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Control of Avian Coccidiosis : Past, Present, and Future

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닭 콕시듐병 방제의 과거, 현재, 그리고 미래

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요 약

현재 콕시듐 병의 방제는 약제 투여, 사육관리, 백신을 이용하거나 혹은 이들 방법을 적당히 혼합하여 실시하고 있다. 약제 투여 방법은 약의 내성이라는 문제점을 내재하고 있지만 아직도 콕시듐 방제에 최우선 방법으로 쓰고 있으며 이러한 약제 내성을 조금이나마 지연시키기 위하여 shuttle, 혹은 rotation program 을 쓰고 있다. 따라서 약제 내성 검사는 각 농장에 어떠한 약제가 효과가 있는지를 알아보는데 가장 중요한 방법이 되어 왔던 것이다. 콕시듐 방제에 있어서 사육관리에 의한 방법은 아직은 완전한 효과적인 방법으로 정착되지 않았는데 그 이유는 소독제 만으로는 콕시듐의 원충을 죽일 수 없기 때문이다. 백신에 의한 방법은 현재까지 살아있는 콕시듐 원충을 제한적으로 쓰고 있는 단계이다. 새로운 형태의 백신개발이 요즈음 연구가 되고 있는데 이 백신은 순화 시킨 콕시듐 원충을 사용하거나 유전공학적 방법 혹은 원충의 일부분에 대한 항원을 추출하여 백신으로 이용하는 것이다.

INTRODUCTION

The introduction of sulfa compounds in the late 1930s for the treatment and prevention of coccidiosis was thought by many producers to be the solution to the devastating losses caused by that disease. Over fifty years and numerous anticoccidials later, coccidiosis remains the major parasitic problem in poultry. Granted, losses from mortality have been markedly reduced but morbidity from improper growth and utilization of feed by the modern broiler has increased.

Currently three methods are used to control coccidiosis ; medication, management, and immunity. Prophylactic medication remains the major control method in broilers where as immunity is the main method used in breeders. We

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are currently on the threshold of developing several new methods for coccidiosis control including several new means of vaccination. Advances in these techniques should markedly reduce the economic impact of this continuing disease in poultry.

MEDICATION IN BROILERS

There are over 25 anticoccidial compounds or combinations currently approved for use in the feed for the prevention of coccidiosis in various countries. It is safe to say that the modern poultry industry could not exist without these compounds for the control of coccidiosis. Drug resistance became a problem shortly after the first anticoccidials were introduced and has continued to contribute to the declining efficacy of medications being experienced today(Jeffers, 1989). Although drug resistance has been slow to develop with some drugs such as monensin, it has never the less appeared in all medications developed to date. Drug resistance to compounds such as the quinolones and robenidine can appear rapidly, i.e., in a single grow out.

Several medication schedules have been used to delay the development of resistance. These are rotation programs, the changing of compounds after several grow outs and shuttle programs, the use of several successive compounds with in a single grow out. There has been a tendency for poultry firms to continue to use good compounds until resistance has greatly reduced or destroyed the efficacy. A good example of where this has not occurred is nicarbazin. In the field, the use of the drug is halted in the late spring summer and fall because of the problem of heat stress susceptibility in birds given this medication. This forced rotation away from nicarbazin has extended the useful life of this compound for many years.

THE VALUE OF SENSITIVITY TESTING WITH AVIAN COCCIDIA

The development of drug resistance by the coccidia has made sensitivity testing with anticoccidials of significant value to the poultry industry(Ruff, 1992). Sensitivity testing provides two important pieces of information; 1) the detection of developing drug resistance and 2) the determination of what drugs will be effective if used. The only way to accurately determine what drugs will control the coccidia in a specific location is to recover the coccdia that are present and test them against several anticoccidials. This is done by feeding unmedicated chickens litter samples and recovering any oocysts produced. These in turn, are used to inoculate medicated chickens and unmedicated controls. Challenge doses of the coccidia must be high enough to produce measurable adverse effects in unmedicated, inoculated control birds and unmedicated uninoculated controls are also essential for meaningful comparisons of medication efficacy.

