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USE OF NICARBAZINEE IN THE CONTROL OF URBAN PIGEON COLONIES IN ITALY IN 1990-2007

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Abstract
Objectives. Verify the effects of Nicarbazinee in birth control in urban pigeons from the early 1990 and following a series of trials with pigeons bred for racing. . *Methods.* .The treatment, which involved 552 colonies (85562 pigeons), consisted of 8-10 g of maize grains enriched with 800 ppm of Nicarbazinee per day and per pigeon, 5 days a week from March to October. *Results.* The resulting infertility (which was complete in dovecot pigeons) can explain the significant reduction (from 28% to 71%) of the number of pigeons in urban colonies from the first treatment. In treated urban colonies the dispersion of a significant number of pigeons possibly involves the sub-adults and non dominant individuals that may be attracted by non-treated colonies with young birds in the nests. The positive results of the tests soon resulted in a veterinary drug with no effects on the environment, non-target species and birds of prey The regular use of this drug could be part of a cheaper and more effective management of problematic colonies of urban pigeons.

Keyword*s:* Nicarbazinee, pigeon, *Columba livia,* control, birth

Introduction

Nicarbazine is a drug that is widely used to control coccidiosis in poultry and whose side effects on egg laying precluded its use in laying hen holdings (Sherwood et al., 1957; Weiss et al., 1960; Hurwitz et al., 1975; Mc Loughlin et al., 1978; Polin, 1978; Hughes B.L.et al, 1991; Valfré et al, 1990). The fertility of captive pigeons was verified in preliminary experimental pharmacotherapy studies (Martelli et al., 1992,1993) and its use on urban pigeons was proposed (Zannetti, 1992). The purpose of this presentation is to highlight the results of studies and field applications carried out by the AA. in the pharmacological control of the reproductive activity of urban pigeons.

In Italy many towns have significant problems caused by the excessive numbers of urban pigeons. By evaluating the costs associated with the impact of urban pigeons, Zucconi et al. (2003) calculated the cost of cleaning out guano from pavements and other places in about € 7.75-10.5/pigeon/year, while the cost of restoring monuments amounted to € 16-23/pigeon/year and estimated additional annual costs of €3.4-4.5 million for 103 italian towns. Other costs (€ 20-43 million/

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year) result from damage caused by pigeons to farming production, with an average loss of 0.5-1% of cereal crops.

No data are available on the total cost in terms of public health and hygiene, despite the fact that the risk of zoonoses from urban pigeons is high (Haag-Wackernagel *et al.*, 2004; Haag-Wackernagel, 2005, Gelati et al. 2007).

There are many methods for the control of overabundant urban pigeons, some of which cannot be applied in urban environments (shooting with firearms, etc.); for such purposes, some urban authorities rely on the capture and surgical sterilisation or translocations while some others rely on the "capture and euthanasia" of pigeons, which generates some negative reaction in the population and involves elevated costs with poor results (Sol & Senar, 1995). Fertility control using Nicarbazine, a well-known drug, could offer a humane, effective method to manage overabundant populations of urban pigeons.

Materials and Methods

Nicarbazine. Nicarbazine-treated food to be administered to free-living urban pigeons was formulated as maize grains containing 800 ppm of Nicarbazine (Ovistop® ACME) . The drug is attached to the grains by means of a patented process that prevents its release into the environment and is delivered in doses of 8-10 g per pigeon per day.

Methods. This treatment has been applied on urban pigeons in different italian cities and towns, in accordance with the following guidelines:

- The choice of colonies to be treated with Nicarbazine was fixed on the basis of problems for urban hygiene and monument protection referred by local municipalities and dues to pigeons pollution
- Identification, localisation and quantitative assessment of turban pigeons and their colonies (Bibby and Burgess, 1988);
- Locations of the points of urban areas where the drug is to be distributed and the times and intervals of administration;
- Staff training for distribution of Nicarbazinee and for data finding and monitoring from the colonies of pigeons during the treatment;
- Distribution of 6.4-8 mg of Nicarbazinee per pigeon per day, 5 days a week, from mid march to mid october, thus covering the whole reproductive cycle of urban pigeons.

