	Traditional	1,804	2,075	2.5	2.5-3.0	2,334	3,034
	Commercial	343	350	2.0	2.0-2.5	385	481
	TOTAL	2,147	2,425	2.2	2.4-2.9	2,719	3,515
SHEEP		32	30	-1.1	0-2.0	30	36
GOATS		325	394	2.9	3.0	453	571
PIGS		122	156	4.6	4.0	187	249
POULTRY		12,183	12,800	0.8	3-5	14,720	22,080
DAY-OLD CHICKS		13,393	16,000	3.2	3	18,400	23,900

* Source Census Figures, Ministry of Agriculture and Water Development

ANNEX 4

Poultry Production, Zambia 1970 - 1980

Years	DAY-OLD CHICKENS Numbers	%-Change	POULTRY Numbers	%-Change
1970	4,986	N/D	4.011	N/D
1971	5,603	12.4	4,370	8.9
1972	6,695	19.5	5.234	19.7
1973	7,508	12.1	5,827	11.3
1974	9,661	28.7	8,020	37.6
1975	13,301	37.8	11,640	15.1
1976	15,641	17.6	14,278	22.8
1977	14,253	-8.9	13.041	-8.7
1978	13,341	-6.4	12,420	-4.8
1979	13,393	0.4	12,183	-1.9
1980	15,338	14.5	12,500	+2.6

ANNEX 5

Quantities of vaccines produced by Central Veterinary Research Institute

VACCINES	1980	1982	1983	1984	1985	1985 US $ Value
Haemorrhagic septicaemia	32,000*	159,480	352,000	455,280	538,200	80,730
Blackquarter	-	230,000	230,000	305,000	378,406	45,048
Brucella S19	-	147,860	90,700	77,220	39,800	11,940
Rabies	-	18,000	10,000	-	11,900	21,063
						158,781

* doses

ANNEX 6

Quantities of vaccines sold by Coopers (Zambia Ltd) 1983 - 1985

	NO. OF DOSES			VALUES IN US $ 1985	
VACCINES	1982/83	1983/84	1984/85	per dose	TOTAL
Anthrax	95,900*	140,800	110,000	0.11	12,100
Blackquarter/Anthrax	117,750	116,600	112,650	0.274	30,866
Fowl Typhoid	164,000	100,000	113,800	0.117	13,314
Newcastle	100,000	7,420,000	5,192,000	0.00300	20,248
Fowl Pox	226,000	0	423,500	0.030	16,546
Blue Tongue	0	2,000	1,000	0.20	200
Rift Valley Fever	0	9,750	33,450	0.268	8,964
Gumboro	576,000	0	712,000	0.033	23,406
TOTAL					127,704

* doses

ANNEX 7

Quantities of vaccines sold by Hoechst (Zambia Ltd) 1983 - 1985

VACCINES	NO OF DOSES 1983	1984	1985	VALUES US $ 1985 per dose	TOTAL
Anthrax	40,000*	25,000	30,000	0.15	4,500
Blackquarter/Anthrax	42,500	100,000	20,000	0.17	3,480
Blackquarter/Septic.	125,000	175,000	175,000	0.16	28,700
Campylobacter	12,350	0	0	0.50	0
Rift Valley Fever	25,500	1,750	10,000	0.17	1,700
Newcastle	100,000	450,000	1,000,000	0.003	2,860
African Horse Sickness	500	0	400	3.60	1.440
Clostridial 7:1	100,000	0	12,800	0.33	4.224
Fowl Pox	250,000	500,000	0	0.08	0
TOTAL					46,904

* doses

ANNEX 8

Vaccines applied directly by Department of Veterinary and Tsetse Control Services 1983 - 1985

VACCINES	NO. OF DOSES X000 1983	1984	1985	VALUE US $ 1985 per dose	TOTAL
Anthrax	49	50	31	0.15	4,622
Blackquarter	602	244	532	0.12	63,907
Haemorrhagic septic.	ND	492	258	0.15	38,715
Brucella S19	23	16	34	0.88	30,158
FMD	185	137	137	0.50	68,500
Rabies	43	106	37	1.77	66,361
Newcastle	22	34	12	0.00286	35
Fowl Pox	11	0	4	0.038	141
Fowl Typhoid	6	0	0	0	0
CBPP	16	37	28	0.30	8,552
Rinderpest	0	124	10	0.30	3,216
TOTAL					286,218

ANNEX 9

Recent Usage and estimated national requirements for priority vaccines (Zambia 1985-2000)