Parameters used to determine susceptibility or resistance to a specific medication include mortality, weight gain, feed conversion ratio, dropping score, or lesion score. Some researchers use these parameters individually; others combine two or three parameters into an anticoccidial index. Regardless of the method used, the end point selected should indicate reduced control or a lack of control that will produce economically significant effects in the field.

Arbitrarily changing to a different drug is no assurance of efficacy if sensitivity testing has not been done. The drug picked may be no more

effective than the one used previously. Waiting to see a problem before conducting sensitivity testing is a bad idea for several reasons. The idea behind sensitivity testing is to identify problems in coccidiosis control before they reach crisis proportions. Waiting until you have obvious coccidial breaks is too late since the tests take several weeks to complete by which time the damage is done.

Table 1 summarizes the results of some sensitivity testing in the United States. Such studies readily show that resistance is increasing with time. For example, all field isolates tested by Jeffers in 1978 were susceptible to monensin but by 1986 only one third were sensitive(McDougald et al., 1986). This problem is not limited to the ionophores but occurs with synthetic chemical drugs as well. Mathis and McDougald(1982) found no resistance to halofuginone, yet in 1989 our Laboratory found 9 out of 11 isolates from the Eastern Shore resistant to that drug(unpublished data). Table 2 shows that similar patterns of developing resistance are found in foreign countries. Note also that many of the isolates shown in Tables 1 and 2 are resistant to more than one drug in a family of compounds, a phenomenon known as cross resistance. There are examples of exposure to one medication producing cross resistance to a related compound, even though the second was not used. For example, resistance does not readily develop following exposure to the experimental drug diclazuril. Conversely, when coccidia are only exposed to toltrazuril, a related compound, resistance readily develops, often with cross resistance to diclazuril.

It isn't practical to continually monitor the pattern of resistance on all farms during each and every grow out because the cost and volume of testing would be prohibitive, nor is such detail necessary. The idea is to know what is happening to the control of coccidiosis, to be able to forecast resistance before it becomes a major problem, and to select effective compounds as alternatives. Generally speaking, the pattern of resistance is similar within a single complex, provided that medication use has been the same throughout. Routine testing can usually be scheduled at 6 month intervals. Problem farms might be checked more frequently.

Because sensitivity testing uses the actual coccidia from the farms in question, the results for resistance and susceptibility to medications

Table 1. Results of selected domestic trials testing for anticoccidial sensitivity

No. isolates	Anti-coccidial	Number of isolates			Reference
		Sens.	Red. Sen.	Resist.	
73	Monensin	73	0	0	Jeffers(1978)
52	Monensin	27	25	0	McDougald(1981)
	Lasalocid	29	23	0	
	Salinomycin	33	19	0	
99	Monensin	33	29	38	McDougald et al.
	Salinomycin	53	18	29	(1986)
	Nicarbazin	67	13	20	
	Amprolium+	39	15	46	

Sens = sensitive; Red Sen = reduced sensitivity, Resist = resistant.

Table 2. Results of selected foreign trials testing for anticoccidial sensitivity

Country	No. Isol.	Anti-coccidial	Number of isolates			Reference
			Sens	Rd Sen	Rst	
Brazil & Argentina	60	Monensin	24	9	27	McDougald et al. (1987)
		Narasin	28	14	18	
		Salinomycin	32	15	13	
		Maduramicin	58	2	0	
		Clopidol	32	11	17	
		Amprolium	10	11	39	
		Nicarbazin	56	4	0	
Czechoslo-vakia	2	Monensin	0	0	2	Bedrnik et al. (1989)
		Lasalocid	2	0	0	
		Salinomycin	0	0	2	
		Narasin	0	0	2	
		Maduramicin	2	0	0	
Japan	72	Decoquinate	43	–	29	Katae et al. (1989)
		Nicarbazin	61	–	11	
		Lasalocid	14	–	58	
		Salinomycin	13	–	59	

Sens = sensitive; Rd Sen = reduced sensitivity, Rst = resistant.