Drugs were generally distributed in the corners of squares, in pavements, flowerbeds or restricted areas of public parks where the pigeons were already used to gathering, although in some cases platforms (and also raised platforms) were used in order to avoid the usual types of disturbance generally found in squares and to ensure that the birds ingested the dose of medicine both quietly and quickly. The best time for administration was identified as shortly after sunrise; in any case, 6:00 a.m. was a suitable time because of the absence of both disturbing passers-by and traffic and because it preceded the possible arrival of people willing to provide urban pigeons with food (bread, pasta, rice and grain). The drug was distributed directly on the ground so as to ensure ease of access, which ensured the complete and rapid intake of the scheduled dose. This method (Ferraresi *et al.*, 1997) also eased the tasks of the staff, i.e. spotting any drug residue 20-30 min. after baiting and noting any substantial variation in the consumption and time. This point is pivotal to ensure that the dose provided is adjusted on a daily basis according to the numbers of pigeons feeding at the location. The drug was administered 5 days per week and was suspended at weekends.

Data collection and processing. Data on the number of treated colonies was collected immediately prior to treatment with Nicarbazine; in many cases, we collected data at the end of treatment and, in some cases, we collected data also during post-treatment.

Pigeon counts were carried out prior to the start of treatment, by visiting colonies in the areas of high pigeon concentration (where pigeons were attracted by food provided by passers-by) immediately after dawn, in order to determine the correct dose of Nicarbazinee preparation to be administered. in certain cases, counts were repeated at the end of october, upon termination of the seasonal program. in some cities, data on colony density was collected every 12 months (once a year, prior to the start of treatment) while in other cities, it was possible to repeat the count every 6 months (at the start and at the end of the yearly treatment). A graph showing the historic pigeon counts in the cities monitored over various years has been created. Data on the six-monthly or annual pigeon counts between cities was interpolated with regression lines in order to estimate change trends in population numbers, as observed over the course of time. Analyses were carried out using the sa 1999/2000 SAS/STAT^{tm} statistical package. For those cities for which pluriannual pigeon quota statistics were not available, we used the t test for paired data to test the statistical significance of pre/post-treatment numerical variations. Counts of pigeons were square root- transformed to normalize the data (Fowler *et al.* 1986).

Results

Besides monitoring the use of Nicarbazine to control fertility in dovecot pigeons, its use on urban pigeon colonies in many cities and towns was also monitored, often over extended periods (**Fig. 1, 2, 3, 4**), thus permitting us to acquire data on 552 treated colonies. During the 1996-2007 period, the pre-treatment count of pigeons in such colonies was 85562. The pre- and post-treatment counts of many monitored cases in the same calendar year (27054 pigeons in 400 colonies) are also available, while in some cases counts are taken just once a year (58508 pigeons in 152 colonies); These counts were usually taken immediately prior to administration of the drug, particularly in cities and towns with very complexes technical or human situations whose census-related difficulties require some modification of the management protocol.

In general, we observed that there were consistent numerical reductions in treated colonies in the initial phase of administration, while subsequent treatment stabilised the average size of the pigeon colonies of the observation interval.

For some cities, a series of pluriannual colony monitoring data is available, with both six-monthly and annual data (**Tab. 1**). In such cases, we used the regression coefficient to express the reduction in the number of pigeons for every 6-month and yearly period, analysing how this occurred in a consistent manner and confirming a significant negative trend for the timeframe in question. Some cities in particular, such as Mantova and Como, recorded reductions in excess of 200 units over a one-year period and Florence recorded changes in excess of 1000 units. In relating such variations to the average size of the entire pigeon population in the periods in question, we obtained the average reduction percentages, net of the various pigeon community numbers. The average variation was in excess of 10% in all cities except Carpi and Udine.