Vaccines	USED x000 doses 1985	ESTIMATED REQUIREMENTS x000 doses 1986	1990	2000
Anthrax	313	485	544	703
Blackquarter	840	1,200	1,350	1,750
Haemorr.Septic	258	250	250	250
Brucella S19	34	240	264	316
Rinderpest	62	40	44	58
FMDV	137	350	385	470
Rabies	37	250	250	250
CBPP	28	16	17	22
Newcastle	6,204	16,000	18,500	24,000
Fowl Pox	427	1,500	2,200	3,000
Marek's	0	8,000	9,000	12,000
Fowl Typhoid	0	1,500	2,200	3,000

ANNEX 10

BLACK QUARTER VACCINE (Cl.chauvoei)

STARTING MATERIALS

1. **Master Culture (MC)**

Origin: Cl.chauvoei strain 16 obtained from Weybridge, England as freeze-dried culture.

Storage: Freeze-dried at 4-8°C.

2. **Master Seed Culture (MSC)**

2.1 Preparation from MC

Microscopic Examination	Freeze-dried MC is opened and reconstituted with nutrient broth. Sampled and used as inoculum for CM medium in flask.
Purity	Incubated for 48-72hrs.
Purity Identity Pathogenicity	Culture sampled and stored at 4-8°C pending on tests.
	Suitable culture is dispensed into ampoules and freeze-dried to form MASTER SEED CULTURE stored at 4-8°C.

3. **Preparation of Seed Culture (SC) from MSC**

	Freeze-dried MSC is opened and reconstituted in nutrient broth and used to inoculate CM medium.
Purity Identity	Incubated at 37°C for 24hrs and sampled. Kept at room temperature pending on tests.
	Suitable culture is dispensed into universal containers and stored at 4-8°C to represent SEED CULTURE.

4. Media, Solutions etc

Production Medium (CM Medium)

Liver 2.5Kg, Peptone 80g, Meat extract 80g, NaCl 40g, Na_2HPO_4 22.56g, KH_2PO_4 13.92g, glucose 80g, distilled water 8000ml.

Nutrient Broth

Triptose Blood Agar

For purity.

Peptone water plus various sugars

(Glucose, lactose, sucrose, maltose, marmital, solicin and insulin). For identity.

Roberts Cooked Meat Medium (CM Medium)

For sterility.

PRODUCTION PROCEDURES AND CONTROL TESTS

Preparation of Production Seed Culture (PSC)
from Seed Culture for the inoculation of medium in culture vessel.

Suitable Seed Culture is removed from 4-8°C and used to inoculate 15 x 500ml flask containing 300ml of CM medium.

Incubated at 37°C for 20hrs sampled.

Microscopic Examination

Purity

Suitable PRODUCTION SEED CULTURE is used to inoculate medium in culture vessel.

Preparation of Production Culture

Production medium is inoculated with suitable (20-22hrs) Production Seed Culture, 300ml/8L CM-medium in 10L glass cylinders.

Incubated at 37°C for 24hrs. Each culture is sample and incubated for an additional 24hrs at 37°C. During this pH is adjusted to pH7.1 - 7.3.

Microscopic Examination

pH

Purity

Suitable Production Cultures are pooled to make ca. 10 lots and 0.6% formalin added.

Incubated at 37°C for 4 days shaking it periodically (at least twice daily).

After 4 days at 37°C pooled antigen concentrate is transferred at 4-8°C and sampled.

Sterility

Innocuity

Suitable INACTIVATED ANTIGEN CONCENTRATE is ready for adjuvant.

Preparation and Testing of Bulk Vaccine

Suitable inactivated antigen concentrate is precipitated with Potassium aluminium sulphate (10%) to a final concentration of 1%.

Kept at room temperature for 18hrs then adjust pH7.2. Sample.

Sterility

Bulk vaccine stored at 4-8^{0}C pending on test for filling out.

Filling and Testing of Final Containers of Vaccine

Suitable Bulk Vaccine is filled out into 100ml bottles (20 doses).

Filled and closed bottles sampled (1%) and stored at 4-8^{0}C pending tests for release.

Sterility
(each filling lot)

Innocuity
(abnorm.toxicity)

Safety
(pooled sample)

Potency

Footnote

A batch of this vaccine (BQ) consists of 35-45 conical bottles, 10L each, containing 8L of medium. Eight litre culture yields ca. 1700-1800 doses of the vaccine. A production cycle is 63 days between media preparation and issue of vaccine. Five to 6 batches p.a. are prepared yielding a total of ca. 500,000 doses.

QUALITY CONTROL TESTS (IN-PROCESS AND FINAL)

Microscopic Examination

A smear of sample is stained by Gram's method and examined microscopically.