reflect the true potential in the field. The objection is sometimes raised that drug resistance identified by sensitivity testing does not always correlate with problems in the field, i.e., no problems are seen even though the strains present are resistant to the medication being used. This is possible when the level of coccidial exposure is quite low as might occur in dry summers when litter conditions are not conducive to sporulation and survival of coccidial oocysts. Similarly, the shedding of low numbers of oocysts because the flock is generally immune will give a mild exposure. In these cases, the medication may not be working, but the failure is not readily apparent. If, however, conditions change so that moisture levels in the house rise, a heavy exposure will not be controlled. We can safely say that a drug that does not control a battery challenge will not control a heavy field challenge.

It is becoming increasingly common to find that only a few compounds have reasonable efficacy against the coccidia present in a particular complex(M. Segraves, Rhone-Poulenc, personal communication). In such instances, it is critical to save the most effective medication for those periods of heavy coccidial exposure(winter or unusually wet springs or falls), rather than risk the development of drug resistance by use during periods of low exposure(summer and dry years). In some field cases, the same drug will produce an improvement of 10 points in feed conversion during periods of heavy exposure, but only a 1 or 2 point improvement in periods of low exposure.

Loss of drug resistance in populations of

coccidia can occur but it may take 5 or more years without exposure to the drug in question. For example, resistance rapidly developed to robenidine in the mid 1970s resulting in an almost complete cessation of its use by the late 1970s. By the early 1980s, strains of coccidia from the field were again showing susceptibility to this compound and the drug now has a place for limited use in control programs. Lose of resistance permits the careful use of older anticoccidials, but shifting to one of these older compounds could be a serious mistake if sensitivity has not returned to the coccidial populations. Only sensitivity testing can provide the answer.

MEDICATION IN PULLETS

In pullets, medication is often given during the early growing period to protect against coccidiosis while immunity develops. This sometimes involves the use of a "step down" program where the drug level in the feed is progressively reduced to allow the birds to experience a continuing low level of infection that progressively stimulates immunity until solid protection is obtained at 8 to 12 weeks of age. The severity of the exposure to coccidial oocysts, the degree of infection, the type and level of medication, and the feeding regimen are all important for the development of the desired level of immunity.

The type of feeding regimen can influence both the degree of protection to a primary infection and the level of immunity developed. Ruff and Chute (1980) showed that some anticoccidials are less effective when fed in a restricted feeding program(skip-a-day) than when medicated feed was given ad libitum(Table 3). In addition, potent anticoccidials, such as monensin was 10 years ago, can be too effective in controlling the initial infection with ad libitum feeding and thus not allow sufficient exposure to stimulate immunity(Table 4). This is not a problem with a restricted feeding program because the intermittent presence of the drug allows for sufficient infection to stimulate development of immunity. It has recently been suggested that the gradual appearance of resistance to monensin might alleviate this problem in ad libitum fed pullets.

Table 3. Efficacy of various anticoccidials in controlling a primary coccidial infection in pullets

Feeding regimen	*Eimeria*	AMP	CLP	MON
Ad libitum	*E. tenella*	HE	HE	HE
	E. acervulina #1	PE	HE	HE
	E. acervulina #2	PE	PE	PE
	E. maxima	NE	NE	HE
Restricted	*E. tenella*	HE	NE	HE
	E. acervulina #1	NE	PE	HE
	E. acervulina #2	PE	PE	PE
	E. maxima	NE	NE	NE

AMP = amprolium, CLP = clopidol, MON = monensin. HE = highly efficacious,
PE = partially efficacious, NE = not efficacious.

Table 4. Immunity of pullets medicated with various anticoccidials and exposed to coccidiosis by contaminated litter in floor pens. Pullets were later moved to cages, given unmedicated ration, and challenged with the same species of coccidia to test for immunity

Feeding regimen	*Eimeria*	AMP	CLP	MON
Ad libitum	*E. tenella*	N	N	N
	E. acervulina #1	S	S	S
	E. acervulina #2	S	N	N
	E. maxima	S	S	N
Restricted	*E. tenella*	S	S	S
	E. acervulina #1	S	S	S
	E. acervulina #2	S	S	S
	E. maxima	S	S	S

AMP = amprolium, CLP = clopidol, MON = monensin. S = solidly immune,
N = only partial immunity developed.