The significance of the statistical analysis models adopted is attested by the values of regression coefficients. Moreover le values of R^2 (model goodness-of-fit index), included between 40% and even 80%, are relevant. In one particular case (city of Como), the regression coefficient turned out to be insignificant due to the fact that it was estimated on the basis of 3 data sources, and therefore with a non significant number of observations, in order to attain statistical significance, considering that even in such a case the average reduction was highly significant (39%). Furthermore, even in the first 18 months of treatment (2 seasonal cycles of treatment, **Tab. 2**), the average expected variation was of 30 % (35% average of the 4 variations): in these cases the t test for paired data equaled 7.21 with $P< 0.01$ (result not reported in table), therefore with average variations that significantly differed from 0 and which indicate that treatment with Nicarbazine was significant in reducing the average pigeon numbers.

In some cities the effects of the suspension or interruption of the administration of Nicarbazine were also recorded, and in these cases, such as in the city of Carpi (**Fig. 1**), it was found that the pigeon populations increased in number; the graph showing the number of pigeons in these cities during the timeframe in question shows the abrupt increase in pigeon numbers during the period in which treatment was suspended (1998, 1999), from just under 400 units as recorded in October 1997 to dore than 1200 units in March 2000. Similar results were also found in the city of Mantova (**Fig. 2**), where the interruption of treatment in October 2004 determined an abrupt increase in the number of pigeons, confirming the fact that if treatment tends to reduce the number of pigeons, interrupting treatment determines a return of fertility, which tends to restore initial pigeon numbers.

Discussion

The data provided clearly shows the efficacy Nicarbazine in urban pigeon fertility treatment. our overall experience of the pharmacological treatment with this drug in dovecot and in urban pigeon colonies (Ferraresi et al., 1997; Ferraresi et al., 1998; Gelati et al, 2000; Ferraresi et al., 2000; Zannetti, 2000; Bursi et al., 2001; Ferraresi and Gelati, 2001), spanning several years (1996-2007), highlighted some peculiarities that deserve individual mention:

Efficacy: The initial tests (Martelli et al., 1992,1993) with the Nicarbazinee in the pigeons highlighted differences in individual susceptibility in terms of efficacy in reducing pigeon fertility with Nicarbazine doses ranging from 50 ppm to 230 ppm in pelleted (extruded) feed, whereas the overall fertility (living and lively nestlings / expected nestlings) of the pairs treated with 400 ppm was 0%. Anatomo-pathological examinations underlined morpho-functional alterations of the reproductive system (hypoplasia of spermatogonial cells, follicles without oocyte), but without jeopardising the overall health of the pigeons

In the successive researchs, a substantial drop in the number of pigeons in the colonies treated was recorded and, particularly, the density recorded over that time was much lower than that of pre-treatment levels. furthermore, in a few cases, treated and non-treated colonies were simultaneously monitored, revealing that, while the number of pigeons in treated colonies were reduced, non-treated colonies (control colonies) remained stable or saw an increase (in Carpi, Ferraresi *et al.,* 1997; in Genoa, Albonetti *et al.,* 2003), while in the event of interruption or suspension of the administration of Nicarbazinee, an increase or stabilisation of colony numbers was noted (**fig. 1, 2**). The evaluation of a regression coefficient in the reduction of pigeon numbers in the towns where annual or six-monthly counts are taken, confirm a significant negative trend (**tab. 1**) during this period. Some towns, such as Mantova and Como, reported reductions of more than 200 units in the space of one year; in Florence, changes by more than 1000 have been reported. Comparison of this data to the average size of the entire population shows that the average reduction percentage over all towns taking part in the trial is consistently above 10%, with the exception of Carpi and Udine. The statistical significance of the models was confirmed (**tab. 1**) by r^2 values of between 40% and even 80%. The regression coefficient for the town of Como is not significant since it is calculated on the basis of just 3 data sources, and does not attain sufficient significance, although the average reduction in pigeons numbers is significant (39 %). Finally, some towns with a series of six-monthly data records, are evaluated on the regression coefficient and r^2 in the first 18 months of treatment with Nicarbazine (**tab. 2**), confirming that during this time the numerical reduction effect on the treated pigeon colonies is more active; subsequently, there is a marked tendency to the stabilisation of an average number of pigeons in the same colonies, with a characteristic plateau in the more advanced periods of post-treatment observation