Pass: typical cell and spore morphology of Cl.chauvoei.

Identity

Biochemical tests, sugars etc.

Pass: Reactions typical of Cl.chauvoei.

Purity

Two Triptose blood agar plates are inoculated with sample, one incubated anaerobically, the other aerobically at 37°C for 24hrs. Smears are made of selected colonies and stained by Gram's method.

Pass: Absence of contaminating colonies on both plates and Gram's stain exhibits cells typical of Cl.chauvoei.

Pathogenicity Test (it is not done routinely only occasionally)

Two mice and 2 Guinea-pigs are inoculated (IM), 0.25ml for mice and 0.5 for G-P.

Pass: Death within 24hrs and hystology (tissue impression for Gram's stain) giving characteristic cell morphology.

Potency Test

Carried out on pooled samples of a filling lot. Ten Guinea-pigs are each inoculated with 2ml of the vaccine S/C. Injections are repeated 21 days later.

Ten days after 2nd inoculation the 10 vaccinates with three non-vaccinates are challenged with a 24hr old culture of Cl.chauvoei representing 50 MLD_{50}. The 3 controls are challenged with 10 MLD_{50}. Observed for 5 days.

Pass: All three controls die in 48hr (or less) and 80% of vaccinates survive.

Test for Innocuity (freedom from abnormal toxicity)

Each of 2 healthy mice are injected (S/C) with 0.5ml of test material. Each of 2 Guinea-pigs are also injected with 0.5ml of test substance (I/M) consisting of 1ml test sample and 0.5ml of 5% $CaCl_2$.

Animals are observed for 10 days.

Pass: Animals should remain clinically normal.

Safety Test

A pool of samples of filled vaccines is used. Each of 2 calves are inoculated (S/C) with 10ml of the sample and observed for clinical reactions, temperature changes and local reactions for not less than 10 days.

Pass: Minimal local reactions (not progressive). Temperatures not exceeding 39.5°C.

ANNEX 11

ANTHRAX (living) SPORE VACCINE

STARTING MATERIALS

1 Master Culture (MC)

1.1 Bacilus anthracis $34F_2$

1.2 Origin: Central Veterinary Laboratories, Weybridge, U.K.

1.3 Maintenance: Freeze-dried at 4-8°C.

2 Master Seed Culture (MSC)

2.1 Preparation of MSC from MC

	Open vial of MC and take up in a small volume of Nutrient Broth (NB).
Microscopic Examination	Dilute NB suspension of MC as required for the inoculation of 5 Roux flasks and sample.
Test for Purity	
	Inoculate each Roux flask with ca. 5ml of suspension of MC and incubate inverted flasks at 37°C for 72hrs. Inspect daily for growth and contamination.
Test for Sporulation	Sample each flask and transfer them at ambient temperature for an additional 2-3 days and sample daily.
Test for purity	Satisfactory flasks are harvested each with ca. 30ml of Weybridge buffer containing sterilized glass beads and harvest collected separately.
	Each harvest heated at 60°C for 60mins, cooled and sampled.
Test for purity	

Satisfactory harvests are pooled stored at 4°C ready for freeze drying.

Harvest removed from 4-8°C and filled out and freeze-dried.

Each filling lot of 15 vials are sampled.

Test for purity

Test for Viability

Satisfactory vials are stored at 4-8°C as MASTER SEED CULTURE to be used as inoculum for SEED CULTURE.

N.B. Acceptance of Master Seed Culture is also subject to satisfactory potency assay and safety test of the first batch of vaccine prepared with it.

3 Media Solutions etc

3.1 Production Medium: (OMS/FAO recommended medium) Bacto casitone (or Triptone) 2%, yeast extract 0.3%, salts and agar, pH7.2. Poured into Roux flasks and autoclaved at 121°C for 30mins.

3.2 Nutrient broth

3.3 Nutrient agar

3.4 Blood agar

3.5 Buffer (Weybridge)
Bacto casitone 50g
Sacharose 100g
Sodium glutamate 40g
Made up with distilled water 1000ml
Sterilised at 121°C for 30mins.

3.6 Sterile saline

3.7 Inactivating agent
Heat at 60°C for 60mins.

3.8 Preservative : Glycerol

3.9 Adjuvant
0.5% Saponin

PRODUCTION PROCEDURES AND CONTROL TESTS

Preparation of Production Seed Culture (PSC)
From Master Seed Culture (MSC)

One vial of MSC is opened and reconstituted in a small volume of NB then diluted with sufficient NB to inoculate 2-3 Roux flasks.