MANAGEMENT

Good management practices can reduce, but not eliminate, the transmission of coccidiosis among individual birds in the flock. Cage raising of broilers, if ever developed, would help reduce transmission and might eliminate the need for much of the medication used. Several aspects of the coccidial life cycle account for the difficulty in controlling the infection by present management practices. First of all, the reproductive potential is enormous. A single oocysts can give rise to 20,000 to 200,000 oocysts six to eight days later. When the oocysts are passed in the feces, moisture and warm temperatures are conducive to the sporulation process, thus, wet spots around waterers should be removed.

The oocysts stage possesses a thick wall that is extremely resistant to disinfectants and changes in the environment such as drying. As a result, cleaning and disinfecting a poultry house will reduce the number of oocysts but will not eliminate them from the premises. The potential remains for a rapid buildup in the litter. Even new poultry houses will develop a resident population of coccidia within 5 to 6 weeks. A combination of coccidiacide and disinfectant is marketed in Europe under the name "OO -cide".

VACCINATION

Vaccination has been used for the control of many diseases in livestock and poultry. In general, vaccination against parasitic diseases has been less successful than vaccination against viral or bacterial diseases. With the advancements in biotechnology over the last 5 to 7 years, vaccination for coccidiosis has received a great deal of attention. Several approaches for vaccination are being investigated including the use of live organisms, parasite fractions, and genetically engineered antigens.

The only vaccines currently available for the control of all species coccidiosis are sold under the names of CocciVac or Immucox. Both of these products utilise controlled dosages of viru-

lent coccidia and depend on recycling of several generations of the parasite in the litter. In addition, treatment must be given in the drinking water if the immunizing infections become too severe. These vaccines are used in replacement pullets to stimulate the development of the immunity that is relied on for control. Recently, some poultry firms in the U.S. have had success with these live vaccines in heavy broilers. Problems can arise because all birds do not receive an equal exposure, thus some birds remain susceptible where as others develop clinical coccidiosis.

Elanco has provided a live vaccine(Elanco Vac M) for the control of *E. maxima* in combination with the ionophore anticoccidials that the company markets. This vaccine gives a dose of live oocysts at the hatchery, usually with the beak-o-vac machine. Protection depends on two characteristics of this coccidial species and ionophore medication. First, because *E. maxima* is the most immunogenic of the avian coccidia, the relatively few oocysts given are sufficient to stimulate a degree of protection against subsequent infections with that species. Second, the ionophores act against the sporozoite stage in the lumen of the intestine, therefore, the delay in medication until the chicks are placed in the broiler house allows this initial infection to develop, yet protects against subsequent exposure in the facilities. Similar vaccines have been tested against other species but have not been as effective.

It has been known for many years that a series of very mild infections from a "trickle exposure", a few oocysts over a period of time, can induce solid immunity without the development of adverse effects from clinical coccidiosis. Recently, there has been renewed interest in this method by encapsulating live oocysts in alginate or fat-water beads that are added to the feed(Parry et al., 1989). Although this allows for a continued, low-level of exposure to coccidia, it precludes medication because the coccidia must grow in the bird for immunity to develop.

A more recent modification in live vaccines involves the use of strains of coccidia that have been attenuated by passage through chick embryos or by selection for precociousness(Shirley and Long, 1990). The shortened prepatent period of the precocious lines is often due to a loss of one or two asexual generations which still allows immunogenicity but reduces pathogenicity. This vaccine is being marketed in Europe under the name "Paracox". Once again, medications cannot be used with this method because growth of the parasites in the birds is required for immunity to develop.

FUTURE METHODS OF COCCIDIOSIS CONTROL

An "old" vaccination technique that is being reinvestigated is the use of x-irradiated oocysts to induce protection without pathological development(Jenkins et al., 1991a). Certain radiation levels inhibit the development of the parasites without restricting invasion of the host cells or the immune response to the infection. These irradiated parasites will not be used as vaccine antigens, but are a powerful tool to provide information on specific antigens that can be targeted for genetic engineering.