<u>*Toxicity*</u>: No toxic effects were observed during the above mentioned research and the mortality in the treated colonies remained within usual and predictable limits in all the moments of the single tests, as is confirmed with numerous tanatological controls in pigeons died pre-, post- and during treatments with Nicarbazinee.

These findings are in accord with the pharmacological and pharmacokinetic observations of Ubaldi and Fusari (2000), which revealed the following:

- the highest Nicarbazine concentration in blood is recorded 9 hours after ingestion;
- the absorption rate is so high that 14% of the drug ingested is absorbed within one hour;
- 70% of the drug is absorbed from the digestive tract into the blood within Tmax (9 hours);
- 8 hours after ingestion, half of the drug absorbed has already been eliminated;
- after 56 hours, over 99% of the drug has been eliminated.

More toxicity studies (ACME dossier for commerce authorisation, 1997-2002) on this drug highlighted that with a daily supply of 8-10 g per head, which means providing 6.4-8 mg of Nicarbazine per head per day, a good tolerability was recorded for correctly performed interventions throughout the peak reproductive period (180 days). In addition, trial monitoring showed no change in mortality as regards treatment with a Nicarbazine dose of 24 mg per head per day, equivalent to 30 g of commercial product (Ovistop®) for 180 days, which by far exceed the recommended dose; the highest non-lethal dose was estimated to be 430 mg/kg, equivalent to about 210 g of Ovistop® per pigeon per day. Consequently, so long as it is used as recommended, the drug could be considered well tolerated by and harmless to the pigeon colonies undergoing the treatment..

Effects reversibility***:*** the population increase recorded after suspension of the programme (Carpi, Florence, Mantova; **fig. 1,2,5**) once again proves the reversibility of the pharmacological effect of Nicarbazinee. . This is an important element for a pharmacological approach to birth control in a wildlife population, also crucial for assure the safe use for any product or drug to be used on the wild or non domestic animals without negative environmental impact, also as regard the EU and italian rules and laws.

Treatment failure and unexpected results: in a few cases, not only did fertility not decrease as expected, but in fact increased (**figures 1,2,5**), but other observations gived good explanations for these failures as, for example, delay in initiating administration programme due to unexpected logistic and organisational problems (for example, discontinuous or irregular distribution of Nicarbazinee during the established period or errors in evaluation of pigeon population or location of the colonies: Giunchi *et al*., 2007).

In some cases (about 60 % of the colonies treated with Nicarbazinee), we observed a dramatic drop (up to 71 %) in the relative pigeon populations, also in absence of real increase in mortality rates, as if the same colonies partially "disintegrate" (Ferraresi et al., 1997). The AA. believe that this partial disintegration" of a treated colony can be attributed to migration and to the non-migration of many young and subdominant pigeons, that usually account for the majority of the population (Johnston and Janiga, 1995). The resulting infertility and the absence of nestlings in colonies treated with Nicarbazinee, presumably eliminates or greatly reduces reproductive and social stimulation and competition, in pigeons whose physical conditions are likely to have been improved by the antiparasitic effect of the drug. A lack of interest in the colony and abandonment of the colony without fertile eggs and chicks have already been observed in the ring-billed gull (*Larus delawarensis)* during trials involving of "egg oiling" for demographic reduction (Pochop *et al.* 1998) and it has generally been noted that birds leave areas where survival or reproductive prospects are poor, searching other areas where conditions are better (Newton, 1998).

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TABLE 1: Regression analysis results on the number of pigeons in the timeframe in question, broken down by city.