Incubate inverted Roux flasks for 24hrs at 37°C.

Harvest each flask separately in ca. 30-40ml NB containing glass beads. Sample each, and store at 4°C pending tests.

Microscopic Examination

Test for Purity

Satisfactory individual harvests are pooled to form the SEED CULTURE to be used as inoculum of PRODUCTION CULTURE

Preparation of Production Culture (PC)

Satisfactory SC is removed from 4-8°C and ca.5ml used to inoculate each Roux flask.

Incubate at 37°C for 72hrs.

After 72hrs incubation 3-4 flasks are sampled daily for an additional 2-3 days for sporulation.

Test for Sporulation

After about 5 days of incubation (pending a satisfactory sporulation) each flask is harvested in ca.30ml of saline, containing glass beads, and harvests of 3-4 flasks are pooled. Each pool is sampled before two-times its own volume of sterile glycerol is added and stored at room temperature in the dark pending purity test.

Test for Purity

Viability Test Safety in the Target Species	After ca. 3 weeks at room temperature satisfactory groups of harvests are pooled to form the BULK CONCENTRATE. Sampled and stored at 4°C pending tests.

Preparation and Testing of Bulk Vaccine

Satisfactory Bulk concentrate is diluted in equal parts of sterile saline and glycerol, containing 0.5% Saponin, to give 1 x 10^7 spores per 1ml dose. Stored at 4-8°C until filled out.

Filling and Testing of Final Containers of Vaccine

Bulk vaccine is removed from 4-8°C and filled out into 50 dose glass containers (rubber stopper, aluminium cap).

Test for Purity Test for Potency	Each filling lot of ca.20L give ca.400 bottles, 1% is taken as sample. Stored at 4°C pending tests for release.

Footnote to Anthrax spore vaccine production

A typical batch of this vacicne consists of the growth yield from 30-50 Roux flasks. Each flask yields ca. 3000 doses. Two to 3 batches p.a. are produced, a total output of 200,000 doses p.a.. Production of a batch from media preparation to issue of vaccine takes 77 days.

The "suite" devoted for the production of this vaccine is shared with that for the productio of Brucella abortus S19 on a campaign basis.

QUALITY CONTROL TESTS

Anthrax Spore Vaccine

(live for veterinary use)

Microscopic Examination

Colonies from blood agar plates (from purity test) are sampled and smears are prepared for staining by Gram's method. These are examined under the microscope.

Pass: cells exhibiting morphology of B.anthracis.

Test for Purity

3 nutrient agar (NA) and 3 blood agar (BA) plates are inoculated with the sample. All but one BA plate are incubated aerobically at 37°C for 48hr. One BA plate is incubated anaerobically at 37°C for 48hr. All plates are examined macroscopically and some of the colonies are sampled for microscopic examination.

Pass: Colonies on all the plates exhibit morphology compatible with those of acapsular B.anthracis. Microscopic examination of selected colonies in support of macroscopic examination.

Test for Sporulation

Smears of samples stained by Ziehel-Neelsen stain and count proportion of spore bearing cells (spores stained bright red).

Pass: 60-70% sporulation (Master Seed Culture)
90% sporulation (Bulk Concentrate).

Test for Viability

Serial 10-fold dilution of the samples are prepared in nutrient broth. One ml of 10^{-7}, 10^{-8} and 10^{-9} dilutions respectively are mixed with 20ml of molten Bacto-casitone medium (cooled to 45°C). Each 20ml volume, representing either 10^{-7} or 10^{-8} or 10^{-9} dilutions, are split and poured into three petri dishes. Incubated at 37°C for 48hrs and colony counts carried out.

Pass: for bulk concentrate not less than 2×10^8 cells/1ml (ie at least 20 doses per ml of bulk concentrate).

Safety in the Target Species

Each of two goats and each of two sheep are inoculated with 10ml of the bulk concentrate S/C (receiving ca. $2\text{-}5 \times 10^9$ spores) and observed for 10 days for systemic and local reactions. Also take rectal temperatures daily for 10 days.

Pass: Absence of progressive oedema. Elevated temperature of up to 40°C for the first 3 days is acceptible. Progressive oedema not acceptible.

Purity (bacterial contamination)

This test is carried out on filled vaccine. Two nutrient broth and 2 thyoglycollate media are inoculated with the sample and incubated at 37°C for 7 days. From each culture smears are prepared for Gram stain and also inoculated onto 3 blood agar plates, 2 incubated aerobically and 1 anaerobcally at 37°C for 48hrs.