Although many past attempts to immunize with killed coccidia were unsuccessful, recent trials using either sporulated oocyst extracts or isolated surface protein fractions have demonstrated protection(Murrey et al., 1986). It was also possible to show cross protection against *E.*

tenella and *E. maxima* challenge following vaccination with *E. acervulina* antigens, an observation markedly different from the species-specific immunity which follows inoculation with sporulated oocysts. An example of protection induced by vaccination with an oocyst extract of *E. tenella* is shown in Table 5.

An area that has received considerable attention over the last few years is the potential development of genetically engineered coccidial antigens for use in "subunit" vaccines. Immune sera or monoclonal antibodies are used to detect coccidial genes that have been cloned into some expression vector such as *E. coli*. In general, many of these proteins can elicit some protection against coccidial challenge(Danforth and Augustine, 1990). Complete protection has only been obtained in battery trials by combining several different antigens(Danforth et al., 1989). Cross-species protection is also seen but it is not complete. One major advantage of subunit vaccines is that immunity does not depend on growth of the parasite, thus medication can be given to provide protection during the period while immunity is developing. This can be important in locations where heavy early coccidial exposure may exist because one day old chicks are fully susceptible to infection.

A method being tested to enhance the immune response of the bird to these antigens involves the incorporation of the coccidial genes into delivery vectors such as pox virus or *E. coli*. The rationale is that these organisms will deliver the antigen directly to the intestine and persist for a period of time to provide better antigenic stimulation of the host's immune system. Protection has been demonstrated against challenge infections following vaccination with *E. coli* that contained coccidial genes(Jenkins et al., 1991b). Coccidial antigens have also been introduced directly into the 18 day old embryo to stimulate partial protection on challenge (Fredericksen et al., 1989).

There are two other areas that have potential for control of coccidiosis in the future. One of these is the blockage of the receptor or recognition sites used by the parasites for invasion of host cells(Augustine and Danforth, 1985). The second would involve the identification of the avian genes responsible for resistance to coccidiosis and the insertion of those genes into commercial lines of poultry(transgenics). This latter approach is still theoretical but advances are being made that may allow the success of this method in the future as resistant and susceptible lines of poultry have already been identified.

CONCLUSIONS

Control of coccidiosis has been a significant problem in poultry for many years. In the past,

Table 5. Effect of challenge infections in chickens immunized by intramuscular injections of *E. tenella* oocyst extracts given at 7 and 14 days of age

μg protein	Immunized	Challenged	Gain(g)	Lesion score
0	−	−	126[a]	0[a]
0	−	+	109[c]	2.3[c]
1	+	+	129[a]	0.9[b]
10	+	+	125[a]	0.6[b]
100	+	+	118[b]	1.8[c]

control in broilers depended mainly on prophylactic chemotherapy, but the ability of the coccidia to develop drug resistance has seriously hampered these efforts. Vaccination was previously limited to live virulent vaccines that have had limited application only in replacement birds or heavy broilers. New advances include the development of attenuated strains and nonliving subunit vaccines that use genetically engineered antigens. Future control of coccidiosis will best be accomplished by using a combination of these methods based on the specific situation on individual production facilities.

ABSTRACT

Control of coccidiosis depends on medication, management, immunity, or some combination of these methods. Prophylactic medication with anticoccidials remains the primary method of control, although the development of drug resistance is a major problem. Shuttle and rotation programs are increasingly used to delay the onset of drug resistance. Sensitivity testing has become an important tool in the identification of what specific medications will be effective in specific poultry operations. Management techniques for the control of coccidiosis have not been totally effective because most disinfectants do not kill the coccidial oocysts. Present methods for vaccination are currently limited to the use of controlled doses of live coccidia. New vaccination methods under investigation include vaccination with attenuated strains or parasite antigens produced either by fractionating the parasites or genetically engineered sub unit vaccines.

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