CITY	TIME INTERVAL	REGRESSION COEFFICIENT	R^2 MODEL %100	Average population	Average % reduction
6-monthly data					
Carpi	2000-2007 a	-38**	46	410	9
Mantova	2000-2004 b	-125**	59	781	16
Senigallia	2004-2007	-82*	65	468	18
Udine	2000-2007	-32**	42	510	6
Annual data					
Florence	1999-2004	-1478*	74	9390	16
Como	2005-2007	-280	85	722	39

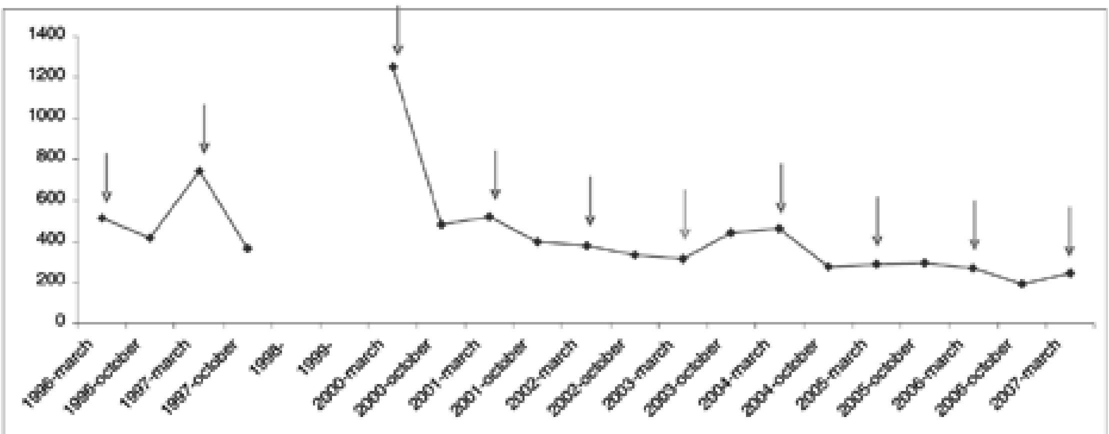
a: the pre-2000 period is not included in the analysis (suspension of treatment)
b: the post-2004 is not included in the analysis (interruption of treatment)
*: P<0.05; ** P<0.01

Table 2 Effect of the treatment in the first 18 months (2 treatment cycles).

City	Time interval	Regression coefficient	R^2 model %100	AVERAGE POPULATION	AVERAGE % REDUCTION
CARPI	2000-2001	-252	68	510	50
MANTOVA	2000-2001	-348*	71	1224	28
SENIGALLIA	2004-2005	-177	80	556	32
UDINE	2000-2001	-197	52	623	32