Pass: Microscopic examination: Gr+-ve cells exhibiting morphology of B.anthracis. Blood agar plates: growth exhibits colony morphology compatible with that of acapsular B.anthracis.

Potency Test

This test is carried out on all of the filling lot. One per cent of the filling lot is taken as samples - usually four containers. These are pooled and each of 10 guinea-pigs (350-400g each) are inoculated S/C with 0.5ml of the vaccine. Twentyone days later these and 3-5 non-vaccinates are challenged S/C with 200 MLD_{50} of spores of B.anthracis 17JB. Observation period is 10 days.

Pass: 80% survival of vaccinates.
100% death of challenge controls.

ANNEX 12

BRUCELLA S19 (living) VACCINE

STARTING MATERIALS

1. **Master Culture (MC)**

   1.1 Br.abortus S19 freeze-dried

   1.2 Origin: WHO Reference Laboratory (Ames Iowa).

   1.3 Maintenance: Freeze-dried at 4-8°C.

2. **Master Seed Culture (MSC)**

   2.1 Preparation of MSC: Not applicable since MC is used as MSC.

3. **Media, diluents etc**

Potato-Infusion Agar Medium (Production medium)

Peptone 10g, NaCl 5g, Lab-Lemco 5g, Neutral glycerin 20ml, dextrose 10g made up to 1000ml using watery potato extract (250g of potato extract in 1000ml of distilled water). Sterilized at 15 lbs for 30mins. Final pH 7.2-7.3.

Glycerol Dextrose Agar

Neutral glycerol 20ml, dextrose 10g, Agar 25g made up to 1000ml with distilled water. Sterilize at 15 lbs for 30mins. Final pH 7.5.

Dextrose Andrade's Medium

Peptone 10g, NaCl 5g, made up to 1000ml with distilled water, pH 7.2-7.3. Sterilise at 15 lbs for 30mins. Add 1% Andrade's indicator and 1% sterile dextrose solution.

Cooked Meat Medium (CM-medium)

Phosphate Buffered Saline (PBS)

Reagents

Andrade's indicator

0.5% acid fusion solution in N/1 NaOH.

Acriflavin (neutral)

10mg of Acriflavin per universal bottle. Stored in the dark and dissolved in distilled water to 1% concentrate just before use.

PRODUCTION PROCEDURES AND CONTROL TESTS

Preparation of Production Seed Culture (PSC)
(from Master Culture)

Open one vial of MC and reconstitute it in diluent provided by WHO.

Inoculate onto potato agar slopes (2) and incubate at 37°C for 48h. Examine daily for growth and contamination.

Harvest each slope separately in PBS and sample. Pending microscopic examination and acriflavin tests, satisfactory harvests are pooled to form PRODUCTION SEED CULTURE and used to inoculate Production Media.

Microscopic Examination

Purity

Dissociation

Acriflavin Test

Aerobic contamination

Anaerobic Contamination

Preparation of Production Culture (PC)

Satisfactory PSC is removed from 4°C, poured into seeding aspirator complete with magnetic bar for steering. Inoculate each of 35 Roux flasks with ca. 5ml of inoculum.

Incubate inverted Roux flasks at 37°C for 48hrs and examine daily for growth and contamination, avoiding contact between inoculum and surface growth.

Group Roux flasks in fives and pour off inoculum. Introduce 25ml of PBS into each flask and harvest growth collecting contents of 5 flask into one pool. Sample and put harvests at 4°C.

Microscopic Examination

Purity

Acriflavin Test

Dissociation/2

Aerobic Contamination

Anaerobic Contamination

Pool satisfactory pools of five harvests to form a single pool of BULK CONCENTRATE and sample.

Density

Viability

Inocuity

Store BULK CONCENTRATE at 4°C pending test results.

Filling and Testing of Final Containers of Vaccine

Bulk concentrate is diluted in PBS to contain 3×10^9 vialbe cells per 2ml dose.

Under constant steering diluted vaccine is dispensed into glass containers holding 25 doses per bottle.

Sample 1% of each filling lot and store at 4^0C pending test results.

Density

Viability

Inocuity

Safety

Footnote to Br.abortus S19 Vaccine Production

Standards and recommendations of WHO Monograph No. 55 (1977) were aimed for.

This vaccine is produced on solid medium in Roux flasks. A typical batch may consist of 35 Roux flasks. The following operations are carried out in cubicle (use of which is shared between production of Brucella S19 and B.anthracis cultures on a campaign basis): preparation of seeds, inoculation and harvesting of growth of Roux flasks, all inoculation of media for quality control, bulking and diluting of antigen concentrate and filling of vaccine (hand operated) for lyophilisation. Incubation of Roux flasks is in a free standing incubator, shared with Anthrax vaccine. Each batch of 35 Roux flask would yield ca. 50,000 doses of vaccine (issue density is ca 1/10 of that specified by B.Vet.Codex) and 3-4 batches per annum are produced yielding ca. 150,000 doses.

Production cycle takes 36 days from media preparation to issue of the vaccine.

TESTS FOR QUALITY CONTROL
Br.abortus S19 (living) vaccine

Microscopic Examination

A smear of seed culture is stained by Gram's stain and examined microscopically.

Pass: typical small gram negative bacilli.

Purity

Glycerol dextrose agar plates (2-3) are inoculated to give isolated colonies and incubated for 4 days at 37°C before visual inspection for contaminants.

Pass: Absence of contaminating growth.

Dissociation

Four days old isolated colonies on glycerol dextrose agar are inspected under a plate microscope in oblique lights.

Pass: Less than 5% rough colonies and not more than 15% semi-rough colonies.

Acriflavin Test

Take one loopful of acriflavin and place onto a clean microscope slide and mix with a small amount of cell suspension. Examine under plate microscope for agglutination.

Pass: absence of agglutination.

Aerobic Contamination

Dextrose Andrade's broth is inoculated and incubated at 37°C for 7 days and growth examined on days 2, 4 and 7 for presence of acid (red) and gas or both.

Pass: absence of red colour (acid) and gas.

Anaerobic Contamination

Cooked meat medium is inoculated and incubated at 37°C for 7 days. Plate out onto BA and incubate anaerobically and look for contaminants.

Pass: absence of colonies other than those of S19.

Density

Use 4 Hopkins' tubes containing 4.5ml of distilled water and add 0.5ml of test material. Centrifuge for 75mins at 2500rpm and read packed cell volume.

Pass: no pass value, "done for the record".

Viable Counts

Serial 10-fold dilutions in PBS are prepared and dilutions of 10^{-8}, 10^{-9} and 10^{-10} glycerol are plated onto dextrose agar plates 0.1ml per plate. Incubated at $37^{o}C$ for 4 days and count colonies and calculate viable cells per 1ml.

Inocuity

Inoculate two mice each with 0.5ml of the vaccine S/C and observe for 10 days.

Pass: no abnormal reaction.

Potency

None done

Safety

Each of two cattle are inoculated with twice the recommended dose of the vaccine (4ml) subcutaneously and observed for 10 days.

Pass: no abnormal local or systemic reactions other than transient fever.

N.B. Issue density of the vaccine is slightly less than 1/10 of that recommended by BP as the minimum.

ANNEX 13

HAEMORRHAGIC SEPTICAEMIA VACCINE

STARTING MATERIALS

1. Master Culture (MC)

1.1 Pasteurella multocida 6:E, a local isolate.
(Type verified by Carter)

1.2 Maintenance: a) freeze-dried and held at 4-8°C.
b) over liquid nitrogen in blood.

2. Master Seed Culture (MSC)

2.1 Preparation

Freeze-dried MC is opened and reconstituted with Nutrient broth.

Incubated at 37°C for 6hrs.

Susceptible calf is inoculated with 6hr broth culture I/V.

Just prior to death calf is bled out into flasks, containing glass bead or sodium acetate and blood is defibrinated.

Defibrinated blood is sampled, 10% glycerol is added then distributed in small volumes and stored in a liquid nitrogen refridgerator to represent MASTER SEED CULTURE pending on tests.

Test for Purity

Identity

Viability

Pathogenicity

Capsulation

2.2 Maintenance:

a) as a suspension of infected blood in liqid nitrogen refridgerator.

b) freeze-dried.

2.3 Verifications:

Purity, Viability, Pathogenicity, Encapsulation.

3. **Media, Solutions etc**

Media :

For preparation of Seed Culture : Nutrient Broth

For production: Nutrient broth (autoclaved) supplemented with 1% yeast extract and 0.2% sucrose (filter sterilised)

For various tests : Blood agar, MacConkey agar, Nutrient broth, thraglycolate medium.

Tests on Media

pH and sterility

PRODUCTION PROCEDURES AND CONTROL TESTS

Preparation of Production Seed Culture (PSC)
from Master Seed Culture (MSC)

	Master Seed Culture removed from storage over liquid nitrogen, thawed and inoculated onto blood agar plate.
	Incubated at 37°C for 18hrs.
Microscopic Examination	Colonies from blood agar harvested into small volume of nutrient broth and sampled.
	Suitable nutrient broth suspension used to inoculate a larger volume of nutrient broth.
	Incubated at 37°C statically for 6-10hrs.
Microscopic Examination Purity Test	6-10hrs old culture is sampled and pending result of microscopic examination it becomes PRODUCTION SEED CULTURE ready for inoculation into final culture.