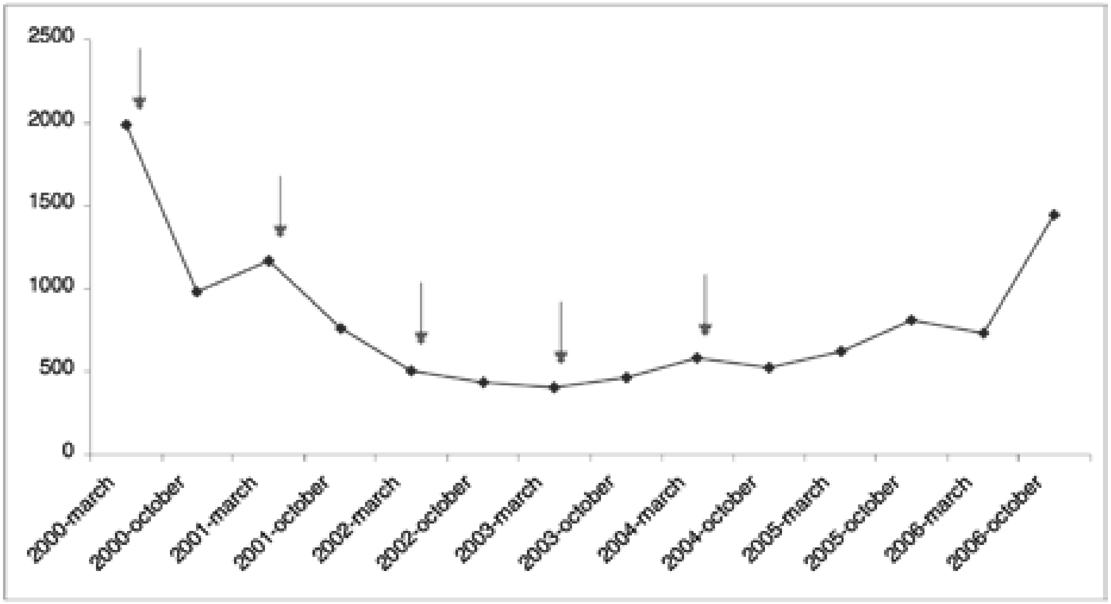
*: P<0.05

FIGURE 1: Pigeon number trend in the period in question (city of Carpi – six-monthly data). estimated equation: y = -38.082 x + 714.72



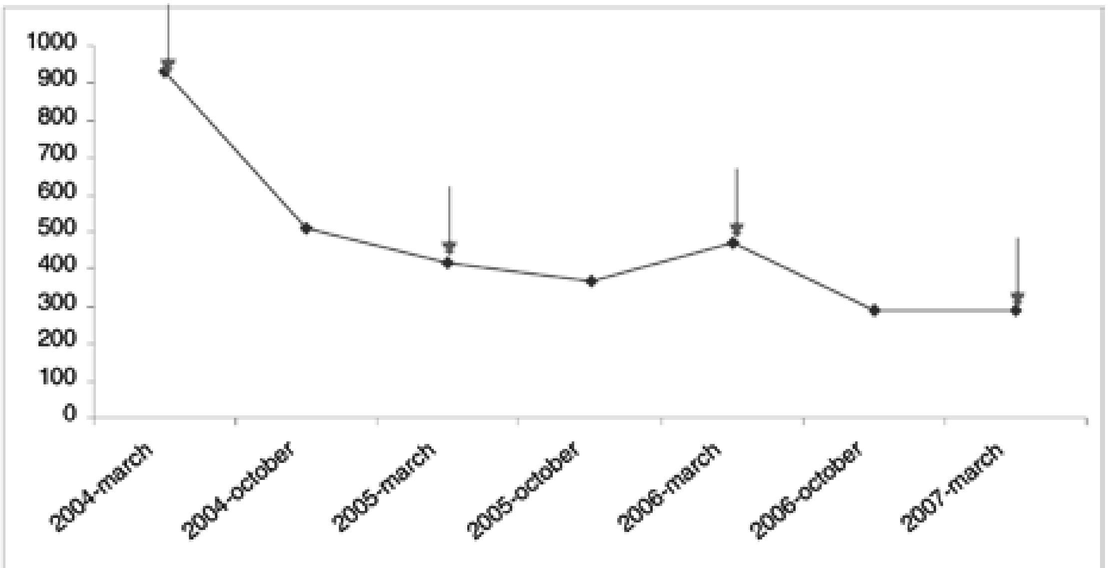
Note: black arrows: treatment starts with Nicarbazine gray arrow: some problems of Nicarbazine distribution to pigeons

Figure 2 Graph of the pigeon number trend in the period in question (city of Mantova – six-monthly data). Estimated equation: y = -124.85 + 1467.5