Preparation of Production Culture

Suitable seed culture is inoculated into 5-10L production medium contained in a 10-20L glass cylinder equipped with a magnetic stirrer for aeration.

Incubated at 37°C for 24hrs whilst constantly agitated by magnetic stirrer whilst aerated through 0.22u millipore filter cartridge.

After 24hr of incubation sample taken and formalin is added to the rest of the culture to final concentration of 0.5%.

Viable Count

Purity Test

Incubated at 37°C for 48hrs and sampled.

Test for Sterility

Innocuity

Transferred to 4°C as inactivated ANTIGEN CONCENTRATE pending on tests.

Processing of Bulk Antigen Concentrate after Inactivation

Suitable bulk antigen concentrate is removed from 4-8°C and allowed to warm up to room temperature.

Diluted with sterile saline to contain 1 x 10^{10} cells per 2ml dose.

Precipitated by the addition of 10% potassium aluminium sulphate to a final concentration of 1% and left overnight at room temperature. After ca. 18hrs of incubation pH adjusted to pH6.5-7.2

Alum precipitated vaccine is sampled and stored at 4-8°C as bulk vaccine ready for filling out pending on tests.

Sterility

Innocuity

Filling of Bulk Vaccine

Suitable bulk vaccine is removed from 4-8°C and, after introduction of a magnetic stirrer into the vaccine, is ready for filling.

Whilst being constantly stirred it is filled out into 100ml volumes.

Sterility

Innocuity

Safety
(pooled sample)

Potency
(pooled sample)

1% of filled containers taken as samples and the rest is stored at 4-8°C pending on tests for release.

Footnote

This vaccine is produced in 10 and 20L glass bottles. For aeration magnetic stirrers are used and filtered air is supplied through a hole in the stopper. An average production batch may consist of 2 x 10L bottles and 2 x 20L bottles containing 30L medium in all.

Preparation of inoculum, seeding of production cultures, all sample collections, inactivation and bulking of cultures, adjuvanting the antigen, filling and closing of bottles (both hand operations) are carried out in a cubicle devoted to this purpose. Incubation of glass bottles is in the walk-in incubator, where the Cl.chauvoei cultures are also incubated. Each batch of ca. 30L culture yields between 100,000-120,000 doses and five batches p.a. are produced, a total of ca. 500,000 doses.

Production cycle is 50 days from media production till issuing vaccine.

TESTS FOR QUALITY CONTROL (IN-PROCESS AND FINAL)

Microscopic examination

Smear of sample stained by Gram's method, then examined microscopically.

Pass: typical morphology of P.multocida.

Test for Purity

Two blood agar and 2 MacConkey agar plates inoculated with specimen and 1 blood agar incubated anaerobically at 37°C for 48hrs.

Pass: absence of any contaminating organism.

Test for Identity

Smear stained by Gram's method and examined microscopically.

Pass: typical bipolar staining cocco-bacilli.

Test for Viability

Serial 10-fold dilutions in nutrient broth from 10^{-1} - 10^{-10} and 10^{-7}, 10^{-8} and 10^{-9} dilutions - 0.1ml of - inoculated per each of 3 blood agar plates. Incubated 37°C for 24hrs before counts are taken.

Pathogenicity Tests

Inoculate each of 2 healthy 12-15g mice S/C with 0.5ml of test sample and one rabbit S/C with 1ml of test sample. Observed for 48hrs.

Pass: All inoculated animals die and on post-mortem inoculated organism is recovered from spleen and blood on blood agar.

Encapsulation

Moller method of capsular stain is applied on sample.

Pass: 90-100% encapsulation.

Test for Sterility

Inoculate 2 blood agar plates and incubate one anaerobically, the other aerobically at 37°C for 48hrs. Also inoculate nutrient broth and thyoglycolate media with sample. Incubate at 37°C for 7 days. After 7 days nutrient broth and thyglycolate media are plated onto 2 blood agar plates and incubated for 7 days at 37°C.

Pass: absence of growth in all cultures. In case of failure a repeat test is carried out on twice the original scale. If same organism is recovered, sample failed.

Innocuity

Two mice per sample, 0.5ml S/C and observe for 10-14 days.

Pass: survival of both mice.