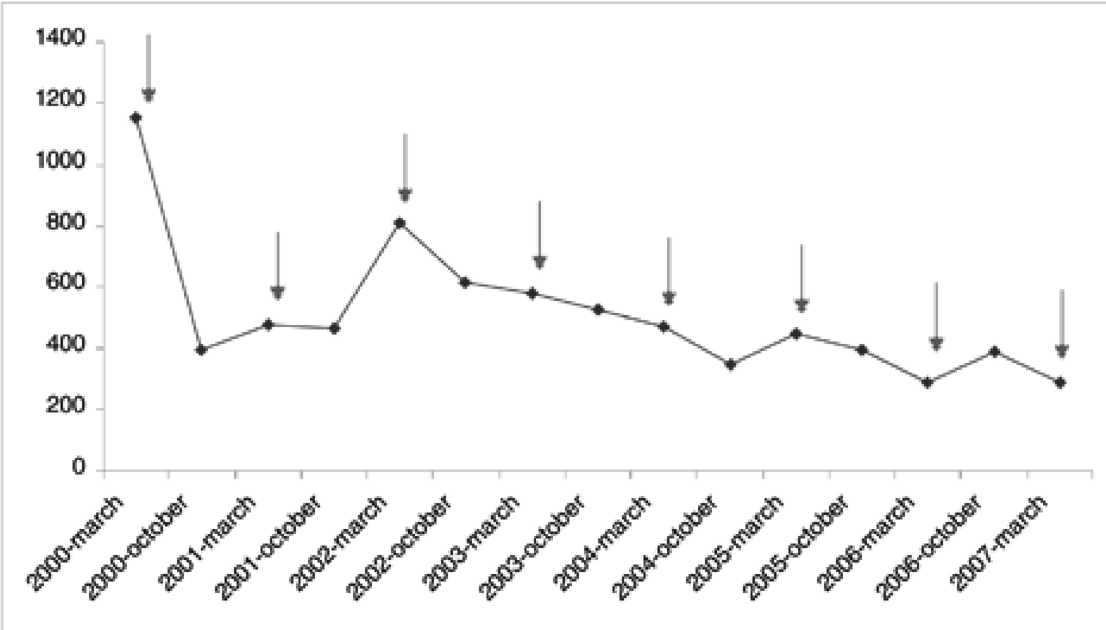
Note: black arrows: start of treatment with Nicarbazine

FIGURE 3 Pigeon number trend in the period in question (city of Senigallia – six-monthly data). estimated equation: y = -82.25 x + 797.14



Note: black arrows: start of treatment with Nicarbazine

FIGURE 4 Pigeon number trend in the period in question (city of Udine – six-monthly data). estimated equation: y = -32.189 x + 768.05



Note: black arrows: start of treatment with Nicarbazine

FIGURE 5. Pigeon number trend in the time interval (city of Florence – annual data). treatment is commenced immediately after the annual count. estimated equation: y = -1471.8 x + 14563

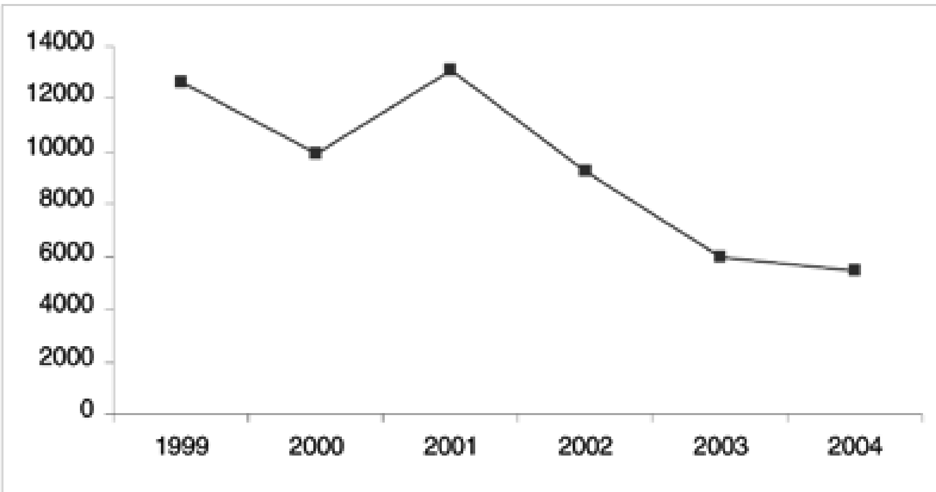
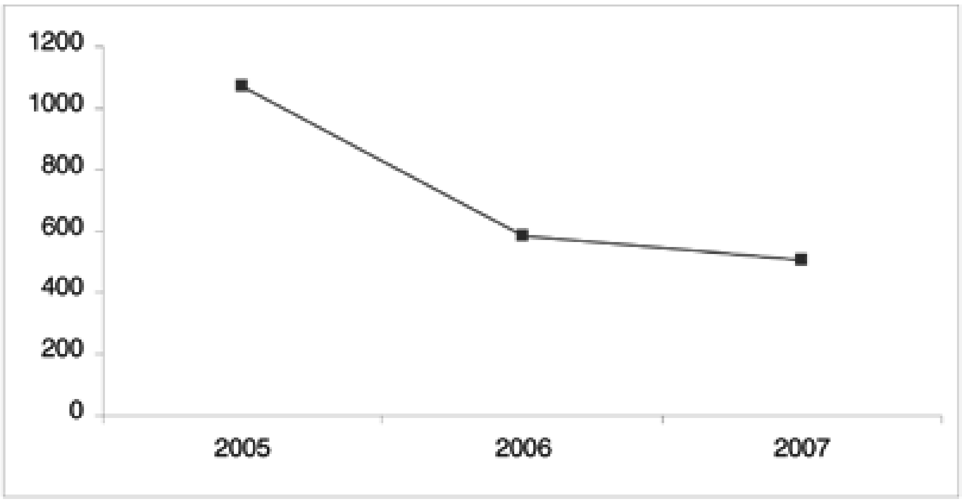


Figure 6 Pigeon number trend in the time interval (city of Como, annual data)). Treatment is commenced immediately after the annual count. Estimated equation: y = -280 x + 1283



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MUCOKYNETIC DRUGS: IN VITRO RHEOLOGICAL STUDY ON HORSE MUCUS

FARMACI MUCOCINETICI: ATTIVITÀ REOLOGICA IN VITRO SUL MUCO DI CAVALLO

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Structured Summary

Objectives - To evaluate the mucolytic activity of N-acetylcysteine (NAC) at different concentrations (2.5%, 5%, 10%, 20%) by measuring, *in vitro*, variations in viscosity and elasticity of mucus samples using a dynamic viscosimeter.

Methods - Mucus samples were collected from 60 adult saddle horses (9-17 years old, mean 14.6) belonging to different breeds, with clinical signs of IAD (inflammatory airway disease), destined for slaughter. The activity of mucolytic agent on rheological properties and viscoelasticity of tracheobronchial secretions was evaluated in comparison to physiological saline solution. Further evaluations were carried out comparing parameters with Bromhexine (0.3%) and with Dembrhexine (0.5%).

Results – Equine mucopurulent tracheobronchial secretions are characterized by high viscosity and elasticity. Mucus viscoelasticity was greatly reduced by 0.9% physiological saline solution: 39-49% viscosity *vs* 31-43% elasticity. NAC resulted in a significant difference ($p<0.05$) between the samples diluted with mucolytic solutions at higher concentrations in comparison to the samples diluted with saline solution alone; the trend for elasticity was similar. Bromhexine and Dembrhexine caused a drop in viscosity and elasticity in the samples diluted with either saline solution or other drugs, however, compared to that of 0.9% physiological solution, no significant difference was observed (Physiological sol. *vs* Bromhexine: $p=0.336$; Physiological sol. *vs*: Dembrhexine: $p=0.503$). The same trend was observed with the elasticity data (Physiological sol. *vs* Bromhexine: $p=0.260$; Physiological sol. *vs*: Dembrhexine: $p=0.560$).

Clinical Significance - The endotracheal administration of mucokinetic agents may be recommended for an improved therapeutic response aimed at eliminating stasis and accumulation of mucus. The choice should, however, be made for direct acting mucolytic agents, such as N-acetylcysteine, and at high concentrations.

Keywords: horse; N-acetylcysteine; bromhexine; dembrhexine; respiratory diseases.

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