Test for Safety

Two calves inoculated with test samples, ie twice the recommended dose -4ml- S/C. Observed for 10-14 days. Rectal temperatures taken daily and local reactions looked for.

Pass: Both animals should be free of severe clinical symptoms. Slight temperature increases for the first 2-3 days is permitted as is slight nodule (an inch in diameter).

Test for Potency

Forty-eight mice are vaccinated S/C with 0.5ml of the vaccine and injections repeated 10 days later. Ten days after 2nd injection mice are divided into 8 x 6 and challenged, together with 6 x 5 non-vaccinated mice, S/C with 6-10hr broth culture diluted 10^{-1}-10^{-8}. For vaccinates 10^{-1}-10^{-8} are inoculated per group of 6. For non-vaccinates 10^{-5}-10^{-10} dils. are inoculated. Observed for 7 days and take mortality daily.

Pass: Compare LD_{50} in vaccinates and non-vaccinates. Minimal difference is five logs or over.

DATA ON MANUFACTURING FACILITIES AT COOPER (ZAMBIA) LIMITED

Cooper Zambia Ltd is a subsidiary of the Wellcome Foundation Ltd, U.K. The company has a work force of 70 people 2 of whom are expatriates. It has a warehouse with a cold-room and a factory which produces the following range of products:-

Cattle dips, anthelmintics, rodenticides, insecticides, dairy hygiene products, disinfectants and aerosols. The factory has dedicated plants for the production of dips (Miscible Oil Products), disinfectants, powders and aerosols, with the following capacities: Miscible Oil Products (M.O.P) 1.2m litres

Disinfectants	600000 litres
Powders	120000 Kg
Aerosols	2.5 million cans
	(all annual capacities)

Owing to various constraints which are beyond the control of the company the actual through-put for the period September 1985 to August 1986 was:

M.O.P.	100000 litres
Disinfectants	50000 "
Powders	25000 Kg
Aerosols	320000 cans

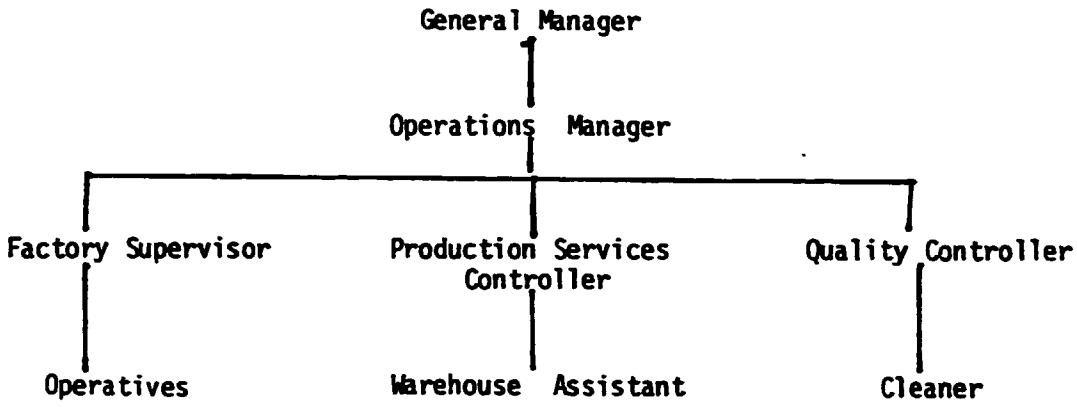
The factory is fairly well equipped for the purpose. The equipment includes;

4 x 2500 litres	mixing tanks
1 x 1000 litres	" tank
2 x 800 "	" tanks
1 x 700 "	" tanks
4 x 400 "	" tanks
1 x 100 Kg	concrete mixer for powders

There is a quality Control Laboratory on site which is capable of carrying out the necessary tests to ensure conformity of the products to acceptable Coopers standards. The Laboratory also carries out dip tank analyses as a service to farmers. The equipment in the laboratory includes:-

1 x Pye Unicam Gas - Liquid Chromatograph with a dual pen recorder, 1 x Sauter Analytic Balance, 2 x pH Meters, 1 x Centrifuge, 1 x Oven, 1 x Mettler Balance and 1 x Water Deioniser.

The organisation chart of the factory is as follows:-



The Operations Manager, a registered pharmacist, heads the factory and he is assisted by a Factory Supervisor, who has 15 years experience in the job, a Production Services Controller, a BSc graduate, who is responsible for material control and monitoring productivity and a Quality Controller who is a qualified Laboratory Technician.

Strict G.M.P. (Good Manufacturing Practice) procedures are implemented to ensure the safe handling of toxic materials.